Journal of Applied Biosciences 51: 3633– 3641

ISSN 1997–5902

Effect of *Pleurotus tuber-regium Singer* on degradation of rattan wood and maize stovers

***Adenipekun, C.O., Okunlade, O.A. and Ogunjobi, A.A.**
Department of Botany and Microbiology, University of Ibadan, Nigeria.
*Corresponding author: Adenipekun, C.O.: oyinpek@yahoo.com +234-8055265186
Originally Submitted on 5th January 2012. Published online at www.m.elewa.org on March 29, 2012.

ABSTRACT

Objective: To investigate the potential of *Pleurotus tuber-regium* in the degradation of rattan wood and maize stovers.

Methodology and results: Twenty five (25g) each of the substrates rattan wood and maize stovers were weighed after the addition of 75ml of distilled water. They were covered with aluminum foil and sterilized at 121^0C for 15minutes. Later they were inoculated with two 5mm agar diameter mycelia and incubated at 28 ±2 ^{0} C. The controls were not incubated. This was replicated four times and harvested after 30, 60 and 90days.The proximate composition; percentages of nitrogen, carbon and potassium, pH, loss of organic matter, digestibility and metabolisable energy and *in-vitro* gas digestibility were carried out on the substrates degraded by *P.tuber-regium*. In degraded rattan wood, crude protein increased significantly from 1.60% to 5.90% and on maize stovers 2.75% to 8.74%, crude fibre decreased significantly from 44.68% to 20.92% for rattan wood and maize stovers 32.33% to 13.03% after 90 days of incubation .In both substrates ether extract, ash and dry matter contents also decreased but moisture contents increased from 0-90days.Carbon, Nitrogen and Potassium increased significantly as the incubation period increased. The pH decreased with the least value being 4.55. Loss of organic matter of the substrates reduced significantly as the period of incubation increased. There was also significant decrease in loss of water as the incubation period increased, the lowest being 32.25 % in rattan wood. There was a reduction in fibre analysis and enzyme production but the organic matter digestibility and metabolisable energy increased with the incubation period .Gas production for *in-vitro* gas digestibility increased at three hour intervals with highest volume being 29.00ml at 24hours for maize stovers.

Conclusion and applications of findings: The results showed that environmental wastes can be recycled and controlled by biodegradation and the product would be of nutritive value in compounding ruminant feeds.

Key words: Biodegradation, Rattan wood, Maize Stovers, *Pleurotus tuber-regium.*

INTRODUCTION

There is increased awareness that agricultural and industrial wastes products contribute to increased eutrophication due to excessive discharge of nutrients. Microbial contamination can impair the quality of receiving water bodies by producing offensive odors caused by anaerobic decomposition of organic residues, resulting in unsightly waste storage at land disposal sites (Hamza, 1989). Smith *et al.* (1983) observed that agricultural wastes are the most abundant on earth comprising of 50% of all biomass with an estimated annual production of 50 billion tons. Logging operations in plantations forest usually generate abundant amount of wastes such as residual wood, branches/twigs, leaves and barks. The wastes account for more than 60% of the total

biomass (Kuhad *et al*., 1997).One of the strategies to utilize agricultural wastes and big products is to grow edible fungi such as edible mushrooms that will not only reduce the fibre but also help in obtaining protein rich substrates.

Buswell *et al*. (1996) stated that edible mushrooms are able to bio-convert a wide variety of lignocellulosic materials due to their secretion of extracellular enzymes. In addition to this, Alofe *et al*. (1998) had showed that mushrooms are capable of transforming nutritionally worthless wastes into protein rich food and have been confirmed to be source of single cell protein. The successful utilization of agro-wastes for both mycelia and sporophore formation of macrofungi, supplies the nutrients needed by these fungi to convert them to protein- rich palatable food and this helps in reducing the environmental and health hazards posed by indiscriminate dumping of wastes (Villario *et al*.,1995).

