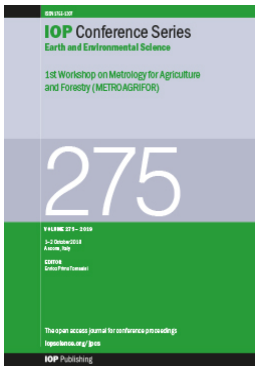

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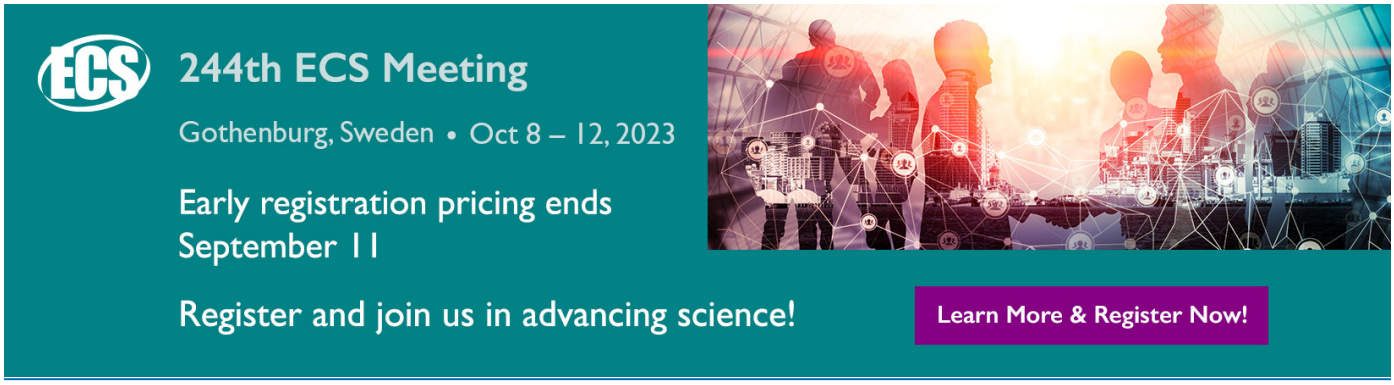

Utilization of corncob waste as bioethanol raw material by activities of *Dekkera Bruxellensis* and *Saccharomyces Cerevisiae*

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Abstract. Corncob waste is not managed properly has the potential to environmental pollution. The purpose of this reasearch is to utilize corncob waste as a potential biomass into bioethanol. Process of making bioethanol is carried out in 4 steps, namely delignification, hydrolysis, fermentation, distillation. Corncob as substrate were prepared mechanically to become size powder 40 mesh. Delignification by fungi *Dekkera Bruxellensis* was varied with substrate ratio of 5,10,and 15 grams at contact times of 3,5,and 7 days.Hydrolysis with 2% sulfuric acid (H_2SO_4) at contact times of 30,45,and 60 minutes. Glucose concentration was measured uing 3,5-dinitrosalicylate (DBS) method. Variations of fungi:ragi = 1:1 at contact times of 3,5,and7 days. Distillation at temperature of 78°C using Gas Chromatography–Mass Spectrophotometry (GC-MS). This result in optimum delignification at substrate variation of 5 grams at contact time of 7 days, with a lignin removal 20,72%. The optimum glucose concentration in hydrolysis produced 7,561 g/L glucose at 2% Sulfuric acid (H_2SO_4). The highest ethanol content of 2,95% occured in ratio fungi:yeast 1:1 with substrate of 5 grams during 7 days. Based on pilot scale calculation with diameter 1,9 m and height of 0,9 m, then for 100 kg of corncobs can be produce 4,133L total ethanol.

Keyword: Bioethanol, Corncob, Dekkera Bruxellensis, Delignification, Saccharomyces Cerevisiae

1. Introduction

Corn is one of the most widely produced agricultural product in Indonesia. The high corn production can cause increase in the amount of corn waste produced. Each corn harvest produces about 70% husks and corn seeds, and 30% from corncobs [1]. The amount of corn in 2018, Indonesian state has the potential to produce 9.016.686 tons of corncobs. Corncobs are agricultural residues [2]. In Indonesia, the use of corncob waste has not implemented optimally by community so it is feared can be pollute environment.

The other side, current problem in Indonesia is the increasing consumption of energy sources causing the depletion of fuel oil reserves or fossil fuels. The use of renewable fuel is environmentally friendly. Bioethanol can be replace for 0ne of the alternative energy sources [3]. Bioethanol is a biochemical liquid from fermentation process of glucose, cellulose, and starch by utilizing the enzymatic activity of microorganism [4]. The advantage of using bioethanol it can be produced continuosly and environmentally friendly. The cost of producing bioethanol is also relatively low, because the source of the raw material is agricultural waste with low economic value [5].

