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Research Paper

Bioethanol Production from Corn Cobs Wastes as Biofuel

[1]Tambuwal, A. D., *[2]Baki A. S. and [3]Bello, A.

[1]Department of Chemistry, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria.
[2]Department of Microbiology, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria.
[3]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria.
*Corresponding Author E-mail: bakisbaki2000@yahoo.com

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Bioethanol is the ethanol from biomass through hydrolysis and fermentation processes. Raw materials of bioethanol are sugary, starch and fibrous materials. Wastes of corn cobs are one of many sources of fibrous materials. In this study, the effect of enzymatic hydrolysis was determined using corn cobs wastes in bioethanol production. Thirty gram of powdered corn cobs wastes were used for enzymatic hydrolysis. Pretreatment was carried out through hydrolysis and then it was fermented. Parameters such as reducing sugar, quality, concentration, density and viscosity of bioethanol production were determined. 0.167mg/ml, 76cm^3, 0.331mg/l, 0.9995g/cm^3 and 14.1g/cm^3 respectively.

Keywords: Bioethanol, corn cobs, enzymatic hydrolysis, wastes materials

INTRODUCTION

Corn cob, a waste product of corn contains large amount of sugars that can be further utilized to produce various compounds (Cao *et al*., 1996). The bioconversion of lignocelluloses to biofuel from cheap non-edible materials such as corn cob for renewal energy is imperative. Thus, by varying temperature conditions during the fermentation process, maximum productivity of biofuel on an industrial scale can be optimized. In the brewing industry, production of biofuel is carried out by the fermentation of starchy materials, in which case, sugars are converted into bioethanol with carbon dioxide and water (Hongguang, 2006) as byproducts. For waste plant materials to be valuable, it must be converted to fuel as a sustainable substitute to fossil fuel. Therefore, there is a need for renewable energy resources from non-edible agricultural sources such as corn cob to replace fossil forms. This is because gas emissions from plant feedstock fuel are less than those emitted by fossil forms and thus beneficial to the environment and global warming (Demirbas, 2005; Hongguang, 2006). Bioethanol produced from corn uses only a small part of the plant material, whereby only the starch from the kernel is transformed into bioethanol (Cao *et al.,* 1996). Several research studies have been carried out on the production of bioethanol from corn cobs through simultaneous saccharification and fermentation of lignocelluloses agricultural wastes by Kluyveromyces marxianus 6556 (Zhang *et al.,* 2009), using *Aspergillums Niger* and Saccharomyces cerevisae in simultaneous saccharification and fermentation (Zakpaa *et al.,* 2009) and from Lignocellulosic Biomass (Kumar *et al.,* 2009). Corn however, is a main staple food in South Africa with an annual production of 8.04 million tons (Adesanya and Raheem, 2009). The cobs produced from corn are mainly used as manure for agricultural production. According to the report of Latif and Rajoka (2001), modern biotechnology allows the use of such lignocelluloses substrates as corn cobs in the production of chemicals and fuels, utilizing microorganisms. It has been shown that when corn is used for bioethanol production at higher temperatures, yeast cells die resulting in a decrease in alcohol yield when the pulp is concentrated, while optimal temperature for maximum productivity occurs at 32°C (Araque *et al.,2008*). It is therefore, necessary to select the optimum temperature at which yeast strains can ferment the sugars from lignocelluloses material. The Simultaneous Saccharification and Fermentation (SSF) process has been identified as economically viable for the conversion of these substrates to fermentation products (Cao *et al.,* 1996). Conversion of glucose and xylose to ethanol by coyeast strains has been successfully obtained by (Taniguchi *et al.,* 1997) using a respiratory deficient mutant of Saccharomyces cerevisiae and Pitchiastipitis. Pichiastipitis strains ferment xylose at

a high capacity of 57 g L–1 than any other yeast, provided the pH is maintained at between 4.5 and 6 and temperature of 25-26°C (Jeffries *et al.,* 2007). According to Jeffries *et al.* (2007), maximum yield of ethanol is obtained when a mixture of S. cerevisiae and P. *stipitis* are introduced into a medium containing both glucose and xylose. The amount of bioethanol produced therefore, depends on the optimal temperature which, invariably influence sugar utilization by yeast cells (Mwesigye and Barford, 1996). It is obvious several microorganisms have used in the production of bioethanol but none has utilized a combination of S. cerevisiae and P. *stipitis* in the production of bioethanol from corn cobs. This study, therefore, utilized an agricultural waste material (corn cobs) in the production of bioethanol as a cheap but effective alternative fuel source to power automobile. Furthermore, time and temperature in the bioethanol production process using the two yeast strains (*S. cerevisiae* and *P. stipitis*) were optimized. The main aim of this research is to produce bioethanol from corn cobs wastes.

