CONVERSION OF PINEAPPLE PEEL GLUCOSE INTO BIOETHANOL USING SIMULTANEOUS SACCHARIFICATION AND FERMENTATION (SSF) METHOD AND SEPARATE HYDROLYSIS AND FERMENTATION (SHF) METHOD

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Abstract

This study aims to determine the bioethanol yield and characteristics from pineapple peel with two methods such as Simultaneous Saccharification and Fermentation (SSF) and Separate Hydrolysis and Fermentation (SHF). The percent yield of bioethanol produced from the fermentation of pineapple peel (*Ananas comosus*) with the Simultaneous Saccharification and Fermentation method was 63.50%, while the yield of bioethanol from the Separate Hydrolysis and Fermentation method was 58.75%. The physical characteristics of bioethanol pineapple peel waste using Simultaneous Saccharification and Fermentation method has a density of 0.8237 w/v whilst with Separate Hydrolysis and Fermentation is 0.8858 w/v. Furthermore, viscosity of bioethanol using Simultaneous Saccharification and Fermentation is 1.05 Cp whereas using Separate Hydrolysis and Fermentation is 1.02 Cp. Pineapple peel bioethanol method of Simultaneous Saccharification and Fermentation has concentration of 13% while Separate Hydrolysis and Fermentation method has concentration of 7.92%. FTIR spectra from SHF bioethanol missing the peak correlated with CH, CH_3, and CO. These missing peaks is due to the high percentage of water. Furthermore, bioethanol from SSF method showed peaks corresponding to CH, CH_3, and CO functional groups. It can be concluded that SSF method give bioethanol with optimum result.

Keywords: *Ananas comosus, separate hydrolysis and fermentation, simultaneous saccharification and fermentation*

Introduction

Petroleum fuel is the most important energy source used in industry, transportation, or households. However, with its use as an energy source, the need for petroleum fuel is higher and not proportional to the amount of production (Kurniati et al., 2021). Based on government regulation No.79/2014 on the National Energy Policy states that 2025 the target role of new and renewable energy is at least 23% in 2025 and 31% in 2050 offset by reducing the role of petroleum by less than 25% in 2025 and less than 20% by 2050. The development of renewable energy that is more efficient and environmentally friendly as a substitute for petroleum is encouraged by this (Rajauddin Amin et al., 2020). So, biofuels are a solution that allows people to reduce the use of fossil oil. One of the important by biofuels is bioethanol (Febriasari et al., 2021).

The raw material for making bioethanol comes from crops such as sugarcane, cassava, and corn. This means organic waste can be used as an alternative raw material for bioethanol because of its availability everywhere, such as vegetable, fruit, and leaf residues. All these vegetable residues contained lignocellulose components (Nuraini & Ratni, 2021). According to the National Standardization Agency (BSN), the export volume of pineapple fruit in 2019-2021 is the highest compared to other fruits. In 2021, pineapple export volume reached 2,886,417 tons, an increase compared to the previous year of 2,196,458 tons. Therefore, the higher the production of pineapple, the more waste produced. To add economic value to pineapple skin waste and because of the high sugar content, it is used as raw material for making ethanol.

The process of producing bioethanol can be defined as the process of converting raw materials in the form of lignocellulose into simple sugars such as glucose to be further fermented to produce bioethanol. The process of producing bioethanol from lignocellulosic involves four stages: pretreatment, hydrolysis, fermentation, and separation/purification. Converting lignocellulose to bioethanol is significantly more difficult than simple sugars due to the nature of the lignocellulosic material, which is composed of a very regular and complex structure where the lignin-hemicellulose-cellulose matrix is a very dense and protected by lignin (Rahkadima & fitri, 2022).

Bioethanol is obtained from the reaction of fermentation of vegetable glucose with yeast. The yeast that commonly used *saccharomyces*. *Saccharomyces* is a species of microorganism that is good for making bioethanol. *Saccharomyces cerevisiae* produces zymase enzymes and invertase enzymes. Sucrose is broken down into glucose and fructose (monosaccharides) by using zymase. Meanwhile, invertase converts glucose and fructose to form bioethanol. There are several constraints for using yeast cells. First, it is resistant to high concentrations of glucose and bioethanol. Second, it is resistant with high salt concentration. Third, low optimum pH and temperature for fermentation (Setyawati & Rahman, 2017).

As an alternative fuel, bioethanol has several advantages compared to fossil oil (BBM). First, bioethanol has a higher octane rating, ranging from 106 to 110. Second, the use of bioethanol can increase combustion efficiency and reduce emissions of pollutants such as oxides, nitrogen, and sulfur because it has a high oxygen content of 34% and a very low sulfur content of 0% (Sudiyani & Aiman, 2019).