Corncob are agricultural waste that contain lignocellulose and produce higher glucose concentration than other corn residues, like corn stalks or leaves [6]. The chemical properties and physical characteristics of corncob can be suitable raw materials for bioethanol production. Process of making bioethanol from corncob containing lignocellulosic substrates include delignification as a pretreatment, hydrolysis, fermentatuon, and distillation processes to separate ethanol and water [7]. Pre-treatment is an important part of converting biomass containing lignocellulose to biofuel [8].

The purpose of this reasearch is to process corncob waste into bioethanol by utlizing the activity from fungi *Dekkera Bruxellensis* and yeast *Saccharomyces Cerevisiae*. The research objectives were to determine the optimum condition in corncob delignification process in breakdown lignin using fungi *Dekkera Bruxellensis*, to determine the optimum conditions for hydrolysis process using sulfuric acid (H_2SO_4) to obtain the optimum glucose content, and to analyze the contact time in fermentation process

to obtain the most bioethanol by utilizing fungi *Dekkera Bruxellensis* and yeast *Saccharomyces Cerevisiae*

2. Methodology

2.1. Plant Material

The materials used for this reaearch were corncobs of 40 mesh powder, corncobs obtained from shelled corn waste in Taram, West Sumatera, fungi *Dekkera Bruxellensis, Saccharomyces Cerevisiae*, and growt media of Nutrient Broth (NB)

2.2. Corncob Waste Preparation

The material used as a substrate for bioethanol production is corncobs waste from agricultural waste. Corncob substrate was cut to 2-3cm in size and then dried in the sun for 2 days. Dried corncobs were sieved using 40 mesh to become corncob powder which will be used as a substrate in this study.

2.3 Cultivation of Fungi *Dekkera Bruxellensis*

Dekkera Bruxellensis fungi from collection of the Environmental Microbiology Laboratory, Department of Environmental Engineering, Trisakti University was growth in Nutrient Broth (NB) media. The growth of *Dekkera Bruxellensis* fungi was observed for 14 days and stored at room temperature until it reached the exponential phase.

2.4 Delignification Step

Delignification step to make lignocellulosic structures as to breakdown polysaccharide polymers into sugar monomers. This step is intended to determine of *Dekkera Bruxellensis* fungi in delignification process with certain contact time to produce rhe maximum starch. This process begins with insert corncob powder as a substrate and *Dekkera Bruxellensis* fungi into an erlenmeyer containing Nutrient Broth (NB) media with pH of 1,5 – 2 at temperature of 27°C - 30°C. This process was observed during contact times of 3,5,and7 days. Analysis of lignin content by gravimetric method. The percentage of lignin removal can be calculated using Equatin (1).

$$\%\text{Lignin Removal} = \frac{\%\text{Initial Lignin content} - \%\text{Final Lignin content}}{\%\text{Initial Lignin content}} \quad \ldots\ldots (1)$$

2.5 Hydrolysis Step

The hydrolysis step is carried out to break the starch polymer chains into reducing sugar unit. This step use sulfuric acid (H2SO4) with concentration 2% and contact times of 30,45,and 60 minutes and heated at 121°C temperature. Analysis using 3,5-dinitrosalicylate (DNS) method. The percentage incrase in glucose concentration can be calculated using Equation (2).

$$\text{Increased glucose concentration} = \frac{Ce\text{-}Co}{Ce} \times 100\% \quad \ldots\ldots (2)$$

Information:
Co = Concentration of glucose before hydrolysis (g/L)
Ce = Glucose concentration after hydrolysis (g/L)

2.6 Fermentation Step

The optimum results from delignification and hydrolysis processes were filtered using filter paper to reduce the residue from hydrolysis. The filtered filtrate will be fermented using fungi *Dekkera Bruxellensis* and yeast *Saccharomyces Cerevisiae* with contact times of 3,5,and7 days at 27°C - 30°C temperature. The velocity of ethanol as a fermentation product can be calculated using Eq (3).

$$v = \frac{d[P]}{t} \quad \ldots\ldots (3)$$

Information:
t = Fermentation contact time (hours)
[S] = Substate / glucose concentration (g/L)
[P] = Concentration of product / ethanol (g/L)
v = Reaction rate (g/L.jam)

2.7 Destillation Step

Destillation is used to separate water content from ethanol contained in fermentation solvent. The solvent was heated on hotplate and a series of other distillation equipment at 78°C for a certain time until final product ethanol.

2.8 Ethanol Sample Test

After obtaining the final result in form of ethanol liquid, then ethanol content and characteristics of bioethanol were tesyed using GC-MS)Gas Chromatography-Mass Spevtrophotometry) method.

3. Result and discussion

The result of preparation of corncobs to obtain a powder measuring 40 mesh as shown in Figure 1.

