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Production of Bioethanol from Kepok Banana Peels (*Musa acuminata x Musa balbisiana*) using Different Types of Yeast

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ABSTRAK

Kulit pisang tergolong sebagai substrat lignoselulosa yang dapat dimanfaatkan sebagai bahan baku bioethanol. Yeast berperan penting dalam fermentasi bioethanol. Penelitian ini bertujuan membandingkan yield etanol menggunakan dua yeast yang berbeda: Saccharomyces cerevisiae and Rhizopus oryzae dengan konsentrasi yeast 2, 3, dan 5 g/L. Fermentasi dilakukan pada suhu ruang selama 120 jam. Hasil penelitian menunjukkan bahwa R. oryzae menghasilkan yield etanol yang lebih tinggi dari pada S. cerevisiae. 2 g/L yeast menghasilkan yield etanol terbesar. Konsentrasi yeast tidak berpengaruh terhadap produksi bioethanol ($p > 0.05$).

ABSTRACT

Banana peels belong to lignocellulosic substrates, which can be used as raw material for bioethanol. Yeast plays an essential role in bioethanol fermentation. The study aims to compare ethanol yield using two different yeasts: Saccharomyces cerevisiae and Rhizopus oryzae, with yeast concentrations of 2, 3, and 5 g/L. The fermentation is run at room temperature for 120 hours. Results show that R. oryzae generates higher ethanol compared to the yeast of S. cerevisiae. Yeast of 2 g/L obtains the highest yield of ethanol. Yeast concentrations do not affect bioethanol production ($p > 0.05$).

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INTRODUCTION

Indonesia has quite a large of organic waste. Converting organic waste into alternative energy is one method to reduce waste accumulation. Banana peels are found widely in Indonesia, considering that banana production in Indonesia is more than 7 million tons (Nur et al., 2021). The banana peel has still not been widely handled and treated duly. Thus, it pollutes the environment

Alternative fuels such as bioethanol lessen dependency on fossil fuels. Bioethanol is generally produced through microbial fermentation that converts sugars (e.g., glucose, galactose, and fructose) to ethanol, carbon dioxide, and other by-products under both aerobic and anaerobic conditions (Tse et al., 2021). Bioethanol from lignocellulosic biomass is estimated as one of the most promising and eco-friendly biofuels (Duque et al., 2021). Banana peels belong to lignocellulosic substrates, which are composed of carbohydrate components (Madhumala et al., 2020). Nevertheless, lignocellulosic biomass contains hemicellulose and lignin. Both constituents are considered an obstacle towards the accessibility of cellulose (Shitophyta et al., 2022).

Pre-treatment processes are required to split the hemicellulose that couples the cellulose molecules into fibres. In the succeeding hydrolysis processes, the removal of the lignin and hemicellulose improves the access of the hydrolytic reagents to the cellulose molecules (Shukla et al., 2023). The hydrolysis of lignocellulosic biomass aims to convert monomeric sugar, which is required for the metabolism of microbes (Bušić et al., 2018). Acid hydrolysis can perform this process. Yeast contributes significantly to bioethanol fermentation. The aptitude of yeast in bioethanol production is to break down six-carbon molecules, such as glucose into two-carbon constituents like ethanol, without continuing to the ending oxidation product (CO_2) (Mohd Azhar et al., 2017).

Previous studies on the production of bioethanol from banana peels have been investigated. Munfarida et al. (2021) investigated bioethanol production from Raja banana peel, Agung banana peel and Nangka banana peel using different concentrations of *Saccharomyces cerevisiae*. 5% of *Saccharomyces cerevisiae* resulted in the highest bioethanol yield. (Rahmanto et al., 2022) studied bioethanol production from banana peels using different concentrations of baker's yeast. Another study was reported by Hamzah et al. (2019) who researched bioethanol production from banana peels at different pH. However, there is no study in the previous literature on utilising two types of yeast (*Saccharomyces cerevisiae* and *Rhizopus oryzae*) in the bioethanol production of Kepok banana peels. Strains of *Rhizopus oryzae* generate extracellular amylase and thaws starch into oligosaccharides and glucose (Maheswari & Kalaikodi, 2011). *Rhizopus oryzae* in this class of fungi are also able to assimilate both hexoses and pentoses and produce ethanol (Abedinifar et al., 2009). Yeast of *Saccharomyces cerevisiae* is broadly used for ethanol fermentation (Joshi et al., 2019). *S. cerevisiae* is more supreme than fibrous fungi, bacteria and other yeast in its diverse physiological characteristics for ethanol production at the industrial scale. It also can put up with a wide range of pH (Bhadana & Chauhan, 2016). This study pointed toward comparing bioethanol production using two various yeasts in different concentrations.

