Utilization of Banana Peel for Bioethanol Production Using Baker's Yeast Starter

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ABSTRACT
Research on the second generation of bioethanol production continues to be developed in the world. Second generation bioethanol has been produced from non starch substrates like cellulose, hemicellulose and bounded lignin as raw material. This research produced bioethanol using Ambon banana peels that contain high cellulose. The delignification process on Ambon banana peel was carried out by immersing the raw material in a 10% NaOH solution with a solution mass ratio of 1:10 (w/v) for 12 hours. The delignified raw materials were then tested for lignocellulosic content using the Chesson Datta method. After delignification process, the substrate was continued at the hydrolysis stage using 2 M H_2SO_4 concentration, then continued with the fermentation process for 2-5 days. The result showed that the delignification process can reduce the lignin of Ambon banana peel up to 12.93%. The highest concentration of bioethanol content was obtained on 0.8% baker's yeast starter during 3 days fermentation resulting in 17.98% of bioethanol. These treatments resulted in the highest score (4.00) for effectiveness test value that had 16.13% distillation volume, 2.91% pure ethanol volume and 15.75 ml/hour distillation rate per 200 ml substrate. It can be recommended that Ambon banana peel has great potential as a raw material for second-generation ethanol production.

Keywords: *Ambon banana peel, Baker's yeast, Bioethanol, Fermentation.*

1. INTRODUCTION

Research on the production of second-generation bioethanol has been widely developed in R&D institutions and universities. First generation bioethanol was produced from the sugar cane juice, namely molasses, that was produced on a large scale basis for more than 40 year. While second generation bioethanol was produced from lignocellulosic biomass (cellulose, hemicellulose and lignin) that converted its transformation into fermentable sugars as substrate to produce bioethanol [1].

Second generation bioethanol has been used as a liquid fuel in several countries. China has built a second generation bioethanol with a capacity of 5000 tons/year in Zhaodong city, while Brasilia has produced 16 billion liters of bioethanol in 2005. Indonesia's seriousness in developing second-generation bioethanol research is evidenced by the increasingly massive university-level research directed at new and renewable energy. Bioethanol can reduce almost 90% of CO_2 emissions compared to gasoline [2].

The second generation of bioethanol production comes from agricultural waste which contains a lot of lignocellulose. Lignocellulose consists of three polymers, namely cellulose, hemicellulose, and lignin. One of the agricultural waste is banana peel [1]. Indonesia's banana production is quite high, reaching more than seven million tons per year (Central Bureau of Statistics/BPS, 2020). Among the factors that support a successful bioethanol fermentation process are suitable substrate preparation, concentration of starter used and fermentation conditions [3]. This research produced the second generation bioethanol from banana peel using baker's yeast and fermentation time. Baker's yeast utilization was due to more applied during preparation of starter, the fermentation must be determined at the optimum time.

2. MATERIALS AND METHOD

2.1. Materials and Equipment

The raw materials for the production of bioethanol were Ambon banana peels from Burno Sari SME, at

Lumajang Residence, East Java. The banana peel delignification process used 10% 2M NaOH, while the hydrolysis process used 10% 2M H_2SO4. The bioethanol starter used Saccharomyces cerevisiae from baker's yeast (Fermipan from Sangra Ratu Boga Company) and added urea for nutrient enrichment.

The equipment for bioethanol fermentation were i.e analytical balance (Ohaus with 0.1mg accuration), pH meter (Hanna with 0.1 accuration), filter paper (Whatman 42), refractometer (Atago), Erlenmeyer (Pyrex), distillation apparatus (Iwaki). Alcohol measurement was carried out using alcohol meter (0-100 Richter- Tralles - Alla France – 0560FG000-20-QP) and pycnometer (5 ml Iwaki).

