



Research Paper

Production of bioethanol from millet husk using fungal consortium

¹Umar, S., ²Baki, A. S. and ^{*3}Bello, A.

¹College of Life Science and Technology, Beijing University of chemical technology, Beijing, 100029, China.

²Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University, Sokoto State, Nigeria.

³Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto State, Nigeria.

*Corresponding author E-mail: abccrcfge28@gmail.com

Received 4 November 2017; Accepted 13 December, 2017

The study was aim to evaluate the potential of millet husk in the generation of bioethanol through fermentation using *Saccharomyces cerevisiae*. Samples of millet husks were obtained from Rugga Waru shafting center of Bado area in Wamakko Local Government and was sun dried. The sample was pre- treated using 600 mls of dilute-sulfuric acid and was later hydrolyzed using the enzyme *Aspergillus niger* for five days. The reducing sugar content of the hydrolyzed sample was also obtained to be 0.02 mg/ml. The hydrolyzed samples were fermented using

reactivated yeast (*Saccharomyces cerevisiae*) for five days at 35°C. The fermented broth was finally distilled at 78°C to obtain the bioethanol. The quantity of the bioethanol was (79 g/l) and the concentration of the bioethanol produced was obtained to be (0.621%). The thermal property of the millet husk was also obtained to be -7.907 mg, and the rate of production was high.

Keyword: *Aspergillus niger*, bioethanol, millet husk, *Saccharomyces cerevisiae*

INTRODUCTION

With concerns over issues relating to global warming and depletion of fossil fuel reserve, search for sustainable and renewable energy sources has become a matter of global interest (Oliviera *et al.*, 2005). Ethanol is an oxygenated fuel with high octane value like that of petroleum fuels. Ethanol is known to run combustion engines at higher compression ratios and thus superior performance. Ethanol is widely used as a partial gasoline replacement in United State of America and other parts of the world such as Canada and Brazil. It can also be used as cooking, heating and lightning appliances. Fuel ethanol that is produced from corn has been used in gasoline or oxygenated fuel since 1980. The blending of ethanol into petroleum-based automobile fuels can significantly decrease petroleum use and release of greenhouse gas emissions. Ethanol that is blended directly with gasoline in a mix of 10% ethanol and 90% gasoline is called gasohol. The world population is estimated to increases from 6.7 billion to 8 billion by 2030. On the other hand, global oil production is expected to decline from 25 billion barrels to 5 billion barrels by 2050. Thus the energy

demand of the future is likely to play a key role in geo-political economics. Given this reality, nations around the world are investing in alternative sources of energy, including bioethanol. The leading nations in bioethanol production are Brazil and the USA, USA is the world's largest producer of bioethanol. Asian countries altogether account for about 14% of world's bioethanol production (Joshi *et al.*, 2011).

The natural energy resources have been estimated to last only a few years. Therefore alternative energy source such as ethanol, methane and hydrogen are being considered. Some biological processes have rendered possible routes for producing Bioethanol and methane in large quantities. A world wide interest in the utilization of Bioethanol as an energy source has stimulated studies on the cost and efficiency of industrial process for ethanol production (Tanaka, 2006). Ethanol can be a safer alternative to the common additive, methyl tertiary butyl ether (MTBE) in gasoline. MTBE is toxic and is a known contaminant in groundwater. Thus ethanol can be a substitute to mitigate the problems associated with the

rising energy demands across the world as well as a way to reduce greenhouse gas emissions to an extent of 85% (Joshi *et al.*, 2011).

Ethanol may be produced either from petroleum products or from biomass. Today most of the ethanol produced comes from renewable sources. Although currently most of the ethanol produced from renewable resources comes from sugarcane and starchy grains, significant efforts are being made to produce ethanol from lignocellulosic biomass (almost 50% of all biomass in the biosphere) such as agricultural residues (Bothast and Saha, 1997). Bioethanol production from sugarcane and starch rich feed stocks such as corn, potato, etc., is considered first generation process and it has already been developed. The long-term viability of this process is in question because it will require significantly increased amounts of cultivatable land and significant hike in food prices that will ultimately lead to food insecurity (Mitchell, 2008). Estimates clearly point to the fact that first generation ethanol production process can not sufficiently meet the global energy needs. Therefore, second generation processes to produce bioethanol are gaining momentum. The second generation processes will use lignocellulosic materials for this purpose and biosphere clearly has sufficient supplies of lignocellulosic materials. The production of ethanol from lignocellulosic biomass (Stover, wheat straw, sugarcane bagasse, rice straw, rice hull, corn cob, oat hull, corn fiber, woodchips and cotton stalk; energy crops such as switch grass and Alfa-Alfa and various weeds such as *Saccharum spontaneum*, *Lantana camara*, *Eichhornia crassipes* (water hyacinth) etc) has become one of the best alternatives, because these sources have widespread abundance and the cost of their procurement is relatively cheap (Mitchell, 2008).

