

Delignification Of Bioethanol Production From Saba Banana Peel (*Musa Paradisiaca* *Formatypica*) By *Aspergillus Fumigatus*

Bunga Faradhani, R. Ratnaningsih, Astri Rinanti

Abstract: Banana peel is a waste, and a lignocellulose biomass used as an alternative raw material to bioethanol by utilizing the enzymatic ability of *Aspergillus fumigatus*. This study therefore was conducted to test the potential of banana peel to be converted into bioethanol. The research started by cultivating *A. fumigatus* on Potato Dextrose Broth media with the banana peel mechanically converted into powder and used as a substrate. This was followed by the pretreatment process by adding *A. fumigatus* to the substrate container in ratios of 1: 1, 1: 5, and 1:10, respectively. Furthermore, the gravimetric method was used to determine the level of lignin due to pretreatment with contact times of 24, 72, and 120 hours. This was preceded with the hydrolysis stage using the DNS method to measure the amount of sugar produced, with the removal of the highest lignin content at the pretreatment stage of 7.3% and the highest sugar level at the hydrolysis stage of 1.353 g/L. This research shows that banana peel contains lignocellulose and has the ability to be used as raw material for bioethanol. Therefore, to increase the levels of bioethanol derived, it is necessary to carry out a fermentation process and further research.

Index Term: Bioethanol, *Aspergillus fumigatus*, Saba Banana Peel, Pretreatment, Delignification, Sugar Production, Hydrolysis.

1 INTRODUCTION

Banana is one of the main fruit produced in Indonesia. Its production increased from 7,162,678 tons in 2017 to 7,264,379 tons with a 1.4% increase, in 2018[1]. Initially, studies were on the various processing methods of the fruit, with none on its peel which was either fed to animals or thrown away, thereby, leading to environmental pollution. Furthermore, the world is faced with problems related to energy scarcity which has led various countries into seeking alternative sources. However, in Indonesia, banana peel is a renewable energy source with great development potential. It has the ability to be used as a raw material in making bioethanol, and as an alternative fuel [2]. Bioethanol is produced from carbohydrates through the fermentation of glucose by the enzymatic activity of yeast known as *Saccharomyces cerevisiae* [3], with ethanol used as a solvent due to its high economic value[4].

Ethanol (C₂H₅OH) is a liquid obtained from the fermentation process of sugar which is derived from the enzymatic activities of microorganisms. Its carbohydrate sources are obtained from materials containing cellulose, polysaccharides, and monosaccharides. Bioethanol are chemicals from the production of foods containing starch, such as cassava, sweet potatoes, corn, and sago. According to research [5] Ethanol is produced from sugar fermentation obtained from various

biomass sources, which are broadly classified into three plant types, sugar such as sweet sorghum, starch such as corn, wheat, and cellulose biomass. Ethanol derived from sugar and starch are referred to as first-generation biofuel, while those from cellulose biomass is known as the second generation. Furthermore, the raw material consists of hemicellulose and lignin, which are often referred to as lignocellulose biomass such as agricultural residues, forestry waste, and energy crops. It provides low-cost benefits, abundant in nature, and, most importantly, it is a non-food source for biofuel production. Four major factors are responsible for bioethanol production, namely, raw materials, conversion technology, hydrolysis processes, and fermentation configurations. [6]. Banana peel has the ability to be used as an alternative fuel because of its relatively high starch content of 59% [7]. It is a D-glucose polymer and is found to store carbohydrates in plants, such as cassava, trees, bananas, corn, and others. Carbohydrates are first separated through the hydrolysis process and fermented using *Saccharomyces cerevisiae* into alcohol, which acquires the final result. Therefore, it is necessary to further investigate the use of Saba banana peel in bioethanol production, to obtain ethanol as a renewable energy.

2. RESEARCH METHODOLOGY

2.1 Materials and microorganism

The banana peel obtained from the small Saba banana processing industry was dried and cut into smaller pieces, dried in an oven at 180° C, and crushed with a blender to obtain powder. This was followed by the *pretreatment* stage, which delignified the powder using *Aspergillus fumigatus* obtained from the Environmental Biology/Microbiology Laboratory, Trisakti University. *A. fumigatus* fungi were grown on *potato dextrose agar* (PDA) media for 5 days at 30° C till perfect sporulation was formed in the exponential phase. After 5 days, the PDA containing the fungus is stored at 40° C [8].