MATERIALS AND METHODS

The materials to be used in this research work include; corn cob, test tubes, potato dextrose agar plates, conical flasks, measuring cylinder, filter paper, spectrophotometer, incubator, wire loop, autoclave, anaerobic jar, water bath, viscometer, and distillation machine, hot-plate, *Saccharomyces cerevisiae* (Baker's yeast), *Zymomonas mobilis Aspergillums Niger*, Potato Dextrose Agar (PDA), 5% H_2SO_4 .

Sample collection

Sample was collected in Polyethylene bags from Dangeshuni local government area and Dundaye village Sokoto state, Nigeria and transported to Microbiology Laboratory in Usmanu Danfodiyo University Sokoto for analysis (Oyeleke *et al.,* 2012).

Sample preparation

The sample (corn cobs) was sun dried and grinded into powder by using wooden pestle and mortar. The powdered form i.e. (corn cob) was sieved several times using a sieve. The sieve materials was further dried in hot air oven at 70°C for 24 h and stored at room temperature until time for the experiment (Beomsoo *et al.,* 2012).

Pretreatment of the sample

The pre-treatment was done using 5% of H_2SO_4 as it was done by (Abba *et al.,* 2013). 50 g of the powdered sample was poured into two different 1000 cm^3 volumetric flasks; 500 cm^3 of 5% H_2SO_4 was added into each flask (to obtain a ratio of 50:50 W/V). Aluminum foil was used to cover it for 24 h. The sample was decanted to remove fat extractives and the residue left, was washed using distilled water.

Potatos dextrose agar (PDA)

Thirty nine (39 grams) of potato dextrose agar powder was dissolved in 1 litre of distilled water in conical flask. It was shaken to dissolve properly and heated with hot plate; the mixture was prepared at 121°C for 15 min in an autoclave (Oyeleke and Manga, 2008).

Process of bioethanol production

Isolation of *Aspergillums Niger*

Floor dust was collected using sterilized spatula from Science Lecture Theater (SLT) in Usmanu Danfodiyo University Sokoto, and transferred to a sterilized sample container. The soil was serially diluted; a sample suspension was prepared by adding 1.0 g of sample to 10 ml of distilled water and mixed well for 10 min. The suspension was diluted serially 10^{-1}, 10^{-2} and 10^{-3}. 1 ml (from the third dilution factor) was measured using a syringe and inoculated into a Potato Dextrose Agar (PDA), a glass spreader was used to spread and incubated at 280°C in an incubating room for 5 days for colony observation (Jasuja *et al.,* 2013).

Isolation of *Zymomonas mobilis*

Isolation of *Zymomonas mobilis* was done from Roselle juice, which was carried out by the method described by (Obire, 2005). The sample was washed and cooked to obtain the juice. The juice was serially diluted from 10^1 up to the fifth 10^5. From the third dilution factor 0.1 ml was taken and inoculated into nutrient agar medium by using spread plate method. The plates was incubated in an incubator at 37°C for 24 h. Colonies suspected to be *Zymomonas mobilis* was characterized on the basis of morphological and biochemical characteristics. The isolates was purified by streaking on a freshly prepared media and incubated at 37°C for 24 h. The ability of *Zymomonas mobilis* to ferment various carbohydrates using glucose, fructose, sucrose, maltose and lactose was determined by growing the isolates in liquid standard medium (glucose broth) containing 1% (w/v) of the particular carbohydrate (Obire, 2005).

Re-activation of Bake's yeast (Saccharomyces cerevisiae)

1 g of 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 5 days to obtain colony of *Saccharomyces cerevisiae* (Gou *et al.,* 2014).

