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The Optimazion of Bioethanol Production from Rice Husk using Simultanous Saccharification and Fermentation Methods

Yuliana Wanda Puspitasari[1] & Senam[2]
[1,2] Chemistry Study Program, Universitas Negeri Yogyakarta, Indonesia

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Corresponding Author:

Yuliana Wanda Puspitasari,
Chemistry Study Program, Universitas Negeri Yogyakarta

Email:
uliana6321fmipa2016@student.uny.ac.id

ABSTRACT

The optimum condition of bioethanol production was studied by determining NaOH concentration, leaving time, and bioethanol content. The research started with delignification reaction using NaOH (alkaline pre-treatment), continued by saccharification process with Aspergillus niger and fermentation process with Saccharomyces cerevisiae. The resulted bioethanol was purified using destilation process and characterized with Gas Chromatography (GC). The result showed that optimum NaOH concentration was 3%, the leaving time optimum at 105 minutes, and the bioethanol content was 10.9966%.

Keyword: *: bioethanol, simultaneous saccharification and fermentation, alkaline pre-treatment, NaOH, rice hull ask*

1. INTRODUCTION

Fuel is an important need in human life, as for cooking, lighting, and transportation (Bali, et al, 2015). Energy sources that are used continuously cause fuel supplies became limited. Bioethanol is a gasoline substitution fuel derived from anaerobically fermented glucose, and is an environmentally friendly alternative energy (Hambali, et al, 2007). Bioethanol is an alcohol compound that can be obtained through a biomass fermentation process with the help of microbes, such as yeast (Khofiya, et al, 2019). The used biomass can come from agricultural and plantation crops, agricultural waste, and domestic waste (Kristina, 2012). Indonesia is an agricultural country with commodity crops such as rice. When agricultural and plantation products are harvested, they produce lignocellulose material which will be left as agricultural waste that is not utilized, such as rice husks (Khofiya, et al, 2019; Naufala, 2015).

Rice husk is one of the high-quality lignocellulose biomass which is abundantly and cheaply available in Indonesia, so it has the potential to be converted into bioethanol. Rice husk has a cellulose content of 42.2%; hemicellulose 18.47%; and lignin 19.4% (Novia, 2014). Utilization of rice husks as raw material for bioethanol is expected to increase the economic value of agricultural waste to meet the energy needs of the community. One of the difficulties in making bioethanol from lignocellulose is the difficulty of the hydrolysis process because it contains lignin. The pre-treatment process toward lignocellulose polymer is carried out to open the structure of the cellulose polymer, thus the enzyme can break down cellulose into glucose (Oloffson, 2008). Alkaline pre-treatment using NaOH is one of the commonly used methods because it is significantly effective in removing lignin (Riyanti, 2009).

Simultaneous Saccharification and Fermentation (SSF) is a method commonly used in the production of high lignocellulosic bioethanol (Rosyida, et al, 2019). In this process, hydrolysis is carried out simultaneously and continuously without a long grace period. The acid commonly used in hydrolysis is HCl. The advantage of this process is that the converted polysaccharides into monosaccharides will not return to polysaccharides because the monosaccharides are directly processed into ethanol [6]. This article will report about the synthesis of bioethanol from rise husk through SSF method with applying alkaline pre-treatment.

2. RESEARCH METHOD

2.1 Materials

The materials used were rice husks, potato tubers, A. niger mold, yeast S. cerevisiae, distilled water, dextrose, HCl, $MgSO_4.7H_2O$, H_2SO_4, $(NH_4)_3PO_4$ and NaOH.

2.2 Procedure

Culture of S. cerevisiae

S. cerevisiae cultures were incubated at room temperature on a shaker at 120 rpm for 48 hours. Measurement of optical density (OD) was carried out every 3 hours from 0 to 48 hours. The number of S. cerevisiae cells was carried out by measuring OD600 using Spectronic 20 at a wavelength of 600 nm with sterile liquid YPD as the blank solution. The results of the sample were compared to the blank solution to determine the absorbance value.

Pre-Treatment the Rice Husk at NaOH Variation

Rice husks were washed, boiled for 30 minutes and dried in an oven to a constant weight. The rice husks are then crushed. A total of 7 500mL erlenmeyer was prepared and 25 g of mashed rice husks were added each. NaOH solution with a concentration of 0%; 0.5%; 1%; 1.5%; 2%; 2.5%; and 3% (w/v) with a volume of 100 mL, then mixed with rice husk in an erlenmeyer. The sample was allowed to stand for 1 hour, filtered and rinsed until it was clean and reached a neutral pH. Samples were dried in an oven to constant weight.