Kuforiji & Fasidi (2008) reported that mushroom hyphae secrete large amounts of cellulase, lipase, amylase carboxymethylcellulase, proteinase and peroxidase which brought about the degradation of macromolecules such as cellulose, hemicelluloses, lignin and protein in the substrates. The degradation of plant organic wastes using an edible fungus in a recycling technology may be adopted by farmers in developing countries. Therefore, the study was undertaken to investigate the potential of *Pleurotus tuber-regium* in degradation of maize stovers and rattan wood. The study which aims at ascertaining the *in-vitro* gas digestibility, the nutrient contents of the substrates after degradation process also determined the enzyme activities involved in the degradation.

MATERIALS AND METHODS

Pieces of rattan wood were collected from Bodija market, Ibadan, Nigeria and maize stovers from Agriculture farm, University of Ibadan. Freshly harvested rice straw obtained from International Institute of Tropical Agriculture (I.I.T.A) Ibadan, was sun-dried for two weeks then cut into 5mm size with a guillotine. Wheat bran was obtained from Bodija market, Ibadan, Nigeria. The pure culture of *Pleurotus tuber-regium* was obtained from Plant Physiology Laboratory of Department of Botany, University of Ibadan. Fresh cultures were by repeated sub-culturing on Potato Dextrose Agar (PDA).

Preparation of Pure Isolates Mycelia: *P.tuber-regium* was maintained on Potato Dextrose Agar (PDA) plates at $28^0C \pm 2^0C$. All the conical flasks were inoculated with 5mm diameter mycelia discs (Jonathan &Fasidi, 2001)

Experimental Design: A randomized factorial experiment comprising one fungus *Pleurotus tuber-regium*, the two substrates, rattan wood and maize stovers, incubation periods being 0,30,60 and 90 days. An ANOVA table was prepared following the Duncan's multiple range test for the first series of experiment on lignin-degrading abilities of the white-rot fungus. Each treatment was replicated four times.

Digestibility Test: The method of Adenipekun and Fasidi (2005) was employed. Twenty five grams (25g) of each of the dry wastes of the rattan wood and maize stovers were weighed into each conical flask and squeezed out after 75ml of distilled water was added. The flasks were covered with aluminium foil then sterilized at 121^0C for 15 minutes and later inoculated with two 5mm agar diameter mycelia at the center and covered immediately except for the controls which were not inoculated. The flasks were incubated at 30 ± 2^0C and 100% relative humidity. The controls which were not inoculated were put in an oven at 100 ± 2^0C for 48 hours to determine the initial dry weight of the substrates. The experiment was replicated four times and conical flasks were observed daily for sclerotium development. The conical flasks were harvested after 30, 60 and 90 days. 5g of the degraded substrates was aseptically weighed out for enzyme analysis and then placed in the oven for 48 hours at 100 ± 2^0C to determine dry weights.

Determination of *in-vitro* gas Production: The *in-vitro* gas production was determined following the procedure of Menke and Steingass (1988). A sensitive scale was used to measure out 200mg of the milled samples (rattan wood and maize stovers); these were placed into 120ml graduated syringes. Two replicates were prepared. Rumen fluid was obtained from two West African dwarf female goats and one West African dwarf male goat. The method for collection (Babayemi and Bamikole, 2006a) involved using suction tube from goats which were fed with concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 15 common salt, 3.75% oyster shell

and 0.25% fish meal. Incubation procedure was carried out as reported by Menke and Steingass (1988) using 120ml calibrated syringes in triplicate at 39^0C.

Thirty (30)ml of the inoculums containing rumen liquor and buffer (g/litre) of 9.8 $NaHCO_3$ (Sodium hydrogentrioxocarbonate(IV)) + 2.77 Na_2 $HP0_4$ (disodium hydrogentetraoxophosphate(V)) + 0.57 Potassium chloride + 0.47 sodium chloride + 0.12g magnesium sulphate $7H_20$ + 0.16g $CaCl_2.2H_20$ (calcium chloride) 1:4 v/v) under continuous flushing with carbon dioxide ($C0_2$), was strained using cheese cloth and dispensed using another 50ml plastic calibrated syringe. The syringe was tapped and pushed upward by the piston in order to completely eliminate air in the inoculums. The silicon tube in the syringe was then tightened by a metal clip so as to prevent escape of the gas. Incubation was carried out at 39 ± 1^0C and the volume of gas production was measured at 3, 6,9,12,15,18,21 and 24hrs. At post incubation period, 4ml of sodium hydroxide (10M) was introduced to estimate methane production. The post incubation parameters such as Metabolisable Energy (ME) and Organic Matter Digestibility (OMD %) were estimated MJ/kgDM according to the method described by Menke and Steingass (1988).

