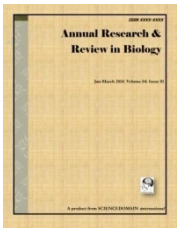

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Study on Production of Biogas and Bioethanol from Millet Husk

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Authors' contributions

This study was carried out in collaboration between all authors. Author UZF designed the work and supervised the laboratory analyses; author AA carried out the laboratory protocol and wrote the draft manuscript; authors UABY and MBY oversaw the literature and monitored the quality of the work while author KJU was responsible for the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The potentiality of millet husk in production of biogas and bio-ethanol was investigated. Proximate analysis was carried out on the millet husk and the husk had the following proximate composition: ash (33.83±0.67%), moisture content (10±0.03%), organic matter (56.17%) and Carbon-Nitrogen ratio (42.31%). It was observed that, addition of cow dung improved the quality and quantity of biogas generated. The volume of biogas in seeded digester was found to be higher; it had total volume of 7333.33 cm^3 and 5733.33 cm^3 of pure methane against 5400 cm^3 total volume and 4200 cm^3 of pure methane, in unseeded digester. Different concentrations (1-5%) of sulphuric acid were used to determine the yield of total reducing sugars and ethanol when the sugar was fermented, the result reveals that 3% sulphuric acid concentration yielded higher percentage of reducing sugars (21.40%) while the highest volume of distillate of ethanol by fermentation of hydrolysate was obtained with 3% H_2SO_4 (21.7 cm^3) with 0.70% purity. Based on cheap cost of production, easy production and use, biogas was found to be better than bio ethanol.

Keywords: Biogas; bioethanol; millet husk; reducing sugars; acid hydrolysis fermentation.

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1. INTRODUCTION

Today, the world relies heavily on fossil fuels for both domestic and commercial energy needs. The depletion coupled with the environmental effects of these fossil fuels has influence research in the development of alternative energy sources [1]. One of the alternative sources of energy is bio-energy. It represents the utilisation of biomass as starting material for the production of sustainable fuels and chemicals. The common fuels obtainable from biomass are bio-ethanol and biogas.

Bio-ethanol is produced from biomass through hydrolysis and fermentation while biogas can be generated when organic matter is fermented with the assistance of micro organisms in the absence of air or oxygen [2]. Bio-ethanol and biogas play an important role in reducing greenhouse gases emissions. Both processes of production only use energy from renewable energy sources. Hence, no net carbondioxide is added to the atmosphere, making them environmentally benign energy source. This paper compares the yield and cost of production of bio-ethanol and biogas from millet husk with the aim of ascertaining the most economical obtainable bio-fuel.

2. MATERIALS AND METHODS

2.1 Collection of Sample and Its Treatments

The material for this research is millet husk. The sample was collected from waste dumping sites in Jega metropolis of Kebbi state, Nigeria. The sample collected was air dried before drying in an oven at 110ºC. The dried sample was ground to fine powder and sieved to obtained higher surface area for the reaction. The powder was stored in a black polyethylene bag until required.

2.2 Determination of Moisture Content

A crucible was weighed and its mass was recorded as M_1, 2 g of the sample was transferred to the weighted crucible, placed in an oven at temperature of 105ºC and heated for 24 h. The process was repeated at one hour interval until a constant weight was attained. Thereafter, the crucible was removed from the oven, cooled in a desiccator containing silica gel and weighed as M_2 [3].

The percentage moisture content was calculated as:

$$Moisture\,Content\,(\%) = \frac{Lost\,on\,ignation}{Weight\,of\,Sample\,(2g)} x\,100$$

The percentage loss in weight indicated the percentage moisture content.

