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Bioconversion of Sawdust and Paper Treaded with Edible Fungus (*Pleurotus pulmonarius*)

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Authors' contributions

This work was carried out in collaboration between both authors. Authors JME and SAL designed the study. Author JME performed the study and wrote the first draft of the manuscript. Author SAL supervised the study. Both authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to determine the effect of *Pleurotus pulmonarius* on proximate composition and solid-state fermentation of sawdust and waste paper, and to quantify the soluble sugar produced in the biodegraded samples. Studies were carried out on the biodegradation of sawdust and paper using *P. pulmonarius* in cultures incubated for 90 days. The results of proximate analysis showed decrease in moisture content crude fiber and hemicellulose, while ash and cellulose were increased. Sawdust has higher soluble sugar content at 30-90 days of fermentation. The crude fibre decreased significantly from 45.5% to 32.4%, 48.2% to 32.9%, 55.5% to 32.8%, 53.4 to 32.1%, 44.5% to 27.5% for sawdust (SD), paper-waste (PW), and three proportional ratio mixtures (PW1:SD3, PW3:SD1, PW1:SD1) substrates respectively. The nutrient contents, pH values, (%) fiber fractions showed significant differences of ($P \leq 0.05$) in the five substrates as the fermentation. Lignin contents of treated paper waste and sawdust were degraded by *P. pulmonarius* and a decrease was observed. There were also consistent significant decreases ($P < 0.05$) in the values obtained for NDF, ADF, ADL. Percentage NDF 75.4% to 57.32%, 68.1% to 57.05%, 67.21% to 56.8%, 75.49% to 58.36%, 74.3% to 59.13% for SD, PW, PW1:SD3,

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PW3:SD1, and PW1:SD1 substrates, respectively. Significant differences were observed in the hemicellulose and cellulose contents. The results obtained from the bioconversion process of selected wastes reveals the potential of extracellular enzymes produced by *P. pulmonarius* as a biotechnological tool for the transformation of waste in biological product.

Keywords: Pleurotus pulmonarius; solid-state fermentation; sawdust; waste-paper; bioconversion.

1. INTRODUCTION

Agriculture wastes, which include sawdust and paper are among the cause of environmental pollution, their conversion to useful product may ameliorate the problems they cause. Proper biotechnological civilization of these wastes in the environment will eliminate pollution and convert them into useful by products [1]. Most agricultural residues are rich in lignocellulosic compounds whose handling and disposal are often problematic due to their chemical structure and decomposition properties. Cotton waste including stem-leaf residues and gin trash, wheat straw, paper waste and sawdust from timber industry are of particular interest to the agricultural economy of temperate and sub-tropical countries, since they are produced in large quantities and their post-harvest treatment is mainly accomplished through burning or incorporation into the soil.

Bioconversion refers to the use of life organism often microorganism to carry out a chemical reaction that is costlier or not feasible non-biologically. The conversion of organic materials (such as wastes) into an energy source (such as methane), by bioprocessing (such as fermentation) involve the use of whole microorganisms or their components such as enzymes. These microorganisms convert a substance to a chemically modified form to produce energy, a catabolic process by living organism [2]. Mushroom is a macro fungus with a distinctive fruiting body, which can either be epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand. Mushrooms are saprophytes and grow well on organic matter [3]. In particular, edible mushroom fungi which possesses appropriate enzymatic mechanisms for the transformation of complex organic macromolecules into simple compounds, has been exploited as the means for the biodegradation of a wide range of plant litter due to their particular ability for selective delignification [4].

The white-rot fungus *P. pulmonarius*, is a wood decaying basidiomycete 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 [5], and this feature make it find application in different biotechnological processes. The efficiency of the biodegradation process depends on the potential of the organisms introduced in wastes and cultivation conditions. Sawdust is a very common agricultural waste in some regions and is a prospective substrate for the bioconversion into fungal biomass and enzymes due to chemical composition [6].