The major aim of the research is to evaluate the potential of millet husk in the generation of bioethanol through fermentation using *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Sample collection

Millet husks were collected in a clean polythene bag from Rugga Waru shafting center of Bado area in Wamakko Local Government of Sokoto State. The samples collected were air dried for one week and transported to Microbiology Laboratory, Faculty of Science, Usmanu Danfodiyo University, Sokoto for analysis (Rabah *et al.*, 2011).

Sample processing

The dried samples were ground to powder using pestle mortar and sieved for obtaining higher surface area for the reaction. The samples were stored until required.

Microbial analysis

Isolation of *Aspergillus niger*

Aspergillus niger was isolated from soil collected from the female section of the central mosque of Usmanu Danfodiyo University Sokoto. The soil was serially diluted on Potato Dextrose Agar. A sample suspension was prepared by adding 1.0 g sample to 10 ml distilled water and mixed well for 15 min. the suspension was serially diluted 10^{-1} to 10^{-3} . 0.1ml (from the dilution factor 3) was pipette onto the PDA plate; it was then spread with a glass spreader and incubated at 28°C for observation (Jasuja *et al.*, 2013).

Identification of the fungi

The fungal isolate was identified by morphological examination and its characteristics. Morphological characteristics were examined under the microscope (Jasuja *et al.*, 2013). Re-activation of baker's yeast (*Saccharomyces cerevisiae*). The baker's yeast was directly inoculated into a test tube containing glucose broth, it was allowed to dissolve. It was then inoculated unto the already prepared PDA plates by streaking and then the plates were incubated for 3-5 days.

Process of bioethanol production

Pre-treatment of the sample

Pre-treatment of the millet husk was done using dilute H_2SO_4 . 30 g of millet husk was weighed and placed into a conical flask and 600 mls of dilute H_2SO_4 was added into the conical flask containing the sample. It was then autoclaved at 121°C for 30 min and was filtered using Whatman paper. The residue was washed for 30 min until neutral pH and oven dried at 100-105°C (Nicholas and Wayman, 2012).

Enzyme hydrolysis

Enzymatic hydrolysis was carried out according to the method described by (Gupta *et al.*, 2009). Four 500 ml capacity conical flasks were used for the enzymatic hydrolysis. The conical flasks were labeled A, B, C and D. 10 g of the millet husk was added in the flasks and 100 ml of distilled water was added. The flasks were plugged with cotton wool and aluminium foil, and were then sterilized at 121°C for 15 min. Each flask was inoculated with 0.5 ml suspension of the cellulolytic fungi *Aspergillus niger*. The flasks were incubated at 37°C for 5 days. After the 5 days period, the samples were filtered

through Whatman filter paper NO 1 and the filtrate was used for fermentation (Gupta *et al.*, 2009).

Determination of reducing sugar content.

The reducing sugar content of the hydrolyzed millet husk was determined using the Dinitrosalicylic acid colorimetric method of (Miller 1959). With glucose as standard. It was assayed by adding 2 ml of 3, 5-DNS reagents to 1ml of the sample. The mixture was heated in boiling water for 10 min to develop the red-brown color. Then 1ml of 40% potassium sodium tartrate solutions was added to stabilize the color, it was then cooled to room temperature under running tap water. The absorbance of the sample was measured at 491 nm using ultraviolet (UV-VIS) spectrophotometer. The reducing sugar content was determined by making reference to a standard curve of known glucose (Rabah *et al.*, 2011).

$$\text{Concentration of sugar} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

Fermentation

The fermentation of the samples hydrolyzed was carried out according to the methods described by Oyeleke and Jibrin, (2009). 100 ml of the millet husk hydrolysates were dispensed in a 500ml capacity conical flask, the flask was then covered with cotton wool, and wrapped with aluminium foil; it was then autoclaved at 121°C for 15 min. the tubes were allowed to cool at room temperature and were then inoculated aseptically with the fermentative organism. The conical flask was inoculated with *Saccharomyces cerevisiae*. The flask was incubated anaerobically at 35°C for 5 days. The hydrolysate was then distilled according to standard method Oyeleke and Jibrin (2009).