(a) (b) (c) (d)

Figure 1. (a) Whole corncobs; (b) 2-3 cm corncobs; (c) 0,5 – 1 corncobs; (d) corncobs powder 40 mesh

Cultivation of fungi *Dekkera Bruxelensis* was controlled at 25-30°C using shaker incubator at speed 150rpm. Exponential phase of fungsi *Dekkera Bruxellensis* as shown in Figure 2.

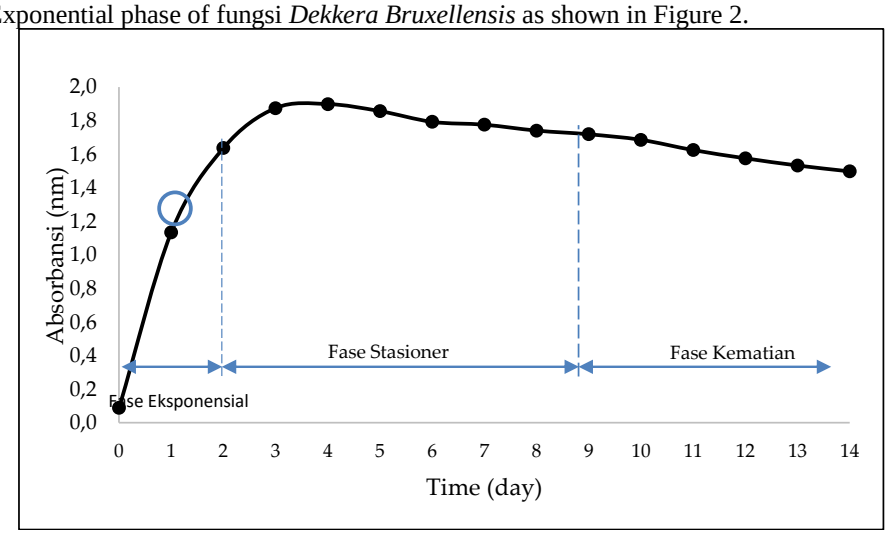

Figure 2. Fungi *Dekkera Bruxellensia* Growth Curve

Based on Figure 2. fungi *Dekkera Bruxellensis* shosws a growth period of 14 days. The growth of *Dekkera Bruxellensis* in NB media from 0 day to 2 days indicated a rapid increase in growth. The exponential phase chosen in day 1 of the growth fungi will be used in delignification and fermentatiokn step. On 10 day, the growth of fungi decreased because the research was carried out in a batch system so souce of nutrients in NB media was reduced.

First step corncob sample entered delignification step. Delignification is carried out using fungi *Dekkera Bruxellensis* because it has ability to grow in extreame conditions [9]. The highest percentage removal was obtained at 20,72% on 5 gram corncob substrate with contact time of 7 days. Yhe lowest percent removal was obtained at 1,83% occured in variation contact time of 3 days with substrate of 15 grams.

The hydrolysis process has been carried out using 2 independent variable indicators, variations corncob substrates of 5,10,15 grams and variations in contact time of 30,45,60 minutues. The result glucose concentration can be calculated using standard curve with R2 = 0,9952. The hydrolysis process was carried out using sulfur acid with concentration 2% with contact time of 30,45,60 minutes. The result showed that

the cellulose plymer will react highly after heating for 60 minutes, because at the contact time of 30 minutes glucose level formed only reached 4,230 g/L. Glucose optimum up to 7,561 g/l at 60 minute.

The decrease in glucose concentration after fermentation is shown in Table 1.

Table 1. Fermented Glucose Concentration

Substrate (gram)	Ratio 1:1								
	3 days			5 days			7 days		
	Co	Ce	Cr	Co	Ce	Cr	Co	Ce	Cr
5	4,162	3,412	18,021	4,042	2,389	40,906	5,940	1,481	75,066
10	4,407	3,516	20,218	4,842	3,334	31,154	5,107	1,717	66,375
15	7,022	6,856	2,361	6,467	4,407	31,851	7,561	2,869	62,048

Based on Table 1. It can be seen that the highest glucose removal at the contact time of 7 days. This study is using kinetic equation of the Michaelis Menten method to determine of ethanol in units of time when building a pilot scale. The delignification and hydrolysis processes of fungi has a good role as a biocatalyst. The ratio to the substrate by 1:1 in the pretreatment processs is the most suitable for delignification, as evidenced by the highest lignin removal [10]. The study proves that addition of substrate at a ratio of 1:1 in the delignification process is the most applicable when compared with others, this is because lignin removal occurred optimally at this proportion, this is accordance with previous research [11]. The advantage over other microorganism is can produce up to 2,31% ethanol in 7 days of fermentation [12]. The results of calculation of comparison of fungi and yeast are presented in Table 2.

