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Production of Bioethanol from Sugarcane Bagasse Using Saccharomyces Cerevisiae

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Authors' contributions

This work was carried out in collaboration between all authors. Author MG designed the study, author NA performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SG and SBU managed the analyses of the study. Author HMM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Ethanol is an alternative fuel derived from renewable biological resources. It's a good substitute for gasoline in spark ignition engines. In this study, the sugar cane bagasse was chemically pretreated with 1% NaOH at room temperature for 2 hours. Dilute acid H_2SO_4 and *Aspergillus niger* was used to hydrolyse the biomass to sucrose. Fermentation of the hydrolysed sample was done using *Saccharomyces cerevisiae*. The fermented product was purified by distillation process at 78^oC, and the fraction was collected, and the ethanol was determined by measuring the specific gravity. The production of ethanol from sugar cane bagasse with *Saccharomyces cerevisiae* was determined after the inoculation into sample A_1, A_2 and B_1 and B_2 and highest ethanol produced were from B_1 with 0.090 followed B_2 0.074, A_2 with 0.069% and D 0.116. The use *of Saccharomyces cerevisiae* gives a better yield. The result of this study can be of a better application in the large production of biofuel from sugar cane bagasse which is renewable and highly abundant, it is saving costs by

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recycling of wastes, and it also helps to alleviate environmental problem such as an excessive release of greenhouse gases from combustion of non-renewable fossil fuel. From the chromatograph, when the peaks spectrum wave analysed by mass spectrometer of the three volatile organic compounds, two were common to both samples, A contains the abundance of Acetic acid 22.37%, Ethyl alcohol 13.55% isobutene 64.08%. While that of Sample B contains the abundance Acetic acid 17.43%, Ethyl alcohol 7.12% and Propane 75.4.according to Pasteur this is due to Microbial oxidation of ethanol to acetic acid that decreases metabolic toxicity to the yeast cells. This study has proven the efficiency of *Saccharomyces cerevisiae* for the production of bioethanol.

Keywords: Bioethanol; sugarcane bagasse acetic acid; chromatogram; fermentation; volatile organic compound; retention time.

1. INTRODUCTION

Since the implementation of Brazilian alcohol program, a large scale of bioethanol has been utilised as biofuel [1]. The production of bioethanol from lignocellulosic materials are also called Second-generation bioethanol, is proposed as an alternative without such adverse effects. The lignocellulosic materials include agricultural residues, municipal solid waste, pulp mill refuse, switch grass and garden wastes could be an environmentally-friendly alternative [2]. Ethanol is an alternative fuel derived from renewable biological resources. It's a good substitute for gasoline in spark ignition engines [3].

Sugarcane bagasse (SCB) is the most abundant agricultural wastes in the world, creating 540 million tons of biomass per year [4]. Bioethanol is simply ethanol is a renewable energy source which is made by fermenting the sugar and starch components of plant. It is produced from the agricultural product such as corn, sugarcane, potatoes, rice, beetroot and recently using grapes, banana, dates and other wastes [5]. Yeasts are most often used in the fermentation process and obtain energy from various carbon sources. Yeasts are the most common microorganisms for ethanol fermentation. Among the yeast kingdom, *S. cerevisiae* is one of the well-known ethanol producers. Ethanol is a vital chemical that is been used as a raw material for a vast range of applications including chemicals, fuel (bioethanol), beverages, pharmaceuticals and cosmetics. *S. cerevisiae* has short germination time and is easily cultured in large scale processes [6].

The world population and increased urbanisation have directly or indirectly influenced the energy demand presently in most countries of the world, the problems associated with petroleum and fossil fuel sources are that they are limited in supply and cannot be renewed hence depletes on usages. It is therefore evident that there is the need to search for alternative fuel sources which can be renewed with time, and ethanol has been found as one of such fuels sources [7].

The major environment advantage of using ethanol is sustainability when using renewable resources as raw material, hence encouraging the independence of fossil fuel, since it does not give any net addition of carbon dioxide to the atmosphere [8]. Bio-fuel has been a source of energy that human beings have used since ancient times, increasing the use of biofuels for energy generation purposes of particular interest. Nowadays because they allow migration of greenhouse gases, provide means of energy independence and may even after new employment possibilities. Bio fuels are being investigated as a potential substitute for current high pollutant fuels obtained from conventional sources.

