Biodegradation of agricultural wastes (rice straw and sorghum stalk) into substrates of utilizable products using white rot fungus(*Pleurotus florida).*

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Abstract: Problem of disposal of solid wastes such as agro-industrial wastes and other pollutants is a major concern in the developing countries of the world. In this study, emphases were focused on conversion of agricultural wastes such as rice straw and sorghum stalk into substrates of utilizable products (wastes to wealth). These wastes were subjected to solid state fermentation for 40 days using *Pleurotus florida* (a white rot fungus from Nigeria). Nutritional analyses were carried out on both fermented and non fermented substrates (control).Nutrients in the biodegraded samples were observed to be increasing with the days of incubation. The results also showed that fermented rice straw and sorghum stalk were richer in nutrients than none fermented substrates. It was also observed that the biodegraded samples enhanced in-vitro digestibility in the tested animals. Biologically treated sorghum stalk were found be richer in nutrients than the treated rice straw. The implications of these observations were discussed.
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1. Introduction:

As long as the earth will be in existence, there will be abundance of wastes from homes, market places, agric farmland, industries etc. Wastes are the so called 'useless' or 'unwanted' remnants after the useful products have been annexed. Sallam *et al.*, (2007), suggested that the agricultural wastes will continue to increase due to increase in agricultural produce as a result of increasing population of the world.

Among common agro –industrial wastes are wood wastes, brewing wastes, paper wastes, cassava wastes, cotton wastes and cereal wastes(Gbolagade,2006;Jonathan *et al.,*2008) In west Africa especially Nigeria,rice,maize,millet and sorghum are among the staple food of the inhabitants. After the harvest of main grains, the remains of these cereals usually constitute nuisance to agricultural environment and are often being dispose off by burning which further compound environmental pollution inadequacy and kill useful microorganisms in the soil which usually maintain soil fertility cycle(Jonathan *et al*.,2011). Small portion of these wastes are being utilised as animal feeds which is highly deficient in necessary nutrients. Agricultural wastes possessed high level of hemicelluloses and recalcitrant lignin which are not easily digestible by the ruminants.

Lignin, which physically and chemically forms a complex with cellulose and hemicellulose, make polysaccharides less accessible to ruminal digestion by blocking access to rumen bacteria and their enzymes (Karunanandaa *et al.,* 1995). Besides, these rumen microorganisms do not perform ligninolytic activity (Zadrazil *et al*.,1995;Zadrazil and Isikhumhen,1997).

Microbiological de-ligninfication is required to be less energy consuming process where the amount of carbohydrate consumption by the organisms needs to be minimum in respect of deligninfication rate. This method is being in use for improving ruminant feed value as well as its digestibility, thereby upgrading the economic value of lignocellulosic wastes (Zadrazil, 1982; Karma *et al*., 1993; Dhanda *et al.,* 1994; Reid, 1995).

Pleurotus florida is an edible mushroom that was first cultivated in Germany as a subsistence measure during World War I.This fungus usually grow in their large numbers during the rainy season (Gbolagade *et al.* , 2006). Mushrooms are rich in proteins, vitamins, and minerals and popularly called as the vegetarian's meat (Gbolagade, 2005;Aina *et al*.,2012). Mushroom proteins are considered to be intermediate between that of animals and vegetables (Gbolagade *et al*.,2006, Jonathan and Adeoyo,2011) as it contains all the nine essential amino acids required for human body (Aina *et al*., 2012). Cultivation of this Mushroom is very simple and low cost production technology, which gives consistent growth with high biological efficiency.

The cultivation of edible mushrooms offers one of the most feasible and economic method for the

bioconversion of agro-lignocellulosic wastes (Gbolagade,2005;Gbolagade,2006;Jonathan *et al*.,2008). The technology can also limit air pollution associated with burning agriculture wastes as well as to decrease environmental pollution due to unutilized agricultural wastes. The concept of preferential delignification of lignocellulose materials by white rot fungi has been applied to increase the nutritional value of forages (Akinfemi *et al.,* 2005; Zadrazil and Isikhuemhen, 1997). Since studies have shown that the cultivation of edible fungi on these wastes help in their bioconversion and there is dearth of this information on this matter, it is therefore necessary to examine the influence of cultivating *Pleurotus florida* on the chemical composition and invitro digestibility of sorghum stalk and rice straw.

