International Scholars Journals

African Journal of Botany ISSN: 3519-3824 Vol. 8 (4), pp. 001-004, April, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Utilization of millet and guinea corn husks for bioethanol production

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Accepted 15 November, 2019

In this study, the rumen of ruminant animals served as a source for isolation of bacteria used in the hydrolysis of millet husks and guinea corn husks prior to fermentation. The isolates were characterized and identified as *Bacillus firmus, Bacillus circulans, Escherichia coli, Proteus rettgerii, Paenibacillus macerans* and *Serratia marcescens*. *Zymomonas mobilis* and *Saccharomyces cerevisiae* isolated from a previous study were used as the fermentative organisms. The production of bioethanol was done by hydrolysis and fermentation. The results obtained revealed that highest yield of reducing sugar of (4.10%) was obtained from guinea corn husk while the reducing sugar yield of millet husk was 3.21%. The highest concentration of bioethanol of (2.33%) was produced using the combination of *S. cerevisiae* and *Z. mobilis* for fermentation of guineacorn husks. Similarly, the lowest concentration of 0.79% was obtained when *Z. mobilis* was used on hydrolysates from millet husk. The results of the study revealed the potentiality of the two agro wastes to produce bioethanol. However, guinea corn husk is more promising than millet husk as it produces more bioethanol and can be used for large scale production.

Key words: Bioethanol, millet husk, guinea corn husk, *Saccharomyces cerevisiae*, *Zymomonas mobilis*.

INTRODUCTION

The burning of fossil fuels at the current rate is likely to create an environmental crisis globally through the generation of carbon (IV) oxide (CO_2), methane (CH_4), and a significant quantity of nitrous oxides. Most of these harmful gases are formed due to incomplete combustion of fossil fuels. As a result of this, there is a growing international quest for an alternative energy source. Ethanol produced from biomass through fermentation contains 35% O_2 that may result in a more complete combustion of fuel and thus reduces tailpipe emissions (Chandel et al., 2007). Moreover, biomass energy can play an important role in reducing greenhouse gases emissions. Ethanol production process only uses energy from renewable energy sources. Hence, no net carbondioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source. The world interest has been shifted to utilization of agricultural wastes for bioethanol production. The long-term benefits of using waste residues as lignocellulosic feedstocks will be to introduce a sustainable solid waste management strategy for a number of lignocellulosic waste materials. This will contribute to the mitigation of greenhouse gases through sustained carbon and nutrient recycling to reduce the potential for water, air and soil pollution associated with the land application of organic waste materials and to broaden the feedstock source of raw materials for the bioethanol production industry (Mtui, 2009). Therefore, the objective of this research was to screen millet husk and guineacorn husk for their bioethanol production potentiality.

MATERIALS AND METHODS

Collection and processing of samples

One kilogram (1 kg) each of millet and guinea corn husks were collected in a clean polythene bags from local milling centers in

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Sokoto metropolis of Sokoto State, Nigeria. The wastes were powdered using pestle and mortar, and sieved. Different parts of rumen were purchased from Sokoto Central Abattoir for the isolation of cellulolytic microorganisms. The rumen samples were placed in clean sterile polythene bags and transported immediately to the laboratory for analysis. *Z.mobilis* and *S. cerevisiae* employed in the fermentation process were isolated from a previous study.

Isolation of cellulolytic bacteria from rumen content of ruminants

The cellulolytic bacteria were isolated in accordance with the method described by Oyeleke and Okusanmi (2008). The rumen of the ruminant animal was sliced and swabbed with a swab stick. The sample was then inoculated on Nutrient agar (NA) and MacConkey agar (MA) plates. The plates were incubated aerobically and anaerobically using candle jar. The plates were incubated at 37°C for 24 h. Colonies developed after the incubation periods were sub-cultured to obtain pure cultures which were maintained on agar slants for further characterization and identification. The pure isolates were then subcultured on cellulose agar plates and were incubated aerobically and anaerobically for seven days to test for their ability to hydrolyze cellulose. Hydrolysis of cellulose was indicated by the appearance of clear zones around the colonies of the organisms. The bacterial isolates were characterized and identified using standard methods as described by Cheesebrough (2003).

