

The Effect of Yeast, NPK and Fermentation Time in Bioethanol Fermentation from “Abacaxi” Pineapple Pericarp

Nery Sofiyanti^{1*}, Dyah Iriani¹, Putri Intan Wahyuni¹, Nurul Idani¹, Puji Lestari¹

¹Department of Biology, Faculty of Math and Natural Science, Universitas Riau, Kampus Bina Widya Panam, Pekanbaru, Riau, Indonesia.

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*Corresponding Author:

Nery Sofiyanti,

Department of Biology,
Faculty of Math and Natural
Science, Universitas Riau.
Kampus Bina Widya Panam,
Pekanbaru, Riau, Indonesia;
Email: nery.sofiyanti@lecturer.
unri.ac.id

Abstract: Bioethanol is promising alternative fuel due to its less effect to environment. It is produced from bio-sources, such plant materials. Yeast and NPK have been reported as the ingredients in affecting bioethanol fermentation. The aim of this study was to examined the effect of yeast and NPK in bioethanol fermentation using the pericarp of “Abacaxi” pineapple cultivar (*Ananas comosus* cv. “Abacaxi”). The pericarp juice of “Abacaxi” pineapple was made by blending the fresh pericarp and aquadest (1 : 1.5). The juice was then mix with dry commercial yeast (1 g, 2.5 g and 5 gr) and NPK (1 g, 2 g, 3 g). A total of 9 treatments were used in fermentation. Each treatment was replicated three times, brought the total sample was 27. The percentage of bioethanol for each treatment was measure using alcoholmeter for six days (24 h, 48 h, 72 h, 96 h, 120 h and 144 h). The result show that the percentage of bioethanol produced in this study varies among different treatment and fermentation time. Yeast and NPK gave significant effect in bioethanol fermentation, with the combination of 5 g yeast and 3 g NPK gave the highest percentage of bioethanol in 144 h of fermentation. Based on the result of this study, the pericarp of “Abacaxi” pineapple is potential bio-source for bioethanol fermentation.

Keywords: abacaxi, bioethanol, fermentation, pineapple, pericarp

Introduction

Bioethanol is produced from bio-sources, and well-known as ethyl alcohol or ethanol. Bioethanol can be used as promising alternative liquid fuel (Zabochnicka-Świątek & Slawik 2010), that may aid fuel supply (Setyawati et al., 2015). This alternative fuel is environment friendly (Susilowati et al., 2022) and less effect due to oxygen content in ethanol (George, 2020). On the other hand, fossil fuel may produce carbon dioxide that harmful for environment and contribute in climate change (Tse et al., 2021). Therefore, the study on alternative energy such as bioethanol increases.

Bio-sources used in bioethanol can be grouped into three type, i.e. sugar and starch-based feedstock, waste feedstock and algal biomass feedstock (Tse et al., 2021). Sugar is crystalline carbohydrate (Okonkwo et al., 2013), that can be fermented into alcohol (Muhammad

2013). Sugar is converted to alcohol and carbon dioxide by yeast in the alcoholic fermentation process (Zentou et al. 2021), especially by strains of *Saccharomyces cerevisiae* (Walker & Stewart, 2016).

Various biosources had been tested their bioethanol potential, such as marine algae (Nahak et al., 2011), sugarcane (Setyawati et al., 2015), banana (Khaliq et al. 2020), pumpkin (Chuoabi et al., 2020), manihot (Moshi et al. 2020) and *Bombax* (Gjazafar et al., 2022). The study on bioethanol fermentation from pineapple (*Ananas comosus*) had also been previously reported by Maynard et al., (2015) and Hilma (2017). Pineapple contains sugar with the dominant lactose, fructose, and sucrose (Cordennunsi et al., 2010). Therefore, this plant is a potential ingredient in bioethanol fermentation.

Pineapple is tropical fruit plant that poses various morphological characteristics. Pineapple

cultivars are mainly group into four classes, i.e. “Abacaxi”, “Queen”, “Smooth Cayenne” and “Red spanish” (Joy & Anjana 2014). However, the high morphological variations are found in each group. “Abacaxi” pineapple (*Ananas comosus* cv. “Abacaxi”) is one of tropical fruit plant that widely cultivated in Riau Province. This cultivar is characterized by having pyramidal-shaped fruit, with yellow pulp inside. The pulp is freshly consumed or used in various process forms such as pineapple jam, juice and chips. However, the pericarp (fruit peel) usually becomes organic waste. Therefore, in this study the pericarp of “Abacaxi” pineapple is used for bioethanol material. The aim of this study was to examined the effect of yeast and NPK in bioethanol fermentation from pericarp of abacaxi pineapple.