Nutrient Content Analysis: Crude fibre (CF), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL) Dietary Fibre (DF), Loss of organic matter (LOM), Loss of water (LW) and pH were determined as described by Zadarazil and Brunnert (1982).The method of Association of Official Agricultural Chemists (A.O.A.C. 2003) was used to determine organic carbon, organic matter, percentage nitrogen, phosphorus, potassium and enzymes assay.

RESULTS

Table 1 shows the proximate composition g/100gDM) of *Pleurotus tuber-regium* on degraded rattan wood and maize stovers incubated for 0-90 days.

Table 1: Proximate composition (g/100gDM) of rattan wood and maize stovers degraded by *Pleurotus tuber-regium.*

Substrate	Days	CP	CF	EE	Ash	DM	MC
Rattan wood	0	1.60^{e}	44.68^{a}	0.24^{c}	7.38^{a}	92.28^{a}	6.73^{d}
	30	1.75^{e}	38.44^{b}	0.18^{c}	6.42^{b}	90.64^{bc}	7.37^{bc}
	60	4.27^{cd}	27.52^{d}	0.16^{c}	4.05^{c}	90.27^{c}	8.74^{bc}
	90	5.90^{bc}	20.92^{e}	0.15^{e}	2.96^{d}	89.85^{c}	9.15^{b}
Maize stovers	0	2.75^{de}	32.33^{c}	0.77^{a}	4.83^{c}	91.75^{ab}	8.25^{cd}
	30	4.49^{c}	25.83^{d}	0.42^{b}	4.49^{c}	90.10^{c}	8.79^{b}
	60	6.22^{b}	17.62^{e}	0.22^{cd}	2.29^{de}	88.90^{d}	9.63^{a}
	90	8.74^{a}	13.03^{e}	0.17^{de}	1.60^{e}	80.42^{bc}	9.89^{bc}

Each value is a mean of 4 replicates

Means of different superscripts in each column are significantly different at P≤ 0.05 according to Duncan's multiple range tests. CP = Crude Protein, CF = Crude Fibre, EE = Ether Extract, DM = Dry Matter and MC = Moisture Content

The crude protein contents of the fungal treated substrates increased significantly, throughout the incubation period, from 1.60% in the control to 5.90% in the rattan wood, and in maize stovers from 2.75%(control) to 8.74%.The Crude Fibre (CF) decreased significantly compared to untreated substrates as the rate of incubation increased. Rattan wood treated with *P.tuber-regium* reduced from 44.68% in the control to 20.92% after 90 days compared to 32.33% to 13.03% in maize stovers treated with the fungus. Ether extract content in rattan wood decreased from 0.24% in the control to 0.15% and in maize stovers from 0.77% in the control to 0.17% .There was a reduction in the ash content of rattan wood from 7.38% in the untreated substrate to 2.96% and in maize stovers from 4.83% to 1.60%. The dry matter decreased in treated rattan wood from 92.28% in the control to 89.85% and maize stovers from 91.75% (control) to 80.42%. Moisture content increased with incubation period in rattan wood from 6.73% (control) to 9.15% and in maize stovers from 8.25% in the control to 9.89%. The percentages of nitrogen, carbon and potassium of the substrates are shown in Table 2.

Table 2: Percentage of Nitrogen, Carbon and Potassium of rattan wood and maize stovers degraded by *Pleurotus tuber-regium*

Substrate	Days	% N	% C	% K
Rattan wood	0	0.26^{e}	3.32^{c}	3.00^{d}
	30	0.28^{e}	2.98^{c}	6.41^{c}
	60	0.69^{cd}	7.54^{b}	8.20^{b}
	90	0.95^{bc}	10.15^{b}	9.45^{a}
Maize stovers	0	0.44^{de}	4.83^{c}	1.98^{d}
	30	0.72^{c}	8.00^{b}	5.43^{c}
	60	1.10^{b}	10.28^{b}	8.12^{b}
	90	1.40^{a}	14.63^{b}	9.29^{a}

Each value is a mean of 4 replicates.
Means with different superscripts in each column are significantly different at $P \leq 0.05$ according to Duncan's multiple range test N= Nitrogen, C = carbon and K = Potassium.