Enzymatic hydrolysis of sample

Pretreated sample is normally used in enzymatic hydrolysis. Here, 10 g of the pretreated sample was poured in twenty different 250 ml conical flasks containing 100 ml of distilled water, the pH was adjusted in triplicates, 3 samples were adjusted to pH 6, 7, 8 using potassium hydroxide (KOH) and Salicylic acid, and two samples were left at their normal pH, where each of them will serve independently as control for a distinct fermentation. The samples were labeled sample A (10 samples; 3 of pH 6, 7, 8 and 1 control), and sample B (10 samples; 3 of pH 6, 7, 8 and 1 control). The samples were sterilized at 121°C for 15 min, using autoclave, then the conical flasks were allowed to cool and then inoculate with *Aspergillums Niger*, cover with cotton wool and wrap with aluminum foil and incubate at 37°C for five days, (Humphrey and Caritas, 2007).

Determination of reducing sugar

After hydrolysis, reducing sugar was determined using Bertrand method and spectrophotometry.This was carried out by adding 2 ml of Dinitrosalicyclic acid (DNSA) reagents to 1 ml of the sample in a test tube. The mixture was heated in boiling water for 5 min and thereafter cools under running tap water. The concentration of the reducing sugar was determined using the formula (Rabah *et al.,* 2011).

$$\text{Conc. of reducing sugar} = \frac{\text{Absorbance of sample} \times \text{Conc. standard}}{\text{Absorbance of glucose}}$$

The absorbencies of the samples were measured at 491 nm using ultraviolet (UV-VIS) spectrophotometer. The absorbance at 491nm of the resulted coloured solution (slightly brown) was read in a spectrophotometer against a blank reagent (Rabah *et al.,* 2011). The blank reagent was prepared by substituting the hydrolyzed sample with distilled water. The reducing sugar was subsequently determined by making reference to a standard curve of a known glucose concentration (Rabah et al., 2011).

Fermentation of sample by Saccharomyces cerevisiae (Bake's yeast)

The fermentation of the samples was carried out according to the methods described by Oyeleke and Jibrin, (2009). The hydrolyzates (samples) in 250 ml capacity conical flasks were inoculated aseptically with Saccharomyces cerevisiae (Baker's yeast); the flasks were then covered with cotton wool, and wrapped with aluminum foil. The flasks were incubated anaerobically at 35°C for 5 days.

Fractional distillation

The fermented samples in 250 ml capacity conical flasks were placed on a heating mantle fixed to a distillation column connected to a cooling chamber from the above. Another set of conical flasks were fixed to the other end of the distillation column to collect the distillate (Ethanol). A heating mantle with the temperature adjusted to 78°C as the standard temperature for ethanol production was used to heat the round-bottom flask containing the fermented samples (Oyeleke and Jibrin, 2009) for a period of 40 min to give room for comparison.

Determination of bioethanol quantity produced

The distillate (Ethanol) collected over a slow heat at 78°C (for a period of 40 min.), was measured using a measuring cylinder, and expressed as the quantity of ethanol produced in g/l by multiplying the volume of distillate collected at 78°C by the density of ethanol (0.8033g/ml). The g/l is equivalent to the yield of 100 g of the dried substrate (Humphrey and Caritas, 2007).

Determination of bioethanol concentration

This was carried out using UV-visible quantitative analysis of alcohols using potassium dichromate reagent. 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. 2 ml of chromium reagent was added to each one of the ethanol concentrations, and allowed to stand for an hour for colour development. The absorbance of each concentration was measured at 540 nm using UV-visible spectrophotometer. Then 5 ml of each produced Bioethanol samples were poured in test tubes then 2 ml of chromium reagent was also added. The mixture was allowed to standard for an hour and the absorbance was measured at 540nm using the UV-VIS spectrophotometer (Oyeleke and Jibrin, 2009).

Determination of viscosity of bioethanol

Determination of the viscosity of Bio-ethanol viscosity is a measure of the resistance of a fluid being deformed by either shear stress .or resistance to flow. There are two quantities that are called viscosities which are dynamic

Table 1. Quantity of substance present in corn cob.

