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UPGRADING OF SUGARCANE BAGASSE BY SOLID STATE FERMENTATION WITH *PLEUROTUS SAJOR-CAJU* AND *PLEUROTUS FLORIDA* AND THE IMPACT ON THE CHEMICAL COMPOSITION AND *IN VITRO* DIGESTIBILITY

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Abstract: Solid fermentation using celllolytic fungi: *Pleurotus sajor-caju* and *Pleurotus florida* for upgrading of sugarcane bagasse to value-added ruminant feed were investigated. The fermentation of the substrate lasted for 21 days after which the changes in the chemical and mineral composition, and the *in vitro* gas production were evaluated. The results obtained showed an increase in the crude protein (%) from 6.43 (control) to 9.82 for *Pleurotus sajor* treated substrate (PSB) and 10.05 for *Pleurotus florida* treated substrate (PFB). The treatment effect on crude fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was significant. Fungal treatment decreased crude fiber (%) from 37.49 (control) to 31.67 (PFB), NDF (%) from 65.92 (control) to 53.34 (PSB), ADF (%) from 49.94 to 34.79 (PSB), and ADL (%) from 15.13 to 9.74 (PSB). Most of the major and trace minerals were higher in the untreated bagasse with the exception of phosphorus (0.15g/kg), magnesium (1.80g/kg), potassium (2.70g/kg) and zinc (21.60g/kg). The degradation of the insoluble but degradable fraction (b, ml) was higher in the control (19.00) followed by PSB (16.00). The estimated organic matter digestibility (%), short chain fatty acid (μ, mol) and metabolisable energy (MJ/Kg DM) increased from 38.77-50.06, 0.56-0.75 and 5.33-6.80 respectively. The gas volumes at 24h, 48h and 72h as affected by treatment was significant ($P<0.05$) with more volumes of gas produced in the treated bagasse. The result obtained in this study showed that fungal treatment of bagasse improved the nutrient contents and digestibility.

Key words: Solid state fermentation, cellulolytic fungi, sugarcane bagasse, ruminant, *in-vitro*-gas production.

Introduction

Annually, huge amounts of agricultural wastes and industrial by-products are produced worldwide from farm practices and industrial food product. In spite of this large amounts of wastes and industrial by-products, the problems of inadequate all year round nutrition and the prohibitive cost of conventional feedstuffs during the dry seasons in Nigeria remained unsolved. One of such agro industrial by-product is bagasse. Bagasse consists mainly of cellulose and hemicellulose. Even though bagasse contains enough cellulose to make it an excellent source of energy for ruminants, it is a poor quality feed in its natural state. It main problems as animal feed are low digestibility, low protein content, poor palatability and bulkiness *(Abdullah et al., 2006)*. The poor digestibility of bagasse is linked to the lignin complex with the cellulose thus baring it from been accessible to rumen micro organisms. It is therefore clear that an intervention must be applied that will overcome this limitations before it can be harnessed as ruminant feed. Thus, considerable effort is being made to find an economic use of this vast bulk of agricultural waste materials most of which is currently burnt as fuel *(Garg et al., 1982)*. In view of this, the objectives of this study were to study the biodegradation of these wastes by *Pleurotus sajor caju* and *Pleurotus florida* for its use as ruminant feed and the resulting impact on the chemical composition and *in vitro* digestibility.

Materials and Methods

Preparation of experimental samples

Dried samples of bagasse straw were collected from the Teaching and Research Farm, Nasarawa State University, Shabu-Lafia, Nigeria. The materials were milled and oven-treated at 65^0C to constant weight for dry matter determination.

The fungus. The sporophores of *Pleurotus sajor-caju* and *Pleurotus florida* growing in the wild were collected from University of Ibadan botanical garden. These were tissue cultured to obtain fungal mycelia *(Jonathan and Fasidi, 2001)*. The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

Degradation of bagasse wastes by *Pleurotus sajor-caju* and *Pleurotus florida*

Preparation of substrate. The jam bottles used for this study were thoroughly washed, dried for 10min. at 100^0C. 25.00g of the dried milled substrates were weighed separately into a jam bottle and 70ml distilled water were added. The

bottle was immediately covered with aluminum foil and sterilized in the autoclave at 121^0C for 15 min. Each treatment was in triplicates.

Inoculation. Each bottle was inoculated at the center of the substrate with 2, 10.00mm mycelia disc and covered immediately *(Adenipekun and Fasidi, 2005)*. They were kept in the dark cupboard in the laboratory at 30^0C and 100% relative humidity (RH). At day 21 day of inoculation, the experimental bottles were autoclaved to terminate the mycelia growth. Samples of biodegradation were oven dried to constant weight for chemical analysis and *in vitro* digestibility.