Fungal treatment of sawdust will not only help to solve the problem of burning but will also assist in converting the waste into a useful form (such as feed) which can be utilized by ruminant animal, and the process is environmentally friendly and can be adopted in the developing countries. White-rot fungi has been used in converting masonia tree sawdust and agricultural wastes into value added ruminant feed [7,8]. Thus, the aim of this study was to investigate the potentials of fungal treatment of sawdust and paper wastes into utilizable ruminant feed and the impact on the proximate composition and the nutrient contents of the biodegraded fractions.

2. MATERIALS AND METHODS

2.1 Sample Collection

Dried samples of agricultural wastes (sawdust) were collected from sawmills at Garin-alimi in Ilorin, Kwara state, Nigeria. The paper pulp was collected from paper mills at Opomalu, Ilorin. Each of these raw materials was dried and grounded to pass through a 30 mm mesh sieve.

2.2 Media Preparation

Freshly prepared Potato Dextrose Agar (PDA) was freshly prepared in in the lab and poured into the Petri-dish. This was inoculated with *P. pulmonarius* and kept in the sterilized inoculation chamber.

2.3 Preparation of Substrates

The method of Adenipekun and Fasidi [9] was employed. Briefly, 50 g of each dried samples were weighed into three sterile jam bottles, in ratio 1:0, 0:1; 1:1, 3:1,1:3 for sawdust and paper respectively and 80 ml of distilled water were added. The bottles were immediately covered with aluminum foil and sterilized in the autoclave at 121ºC for 15 minutes.

2.4 Inoculation

Each of the bottles was inoculated at the center of the substrate with two agar plugs (7 mm in diameter) of vigorously growing mycelia disc and covered immediately. They were kept in a clean dark cupboard in the laboratory at 30ºC and 100% relative humidity. The experiment was replicated three times and the bottles were harvested after 30, 60, and 90 days, and the dry weight was determined. The experimental bottles were harvested by autoclaving to terminate the mycelia growth. Biodegraded samples were oven dried at 80ºC to constant weight for chemical analysis.

2.5 The pH Determination

The pH determination was carried out by adding 80 ml of distilled water to 4 g of the substrate in clean bottles. After 18 hrs at room temperature, the pH of the suspension was measured using electronic pH meter. The experiment was done in triplicate.

2.6 Sample Analysis

The moisture content, crude fibre and ash content (AC) were determined according to AOAC method [10]. Neutral detergent fiber (NDF), Neutral detergent solubles (NDS), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were determined using the method described by van Soest et al. [11]. The %NDS was evaluated by subtracting %NDF from 100%. Hemicellulose was calculated as the difference between NDF and ADF while cellulose content was the difference between ADF and ADL [12].

2.7 Soluble Sugar

Cellulose was assayed using 3, 5 dintrosalicylic acid (DNSA) and the amount of reducing sugar formed were determined according to Denison and Koehn [13].

2.8 Data Analysis

The data were obtained in triplicates and subjected to analysis of variance and test of ANOVA significance was carried out by Duncan's multiple range tests.

3. RESULTS AND DISCUSSION

The result showed pH range between 7.69 - 5.49 for the incubation period as shown in Fig. 1. It was observed that the pH decreased gradually as the incubation days were elongated, although the fall in pH was more between day 30 and 60 than any other period of sampling in this study. 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. Study has showed that biological treatment reduces the pH of the fermented samples [14].

One goal of biological delignification or bioconversion using lignin degrading fungi is for effective digestibility of the substrate carbohydrate. This can be achieved by rapid lignin degradation [9]. In this study, *P. pulmonarius* degradation of the substrates; sawdust (SD), paper-waste (PW), and their three proportional ratio mixtures (PW1:SD3, PW3:SD1, PW1:SD1), caused changes in composition of the substrates as shown in Table 1. The moisture content decreased with increase in incubation period (I.P) in the order of while the total ash increased with increase in incubation period in the order of SD> PW> PW1:SD3> PW3:SD1> PW1:SD1, notwithstanding, moisture content and percentage of crude fiber (CF) as they both decreases with increase in incubation period (I.P).