This study was therefore conducted to provide information on the possibility of converting sorghum stalk and rice straw into value added feedstuff for ruminant feed through solid state fermentation of these wastes and to have a comparative study on the degradability and digestibility of the two wastes through invitro digestibility.

2. Materials and Methods

2.1 The substrates:

The agricultural substrates sorghum stalk and rice straw were obtained from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. They were cut into small pieces and dried in the oven (55^0 C) for 24hrs

2.2 Fungi:

Pleurotus florida used for this study was obtained from the University of Ibadan Botanica;l Gardens and tissue cultured using the method of Gbolagade *et al*.,2006).The pure culture thus obtained were maintained on slants of PDA(oxoid)

2.3 Preparation of substrates:

Twenty five grams (50.0g) of the substrates were weighed into each jam bottles and 1400ml of distilled water was added to moisten and then squeezed out into the bottles using cheese cloth. The bottles were covered with aluminium foil and sterilized in the autoclave at 121^0 C for 15 minutes. Each treatment was done in triplicate.

2.4 Inoculation:

Inoculation was done with 10g spawn of *Pleurotus florida* from actively growing culture (Jonathan *et al*. ,2012). The bottles were incubated in the dark at 30^0C at 100% relative humidity for 40days, but samples were analyzed at every interval of 10 days from the day of inoculation. The fermented samples were oven dried at 65^0C .

2.5 Microbiological examination:

Microbiological evaluation of the substrates were carried out using the procedure of Gbolagade (2006). One gram (1.0g) of biodegraded and non-biodegraded substrates were serially diluted and plated out using plate count method, and the microorganism isolated were counted and expressed as colony forming unit (CFU/ml).

2.6 Proximate compositions:

The nutrients evaluated were Dry matters (DM) . This was quantified using the method of Pearson (1975). Crude protein (CP), ether extracts (EE) (Crude fat) and ash content (AC) were analysed using the method of AOAC method (1995). Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were also evaluated (Van Soest *et al.* 1991). Hemicellulose was calculated as the difference between NDF and ADF while cellulose was also determined using the procedure of Zadrazil(1982). The difference between ADF and ADL was taken as cellulose composition

2.7 In vitro gas production:

Gastric fluid were obtained from experimental goats using the method of Babayemi and Bamikole (2006). Suction tube were used to obtain the rumen fluid from goats that have been previously fed with 40% concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal) . The rumen fluid was collected into the special flasks that had been pre warmed to a temperature of 39°C from the before they were offered the morning feed (Babayemi, 2007).

Incubation was carried out using the standard method of Menke and Steingass (1988).Three replicates of 200mg each of the sample were weighed into a pre weighed bags and sealed(Jonathan *et al,*.2012). 30 ml inoculua containing cheese cloth strained rumen liquor and buffer (g/liter) of 9.8 NaHCO3 + 2.77 $Na2HPO_4$ + 0.57 KCl + 0.47 NaCl + 2.16 $MgSO_3$ $7H_2O$ + 16 $Cacl_2$. $2H_2O$) (1:2 v/v) was dispensed using another 50 ml plastic calibrated syringe. The syringe was tapped and pushed upward by the piston in order to completely eliminate air in the inoculums. The infusion clip in the syringes was then locked so as to prevent escape of gas. Incubation was carried out at 39 ± 1°C and the volume of gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24h. After incubation, methane production was quantified using the method of Fievez *et al*. (2005). The bags introduced to the rumen were removed, washed and dried in the until constant weight was obtained to quantify percentage digestibility using the formula:

$$\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

After incubation, metabolisable energy, organic matter digestibility and short chain fatty acids were quantified using the method of Menke and Steingass (1988). Gas production after 24hrs was also determined(Akinfemi *et al*.,2009).The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample against the incubation time and from the graph, the gas production characteristics were estimated using the equation Y = a+b (1-e-ct) as described by Orskov and McDonald (1979). Where Y = volume of gas produced at time t, c, = intercept (gas produced from the insoluble fraction (b), t = incubation time. Metabolisable energy (ME) was calculated as ME = 2.20 + 0.136Gv + 0.057CP + 0.0029 CF (Menke and Steingass, 1988), while organic matter digestibility (OMD) (%) was assessed as OMD = 14.88 + 889Gv + 0.45CP + 0.651XA (Menke and Steingass, 1988). Short chain fatty acids (SCFA) were estimated according to the procedures Getachew *et al.,* (1999) w Crude protein, crude fiber and ash were determined using AOAC method (1995).