Bioethanol can be produced by two methods, namely saccharification and simultaneous fermentation (SSF) and separate hydrolysis and fermentation (SHF). The separate hydrolysis and fermentation (SHF) method is carried out indirectly by separating the hydrolysis and fermentation processes, while the saccharification and simultaneous fermentation (SSF) methods are carried out directly together by combining the hydrolysis and fermentation processes (Kartika Widyastuti et al., 2020). (Nungky Villarul et al., 2017). Previous research was conducted that used calcium oxide adsorbent to adsorb water from bioethanol.

Based on the description above, researchers are interested in carrying out the glucose conversion process of pineapple skin waste (*Ananas comosus*) for the manufacture of bioethanol by comparing the results of yield analysis and bioethanol characteristics from the simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) methods.

Research Methods

Materials

Pineapple Peel (*Ananas comosus*), NaOH (Merck, Germany, 0.1 M), HCl (Merck, Germany, 0.5 M), CH_4N_2O (Merck, Germany), H_2O, Cellulase enzymes, Baker's Yeast.

Instrumentation

Analytical Balance (FSR-A320 FUJITSU), hot-plate (Cimarate), autoclave (GEA YX-182M), incubator shaker (memmert INE-400), pycnometer (Pyrex), viscometer (Pyrex), UV-Visible spectrophotometer (LaMotte SMART Spectro), and FT-IR (Fourier Transform Infra-Red) (Perkin Elmer Spectrum Two), and other supporting glasses were used.

Procedure

1) Preparation of raw materials
   Pineapple skin is cut into small parts, then pineapple skin is washed with water, then dried for 3 days under the hot sun, after drying it is mashed with the help of a grinder and sifted until fine pineapple skin flour is obtained using an 80-mesh sieve, the smooth pineapple skin is stored in a closed container (Muhammad Rijal, 2019).
2) Pretreatment
   Measuring fine flour pineapple peel and NaOH 0.1 M with a ratio of 1:10 (w/v) in a beaker and then heated at 120°C for 4 hours. After heating, the pineapple peel powder is separated from the filtrate and rinsed using H_2O until the pH is neutral. Dried in the oven at 110°C (Fachry et al., 2013).
3) Making bioethanol by SHF method
   Considering the results of delignification as much as 40 grams, then the results of delignification were hydrolyzed with 0.5 M HCl acid as much as 100 ml. Then the hydrolysis solution is heated in an autoclave with a temperature range between 121°C for 1 hour. The results of the hydrolysis filter in the form of liquid are put into the Erlenmeyer to proceed to the fermentation stage and added HCl and NaOH up to pH 5, then added 20% *Saccharomyces cerevisiae* immobilization cells. The fermentation process is carried out for 96 hours at a temperature of 35°C in an incubator (Fachry et al., 2013).
4) Making bioethanol by SSF method
   Weighing 40 grams of delignification results, 0.4 grams of cellulase enzymes, and pH 5 acetate buffer to maintain fermentation pH (400 mL), and added 20% *Saccharomyces cerevisiae* cell immobilization for the fermentation process, then incubated in an incubator shaker for 96 hours at 35°C. Furthermore, the fermentation results are filtered to take the filtrate and centrifuged to separate solids (Amalia & Rezeki Muria, 2014).
5) Purification stage of fermentation results
   The purification process is done by assembling the distillation apparatus and turning on the distillation equipment. The filtered fermented products are introduced into the flask. Then, the pump is assembled on the distillation device. This process begins by heating the fermented filtrate on a hot plate at 78-85 °C. Distillates are accommodated and stored in remove to a closed container (Novia et al., 2017). The distillate is a closed container for calcium oxide adsorption. The ratio of CaO to Bioethanol is 1:2, with a soaking time of 12 hours. After adsorption, it was followed by a positivity bioethanol test (Anas Ibrahim et al., 2022).
6) Data analysis
   Bioethanol is characterized based on physical properties (Density, Viscosity, State at room temperature, Color, Content, and Yield), UV-Vis, and FTIR spectrophotometry.

Results and Discussion

Characterization of bioethanol using SHF and SSF methods

Bioethanol from pineapple peel using SHF and SSF method is characterized using FTIR. FTIR will give information related to the function of bioethanol. Meanwhile, the concentration of bioethanol is acquired using UV-visible spectrophotometry. Furthermore, the physical characteristics of bioethanol are determined. Table 1 indicates all the physical characteristics of bioethanol.