The decrease in CF could be due to cellulase and hemicellulose (HC) being secreted by cellulolytic fungi for extensive utilization of cellulose and hemicellulose. Safari et al., [15] has reported crude fiber loss in wheat, barley and rice straw incubated with *P. chrysosporium,* while degradation of banana leaves decreased in crude fibre fractions due to the production of various enzymes during the vegetative and reproductive phases with lignocelluloses degrading properties [16]. Additionally, the decrease in CF fraction may due to the fungal utilization of part of the produced fermentable sugar produced by the action of hydrolyzing

enzyme fungus on the substrate of amylase which then stimulates the fungus to produce high enzyme activities. The biodegradation of waste by associated enzyme has been reported by Arotupin [17].

As shown in Table 1, the percentage of NDF decreases with preceding in the incubation periods for all the substrates in the order of PW3:SD1> PW1:SD1> SD> PW> PW1:SD3 while the reverse results for NDS as they both increased with increase in incubation days in the order of PW1:SD3> PW> SD> PW3:SD1> PW1:SD1. Hemicellulose was calculated as NDF-ADF and cellulose as ADF – ADL. The cellulose content increased while the hemicelluloses content was found to be decreasing with an increase in fermentation time. This preferential changes in cellulose and hemicellulose could be as a result of the type of substrate, duration of degradation and physiological behaviours of the fungi used as proposed by Jonathan et al. [18], and Chen et al. [19].

The %ADF, %ADL, silica, and hemicellulose decreases, with increase in incubation period while the cellulose increase slightly (Table 1). The decreased observed in neutral detergent fibre (NDF) after 4 weeks (30 days) of fermentation may be due to the extensive utilization of hemicellulose by the test fungus and this result corroborate the report of Chen *et al.*, [19]. The crude fibre (CF) and acid detergent fibres (ADF) values which decreased in the treated substrates and similar observation was report by Nasreen et al. [20]., The decrease observed in ADF and ADL for each of the substrate as the fermentation day increases showed similar decrease reported in previous studies [18,21,22]. This decrease could be due to the production of various lignocelluloses enzymes such as lignin peroxidise, manganese peroxidase and laccase, during the vegetative and reproductive phases as reported by Isikhuemhen and Nerud [23].

In this present work the highest soluble sugar content was observed to be at incubation period of 90 days and the substrate PW1:SD3, followed by PW1:SD1 as shown in Fig. 2. The amount of soluble sugar produced by each substrate differs depending on the amount of carbon source utilized by the organism. It was observed that soluble sugar increased significantly all through the period of incubation on the five substrates with increase in incubation time until an optimum production was attained. Subsequent increase in incubation time beyond the optimum led to a decline in production. This result showed that nutritional improvement of sawdust and waste paper is possible and the inclusion of its subsequently biodegraded product in animal feed can be pursued with expected better utilization.

