BIODEGRADATION OF AGRO-WASTES BY SOME NIGERIAN WHITE-ROT FUNGI

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Three white-rot fungi: *Daedalea elegans*, *Polyporus giganteus*, and *Lenzites betulina* were screened for their lignin degrading abilities on rice straw, maizecob, sawdust of *Terminalia superba*, and sugarcane bagasse at different time intervals (30, 60, and 90 days). All the fungi demonstrated varying levels of ligninolytic capability with different degrees of lignin degradation in all the fermented substrates. A significant difference ($p<0.05$) was observed in the mycelia extension of *Daedalea elegans* grown on the different agro-industrial wastes. *D. elegans* gave maximum extension of 4.5 cm on sugarcane bagasse. The highest lignin reduction of 92.9% ($p<0.05$) was recorded in maize cob fermented with *Daedalea elegans* after 90 days. On the basis of lignocellulosic material degraded, it is concluded that the white-rot fungi offer a better alternative to conventional ways of disposing these waste substances. This paper considers the ability of indigenous white-rot fungi to degrade lignin as a way of using them in effective waste management.

Keywords: Lignocellulosic biomass; White-rot fungus; Agro-wastes; Substrates

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INTRODUCTION

Lignocellulosic biomass from plants is the most abundant class of agricultural residues in the world (Kuhad *et al.* 1997). It accounts for more than 60% of the total biomass production (Kuhad *et al.* 1997). The accumulation of these lignocellulose-containing wastes in our environment is on the increase and poses significant environmental problems. Up to this point, there has not been any major effort towards remediating these wastes, apart from the burning of biomass (Levine 1996). Biomass burning and landfill are the common major routes of disposing these wastes in Nigeria (Okonko *et al.*, 2009). A lot work has been directed towards optimization of the physical, chemical, and biological processes for the conversion of abundant agro-waste for value-added products (Okonko *et al.* 2009; Abbot *et al.* 2009). The most ambitious of these have been the conversion of lignocelluloses to alternative energy carriers (Kaylen *et al.* 2000). Others have been aimed at improving digestibility of nutritionally poor forage (Adenipekun and Fasidi, 2005).

Lignocelluloses bioconversion is naturally slow and limited to a few microorganisms, due to its complex heterogenous structure (Marcelo *et al.* 2001). The most efficient lignin-degrading microorganisms are white-rot fungi (Falcon *et al.*, 1995; Orth and Tien 1995). Although several studied have been done on the ability of temperate exotic white-rot fungi to degrade lignin in lignocellulosic wastes, few studies has been done on tropical white-rot fungi, especially Nigerian ones. This present study

was therefore undertaken to determine the ability of some Nigerian white-rot fungi to degrade lignin in lignocellulosic wastes.

EXPERIMENTAL

The Fungi

The pure cultures of *Daedalea elegans, Pleurotus florida, Polyporus giganteus, Schizophyllum commune, Lenzites betulina,* and *Corilopsis occidentalis* were obtained from the Plant Physiology/Biochemistry Laboratory, Department of Botany and Microbiology, University of Ibadan.

The substrates used for this study were rice straw, maizecob, sawdust of *Terminalia superba*, and sugarcane bagasse. The rice straw was collected from the International Institute Tropical Agriculture (IITA), Ibadan. The maize cob and sugarcane bagasse were collected from local corn and sugarcane sellers in Bodija Ibadan, and the sawdust was obtained from Mobalufon sawmill in Ijebu-ode, Nigeria. The agro wastes were sun-dried and milled into 5mm diameter pieces.

