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Production of Bioethanol from Lignocellulosic Materials

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Abstract

The work is at investigated the potential of cheap raw materials as substrates (sawdust, rice straw and maize straw) for the production of bioethanol. Lignocellulosic substrates were hydrolysed using *Pleurotus tuber-regium* for 42 days. Samples were taken every 14 days and production of lignin, hemicellulose, cellulose, pH, reducing sugar and total solids were determined. Hydrolysates of lignocellulosic substrates were fermented using *Saccharomyces cerevisiae* and Baker's yeast for 10 days in with pH, reducing sugar and ethanol content determined at 2 days interval. Effect of supplementation (maize pomace and corn steep liquor) on pH, reducing sugar and production of ethanol were determined every 2 days for 10 days. Significant decrease in lignin and cellulose content was recorded in some samples while the hemicellulose content decreased in all the samples hydrolysed with increase in incubation time. Sawdust from *Amphimas pterocarpoides* (SD1) and Rice straw (RS) had highest (21.50%) and lowest (5.94%) lignin content respectively. Maize Straw (MS) had the highest hemicellulose content (25.37%) and lowest cellulose content (42.90%). Total solid significantly increased with increase in incubation time. The highest ethanol content (1.8%) was recorded on the 2nd day of fermentation in filtrates from unsupplemented substrates (sawdust from *Ceiba pentandra* (SD2) and Maize straw) fermented with *Saccharomyces cerevisiae*. Ethanol production was higher in unsupplemented substrates (1.8%) than supplemented substrate (0.9%). Reducing sugar and pH decreased with increase in fermentation time. Substrate supplemented with corn steep liquor produced higher quantity of ethanol than substrates supplemented with maize pomace. Hydrolysates from untreated maize straw (MS) fermented with *Saccharomyces cerevisiae*, Baker's yeast and the co-inoculation of both within the range of 3.7 – 6.0 for 2 days can be used for bioethanol production. Likewise, filtrates from treated sawdust from *Ceiba pentandra* (SD2) fermented with *Saccharomyces cerevisiae*, Baker's yeast and the co-inoculation of both within the pH range of 4.3 – 5.4 for 2 days can also be used for bioethanol production but appreciable quantity can be produced from untreated (autoclaved) MS with *Saccharomyces cerevisiae* (Baker's yeast) only.

Introduction

Various fossil energy sources (oil, coal, natural gas etc) which are used for production of fuel and electricity control world's present economy (Uihlein and Schbek, 2009). The level of greenhouse gases in the atmosphere has drastically increased due to high levels of pollution generated from consumption of fossil fuels (Ballesteros *et al*., 2006). Global energy consumption increase with the expansion of human population and increase in industries. There will be decline in annual global oil production in future

Keywords: Bioethanol, *Pleurotus tuber-regium*, *Saccharomyces cerevisiae*, Baker's yeast Lignocellulosic substrates

(Campbell and Laherrere, 1998). Production of bioethanol from biomass is a way of reducing continuous rising petroleum costs and dependence on fossil fuel (Lang *et al*., 2001).

Microorganisms like brown rot, white rot and soft rot fungi can degrade lignocellulosic complex to liberate sugars (Isroi *et al*., 2011). The released sugars can be fermented by yeast to bioethanol. Biomass is made up of lignocellulose which is a complex of cellulose (30-70%), hemicellulose (15-30%) and lignin (10-25%) (Monlau *et al.*, 2013). Lignocellulosic biomass can be used to produce 442 billion litres of bioethanol per year which is about 16 times higher than the actual world bioethanol production (Kim and Dale, 2004). Lignocellulose is renewable, cheap and always available in abundant amount which could be effectively utilized by conversion to bioethanol. This research work aimed at investigating the potential of cheap raw substrates such as sawdust, rice straw and maize straw for the production of ethanol.

Materials and Methods

Raw materials collection and processing

The raw materials used for this study include sawdust, rice (*Oryza sativa*) straw and maize (*Zea mays*) straw. Samples of saw dusts were obtained from three different types of trees [*Ceiba pentadra* "Igi araba", *Ricinodendron heudolotii* subspecies *africanum* "Omodon" and *Amphimas pterocarpoides* "Tukuna"] from a saw mill factory. Freshly harvested rice straw was collected from International Institute of Tropical Agriculture (I.I.T.A) in Ibadan, while maize straw was collected from a local farm in Ondo, Ondo State. Rice straw and maize straw were sun-dried and milled using a milling machine. The raw materials were stored in a cool and dry place.

Maintenance of the cultures

Pure culture of *Pleurotus tuber-regium* used for microbiological pre-treatment of the substrates was obtained from Plant Physiology Laboratory of Department of Botany, University of Ibadan. Fresh cultures of *Saccharomyces cerevisiae* used for fermentation was obtained from the Department of Microbiology, University of Ibadan, and maintained on Yeast Extract Agar medium, while Baker's yeast was purchased from Bodija market in Ibadan.

Microbiological pre-treatment of the substrates

Fifteen grams (15g) of each of the substrates were weighed into clean flask and 45ml of distilled water was added. The flasks were covered with aluminium foil then sterilized at 121°C for 15 minutes and later inoculated after cooling (at the centre) with 2 agar blocks (5mm diameter) containing actively growing mycelia of *Pleurotus tuber-regium* and covered immediately except for the controls which were not inoculated. The flasks were incubated at 30±2°C for the duration of 42 days and sampled every 14 days to determine the chemical qualities, pH, total solids and reducing sugar. This was done according to the method of Adenipekun and Fasidi (2005).

Estimation of Total solids

This was done according to the method of Ehrman (1994). Aluminium weighing dishes were pre-dried by placing them in a 105±3°C drying oven for a minimum of four hours. Dishes were cooled in a desiccator and 2g of solid residue was weighed out into the weighing dish. The weight of the sample plus weighing dish was recorded. The sample was placed in a convention oven at 105±3°C for a minimum of four hours. Sample was removed from the oven and allowed to cool to room temperature in a desiccator. The dish containing the oven-dried sample was weighed to the nearest 0.1mg and weight was recorded. The sample was placed back into the convection oven at 105± 3°C and dried to constant weight. Constant weight is defined as ±0.1% change in the weight percent solids upon one hour of re-heating the sample. The percentage total solids on a 105°C dry weight basis was calculated as follows:

$$\text{Total Solids} = \frac{\text{Weight of sample plus dish} - \text{Weight of dish}}{\text{Weight of sample as received}}$$

Estimation of Lignin, Hemicellulose and Cellulose content

Acid Detergent Fibre (ADF), Neutral Detergent Fibre (NDF) and Acid Detergent Lignin (ADL) were determined according to modified method of

Clancy and Wilson (1966), modified method of Robertson and van Soest (1977) and AOAC (1997) respectively. Percentage of Hemicellulose, Cellulose and Lignin were estimated as follow:

%Hemicellulose	=	%NDF - %ADF
%Cellulose	=	%ADF - %ADL
%Lignin	=	%ADL

Estimation of reducing sugars

Reducing sugar was estimated using the method of Miller (1959).

