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Utilization of Sugarcane Bagasse for Bio-Alcohol Production Using *Saccharomyces cerevisiae*

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ABSTRACT

Bagasse is another by-product of the sugar industry, consisting of the diverse fibrous material left behind after the sugarcane stalks are crushed to extract the juice. As the demand for sustainable and renewable energy sources increases, sugarcane bagasse has attracted significant attention as a promising raw material for bio-alcohol production. The objective of this study was to explore the feasibility and efficiency of using sugarcane bagasse as a raw material for bio-alcohol production. The utilization of sugarcane bagasse for bio-alcohol production involves several processes, including pretreatment, hydrolysis, fermentation, and distillation. In this study, NaOH solution was used during the pretreatment stage, while H_2SO_4 solution was employed in the hydrolysis stage. During fermentation, *Saccharomyces cerevisiae* was added in quantities of 4 grams and 5 grams, with fermentation periods of 3, 5, 7, 11, and 13 days. Each treatment was conducted in triplicate. The results indicated that the highest alcohol content was achieved with 5 grams of yeast and a fermentation time of 7 days, yielding bio-alcohol content of 3.08% (v/v). This study plays a role in furthering the development of sustainable and renewable energy innovations.

Keywords: bio-alcohol; sugarcane bagasse; yeast

INTRODUCTION

Sugarcane bagasse, the fibrous residue remaining after the extraction of juice from sugarcane, is a significant by-product of the sugar industry. The Indonesian Ministry of Agriculture reports that the national sugarcane production is 2.5-3.0 million metric tons per year. This results in the generation of around 1 to 2 metric tons of bagasse annually. [1] Overall, bagasse waste remains largely unutilized and poorly managed. Traditionally, this biomass has been used as a fuel for boilers in sugar mills or as a raw material for producing paper and animal feed. [2] Research conducted by Hidayati *et al.* (2016) revealed that 50% of bagasse from sugarcane milling is used as boiler fuel, while the remainder is stockpiled as low-value waste. This stockpiling poses environmental risks due to its flammability and occupies significant land area for storage. Proper management and utilization strategies are needed to address these issues and harness the potential of bagasse more effectively. [3] However, with the growing emphasis on sustainable and renewable energy sources, sugarcane bagasse has gained attention as a potential raw material for bio-alcohol production.

Bioalcohol is produced biologically using microorganisms and enzymes to ferment sugars derived from various sources, including wheat, corn, sugarcane, molasses, and starchy materials like potatoes and fruit scraps. In Indonesia, the abundant

availability of these raw materials makes bioalcohol production highly feasible. Potential sources include high-carbohydrate plants such as sugarcane and its bagasse, banana stems, aren palm, coconut, cassava, sorghum, cashew waste, arrowroot, sweet potato, corn, corn stumps, straw, and other agricultural residues. These materials offer significant opportunities for sustainable bioalcohol production and development. Recent studies highlighted the potential of agriculture waste for bioethanol production. Munfarida *et al*. (2021) reported that banana peels from Indonesia can serve as a viable raw material for bio-alcohol production. [4] Ethanol, in particular, has been widely studied and utilized as a biofuel, either on its own or blended with gasoline. The production of bio-alcohols from biomass like sugarcane bagasse not only helps in reducing dependence on non-renewable energy sources but also adds value to agricultural residues, promoting a circular economy. Recent studies highlighted the potential of sugarcane bagasse for bioethanol production. They explored various pretreatment methods, including autohydrolysis and dilute acid hydrolysis, to enhance glucose recovery and ethanol yield. Their findings showed that sugarcane bagasse can be effectively converted to ethanol, achieving high yields under optimized conditions. [5,6,7] Previous studies in Indonesia have shown that sugarcane bagasse has the potential as a significant feedstock for bio-alcohol. One study investigated the use of detoxified sugarcane bagasse hydrolysate for bioethanol production, highlighting the efficiency of utilizing sugarcane bagasse in biofuel applications.[8] Another study focused on optimizing the delignification process of sugarcane bagasse to enhance glucan content for bioethanol production, demonstrating its potential as a significant feedstock for bio-alcohol. [9] While studies like those by Setyawati *et al.* (2021) and Oktaviani *et al.* (2016) have explored various pretreatment methods, further optimization is required to enhance the efficiency and cost-effectiveness of these processes. The current methods need improvements to increase the yield of fermentable sugars and reduce the formation of inhibitors that can affect fermentation efficiency. To enhance the productivity of bio-alcohol from sugarcane bagasse, we conducted this study. The objective is to assess the feasibility and efficiency of utilizing sugarcane bagasse as a raw material for bio-alcohol production.