Material and methods

Research time and place

This study had been carried out from April to May 2022. Sample was collected from pineapple farmer, Kampar, Riau Province. The bioethanol fermentation had been carried in Botany Laboratory, Department of Biology, Faculty of Math and Natural Science, Universitas Riau.

Juice preparation

Fresh pericarp of “Abacaxi” pineapple was cleaned by using sterilized water and mix dDH₂O (ratio 1 : 1.5). The sample was then blended using herb blender to make pericarp juice.

Bioethanol fermentation

A total of 150 ml pericarp juice was put in fermenter bottles, mixed with commercial yeast (Mauripan) and NPK Mutiara 16:16:16). Mix well the mixture using laboratory spoon, and tighten the bottle seal. Table 1 shows the composition of yeast and NPK used in this study.

Table 1. Composition of yeast and NPK

No	Sampel code	Yeast (g)	NPK (g)	NR
1.	NE1A	1	1	3
2.	NE1B	1	2	3
3.	NE1C	1	3	3
4.	NE2A	2.5	1	3
5.	NE2B	2.5	2	3

No	Sampel code	Yeast (g)	NPK (g)	NR
6.	NE2C	2.5	3	3
7.	NE5A	5	1	3
8.	NE5B	5	2	3
9.	NE5C	5	3	3
Total				27

The percentage of bioethanol was measured using alcoholmeter for six days (24 h, 48 h, 60 h, 72 h, 96 h, 120 h and 144 h).

Data analysis

Data were analyzed by using ANOVA in SPSS, to know the statistically significant different of independent variable affect the dependent between the mean of combination independent groups. If the result gave significant different in ANOVA test, a further analysis was then carried out. Pos Hoc Tukey HSD (Honestly Significant Different) test was used in this study for independent variable with significant different in ANOVA.

Result and Discussion

The demand of energy fermentation especially alternative fuel that friendly to environment increases (Tse et al., 2021). Therefore, may studies on the ecofriendly energy become attractive for many scientist. Bioethanol that containing 35% oxygen can keep down the emission during combustion (Zabed et al., 2014). During bioethanol fermentation, free sugar can be converted directly into ethanol by the fermentation process with microorganism (Zabed et al., 2014; Jalil & Hossain 2015) such as yeast (Zentou et al., 2021). The main strain of yeast that used in bioethanol fermentation is *Saccharomyces cerevisiae* (Walker & Stewart, 2016) that may generate ethanol as main fermentation product (Tessfaw & Assefa 2014).

This study used a total of three groups of commercial dry yeast (*Saccharomyces cerevisiae*) (1 g, 2.5 g and 5 g) used in bioethanol fermentation. *S. cerevisiae* is a microorganism commonly used during bioethanol fermentation (Ciani et al., 2008). This yeast was chosen because of its ability to withstand conditions of low pH, high ethanol, anaerobic conditions or scarce oxygen availability (Ciani et al., 2008; Albergaria & Arneborg 2016). In addition,

strains of *S. cerevisiae* can produce ethanol faster than other microorganisms (Valera et al., 2020).

The percentage of bioethanol in this study varies among different treatments of yeast and NPK composition, as well as fermentation time. Figure 1.A - C shows the percentage of bioethanol fermentation from “Abacaxi” pineapple using 1 g, 2.5 g and 5 g yeast (Fig. 1A, Fig. 1B, Fig. 1C, respectively). The bioethanol percentage after fermentation using 1 g yeast is lower than 10 % in all of the combination of NPK (1 g, 2 g, and 3 g) and fermentation time (24 h, 48 h, 72 h, 96 h, 120 h and 144 h).

The highest percentage of bioethanol (9.33 %) after fermentation is found in the treatment of 1 g yeast and 3 g NPK after being fermented for 72 h. The previous research on bioethanol fermentation from pineapple pericarp had been reported by Mandari et al., (2022). Their result showed that the percentage of bioethanol was also lower than 10% (with the highest percentage was 8 %). However, their treatment is different from this study and only used 24 h for fermentation time.