Distillation

The fermented broth was dispensed into round bottom flask fixed to a distillation column enclosed running tap water. A conical flask was fixed to the other end of the distillation column to collect the distillate. A heavy mantle was used to heat the round-bottom flask containing the fermented broth by adjusting the temperature to 78°C (Oyeleke and Jibrin, 2009).

Determination of quantity of ethanol produced

The distillate was measured using a measuring cylinder and expressed as the quantity of ethanol produced in g/l

by multiplying the volume of distillate by the density of ethanol (0.8033 g/l). g/l is equivalent to the yield of 100 g of dried substrate (Humphrey and Okafogau, 2007).

Determination of concentration of bioethanol produced

This was carried out using UV-visible quantitative analysis of alcohols using chromium VI reagent according to the methods described by Patel *et al.* (2007). 1 ml of standard ethanol was diluted with 100 ml of distilled water to give a concentration of 1%. Then 0, 2, 4, 6 and 8 ml each of the 1% ethanol was diluted to 10 ml with distilled water to produce 0, 0.2, 0.4, 0.6 and 0.8% of the ethanol. To each of the varying ethanol concentrations, 2 ml of chromium reagent was added and allowed to stand for an hour for color development. The absorbance of each concentration was measured at 588 nm using UV-visible spectrophotometer (UV-1650pc, Shimadzu) and the readings used to develop standard ethanol curve. Then five 5 ml of each bioethanol samples were put in test tubes and treated with 2 ml of the chromium reagent. The mixture was allowed to standard for an hour and the absorbance was measured at 540 nm using the UV-VIS spectrophotometer (UV-1650pc, Shimadzu) (Rabah *et al.*, 2014).

Determination of density of bioethanol produced

Density of bioethanol produced was measured by using the formula of calculating density. The quantity of 10 mls of the bioethanol was measured using measuring cylinder, by weighing in a beaker on a weighing balance to obtain the mass (Manual of weighing applications, 1999).

$$\text{Density} = \frac{\text{mass}}{\text{Volume}}$$

Thermo gravimetric analysis of millet husk

Thermo gravimetric analysis or thermal gravim analysis is a method of thermal analysis in which changes in physical and chemical properties of materials are measured as a function of increasing temperature (with constant heating rate), or as a function of time (with constant mass loss). The TGA instrument continuously weighs a sample as it is heated to temperatures of up to 2000°C for coupling with FTIR (Fourier transform infrared spectroscopy) and mass spectrometry gas analysis. As the temperature increases, various components of the sample are decomposed and the weight percentages of each resulting mass change are measured (Coats and

Table 1. Concentration of bioethanol produce from *Saccharomyces cerevisiae*.

Sample	Type	Conc. mg/ml	WL540.0	Wgt.Factor
1.	Standard	0.200	0.001	1.000
2.	Standard	0.400	0.021	1.000
3.	Standard	0.600	0.079	1.000
4.	Standard	0.800	0.070	1.000

Table 2. Concentration of bioethanol produce from other biomass.

Sample	Type	Conc. mg/ml	WI540.0
A	Unknown	0.569	0.052
B	Unknown	0.741	0.075
c.	Unknown	0.552	0.050

Redfern, 1963).