Table 1. Characteristics of Bioethanol

Characteristics	Bioethanol	
	SHF Method	SSF Method
Density	0.8858 w/v	0.8237 w/v
Viscosity	1.02 cp	1.05 cp
Concentration	7.92 %	13 %
Yield	58.75 %	63.5 %
Exists at room temperature	Liquid	Liquid
Color	Clear	Clear

Bioethanol characteristics compared to standard pure ethanol

The density value of bioethanol in the SHF method is greater than the density value in the SSF method. Where it can be seen in figure 1 that the bioethanol density of the SHF method produced is 0.8858 w/v, while the SSF method give density of 0.8237 w/v. The density of bioethanol obtained in this study is still greater than the density of pure ethanol (Bahri et al., 2019). It is well known, which is 0.79 w/v. It can be known that if the lower the density of ethanol, the better the quality (Zikri, 2023).

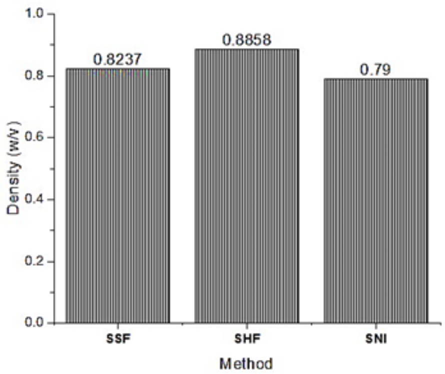

Figure 1. Comparison of Bioethanol Method to Bioethanol Density

The highest viscosity value is 1.05 Cp with SSF method treatment, while the lowest viscosity is 1.02 Cp from SHF method. Based on the quality standard, the viscosity of ethanol is 1.17 Cp. The viscosity results from this experiment are close to the standard level.

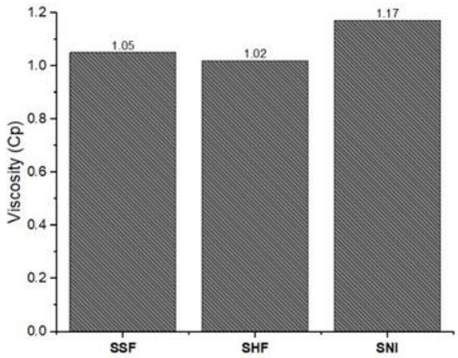

Figure 2. Comparison of the Bioethanol Method to Bioethanol Viscosity

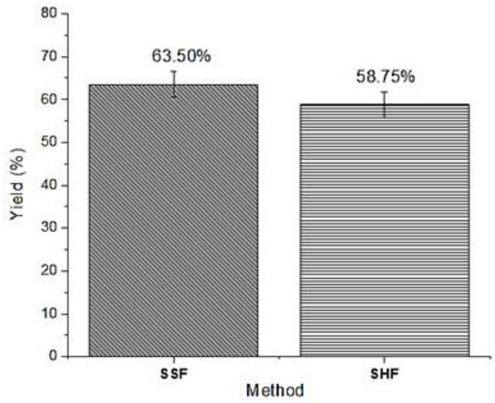

Figure 3. Comparison of the Bioethanol Method to Bioethanol Yield

The bioethanol yield value in the SSF method is greater than the bioethanol yield value obtained by the SHF method. This is because the SSF method uses a saccharification and fermentation process that is carried out directly so that it can increase the yeast population quickly, that is because the yield of bioethanol with the SSF method is greater at 63.50%. Bioethanol yield in SHF method is lower at 58.75%. Bioethanol yield in the SSF method is greater because it uses a saccharification and fermentation process that is carried out directly so that it can increase the amount of sugar consumption by yeast and increase the yeast population fast. Yeast with a smaller amount of sugar consumption is able to produce higher growth yield (Jayus et al., 2017).

FTIR analysis of bioethanol SSF and SHF methods

These are 3 types of experiment conducted in this study. The first one, is bioethanol produced using SSF method with urea. In those first experiment all functional groups related to bioethanol are visible. The functional group that are related to bioethanol are OH (3441.01 cm^{-1}), CH (2630.91 cm^{-1}), CH_2 (1635.64 cm^{-1}), CH_3 (1388.75 cm^{-1}), and CO (1014.53 cm^{-1}). The second experiment is using SHF method with urea. There are only two visible functional group that are OH (3339.72 cm^{-1}) and CH_2 (1635.38 cm^{-1}). Furthermore, the third experiment using SHF method without of urea. In this experiment, only two visible functional group that are OH (3340.34 cm^{-1}), and CH_2 (1635.03 cm^{-1}). FT-IR spectra of each are presented in Figure 4. Based on the interpretation of the FTIR spectra of Putri and Supartono's research, 2015, namely the wavenumber in the absorption of the –OH functional group is 3441.01 cm^{-1}, the absorption of the –CH functional group at the wavenumber 2630.91 cm^{-1}, the absorption of the $–CH_2$ functional group at the wavenumber 1635.64 cm^{-1}, the absorption of the $–CH_3$ functional group at the wavenumber 1388.75 cm^{-1}, and the absorption of the –CO functional group at the wavenumber 1273.02 cm^{-1} (Putri & Supartono, 2015).