***In vitro* gas production.** Rumen fluid was obtained from three West African Dwarf female goat through suction tube before the morning feed. The animals were fed with 40% concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fishmeal) and 60% Guinea grass. Incubation was carried out according to *(Menke and Steingass 1988)* in 120ml calibrated syringes in three batches at 39^0C. To 200mg sample in the syringe was added 30ml inoculum that contained cheese cloth strained rumen liquor and buffer (9.8g $NaHCO_3$ + 2.77g Na_2HPO_4 + 0.57g KCL + 0.47g NaCL + 0.12g $MgSO_4$. $7H_20$ + 0.16g $CaCI_2$. $2H_20$ in a ratio (1:4 v/v) under continuous flushing with CO_2. The gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24h. The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. The volume of gas production characteristics were estimated using the equation $Y = a + b\ (1 - e^{ct})$ *(Ørskov and McDonald, 1979)*, where Y = volume of gas produced at time't', a = intercept (gas produced from the soluble fraction), b = gas production from the insoluble fraction, (a+b) = final gas produced, c = gas production rate constant for the insoluble fraction (b), t = incubation time. The post incubation parameters such as metabolizable energy (ME, MJ/Kg DM), organic matter digestibility (OMD %) and short chain fatty acids (SCFA) were estimated at 24h post gas collection *(Menke and Steingas, 1988)*.

$$ME = 2.20 + 0.136* Gv + 0.057* CP + 0.0029*CF;$$

$$OMD = 14.88 + 0.88Gv + 0.45CP + 0.651XA;$$

$$SCFA = 0.0239*Gv - 0.0601;$$

Where Gv, CP, CF and XA are net gas production (ml/200mg, DM) at 24 h incubation time crude protein, crude fibre and ash of the incubated sample respectively.

Statistical analysis. Data obtained were subjected to analysis of variance (ANOVA) and where significant difference occurred means were separated by *Duncan (1955)* using Statistical Analysis System (SAS) package.

Results and discussion

The result of the chemical composition is shown in Table 1. There was wide variation in the chemical composition. The CP (%) increased from 6.43 in the control to 10.05 in *Pleurotus florida* (PFB) treated substrate while the CF (%) decreased from 37.49 (control) to 31.47 (PFB). The increase in CP content may be attributed to increase in microbial biomass in the form of single cell. This view was supported by the findings of other researchers *(Fasidi and Kadiri, 1993*; *Akinyele, 2003)*. Protein content increase could also be as a result of hydrolysis of starch to glucose and its subsequent use by the same organism as a carbon source to synthesize fungal biomass rich in protein *(Bender, 1970; Hammond and Wood, 1985)*. Others, *(Kadiri ,1999; Akinyele, 2003; Akinfemi, 2010)* also reported that protein increase may be due to secretion of certain extracellular enzymes which are proteineous in nature into the waste during their breakdown and its subsequent metabolism. The CF and CF fraction (NDF, ADF and ADL) decrease on the other hand may be as a result of the ability of white-rot fungi to decompose and metabolize all plant cell wall constituents (cellulose, hemicellulose and lignin) by their enzymes *(Erikson et al., 1990)*. Many species of white-rot fungi which are effective lignin degraders have been used to assess their ability to improve the nutritive value of fodder for ruminant nutrition *(Yamakava and Okamnto, 1992; Howard et al., 2003)*. Report *(Chen et al., 1996)* indicated that some white-rote fungi such as the one used in this study are able to decompose free phenolic monomers and to break the bonds with which lignin are cross-linked to the polysaccharides. In our present study, cellulose was depleted by the fungi used while the hemicellulose was most depleted by *Pleurotus ortreatis* (POB). Cellulose and hemicellulose reduction in fungal treated substrates is common. Previous studies *(Akinfemi et al., 2010*; *Akinfemi and Ogunwole, 2012)* support these views. However, differences in cellulose and hemicellulose contents of POB and PFB could be due to strain differences.