2. MATERIALS AND METHODS

2.1 Experimentation

2.1.1 Collection and processing of samples

The sugar cane bagasse was collected and purchased from Sokoto market in Sokoto State Nigeria. It was collected in a clean polythene bag and transported immediately to the microbiology laboratory Sokoto State University, Sokoto. The substrate was dried and pounded using pestle and mortar, sieved using a sieve with mesh size of 0.2mm. Soil sample was obtained in Sokoto State University for isolation of *Aspergillus niger*. Sample of burukutu was purchased from Army Barracks Mammy Market for isolation of

Saccharomyces cerevisiae and the samples were transported immediately to the laboratory for analysis.

2.2 Isolation of *Aspergillus niger* from Soil Sample

Isolation of *Aspergillus niger* was performed by serial dilution 1g of soil from tube 1 to tube 5. The 0.1ml aliquot from the 5th tubes was plated using pour plate method on Potatoes Dextrose Agar (PDA) and incubated at 28°C for 48hours. Thereafter, streptomycin was added to the PDA at 0.05mg/m to inhibit the growth of bacteria. Cultures were obtained and identified by morphological structures using lactophenol staining and viewed under x100 lens [9].

2.3 Isolation and Identification of *Saccharomyces cerevisiae* from Burukutu

Saccharomyces cerevisiae was isolated from burukutu obtained from Army Barrack Mammy market, Sokoto State. An Aliquot of 0.1ml of burukutu was spread on the surface of yeast potatoes dextrose Agar (YPDA) plate and incubated at 30ºC for 3 days colonies observed were suspected to be *saccharomyces cerevisiae* based on colonial characteristics was sub cultured on PDA. A smear of the isolate was examined microscopically after staining. The isolates were identified by comparing their characteristics with those of known taxa using the scheme of as described by [10].

2.4 Pre-treatment and Hydrolysis

The sugar cane bagasses were chemically pre-treated with 1% NaOH and H_2SO_4 at room temperature for a period of 2 hours. For hydrolysis, an Interval of five days to produce a homogenous solution after which the mixture was filtered using filter paper was taken as described by [11]. Additionally, after hydrolysis the pH was neutralised by adding appropriate amounts of NaOH to neutralise the acid before the downstream fermentation processes [12].

2.5 Enzyme Hydrolysis

Pretreated lignocellulosic was scarified enzymatically to get fermentable sugars and *Aspergillus niger* was incubated directly into four sets of the mixture and the fifth set served as the control. These sets up were kept at room temperature (28°C) and shaken at intervals of five days to produce homogenous solution after which the mixture were filtered using filtered paper [13].

2.6 Determination of Reducing Sugar

After 5 days of incubation, the samples were filtered and the presence of reducing sugar in each filtrate was detected using the Benedict test. Spectrophotometer was applied to find the concentration of reducing sugar in each sample [14].

2.7 Benedict Test

Into 5mls of each sample, 1ml of Benedict reagent was added; the mixture was placed into boiling water bath and allowed to stand for 5 minutes. Positive result gives rise to a brown colour [13]. The absorption of a portion of each mixture was then read using spectrophotometer at 477mm. Glucose concentration was then calculated using the formula.

(Test / Standard absorbance) x Standard concentration

2.8 Fermentation

The fermentation of the hydrolysed sample was carried out by dispensing the hydrolysate into four different and well labeled conical flasks. The flasks were then covered with cotton wool wrapped in aluminum foil and autoclaved at 121ºC for 15 minutes. The tubes were allowed to cool at room temperature and aseptically inoculated with the fermentation organisms. The 1st sets of 2 conical flasks were inoculated with *Saccharomyces cerevisiae*, and the 2nd set with S*accharomyces cerevisiae* and the 3rd set were left un-autoclaved to serve as a control. All the flasks were incubated on a shaker incubator at room temperature (28ºC ± 2ºC) for five days [14].

2.9 Distillation

Five grams of calcium oxide powder was added to 250ml of distillate before distillation which was carried out with a distillation apparatus set up for each of the fermented broth. The fermented liquid was transferred into round bottom flask and placed on a heating mantle fixed to a distillation column enclosed in running tap water. Another flask was fixed to the other end of the distillation

column of collect distillate at 78ºC which is the standard temperature ethanol production.

2.10 Determination of Concentration of Bioethanol Produced

Determination of concentration of bioethanol produced was carried out using the method described by [14]. 1ml of standard ethanol was diluted with 100ml of distilled water to give a concentration of 1%. From this stock solution 0, 0.2, 0.4, 0.6 and 0.8% of the ethanol was prepared by diluting it with distilled water. To each of the varying ethanol concentrations, 1ml of chromium reagent was added and allowed to stand for an hour for colour development. The absorbance of each concentration was measured at 540mm using UV-VIS spectrophotometer and the readings sued to developed standard ethanol curve. A quantity of one millilitre (1ml) of each bioethanol samples were put in test tubes and treated with 1ml of chromium reagent. The mixture was allowed to stand for an hour and the absorbance was measured as for standard curve.