With increasing incubation period, the percentage nitrogen of treated rattan wood was from 0.26% in the control to 0.95% and in maize stovers from 0.44% in the control to 1.40%. Carbon content of rattan wood increased from 3.32% in the untreated substrate to 10.15% and in maize stovers from 4.83% (control) to 14.63%.The potassium content in treated rattan wood increased from 3.00% to 9.45% while that of maize stovers from 1.98% to 9.29%. As shown in Table 3, the pH of rattan wood decreased from 5.90 to 4.55 and in maize stovers from 6.53 to 4.90 after 90 days incubation. The percentage loss of organic matter reduced with increase in incubation period in rattan wood from 69.00% in the control to 32.00% and in maize stovers from 40.00% in the control to 20.00%. There was also decrease in the percentage loss of water as the incubation increased. In rattan wood, there was a reduction from 45.50% to 38.25% while that of maize stovers was from 47.50% to 32.25%. There was a consistent reduction in the Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), cellulose and hemicelluloses. The NDF of the treated rattan wood decreased from 70.93% to 31.88% (Table 4).

Table 3: pH, Loss of Organic matter and water of rattan wood and maize stovers degraded by *Pleurotus tuber-regium.*

Substrate	Days	pH	LOM (%)	LOW (%)
Rattan wood	0	5.90^{b}	69.00^{a}	45.50^{ab}
	30	5.48^{bc}	64.00^{a}	44.25^{ab}
	60	5.32^{cd}	41.00^{ab}	41.50^{b}
	90	4.55^{e}	32.00^{ab}	38.25^{b}
Maize stovers	0	6.53^{a}	40.00^{ab}	47.50^{ab}
	30	6.40^{a}	32.00^{ab}	42.50^{ab}
	60	5.20^{cd}	31.00^{ab}	40.50^{a}
	90	4.90^{de}	20.00^{a}	32.25^{a}

Each value is a mean of 4 replicates.
Means with different superscripts in each column are significantly different at $P \leq 0.05$ according to Duncan's multiple range tests., LOM – Loss of organic matter. LOW – Loss of water.

ADF from 62.80% to 28.50%, ADL reduced from 53.90% to 21.90%; cellulose from 8.90% to 6.40% and the hemicelluloses from 8.13% to 3.38%.The NDF of maize showed reduction from 62.22% (control) to 19.26%, ADF of the maize stovers decreased from 54.70% to 15.70%; the ADL, cellulose content and hemicelluloses content of the maize stovers treated

decreased from 45.59% to 13.34%; 9.11% to 3.34% and 15.52% to 3.55% respectively.

Table 4: Fibre analysis (g/100gDM) of rattan wood and maize stovers degraded by Pleurotus *tuber-regium.*

Substrate	Days	NDF	ADF	ADL	Cellulose	Hemicelluloses
Rattan wood	0	70.93^{a}	62.80^{a}	53.90^{a}	8.90^{b}	8.13^{bc}
	30	62.38^{b}	57.38^{ab}	40.94^{b}	7.44^{a}	4.50^{cd}
	60	39.70^{d}	33.35^{cd}	26.85^{d}	6.50^{bc}	4.35^{cd}
	90	31.88^{e}	28.50^{bc}	21.90^{ed}	6.40^{bc}	3.38^{cd}
Maize stovers	0	62.22^{ab}	54.70^{b}	45.59^{b}	9.11^{b}	15.52^{a}
	30	50.56^{c}	40.00^{c}	33.48^{c}	7.80^{b}	10.56^{ab}
	60	25.62^{ef}	20.94^{e}	16.98^{ef}	3.97^{c}	4.68^{d}
	90	19.26^{f}	15.70^{e}	13.34^{f}	3.34^{e}	3.55^{d}

Each value is a mean of 4 replicates.

Means with different superscripts in each column are significantly different at P ≤ 0.05 according to Duncan multiple range test.