2.3 Determination of Ash Content

The ash of the sample was determined by weighing a crucible (W_1) and charged with 2g of the sample. The crucible and its content was put into a muffle furnace and heated at 600ºcfor 3 h, cooled in a desiccator containing silica gel and weighed. The percentage ash content was calculated as:

$$Ash Content\,(\%) = \frac{Amount\ of\ Ash}{Weight\ of\ Sample\,(2g)}\,x\,100$$

2.4 Determination of Nitrogen Content

Procedure: 2g of each dried sample was weighed into Kjeldahl digestion flask and 0.5g of Kjeldahl tablet was added followed by addition of 10cm^3 of concentrated tetraoxosulphate (VI) acid. The content was then heated in Kjeldahl digestion unit until the digest became clear (approximately 2 hours). After the digestion had been completed, the flask was cooled, diluted with 10cm^3 distilled water and filtered with a Whatman No.1 filter paper into a 100cm^3 volumetric flask and made up to the mark with distilled water. 10cm^3 of homogeneous aliquot solution was pipetted into distillation flask and 20cm^3 of 45% NaOH solution was added. The content was diluted to about 200cm^3 with distilled water and distilled using micro-Kjeldahl distillation apparatus. The distillate was collected in receiving flask containing 10cm^3 boric acid indicator solution. After the distillation, the distillate was titrated with standardized 0.01M HCl to the end point. Blank was determined using all the reagents in the same quantities as described above. The process was carried out in triplicate and the crude protein (CP) calculated using equation:

$$\text{Protein}\,(\%) = \frac{(a-b) \times 0.01M HCl \times 14\ x\ c}{d \times e} \times 6.25\ x\ 100$$

a = titre value for the digest
b= titre value for the blank
c = volume to which the digest was made up
d = volume of aliquot used in distillation
e = weight of dried sample

2.5 Determination of Organic Matter

The organic matter (volatile solid) was determined by subtracting the percentages of moisture and ash content from 100% [4].

Organic matter = 100% - (% ash + %moisture content).

2.6 Determination of Percentage Carbon

The percentage Carbon was estimated using the equation; %C = 0.58 x Organic matter [5].

2.7 Determination of Carbon- Nitrogen ratio

The Carbon to Nitrogen ratio was evaluated by calculating the ratio of organic Carbon content to that of Nitrogen content [6]:

$$C:N = \frac{\%\ \text{Organic carbon in the sample}}{\%\ \text{Nitrogen in the sample}}$$

2.8 Determination of pH before and After Digestion

A pre- calibrated pH meter (jenway-3015) was used to measure the pH values of each of the slurries before and after the digestion.

2.9 Method of Biogas Production

The digesters were five gallon capacity. A hole was bored on the top of the cap of the digester; tube from a urine bag was inserted through the hole and glued using araldite to make it air tight. The slurry was prepared from the stored sample by taking 400g in separate beaker and 2000 cm^3 of water was added to the substrate to obtain a ratio 1:5 W/V, mixed thoroughly and transferred to the digester [7]. Two groups of digesters were made, group A and B comprising of three digesters each, that is, A_1, A_2 and A_3 and B_1, B_2 and B_3 respectively). Some quantity of cow dung were thoroughly mixed (at ratio of 1: 8 cow dung to millet husk) with the substrate of group B (to serve as source of *methonogen*) [8], while to group A no cow dung was added. The volume of the biogas generated was collected continuously in the urine bags for 30days. Whenever the urine bag is filled its content will be emptied into a fresh urine bag and kept.

2.10 Removal of Carbon (IV) Oxide in the Biogas

The carbon (IV) oxide content of biogas was removed by passing the gas produced through a solution of 4M Sodium hydroxide, NaOH [8]. The reaction occurs in stages as follows:

$$2NaOH_{(aq)} + CO_2 \longrightarrow Na_2CO_{3(aq)} + H_2O_{(l)}$$

$$Na_2CO_{3(aq)} + H_2O_{(l)} + CO_2 \longrightarrow 2NaHCO_{3(s)}$$

Four molarNaOH solutions were dispensed in a 2 gallon plastic container. The container was capped with a double holed rubber cork through which an empty urine bag was connected to one of the hole with the receiving tube above the alkaline solution. The bag containing the generated biogas was then connected through the other hole with the dispensing tube deep into the alkaline solution. The urine bag containing the gas was pressed slowly so that the gas bubbles through gradually in the alkaline solution and collected into another empty urine bag (Plate 1).