3. RESULTS AND DISCUSSION

3.1 Morphological and Characterization of *Aspergillus niger* from Soil

The Morphological and characterisation of *A. niger* revealed a black mycelium on the potato dextrose agar medium, it had septate hyphae, long and smooth conidiophores, and long un-branched sporangiospores with a large and round head.

Agro wastes are cheap materials that are obtained from farm products that have limited values for humans, this waste can be converted to useful substance using biotechnology for ethanol production. The production of bioethanol was achieved using acid hydrolysis, saccharification and fermentation of sugar cane bagasse with *Saccharomyces cerevisiae*.

The determination of reducing sugar was conducted at 1,2,3,4 and 5 after the incubation. The highest reducing sugar was obtained from the day 5 with 3.3% followed by 3.0%, 2.4%, 2.7% and 2.9% on the day 4, 3, 2 and 1 respectively. The result obtained from day 1 which is 2.9% is due to the inhibitory effect of the substrate at their substrate concentration impact.

Production of ethanol from sugarcane bagasse with *saccharomyces cerevisiae* was determined after the inoculation into sample A_1, A_2 and sample, B1 and B_2 and the highest ethanol concentration was obtained from B1 with 0.090% followed by B2 0.074%, A2 with 0.069% and A1 0.116%. the result obtained from the production with A1 0.116% could be an attribute at the fact that, the yeast cells are probably at their lag phase trying to synthesized necessary enzymes for their metabolism thereby converting the readily available sugar to ethanol [15]. The highest may represent the maximum tolerable unit of ethanol by the yeast cells. Ethanol has been reported well known as inhibitors of the growth of microorganism due to its effect on the mitochondrial of yeast cells and some enzyme such as hexokinase and dehydrogenase [16] Nevertheless, some strains of the yeast *Saccharomyces cerevisiae* show tolerance and can adapt to the high concentration of ethanol [17].

The result presentation of Chromatogram shows peak of volatile organic compound for both A and B. From the chomotography, when the peaks spectrum wave analysed by Mass Spectrometer (MS) of the three Volatile Organic Compound, two were common to both sample. The Volatile organic compound profile of sugar cane bagasse fermented using *Saccharomyces cerevisiae* for bioethanol production for sample A contains the abundance of Acetic acid 22.37%, Ethyl alcohol

Table 1. Result of determination of reducing sugar

S/No	Sample sugar cane bagasse	Water ratio	Substrate ratio	Inoculums (ml) size	Reducing sugar (%)
1	A1	150	5	0.5	0.798
2	A2	150	5	0.5	0.840
3	B1	200	10	1	0.641
4	B2	200	10	1	0.700
Total yield					2.97

13.55% isobutene 64.08%. While that of Sample B contains the abundance Acetic acid 17.43%, Ethyl alcohol 7.12% and Propane 75.4 according to Pasteur this is due to Microbial oxidation of ethanol to acetic acid that decreases metabolic toxicity to the yeast cells. The result of the present study has shown or revealed that sugar cane bagasse as a substrate produced more ethanol using *Saccharomyces cerevisiae* [15].

Table 2. Result of determination of reducing sugar

S/No	Sample sugar cane bagasse	Water ratio	Substrate ratio	Inoculums (ml) size	Reducing sugar (%)
1	A1	150	5	0.5	0.798
2	A2	150	5	0.5	0.770
3	B1	200	10	1	0.672
4	B2	200	10	1	0.515
Total Yield					2.66

Table 3. Result of determination of reducing sugar

S/No	Sample sugar cane bagasse	Water ratio	Substrate ratio	Inoculums (ml) size	Reducing sugar (%)
1	A1	150	5	0.5	0.596
2	A2	150	5	0.5	0.614
3	B1	200	10	1	0.702
4	B2	200	10	1	0.500
Total Yield					2.41

Table 4. Result of determination of reducing sugar

S/No	Sample sugar cane bagasse	Water ratio	Substrate ratio	Inoculums (ml) size	Reducing sugar (%)
1	A1	150	5	0.5	0.702
2	A2	150	5	0.5	0.756
3	B1	200	10	1	0.964
4	B2	200	10	1	0.596
Total Yield					3.01