2.2. Experimental Design

The study was designed using a completely randomized design study (CRD) with two factors. The first factor was the variation of the concentration of baker's yeast which consists of four treatment levels: 0.2% (A1), 0.4% (A2), 0.6% (A3), 0.8% (A4). The second factor was the fermentation time of 2 days (B1), 3 days (B2), 4 days (B3), and 5 days (B4) as shown in Table 1. This research was replicated three times. The research stages of making bioethanol made from Ambon banana peels consist of three stages, namely the pretreatment stage which includes delignification and hydrolysis of banana peels, the fermentation stage and the distillation stage.

Table 1. Combination of bread yeast concentration treatment and fermentation time

Baker's yeast concentration (A)	Fermentation time (B)			
	2 days (B1)	3 days (B2)	4 days (B3)	5 days (B4)
0.2 (A1)	A1B1	A1B2	A1B3	A1B4
0.4 (A2)	A2B1	A2B2	A2B3	A2B4
0.6 (A3)	A3B1	A3B2	A3B3	A3B4
0.8 (A4)	A4B1	A4B2	A4B3	A4B4

2.3. Delignification of Banana Peel

Banana peels were cut into smaller sizes and dried to a maximum moisture content of 15% then mashed using a grinder. The banana peel powder was sieved through a 60 mesh sieve. Delignification of banana peel powder used 2M NaOH[4]. A total of 10% w/v NaOH solution was added to the Ambon banana peel powder. The solution was homogenized using a magnetic stirrer at a speed of 100 rpm for 3 minutes, then allowed to stand for 12 hours and throw away the water to get the cake. Furthermore, the cake was rinsed using distilled water until the pH was neutral, filtered and then dried using Memmert oven at 105 °C until the weight was constant. This cake is called delignified banana peel.

2.4. Hydrolysis of Delignified Banana Peel

Hydrolysis of delignified banana peel was carried out using 2 M H_2SO_4. A total of 10% w/v of banana peel delignin powder was added with sulfuric acid and stirred using a magnetic stirrer at 600 rpm for 5 minutes. Furthermore, it was heated under pressure using a pressure cooker 120 °C for 30 minutes after the water was boiling or the pressure cooker was ringing. Furthermore, the hydrolyzate was neutralized with the addition of 2 M NaOH solution until a pH value of 4.5 - 5.5 was reached as a substrate for bioethanol fermentation.

2.5. Bioethanol Fermentation by Baker's Yeast

The substrate was added with 0.5% w/v urea and autoclaved at 120 °C for 15 minutes by using Maxim pressure cooker with capacity of 4 litres. Then cooled and inoculated with baker's yeast at concentrations of 0.2%, 0.4%, 0.6% and 0.8%. Subsequently, they were incubated at room temperature for 2, 3, 4 and 5 days. A commercial pipe (Ø 0.5cm) was installed to drain CO_2 (g) produced during the fermentation process. The gas flowed through a hose that was inserted into water (Figure 2d). The gas intensity was characterized based on the formation of gas bubbles in the fermentation media (substrate).

2.6. Ethanol Distillation

Distillation was carried out using a reverse-cooled distillation apparatus. The fermented slurry was poured into a distillation flask, then heated in a temperature range of 78-80°C as the boiling point of bioethanol. Distillation was carried out for 2 hours. The resulting distillate was measured for its ethanol content using an alcoholmeter (0-100 Richter- Tralles - Alla France-0560FG000-20-QP) with accuration is 1 %.

2.7. Parameter Evaluation

Analysis of the lignin, cellulose, and hemicellulose content of Ambon banana peel powder using the Chesson Datta method[4]. Analysis was carried out on banana peel powder before and after delignification. 1g of sample (a) was added 150 mL of demineralized water, then heated at 100 °C for 1 hour. Slurry was filtered and washed the residue (cake) using hot demineralized water (300 mL), then the residue was dried until the weight was constant (b). Next step, the residue was mixed with 1 N H_2SO_4 (150 mL) and heated in the oil bath at 100 °C for 1 hour, then was filtered and washed with demineralized water. The cake was soaked with 72% H_2SO_4 (10 mL) at room temperature for 4 hours. Then 1 N H_2SO_4 (150 mL) was added into the mixture and refluxed in the oil bath for 1 hour. The cake was washed with demineralized water (400 mL), heated in the oven at 105 °C until the constant weight (d). Finally the cake (solid) was burn to ash and