- Bunga Faradhani, Environmental Engineering student in Universitas Trisakti, Jakarta, Indonesia.
- R. Ratnaningsih, Faculty of Landscape Architecture and Environmental Technology, Department of Environmental Engineering, Trisakti University, Indonesia.
- Corresponding Author is Astri Rinanti, environmental engineering lecturer in biotechnology at Universitas Trisakti, Jakarta, Indonesia. E-mail: astririnanti@trisakti.ac.id

2.2 Pretreatment of banana peels

The *A. fumigatus* fungus, acted as a catalyst in the pretreatment process, and was inserted into a *potato dextrose broth* (PDB) container using an autoclave at 121° C for 15 minutes. Furthermore, the ratio of fungi *Aspergillus fumigatus* to the substrate was 1: 1, 1: 5, and 1:10 (w/w). Observations were made with a contact time of 24, 72, and 120 hours, with the residue, filtered and dried at 70° C.

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis was carried out using cultivated and dried *Aspergillus fumigatus* fungi. The fungus was added to the substrate in ratios of 1: 1, 1: 5, and 1:10 (w/w) with a contact time of 24, 72, and 120 hours. This was followed by the filtration process.

2.4 Analytic Methods

The amount of lignin formed as a result of the delignification process was determined by the gravimetric method. The efficiency of lignin removal was calculated using equation 1 as follows:

$$\% \text{ removal} = \frac{W_{(a)} - W_{(b)}}{W_{(a)}} \times 100 \quad (1)$$

Description:

$W_{(a)}$: Levels (%) of lignin before delignification

$W_{(b)}$: Levels (%) of lignin after delignification

The DNS method is used to determine the estimated sugar production at the hydrolysis stage [9]. The addition of 1 gram DNS, 50 mg ofsodium sulfite, and 1 gram NaOH were carried out in the manufacture of the reagents with 50 mL of distilled water dissolved in a colored volumetric flask to avoid the oxidation process. The sugar production was conducted by adding 3 mL each of the samper and DNS reagent into a test tube covered with aluminum foil. It was further heated at a temperature of 90° C for 15 minutes or till a brownish-red color is formed. Next, 1 mL of Rochelle salt was added, then measured with a spectrophotometer at a wavelength of 575 nm. Rochelle salt is manufactured by dissolving 20 grams of Na-K Tartat in a 50 mL measuring flask.

3. RESULTS AND DISCUSSION

3.1 Effect of Pretreatment

Lignin and carbohydrate removal is conducted to facilitate the hydrolysis process in producing sugar with the removal values shown in Table 1. Table 1 show lignin removal with a variety of fungi ratios on the substrate at various contact times Furthermore, the removal pattern in the substrate is shown in Figure 1.

Table 1: Lignin removal with a variety of fungi ratios on the substrate at various contact times

| Comparison Fungi : Substrate | Contact Time (hours) | Lignin Removal Efficiency (%) |
|------------------------------|----------------------|-------------------------------|
| 1:1 | 24 | 6.5 |
| | 72 | 7.3 |
| | 120 | 5.6 |
| 1:5 | 24 | 4.7 |
| | 72 | 6.3 |
| | 120 | 5.4 |
| 1:10 | 24 | 3.2 |
| | 72 | 4.3 |
| | 120 | 2.8 |

The removal of 7.3% was found to be the best delignification process with fungi to substrates ratio of 1: 1 at 72-hour contact time as shown in Figure 1. These result showed that the ratio of 1: 1 was quit effective in changing the lignocellulose component found in the substrate, and the *Aspergillus fumigatus* had the ability to degrade lignin compounds. The calculation of removal efficiency is shown in equation 1, with a more friendly biological catalysts that do not cause side effects in the process [10]. The purpose of the pretreatment process was to reduce or eliminate various materials/compounds capable of inhibiting the rate of hydrolysis and increasing the bioethanol production from simple sugars derived from cellulose and hemicellulose [11], [12].The selection of the pretreatment method is important because this stage determines the use of hydrolysis and fermentation methods. In the end the selection of this pretreatment greatly affects the overall operational costs of bioethanol production. The use of non-optimal pretreatment methods causes partial degradation of hemicellulose and lignin and produces toxic compounds that inhibit the growth of yeast [13]. Pretreatment is the most expensive process of converting biomass into fuel and has great potential for increasing efficiency and reducing costs through further research and development. [14]. The removal pattern in the substrate is shown in Figure 1.