Sample	Reducing sugar (mg/ml)	Concentration (mg/l)	Quantity (cm3)	Density (g/cm3)	Viscosity (g/cm3)
Corn cob	0.167	0.331	76	0.9995	14.10

viscosity, absolute viscosity or simple viscosity to distinguish it from the other quantity, but are usually just called viscosity of a fluid to sits density (Mott, 2006).

Determination of density bioethanol

Density of bioethanol produced was measured by using the formula of calculation of density of substances. The volume of bioethanol was measured using measuring cylinder. The weight of the bioethanol was measured using a beaker on weighing balance (Amenaghawon *et al.,* 2012).

$$\text{Density} = \frac{(X_2 - X_1)}{(X_3 - X_1)}$$

Where, X_1 = weight (g) of empty density bottle
X_2 = weight (g) of empty density bottle + sample
X_3 = weight (g) of empty density bottle + water

RESULTS

The result in Table 1 shows the analyzed result of the bioethanol obtained, the result shows that, the reducing sugar concentration was 0.167mg/ml, concentration of bioethanol was 0.331mg/L, the quantity obtained was 76 cm^3 viscosity of bio ethanol was 14.10g/cm^3 and density of bio ethanol was 0.9995g/cm.^{3}

DISCUSSION

The result presented in (Table 1) shows that corn cobs wastes hydrolyzed substrate gave a low concentration of bioethanol (0.331mg/l), density of bioethanol (0.9995g/cm^3), the quantity observed was (76cm^3) and reducing sugar was (0.167mg/ml). The result of the research does not correlate with the result obtained from findings of (Oyeleke *et al.,* 2012). In this research work, bioethanol production was studied based on the process of hydrolysis using *Aspergillums Niger* and fermentative production of ethanol using *saccharomyces cerevisiae.* Enzymatic hydrolysis is the main step in biomass to ethanol conversion (Oyeleke *et al.,* 2008). In this study a fungal culture *Aspergillums Niger* was used as source of cellulose enzymes in hydrolysis, step which hydrolysis complex cellulolosic substrate by the release of extracellular cellulose enzymes and release reducing sugars. The amount of sugar released depends on the type and nature of substrate as there was variation in the amount produced by the substrate. The amount of reducing sugar release determines the volume of bioethanol to be produced; this was clearly stated by (Oyeleke and Jibrin, 2009) that cost of cellulose and recovery of fermentable sugar after enzymatic hydrolysis are the important factor which will decide the tangible cost of biomass to ethanol process. Bioethanol production is widely studied process for biofuels production. Other researchers have studied various raw materials and different methods of bioethanol production but, recently it was being observed that lignocelluloses materials are focused for bioethanol production in this research. A low concentration (0.331 mg/l) was produced from corn cobs wastes which are in disagreement with the findings of Oyeleke *et al.,* (2012) who report a high concentration of 17.6 mg/l from cassava peel. This is likely due to the present of high carbohydrate content in cassava peel than in corn cobs. The volume of bioethanol produced from corn cobs using *saccharomyces cerevisiae* at 5 days of fermentation was 29g/l higher than the volume of bioethanol obtained from other plants biomass such as sawdust (12.30g/l), pineapple (26.31g/l) by (Oyeleke and Jibrin, 2009), rice husk (06.22g/l) by Coa *et al*. (1995) and empty fruit branches of palm tree (10.33g/l) by (Oyeleke and Manga, 2009). The differences in volume of bioethanol obtained from different biomass could be associated with the major composition of various feed stocks in addition to fermenting organisms involved in the production process.

Conclusion

The result of this study shows that corn cobs known to contain reducing sugar with concentration 0.167 mg/ml, concentration of bioethanol was 0.331mg/L, the quantity of bioethanol obtained was 76cm^3 the viscosity of bioethanol was 14.10 g/cm^3 and density of bioethanol was 0.9995g/cm^3. Therefore the findings of this work suggest that ethanol can be produced from corn cobs wastes rather than allowing it to contribute a nuisance to the environment.

Recommendations

(a) It is recommended that biomass materials such as corn cobs 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) The utilization of agricultural waste for biofuel production should given more consideration in the society and further researches should be carried out so as to improve the production and usage of biofuel which is cheaper and environmental friendly.

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