weighed (e). The percentage of hemicellulose, cellulose and lignin was calculated using equation as follow

$$\% \text{ hemicellulose} = (c - b)/a \times 100\% \quad (1)$$

$$\% \text{ cellulose} = (d - c)/a \times 100\% \quad (2)$$

$$\% \text{ lignin} = (e - d)/a \times 100\% \quad (3)$$

Bioethanol content was analyzed using the pycnometer method (specific gravity). While the alcohol destilate was measured using an alcohol meter. The rate of distillation was determined based on the volume of distillate divided by distillation time.

The data was processed using descriptive methods which are presented in the form of tables, graphs or histograms equipped with standard deviations. The process effectiveness score was determined using the effectiveness test method [5].

The best treatment for bioethanol production using banana peel substrate and baker's yeast starter was analyzed using the effectiveness index value test [6]. Score giving was carried out on each parameter with a relative number of -1 to 1. The highest score (1.00) was given for ethanol content and distillation rate. While the volume of distilled ethanol was given a score of 0.80. Then was calculated the normal weight, effectiveness value, and product value used these formula:

$$\text{Normal Weight} = \frac{\text{(Value of Parameter Score)}}{\text{(Total Score)}} \quad (4)$$

$$\text{Effectiveness Value} = \frac{\text{(Treatment-lowest value)}}{\text{(Highest-lowest value)}} \quad (5)$$

$$\text{Product Value} = \text{Effectiveness Value} \times \text{Normal Weight} \quad (6)$$

3. RESULTS AND DISCUSSION

3.1. Hemicellulose, Cellulose and Lignin Content of Ambon Banana Peel Powder

The delignification process aims to free cellulose and hemicellulose from lignin so that they are easily hydrolyzed by acid into simple sugars. Physically, the results of the delignification treatment of Ambon banana peel have different colors before and after delignification. Banana powder that has been delignified by 10% NaOH solution [4] looks brighter than the undelignified powder. The lignocellulosic content of Ambon banana peel with treatment before and after delignification is presented in Figure 1.

Figure 1 shows that lignin degradation occurred before and after delignification with 10% NaOH, from 30.90% to 18.05%. The decrease in hemicellulose content occurred by 3.14%. Beroual et al. [7] reported that the delignification process resulted in higher crystallinity and better thermal stability. The delignification method may affect the purity of cellulose without influencing its chemical structure.

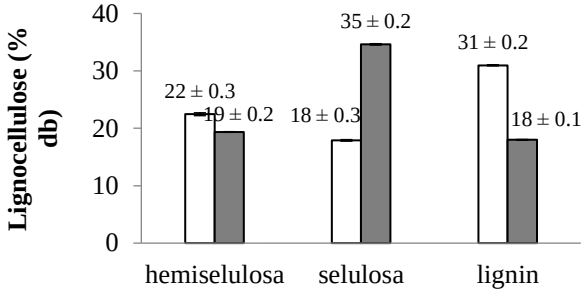

Figure 1. The lignocellulosic content of *Ambon* banana peel with treatment before (□)and after (■) delignification.

The cellulose content of Ambon banana peel was 17.91% and increased to 34.55% after the delignification process. The increase in the percentage of cellulose was due to the degradation of the lignin component that binds cellulose and hemicellulose, so that the cellulose component increased in percentage of the total components in the banana peel. Beroual et al. [7] reported that the cellulose content of date palm frond fiber increased after delignification into 31.4%.