Determination of pH

The pH of samples was determined using pH meter (pHep®, Hanna instruments) throughout the sampling period. The pH meter was calibrated with buffers 4, 7 and 9. The electrode probe was gently dipped aseptically into each sample after it has been washed with 95% ethanol and later rinsed with sterile distilled water.

Pretreatment and Fermentation of Substrates

One hundred and eighty grams (180g) of each substrate was pre-treated using the method of Adenipekun and Fasidi (2005) as earlier described with different incubation periods. After incubation, 1.8L of distilled water was added to each flask and was filtered after vigorous shaking. The filtrate of different substrates was used for fermentation. They were transferred into different sets of flasks correctly labelled, covered, autoclaved at 121°C for 15minutes and allowed to cool. Freshly harvested cells of *Saccharomyces cerevisiae* was aseptically transferred into a set of flasks containing the filtrates at the rate of 1% inoculum (v/v) and Baker's yeast was also added into another set of flask at the rate of 0.2% inoculum (w/v). The two yeast strains were combined into the third set of flasks containing the filtrates. The flasks were corked using cotton wool, mixed gently and incubated at 28±2°C for ten days. The flasks were shaken at interval to produce a homogenous solution and even distribution of the organisms in the substrates mixture. This was done according to the modified method of Oyeleke *et al*. (2012).

Supplementation

One hundred and eighty grams (180g) of substrates were supplemented with Maize pomace and corn steep liquor in the ratio 15g/1000g and 150ml/1000g respectively. Pre-treatment was also done using the method of Adenipekun and Fasidi (2005) as described earlier with different incubation periods. After incubation, 1.8L of distilled water was added to each flask and was filtered after vigorous shaking. The filtrate of different substrates was used for fermentation. They were transferred into different sets of flasks correctly labelled, covered, and autoclaved at 121°C for 15 minutes and allowed to cool. Freshly harvested cells of *Saccharomyces cerevisiae* was aseptically transferred into a set of flasks containing the filtrates at the rate of 1 % inoculum (v/v) and Baker's yeast was also added into another set of flasks at the rate of 0.2% inoculum (w/v). The two yeast strains were combined into the third set of flasks containing the filtrates. The flasks were corked using cotton wool, mixed gently and incubated at room temperature (28± 2°C) for ten days. The flasks were shaken at interval to produce a homogenous solution and even distribution of the organisms in the substrates mixture. This was done according to the modified method of Oyeleke *et al*. (2012).

Determination of Ethanol Content of the Samples

Gravimetric method was used to determine Ethanol content of fermented samples. Specific gravity was determined using density bottle. Heating mantle was used to distil 100ml of each sample. The distillate was poured into a pre-weighed dry bottle with a volume of 25ml and weighed. Equal volume of distilled water was also poured into the dry bottle and weighed. The specific gravity is determined by dividing the weight of the distillate of fermented sample by weight of equal volume of distilled water. This is according to AOAC (1990).

$$Specific\ Gravity = \frac{W1 - W}{W2 - W}$$

Where W1 = Weight of the density bottle plus weight of distillate of fermented sample
W2 = Weight of the density bottle plus weight of distilled water and
W = Weight of empty density bottle

The specific gravity was used to determine the concentration of ethanol using Ethyl Alcohol Conversion Table.

Statistical Analysis

The experimental data were statistically analyses using Analysis of Variance procedure through SPSS statistical package version 21. The treatment means were ranked with the Duncan Multiple Range Test (DMRT) at P≤0.05. Means values are means of duplicates. The level of significance was P≤0.05.

Results

Table 1 shows the effect of pre-treatment of substrates on Lignin, Hemicellulose, Cellulose, pH, Reducing sugar and Total solids. With increasing incubation period, the lignin content in all the samples increased within the first 28 days and decreased by 42nd day of incubation. At all sampling time, sample SD1 (*Amphimas pterocarpoides*) had the highest lignin contents, followed by sample SD3 (*Ricinodendron heudolotii* subspecies africanum) and the least were recorded in sample RS (Rice straw). The lignin content ranged from 20.51 – 22.54% in sample SD1, 10.46–13.61% in sample

Table 1: Effect of pre-treatment of substrates on Lignin, Hemicellulose, Cellulose, pH, Reducing sugar and Total solids

SAMPLE CODE	INCUBATION PERIOD (DAYS)	LIGNIN (%)	HEMICELLULOSE (%)	CELLULOSE (%)	pH	REDUCING SUGAR (mg/ml)	TOTAL SOLIDS (%)
SD1	C	21.70±0.0028^{c}	13.79±0.0042^{d}	59.10±0.0071^{b}	7.7±0.0283^{d}	0.36±0.0056^{b}	13.50±0.0071^{a}
	14	20.51±0.0028^{a}	9.64±0.0028^{a}	65.07±0.0042^{d}	4.6±0.0424^{b}	0.34±0.0028^{b}	19.50±0.0099^{b}
	28	22.54±0.0057^{d}	10.09±0.0028^{b}	58.64±0.0042^{a}	5.3±0.0283^{c}	0.34±0.0028^{a}	25.50±0.0042^{c}
	42	21.24±0.0028^{b}	11.38±0.0014^{c}	59.15±0.0028^{c}	4.2±0.014^{a}	0.36±0.0014^{b}	28.50±0.0028^{d}
SD2	C	10.73±0.0014^{b}	24.29±0.0014^{d}	48.96±0.0028^{a}	8.7±0.0424^{d}	0.56±0.0028^{b}	14.00±0.0071^{c}
	14	10.46±0.0014^{a}	17.49±0.0028^{c}	55.04±0.0042^{d}	7.3±0.0424^{d}	0.58±0.0014^{c}	6.00±0.0028^{a}
	28	13.61±0.0042^{d}	13.75±0.0028^{a}	52.74±0.0057^{b}	6.9±0.0283^{b}	0.60±0.0028^{d}	11.00±0.0028^{b}
	42	12.19±0.0042^{c}	14.78±0.0028^{b}	52.85±0.0014^{c}	6.3±0.0283^{a}	0.44±0.0014^{a}	15.00±0.0042^{d}
SD3	C	14.38±0.0042^{c}	13.94±0.0014^{c}	60.26±0.0057^{d}	8.0±0.0141^{b}	0.32±0.0042^{a}	8.00±0.0028^{a}
	14	12.87±0.0042^{b}	39.97±0.0028^{d}	33.75±0.0014^{a}	9.0±0.0141^{d}	0.42±0.0028^{b}	14.50±0.0042^{c}
	28	17.04±0.0028^{d}	10.11±0.0014^{a}	56.37±0.0042^{c}	8.5±0.0283^{c}	0.74±0.0042^{c}	13.50±0.0014^{b}
	42	12.27±0.0028^{a}	12.54±0.0042^{b}	53.28±0.0028^{b}	5.2±0.0141^{a}	1.44±0.0057^{d}	17.00±0.0028^{d}
RS	C	4.75±0.0042^{a}	12.27±0.0028^{c}	55.70±0.0028^{d}	7.3±0.0283^{b}	1.36±0.0028^{a}	18.00±0.0014^{b}
	14	6.24±0.0028^{c}	18.33±0.0042^{d}	49.89±0.0028^{a}	8.1±0.0283^{d}	2.20±0.0042^{b}	13.00±0.0042^{a}
	28	7.42±0.0028^{d}	7.00±0.0042^{a}	50.03±0.0014^{b}	7.5±0.0283^{c}	2.44±0.0014^{c}	22.00±0.0028^{c}
	42	5.33±0.0028^{b}	10.37±0.0042^{b}	53.04±0.0014^{c}	7.2±0.0141^{a}	2.62±0.0028^{d}	24.00±0.0057^{d}
MS	C	8.21±0.0028^{a}	27.32±0.0042^{c}	40.44±0.0014^{b}	7.1±0.0424^{b}	4.30±0.0028^{d}	9.50±0.0014^{a}
	14	9.10±0.0028^{b}	28.17±0.0042^{d}	39.42±0.0014^{a}	6.3±0.0424^{a}	3.96±0.0014^{c}	12.00±0.0028^{b}
	28	14.01±0.0028^{d}	21.72±0.0042^{a}	44.53±0.0028^{c}	7.8±0.0141^{d}	1.38±0.0028^{b}	18.00±0.0014^{c}
	42	10.34±0.0057^{c}	24.24±0.0014^{b}	47.21±0.0028^{d}	7.3±0.0283^{c}	1.22±0.0014^{a}	19.00±0.0028^{d}