Enzymatic 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 of the agrowastes. The conical flasks were labelled A and B (A: millet husk and B: guineacorn husk). 10 g of each agrowastes was put in the flasks and 100 ml of distilled water added. The flasks were plugged with cotton wool and aluminium foil and then sterilized at 121°C for 30 min. Each flask was inoculated with 0.5 ml suspension of the isolated cellulolytic bacteria. The flasks were incubated at 37°C for 5 days on an orbital shaker. After the 5 days period, the samples were filtered through Whatman filter paper No1 and the filterate were then used for fermentation.

Determination of reducing sugar

The reducing sugar content of the hydrolyzed agrowastes was determined using the dinitrosalicylic acid colorimetric method of Miller (1959) with glucose as standard. It was assayed by adding 3 ml of 3, 5- DNS reagents to 3 ml of the sample. The mixture was heated in boiling water for 10 min to develop the red-brown colour. Then 1 ml of 40% potassium sodium tartarate solution was added to stabilize the colour and cooled to room temperature under running tap water. The absorbance of the samples was measured at 491 nm using ultraviolet (UV-VIS) spectrophotometer. The reducing sugar content was subsequently determined by making reference to a standard curve of known glucose concentrations.

Fermentation

The fermentation of the hydrolysed samples was carried out in accordance with the methods described by Brooks (2008) and Oyeleke et al. (2009). 100 ml of the millet husk hydrolysates was dispensed into three different 500 ml capacity conical flasks. The flasks were then covered with cotton wool, wrapped in aluminium foil and autoclaved at 121°C for 15 min. The tubes were allowed to cool at room temperature and aseptically inoculated with the fermentative organisms. Conical flask A was inoculated with *S. cerevisiae*; B: inoculated with *Z. mobilis* and C: inoculated with *S. cerevisiae* and *Z. mobilis* (combined). All the flasks were incubated anaerobically at 35°C for 5 days. The same procedure was repeated for the guineacorn husk hydrolysates. The hydrolysates were then distillated according to standard method. The fermented broth was dispensed into round-bottom flasks 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 with the temperature adjusted to 78°C was use d to heat the round-bottom flask containing the fermented broth.

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 Oyeleke and Jibrin (2009). 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 colour development. The absorbance of each concentration was measured at 588 nm using UV-visible spectrophotometer 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 588 nm using the UV-VIS spectrophotometer.

Statistical analysis of data

Data generated was subjected to statistical analysis using one-way analysis of variance (ANOVA) using the SPSS (version 14.0) to establish significant differences at 95% confidence limit. Comparison of means were made by the New Duncan's multiple range test (P = 0.05).

RESULTS AND DISCUSSION

The results of the characterization and identification of cellulose utilizing bacteria is presented in Table 1. The bacteria were characterized and identified as *B. firmus, B. circulans, E. coli, P. rettgerii, P. macerans* and *S. marcescens*. The results of the reducing sugar yield of the hydrolysed agrowastes is presented in Table 2. The highest yield of reducing sugar of 4.10% was obtained from guinea corn husk while millet husk produced a total yield of 3.21%. The results of the concentration of the bioethanol produced from fermentation of the agrowastes using *S. cerevisiae, Z. mobilis* and a combination of *S. cerevisiae* and *Z. mobilis* are presented in Table 3. The highest concentration of bioethanol of 2.33% was produced using the combination of *S. cerevisiae* and *Z. mobilis* from guineacorn husk. The lowest concentration of 0.79% was obtained using *Z. mobilis* on millet husk hydrolysates. *Bacillus* species account for most of the isolates that were isolated from the rumen of the ruminants along with some members of the coliform

Table 1. Cellulose-utilizing bacteria isolated from the rumen of ruminants.