Evaluation of Mycelia Growth on Agro-Industrial Wastes

Growth on different substrates was evaluated according to the method of Fasidi and Kadiri (1993) and according to the modified method of Braun *et al.* (2000). 10g each of unfermented sawdust of *Terminalia superba*, hulled maize cob, rice straw, and sugarcane bagasse were soaked in hot water containing 2% (w/v) calcium carbonate for 30 minutes at 80 ^{o}C. The soaked substrate was then pressed to remove excess water until the moisture content was about 60% (w/w) and then put into 10 cm (diameter) Petri dishes. The Petri dishes with the substrates were sterilized at 121 ^{o}C for 15 minutes. After cooling, each substrate was inoculated at the center with a 9 mm agar plug of a 7-day-old actively growing pure culture of *Daedalea elegans, Pleurotus florida, Polyporus giganteus, Schizophyllum commune, Lenzites betulina,* or *Corilopsis occidentalis* mycelia and incubated for 7 days at 30^{o}C$\pm$ 2^{o}C. Each treatment was replicated thrice. The growth diameter was measured as the increase in mean colony diameter along two lines, drawn perpendicularly to each other and mycelia density was compared visually (Fasidi, 1996)

Screening for Lignin Degradation

Substrate pretreatment

Substrate treatment was done according to the modified method of Braun *et al.* (2000). Twenty-five grams of each of unfermented milled dry substrate particle size (1mm) were weighed into screwed-cap bottles. Seventy-five milliliters of distilled water were added to the substrates and covered with aluminum foil to avoid loss of moisture. The fresh weight of the substrate was determined and the bottles were autoclaved at 121^{o}C for 15 min.

The bottles were inoculated with two agar plugs (diameter: 9mm) of white-rot fungi mycelia, in triplicates and were incubated in a very clean, dark cupboard for 30, 60, and 90 days at 30^{o}C$\pm$ 2^{o}C (ambient temperature) at constant moisture level. Similarly

prepared bottles without fungal inoculum were incubated as a control. The controls were put in an oven at 80°C ± 2°C for 48 hours to determine the initial dry weight of the substrate. Harvested samples were also put in the oven for 48 hours at 100°C ± 2°C to remove moisture.

Lignin Determination

The acid detergent lignin (ADL) of the samples was determined according to the method of the Association of Analytical Chemists (AOAC), (2004) as modified by the method of Nahm (1982). Two grams of the oven-dried samples were weighed into 250m glass measuring flasks suitable for refluxing. One-hundred milliliters of cold acid detergent solution made up of 20g of Cetyl trimethyammonium bromide (CTAB) added to 1 liter of 1N H_2SO_4 and 2ml decahydronapthalene were added and the contents were heated to boiling in 5-10 minutes. Refluxing of the sample was done at 40°C for 60 minutes from the onset of boiling and the sample filtered after refluxing using a vacuum pump. The samples were washed repeatedly with acetone until it removed no more colour and then dried at 105 °C for eight hours. This was then allowed to cool in a desiccator to room temperature and the acid detergent fiber (ADF) determined as follows:

$$\text{ADF} = \frac{\text{Weight of crucible with sample} - \text{weight of crucible}}{\text{Original sample weight}} \times (100/1) \qquad (1)$$

The acid detergent fiber (ADF) sample obtained was covered with 72 % H_2SO_4 and stirred with a glass rod to a smooth paste, by breaking all the sample lumps. This was allowed to stand for three hours, after which the acid was filtered off as much as possible with a vacuum pump. The sample residue was washed with hot water (85°C to 95°C) until it was free from acid. This was dried in a crucible overnight at 100°C and re-weighed. The crucible with the content was ignited in a muffle furnace at 500°C for 3 hours. The samples were allowed to cool to 250°C and then transferred to a desiccator to cool to room temperature and the weight determined. The lignin content was thus calculated as:

$$\text{ADL}(\%) = \frac{\text{Dry weight of crucible + lignin} - \text{Dry weight of crucible + ash} \times 100\%}{\text{Original weight of ADF sample}} \qquad (2)$$

pH Determination

pH determination was carried out according to the method of Braun *et al.* (2000). 4g of each of the substrates were soaked in 80ml distilled water for 18hours at room temperature.