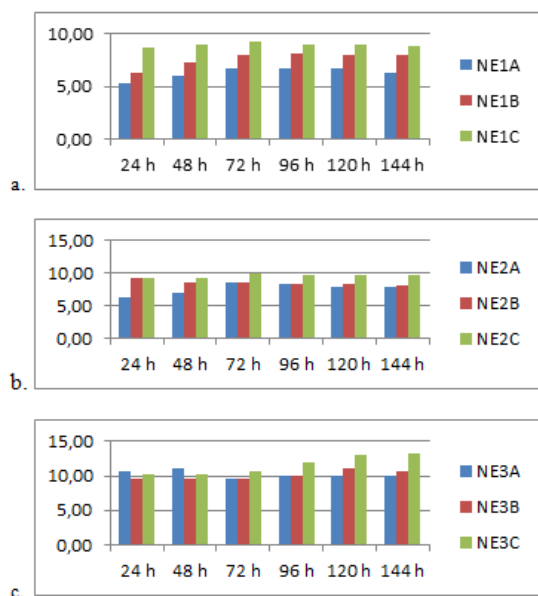


Figure 1. The percentage rate of bioethanol fermentation from pericarp of “Abacaxi” pineapple (*Ananas comosus* cv. “Abacaxi”). a. 1 g yeast, b. 2.5 g yeast and c. 5 gr. Yeast. (NE1 = 1 g NPK; NE2 = 2 g NPK; NE5 = 5g NPK; A = 1 g NPK, B = 2 g NPK, C = 3 g NPK).

In this study, the treatment using 2.5 g yeast showed that the percentage of bioethanol

range from 6.33 to 10 %. The lowest percentage is also found in the treatment of 2.5 g yeast and 1 g NPK) at 24 h, and the highest percentage showed by the combination of 2.5 g yeast and 3 g NPK, after 72 hour of fermentation. The treatment using 5 g yeast showed that the lowest percentage is also found in the treatment of 1 g NPK, and the highest percentage (13.33 %) showed by the combination 3 g NPK, after 144 hour of fermentation. However, this percentage of bioethanol is lower than the study of Fitria and Lindasari (2021). Their result recorded that the highest percentage of bioethanol was 28,5%. It was obtained by the addition of sugar and urea in pineapple pulp.

Based on the data presented in figure 1, the percentage of bioethanol fermentation increases as the weight of yeast. Yeast is used during the bioethanol fermentation to produce enzymes. This enzymes will break down sugar in order to form pyruvate molecules (Malakar et al., 2020). This process is known as glycolysis (Valera et al., 2020). The pyruvate molecules are then reduced into ethanol and carbon dioxide (CO₂) (Malakar et al., 2020). Therefore, the higher of yeast amount during the fermentation, the higher is level of bioethanol (Hapsari & Pramashinta 2013).

The addition of NPK during bioethanol fermentation in this study, was also affect the percentage of bioethanol. The addition of NPK in bioethanol fermentation was also reported by Utomo and Palupi (2013) and Hastuti et al., (2015). Their studies reported that the addition of NPK in bioethanol fermentation using tuber of *Canna edulis* and water guava increase the level of ethanol. In yeast growth and metabolism, nitrogen is essential. According to Mendes-Ferreira et al., (2011) and Christofi et al., (2022), wide range of nitrogen-containing compound can be used by yeast as sole nitrogen sources. During the first part of growth phase of nitrogen compound will transported into the cells (Mendes-Ferreira et al., 2011).

The percentage of bioethanol fermentation data in all of the treatments presented in Figure 1, was analyzed using ANOVA (Analysis of Variance). This test aims to identify which treatment that gave significant effect in the percentage of bioethanol after fermentation step. Table 2 shows Two Way -ANOVA test result in this study.

The result of Two Way-ANOVA test presented in Table 2 shows that based on the Type III, yeast is the most important factor in bioethanol fermentation from “Abacaxi” pineapple pericarp. This is indicated by the highest number of Type III (85.182). The model, yeast and NPK composition gave the significant effect on the percentage of bioethanol fermentation from “Abacaxi” pineapple pericarp, indicated by significant F-test (marked with asterisk symbol), with α values of these treatments are 0.000. On the other hand, the fermentation time did not significantly affect in bioethanol fermentation indicated by non-significant F-test in fermentation time (α values is 0.084, more than 0.05).