2.8 Statistical analysis:

The data obtained from these studies were subjected to analysis of variance and test (ANOVA) and test of significance was carried out by Duncan's multiple range tests.

3. Results and Discussion

Microbiological analyses of fermented and non fermented substrates were presented on Table1. It was observed that there were no significant increases in all the parameters considered (total aerobic count, total coliform and *Escherichia coli* count) in the fermented substrates. This results indicate that secondary metabolites must have been produced by the fermenting microorganisms discouraging other microbes from growing((Gbolagade,2006)

Table 1. Microbial Population of untreated substrates and 40 days treated substrates.

	Total aerobic count	Total coliforms	*Escherichia coli*
URS	150	39	22
RS	0	0	0
USS	125	34	14
SS	0	0	0

URS =Untreated rice straw, RS = rice straw, USS = Untreated sorghum stalk, SS = sorghum stalk.

Table 2 and 3 represents the chemical compositions (g/100gDM) of the treated and the untreated substrates according to their days of harvest, which showed significant difference of (P<0.05). The crude protein and ether extracts increased for the 10 – 40 days treatment. Rice straw increased from 4.50% to 4.52%, 4.60%, 5.19% and 7.03% while sorghum stalk increased from 5.31% to 5.47%, 5.61%, 6.30% and 7.32% respectively. Ether extracts for rice straw increased from 1.36% to 1.47%, 1.49%, 1.59% and 1.82% while sorghum stalk increased from 1.17% to 1.26%, 1.37%, 1.49% and 1.70% respectively. The improved crude protein may be as a result of the addition of fungal biomass to fermented substrates (Jonathan *et al*., 2008). It could also be due to the capture of excess nitrogen by aerobic fermentation (Sallam *et al*., 2007, El-shafie *et al*., 2007). The ether increase indicates energy availability to the animals, as this agrees with (Verma 2006, Odedire and Babayemi 2008). Ether extracts are referred to as lipids and act as stores of energy.

Table 2. Chemical composition (g/100g DM) of untreated and 10 – 40days *Pleurotus florida* treated rice straw.

Parameters	**URS**	**10 days**	**20 days**	**30 days**	**40 days**
Dry matter	74.2^{a}	70.9^{a}	66.3^{b}	60.5^{c}	54.7^{c}
Crude protein	4.50^{c}	4.52^{c}	4.60^{c}	5.19^{b}	7.03^{a}
Ether extract	1.36^{d}	1.47^{c}	1.49^{c}	1.59^{b}	1.82^{a}
Ash content	12.2^{a}	10.2^{b}	6.25^{c}	4.97^{d}	3.44^{d}
Crude fiber	41.2^{a}	38.5^{b}	35.2^{c}	32.4^{c}	19.5^{d}
NDF	68.9^{a}	67.4^{a}	64.7^{b}	59.9^{c}	52.6^{c}
ADL	26.4^{a}	24.3^{a}	20.6^{b}	17.3^{b}	14.7^{c}
ADF	51.1^{a}	48.5^{a}	42.3^{b}	36.6^{c}	30.4^{c}
Cellulose	24.7^{a}	24.2^{a}	21.7^{a}	19.3^{b}	15.7^{c}
Hemicellulose	17.8^{b}	18.9^{b}	22.2^{a}	23.3^{a}	22.2^{a}

$^{a, b, c}$, means values followed on the same row for each substrate are significantly different by Duncan's Multiple range test (P<0.05). URS = Control for Rice straw,

The crude fiber and crude fiber fractions (NDF, ADL and ADF) decreased in the treated substrates as the days of fermentation were increasing, and this agrees with Sommart *et al*. (2000). This could be as a result of production of various enzymes that degrade the lignin content of the substrates as this agrees with (Tamara *et al*., 1996). The decrease in the CF content showed that the lignin content of the treated substrates was degraded. The degradation of lignin, a complex polymer (Kuforji *et al.,* 2003) is important because using lignin degrading fungi is to make as much as possible the digestibility of the substrates degraded (Akinfemi *et al*., 2009). Karunanandaa *et al.* (1995), have shown that increased digestibility was associated with the degradation of structural carbohydrates

The cellulose and hemicellulose for rice straw ranged from 24.7% to 24.7%, 24.2%, 19.3%, 15.7% and 17.8% to 18.9%, 22.2%, 23.3% and 22.2% respectively while sorghum stalk cellulose and hemicellulose ranged from 17.3% to 19.2%, 19.8%, 20.9%, 22.5% and 19.1% to 18.1%, 18.4%, 19.4%, 15.9% respectively. The decrease of the cellulose and hemicellulose in the two treated substrates may be as a result of extensive utilization of them as energy source, as this agrees with (Jonathan *et al*., 2012).