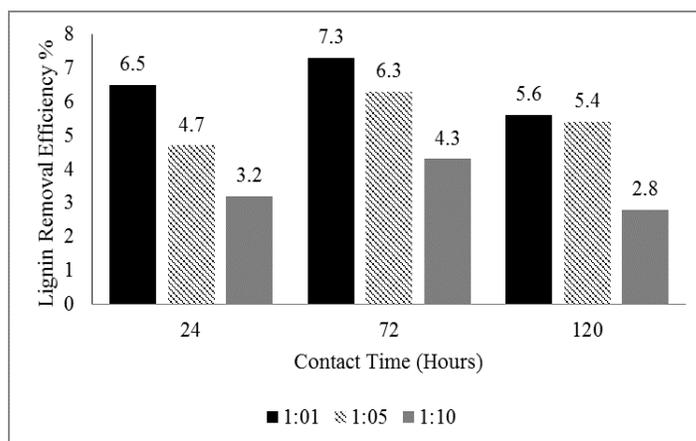


Figure 1: Removal of lignin content in banana peel waste

3.2 Hydrolysis Process

The production of fermented sugar is very influential in the formation of bioethanol. The results of sugar production at the hydrolysis stage show in Figure 2.

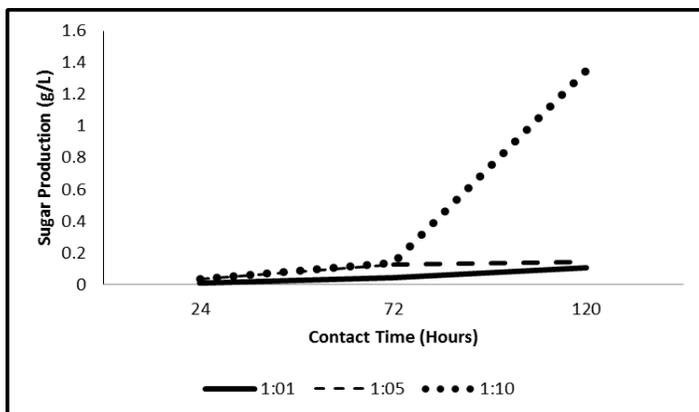


Figure 2. The results of sugar production at the hydrolysis stage

After the delignification is completed, the biomass is processed to produce sugar. The hydrolysis is conducted by using acids or bases and the enzymatic process. Table 2 show a results of sugar production at the hydrolysis stage

Table 2. Results of sugar production at the hydrolysis stage

| Comparison Fungi : Substrate | Contact Time (hours) | Sugar (g/L) |
|------------------------------|----------------------|-------------|
| 1:01 | 24 | 0.010 |
| | 72 | 0.041 |
| | 120 | 0.107 |
| 1:05 | 24 | 0.033 |
| | 72 | 0.128 |
| | 120 | 0.145 |
| 1:10 | 24 | 0.035 |
| | 72 | 0.136 |
| | 120 | 1.353 |

Figure 2 and Table 2 show the enzymatic hydrolysis process which produced the highest sugar at a ratio of 1:10 with a contact time of 120 hours and yield of 1,353 g/L. The results of this study are higher than those obtained using the *Cytophaga hutchisonii* bacteria which produced sugars of 0.148 g and 0.123 g through isolated cultures [15].

In the hydrolysis process, the cellulose present in the substrate is converted to ethanol using *Aspergillus* fungi after the pretreatment process [16]. In many researches, bacteria were used in the hydrolysis compared to chemical enzymes because it is expensive and increases the cost of producing bioethanol [12], [16], [17]. The hydrolysis is carried out at neutral pH.