N.D.F. = Neutral Detergent Fibre; A.D.F = Acid Detergent Fibre, ADL = Acid Detergent Lignin.

Table 5 shows the production of amylase, cellulase, ligninase and peroxidase by *P.tuber-regium* for 0-90 days where a decrease in enzyme production as the period of incubation increased was observed.

Table 5: Enzyme Production (Unit/ml) during biodegradation of rattan wood and maize stovers by *Pleurotus tuber-regium.*

Substrate	Days	Amylase	Cellulase	Ligninase	Peroxidase
Rattan wood	0	0.00	0.00	0.00	0.00
	30	0.24^{d}	0.16^{e}	0.36^{c}	0.18^{b}
	60	0.17^{d}	0.14^{ed}	0.25^{d}	0.17^{d}
	90	0.13^{e}	0.12^{e}	0.18^{e}	0.16^{e}
Maize stovers	0	0.00	0.00	0.00	0.00
	30	0.27^{a}	0.51^{b}	0.51^{b}	0.38^{c}
	60	0.24^{d}	0.25^{cd}	0.26^{d}	0.19^{e}
	90	0.12^{e}	0.16^{e}	0.17^{e}	0.15^{e}

Each value is a mean of 4 replicates.

Means with different superscripts in each column are significantly different at P ≤ 0.05 according to Duncan's multiple range tests.

The amylase produced in treated rattan wood reduced from 0.24 unit/ml after 30 days to 0.13 unit/ml after 90 days; cellulase from 0.16 unit/ml after 30 days to 0.12unit/ml while ligninase and peroxidase decreased from 0.36 unit/ml to 0.18 unit/ml and 0.18 unit/ml to 0.16 unit/ml after 90 days incubation. The amylase produced in maize stovers decreased from 0.27 unit/ml after 30 days to 0.12 unit/ml for 90 days of incubation while cellulase, ligninase and peroxidase reduced from 0.51 unit/ml to 0.16unit/ml; 0.51unit/ml to 0.17 unit/ml and 0.38 unit/ml to 0.15 unit/ml after 90 days of incubation respectively. As shown in Table 6, the organic matter in treated rattan wood increased from 5.57% in control to 17.50% and in maize stovers from 8.32% to 25.22% while metabolisable energy in rattan wood was enhanced from 2.35 MJ/KGDM to 2.56MJ/KGDM and in maize stovers from 2.20MJ/KGDM to 2.61MJ/KGDM.

Table 6: Organic Matter, Digestibility and Metabolizable Energy of rattan wood and maize stovers degraded by *Pleurotus tuber-regium.*

Substrate	Days	Organic matter	Metabolizable energy
Rattan wood	0	5.57^{c}	2.35^{e}
	30	6.14^{c}	2.48^{cd}
	60	13.14^{b}	2.52^{c}
	90	17.50^{b}	2.56^{b}
Maize stovers	0	8.32^{c}	2.20^{g}
	30	13.79^{b}	2.28^{f}
	60	17.72^{a}	2.46^{d}
	90	25.22^{a}	2.61^{a}

Each value is a mean of 4 replicates. Means with different superscripts in each column are significantly different at P ≤ 0.05 according to Duncan's multiple range tests

Table 7 shows the *in-vitro* gas production of degraded rattan wood and maize stovers. It was observed that gas production increased at 3 hours interval for 24 hours and that the methane gas production increased with increasing incubation period. Treated rattan wood produced methane at 0,30,60 and 90 days with values 7.00ml, 9.00ml, 9.00ml and 14.00ml respectively while in maize stovers 6.00ml, 9.00ml, 9.50ml and 18.00ml of methane were produced at 0,30,60 and 90 days respectively. The highest volume of gas (methane gas) was produced at 90 days incubation.