RESULTS AND DISCUSSION

The result in (Tables 1 and 2) shows the analyzed results of the bioethanol obtained, in sample one, the reducing sugar concentration was 0.02 mg/ml, concentration of the bioethanol was 0.621 mg/l, the Quantity obtained was 79 g/l, Viscosity of 11.1 mpas, density of 0.841g/ml and thermal analysis of the millet husk used in the production of bioethanol was -7.907 mg. The high volume of bioethanol obtained from millet husk using *Saccharomyces cerevisiae* at 5 days of fermentation was (79 g/l) higher than the volume of ethanol obtained for other plants biomass such as guinea corn husk (26.31 g/l), sawdust (12.30 g/l) by Oyeleke and Jibrin, (2009); sweet potato peels (16.47 g/l), rice husk (06.22 g/l) by Nikzad *et al.*, (2013) and empty fruit branches of palm oil tree (10.32 g/l) by Mohd *et al.* (2011). These differences in volume of bioethanol obtained from the different plant biomass could be associated with the major composition of the various feed stocks in addition to the fermenting organisms involved in the production process. A low yield of reducing sugar of 0.02mg/ml was obtained which is in disagreement with the findings of Rabah *et al.* (2011), who obtained a high yield of 3.21% from bacterial hydrolyzed millet husk, which may be because the isolates used for the hydrolysis may not be able to break the reducing sugar easily from its structural components. Since little amount of reducing sugar was available for the fermentative organism, less concentration of bioethanol was produced (Rabah *et al.*, 2011). The higher the sugar content the more the ethanol can be produced (Yamba *et al.*, 2007).

Reducing sugar

This was carried out as described by (Rabah *et al.*,

2011). Using the formula

$$\text{Concentration of sugar} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

$$\text{Absorbance of standard} = 0.183$$

$$\text{Concentration of standard} = 0.02\text{mg/ml}$$

$$\text{Absorbance of standard} = 0.160$$

$$\text{Concentration of Sugar} = \frac{0.160 \times 0.02}{0.183} = 0.017 = 0.02 \text{ mg/ml}$$

Quantity of bioethanol produced

According to the method described by Oyeleke *et al.*, (2012) as

Quantity of ethanol produced = distillate collected x density of ethanol

$$\text{Distillate collected} = 98 \text{ mls}$$

$$\text{Density of ethanol} = 0.803\text{g/ml}$$

$$\text{Quantity of ethanol produced} = 98 \times 0.8033 = 78.72 = 79\text{g/l}$$

Standard deviation

$$\text{Standard Deviation} = \frac{\sqrt{\sum(x - \bar{x})^2}}{N}$$

Values of X=

$$0.569$$

$$0.741$$

$$0.552$$

$$N=3$$

$$\bar{x} = \frac{0.569 + 0.741 + 0.552}{3}$$

$$= \frac{1.862}{3}$$

$$\bar{x} = 0.621$$

$$S = \sqrt{\frac{(0.569 - 0.621)^2 + (0.741 - 0.621)^2 + (0.552 - 0.621)^2}{3}}$$

$$S = \sqrt{\frac{(-0.052)^2 + (0.12)^2 + (-0.069)^2}{3}}$$

$$S = \sqrt{\frac{0.003 + 0.014 + 0.005}{3}}$$

$$S = \sqrt{\frac{0.022}{3}}$$

$$S = \sqrt{0.007}$$

$$S = 0.084$$

Concentration of sample

Average concentration =

$$\frac{0.569 + 0.741 + 0.552}{3}$$

$$= \frac{1.862}{3} = 0.621 \text{ mg/l}$$

Density of bioethanol produced

Weight of sample measuring 10 mls

A = 8.44g

B = 8.40g

C = 8.39g

Average mass =

$$\frac{8.44 + 8.40 + 8.39}{3} = 8.41 \text{ g}$$

$$\text{density} = \frac{8.41}{10}$$

$$= 0.841 \text{ g/ml}$$

A low concentration of 0.0621mg/l was produced from millet husk which is in disagreement with the findings of Oyeleke *et al.* (2012), who reported a high concentration

of 17.6 mg/l from cassava peel. This is likely due to presence of more carbohydrate content in cassava peel than in millet husk. Zakpaa *et al.* (2009) reported that as toxic compounds such as lignin residues, acids and aldehydes accumulated in the fermentation medium, the concentration of bioethanol tend to decrease. Similarly, the fermentative organisms' inability to ferment pentoses more especially xylose which is the main component of Hemicellulose fraction of lignocellulose may be another attributable factor for low bioethanol concentration.

Conclusion

Fermentation of the hydrolyzed millet husk after five days of fermentation and distillation yielded bioethanol quantity of (70.0 g/l). The bioethanol produced has a concentration of (0.621 mg/l), viscosity (11.1 mpas), and density of (0.841 g/ml). It can be concluded that it is possible to successively use millet husk for bioethanol production. The use of millet husk is a means of controlling environmental pollution since bioconversion of cellulose biomass into fermentable sugars for production of bioethanol was done using cellulose degrading microorganism, thus, making bioethanol production economically and environmentally friendly.

