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Full Length Research Article

PRODUCTION OF BIO-ETHANOL FROM SULFURIC ACID PRE-TREATED RICE HUSK USING CO-CULTURE OF *SACCHAROMYCES CEREVISAE* AND *ASPERGILLUS NIGER*

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ABSTRACT
This study investigates the potential of bioethanol production from agro wastes. Agro waste from rice husk was subjected to a pretreatment process using acid hydrolysis. 20g of rice husk was soaked in 2 %, 6 % and 10 % H_2SO_4 acid concentration for 2 hours, followed by filtration using 1 Whatman filter paper. The pH of the filtrate sample was adjusted to pH of 4.5 using 10 % NaOH, and co-culture of Saccharomyces cerevisiae and A. Niger was introduced into the filtrate and stored for 5 days. Distillation was carried out at 78 °C. The ethanol yield, sugar content and ethanol content of all the samples obtained was analysed. The results obtained shows that 10 % H_2SO_4 pretreated sample resulted into maximum ethanol yield (32.13 g/ml) and sugar content (13.4 %). The colour change to green colour, indicates that ethanol is present in the samples.

Keywords: Rice husk, pretreatment, hydrolysis, alcohol, sugar, specific gravity, fermentation.

INTRODUCTION

Human activities generate large amounts of waste such as crop residues, solid waste from mines and municipal waste. They may become a nuisance and sources of pollution. It is therefore important to handle them judiciously to avoid health problems, since these wastes may harbor pathogenic microorganisms (Ledward *et al*., 2003). Agricultural wastes, including wood, herbaceous plants, crops and forest residues, as well as animal wastes are potentially huge source of energy. In Nigeria, large quantities of these wastes are generated annually and are vastly underutilized. The practice is usually to burn them or leave them to decompose. However, studies have shown that these residues could be processed into liquid fuel such as biogas and bio-ethanol, or combusted to produce electricity and heat (Soltes 2000). Ethanol production involves use of energy from renewable sources and there is no net CO_2 emission to the atmosphere, thus making ethanol an environmentally beneficial energy source. In addition, ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the greenhouse gas effect. This reduction of greenhouse gas emission is the main advantage of utilizing biomass conversion into ethanol (Oyeleke & Jibrin 2009).
Bio-ethanol is an alcohol produced by fermentation, mostly from carbohydrates or starch crops such as corn, rice, sugarcane, or sweet sorghum. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also being developed as a feedstock for ethanol production (Ledward *et al.,* 2003). Cellulosic crops offer alternative feedstock's for ethanol production with key advantages being their abundance, diversity and lower cost. These crops include most plant matter consisting almost entirely of cellulose, hemicelluloses and lignin. Cellulose and hemicelluloses can be converted into simpler sugars like glucose and xylose, while lignin can be combusted into heat energy. Commercial cellulose-to-ethanol production is rare, and today, huge amounts of cellulosic biomass like sugar cane crop is left in the fields or commonly burnt (Hahn-Hagerdal *et al*., 2007; Abbas & Ansumali 2010).
The aim of this research was to synthesize bio-ethanol from rice husk (feedstock) by acid hydrolysis using different concentrations of sulfuric acid, fermentation process using co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* and distillation method. Sulfuric acid was used for the pretreatment of the rice husk samples because of its low cost, availability and it is environmental friendly.

MATERIALS AND METHODS

Collection of Samples

Rice husk was collected from rice processing mill located at station market, Kaduna, Nigeria. The collected rice husk sample was packed in an air tight polyethylene bags and was taken to the laboratory for further analysis.

Pretreatment of the Samples

Rice husk samples were dry at 100 °C overnight. The dried husks was then grinded into powder form and sieved by passing through a 1mm screen to standardize the particle size range of 1mm. The sample was kept in tightly closed container at room temperature and pre-treated (acid hydrolysis) with different concentrations of sulfuric acid. The hydrolysis conditions and different concentrations of sulfuric acid were selected based on literature results on acid pretreatment of rice husks according to (Saha *et al.,* 2005) and other open literatures. Agrowastes can be used as a fermentation feedstock only after being subjected to an effective pretreatment. Pretreatment is required to alter the biomass macroscopic and microscopic size, structure as well as its submicroscopic chemical composition so that hydrolysis of carbohydrate fraction to monomeric sugars can be more rapid with greater yields. Pretreatment affects the structure of the biomass by solubilizing hemicellulose, reducing crystallinity and increasing the available surface area and pore volume of the substrate. Pretreatment has been considered to be one of the most important processing steps in biomass to fermentable sugar conversion (Mtui 2009).