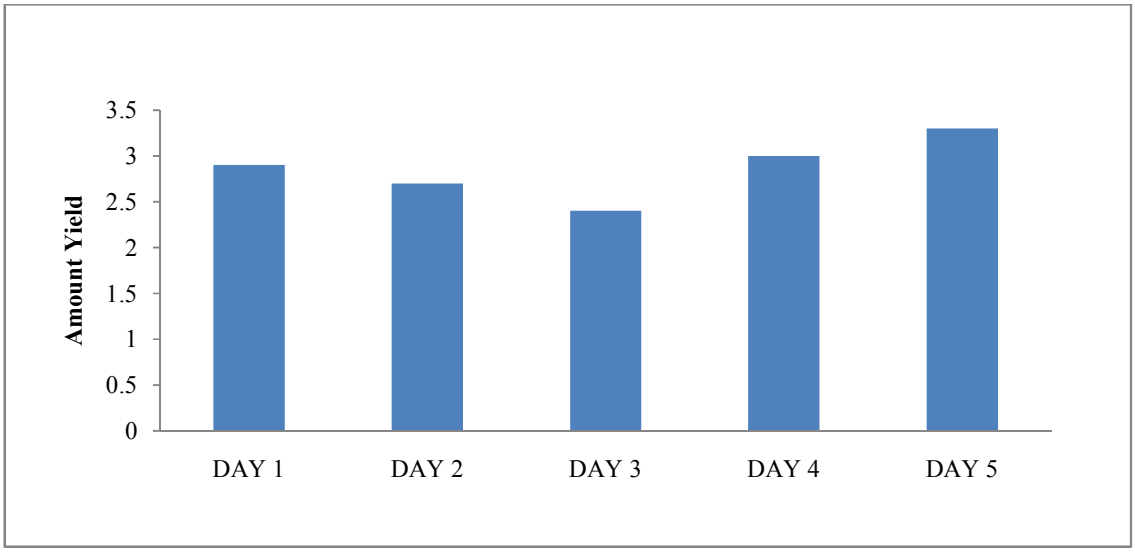

Fig. 1. Determination of reducing sugar

Table 5. Result of determination of reducing sugar

S/No	Sample sugar cane bagasse	Water ratio	Substrate ratio	Inoculums (ml) size	Reducing sugar (%)
1	A1	150	5	0.5	0.731
2	A2	150	5	0.5	0.826
3	B1	200	10	1	0.870
4	B2	200	10	1	0.820
Total yield					3.24

Table 6. Determination of concentration of bioethanol produced

Sample	Test mg/ml	Standard concentration	Standard absorbance	Total yield
A1	0.291	0.2	0.5	0.116
A2	0.173	0.2	0.5	0.069
B1	0.225	0.2	0.5	0.090
B2	0.187	0.2	0.5	0.074

[1]*Derived Concentration from Standard Concentration Curve*

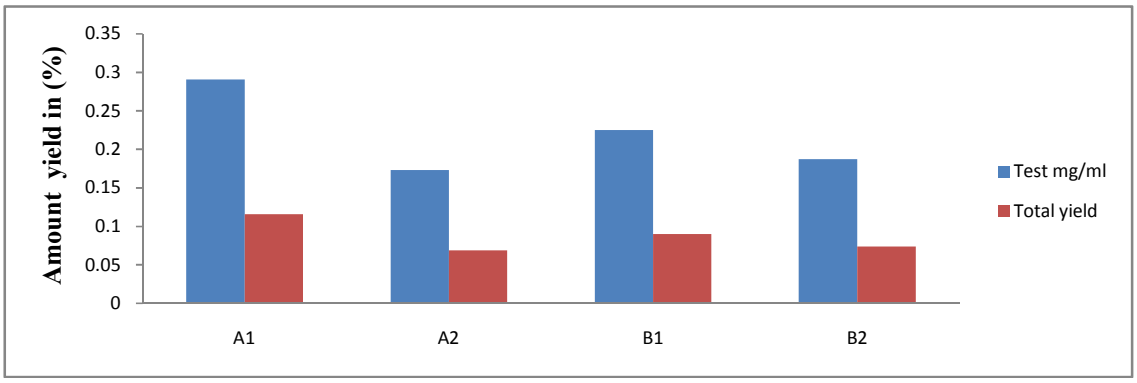

Fig. 2. Determination of concentration bioethanol produced from sugar cane bagasse