Values are means of duplicate reading ± SD. Mean value with different alphabets in superscript along the column are significantly different (P≤0.05)

Key:
SD1 = Sample of saw dust from *Amphimas pterocarpoides*
SD2 = Sample of sawdust from *Ceiba pentandra*
SD3 = Sample of sawdust from *Ricinodendron heudolotii* subspecies *africanum*
RS = Rice Straw
MS = Maize straw
C = Control/un-inoculated sample

SD2 (*Ceiba pentandra*), sample SD3 (12.27–17.04%), sample RS (4.75 – 7.42%) and 8.21 – 14.01% in sample MS (Maize straw).

The percentage of hemicellulose content in all the substrates decreased with increase in incubation period for the first 28 days and increased by 42nd day. Highest hemicellulose content was observed in the 14th day of incubation in all substrates except substrates SD1 and SD2 which had their highest percentage in the untreated (control) samples. On the 14th day, sample SD3 had the highest percentage hemicellulose (39.97%) followed by sample MS (28.17%) and the least (9.64%) recorded in sample Sd1.

Cellulose content increased significantly ($P \leq 0.05$) with incubation time in samples SD1 (59.10 –59.15%), SD2 (48.96 – 52.85%) and MS (40.44 – 47.21%), but decreased in samples SD3 (60.26 – 53.28%) and RS (55.70 – 53.04%) after 42 days of incubation. Highest percentage of cellulose (65.07%) was observed in sample SD1 and followed by SD3 (56.37%).

During the incubation period, the pH of filtrates from samples SD1, SD2, SD3 and RS decreased from 7.7, 8.7, 8.0 and 7.3 to 4.2, 6.3, 5.2 and 7.2 respectively but the pH in sample MS increased from 7.1 to 7.2 as compared with the controls. Reducing sugar in substrates SD1, SD2, SD3, RS and MS ranged from 0.34 – 0.36mg/ml, 0.44 – 0.60mg/ml, 0.32 – 1.44mg/ml, 1.36 – 2.62mg/ml and 1.22 – 4.30mg/ml respectively. Total solid increased significantly ($P \leq 0.05$) all through the period of incubation. Highest percentage of Total solids (28.50%) was observed in sample SD1 after 42 days of incubation while lowest (6.00%) was observed in sample SD2 on the 14th day.

Table 2: pH of fermenting filtrates from all the substrates

Organism	Sample	Fermentation Period (day)					
		0	2	4	6	8	10
Saccharomyces cerevisiae	SD1 (0)	5.0^{a}	5.3^{a}	5.5^{a}	5.6^{b}	5.7^{b}	5.5^{b}
	SD2 (28)	6.4^{b}	5.4^{b}	5.5^{a}	5.4^{a}	5.6^{a}	5.6^{c}
	SD3 (42)	6.5^{c}	6.9^{d}	6.1^{b}	6.2^{c}	6.2^{c}	6.0^{d}
	RS (42)	7.3^{d}	8.0^{e}	7.1^{d}	6.9^{e}	6.8^{d}	6.8^{e}
	MS (0)	7.4^{e}	6.0^{c}	6.3^{c}	6.5^{d}	6.2^{c}	5.2^{a}
Baker's yeast	SD1 (0)	5.0^{a}	4.3^{b}	4.7^{c}	4.9^{c}	4.9^{c}	4.4^{b}
	SD2 (28)	6.4^{b}	4.3^{b}	4.5^{b}	4.4^{b}	4.5^{b}	4.5^{c}
	SD3 (42)	6.5^{c}	5.5^{c}	5.6^{d}	5.8^{d}	5.8^{d}	5.6^{d}
	RS (42)	7.3^{d}	6.5^{d}	6.4^{e}	6.4^{e}	6.3^{e}	6.2^{e}
	MS (0)	7.4^{e}	3.9^{a}	3.8^{a}	3.7^{a}	3.9^{a}	3.9^{a}
Saccharomyces cerevisiae + Baker's yeast	SD1 (0)	5.0^{a}	4.3^{b}	4.5^{c}	4.8^{c}	4.8^{c}	4.4^{b}
	SD2 (28)	6.4^{b}	4.4^{c}	4.4^{b}	4.5^{b}	4.6^{b}	4.6^{c}
	SD3 (42)	6.5^{c}	5.4^{d}	5.6^{d}	5.7^{d}	5.7^{d}	5.7^{d}
	RS (42)	7.3^{d}	6.4^{e}	6.2^{e}	6.3^{e}	6.2^{e}	6.3^{e}
	MS (0)	7.4^{e}	3.7^{a}	3.7^{a}	3.7^{a}	3.7^{a}	3.8^{a}

Values are means of duplicate readings ± SD. Mean value with different alphabets in superscript along the column are significantly different (P=0.05)

Key:
SD1 (0) = Filtrate sample of sawdust from *Amphimas pterocarpoides* after 0 day of pre-treatment
SD2 (28) = Sample of sawdust from *Ceiba pentandra* after 28 days of pre-treatment
SD3 (42) = Sample of sawdust from *Ricinodendron heudolotii* subspecies *africanum* after 42 days of pre-treatment
RS (42) = Filtrate sample from rice straw after 42 days of pre-treatment
MS (0) = Filtrate sample from maize straw after 0 day of pre-treatment

Ethanol content (%) of fermented filtrates is shown in Table 4. Ethanol was not produced at the onset of fermentation (0 day). Maximum ethanol production (1.8%) was recorded on the 2nd day of fermentation in samples SD2 and MS. There was fluctuation in the quantity of ethanol produced at different sampling time and the least production (0.5%) recorded in day 4 filtrate of most substrates.