Table 2. Two Way -ANOVA test result

Source	TSS	df	MS	F	Sig.
Model	4472.646 ^a	10	447.265	956.473	.000*
Yeast	85.182	2	42.591	91.081	.000*
NPK	36.714	2	18.357	39.257	.000*
FT	4.904	5	.981	2.097	.084
Error	20.575	44	.468		
Total	4493.221	54			

R Squard= .995 (Adjusted Squared = .994)

Note: TSS = Type III Sum of Squares, MS = mean square

Further analyzes were only performed for yeast and NPK. The value of R squared in this study was 0.995, indicating a strong relationship between all independent variables (yeast, NPK and fermentation time) and the percentage of bioethanol. This research was conducted post hoc test (Tukey HSD - Honestly Significantly Different) then further analysis was carried out for yeast and NPK treatment. This test provides multiple comparisons and a homogeneous subset. Table 3 presents the Post Hoc Test for Multiple Comparison in this study.

The term Pos Hoc comes from Latin word, meaning after an event (Teigen 2010). Pos Hoc Tukey Test is a test to find out the significant difference between a pair of means (Kim 2015). Therefore, this test is also known as multiple comparison test. The result of Pos Hoc test in this study that presented in Table 3 shows that each group of yeast weight gave significant different from each other. In this test, a total of 6 pairs of yeast weight (1 and 2, 5; 1 and 5 g; 2.5 and 1 g;

2.5 and 5 g; 5 and 1 g; 5 and 2.5 g yeast) were compared.

Table 3. Summary of Pos Hoc Test for Multiple comparison

Dependant Variable : Percentage of bioethanol				
(I)	(J)	MD (I-J)	Std. Error	Sig.
Yeast				
1 g	2.5 g	-1.0130*	.22794	.000
	5 g	-3.0222*	.22794	.000
2.5 g	1 g	1.0130*	.22794	.000
	5 g	-2.0093*	.22794	.000
5 g	1 g	3.0222*	.22794	.000
	2.5 g	2.0093*	.22794	.000
NPK				
1 g	2 g	-.7000*	.22794	.010
	3 g	-1.9907*	.22794	.000
2 g	1 g	.7000*	.22794	.010
	3 g	-1.2907*	.22794	.000
3 g	1 g	1.9907*	.22794	.000
	2 g	1.2907*	.22794	.000

Note: MD = mean different

All of the treatment pairs have significant mean different that marked by asterisk symbol (*) and significant values are 0.000. For NPK group, a total of 6 pairs of NPK weight were also compared in this test. Two pairs (1 and 2 g; 2 and 1 g NPK) gave significant value 0.01, and the rest is 0.00. This result indicated that each pair of NPK group showed significant different from each other. Table 4 shows the Homogenous Subset Tukey HSD Test Result.

Table 4. Homogenous Subset of Tukey HSD Test

Yeast (g)	N	Subset		
		1	2	3
1,0	18	7.6259		
2.5	18	8.6389		
5,0	18	10.6481		
sig.		1.000	1.000	1.000
NPK	N	Subset		
		1	2	3
1 g	18	8.0741		
2 g	18	8.7741		
3 g	18	10.0648		
Sig.		1.000	1.000	1.000

The Homogeneous subsets indicated which groups that have the same or different mean. Groups with the same mean will have the same subset. Otherwise, groups that have

different mean will show different subset. The homogenous subset presented in table 4, shows that each group of both yeast and NPK groups placed in the different subset. Therefore, each group has significant different.

The ANOVA analysis in this study shows that yeast and NPK gave significant effect on bioethanol fermentation, and yeast is the most important aspect in this process. On the other hand, fermentation time did not significantly affect in bioethanol fermentation. Based on the result of this study, the pericarp of “Abacaxi” pineapple can be used for bioethanol bio-source. However, a further study in various composition of yeast and other materials is pivotal in order to get the higher percentage of bioethanol.

Conclusion

The pericarp of “Abacaxi” pineapple is potential for bioethanol fermentation. The percentage of bioethanol produced in this study varies among different treatment and fermentation time. Yeast and NPK gave significant effect in bioethanol fermentation. On the other hand, fermentation time did not give significant effect in this study. The result of this study provide the additional information on the effect of yeast, NPK and fermentation time in bioethanol fermentation using pericarp of “Abacaxi” pineapple.

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