Table 2. *Michaelis Menten* Fermentation Kinetic Calculation

Calculation	Ratio 1:1		
	5 gram	10 gram	15 gram
Slope	7,649	10,335	20,941
Intercept	0,0007	0,0004	-0,0005
$1/[v_{maks}]$	0,0007	0,0004	-0,0005
v_{maks} (mg/L.jam)	1.429	2.500	2.000
K_m (mg/L)	10.991	25.838	-41.882
Persamaan	1/v = 0,0007 + 7,649 (1/S)	1/v = 0,0004 + 10,335 (1/S)	1/v = -0,0005 + 20,941 (1/S)

Based on Table 2. The best conditions on 5 and 10 grams substrates, namely Michaelis Menten constant was positive (+) indicating that the fermentation process was moving toward product and perfectly progress. However, the product result is more on 5 gram corncob substrate.

Bioethanol has been separated from fermentation through a distillation process and analyzed using GC-MS. The result of analyzed ethanol content can be show in Figure 3.

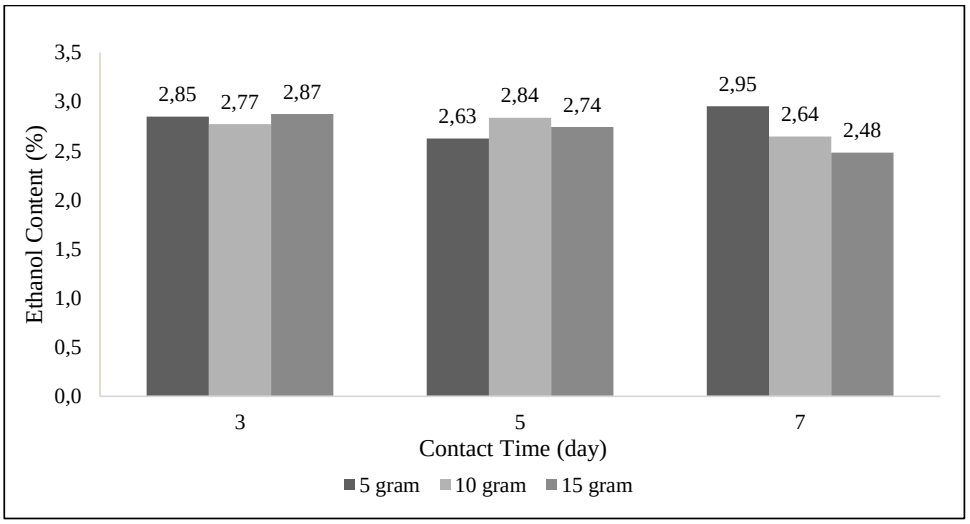

Figure 3. Ethanol Content

The result of GC-MS analysis is the highest ethanol content was obtained in 5 grams of corncobs with fermentation time of 7 days, which was 2,95%. The analysis using GC-MS produced ethanol which was not higher than the previous study, because the sample analyzed was only 10 mL. The result of this research have been found used for bioethanol as raw material for corncobs as 100 kg. The highest GC-MS results ethanol content of 2,95%.

The results of ethanol content in GC-MS analysis were convereted into volume of 0,295 mL. To produce 100.000 gram or 100 kg of biomass, approximately 3.600L of NB solution and 400 L of *Dekkera Bruxellensis* are needed. On laboratory scale, used for ethanol with 5 grams of corncob as a substrate is 250 mL. The calculation results show that 5.000.000 mL or 5.000L is needed. Generally, the industry will need 2 reactors in the system that are useful for the repair or maintanance process so it will not stop the production process. That is recapitulation of need for pilot scale bioethanol production show in Table 3.

Table 3. Recapitulation Pilot Scale

Process	**Requirement**	**Score**	**Unit**
Delignifikasi	Tongkol Jagung	100	kg
	Dekkera bruxellensis	400	L
	Nutrient Broth (NB)	3.600	L
Hidrolisis	H_2SO_4 2%	1.000	L
Fermentasi	*Dekkera bruxellensis*	200	L
	Saccharomyces cerevisiae	200	L

Table 3 describes the result of recapitulation required to convert 100 kg of corncob waste into 4.133 L of ethanol.

4. Conclusion

Based on the research, it can be concluded that corncob waste containing 47,10% lignin has potrential as raw material for ethanol. The optimum efficiency of corncob lignin removal by *Dekkera Bruxellensis* reached 20,72% with 5 grams of substrate for 7 days. Hydrolysis using 2% H_2SO_4 produced an optimum glucose level of 7,561 g/L at contact time 60 minutes. Fermentation using the *Dekkera Bruxellensis* and *Saccharomyces Cerevisiae* at ratio of 1:1 an optimum ethanol of 2,95% with substrate of 5 grams for 7 days.

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