Analysis of Data

Data represented above are means of 3 replicates. Values followed by the same letter along each vertical column are not significantly different by Duncan's multiple range test ($p \leq 0.05$)

RESULTS AND DISCUSSION

Higher Fungi Used

The six fungal species were used for these studies were as follows: *Daedalea elegans* has sessile fruit body with a short lateral stem (1-2cm long). It has a daedeliod white hymenium when fresh, which becomes brown when dried. *Pleurotus florida* has an oyster shell-shaped cap. They may be sessile, lacking a stalk, or have a very short lateral stalk. *Polyporus giganteus* is white, flashy, and usually attached to a substratum by a narrow stem base. It is not edible because of its leather-like or tough texture. The *Schizophyllum commune* fruit body is xerophytic and leathery, and the cap is grayish white and fan-shaped, usually small, ranging from 1-4cm in width. The fruit body is laterally attached to its substratum. *Lenzites betulina* has a semicircular, leathery annual bracket covered in fine hairs and zoned in shades of brown. The underside has gill-like pores, characteristic of this genus. *Corilopsis occidentalis* sporophores are coriaceous to corky, leathery when fresh, and rigid when dry. The hymenial surface is poroid, and sometimes subdeadaloid near the center. All the white-rot fungi produced white spore prints, which were hyaline and smooth.

Evaluation of Mycelia Growth on Agro-Industrial Wastes

All the fungi showed white mycelia that ramified the entire substrates surface after 7 days of incubation. However in sugarcane bagasse the ramification was faster and showed a denser mycelium than in the other substrates (Table 1). Mycelia growth on rice straw possibly resulted in a reduced fungi mycelia ramification (3.0cm) with *Daedalea elegans* and less dense fungi mycelium on all the substrates (Table 1). These variations were not statistically significant (Table 1).

Screening for Lignin Degradation

All the fungi were observed to induce a significant reduction in lignin content of all the agro-wastes substrates as the incubation period increased (Tables 2-4). At zero days, maize cob fermented with *D. elegans* had the most lignin content (14.0%), which reduced significantly to 1.0% after 90 days. The highest percentage reduction (92.9%) was observed in *D. elegan*s fermented maize cob (Table 2), followed in order by *D. elegans* fermented rice straw (84.1%), and *P. giganteus* fermented sugar cane bagasse (65.8%), while the least lignin content reduction (29.6%) was in *L. betulina* fermented rice straw (Table 3).

The growth of the white rot fungi influenced a decrease in the pH of the agro-wastes as the incubation period increased (Tables 2-4). The lowest pH value of 3.2 was observed in sugarcane bagasse fermented with *P. giganteus* after 90 days of incubation (Table 3). These changes in pH values were observed in other agro-wastes fermented with different white rot fungi. The pH values decreased as the incubation time increased for all the agro-wastes (Tables 2-4).

Table 1. Mycelial Growth of Different White Rot Fungi on Agro-Industrial Wastes

Isolates	Rice Straw		Corncob		Sawdust of *T. superba*		Sugarcane bagasse	
	Mycelial extension (cm)	Mycelial density	Mycelial extension (cm)	Mycelial density	Mycelial extension (cm)	Mycelial density	Mycelial extension (cm)	Mycelial density
S. commune	4.6[a] ±0.01*	+5	3.2[c] ±0.00	+1	4.7[b] ±0.01	+5	5.5[b] ±0.01	+7
D. elegans	3.0[c] ±0.00	+2	4.7[c] ±0.01	+4	4.5[d] ±0.01	+4	4.8[d] ±0.00	+4
L. betulina	4.2[b] ±0.01	+3	5.2[b] ±0.02	+7	4.7[b] ±0.01	+5	5.0[c] ±0.00	+7
P. florida	3.2[c] ±0.00	+1	4.3[d] ±0.00	+3	4.6[cd] ±0.01	+4	5.0[c] ±0.00	+4
P. giganteus	4.2[b] ±0.01	+5	5.8[a] ±0.02	+6	5.3[a] ±0.01	+8	5.8[a] ±0.01	+8
C. occidentalis	4.5[a] ±0.01	+2	4.5[cd] ±0.00	+3	4.6[cd] ±0.00	+5	4.8[d] ±0.00	+5

*=Mean of three replicate± standard error

Initial size of inoculum = 9.0mm; Each value is the mean of three replicates; Values followed by the same letter(s) along each vertical column are not significantly different by Duncan's multiple range test (p≤0.05).