Plate 1. Set up for purification of carbon dioxide

The approximate mass of purified biogas was determined by;

Volume of methane obtained (dm^3) / 22.4 (molar volume of gas) x molar mass of methane while the percentage mass of the gas was calculated as;

Mass of the gas yielded / total mass of substrate x 100

2.11 Method of Bio-ethanol Production

Methods used for production of bio ethanol include acid hydrolysis, fermentation and fractional distillation.

2.12 Pre-treatment

The pre-treatment was done based on the procedure described by Layokun[9]. Into five 1000 cm^3 different volumetric flasks, fifty gram (50g) of the powdered sample were transferred, 250 cm^3 of diethyl-ether was then added into each flask (to obtain ratio of 1:5 w/v), covered with aluminium foil for 24 h in order to remove fat extractives, and the residue left was washed with distilled water.

2.13 Acid Hydrolysis

The pre-treated samples were labelled A, B, C, D and E. 500 cm^3 of 1%, 2%, 3%, 4% and 5% sulphuric acid, H_2SO_4, were added to A, B, C, D and E respectively. The flasks were covered with cotton wool, wrapped in an aluminium foil, heated for 2 h on a hotplate at temperature close to boiling. The flasks were allowed to cool, filtered through double-layered muslin cloth then through No 1 Whatman filter paper [10].

2.14 Quantification of Reducing Sugars

The reducing sugars were estimated by using DNS reagent [11]. The glucose standard was prepared by dissolving 0.1g of glucose into 100 cm^3 of distilled water, 10 cm^3 portion of the first standard was pipetted and transfer into another 100 cm^3 volumetric flask and made to the mark using distilled water. To each test tube 3 cm^3 of DNS reagent was added. The content of each tube was placed in boiling water bath for 10 min to develop red brown colour. Then 1 cm^3 of 40% potassium sodium tartrate solution was added to stabilize the colour while hot then cooled at room temperature. The absorbance was measured at 508nm with a UV-visible spectrophotometer.

2.15 Acid Hydrolysis Fermentation

The fermentation was carried out based on the method describe by Kroumor et al. [12] and Oghgren et al. [13]. In to each flasks containing the hydrolysates, 3.0g of *Saccharomyce cerevisiae,* (Baker's yeast) was aseptically inoculated and supplemented with 1.0g of ammonium sulphate, $(NH4)_2SO4$, as nutrient and incubated at room temperature for seven days [14]. At the end of the fermentation period, the contents were distilled. The volume of the distillate was measured and the ethanol was subjected to quantitative test using spectrophotometric method.

2.16 Quantitative Estimation of Ethanol

This was carried out using UV-visible quantitative method of alcohol using saturated chromic (VI) reagent [11]. The ethanol standard was prepared by diluting 1 cm^3 of absolute ethanol (98% (v/v) AR) to 100 cm^3 using distilled water (1% ethanol stock solution). To each tube, 2 cm^3 of saturated acid - dichromate reagent was added to make the volumes to 10 cm^3. The content of each tube was then heated in a boiling water bath for 5min. for reaction to complete and developed colour. The absorbance of each concentration was measured at 581nm using UV- visible spectrophotometer. The mass of ethanol yield was determined by multiplying the volume obtained by the density of ethanol (0.8033g/ml) and the percentage yield of the ethanol was calculated by[10]:

Mass of ethanol yielded / total mass of substrate X 100

3. RESULTS AND DISCUSSION

3.1 Results

The results obtained in this study are presented in Tables 1-5 all values are mean±S.D. of triplicate measurement.