3.2. Hydrolysate of the Delignified Banana Peel

The delignified banana peel powder was hydrolysed using 2 M H_2SO_4 under pressure heating for 30 minutes. Hydrolysis must be carried out to convert cellulose and hemicellulose into fermentable materials, namely glucose. Other researchers used 1% of sulfuric acid to hydrolyse banana peel on the bioethanol production Palacios et al.[8]. The hydrolysis process resulted in a 16 °Brix of hydrolysate. Gil et al. [9] reported that hydrolysis of cellulose or hemicellulose into alcohol can produce brix values of 15-18 °Brix. Jayus et al. [10] reported that molasses hydrolysate must be diluted into 28 °Brix before being used as substrate for bioethanol production.

Table 2. Observation during bioethanol fermentation using banana peels as substrate and baker's yeast starter

Fermentation day	Colour of substrate	Gas
First	Black	+ +
Second	Black	+ + +
Third	Brighter Black	+ + + +
Fourth	Bright	+ +
Fifth	Strong bright	no

Furthermore, neutralization was carried out to pH 4.5 - 5.5 by adding 2M NaOH. The substrate was enriched with the addition of 0.5% w/v urea and sterilized at 121

°C for 15 minutes. Table 2 shows the results of the hydrolysis process. It is then cooled and ready to be continued in the fermentation process. The pH conditions of the hydrolysate before and after adding NaOH are presented in Table 2.

3.3. Bioethanol Fermentation Using Baker's Yeast with Ambon Banana Peel Substrate

The fermentation process was carried out using baker's yeast [10] in a facultative anaerobic way with a fermentation vessel using alufo and wax so as not to be contaminated with environmental air as shown in Figure 2. The fermentation process was carried out for 2 days, 3 days. 4 days, and 5 days with descriptive observations regarding turbidity (turbidity) and air bubbles formed. (a B C) (d) (e)

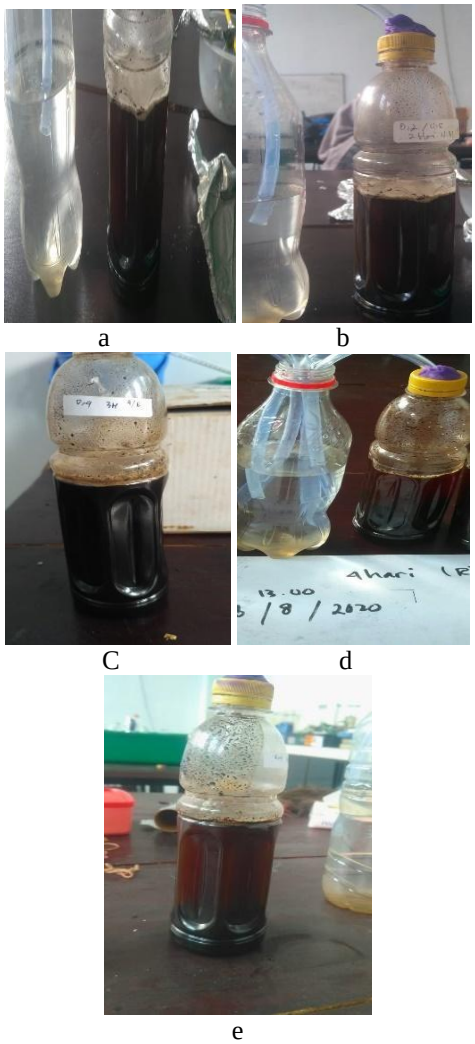

Figure 2 Fermentation of bioethanol with Ambon banana peel as a substrate for 1 day (a), 2 days (b), 3 days (c), 4 days (d), and 5 days (e)

Figure 2 showed that fermentation occurred on the first day to the fourth day with the formation of gas bubbles. On the first and second day the color of the substrate was still dark brown, on the third and fourth day it had started to become light brown and on the fifth day the color of the substrate became bright. This is due to the occurrence of deposition on the substrate due to the fermentation process taking place singly without being accompanied by agitation. In addition, the substrate nutrients have also begun to be consumed by the starter microbes. Ndukwe et al. [11], explained that simulations of the presented model showed that YGE, energy intake (EI), and their produced ethanol energy (PEE) are always balanced during the fermentation process according to the law of conservation of energy.