Figure 1 shows the effect of maize pomace supplementation on the pH of fermented filtrates from selected high ethanol produced substrates. The pH decreased with increase in fermentation time and increased on the 10th day of fermentation except in sample SD2 fermented with *Saccharomyces cerevisiae*. The pH of two samples (SD2 and MS) fermented with Baker's yeast and the co-inoculation of *Saccharomyces*

Table 3: Reducing sugar (mg/ml) of fermenting filtrates from all the substrates

Organism	Sample	Fermentation Period (day)					
		0	2	4	6	8	10
Saccharomyces cerevisiae	SD1 (0)	0.62±0.0014^{b}	0.84±0.0028^{c}	0.56±0.0042^{a}	0.50±0.0028^{a}	0.54±0.0014^{a}	0.46±0.0028^{a}
	SD2 (28)	0.78±0.0028^{c}	0.64±0.0014^{a}	0.60±0.0028^{b}	0.62±0.0014^{c}	0.58±0.0028^{b}	0.62±0.0014^{c}
	SD3 (42)	0.58±0.0014^{a}	0.68±0.0042^{b}	0.66±0.0028^{c}	0.60±0.0014^{b}	0.60±0.0042^{c}	0.54±0.0028^{b}
	RS (42)	1.18±0.0042^{e}	1.28±0.0014^{e}	1.14±0.0028^{e}	1.18±0.0042^{e}	1.18±0.0014^{e}	1.22±0.0028^{e}
	MS (0)	0.96±0.0028^{d}	0.96±0.0014^{d}	0.74±0.0042^{d}	0.64±0.0014^{d}	0.82±0.0028^{d}	0.86±0.0042^{d}
Baker's yeast	SD1 (0)	0.62±0.0014^{b}	0.78±0.0028^{a}	0.78±0.0014^{b}	0.72±0.0042^{a}	0.74±0.0014^{a}	0.54±0.0042^{a}
	SD2 (28)	0.78±0.0028^{c}	0.84±0.0014^{b}	0.74±0.0042^{a}	0.78±0.0014^{c}	0.76±0.0042^{b}	0.74±0.0028^{b}
	SD3 (42)	0.58±0.0042^{a}	0.86±0.0014^{c}	0.84±0.0028^{c}	0.82±0.0042^{d}	0.82±0.0028^{d}	0.78±0.0042^{c}
	RS (42)	1.18±0.0014^{e}	1.42±0.0042^{e}	1.38±0.0028^{e}	1.36±0.0014^{e}	1.02±0.0028^{e}	0.86±0.0014^{e}
	MS (0)	0.96±0.0042^{d}	1.24±0.0028^{d}	0.88±0.0014^{d}	0.74±0.0028^{b}	0.78±0.0014^{c}	0.82±0.0028^{d}
Saccharomyces cerevisiae + Baker's yeast	SD1 (0)	0.62±0.0014^{b}	0.82±0.0042^{a}	0.80±0.0028^{b}	0.76±0.0042^{a}	0.66±0.0014^{a}	0.68±0.0028^{a}
	SD2 (28)	0.78±0.0042^{c}	0.86±0.0014^{b}	0.76±0.0028^{a}	0.78±0.0014^{b}	0.78±0.0028^{b}	0.80±0.0042^{b}
	SD3 (42)	0.58±0.0014^{a}	0.86±0.0028^{b}	0.82±0.0042^{c}	0.84±0.0014^{c}	0.84±0.0042^{c}	0.68±0.0014^{a}
	RS (42)	1.18±0.0028^{e}	1.34±0.0042^{d}	1.08±0.0028^{e}	1.00±0.0014^{e}	0.90±0.0028^{d}	1.16±0.0042^{d}
	MS (0)	0.96±0.0042^{d}	1.16±0.0014^{c}	0.92±0.0042^{d}	0.90±0.0028^{d}	0.96±0.0014^{e}	0.86±0.0042^{c}

Values are means of duplicate readings ± SD. Mean value with different alphabets in superscript along the column are significantly different (P=0.05)

Key:
Same as Table 2

Table 4: Ethanol content (%) of fermented filtrates

Organism	Sample	Fermentation Period (day)					
		0	2	4	6	8	10
Saccharomyces cerevisiae	SD1 (0)	0.0±0.0000	0.0±0.0000^{a}	0.0±0.0000^{a}	0.5±0.0141^{b}	0.5±0.0283^{b}	0.0±0.0000^{a}
	SD2 (28)	0.0±0.0000	1.8±0.0283^{c}	0.0±0.0000^{a}	0.5±0.0283^{b}	0.0±0.0000^{a}	0.0±0.0000^{a}
	SD3 (42)	0.0±0.0000	0.5±0.0424^{b}	0.0±0.0000^{a}	0.0±0.0000^{a}	0.0±0.0000^{a}	0.0±0.0000^{a}
	RS (42)	0.0±0.0000	0.5±0.0141^{b}	0.5±0.0283^{b}	0.0±0.0000^{a}	0.5±0.0424^{b}	0.5±0.0141^{b}
	MS (0)	0.0±0.0000	1.8±0.0141^{c}	0.0±0.0000^{a}	0.0±0.0000^{a}	0.0±0.0000^{a}	0.0±0.0000^{a}
Baker's yeast	SD1 (0)	0.0±0.0000	0.5±0.0424^{b}	0.5±0.0141^{b}	0.5±0.0424^{b}	0.0±0.0000^{a}	0.0±0.0000^{a}
	SD2 (28)	0.0±0.0000	0.9±0.0283^{c}	0.0±0.0000^{a}	0.5±0.0283^{b}	0.0±0.0000^{a}	0.5±0.0424^{b}
	SD3 (42)	0.0±0.0000	0.0±0.0000^{a}	0.5±0.0141^{b}	0.5±0.0424^{b}	0.0±0.0000^{a}	0.5±0.0141^{b}
	RS (42)	0.0±0.0000	0.5±0.0141^{b}	0.5±0.0283^{b}	0.5±0.0141^{b}	0.5±0.0283^{b}	0.5±0.0424^{b}
	MS (0)	0.0±0.0000	1.8±0.0424^{d}	0.0±0.0000^{a}	0.0±0.0000^{a}	0.0±0.0000^{a}	0.0±0.0000^{a}
Saccharomyces cerevisiae + Baker's yeast	SD1 (0)	0.0±0.0000	0.5±0.0283^{b}	0.5±0.0141^{b}	0.0±0.0000^{a}	0.0±0.0000^{a}	0.0±0.0000^{a}
	SD2 (28)	0.0±0.0000	1.8±0.0141^{d}	0.0±0.0000^{a}	0.5±0.0424^{b}	0.0±0.0000^{a}	0.0±0.0000^{a}
	SD3 (42)	0.0±0.0000	0.0±0.0000^{a}	0.5±0.0283^{b}	0.5±0.0141^{b}	0.5±0.0424^{b}	0.5±0.0283^{b}
	RS (42)	0.0±0.0000	0.5±0.0141^{b}	0.5±0.0283^{b}	0.5±0.0141^{b}	0.5±0.0424^{b}	0.5±0.0283^{b}
	MS (0)	0.0±0.0000	1.4±0.0283^{c}	0.0±0.0000^{a}	0.5±0.0283^{b}	0.0±0.0000^{a}	0.0±0.0000^{a}

Values are means of duplicate readings ± SD. Mean value with different alphabets in superscript along the column are significantly different (P=0.05)

Key:
Same as Table 2

cerevisiae and Baker's yeast were similar at most time. The pH of filtrates fermented with Baker's yeast and the co-inoculation of *Saccharomyces cerevisiae* ranged from neutral to slightly acidic while that fermented with *Saccharomyces cerevisiae* were slightly alkaline.