Pre-Treatment the Rice Husk at Leaving Time Variation

Rice husks were washed, boiled for 30 minutes and dried in an oven to a constant weight. The rice husks are then mashed using a blender. A total of 7 500mL Erlenmeyer were prepared and added 25 g of mashed rice husks each. 3% (w/v) NaOH solution with a volume of 100 mL, then mixed with rice husks in each erlenmeyer. The samples were then allowed to stand for 15, 30, 45, 60, 75, 90, and 105 minutes. The sample is then filtered and rinsed until it is clean and reaches a neutral pH. Samples were dried in an oven to constant weight.

Proses SSF

A total of 2.5 g of rice husk powder was put into a 100 ml Erlenmeyer then added with nutrient media (a mixture of 1 g/l (NH4)3PO4 and 0.05 g/l MgSO4.7H2O) and 0.05 N HCl solution to pH 5 0.0 then sterilized in an autoclave at 121oC for 15 minutes. The sample was cooled at room temperature and then added with starter A. niger and S. cerevisiae with a concentration of 20%:20% (v/v). The samples were incubated in a water bath at 32°C for 4 days (96 hours). The incubation filtrate is separated by distillation. The samples were then tested by gas chromatography.

3. RESULTS AND DISCUSSION

Rice husk mass before and after pre-treatment at various concentrations of NaOH is presented in Table 1. Rice husk mass before and after pre-treatment at various leaving time is listed in Table 2. NaOH can degrade lignin by breaking ester cross-links in lignin and increasing the porosity of biomass. The black liquor that appears indicates the lignin has dissolved and has separated from the cellulose [13]. Rice husks after pre-treatment changed color to a paler color which could be concluded that the lignin content had been lost and cellulose was obtained which was used in the SSF process. The concentration

of resulted ethanol from the SSF process presented in Table 3. The standard ethanol concentration was shown in Figure 1.

Tabel 1. Mass of rice husk before and after NaOH variation

NaOH concentration (%)	Mass before *Pre-Treatment* (g)	Mass after *Pre-Treatment* (g)
0	25	20,720
0,5	25	20,576
1	25	20,794
1,5	25	19,976
2	25	19,644
2,5	25	19,602
3	25	18,674

Tabel 2. Mass of rice husk before and after leaving time variation

Leaving time (min)	Mass before (g)	Mass after (g)
15	25	19,274
30	25	19,258
45	25	18,766
60	25	18,566
75	25	18,410
90	25	18,562
105	25	17,022

Tabel 3. Standard Ethanol Concentration

No	Standard Ethanol Concentration (%)	Retency	Area
1	1	0,440	562,5204
2	5	0,443	1334,0415
3	10	0,440	2461,9564

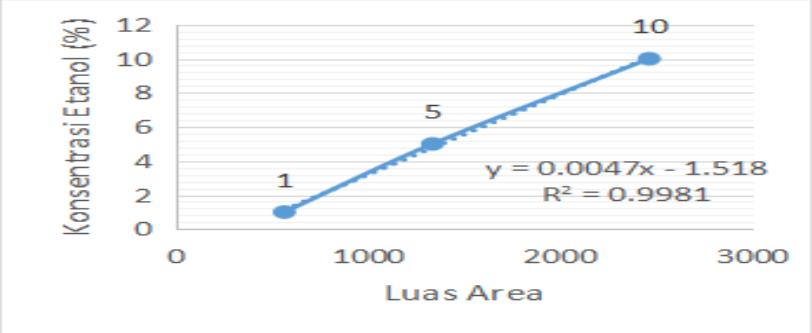

Figure 1. Standar Ethanol Calibration

Based on the resulting calibration curve, the linear regression equation y = 0.0047158x - 1.5183511 is obtained. This equation is used to calculate the bioethanol content by substituting the x value with the area of the gas chromatography data. The value of y obtained is the level of bioethanol produced. The determination of ethanol content for leaving time variation was done using the same method.