3.4. Bioethanol Levels During the Fermentation Process

At the end of the fermentation process, the substrate residue is distilled to obtain bioethanol. The distillation process was carried out at a temperature of 95-96 °C for 2 hours. Figure 3 showed the volume of distillate produced within 2 hours of distillation.

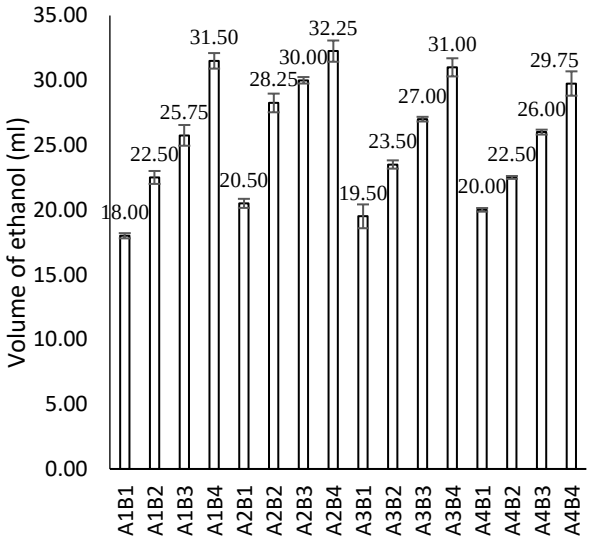

Figure 3. Volume of bioethanol during 2 hours destilation (Data represent the mean ± standard deviation from n=3)

Increasing the amount of starter added from 0.2% to 0.8% resulted in an increase in the volume of the distillate. Figure 3. showed the addition of 0.8% starter to produce the highest volume of distillate. The result showed that the 3 days fermentation has a higher volume of distillate ethanol than the other day fermentation. A2B1 treatment produced a volume of ethanol as much as 22.50 ± 2.12 and increased in A2B2 treatment which resulted in a volume of ethanol from distillation as much as 28.25 ± 1.06. A3B3 and A2B4 treatments decreased, respectively, with the volume of distilled ethanol as much as 23.50 ± 0.71 and 22.50 ± 0.09.

The ethanol content increased with more yeast starter added. The 3 day fermentation of bioethanol resulted in higher ethanol content than the other treatments, namely 12.12 ± 0.44% at 0.2% yeast concentration and 17.98 ±

0.64% at 0.8% yeast concentration. Komarayati et al. [12] also reported that the amount of yeast needed in fermentation varies with the ethanol content produced. According to Chen & Fu [13], the amount of yeast used in bioethanol fermentation affects the alcohol content produced. The higher the amount of yeast used, the higher the alcohol content produced. There is a limit to the amount of yeast that can be used to get the optimal alcohol content. Chang et al. [14] stated that excess yeast will actually inhibit alcohol production because glucose is used more for the growth of microbial biomass.

Another factor that affects the bioethanol content is the length of fermentation. Two days fermentation produced a lower ethanol content (10.44 ± 0.11%) than three fermentation (12.12 ± 0.44%) at the same amount of starter. However, four and five days fermentation resulted in lower ethanol content than the 3-day fermentation at 18 ± 0.86% and 7.07 ± 0.05%, respectively. This is because the effective fermentation time was 3 days with the ethanol content having a peak point marked by the increasing number of microorganism spot reactions from the fermenter. The fourth and fifth days on each substrate showed a decrease in the bioethanol content. According to Nurhayati et al. [15], the decreasing is due to the availability of substrates that are running low during fermentation, as well as the presence of toxic ethanol which causes yeast to work not optimally and because microorganisms have gone through an exponential phase leading to the death phase.

3.5. Bioethanol Distillation Rate from Banana Peel Powder

The distillation rate is calculated by dividing the distillate volume obtained for each treatment by the time of distillation process. The higher distillation rate and the higher ethanol content were at the same time and temperature. Figure 4 showed the distillation rate along with Ambon banana peel substrate and baker's yeast starter.