Recommendation

(a) It is recommended that biomass feed stock such as millet husk should be enhanced towards bioethanol production industries that will serve as a source of income and also to overcome the effect of global warming and environmental pollution.

(b) It is recommended that bioethanol policy is implemented which can be helpful in improving environmental and rural economic development and sustainable agricultural practice.

REFERENCES

- Humphrey CN, Okafoagu UC (2007). Optimization of ethanol production from garcinia kola (bitter kola) pulp Agro Waste. *African Journal of Biotechnology* 6(17): 2033-2037.
- Jasuja ND, Saxena R, Chandra S, Joshi SC (2013). Isolation and Identification of Microorganisms from Polyhouse Agricultural Soil of Rajasthan. *African Journal of Microbiological Research* 7(41):4886-4891.
- Joshi B, Bhatt MR, Sharma D, Joshi J, Malla R, Sreerama L (2011). Lignocellulotic ethanol production: Current practices and Recent Developments: A review. *Biotechnology and Molecular Biology Review* 6(8):172-182.
- Manual of weighing Applications (1999). Sartorius Marketing Weighing Technology Part 1, pg 2.
- Miller GL (1959). "Use of Dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemist*, vol. 31, pp. 426.
- Mohd AK, Loh SK, Nasrin A, Astimar A, Rosnah MS (2011). "Bioethanol

- production from empty fruit bunches hydrolysate using *Saccharomyces cerevisiae*". *Research journal of environmental science*, **5**(6):573-586.
- Nicholas JR, Wayman EE (2012). Dilute sulfuric-acid: A cheap acid for optimization of bagasse Scint (Lahore), **24**(1):41-45 ISSN103-5316.
- Nikzad M, Movagharnjad K, Najafpour GD, Talebnia F (2013). "Comparative studies on effect of pretreatment of rice husk for enzymatic digestibility and Bioethanol production". *International Journal of engineering*. Transactions B:Applications, **26**(5):455-464.
- Oliviera, De MED, Vaughn BE, Rykeiel Jr, EJ.(2005). Ethanol as Fuel Energy, Carbon dioxide balances and Ecological foot print. *Biosciences*.**55**:593-602.
- Oyeleke SB, Jibrin NM (2009). Production of Bioethanol from Guinea corn husk and Millet husk. *African Journal of Microbiology Research* **3**(4):145-152.
- Oyeleke SB, Dauda BEN, Oyewale OA, Okoliegbe IN, Ojebode T (2012). Production of Bioethanol from Cassava and Sweet Potato Peels. *Advances in Environmental Biology* **6**(1):241-245.
- Patel SJ, Onkarappa R, Shobha KS (2007). "Fungal pretreatment studies on rice husks and bagasse for ethanol production," *Electronic Journal of Environmental, Agricultural, and Food Chemistry*, vol. 6, **4**, pp. 1921-1926.
- Rabah AB, Oyeleke SB, Manga SB, Hassan LG (2014). Utilization of Rice husks and Groundnut shells for Bioethanol Production. *International Journal of Environmental Science and Development*, **5**(4):367-368
- Rabah AB, Oyeleke SB, Manga SB, Hassan LG (2011). Utilization of millet and guinea corn husks for Bioethanol production. *African Journal Microbiological Resource*; **5**(31): 5721-5724.
- Tanaka L (2006). Ethanol fermentation from biomass current state and prospects. *Applied Microbiological Biotechnology* **69**: 627-642.
- Yamba FD, Wamukwamba CK, Matsika E, Sangiso M (2007). Investigation into the Production and Use of Bioethanol from Sweet Sorghum as an Alternative Fuel. Department of Mechanical Engineering, School of Engineering, University of Zambia, Lusaka. Pg 4.
- Zakpaa HD, Mak-Mensah EE, Johnson FS (2009). "Production of Bioethanol from corncobs using *Aspergillus niger* and *Saccharomyces cerevisiae* in simultaneous saccharification and fermentation," *African Journal of Biotechnology*, **8**(13):3018-3022.