Table 1. Proximate analysis of millet husk

Parameter	Results (%)
Moisture	10±0.03
Ash	33.83±0.67
Organic matter	56.17±0.88
Carbon	32.58±1.10
C:N	42.31
% degraded mass (unseeded)	20.68±0.56
% degraded mass (seeded)	30.80±0.80

Table 2. Biogas production for 30 days using millet husk substrate

Days	Vol. of Biogas (cm^3)*	Vol. of methane (cm^3)	Vol. of CO_2 (cm^3)	Methane-biogas ratio
1-8	2000	1400±8.17	600	0.7
9-18	2000	1600±11.55	400	0.8
19-30	1400±14.14	1200±14.14	200	0.86
Total vol.	5400	4200	1200	0.78
Amount of methane by volume (%)		77.78	22.22	
Amount of methane by weight (%)		0.75	0.59	

**Daily Temperature (^oC) = 35-36^oC*

Table 3. The Biogas production of seeded Millet husk in 30 days

Days	Vol. of Biogas (cm^3)	Vol. of methane (cm^3)	Vol. of CO_2 (cm^3)	Methane-biogas ratio
1-9	2000	1366.67±10.54	633.33	0.7
10-15	2000	1533.33±10.54	466.67	0.8
16-19	2000	1700	300	0.9
20-30	1333.33±1105	1133.33±13.33	200	0.8
Total vol.	7333.33	5733.33	1600	0.78
Amount by volume (%)		78.18	21.82	
Amount by weight (%)		1.02	0.78	

**Daily Temperature (^oC) = 34-36^oC*

Table 4. Percentage yield of reducing sugars of the hydrolysed millet husk

Acid concentration (%)	Concentration(g/l)	Yield per mass of substrate (%)
1	7.01±0.30	14.00
2	8.34±0.30	16.00
3	10.70±0.30	21.40
4	8.34±0.40	16.70
5	5.30±0.30	10.60

Table 5. Volumes of ethanol obtain by fermentation of hydrolysate

Sample	Volume of distillate (cm^3)	Percentage (v/v)
A	3.8±1.20	0.007
B	18.2±20	0.80
C	21.7±1.70	0.70
D	12.3±1.10	0.30
E	4.7±1.60	0.008

3.2 Biogas

The results indicated that millet husk is a good feedstock for generation of biogas. Tables 2 and 3 show total volume of 5400 cm^3 for generated biogas and 4200 cm^3 of purified methane gas. Biogas volume of 7333.33 cm^3 and 5733.33 cm^3 of pure methane gas obtained from digester seeded with cow dung implies that, seeding can improve the total output of biogas generation. It was observed that, the production of biogas at the early stage is faster in the unseeded digester (day 1-9). This is because of high pH in the seeded digester due to the digestion of organo-nitrogeneous compounds in the dung which leads to the release of NH_3, but when the methane production level is stabilized it remain buffered between 7.2 – 8.2 [15]. The methane gas to total gas (volume) ratio increases as days pass by in both experiments (Tables 2 and 3), which could be attributed to the gradual development of *Methanogenic* bacteria as the digester became more anaerobic [8]. The rate of Biogas production was also observed to be gradually increasing at the early stage and reached peak toward the end of third week of the experimental period (collection of gas decreased), then declined and finally went extremely slowly. This is because at the early stage, the group of synergistic bacteria whose activities depends on release of biogas were not fully developed and hence they have low activity which leads to the low rate of production of the gas. Towards the middle of second week to the end of third week of the experiment, the

bacteria are fully developed and the slurry has its appreciable concentration, hence, maximum rate of production. The rate gas production finally stopped, because the concentration of microbes in the slurry has been reduced lower than the level suitable for the biogas generation (rate of reaction is proportional to concentration).

The masses of slurry degraded show a difference between seeded and unseeded digester. A decrease of 30.80% and 20.68% of original masses were observed at the end of the process for seeded and unseeded digesters respectively. This gives the percentage of substrate degraded for seeded and unseeded digesters, when the experiment was carried out under the same condition. The difference was due to the introduction of bacteria from cow dung to the millet husk.

Similar experiments carried out by Bagudo et al. [7], indicated that, the volume of biogas produced by 400g of Millet husk were 5230 cm^3 and 5640 cm^3 for unseeded and seeded substrate respectively in nine weeks. Both results were lower than the result in this experiment in spite of the longer time it took. This may be due to the fact that the collection method used by Bagudo et al. [7] was an inverted cylinder method which measures the volume of the gas collected by water displacement, and therefore,water must be saturated with gas before it is displaced. Furthermore, there are some components of biogas particularly CO_2 which is soluble in water that can dissolved and lead to the reduction of his experimental volume.

$$CO_{2(g)} + H_2O_{(l)} \longrightarrow H_2CO_{3(aq)}$$

While this experiment used direct collection of the gas in urine bag without passing through water, implying that all the generated gas was collected without any loss due to water solubility.