Table 2. Changes in Lignin Content and pH Values of Agro-Industrial Wastes Fermented with *D. elegans*

Substrates	Days of Incubation	% Lignin value	% Lignin reduction	pH
Rice straw	0	15.8[a] ± 0.01*	0.0[d] ± 0.00	7.5
	30	8.2[b] ± 0.01	48.4[c] ± 1.00	6.7
	60	4.5[c] ± 0.01	71.8[b] ± 2.50	6.4
	90	2.5[d] ± 0.05	84.1[a] ± 2.50	5.2
Maize cob	0	14.0[a] ± 0.01	0.0[d] ± 0.00	6.7
	30	4.1[b] ± 0.05	70.5[c] ± 1.00	4.9
	60	3.5[c] ± 0.05	75.0[b] ± 1.00	4.5
	90	1.0[d] ± 0.05	92.9[a] ± 1.00	4.2
Sawdust of *T. superba*	0	27.5[a] ± 0.01	0.0[d] ± 0.00	5.8
	30	26.9[a] ± 0.01	2.3[c] ± 1.00	4.8
	60	23.8[ba] ± 0.03	13.6[b] ± 1.00	3.9
	90	14.6[b] ± 0.03	46.8[a] ± 1.50	3.6
Sugarcane bagasse	0	18.0[a] ± 0.01	0.0[d] ± 0.00	4.5
	30	9.2[b] ± 0.01	48.7[c] ± 1.00	3.9
	60	8.3[c] ± 0.01	54.1[b] ± 1.00	3.3
	90	7.3[d] ± 0.01	59.7[a] ± 1.00	3.3

*=Mean of three replicate ± standard error
Values followed by the same letter(s) along each column are not significantly different by Duncan's multiple range test (p≤0.05). Each value is an average of three replicates.

Table 3. Changes in Lignin Content and pH Values of Agro-Industrial Wastes Fermented with *L. betulina*

Substrates	Days of Incubation	% Lignin value	% Lignin reduction	pH
Rice straw	0	15.8[a] ± 0.01*	0.0[d] ± 0.00	7.5
	30	8.7[b] ± 0.01	46.1[b] ± 1.00	5.7
	60	8.2[b] ± 0.01	48.0[b] ± 1.00	6.0
	90	6.1[c] ± 0.01	61.6[a] ± 2.50	5.2
Maize cob	0	14.0[a] ± 0.01	0.0[d] ± 0.00	6.7
	30	11.5[b] ± 0.03	25.2[c] ± 1.00	4.7
	60	6.8[c] ± 0.01	58.8[b] ± 2.50	4.8
	90	5.8[d] ± 0.01	65.9[a] ± 2.50	4.3
Sawdust of *T. superba*	0	27.5[a] ± 0.01	0.0[d] ± 0.01	5.8
	30	15.5[b] ± 0.01	43.6[c] ± 2.50	4.2
	60	14.7[c] ± 0.01	50.3[b] ± 2.50	4.4
	90	12.2[d] ± 0.01	55.7[a] ± 3.00	4.2
Sugarcane bagasse	0	18.0[a] ± 0.01	0.0[d] ± 0.01	4.5
	30	17.2[a] ± 0.01	4.6[c] ± 1.00	4.4
	60	12.3[b] ± 0.01	31.8[b] ± 2.50	3.9
	90	10.5[b] ± 0.01	41.7[a] ± 2.50	3.4

*=Mean of three replicate± standard error
Values followed by the same letter(s) along each column are not significantly different by Duncan's multiple range test (p≤0.05). Each value is an average of three replicates.