Acid Hydrolysis

20 g each of rice husk sample was weighed into 4 separate conical flasks, and distilled water, 2 %, 6 % and 10 % of sulfuric acid was added to each conical flask respectively. The flasks were covered with cotton wool, wrapped in aluminum foil, and was heated in a

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water bath for 2 hours at 98 °C, followed by autoclave for 15 mins at 121 °C. The flask was allowed to cool, then followed by filtration using 1 Whatman filter paper, and the residue obtained was subjected to further analysis. The pH of the filtrate sample was adjusted to pH of 4.5 using 10% NaOH. The residual samples was washed with distilled water until a neutral pH was obtained for all treatment. The treated-washed samples were oven dried at 90 °C for 12-18 hours and were used for further studies.
The samples were labelled as follows:
S1 = 20g rice husk + distilled H_2O.
S2 = 20g rice husk + 2% H_2SO_4.
S3 = 20g rice husk + 6% H_2SO_4
S4 = 20g rice husk + 10% H_2SO_4

Fermentation
The fermentation was carried out along with saccharification (simultaneous saccharification and fermentation (SSF), as described by Kroumov *et al*., (2006) and Oghgren *et al*., (2006). The flasks containing the hydrolyzed samples were covered with cotton wool, wrapped in aluminium foil, autoclaved for 15 min at 121 °C, and allowed to cool at room temperature. Co-culture of *A. niger* and *Saccharomyces cerevisiae* (yeast) were aseptically inoculated into each flask and incubated at 30 °C. All samples were removed after 5 days.

Fractional distillation
The fermented broth was dispensed into a round-bottom flasks fixed to a distillation column enclosed in a running tap water. A conical flask was fixed to the other end of the distillation column to collect the distillate. A heating mantle with the temperature adjusted to 78°C was used to heat the round-bottomed flask containing the fermented broth (Kroumov *et al*., 2006).

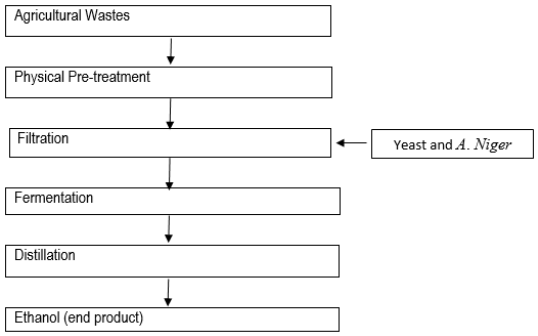

Figure I: Process flow diagram for the production of bio-ethanol

Figure II: Brix refractor meter

RESULTS
The results obtained from the analysis carried out on the collected rice husks samples are represented in the tables below:

Quantity of ethanol produced after analysis
The distillate collected was measured using a measuring cylinder, and the results obtained was used to plot a graph of quantity of ethanol (ml/g) against acid concentration (%) as presented in Figure III below:

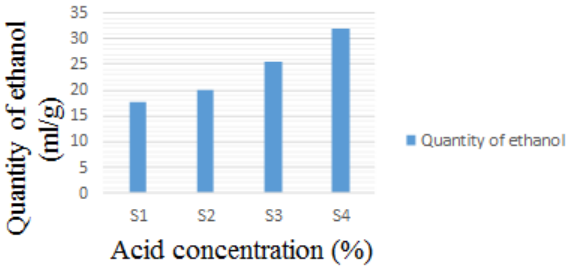

Figure III: Quantity of ethanol produced after analysis at varying H_2SO_4 acid concentrations.

Confirmatory Test for Bio-Ethanol
The result obtained from the confirmatory test is presented in Figure IV below:

TEST	OBSERVATION	INFERENCE
2ml of distillate + pinch of potassium dichromate	The formation of green color	Ethanol present

Percentage sugar content of the filtrate after analysis;
The estimation of percent sugar content produced after pretreatment of sample with water and acid of different concentration was done with the brix refractor meter. The results recorded are presented below:

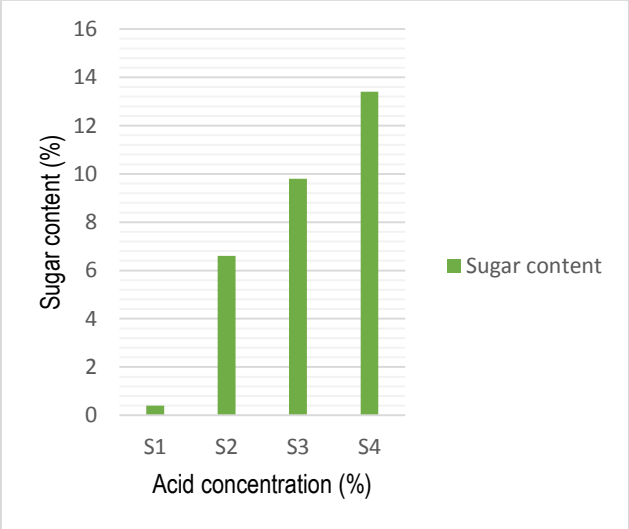

Figure V: Percentage Sugar Content of Filtrate after Analysis at Varying H_2SO_4 concentrations

DISCUSSION
The experimental results reveal that the applied conditions were effective for the hydrolysis of sugars. Insufficient drying of the rice husk samples may encourage fungal growth and cause the husks to lose some of its sugars (Patel *et al*., 2006). Also, enzyme-

producing micro-organisms are capable of breaking down cellulose as observed by (Sun & Cheng 2002).