Tabel 4. Ethanol content at NaOH variation

NaOH concentration(%)	Ethanol content (%)			
	1	2	3	Average
0	2,0068	1,9544	1,9159	1,9590
0,5	7,0377	6,9920	7,1306	7,0535
1	7,5024	7,5185	7,5748	7,5319
1,5	8,1293	8,2311	8,1841	8,1815
2	8,6563	8,8496	8,6896	8,7318
2,5	9,2205	9,3202	9,2776	9,2727
3	9,4805	9,6793	9,5669	9,5755

Tabel 5. Ethanol content resulted from leaving time variation

Leaving time (Menit)	Ethanol content (%)			
	1	2	3	Average
15	7,8942	7,7981	7,8273	7,8399
30	8,1472	8,1867	8,2321	8,1887
45	8,8217	8,7370	8,8005	8,7864
60	9,3654	9,2721	9,3212	9,3196
75	9,7704	9,8112	9,8434	9,8084
90	10,5307	10,5840	10,5471	10,5539
105	10,9036	11,0216	11,0645	10,9966

Figure 2. Relation between NaOH concentration and Bioethanol Content

The relation between NaOH concentration and bioethanol process was shown in Figure 2 which indicated that a higher concentration of NaOH used in the pre-treatment stage resulted in higher levels of bioethanol produced. These results illustrate that the higher the concentration of NaOH, the more broken lignin bonds. The damaged lignin structure can be proven by the decreasing weight of rice husk at each concentration of NaOH. The similar trend was found in the relation between leaving time and ethanol content (Figure 3), which indicated that the longer residence time during pre-treatment resulted in greater levels of bioethanol produced. If the pre-treatment process is continued, the bioethanol content produced can be even higher. This condition is because the longer the residence time at the pre-

treatment stage causes the contact time between the NaOH solution and rice husks to be longer, so that the breakdown of lignin bonds is more perfect [4][6].

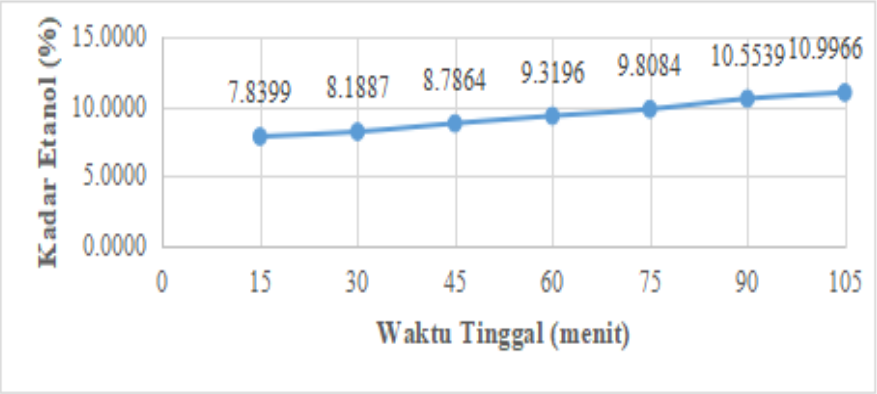

Figure 3. Relation between leaving time with ethanol content

The profile of *S. cerevisiae* growth was then made to study the amount of the cell and the growing rate of *S. cerevisiae* at a media in a specific time.

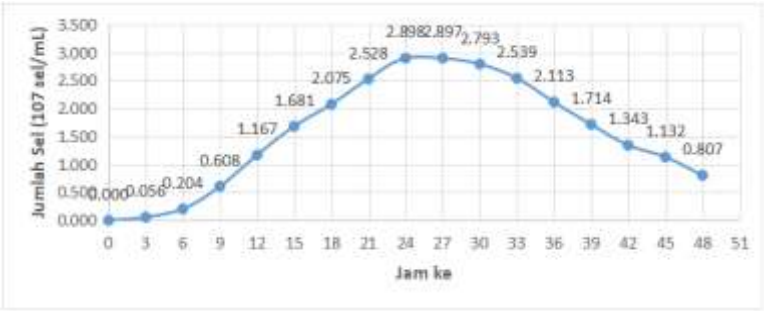

Figure 4. The profile of *S. cerevisiae* growth

In this study, S. cerevisiae had 4 growth phases that formed a pattern, namely the lag phase, the log/exponential phase, the stationary phase, and the cell death phase [3]. The lag phase (adaptation) on the growth profile of S. cerevisiae was shown right after the cells were added to the liquid YPD medium, namely at 0 to 3 hours. The logarithmic phase occurred at the 3rd hour to the 24th hour which was characterized by very fast growth of S. cerevisiae. The stationary phase in this study was not seen significantly and occurred in a short time, namely at the 24th hour to the 27th hour. The last phase, namely the cell death phase, is marked by a decrease in the growth curve at the 27th hour to the 48th hour.

4. CONCLUSION

It can be concluded that the bioethanol can be synthesis from rice husk through Simultaneous Saccharification and Fermentation by applying alkaline pre-treatment with optimum NaOH concentration at 3%, optimum leaving time of 105 minutes. The bioethanol content resulted in an optimum condition was 10.9966%.

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