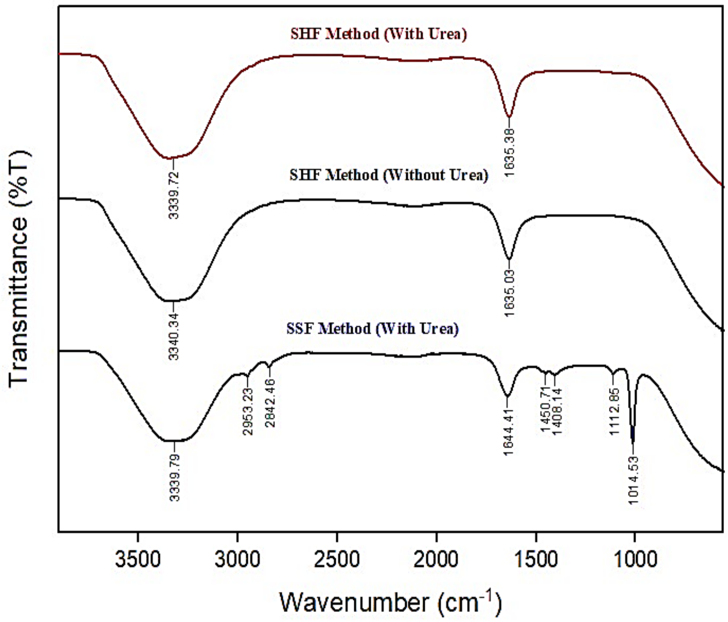

Figure 4. Pineapple Skin Bioethanol FT-IR Spectrum Comparison

FTIR spectrum from SHF bioethanol missing the peak correlated with CH, CH_3, and CO. These missing peaks are due to the high percentage of water, there is no relationship between Beer's law and the complexity of the spectra so that the overlap of peaks, the absence of these functional groups if bioethanol is heated so that the bonds of the compound are broken (Harmi, 2019).

UV-visible spectrophotometer analysis

Distillates that have been adsorbed with calcium oxide are tested using a UV-visible spectrophotometer to determine the level of bioethanol produced. The measurement of the absorbance of standard solutions and blanks obtained a linear regression equation y = 0.3446x + 0.0989 with a regression coefficient value (R^2) = 0.9126 where 0.3446x is the slope and 0.0989 is the intercept. In the treatment of variations in the SSF and SHF methods, the absorbance value of bioethanol samples was obtained with 2 repeats, which can be seen in Table 2.

Table 2. Bioethanol Concentration SHF and SSF Method

Sample (Method)	Average Absorbance	Concentration (%)
SHF	0.2355	7.92 %
SSF	0.325	13 %

The absorbance value obtained from each sample that the largest bioethanol content is in hydrolysis treatment using cellulase enzymes with the SSF method, the SSF method produces bioethanol by 13% by maintaining pH between 4–5 using acetate buffer. Conversely, the SHF method with acid hydrolysis treatment produces bioethanol of 7.92% with conditions between 4–5 using NaOH 0.1M or HCl 0.5M. However, the bioethanol levels produced in this study are very small to be applied as a mixing agent or substitute for fossil fuels (*biofuel*). There are several factors that can affect small bioethanol levels. The first, the delignification process that is not optimal. for example, the use of concentrations of NaOH solutions which are still relatively small, to be used in the breakdown of lignin structures so that lignin breakdown is not complete.

Second, the adding large amounts of urea causes the levels of bioethanol produced are small because urea can form ammonia which is toxic and inhibits the growth of microorganisms if consumed in large quantities and in a short time. Therefore, the optimal urea concentration for nutrients in fermentation based on the research of (Rahmah & Bahri, 2015) is ranging from 0.2 grams to 0.6 grams.

Conclusions

The SSF method is more effective in term of converting pineapple peel into bioethanol. Moreover, Bioethanol from SSF method has a density of 0.8237 w/v whereas the density of SHF bioethanol is 0.8858 w/v. Additionally, the viscosity of SSF bioethanol is 1.05 Cp while the viscosity of SHF bioethanol is 1.02 Cp. The viscosity and density value from both method is not too far off from the SNI value of bioethanol. Finally, the percent yield of the SSF method is 63.50% while the SHF method is 58.75%. Ultimately, the FTIR spectra suggest that the bioethanol produced from pineapple peel using SHF and SSF method still contain a large amount of water. In this case, the future work is finding the most effective way to remove the water from bioethanol.

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Conflict of Interest

The author has no conflict with any interest. The attached data shows the results of the research that has been done.

Author Contributions

All authors contributed to the study. The entire research flow, starting from drafting, writing, and revising the manuscript, is carried out by all authors.

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