Table 7: *In-vitro* gas production in rattan wood and maize stovers degraded by *Pleurotus tuber-regium* Singer

Substrate	Days	Gas (ml)								Gas methane gas
		3rd hr	6th hr	9th hr	12th hr	15th hr	18th hr	21st hr	24th hr	
Rattan wood	0	-	6.00	7.00	10.00	10.00	11.00	12.00	13.00	7.00
	30	-	7.00	9.00	10.00	13.00	14.00	15.00	18.00	9.00
	60	-	4.00	7.00	8.00	8.00	10.00	10.00	11.00	9.00
	90	-	7.00	9.00	11.00	14.00	16.00	18.00	20.00	14.00
Maize stovers	0	-	6.00	7.00	7.00	7.00	7.00	8.00	9.00	6.00
	30	-	6.00	8.00	11.00	14.00	16.00	17.00	20.00	9.00
	60	-	5.00	7.00	7.00	8.00	10.00	11.00	12.00	9.50
	90	3.00	7.00	11.00	14.00	19.00	22.00	24.00	28.00	18.00

Each value is a mean of 4 replicates.

DISCUSSION

White-rot fungi have been identified as the most widely studied lignin-degrading organisms. Capelari and Zadrazil (1997) screened 72 species of white-rot fungi and their ability to increase or decrease the *in-vitro* digestibility using wheat straw substrate at 25 or 30^{0}C after 30 and 60days incubation. *Pleurotus tuber-regium* degraded rattan wood and maize stovers in this study. The crude protein in the treated substrates for both substrates was higher than the untreated substrates throughout the period of incubation and this could be due to secretion of extracellular enzymes (which contain protein substances in nature) into the waste during their breakdown and its subsequent metabolism (Kadiri, 1999). Hammond and Wood (1985) reported that the increase in crude protein could be as a result of hydrolysis of starch to glucose and its subsequent use by the same organisms as a carbon source to synthesis of fungal biomass rich in protein. Similarly, Belewu and Banjo (1999) observed increase in crude protein when rice husk was treated with white rot fungi and was found to compensate for the low and poor protein nutrient of concentrate diets of raw straw and hay consumed by animals in the tropical environment. The decrease in fibre analysis of *Pleurotus tuber-regium* on

degraded rattan wood and maize stovers could be as a result of cellulosic enzymes secreted by cellulolytic fungi. As observed by Belewu and Belewu (2005) the 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. Kuforiji and Fasidi (2009) reported that hemicelluloses and celluloses reduced when *Pleurotus tuber-regium* was used during biodegradation of agro-industrial wastes. They stated that it showed the extent to which the substrates were able to utilize the mushroom. The decrease in crude fibre fractions in the substrate may have been as a result of the fungal ability to produce extracellular enzymes capable of reducing the fibre contents. In another study, Akinfemi *et al.* (2009) were of the view that decreases in the value of detergent fibre (hemicelluloses, cellulose and lignin) and acid detergent fibre (lignin and cellulose) for fungal treated maize cobs could be as a result of extra cellular enzymes produced by *Pleurotus species.*

Karunanandaa and Varga (1996) concluded that lignification of structural polysaccharides would not only inhibit ruminal microbial digestion of polysaccharide by forming 3-D matrix, but also that the presence of highly lignified tissue formed a barrier preventing accessibility of the otherwise highly digestible tissue to the action of hydrolytic enzymes of the rumen microorganisms. Increased digestibility was shown to be associated with the degradation of structural carbohydrates. The decreasing crude fibre and crude fibre fractions could also be as a result of activities of cellulolytic bacteria (Sallam *et al*.; 2007). During fungal growth, part of the cell wall is converted into soluble sugars to provide energy a phenomenon that could be responsible for decrease in a major fibre (cellulose and hemicelluloses components).The increase in percentages of Nitrogen, Carbon and Potassium might be due to the fact that mushrooms contain appreciable amount of mineral elements. Isikhuemhen *et al*. (1996) reported that variation in mineral elements present in a mushroom may not be unconnected with the type of substrates used during duration of fermentation and the specie of fungus used. 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. This agrees with the finding of Adenipekun and Fasidi (2005) observing a change in pH during the degradation of selected agricultural wastes by *Pleurotus tuber-regium* and *Lentinus subnudus.* The increase in amino nitrogen content may be due to hydrolysis of protein within the substrates. The decrease in organic matter during the incubation period may be due to the release of carbon content released by the fungi during the period of degradation of the substrates. This agrees with the report of McDowell (1985) who observed deficiency of calcium and phosphorous in fungal treated substrates of ruminant feeds and thereby led to the decrease in organic matter content and recommended calcium and phosphorous ratio of 1:1.The decrease in organic matter may also be due to complete uptake of carbon content during the favorable condition of biodegradation. Water is essential for supporting the metabolic activities of fungi, thereby enhancing the biodegradation process of the substrates. Tamara *et al* (1996) observed the importance of water content in the solubilization of lignin at the vegetative phase and reproductive phase with lignocellulose degrading properties. This may explain the decrease in the loss of water with increase in the incubation period in the present study.