Another factor that enhanced higher volume of gas includes; lower moisture content of the substrate (10%) compared to (20%) for Bagudo et al. [7], since the higher the moisture content the lower the potential of the substrate to produce biogas [16]. This is because high moisture content means low total solid.

3.3 Reducing Sugars

Millet husk hydrolysed with 3% (v/v) H_2SO_4 (Table 4) released the highest percentage of reducing sugar (21.40%), which indicates that, the best acid concentration for the hydrolysis of the millet husk is 3%. The percentage of reducing sugars obtained from 1% and 2% acid were less than that of 3% H_2SO_4 concentration, this is because 1% and 2% concentration are not strong enough to break through lignin into hemicllulosic and cellulosic part of the substrate which contained the reducing sugars. At 4% H_2SO_4 concentration the percentage of the reducing sugars began to decrease, this suggests that, the reducing sugars released from hydrolysis of hemicellulosic part of the substrate begin to degrade and give compounds such as furan and carboxylic acids beyond 3% acid concentration.

3.4 Bio Ethanol

Result shown in Table 5 indicated a linear relationship between the volume of ethanol produced and the mass of fermentable sugar. Though amount of ethanol can increase with increase in mass of fermentable sugar up to production of 15% ethanol by the total

volume.Beyond this level the ethanol becomes toxic to the fermenting organism and production starts to decline which eventually kill the fermenting organisms (*Saccharomyces cerevisiaes)* before the total sugar present is fermented [17]. It is therefore, impossible to produce ethanol containing more than 15% by volume using only fermentation. In this experiment, the quantity of reducing sugar obtained was diluted in order to ensure complete fermentationof the sugar. The highest volume of ethanol distillate obtained (21.7 cm^3) in this experiment is similar to the highest distillate of ethanol obtained (22.8 cm^3) using millet husk but lower than the volume of ethanol (25.30 cm^3) obtained in guinea corn husk [10].

3.5 Comparison between Biogas and Bio-ethanol Percentage Yield

Table 6 summarizes the percentage yields by mass of highest methane (in biogas) and bio-ethanol produced in the experiment with respect to the total masses of the substrate used. The highest yield of pure methane (1.02%) was obtained after purifying the biogas in seeded digester while the percentage ethanol was (0.24%) the highest obtainable using optimum acid concentration for the cellulose hydrolysis.

Table 6. Comparison between the biogas and bio ethanol percentage yield per mass of sample

Product	%yield
Biogas (methane)(w/w)	1.02
Bio ethanol(w/w)	0.24

From the result, it is clear that, the substrate produced more fuel in form of biogas when compared to ethanol by the 4.25:1. This large ratio simply indicates the viability of the biogas production over bio-ethanol. On the other hand, the bio-ethanol production is more tedious having at least three stages involving hydrolysis, fermentation and distillation while the biogas involved only the digestion because the purification is not very essential as the gas can be used directly without purification since the percentage of CO_2 is small. On comparison of the cost, the bio ethanol is more expensive to produce especially because of usage of H_2SO_4 for hydrolysis and energy is required during distillation. These add to the cost of production of the bio ethanol.

4. CONCLUSION

According to the findings of this research, millet husk can be processed into bio-fuels (biogas and bio ethanol).

The biogas production is more economical judging from the percentage yield, 78.10% of biogas (Table 3) compared to the percentage yield of ethanol 0.7% (Table 5), ease of production and cost implication. Its production is cheaper because:

I. The raw material does not require high level of procession, purification or use of a chemical for commencement of the gas generation.
II. The gas generated can be directly used from the digester.
III. The construction and maintenance of the digester is not difficult, as can be produced and maintained in rural areas.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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