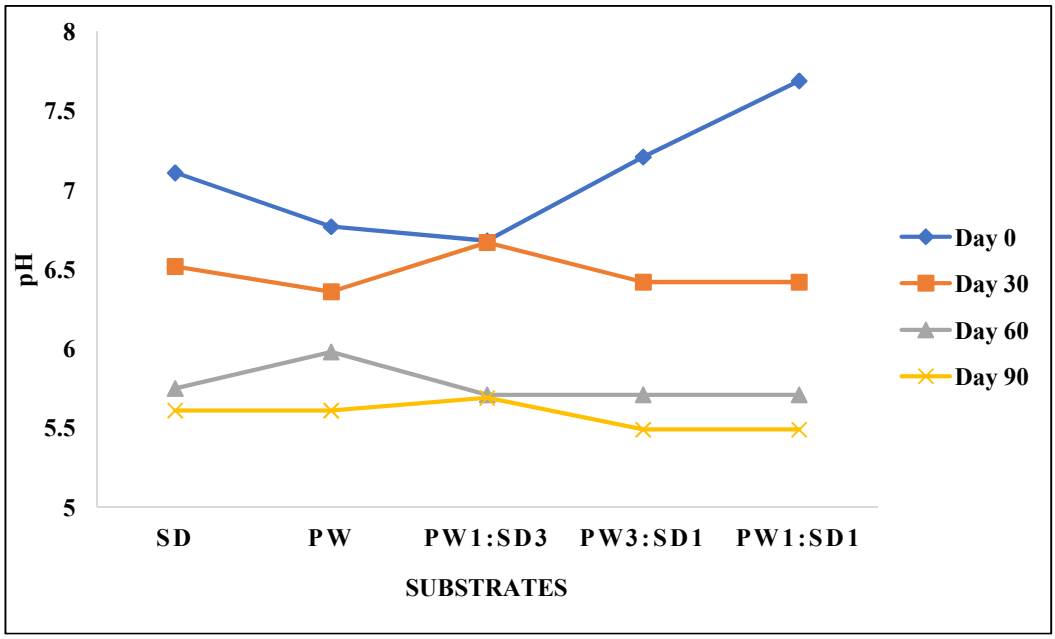

Fig. 1. Effect of *P. pulmonarius* on pH of biodegraded substrates

Table 1. Changes in composition of substrates upon fermentation by *Pleurotus pulmonarius* during 0-90 days incubation

Substrate	I. P. (Days)	Ash	Moisture	%CF	%NDF	%ADF	%ADL	%NDS (100 - %NDF)	Hemicellulose (NDF-ADF)	Cellulose (ADF-ADL)
SD	0	0.50^{a}	3.10^{a}	45.50^{d}	75.40^{d}	59.53^{b}	39.77^{d}	24.60^{a}	15.87^{d}	19.76^{a}
	30	3.10^{a}	1.60^{a}	42.60^{d}	68.39^{d}	55.29^{b}	35.05^{c}	31.61^{a}	13.10^{d}	20.24^{a}
	60	7.80^{b}	1.42^{a}	36.20^{c}	62.37^{c}	47.21^{a}	26.21^{b}	37.63^{a}	15.16^{d}	21.00^{b}
	90	8.40^{b}	1.45^{a}	32.40^{c}	57.32^{c}	43.41^{a}	16.80^{a}	42.68^{b}	13.91^{d}	26.61^{c}
PW	0	0.90^{a}	3.81^{a}	48.20^{d}	68.10^{d}	56.30^{b}	36.20^{d}	31.90^{a}	11.80^{c}	20.10^{a}
	30	1.51^{a}	1.32^{a}	45.28^{d}	66.25^{d}	54.25^{b}	31.40^{c}	33.75^{c}	12.00^{d}	22.85^{b}
	60	5.30^{b}	1.43^{a}	37.33^{c}	62.15^{c}	51.14^{a}	27.30^{b}	37.85^{b}	11.01^{c}	23.84^{b}
	90	7.20^{b}	1.71^{a}	33.90^{c}	57.05^{c}	46.06^{a}	18.50^{a}	42.95^{b}	10.99^{c}	27.56^{c}
PW1:SD3	0	0.30^{a}	3.66^{a}	55.20^{d}	67.21^{d}	62.10^{c}	39.10^{d}	32.79^{b}	5.11^{a}	23.00^{b}
	30	3.40^{a}	1.10^{a}	48.40^{d}	62.17^{c}	57.23^{b}	35.20^{d}	37.83^{b}	4.94^{a}	22.03^{b}
	60	3.80^{a}	0.38^{a}	42.30^{d}	58.13^{c}	54.10^{b}	28.41^{b}	41.87^{b}	4.03^{a}	25.69^{c}
	90	3.08^{a}	1.39^{a}	36.80^{c}	56.80^{c}	49.40^{a}	26.02^{b}	43.20^{b}	7.04^{b}	23.38^{b}
PW3:SD1	0	0.80^{a}	3.76^{a}	53.40^{bd}	75.49^{d}	67.20^{c}	38.30^{d}	24.51^{a}	8.29^{c}	28.90^{c}
	30	0.91^{a}	1.04^{a}	46.70^{d}	71.42^{d}	64.16^{c}	34.70^{c}	28.58^{a}	7.26^{b}	29.46^{d}
	60	1.10^{a}	1.78^{a}	42.80^{d}	65.38^{c}	58.24^{b}	29.60^{b}	34.62^{a}	7.14^{b}	28.64^{c}
	90	1.80^{a}	1.72^{a}	32.10^{c}	58.36^{c}	52.30^{a}	27.20^{b}	41.64^{b}	6.06^{a}	25.10^{c}
PW1:SD1	0	0.43^{a}	3.73^{a}	44.50^{d}	74.30^{d}	60.50^{c}	38.05^{d}	25.70^{a}	13.80^{d}	22.45^{b}
	30	1.01^{a}	0.99^{a}	38.30^{c}	69.36^{d}	57.40^{b}	32.02^{c}	30.64^{a}	11.96^{c}	25.38^{c}
	60	1.01^{a}	1.24^{a}	32.40^{c}	65.22^{c}	54.30^{a}	25.01^{b}	34.78^{a}	10.92^{c}	29.09^{d}
	90	1.02^{a}	1.41^{a}	27.50^{c}	59.13^{c}	49.01^{a}	19.70^{a}	40.87^{b}	10.12^{c}	29.31^{d}