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. Kuforiji and Fasidi (2008) 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. In a similar report, Rajarathman *et al.* (1998) stated that edible mushrooms are able to bioconvert a wide variety of lignocelluloses materials due to secretion of extracellular production with increasing incubation period. The increase in organic matter digestibility and metabolisable energy could be as a result of increase in crude protein and it shows that microbes in the rumen and animals have high nutrient uptake (Chumpawadee *et al*., 2005). Incubation with *Pleurotus tuber-regium* brought about an increase in metabolisable energy in treated substrates. This agrees with the report of Kamra and Zadrazil (1988) where they found higher lignin degradation rates and, consequently, increases in digestibility using cereal straws and substrates compared to wood. Abiola and Tewe (1991) also noted that cocoa husks have dietary fibre content as non-starch polysaccharides. There are many factors that determine the amount of gas during fermentation depending on the nature and level of fibre (Babayemi *et al.*, 2004) and potency of the rumen liquor used for incubation. The gas production is a function and mirror of degradable carbohydrates and therefore

the amount of gas produced depends on nature of the carbohydrates. Methane gas production was observed to be high in treated substrates. Methane gas production has negative effects on the animals because it is energy loss to animal. However when it accumulates in the rumen it causes bloat (Babayemi, 2006). Therefore, a reduction in the methane gas production is saving energy for the animal.

REFERENCES

Abiola SS. and Tewe OO, 1991. Chemical evaluation of cocoa by-product. Tropical Agriculture (Trinidad) 68:335-336.

Adenipekun, CO. and Fasidi IO, 2005. Degradation of Selected agricultural wastes by *Pleurotus tuber-regium* (Fries) Singer and *Lentinus subnudus* (Berk) Nigerian edible mushroom. Advances in Food Sciences, 27.2: 61-64.

Akinfemi, A, Adu, OA, Adebiyi OA, 2009. Use of white-rot-fungi in upgrading maize straw and the resulting impact on chemical composition and *in-vitro* digestibility. Livestock research for rural development 21:10.

Alofe FA, Odu, EA, Illoh HE, 1998. Mushrooms, Man and Nature, edible wild mushroom on decaying wood in Obafemi Awolowo University Campus. The Nigerian Field 63:3-18.

AOAC. 2003. Association of Official Analytical Chemists. Methods of Analysis. 18th edn .Washington D.C.

Babayemi OJ, 2006. Anti-Nutritional Factors, Nutritive value and *in vitro* gas production of Foliage and Fruit of *Enterolobium cyclocarpun* World Journal of Zoology 1.2: 113-117.

Babayemi OJ, Demeyer D, Fievez V, 2004. Nutritive value and qualitative assessment of secondary compounds in seeds of eight tropical browse shrub and pulse legumes comm. Applied Biological Sciences 69.1: 103-110.

Babayemi, OJ. and Bamikole MA, 2006a. Effects of *Tephrosia candida* DC leaf and its mixture with guinea grass on *in-vitro* fermentation changes on feed for ruminants. Pakistan Journal of Nutrition 5.1: 14-18.

Belewu MA. and Banjo NO, 1999. Bio delignification of rice husk and sorghum stover by edible mushroom (*Pleurotus sp*).Tropical Journal of Animal Science 1:137-142.

Belewu, MA. and Belewu, KY, 2005. Cultivation of Mushrooms (*Volvariella volvacea)* on banana Leaves. African Journal of Biotechnology 4.12: 1401-1403.

Buswell JA, Cla, YJ, Chang ST, Perberdy JF, Fu SY ,Yu, ITS, 1996. Lignocellulolytic enzymes profiles of edible mushroom fungi. World Journal of Microbiology 12:537-542.