Table 1. Changes in the chemical composition and crude fiber fractions of fungal treated Bagasse

Parameters	Control	PSB	PFB	SEM
Dry matter	89.87^{c}	90.54^{a}	90.15^{b}	0.006
Crude protein	6.43^{c}	9.82^{b}	10.05^{a}	-
Crude fiber	37.49^{a}	32.94^{b}	31.67^{b}	0.22
Ether extract	2.94^{c}	3.70^{a}	3.41^{b}	0.005
Ash	3.87^{c}	8.04^{a}	7.16^{b}	0.005
Carbohydrate	49.27^{a}	45.49^{c}	47.72^{b}	0.22
NDF	65.92^{a}	53.34^{b}	54.80^{b}	0.41
ADF	49.94^{a}	34.79^{c}	46.85^{b}	0.03
ADL	15.13^{a}	9.74^{c}	13.23^{b}	0.01
Cellulose	34.81^{a}	25.05^{c}	33.62^{b}	0.04
Hemicellulose	15.98^{b}	18.55^{a}	7.95^{c}	0.38

a,b,c, means on the same row with different superscripts are significantly varied ($P < 0.05$) SEM = Standard error of mean,PSB= *Pleurotus sajor-caju* trated bagasse, PFB= *Pleurotus florida* treated bagasse

Table 2 shows the mineral composition of the treated substrates. The results obtained showed that most of the major and mineral elements with the exception of phosphorus, magnesium and zinc were higher in the untreated sample. It is likely the microorganism might have use some of the minerals for their metabolic activities *(Akinyele et al., 2011)*. This observation is consistent with the work of *Frazier and Westhoff (1978)* and *Bannet et al., (2002)* who reported that all living organisms require some mineral elements to maintain some metabolic functions.

Table 2. Some major minerals (g/Kg DM) and trace (ppm) mineral compositions of fungal treated bagasse

Parameters	Control	PSB	PFB	SEM
Major minerals				
Calcium	0.70^{a}	0.17^{b}	0.22^{b}	0.02
Phosphorus	0.13^{c}	0.28^{b}	0.32^{a}	0.003
Magnesium	1.80^{c}	4.50^{b}	4.80^{a}	0.03
Sodium	0.80^{a}	0.32^{b}	0.35^{b}	0.02
Potassium	2.70^{b}	2.73^{b}	3.03^{a}	0.05
Trace minerals				
Iron	19.20^{a}	15.40^{c}	16.37^{b}	0.04
Copper	10.20^{a}	5.40^{b}	4.70^{c}	0.03
Zinc	21.60^{b}	21.57^{b}	22.80^{a}	0.04
Manganese	34.50^{a}	13.14^{c}	14.40^{b}	0.05

a,b,c, means on the same row with different superscripts are significantly varied ($P < 0.05$) SEM = Standard error of mean,PSB= *Pleurotus sajor-caju* trated bagasse, PFB= *Pleurotus florida* treated bagasse

In Table 3, the result of gas production characteristics and estimated metabolisable energy (ME), organic matter digestibility (OMD) and short chain fatty acid (SCFA) is presented. Treatment effect on gas volume at 24h, 48h and 72h is significant. Gas volume ranked from the highest to lowest at 24h, 48h and 72h were POB, PFB and control. Although gas production is a nutritionally wasteful product *(Mauricio et al., 1999)* it nevertheless provides a useful basis from which ME, SCFA and OMD may be predicted *(Babayemi et al., 2006)*.

Table 3. Gas production characteristics, and estimated metabolisable energy (ME), organic matter digestibility OMD and short chain fatty acid (SCFA)

Parameters	Control	PSB	PFB	SEM
b (ml)	19.00^{a}	16.00^{ab}	15.00^{b}	0.61
C (h^{-1})	0.023^{b}	0.038^{a}	0.012^{c}	0.005
OMD (%)	38.77^{c}	50.06^{a}	43.42^{b}	0.42
SCFA (m mol)	0.56^{b}	0.75^{a}	0.59^{b}	0.01
ME (MJ/Kg DM)	5.33^{b}	6.80^{a}	5.86^{b}	0.07
Gv24	21.00^{b}	29.00^{b}	22.00^{a}	0.47
Gv48	38.00^{b}	41.00^{a}	41.00^{a}	0.33
Gv72	45.00^{b}	54.50^{a}	51.50^{a}	0.59

a,b,c, means on the same row with different superscripts are significantly varied ($P < 0.05$) SEM = Standard error of mean,PSB= *Pleurotus sajor-caju* trated bagasse, PFB= *Pleurotus florida* treated bagasse

Menke et al., (1979) suggested that gas volume is a good parameter from which to predict digestibility, fermentation end-product and microbial protein synthesis.

The high volume of gas obtained in the treated substrates may be the results of treatment effects on the cell wall content (NDF and ADF). This findings is in agreement with the assertion elsewhere *(Sallam et al., 2007)* which stated that cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters.

The potential degradation of the insoluble but fermentable fraction (b, ml) ranged from 15 (PFB) to 19 (control). Fast rate (h^{-1}) of gas production was obtained in POB (0.038) followed by the control (0.023). The fast rate obtained in POB could probably be influenced by carbohydrate fractions readily available to the microbial population *(Chumpawadee et al., 2007).*

The higher degradation of the insoluble fraction (b ml) in the control (19.00) than the treated bagasse, though contrary to expectations, has been previously reported *(Teguin et al., 1999*; *Melaku et al., 2003). Melaku et al., (2003)* suggested that this phenomenon could be due to the rapid rate of gas production leading to substrate exhaustion and limitation on the extent of gas production in the control.