Figure 4 shows that with different concentrations of baker's yeast starter with the same fermentation time from the A1B1 treatment, the distillation rate of 9 ml/hour was obtained and it increased until the fifth day of fermentation was 15.75 ml/hour. This indicates that the addition of baker's yeast concentration affects the rate of detailing. In contrast to the fermentation time, with the same baker's yeast starter concentration and different fermentation time from the A1B1 treatment, the distillation rate of 9 ml/hour resulted in an increase in the distillation rate resulting from the A1B2 treatment of 14.13 ml and a decrease in the A1B3 treatment which resulted in a 14.13 ml rate of distillation. distillation of 9.75 ml/hour. This shows that fermentation for 3 days produces the highest distillation rate. The results of fermentation on the fourth and fifth days in a row decreased because the distillation rate was directly proportional to the resulting distillate.

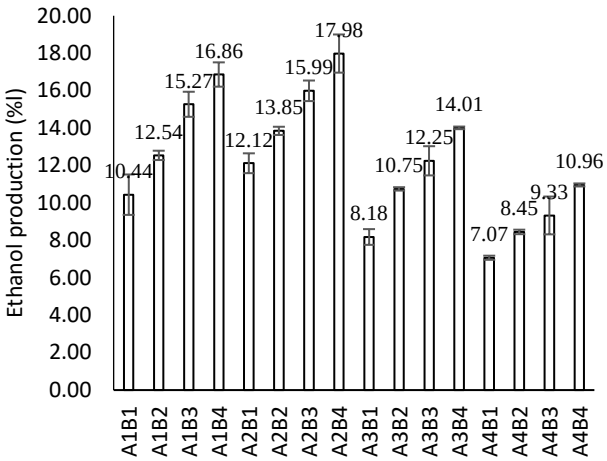

Figure 4. Destilation rate of bioethanol made from banana peel (Data represent the mean ± standard deviation from n=3)

3.6. Effectiveness Index Value of Bioethanol Production from Banana Peel with Baker's Yeast Starter

The effectiveness value determines the best treatment for bioethanol production from banana peels with baker's yeast starter. Reference parameters used in this study include ethanol content, volume of pure ethanol, distillation rate, and volume of distillation. The effectiveness value of each of the best treatments for bioethanol production from banana peels with baker's yeast starter is presented in Table 3.

Table 3. The effectiveness value of treatments of bioethanol production made from banana peel using baker's yeast starter

Treatments	Effectiveness value
A1B1	0.42
A2B1	1.45
A3B1	2.41
A4B1	3.68
A1B2	1.06
A2B2	2.63
A3B2	3.27
A4B2	4.00
A1B3	0.35
A2B3	2.13
A3B3	2.17
A4B3	3.13
A1B4	0.28
A2B4	0.87
A3B4	1.56
A4B4	2.42

The highest effectiveness value for bioethanol production from banana peels with baker's yeast starter, namely 4.00, was found in A4B2 treatment, which was 0.8% concentration with fermentation for 3 days. The lowest effectiveness value is found in the A4B1 treatment, which is 0.28. This shows that the A4B2 treatment is the best treatment in the manufacture of bioethanol made from Ambon banana peel which produces an ethanol content of 17.98%, the volume of ethanol from distillation is 31.50 ml, the volume of pure ethanol is 5.80 ml with a distillation rate of 16.13 ml/hour.

4. CONCLUSION

The delignification pretreatment using 10% NaOH on banana peel substrate was effective in reducing lignin content by 12.93% and increasing cellulose by 16.64%. Making bioethanol from Ambon banana peel is most optimal using baker's yeast with A4B2 treatment, namely the addition of 0.8% yeast concentration with fermentation for 3 days which can produce ethanol content of 17.98%, distillate volume of 32.25 ml, distillation rate of 15 .75 ml/hour, the volume of pure ethanol is 5.80 ml with a distillation rate of 16.13 ml/hour

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