Reducing sugar (mg/ml) content of fermented filtrates from selected substrates supplemented with Maize pomace is as shown in Figure 2. Reducing sugar of filtrates inoculated with *Saccharomyces cerevisiae* only, Baker's yeast and co-inoculation of *Saccharomyces cerevisiae* and Baker's yeast ranged from 0.62 – 1.14mg/ml, 0.70 – 1.12mg/ml and 0.72 – 1.30mg/ml. The least reducing sugar (0.62mg/ml) was recorded in sample SD2 fermented with *Saccharomyces cerevisiae* and highest (1.30mg/ml) reducing sugar was observed in sample MS fermented with co-inoculation of *Saccharomyces cerevisiae* and Baker's yeast.

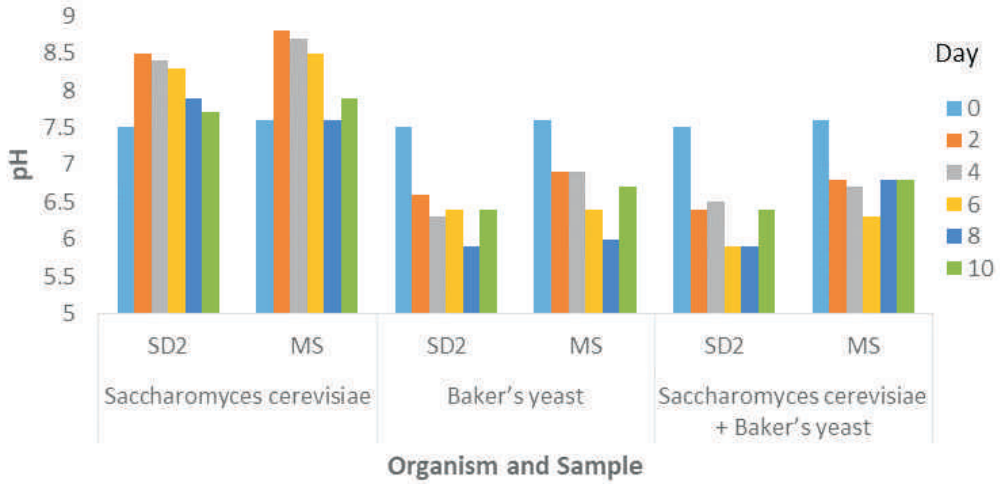

Figure 1: Effect of Maize pomace supplementation on pH of fermented filtrates from selected samples

Key: SD2 = Sample of sawdust from *Ceiba pentandra* supplemented with Maize pomace
MS = Maize straw supplemented with Maize pomace

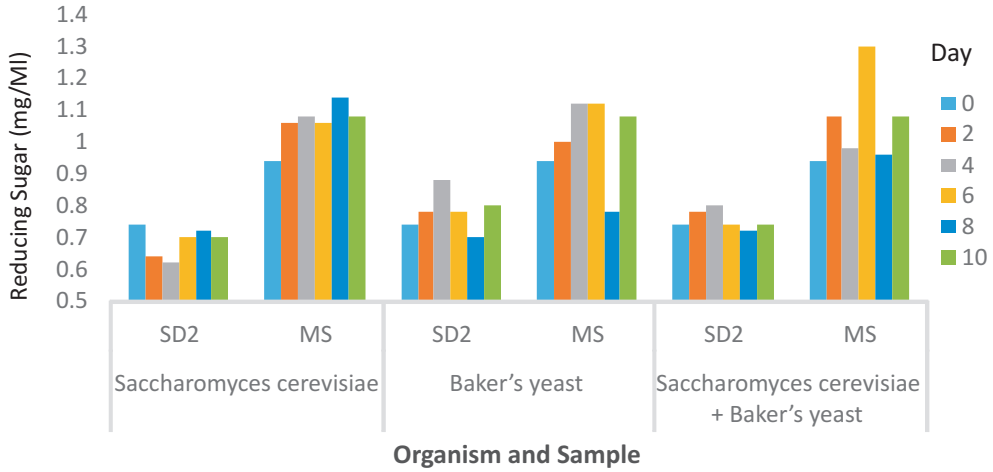

Figure 2: Effect of Maize pomace supplementation on Reducing sugar (mg/ml) of fermented filtrates from selected samples.

Key: SD2 = Sample of sawdust from *Ceiba pentandra* supplemented with Maize pomace
MS = Maize straw supplemented with Maize pomace

Table 5 shows the effect of maize pomace supplementation on the quantity of ethanol produced from the fermented filtrates of selected substrates. There was no ethanol production at the onset of fermentation (0 day). Maximum ethanol production was observed on the 10th day of fermentation in all filtrates except for 6th - day sample SD2 fermented with both *Saccharomyces cerevisiae* and Baker's yeast. There was better ethanol production in filtrates from sample SD2 (ranging from 0.5 – 0.7% in all organisms used at all sampling time) than MS which ranged from 0.3 – 0.6% in all organisms used at all sampling time.

Table 5: Ethanol content (%) of fermented filtrates from selected substrates supplemented with Maize pomace

Organism	Sample	Fermentation Period (day)					
		0	2	4	6	8	10
Saccharomyces cerevisiae	SD2	0.0±0.0000	0.5±0.0141^{c}	0.6±0.0283^{c}	0.6±0.0424^{b}	0.6±0.0141^{b}	0.7±0.0424^{b}
	MS	0.0±0.0000	0.4±0.0283^{b}	0.4±0.0141^{a}	0.5±0.0283^{a}	0.4±0.0424^{a}	0.6±0.0141^{a}
Baker's yeast	SD2	0.0±0.0000	0.5±0.0141^{c}	0.6±0.0283^{c}	0.7±0.0141^{c}	0.6±0.0424^{b}	0.7±0.0283^{b}
	MS	0.0±0.0000	0.5±0.0283^{c}	0.5±0.0424^{b}	0.6±0.0283^{b}	0.6±0.0424^{b}	0.6±0.0141^{a}
Saccharomyces cerevisiae + Baker's yeast	SD2	0.0±0.0000	0.5±0.0283^{c}	0.7±0.0141^{c}	0.7±0.0424^{c}	0.6±0.0141^{b}	0.6±0.0283^{a}
	MS	0.0±0.0000	0.3±0.0141^{a}	0.5±0.0283^{b}	0.6±0.0283^{b}	0.4±0.0141^{a}	0.6±0.0141^{a}

Values are means of duplicate readings ± SD. Mean value with different alphabets in superscript along the column are significantly different (P=0.05).