3.1 Gas volume

Gas production from the treated and untreated substrates were measured at 3, 6, 9, 12, 15, 18, 21 and 24h using invitro gas production technique of (Menke and Steingass, 1988). There are many factors that may determine the amount of gas produced during fermentation, depending on the nature and level of fiber (Babayemi *et al.,* 2004a) and potency of the rumen liquor used for incubation (Babayemi, 2007). There was increase in gas production as the days of fermentation increases with the highest production in maize grain used as a standard. Generally, as cited by Babayemi (2007) gas production is a function and mirror of degradable carbohydrate and therefore, the amount of gas produced depends on nature of the carbohydrates (Demeyer and Van Nevel, 1975, Blummel and Becker, 1997). The two substrates used were improved to a better feed by the fungus (*P.florida)* used indicating increase in digestibility as this agrees with work of Sommart *et al.*(2000) which suggested that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrates by rumen microbes in the *in vitro* system. The difference in gas production of the treated substrates to that of maize grain indicates the difference in their composition, no fiber content of maize grain and presence of lignin which protects carbohydrate from attack by rumen microbes as this confirms the work of (Sallam *et al*., 2007).

Table 3. Chemical composition (g/100g DM) of untreated and 10 – 40days *Pleurotus florida* treated sorghum stalk.

Parameters	USS	10 days	20 days	30 days	40 days
Dry matter	70.7 [a]	68.8 [a]	65.2 [b]	63.7 [b]	56.4 [c]
Crude protein	5.31 [c]	5.47 [c]	5.61 [c]	6.36 [b]	7.32 [a]
Ether extract	1.17 [c]	1.26 [c]	1.37 [b]	1.49 [b]	1.70 [a]
Ash content	7.27 [a]	5.98 [b]	4.24 [c]	3.52	4.75 [c]
Crude fiber	31.2 [a]	28.9 [a]	25.9 [b]	23.9 [b]	21.0 [b]
NDF	64.6 [a]	62.9 [a]	60.9 [b]	56.6 [c]	52.4 [c]
ADL	28.2 [a]	25.6 [a]	22.1 [a]	17.0 [b]	14.0 [b]
ADF	45.5 [a]	44.8 [a]	41.9 [a]	37.2 [b]	36.5 [b]
Cellulose	17.3 [c]	19.2 [b]	19.8 [b]	20.9 [a]	22.5 [a]
Hemicellulose	19.1 [a]	18.1 [b]	18.4 [b]	19.4 [a]	15.9 [c]

[a, b, c] means values followed on the same row for each substrate are significantly different by Duncan's Multiple range test ($P<0.05$). USS = Control for Sorghum stalk.

Estimated methane production from (fig 3) shows low methane production in the treated substrates compared to the maize grain used as standard though they were increasing as the days of fermentation increases. The highest production was seen in treated sorghum stalk while treated rice straw gave the lowest production. Miller *et al* (1995) reported that the reduction in methane production could be due to conversion of CO_2 and H_2 to acetate instead of CH_4. This process mainly occurs when roughages diet containing high proportion of sugars and protein is fed (Leedle and Greeming, 1988).

3.2 Invitro organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acid (SCFA)

Table 4 represent the organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acid (SCFA) estimated for the untreated and

treated substrates. The results showed high values estimated for OMD in all treated substrates with highest values occurring in 30 – 40 days treated substrates. OMD has been shown to have high correlation with gas volume (Sommart *et al.*, 2000; Nitipot and Sommart, 2003). The highest value obtained for OMD was seen in 40 days PFS and PFR. The high volume obtained for OMD in this study implies that the microbes in the rumen and animal have high nutrient uptake (Chumpawudee *et al.,* 2006).

Table 4. Estimated organic matter digestibility (OMD) (%), short chain fatty acid (mol) and metabolizable energy (MJ/Kg DM) of untreated and 10 - 40 days treated rice straw and sorghum stalk.

	OMD	ME	SCFA
URS	31.892	3.664	0.1311
R10	35.874	3.609	0.2745
R20	40.379	5.556	0.4657
R30	40.575	5.854	0.5135
R40	44.159	6.194	0.5613
USS	30.803	3.953	0.1789
S10	37.413	5.036	0.3701
S20	41.285	5.859	0.5135
S30	42.914	6.168	0.5613
S40	47.666	6.758	0.6569

URS = untreated rice straw, R = treated rice, USS = untreated sorghum stalk, S = treated sorghum.