Effect of Sulfuric acid pretreatment at Varying Concentrations
The choice of pretreatment takes into account the sugar release pattern and the compatibility/suitability of these sugars in the overall process of ethanol production. The effect of acid pretreatment of rice husks was carried out by varying the concentrations of the sulfuric acid (2, 6 and 10 % H_2SO_4 acid) at constant time (2 hours). The purpose of the sulphuric acid pretreatment was to examine the optimum condition to break down rigid lignin structure and matrix conformation of cellulose for accessible of enzymatic hydrolysis in the next step. The target was to produce maximum yield of sugar. According to the results obtained from fig: III (Quantity of ethanol produced) and fig: V (Percentage sugar content of filtrate). The maximum sugar content was observed at 10 % H_2SO_4 treated rice husk sample, followed by 6 % H_2SO_4 treated rice husk sample, then 2 % H_2SO_4 treated rice husk sample. The control, i.e. distilled treated rice husk sample has the minimum sugar content yield with a value of 0.4 %. This results indicate that the pretreatment of sample with sulfuric acid was very effective and it was capable of breaking down the lignin and hemicelluloses which increased the cellulose content. The hydrolyzed material was then washed with distilled water to eliminate retained acid and the solid residue is further processed for optimization enzymatic hydrolysis. Any pretreatment with much yield of inhibitors capable of inhibiting cellulose activity or hindering the fermenting organism from growth is usually not considered suitable (Larissa *et al*., 2012). The difference between the amounts of sugar produced after the different pretreatments can be attributed to pretreatment and other by-products these pretreatments produce.

Quantity of ethanol produced after analysis
From fig. III, it can be observed that S1 (control) has the minimum quantity of 17.67 g/ml, followed by S2 (2 % H_2SO_4) with 20.08 g/ml and then S3 (6 % H_2SO_4) with value of 25.70 g/ml. While, S4 (10 % H_2SO_4) has the maximum quantity of ethanol with value of 32.13 ml/g. Also, from the graph it can be deduce that the quantity of the extracted ethanol increases with increased H_2SO_4 acid concentration.

Confirmatory Test for Bio-Ethanol
The presence of bioethanol was determined based on the colour change to green (Berhanu *et al*., 2015). The formation of green color in the results presented in fig. IV, is a strong evidence for existence of ethanol in the crude primary distillate.

Percentage sugar content of the filtrate after analysis;
From the graph presented in fig. V, the maximum sugar content utilized during the process was found in 10% H_2SO_4 treated rice husk sample (S4). The estimation of percentage sugar content produced after pretreatment of the samples with distilled water and sulfuric acid at varying concentrations was done with a Brix refractor meter. The graph shows that the sugar content increased with increased in concentration of acid. Rice husk hydrolyzed with 10 % H_2SO_4 (S4) released the highest percentage of reducing sugar (13.40 %), while 6 % H_2SO_4 (S3) recorded a sugar content of 6.6 %, and 2 % H_2SO_4 (S2) has a sugar content of 9.8 %. The control (S1) has the lowest sugar content of 0.4 %. This indicates that, the best acid concentration for the hydrolysis of the rice husk is 10 % H_2SO_4 and the percentage of reducing sugars obtained from distilled water and 2 % and 6 % acid were less than that of 10% H_2SO_4 concentration treated rice husk samples, this is because distilled water (control), 2 % and 6 % H_2SO_4 concentration treated rice husk samples are not as strong as the 10 % H_2SO_4 treated rice husk samples which was able to break lignin into hemicellulosic and cellulosic part of the substrate containing reducing sugars. Although, Abba *et al.,* (2014) reported that sugar content increased with increase in acid concentration where optimum concentration for yield was 3 % H_2SO_4 using millet husk.

Conclusion
The present study has shown that agricultural waste such as rice husk can be used as a feedstock or substrates for ethanol production. Therefore, from the findings of this work, bio-ethanol production from agricultural wastes can be termed as 'Waste to Wealth' a useful method in waste management rather than allowing it to contribute a nuisance to the environment.

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