Isolates	Gram	Mot	Cat	Glu	Lac	Suc	H_2S	Gas	MR	VP	Cit	Ind	Ure	Oxi	CH	Organism
1	+Rod	+	+	+	-	+	-	-	+	-	-	-	-	-	+	*Bacillus firmus*
2	+Rod	+	+	+	+	+	-	-	-	+	-	-	-	-	+	*Bacillus circulans*
3	-Rod	+	+	+	+	+	-	+	+	-	-	+	-	-	+	*Escherichia coli*
4	-Rod	+	+	+	-	-	-	-	+	-	+	+	+	-	+	*Proteus rettgeri*
5	+Rod	+	+	+	-	+	-	-	-	+	-	-	-	+	+	*Paenibacillus macerans*
6	-Rod	+	+	+	-	-	-	-	-	+	+	+	+	-	+	*Serratia marcescens*

+, Positive; -, negative; Mot, motility; Cat, catalase; Glu, glucose; Lac, lactose; Suc, sucrose; MR, methyl red; VP, voges proskauer; Ind, indole; Cit, citrate; Oxi, oxidase; Ure, urease; CH, cellulose hydrolysis.

Table 2. Glucose yield of the hydrolysed agro-wastes (%).

Agrowastes	Glucose yield (%)	
	Total	Mean±SD
Millet husk	3.21	0.27±0.02
Guineacorn husk	4.10	0.34±0.08

Table 3. Bioethanol yield of the different agro-wastes (%).

Agrowastes	Fermentative organism	Bioethanol yield (%)	
		Total	Mean±SD
Millet husk	*Saccharomyces cerevisiae*	1.51	0.126±0.015
	Zymomonas mobilis	0.79	0.066±0.090
	S. cerevisiae + *Z. mobilis*	1.59	0.133±0.015
Guinecorn husk	*Saccharomyces cerevisiae*	1.27	0.099±0.018
	Zymomonas mobilis	1.19	0.194±0.020
	S. cerevisiae + *Z. mobilis*	2.33	0.106±0.015

bacteria.

This is similar to the findings of Oyeleke and Okusanmi (2008) who reported the isolation of about 37.8% of *Bacillus* species from the rumens of cow, goat and sheep. This agrees with Lynd et al. (2002) who isolated these organisms from the rumen and were implicated in the hydrolysis of cellulose. Evidences based on zones of clearing in cellulose agar led to the conclusion that *Bacillus* possesses firmly bound cellulase. This is in agreement with Colombatto et al. (2002) who describe the cell morphology of *Bacillus* species

as having a thin coat that adheres tightly to plant cell wall. Little quantity of reducing sugar was obtained from the hydrolysates of all the agrowastes. The highest yield of reducing sugar of 4.10% was obtained from guinea corn husks as compared to that of millet husk. This may be attributable to two major problems concerning the use of these agrowastes for bioethanol production. First, the glucose exists as cellulose, which can be difficult to break down into simple sugars. Second, the lignin is a structural component of plants. Lignin serves as a binder for cellulose fibres in plants and adds strength and stiffness to the cell walls. Therefore, 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 organisms, less concentration of bioethanol was pro-duced by these organisms. Coupled with this, *Z. mobilis* only ferment glucose, fructose and sucrose. Therefore, these isolates could not ferment pentose sugars and may not be suitable for the fermentation of cellulosic materials (Brooks, 2008). Also, the agrowaste grits used may sink to the bottom of the fermentation flasks. Thus, becoming unavailable to the fermentative organisms. These findings are in conformity with the work of Epstein et al. (2010) who reported an ethanol volume as low as 0.06 ml/g from apple and grape juices. However, the findings are not in agreement with that of Salvi et al. (2010) who reported an ethanol yield of 24.53 g/L from dilute ammonia treated sorghum.

In conclusion, the agrowastes offer great promise in their usage for production of bioethanol. However, there is need to optimize their usage in the production of bioethanol so as to free more of their reducing sugar content for fermentation.

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