The reduced CF contents (Table 2 –3) of the fungal treated substrates probably influenced improvement in OMD, since high NDF and ADL contents in feedstuffs result in lower fiber degradation (Van Soest, 1988).

SCFA level indicate the energy available to the animal, it contributes nearly 80% of animal daily energy requirement (Fellner, 2004). SCFA was directly proportional to metabolizable energy (Menke *et al.*, 1979). The result agrees with this because the SCFA increases as fermentation increase giving highest values in 40 days treated substrates. The ME was higher in all the treated substrates with highest values coming from the 40 days treated substrates and this indicates that there is an improvement in energy station of the substrates and thus, the potential of been incorporated in conventional feed mixtures.

A relationship exists between total gas production and ME, SCFA and OMD.

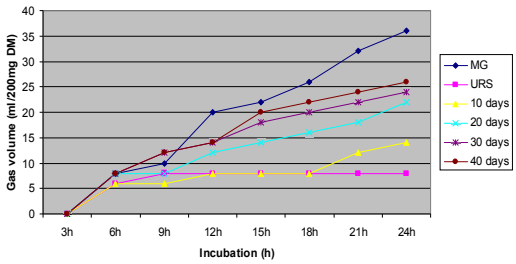

Figure 1: Gas volume for untreated and 10 – 40 days treated rice straw using maize grain (MG) as a standard.
MG = maize grain, URS = untreated rice straw.

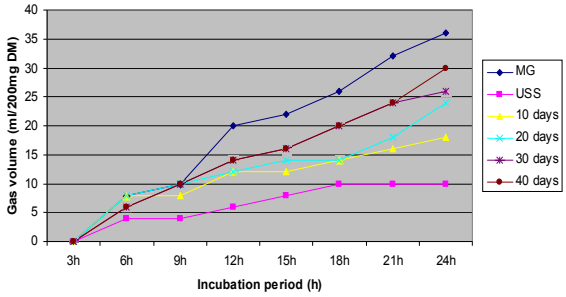

Figure 2: Gas volume for untreated and 10 – 40 days treated sorghum stalk using maize grain (MG) as a standard.
MG = maize grain, USS = untreated sorghum stalk.

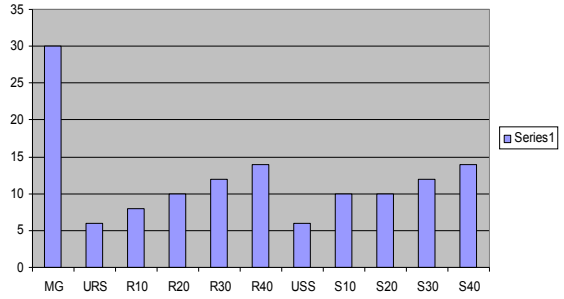

Figure 3: Methane production for untreated and 10 – 40 days treated rice straw and sorghum stalk using maize grain (MG) as a standard. MG = maize grain, URS = untreated rice straw, R = treated rice, USS = untreated sorghum stal

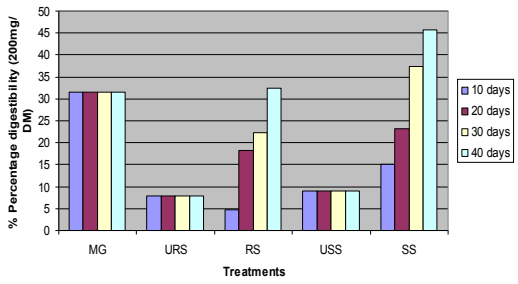

Figure 4: Percentage digestibility (200mg/ DM) of untreated and 10 – 40 Days treated rice straw and sorghum stalks with maize grain (MG) as a standard. URS = untreated Rice straw, USS = untreated Sorghum stalk, RS = Rice straw, SS = *P. florida* Sorghum stalk.

4. Conclusion

Sorghum stalk undergoes more biodegradation as compared to rice straw. This made it to be more digestible by the ruminants. It was observed that as days of fermentation increases, the rate of degradation increases and, this increases the digestibility of the substrates. *Pleurotus florida* proved effective in the process of fermentation and enhanced these two substrates in the process of value added feed for the experimental ruminants.

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