MATERIAL AND METHODS

Preparation for raw material

Banana peels were collected from local markets and food street sellers, then copped and dried under the sun. The dried peels were ground and screened through the 40-mesh sieve. Delignification of banana peels used 5% w/v hydrochloric acid (HCl) solution. The substrates of banana peels and 5% HCl solution was put in the autoclave for 15 minutes at 120°C. Then, the substrates were cooled at room temperature. After that, the cake was washed using distilled water, then filtered and dried for 30 minutes at 40°C.

Hydrolysis process

The dried banana peels were mixed with 10% w/v of HCl solution into 300 mL of three-neck flasks. The hydrolysis process was carried out for 2 hours at a temperature between 80°C and 90°C. Samples of the hydrolysis process were adjusted into the neutral pH by adding NaOH solution, then incubated for 24 hours at a temperature of 50°C using shakers.

Fermentation Process

The hydrolysis samples were inoculated with *Rhizopus oryzae* and *Saccharomyces cerevisiae* at concentrations of 2 g/L, 3 g/L, and 5 g/L. The fermentation was performed for 120 hours (5 days) at room temperature.

Distillation

The fermented substrates were fed into a flask. The distillation process was performed at 80°C for 3 hours. The temperature of distillation was kept constant by flowing cooling water. The vapour generated from the distillation was condensed and collected into a flask (Yousif & Abdulhay, 2017). The ethanol content was measured by an alcohol meter.

RESULTS AND DISCUSSION

Effect of *S. cerevisiae* on Bioethanol Production

The effect of *S. cerevisiae* on bioethanol production was investigated using different concentrations of 0.2%, 0.3%, and 0.5%. Figure 1 presents the ethanol yield obtained by various concentrations of *S. cerevisiae*.

The highest ethanol yield of 57.6% was obtained by *S. cerevisiae* of 2 g/L, followed by ethanol yield of 56.1% and 54.7% at *S. cerevisiae* concentrations of 3 g/L and 5 g/L, respectively.

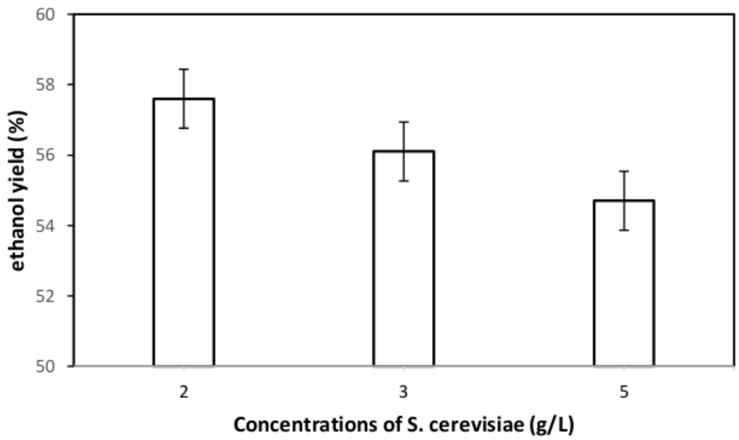

Figure 1. Ethanol Yield on Different Concentrations of *S. Cerevisiae*

The result indicates that increasing concentrations of *S. cerevisiae* has no impact to increase ethanol yield. Statistical analysis also shows that larger concentrations of *S. cerevisiae* do not affect ethanol yield, with a p-value higher than 0.05 (0.988). This phenomenon may occur due to imbalanced microbes. The more microbes, the more rapid bioethanol production, consequently, fewer substrates are consumed by microbes in the succeeding fermentation, which leads to a lessening of bioethanol production (Siregar et al., 2019). A previous study reported ethanol volume decreased at the higher weight of *S. cerevisiae* during bioethanol production from pineapple peels at different weights of *S. cerevisiae* of 20 g, 25 g, and 30 g (Yusmartini et al., 2020). Sheikh et al. (2016) also discovered *S.cerevisiae* of 2 g/L produced ethanol efficiently compared to concentrations of the yeast of 0, 4, 5, and 8 g/L during bioethanol production from potato peels.

Effect of *Rhizopus Oryzae* on Bioethanol Production

Figure 1 represents the influence of concentrations of *Rhizopus oryzae* on bioethanol production. The yeasts concentrations of 3 g/L and 5 g/L generated almost similar ethanol yields of 58.8% and 58.5%, respectively. The greatest ethanol yield of 60.1% was obtained at a yeast concentration of 2 g/L.