Key: SD2 = Sample of sawdust from *Ceiba pentandra* supplemented with Maize pomace
MS = Maize straw supplemented with Maize pomace

Figure 3 shows the effect of Corn steep liquor supplementation on pH of fermented filtrates from selected substrates. As the fermentation period increased, the pH of filtrates steadily decreased in all organisms except for sample MS fermented with *Saccharomyces cerevisiae*. Highest pH (7.2) was observed in sample MS fermented with *Saccharomyces cerevisiae* after 10 days of fermentation. Least pH was recorded on the 2nd day of fermentation except for sample SD2 fermented with *Saccharomyces cerevisiae* where it was observed on the 10th day of fermentation. The pH of sample MS was higher at all sampling times and in all organisms.

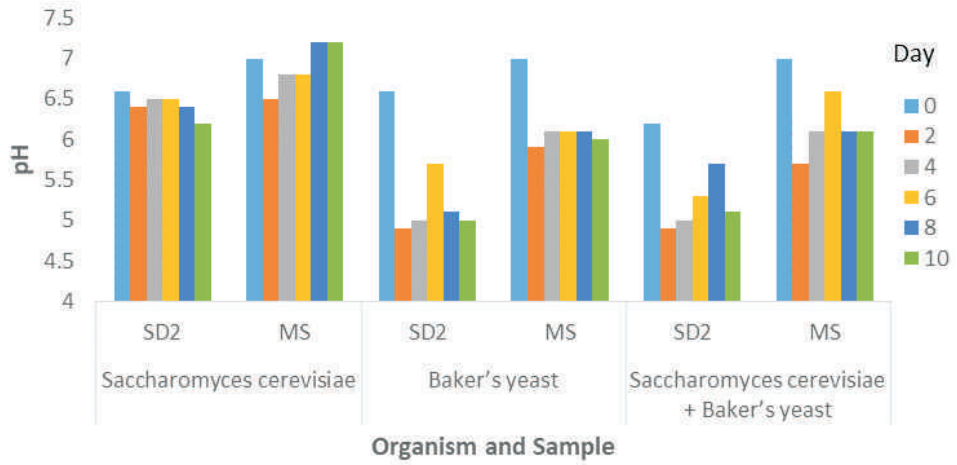

Figure 3: Effect of Corn steep liquor supplementation on pH of Fermented filtrates from selected samples

Key:
SD2 = Sample of sawdust from *Ceiba pentandra* supplemented with Corn steep liquor
MS =Maize straw supplemented with Corn steep liquor

Figure 4 shows the effect of Corn steep liquor supplementation on the Reducing sugar (mg/ml) content of fermented filtrates from selected substrates. Within each organism, sample MS had higher reducing sugar content at all fermenting time. Highest reducing sugar content (1.02mg/ml) was recorded on day 8 in sample MS filtrate fermented with Baker's yeast while the least reducing sugar content (0.60mg/ml) was observed in 10-day fermented filtrates of sample SD2 fermented with *Saccharomyces cerevisiae* and the co-inoculation of *Saccharomyces cerevisiae* and Baker's yeast.

Table 6 shows the effect of Corn steep liquor supplementation on ethanol produced from fermented filtrates of selected substrates. Ethanol was not produced at the onset of fermentation (0 day). There was increase in ethanol content of all fermenting filtrate with increase in fermentation period and highest ethanol content of each substrate was obtained after 10 days of fermentation with an exception of sample MS fermented with *Saccharomyces cerevisiae* where decrease in ethanol content was recorded after 6 days of fermentation. Highest ethanol content (0.8%) was observed in sample SD2 fermented with Baker's yeast and co-fermentation of *Saccharomyces cerevisiae* and Baker's yeast.

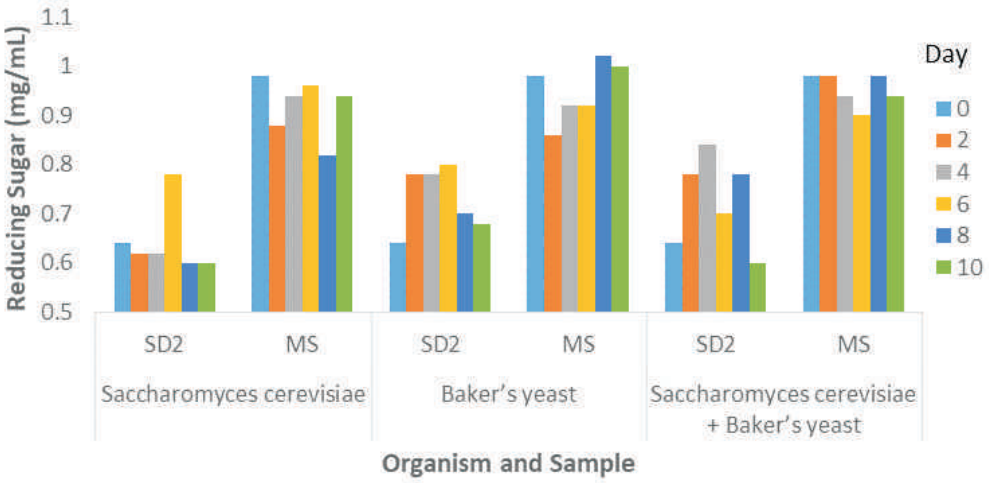

Figure 4: Effect of Corn steep liquor supplementation on reducing sugar (mg/ml) of fermented filtrates from selected samples

Key: SD2 = Sample of sawdust from *Ceiba pentandra* supplemented with Corn steep liquor
MS = Maize straw supplemented with Corn steep liquor

Table 6: Ethanol content (%) of fermented filtrates from substrates supplemented with Corn steep liquor

Organism	Sample	Fermentation Period (day)					
		0	2	4	6	8	10
Saccharomyces cerevisiae	SD2	0.0±0.0000	0.5±0.0141^{b}	0.6±0.0283^{b}	0.6±0.0141^{a}	0.7±0.0424^{b}	0.7±0.0283^{b}
	MS	0.0±0.0000	0.4±0.0141^{a}	0.5±0.0283^{a}	0.8±0.0424^{c}	0.3±0.0283^{a}	0.5±0.0141^{a}
Baker's yeast	SD2	0.0±0.0000	0.7±0.0141^{d}	0.7±0.0283^{c}	0.7±0.0424^{b}	0.7±0.0141^{b}	0.8±0.0283^{c}
	MS	0.0±0.0000	0.5±0.0283^{b}	0.6±0.0424^{b}	0.6±0.0141^{a}	0.7±0.0283^{b}	0.7±0.0424^{b}
Saccharomyces cerevisiae + Baker's yeast	SD2	0.0±0.0000	0.6±0.0283^{c}	0.6±0.0141^{b}	0.9±0.0283^{d}	0.7±0.0424^{b}	0.8±0.0566^{c}
	MS	0.0±0.0000	0.5±0.0424^{b}	0.6±0.0141^{b}	0.6±0.0424^{a}	0.7±0.0141^{b}	0.7±0.0283^{b}