Means with different superscripts in each column are significantly different at P ≤ 0.05 by Duncan's multiple range test

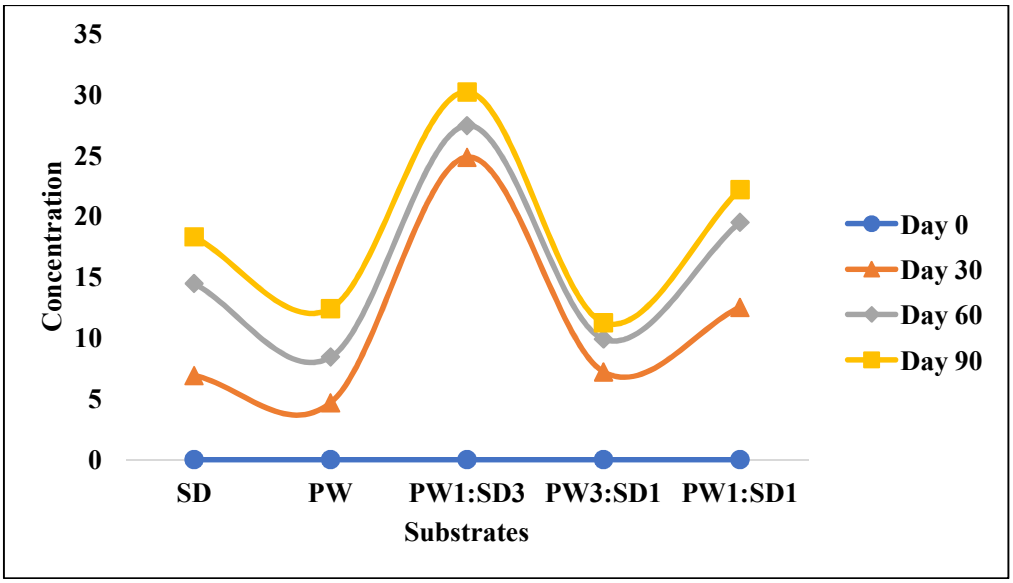

Fig. 2. Effect of *P. pulmonarius* on soluble sugar content of biodegraded sawdust and paper

4. CONCLUSION

The result of proximate analysis revealed that sawdust and paper contain considerable amount of carbohydrate which stimulate the cell to many hydrolytic enzymes one of which is soluble sugar presence. In addition, it contains appreciable amount of utilizable simple sugars which encourages growth initiation and protein which can serve as essential nitrogenous compound. The results of the present study showed that *Pleurotus pulmonarius* can be successfully cultivated on waste materials. This indicates that these wastes contain nutrients that support the growth of this mushroom. This can be attributed to the ability of mushroom substrates to support the growth of mushrooms in the presence of lignin, cellulose and mineral element. Thus, fungal treatment of wood sawdust can be used as feed for ruminants. The bioconversion process of selected wastes reveals the potential of extracellular enzymes produced by *P. pulmonarius* as a biotechnological tool for the transformation of waste in biological product.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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