Table 4. Changes in Lignin Content and pH Values of Agro-Industrial Wastes Fermented with *P. giganteus*

Substrates	Days of Incubation	% Lignin value	% Lignin reduction	pH
Rice straw	0	15.8[a] ± 0.01*	0.0[c] ± 0.00	7.5
	30	10.3[b] ± 0.01	34.9[b] ± 1.50	6.4
	60	8.9[cb] ± 0.01	43.7[ba] ± 1.50	6.7
	90	8.2[c] ± 0.01	48.0[a] ± 1.50	6.3
Maize cob	0	14.0[a] ± 0.01	0.0[d] ± 0.00	6.7
	30	10.4[b] ± 0.01	25.6[c] ± 1.00	6.2
	60	9.5[c] ± 0.02	31.9[b] ± 1.00	6.1
	90	6.3[d] ± 0.02	55.2[a] ± 3.00	5.2
Sawdust of *T. superba*	0	27.5[a] ± 0.01	0.0[b] ± 0.00	5.8
	30	18.0[b] ± 0.01	34.6[c] ± 1.00	4.9
	60	17.8[b] ± 0.01	35.5[c] ± 1.00	4.4
	90	11.9[c] ± 0.01	56.6[a] ± 2.00	4.4
Sugarcane bagasse	0	18.0[a] ± 0.01	0.0[d] ± 0.00	4.5
	30	16.3[b] ± 0.01	9.4[c] ± 0.05	3.9
	60	12.3[c] ± 0.05	31.5[b] ± 1.00	3.2
	90	6.2[d] ± 0.05	65.8[a] ± 3.00	3.2

*=Mean of three replicate± standard error
Values followed by the same letter(s) along each column are not significantly different by Duncan's multiple range test (p≤0.05). Each value is an average of three replicates.

All of the substrates were observed to support mycelial growth of the white-rot fungi. However, sugarcane bagasse supported the fastest mycelial extension, followed in order by sawdust of *T. superba*, maizecob, and rice straw, respectively. The rapid colonization of the different agro-waste by the white-rot fungi mycelia observed in this study may be due to their saprophytic ability. This result is in agreement with the findings of Kadiri (1990), Fasidi and Ekuere (1993), and Jonathan *et al.* (2008), that white-rot fungi as a group of basidiomycetes have high saprophytic ability to grow on agro-wastes. The cultivation of edible mushroom using agricultural residues is a value-added process to convert these materials, which are otherwise considered to be wastes, into human foods.

Significant reduction in lignin content of the agro-industrial wastes was recorded in all the agro-wastes fermented with the white-rot fungi. One of the goals of biological delignification using white-rot fungi is to make as much possible of the digestible substrate carbohydrate (Adenipekun and Fasidi 2005). Zadrazil (1985) and Braun *et al.* (2000) reported loss in organic matter and lignin content of substrates brought about by the growth of white-rot fungi including *Pleurotus sajor-caju*, *Ganoderma lucidium*, and *Lenzites betulina.* In this study loss of %lignin content was higher for substrates fermented by *D. elegans* in comparison to *P. giganteus* and *Lenzites betulina* with increasing incubation time. Results obtained from this study showed that biodegradation rates of maize cob was highest with *D. elegans* and *Lenzites betulina* and sugarcane bagasse with *P. giganteus*. This may probably be due to the fact that lignin biodegradation depends on fungal species, substrates, and incubation time. It has been shown that lignin, C/N ratio, and N contents of crop residues would affect their decomposition rate (Muller *et al.* 1988; Summerell and Burgess 1989; Jonathan *et al.* 2008). Residues like sawdust of *T. superb* with high lignin and low readily available C and N contents generally have slow decomposition rates (Janzen and Kucey 1988; Parr and Papendiek 1978).

The changes in pH values of the white rot fermentation agro-wastes as the period of fermentation increased may be linked to the increase in metabolic products within the substrates. Fungal growth has been known to cause changes in pH of the straw mycelium (Zadrazil 1977).

CONCLUSIONS

1. From this study, it was observed that the white-rot fungi were able to reduce lignin in the agro-wastes significantly.

2. This observation has shown these fungi as potential degraders of recalcitrant substances and this could inform their uses in further research for bioremediation of polluted soils.

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