CONCLUSION

This research proved that the delignification and hydrolysis processes of fungi *Aspergillus fumigatus* by the enzymatic activity has a good role as a biocatalyst. The ratio of *Aspergillus fumigatus* to the substrate by 1: 1 in the pretreatment process is the most suitable for delignification, as evidenced by the highest lignin removal of 7.3% at 72 hours, and at a ratio of 1:10 with a contact time of 120 hours, the highest sugar content was 1,353 g/L. Further research on the fermentation process for the production of Saba banana peel as a raw material in producing bioethanol need to be further investigated.

REFERENCES

- [1] Central Statistics Agency and Directorate General. 2019. Fruit Production by Province in 2016-2018. Ministry of Agriculture Republic of Indonesia.
- [2] Ramayanti, Cindi dan Ketty, R. Giasmara. 2017. Bioethanol Production From Waste Paper Using Separate Hydrolysis and Fermentation. *Ind. J. Chem. Res.*, 2017, 5(1), 429-433.
- [3] Sriwulan, D .2012. Bioethanol production from agricultural wastes: An overview. *Jurnal Renewable Energy*, 37.
- [4] Rinanti, A., Kardena E., Astuti, DI., Dewi, K., 2013. Growth Response and Chlorophyll Content of *Scenedesmus obliquus* Cultivated in Different Artificial Media. Faculty of Civil and Environmental Engineering, Bandung Institute of Technology, Bandung, Indonesia.
- [5] Kumar, Deepak dan Vijay, Singh. 2018. Bioethanol Production From Corn. Chapter 22. Department of Agricultural and Biological Engineering, University of Illinois at Urbana Champaign, Urbana, IL, United States.
- [6] Sarkar, N., Ghosh, S. K., Bannerjee, S. & Aikat, K .2011. Bioethanol production from agricultural wastes: An overview. *Jurnal Renewable Energy*, 37, 19-27.
- [7] Anhwange, B. Ugye, T. and Nyiaatagher, T. 2009. Chemical Composition of *Musa sapientum* (Banana) Peels. *EJEAFChe*, 8 (6) pp. 437-442.
- [8] Das A, Gosh P, Paul T, Gosh U, Pati B R, Mondal K C. 2016. Production of bioethanol as useful biofuel through the bioconversion of water hyacinth (*Eichhornia crassipes*). *Biotech* (2016) 6:70.
- [9] Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31, 426-428.
- [10] N. Mosier, Wyman, C., Dale, B., Elander, R., Lee, YY., Holtzapple, M., Ladisch, M., Hendrickson. Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass, *Bioresource Technology*, 96: 6673-686, 2005.
- [11] Balat, M., Havva B., and Öz C. 2008. Progress in Bioethanol Processing. *Progress in Energy and Combustion Science*, 34(5): 551-573.
- [12] Putri, F., Rinanti., A. 2018. Starch producing in microalga biomass as a raw material for bioethanol. *Matec Web of Conference* 197, 13018. <https://doi.org/10.1051/mateconf/201819713018>
- [13] Galbe, M.; Zacchi, G. Pretreatment of lignocellulosic materials for efficient bioethanol production. *Adv. Biochem. Eng./Biotechnol.* 2007, 108, 41-65.

- [14] Tomas-Pejo E, Alvira P, Ballesteros M, Negro MJ. 2011. Pretreatment Technologies for Lignocellulose-to-Bioethanol Conversion. Di dalam Pandey A (ed.), Biofuels: Alternative Feedstocks and Conversion Processes, pp: 149-176.
- [15] Anwar, Zahid., Gulfraz, M., Asad, Javaid., Imran, M., Akram, Z., Mehmodd, S., Rehman, A., Anwar, P., Sadiq, A., .2012. Bioethanol productions from rice polish by optimization of dilute acid pretreatment and enzymatic hydrolysis. African Journal of Biotechnology Vol. 11(4), pp. 992-998, 12 January, 2012.
- [16] Byadgi, A. Shruti dan Kalburgi, P. B. 2015. Production of Bioethanol from Waste Newspaper. International Conference on Solid Waste Management, 5IconSWM 2015.Procedia Environmental Sciences 35 (2016) 555 – 562.
- [17] Juliarnita, IGA., Hadisoebroto, R., Rinanti., A. 2018. Bioethanol production from mixed culture microalgae biomass with temperature hydrolysis variation Matec Web of Conference 197, 13010. <https://doi.org/10.1051/matecconf/201819713010>