Full Length Research Paper

Bacteriological evaluation of millet husk as substrate for bioethanol production

Umar, S.¹ and Bello, A.²

¹College of Life Sciences and Technology, Beijing University of Chemical Technology, Beijing, China.
²Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto.

Accepted 21 November, 2012

The study was to evaluate the potential of millet husk in the generation of Bioethanol through fermentation using Saccharomyces cerevisiae. Sample of millet husk was obtained from Rugga Waru shafting center of Bado area in Wamakko Local Government and was sun dried. The sample was pre-treated using 600mls 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 0.02mg/ml. the hydrolyzed sample was 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 (79g/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 -7.907mg, and the rate of production was high. The aim and Objectives of the work is to evaluate the potential of millet husk in the generation of Bioethanol through fermentation using Saccharomyces cerevisiae through the production of Bioethanol from millet husk using enzymatic hydrolysis, determination of the concentration, reducing sugar and viscosity of Bioethanol produced and to investigate the thermal properties of millet husk.

Key word: 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 (Oleviera 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 US and other parts of the world such as Canada and Brazil. It can also be used as in a variety of 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 geopolitical 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 worlds 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 (Mitchell, 2008). 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 (corn 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 aim and Objectives of the work is to evaluate the potential of millet husk in the generation of Bioethanol through fermentation using Saccharomyces cerevisiae using enzymatic hydrolysis, determination of the concentration, reducing sugar and viscosity of Bioethanol produced and to investigate the thermal properties of millet husk.

**MATERIALS AND METHODS**

**Sample collection**

Millet husk was collected in a clean polythene bag from Rugga Waru shafting center of Bado area in Wamakko Local Government of Sokoto state. The sample collected was air dried for one week and transported to microbiology laboratory for analysis (Rabah et al., 2011).

**Sample processing**

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

**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 1.0g sample to 10ml 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 $28^\circ C$ 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 $H_2SO_4$. 30g of millet husk was weighed and placed into a conical flask and 600mls of dilute $H_2SO_4$ was added into the conical flask containing the sample. It was then autoclaved at 121$^\circ C$ for 30 minutes and was filtered using Whatman paper. The residue was washed for 30 minutes until neutral pH and oven dried at 100$^\circ C$-105$^\circ C$ (Rabah et al., 2011).

**Enzyme hydrolysis**

Enzymatic hydrolysis was carried out according to the method described by (Gupta et al., 2009). Four 500ml capacity conical flasks were used for the enzymatic hydrolysis. The conical flasks were labeled A, B, C and D. Ten g of the millet husk was added in the flasks and 100ml of distilled water was added. The flasks were
plugged with cotton wool and aluminum foil, and were then sterilized at 121°C for 15 mins. Each flask was inoculated with 0.5ml 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 2ml 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 tartarate 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).

\[
\text{Concentration of sugar} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}
\]

**Fermentation**

The fermentation of the samples hydrolyzed was carried out according to the methods described by Jasuja et al., (2013). 100ml of the millet husk hydrolysates were dispensed in a 500ml capacity conical flask, the flask was then covered with cotton wool, and wrapped with aluminum 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 Jasuja et al., (2013).

**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. (Jasuja et al., 2013).

**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 (0.8033g/l). g/l is equivalent to the yield of 100g of dried substrate. (Humphrey and Okafogu, 2007)

**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 percentage of each resulting mass change can be measured. Results are plotted with temperature on the X-axis and mass loss on the Y-axis (Coats and Redfern, 1963).

RESULT AND DISCUSSION

The result in table 1 shows the analyzed results of the Bioethanol obtained, the sample was one, the reducing sugar concentration was 0.02mg/ml, concentration of the Bioethanol was 0.621mg/l, the Quantity obtained was 79g/l, Viscosity of 11.1mpas, Density of 0.841g/ml and Thermal analysis of the millet husk used in the production of Bioethanol was -7.907mg.

The high volume of Bioethanol obtained from millet husk using *Saccharomyces cerevisiae* at 5 days of fermentation was (79g/l) higher than the volume of ethanol obtained for other plants biomass such as guinea corn husk (26.31g/l), sawdust (12.30g/l) by Jasuja et al., 2013; sweet potato peels (16.47g/l), rice husk (06.22g/l) by Nikzad et al., 2013 and empty fruit branches of palm oil tree (10.32g/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)

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 17.6mg/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 pentose’s more especially xylose which is the main component of Hemicelluloses fraction of lignocelluloses may be another attributable factor for low Bioethanol concentration.

**Table 1. Results of Bioethanol produced.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reducing sugar (mg/ml)</th>
<th>Concentration (mg/l)</th>
<th>Quantity (g/l)</th>
<th>Density (g/ml)</th>
<th>Thermo gravimetric analysis (mg)</th>
<th>Viscosity (mpas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Millet husk</td>
<td>0.02</td>
<td>0.621</td>
<td>79</td>
<td>0.841</td>
<td>-7.907</td>
<td>11.1</td>
</tr>
</tbody>
</table>

CONCLUSION

Fermentation of the hydrolyzed millet husk after five days of fermentation and distillation yielded Bioethanol quantity of (70.0g/l). The Bioethanol produced has a concentration of (0.621mg/l), Viscosity (11.1mpas), and Density of (0.841g/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

Based on the above result, 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. Furthermore, Bioethanol policy is implemented which can be helpful in improving environmental and rural economic development and sustainable agricultural practice.

REFERENCES


acid for optimization of bagasse Scint (Lahore), 24(1):41-45, ISSN103-5316.