Capelari M. and Zadrazil F, 1997. Lignin degrading and *in-vitro* digestibility of wheat straw treated with Brazilian tropical species of white-rot fungi. Folia Microbiol. 42(5):481-487.

Chumpawadee S, Sommart K, Vongpralub T, Pattarajinda, V, 2005. Nutritional evaluation of non-forage high fibrous tropical feeds for ruminant using *in-vitro* gas production technique. Pakistan Journal of Nutrition 4: 298-303.

Hammond JBW. and Wood DA, 1985. Metabolism, biochemistry and physiology in biology and technology of cultivated mushroom ed. PB Flegg, DM Spencer, DA Wood pp:63-80 Chichester Willey p347.

Hamza A, 1989.Utilization of agro-industrial residues in Alexandria: Experience and Prospects. Biological wastes ,29:107-121.

Isikhuemhen OS, Zadrazil F, Fasidi FO, 1996. Cultivation of white rot fungi in solid state fermentation. Journal of Science and Industrial Resource 44: 388-393.

Jonathan SG. and Fasidi IO, 2001. Effect of Carbon, Nitrogen and Mineral Sources on growth of *Psathyyerella* atroumbonata *(Pegler),* a Nigerian edible mushroom. Food Chemistry 72:479-483.

Kadiri M. 1999. Changes in intracellular and extra cellular enzyme activities of *Lentinus subnudus* during sporophore development. Biosciences Resource Comm 11.2: 127-130. Fungi,Elsevier,London, pp 56-63.

Kamra DN. and Zadrazil F, 1988. In: Treatment of lignocellulosics with white-rot fungi. Fungi(eds Zadrazil,F. and Reininger,R.pp 56-63(Elsevier, London).

Karunnanada K. and Varga GA, 1996. Colonization of the straw by white-rot fungi (*Cyathus sterioreus*): Effect on ruminant fermentation Pattern, Nitrogen Metabolism and Fiber utilization during Continuous Culture. Animal Food Science technology 61:1-16.

Kuforiji OO. and Fasidi IO, 2008: Enzymes Activities of *Pleurotus tuber-regium* (Fries) Singer. cultivated on selected agricultural wastes. Bioresource Technology 99: 4275-4278.

Kuhad R.C, Singh A, Eriksson KEC, 1997.Microorganisms and Enzymes involve in degradation of plants fibre cell walls in advances in biochemical engineering biotechnology, Eriksson, K. E.L(ed).Bol.57 *Springer Verlag Germany*. Pp. 46-125.

McDowell LR, 1985. *Nutrition of Grazing Ruminants in warm Climates*. London: Academic Press/Harcourt Brace Jovamovich.

Menke KH. and Steingass H, 1988. Estimation of the energetic feed value from chemical analysis and *in vitro* gas production using rumen fluid. Animal Resource Development. 28:7-55.

Rajarathnam S, Shashirekha M, Bano Z, 1998. Biodegradation and biosynthetic capacities of mushrooms. Present and future strategies. Critical mushrooms. Present and Future strategies. Critical Reviews in Biotechnology 18:91-236.

Sallam SMA, Nasser MEA, El-Waziry AM, Bueno ICS, Abdalla AL, 2007. Use of an *in-vitro* gas production technique to evaluate some ruminant feedstuffs. Journal of Applied Sciences Resource 3.1: 34-42.

Tamara V, Mika K, Annele H, 1996. Lignin peroxidases, Mangenese, peroxidases and other lignolytic enzymes produced by phelebia radiate during solid state fermentation of wheat straw. Applied Environmental Microbiology 3515-3529.

Villario MC, Martcalvo J, Volarsek 1995. Cuban-Mexican experience in the cultivation of *Pleurotus sp* .Dev Ibero.Mycologia 12:9-11.

Zadrazil F. and Brunnert H, 1982. Solid State Fermentation of Lignocellulose containing plant residues with *Sporotrichum pulverlentium.* Nov. and *Dichotimus squalens* (*Karsl)* Reid. European Journal of Applied Microbiology and Biotechnology 16: 45-51.