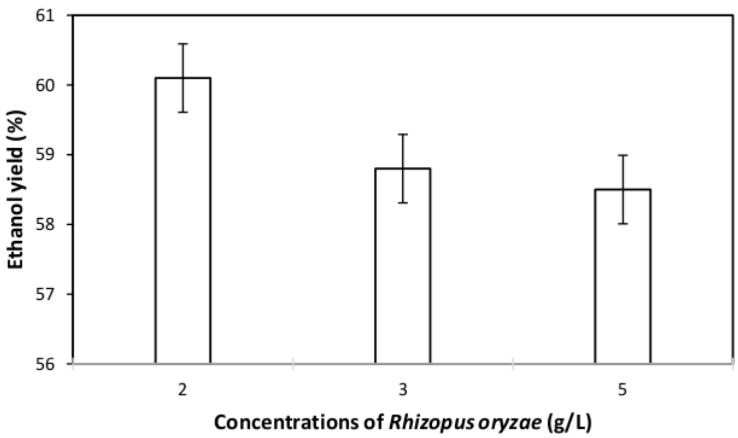

Figure 1. Ethanol Yield on Different Concentrations of *Rhizopus oryzae*

The result of the study denotes that an increase in *Rhizopus oryzae* concentrations can't produce higher ethanol yield. Statistical analysis confirms that the concentration of *Rhizopus oryzae* does not affect ethanol production significantly, with a p-value of 0.865 ($p > 0.05$). The prior study also revealed that the highest yield of ethanol was obtained by the smaller amount of yeast (5%) during the anaerobic fermentation of giant cassava using yeast concentrations of 5% and 10% (Candra et al., 2019).

Too much yeast in substrates establishes severe rivalry in the life of microbes that disrupts the conversion of glucose into alcohol due to the vast mortality of yeast. This condition creates a smaller number of cells and less yield of ethanol. The reduction of ethanol production occurs due to inequity between nutrients and yeast. Consequently, yeast has a lack of food which causes a drop performance of yeast and less ethanol production (Ahmad et al., 2019).

Comparison of Bioethanol Yield using *Saccharomyces Cerevisiae* and *Rhizopus Oryzae*

The difference in ethanol yield generated by two different yeast is presented in Table 1.

Table 1. Bioethanol yield generated by two different yeasts

Yeast	Concentrations of yeast (g/L)	Ethanol yield (%)	Average
	2	57.6	
Saccharomyces Cerevisiae	3	56.1	56.13±1.45
	5	54.7	
	2	60.1	
Rhizopus Oryzae	3	58.8	59.13±0.49
	5	58.5	

Based on Table 1, *Rhizopus oryzae* produced higher ethanol yields than *Saccharomyces cerevisiae*. Both *S. cerevisiae* and *Rhizopus oryzae* produced the highest ethanol yield at the yeast concentration of 2 g/L. Then, ethanol yield decreased with an increase in yeast concentrations.

The lower ethanol generated by *S. cerevisiae* may be due to the incapability of *S. cerevisiae* to grow in media containing high levels of alcohol, causing the inhibition of ethanol production (Mohd Azhar et al., 2017). The reduction of ethanol production is also triggered by HMF (5-hydroxymethylfurfural), which inhibits the growth of yeast and decreases the activities of several yeast enzymes such as aldehyde dehydrogenase, alcohol dehydrogenase and pyruvate dehydrogenase (Adewumi et al., 2022). *S. cerevisiae* metabolize HMF, which is a peculiar example of in-situ detoxification of lignocellulosic hydrolysates (Fazelinejad et al., 2013). HMF is a furan derivative which is formed in hydrolysis of lignocellulosic biomass. HMF inhibits fermentation of hydrolysates during ethanol production (Petersson, 2006).

Inversely to *S. cerevisiae*, fermentation using *R. oryzae* obtained higher ethanol. This condition occurs because *R. oryzae* can grow on many carbon sources, including C5 sugars and has low-growth necessities. Moreover, it can bear the inhibitors existing in acid hydrolysates of lignocellulosic biomass (Meussen et al., 2012). *R. oryzae* secretes a host of digesting enzymes, including amylases, cellulases, and hemicellulases. The cellulase composition in *R. oryzae* consists of two hydrolytic enzymes: extracellular endoglucanase and exoglucanase, hence, cellulosic wastes could be handily and quickly converted into glucose without alkali or acid pretreatments (Uyar & Uyar, 2023). Hydrolysate in the fermentation with *R. oryzae* could be directly used as a carbon source to produce cell biomass, lactic acid, and ethanol. This is because the soluble protein in the hydrolysates provides extra-organic nitrogen sources in the fermentation medium. Extra nitrogen sources assist in promoting cell growth which led to oxygen limitation. This finally switched the fungal pathway to ethanol production (Thongchul et al., 2010).

CONCLUSION

Fermentation with *Rhizopus oryzae* produces higher ethanol yields than *Saccharomyces cerevisiae*. The lowest concentration of yeast obtains the highest ethanol yield. An increase in concentrations of yeast does not affect ethanol production significantly ($p > 0.05$). This study concludes that *Rhizopus oryzae* works better for producing bioethanol from Kepok banana peels.

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