MATERIALS & METHODS

The utilization of sugarcane bagasse for bio-alcohol production involves several processes, including pretreatment, hydrolysis, fermentation, and distillation.

Pretreatment

The sugarcane bagasse was first washed thoroughly and then divided into several portions, which were subsequently dried in the sun. After drying, the bagasse was pulverized using a blender. Approximately 20 grams of the pulverized bagasse was placed into a glass container, to which 400 mL of distilled water and 6 mL of NaOH are added. The container was then covered with cotton and aluminum foil and heated in an autoclave at 120°C for 15 minutes. After autoclaving, the sample was cooled, and the solution and solids were separated. The bagasse was washed with distilled water until it reaches a neutral pH. Finally, the bagasse was dried at 80°C for approximately 2 hours.

Hydrolysis

Twenty grams of sugarcane bagasse was placed into a glass jar for hydrolysis. Next, 200 mL of a 6% H_2SO_4 solution was added. The jar was then sealed with cotton and aluminum foil and heated at 120°C for 60 minutes. After hydrolysis, the mixture was allowed to cool, and the solution and solids were separated and filtered.

Fermentation

The filtered solution of bagasse hydrolysate was adjusted to a pH of 4.5 using NaOH. The solution was then cooled to room temperature. Following this, the equipment was sterilized in an autoclave at 120°C for 15 minutes. *Saccharomyces cerevisiae* was introduced into the fermentor with two weight variations: 4 grams (20%) and 5 grams (25%). The Erlenmeyer flask containing the fermentation media was sealed tightly with plasticine, and a hose was connected to the flask with the other end placed in a bottle filled with water to prevent direct contact with outside air and to release carbon dioxide gas. Fermentation was carried out for 3, 5, 7, 11, and 13 days.

Distillation

The distillation apparatus was prepared for the distillation process. The alcohol-water mixture was placed into a flask, which was then securely attached to the distillation device. The temperature was set to 78°C and maintained for approximately 2 hours. The distilled product was then collected and stored in a tightly sealed bottle to prevent contamination and evaporation.

Data Analysis

The alcohol content is determined by calculating the ratio of the volume of alcohol obtained from the distillation process to the volume of the initial product, using the following formula:

$$Bio-alcohol = \frac{Volume\,of\ Final\ Product}{Volume\,of\ InitialProduct} x100\%$$

Description:

- Volume of Final Product: The volume of the alcohol solution obtained from distillation.
- Volume of Initial Product: The volume of the initial product solution before fermentation.

STATISTICAL ANALYSIS

This study employed SPSS version 25 to perform the Friedman test, a non-parametric statistical test used to analyze differences in data variations across multiple related samples.

RESULT

Bio-alcohol content

Bio-alcohol content is determined by calculating the yield of alcohol produced from the fermentation process. This involves measuring the volume of bio-alcohol obtained (in ml) and dividing it by the volume of the base material or initial product used. The bio-alcohol content is expressed as a percentage of the initial volume. Figure 1 illustrates the fermentation and distillation process, while Table 1 presents the results of the bio-alcohol content analysis.

(a) (b)

Figure 1. Fermentation and Distillation Processess

Table 1. Bio-alcohol content

No	Day	Yeast Weight (g)	Bio-alcohol (%)
1	3rd	4	1,28
2	3rd	5	1,28
3	5th	4	1,66

4	**5th**	**5**	**1,15**
5	**7th**	**4**	**2,46**
6	**7th**	**5**	**3,08**
7	**11th**	**4**	**1,43**
8	**11th**	**5**	**2,31**
9	**13th**	**4**	**1,5**
10	**13th**	**5**	**1,95**

Table 1 illustrates that both the amount of yeast and the fermentation period affect the bio-alcohol yield, with varying results observed across different days and yeast weights. On the 3rd day, using 4 grams and 5 grams of yeast both resulted in a bio-alcohol content of 1,28%. On the 5th day, 4 grams of yeast produced 1,66% bio-alcohol, while 5 grams produced 1,15%. On the 7th day, 4 grams of yeast resulted in 2,46% bio-alcohol, and 5 grams of yeast resulted in the highest bio-alcohol content of 3,08%. On the 11th day, 4 grams of yeast produced 1,43% bio-alcohol, and 5 grams produced 2,31%, and on the 13th day, 4 grams of yeast resulted in 1,5% bio-alcohol, while 5 grams produced 1,95%.

STATISTICAL ANALYSIS

Table 2 presents the statistical analysis based on the results of the Friedman test.