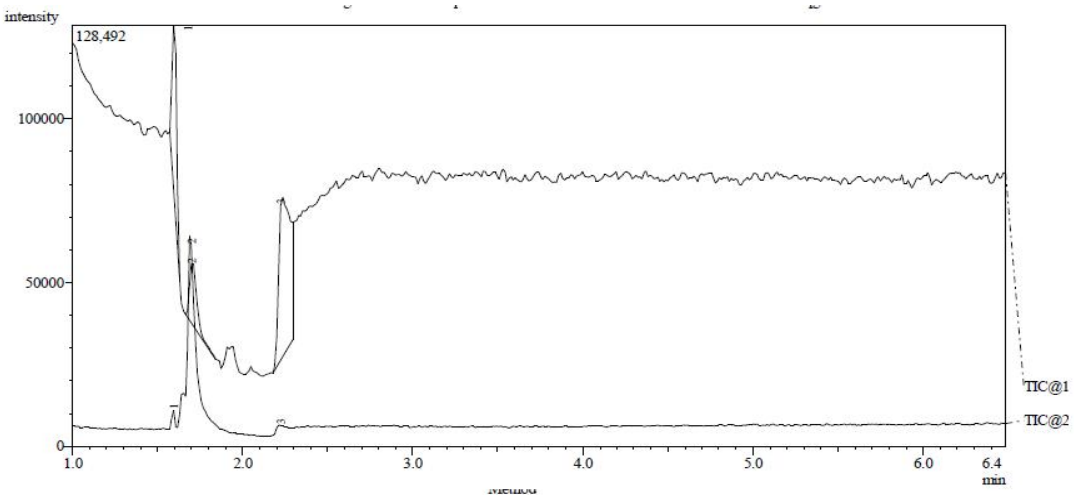

Fig. 3. Chromatogram of A showing peaks of volatile organic compounds in sugarcane bagasse fermented using *Saccharomyces cerevisiae* for bioethanol production.

[1] *Derived Concentration from Standard Concentration Curve*

Table 7. Volatile organic compound profile of sugar cane bagasse fermented using *Saccharomyces cerevisiae* for bioethanol production

RT (min)	Volatile organic compound	Abundance	% (v/v)
		A	B
1.60	Acetic acid	22.37	17.43
1.71	Ethyl alcohol	13.55	7.12
2.24	Isobutane	64.08	-
2.24	Propane	-	75.44

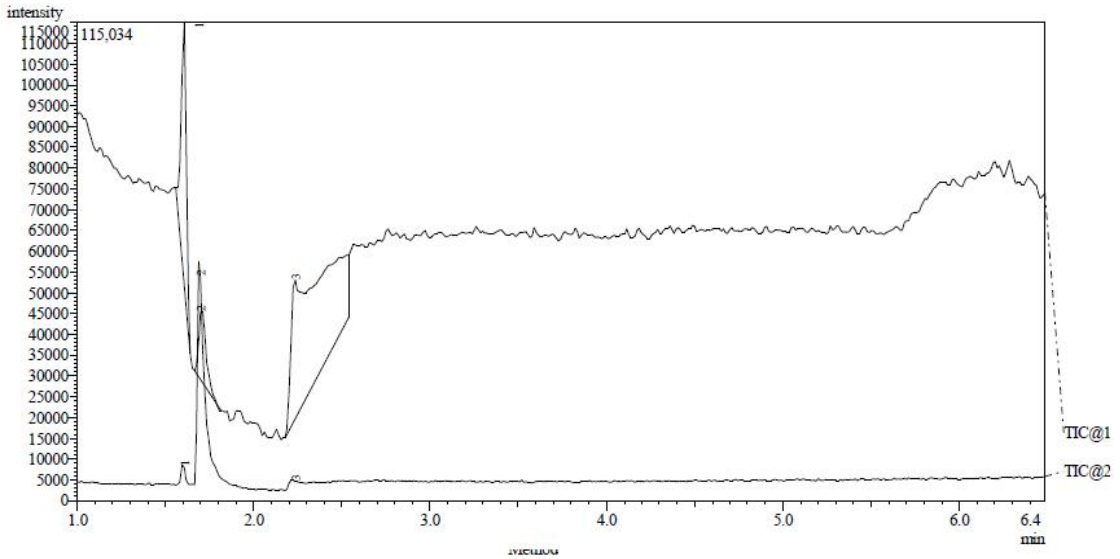

Fig. 4. Chromatogram of B showing peaks of volatile organic compounds in sugarcane bagasse fermented using *Saccharomyces cerevisiae* for bioethanol production.

4. CONCLUSION

The result of this study confirmed that ethanol can be produced from sugar cane bagasse which is an agricultural waste. The use of enzyme hydrolysed sugar cane bagasse is very good for ethanol production, considering its yield cost and it is a means of controlling environmental pollution since bioconversion of cellulosic biomass into fermentable sugar for production of ethanol was done using cellulose degrading of microorganism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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