Table 4. Values for *in vitro* gas production (ml/200mg Dm) at different incubation period

Feed samples	3h	6h	9h	12h	15h	18h	21h	24h
Control	2^{b}	6^{b}	9	9^{b}	10^{b}	10^{c}	16^{b}	21^{c}
PSB	3^{b}	9^{a}	9	11^{a}	11^{ab}	13^{b}	16^{b}	29^{c}
PFB	7^{a}	8^{a}	8	11^{a}	12^{a}	16^{a}	21^{a}	22^{b}
SEM	0.27	0.19	0.27	0.33	0.33	0.19	0.69	0.47

a,b,c, means on the same row with different superscripts are significantly varied ($P < 0.05$) SEM = Standard error of mean,PSB= *Pleurotus sajor-caju* trated bagasse, PFB= *Pleurotus florida* treated bagasse

Higher values of OMD, ME and SCFA were estimated for the treated samples. Since treatment effects on SCFA was in favour of POB and PFB compared with the control, that suggests a potential to make energy available to ruminants *(Babayemi et al., 2006).* The estimated OMD in the treated substrates is comparable to 41.1% estimated by *Sallam et al., (2007)* for bagasse, but higher than that estimated for rice straw, linseed straw and date stone. The ME estimated for POB is high, but not significantly different between PFB and the control, *Menke and Steingass (1988)* reported a strong correlation between ME values measured *in vivo* and predicted from 24h *in vitro* gas production and chemical composition of feed. In addition estimated ME in the present study was found to be lower than that reported by *NRC (2001).*

Conclusion

The fungal treated and untreated bagasse showed variation in chemical and mineral composition, and *in vitro* digestibility. Based on the result obtained in this study, the use of fungi in treatment of bagasse is a potential method to improve the nutritional value of the substrate. However, more research is required to identify the best fungi with the highest degradation properties. It will also be necessary to test this feed on live animals so as to assess their response to the feed.

Unapređenje otpadaka od prerade šećerne trske čvrstom fermentacijom sa *Pleurotus sajor-caju* i *Pleurotus florida* i uticaj na hemijski sastav i *in vitro* svarljivost

A. Akinfemi

Rezime

Ispitivana je čvrsta fermentacija pomoću celulolitičnih gljiva: *Pleurotus sajor-caju* i *Pleurotus florida* za unapređenje otpadaka u preradi šećerne trske kao dodata vrednost hrani koja se koristi u ishrani preživara. Fermentacija supstrata je trajala 21 dan, nakon čega su ocenjivane promene u hemijskom i mineralnom sastavu, kao i produkcija gasa *in vitro.* Dobijeni rezultati su pokazali povećanje sadržaja sirovih proteina (%) od 6,43 (kontrola) do 9,82 za *Pleurotus sajor* tretirane podloge (PSB) i 10,05 za *Pleurotus florida* tretirane podloge (PFB). Efekat tretmana na sadržaj sirove celuloze, NDF, ADF i ADL je bio signifikantan. Tretman gljivama je uticao na smanjenje sirove celuloze (%) od 37,49 (controla) do 31,67 (PFB), NDF (%) od 65,92 (kontrola) do 53,34 (PSB), ADF (%) od 49,94 do 34,79 (PSB) i ADL (%) od 15,13 do 9,74 (PSB). Većina esencijalnih i minerala u tragovima je imala veće vrednosti u netretiranim otpacima šećerne trske, sa izuzetkom fosfora (0,15g/kg), magnezijuma (1,80g/kg), kalijuma (2,70g/kg) i cinka (21,60g/kg). Degradacija nerastvorljive ali razgradive frakcije (b, ml) je bila veća u kontrolnoj grupi (19,00), zatim PSB (16,00). Procenjena svarljivost organske materije (%), masne kiseline kratkog lanca (μ, mol) i metabolička energija (MJ / kg DM) povećana je sa 38,77-50,06, 0,56-0,75 i 5,33-6,80 respektivno. Zapremina gasova na 24h, 48h i 72h je bila pod signifikantnim uticajem tretmana ($P < 0,05$), sa većom zapreminom gasa proizvedenom u slučaju tretiranih otpadaka šećerne trske. Rezultati dobijeni u ovom istraživanju pokazuju da tretman gljivama otpadaka u preradi šećerne trske se poboljšava hranljivi sadržaj i svarljivost.

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