Values are means of duplicate readings ± SD. Mean value with different alphabets in superscript along the column are significantly different (P=0.05)

Key: SD2 = Sample of sawdust from *Ceiba pentandra* supplemented with Corn steep liquor
MS = Maize straw supplemented with Corn steep liquor

Discussion

White-rot fungi have been identified as the most widely studied lignin-degrading organisms (Gold and Alic, 1993; Hamman *et al*., 1999). *Pleurotus tuber-regium* degraded the lignocellulosic materials (sawdusts, rice straw and maize straw) used in this study. There was a significant reduction in lignin, hemicellulose and cellulose content with increase in degradation time. This is in accordance with the work of Adenipekun *et al*. (2012), who degraded rattan wood and maize stovers using *Pleurotus tuber-regium* and reported a reduction in lignin, hemicellulose and cellulose content. Lignin content was recorded to be highest in sawdusts than rice straw and maize straw. This is in agreement with the findings of Kuforiji and Fasidi (2009) who reported that sawdust contains higher quantity of lignin than other agro-waste. Highest cellulose content was recorded in sawdust samples, an observation which agrees with the findings of Apetorgbor *et al*. (2013) who reported highest cellulose content in sawdust during the degradation of some selected plant wastes using *Pleurotus tuber-regium*. This may be due to the dominance of cellulose as the major structural polysaccharide in lignocellulosic material (Saha, 2003; Monlau *et al*., 2013).

The pH of filtrates decreased as fermentation day increased. This is in line with the work of Wakil and Onilude (2011) where a decrease was observed in fermented formulated weaning blends with increase in fermentation period. Decrease in pH observed has also been reported by Wakil and Osamwonyi (2012) to be the characteristics of fermentation.

In Maize straw, reducing sugar was observed to be highest in the un-pretreated substrate throughout the period of incubation. This may be due to maximum release of reducing sugar through the autoclaving process. Hsu *et al*. (2011), in order to avoid the by-products formed through acid treatment (e.g. furfural and 5-hydroxyfurfural) which inhibit the fermentation step, pre-treated corncob by autoclaving and recorded high yields of reducing sugar. They observed that most of the cellulosic components in the corncob were converted to reducing sugar after treatment with heating by autoclaving. Generally, pre-treatment can increase the accessible surface area, modify the lignin structure, reduce the cellulose crystallinity and polymerisation, and reduce the degree of hemicellulose acetylation (Monlau *et al.*, 2013; Zheng *et al.*, 2014).

Total solids (weight loss) increased with increase in incubation time. This result is in agreement with the findings of Albores *et al*. (2006) who reported a decrease in substrate dry weight with increase in incubation time.

Reducing sugar of fermenting filtrates significantly decreased as fermentation day increased. This was also reported by Teck-Yuan *et al.* (2011) who stated that the gradual decrease of reducing sugar level was found to be associated with the production of bioethanol. Rani *et al*. (2006) also reported a decrease in the level of reducing sugar as fermentation progressed.

Reducing sugar was observed to be higher in supplemented substrate than in unsupplemented substrate. This is in accordance with the work of Moonjai *et al*. (2013) who recorded a low amount of reducing sugar in unsupplemented rice husk but observed an increase in the concentration of reducing sugars produced when rice husk was supplemented with rice polish.

The reduction in the reducing sugar contents and increased weight loss (total solids) of biomass as fermentation time increases may be due to the insolubility and resistant structure of lignocellulose which prevent it from being efficiently degraded; and its low nutrient content (nitrogen, phosphorus, trace elements *etc*.) that makes the materials a poor nutrient source for degrading microorganisms (Tsavkelova and Netrusov, 2012; Monlau *et al.*, 2013; Sawatdeenarunat *et al.*, 2015).

The highest ethanol content which is 1.8% (14.22g/L) was recorded in hydrolysates from sawdust and Maize straw substrates fermented with *Saccharomyces cerevisiae*. Gurav and Geeta (2007) recorded ethanol concentration of 0.437g/L

by fermenting the hydrolysates from agro-wastes with *Saccharomyces cerevisiae* which is lower than the ethanol content that was recorded in this research work. Oyeleke and Jibrin (2009) recorded 26.83g/L and 18.31g/L of ethanol from guinea corn husk and millet respectively which are both higher than the ethanol concentration recorded in this research work. Wakil *et al*. (2013a) recorded ethanol concentration of 3.7% by fermenting palm oil mill effluent with *Saccharomyces cerevisiae* which is also higher than the ethanol concentration recorded in this research work.

Ethanol production was higher in unsupplemented substrate (1.8%) than in supplemented substrate (0.9%). This is in contrast with the work of Moonjai *et al*. (2013) who recorded a higher ethanol content in rice husk supplemented with rice polish (1.53%) than in unsupplemented rice husk (0.5%). Wakil *et al*. (2013b) recorded their highest ethanol concentration (2.3%) by supplementing palm oil mill effluent with glucose and sugarcane bagasse. This is higher than the quantity of ethanol recorded in this research work.
This work has shown that bioethanol can be produced from lignocellulosic materials (such as sawdust, rice straw and maize straw) and filtrates from untreated maize straw fermented with *Saccharomyces cerevisiae*, Baker's yeast and the co-inoculation of both within the pH range of 3.7 – 6.0 for 2 days can be used for bioethanol production. Likewise, filtrates from treated sawdust from *Ceiba pentandra* (SD2) fermented with *Saccharomyces cerevisiae*, Baker's yeast and the co-inoculation of both within pH of 4.3 – 5.4 for 2 days can also be used for bioethanol production. Supplementation resulted in increased pH (reduced acidity), increased reducing sugar content of maize straw with little or no effect on sawdust and significant reduction in production of ethanol.

In conclusion, fermentation of filtrate (hydrolysate) from sterile (autoclaved) fresh maize straw with *Saccharomyces cerevisiae* or Baker's yeast (*Saccharomyces cerevisiae*) within the pH range of 3.7-6.0 for 2days can be used for bioethanol production. Further work on the quantitative and qualitative analysis of the sugars present in the filtrate should be carried out.

References

Adenipekun, C. O., Okunlade, O. A. and Ogunjobi, A. A. 2012. Effect of *Pleurotus-regium* singer on degradation of rattan wood and maize stovers. *Journal of Applied Biosciences*. 51: 3633 – 3641.

Adenipekun, C. O. and Fasidi, I. O. 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.

Albores, S., Pianzzola, M. J., Soubes, M. and Cerdeiras, M. P. 2006. Biodegradation of agro industrial wastes by *Pleurotus* spp. for its use as ruminant feed. *Electronic Journal of Biotechnology*. 9(3): 215 – 220.