Table 2. Statistical Analysis Results

Test Statistic	
N	30,000
Chi-Square	21,130
Df	2,000
Asymp. Sig	0,000

The p-value is 0.000, which is less than the conventional alpha level of 0.05. This indicates that there is a statistically significant difference in data variations across the multiple related samples. The results suggest that the observed differences in data variations are statistically significant, leading to the rejection of the null hypothesis, which states that there are no differences among the groups. In conclusion, there is a significant difference in bio-alcohol content based on the amount of yeast and the fermentation time of bagasse as bio-alcohol.

DISCUSSION

Sugarcane bagasse (SCB) is a promising raw material for bio-alcohol production due to its high content of cellulose, hemicellulose, and lignin. Studies have shown that SCB consists of approximately 45-50% cellulose, 25-30% hemicellulose, and 25% lignin, which are essential components for bioethanol production through enzymatic hydrolysis and fermentation processes.[10] The effective conversion of SCB to bioethanol involves several pre-treatment methods to break down the complex lignocellulosic structure and improve the yield of fermentable sugars. These methods include alkaline pre-treatment, steam explosion, and acid/alkali pre-treatment, which enhance the efficiency of enzymatic hydrolysis. For example, alkaline pre-treatment using sodium hydroxide has been shown to significantly increase enzyme accessibility to the cellulose and hemicellulose fractions, leading to higher sugar yields. [11,12] This study utilized sulfuric acid as a catalyst in the hydrolysis process for bio-alcohol production. The addition of sulfuric acid facilitates the breakdown of cellulose and hemicellulose components within the biomass, converting them into fermentable sugars essential for bio-alcohol production. This approach is supported by previous research, which has demonstrated the effectiveness of sulfuric acid in enhancing the hydrolysis process and increasing sugar yields necessary for bio-alcohol production. [13,14]

The alcohol fermentation stage involves decomposing glucose into ethanol and CO_2 through the activity of yeast under anaerobic conditions. During this stage, *Saccharomyces cerevisiae* is introduced into the fermenter in varying amounts: 4 grams

(20% weight variation) and 5 grams (25% weight variation). Recent studies support the efficacy of using *Saccharomyces cerevisiae* in bioethanol production. For instance, a study conducted by Tian *et.al* (2017) discussed how *Saccharomyces cerevisiae* adapts to ethanol stress during fermentation, maintaining high efficiency in ethanol production even under challenging conditions. [15] Another study conducted by Walker & Stewart (2016) highlighted the role of *Saccharomyces cerevisiae* in converting sugars to ethanol and CO_2, emphasizing its importance in industrial fermentation processes for alcoholic beverages. [16] Recently, research conducted by Alabdalall *et.al* (2023) detailed the optimization of ethanol production using immobilized *S. cerevisiae* confirming its effectiveness in bio-alcohol production. [17]

This study demonstrates that the longer the fermentation time, the higher the alcohol content initially observed. However, on the eleventh and thirteenth days, the alcohol content decreased due to several factors, including temperature, oxygen (O_2) levels, pH, and the availability of substrates or nutrients. These fermentation factors play crucial roles in the efficiency of the fermentation process. Additionally, prolonged fermentation affects the breakdown of carbohydrates into glucose, which microbes use to produce alcohol. Microbes have a limited capacity to produce alcohol; if fermentation time is extended beyond their productive phase, the alcohol content will not increase. This is because the microbes enter an inactive or death phase due to substrate depletion, causing the fermentation process to halt. Based on this study, the most effective combination of yeast amount and fermentation time is 5 grams of yeast over 7 days, resulting in the highest observed alcohol content of 3.08% (v/v). Previous studies have corroborated these findings. For example, Zhang *et al.* (2023) demonstrated that extending fermentation beyond optimal times can lead to decreased efficiency due to substrate depletion and microbial death. [18] Similarly, Walker and Stewart (2016) highlighted the importance of maintaining optimal fermentation conditions to prevent the decline in alcohol content resulting from prolonged fermentation periods. [16] Additionally, Albergaria & Arneborg (2016) discussed how the physiological limits of *Saccharomyces cerevisiae* play a crucial role in determining the efficiency of ethanol production, with extended fermentation times leading to reduced productivity due to the death phase of the yeast cells. [19] These studies collectively emphasize the need to optimize fermentation time and conditions to maximize bio-alcohol yield and prevent inefficiencies.

CONCLUSION

This study investigates the feasibility and efficiency of converting sugarcane bagasse into bio-alcohol. The process involves pretreatment with NaOH solution, hydrolysis using H_2SO_4 solution, followed by fermentation and distillation. Yeast quantities of 4 grams and 5 grams were tested across fermentation periods of 3, 5, 7, 11, and 13 days. The findings revealed that the optimal condition for highest alcohol yield was 5 grams of yeast over a 7-day fermentation period, producing an alcohol content of 3.08% (v/v). This research contributes to the advancement of sustainable and renewable energy solutions.

Declaration by Authors

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