AOAC 1997. AOAC official method 973.18, fiber (acid detergent) and lignin in animal feed. Official methods of analysis of AOAC International. 16th ed. *Arlington*: ASA-SSA Inc. pp 28–29

AOAC. 1990. Official Methods of Analysis 11 Edn. Association of Official Analytical Chemist Washington DC.

Apetorgbor, A. K., Dzomeku, M. and Apetorgbor, M. M. 2013. Growth factors and cultivation of *Pleurotus tuber-regium* on selected plant wastes. *International Food Research Journal*. 20(6): 3387 – 3393.

Ballesteros, I., Negro, M. J. Oliva, J. M., Cabanas, A., Manzanares, P. and Ballesteros, M. 2006. Ethanol production from steam-explosion pretreated wheat straw. *Applied Biochemistry and Biotechnology* 130: 496 – 508.

Betts, W. B., Dart, R. K., Ball, A. S. and Pedlar, S. L. 1991. Biosynthesis and Structure of lignocellulose. In Betts (eds) Biodegradation: Natural and Synthetic Materials. *Springer-Verlag*, Berlin, Germany, pp. 139-155.

Campbell, C. H. and Laherrere, J. H. 1998. The end of cheap oil. *Scientific American.* pp 78 – 83.

Clancy, M. and Wilson, R. 1966. Development and application of a new chemical method for predicting the digestibility and intake of herbage samples. *Proceedings of 10th International Grassland Congress.* 445 pp.

Ehrman, T. 1994. Standard Method for Determination of Total Solids in Biomass. Chemical Analysis and Testing Task Laboratory Analytical Procedure. 1: 1 – 8.

Gold, M. H. and Alic, M. 1993. Molecular biology of the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Microbiological Reviews*. 57: 605 – 622.

Gurav, M. S. and Geeta, G. S. 2007. Effectiveness of Fungal pre-treatment of agro residues on ethanol production by yeast and *Zymomonas mobilis*. *Karnataka Journal of Agricultural Sciences*. 20(2): 301 – 304.

Hamman, O. B., de la Rubia, T. and Martinez, J. 1999. The effect of manganese on the production of *Phanerochaete flavido-alba* ligninolytic peroxidases in nitrogen limited cultures. *FEMS Microbiology Letters*. 177: 137 – 142.

Hsu, C. Chang, K., Lai, M., Chang, T., Chang, Y. and DerJang, H. 2011. Pre-treatment and hydrolysis of cellulosic agricultural wastes with a cellulase-producing *Streptomyces* for bioethanol production. *Biomass and Bioenergy*. 35(5): 1878 – 1884.

Isroi, Millati, R, Syamsiah, S., Niklasson, C, Cahyanto, M. N., Lundquist, K. and Taherzadeh, M. J. 2011. Biological pretreatment of lignocelluloses with white-rot fungi and its application: A review. *Bioresources* 6(4):5224 – 5259.

Kim, S. and Dale, B. E. 2004. Global potential bioethanol production from wasted crops and crop residues. *Biomass and Bioenergy* 26:361 – 375.

Kuforiji, O. O. and Fasidi, I. O. 2009. Biodegradation of agro-industrial wastes by an edible mushroom *Pleurotus tuber-regium*. *Journal of Environmental Biology*. 30(3): 355 -358.

Lang, X., Macdonald, D. G. and Hill, G. A. 2001. Recycle bioreactor for bioethanol production from wheat starch II. Fermentation and Economics. *Energy Sources* 23: 427 – 436.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Annals of Chemistry*. 31: 426 -428.

Monlau, F., Barakat, A., Trably, E., Dumas, C., Steyer, J.-P. and Carrère, H. 2013. Lignocellulosic materials into biohydrogen and biomethane: impact of structural features and pretreatment. *Critical Reviews in Environmental Science and Technology,* 43(3): 260-322.

Moonjai, N., Pukahuta, C. Salubchua, J. 2013. Simultaneous saccharification and fermentation of fungal bio-pretreated rice polish to ethanol. Available from http://www.docin.com/p-663140220.html.

Oyeleke, S. B. and Jibrin, N. M. 2009. Production of bioethanol from guinea cornhusk and millet husk. *African Journal of Microbiology Research*. 3(4): 147 – 152.

Oyeleke, S. B., Dauda, B. E. N., Oyewole, O. A., Okoliegbe, I. N. and Ojebode, T. 2012. Production of bioethanol from cassava and sweet potato peels. *Advances in Environmental Biology*. 6(1): 241 – 245.

Rani, E., Sunitha, M. and Devaki, K. 2006. Comparative study of ethanol production by batch fermentation using free cells of *Saccharomyces cerevisiae* and *Zymomonas mobilis*. *Asian Journal of Microbiology, Biotechnology and Environmental Science*. 8: 745 – 749.

Robertson, J. B. and van Soest, P. J. 1977. Dietar fiber estimation in concentrate feedstuffs. *Journal of Animal Science* .45 (1):245.

Saha, B.C. 2003. Hemicellulose bioconversion. *Journal of Industrial Microbiology and Biotechnology.* 30:279-291.

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H. and Khanal, S.K. 2015. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. *Bioresource Technology,* 178: 178-186.

Teck-Yuan, D., Siew-Ling, H., Lisa-Gaik-Ai, O. and Tau-Chuan, L. 2011. Investigating the potential of using coconut husk as substrate for bioethanol production. *International Conference on Biotechnology and Environment Management.* 18(6):29 – 32.

Tsavkelova, E.A. and Netrusov, A.I. 2012. Biogas production from cellulose-containing substrates: A review. *Applied Biochemistry*

and Microbiology, 48(5): 421-433.

Uihlein, A. and Schbek, L. 2009. Environmental impacts of a lignocellulosic feedstock biorefinery system: An assessment. *Biomass and Bioenergy* 33: 793 – 802.

Wakil, S. M. and Onilude, A. A. 2011. Time related total lactic acid bacteria population diversity and dominance in cowpea-fortified fermented cereal-weaning food. *African Journal of Biotechnology*. 10(6): 887 – 895.

Wakil, S. M. and Osamwonyi, U. O. 2012. Isolation and screening of antimicrobial producing lactic acid bacteria from fermented millet gruel. *International Research Journal of Microbiology*. 3(2): 72 – 79.

Wakil, S. M. Adelabu, A. B., Fasiku, S. A. and Onilude, A. A. 2013a. Production of Bioethanol from Palm Oil Mill Effluent using Starter Cultures. *New York Science Journal*. 6(3): 77 – 85.

Wakil, S. M. Fasiku, S. A., Adelabu, A. B. and Onilude, A. A. 2013b. Production of Bioethanol from Spontaneous Fermentation of Palm Oil Mill Effluent (POME). *Researcher* 5(2): 28 – 35.

Zheng, Y., Zhao, J., Xu, F. and Li, Y. 2014. Pretreatment of lignocellulosic biomass for enhanced biogas production. *Progress in Energy and Combustion Science,* 42: 35-53.

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