International Journal of Technology (2015) 7: 1110-1118
ISSN 2086-9614 © IJTech 2015

EFFECT OF AERATION AND NUTRIENTS ON *Saccharomyces cerevisiae* CULTIVATION USING LIGNOCELLULOSIC HYDROLYSATE FROM EMPTY FRUIT BUNCH

Heri Hermansyah[1*], Adinda Putri Wisman[1], Diki Firdaus[1], Rita Arbianti[1], Tania Surya Utami[1], Annisa Kurnia[1]

[1] *Bioprocess Engineering, Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Kampus Baru UI Depok, Depok 16424, Indonesia*

(Received: June 2015 / Revised: September 2015 / Accepted: September 2015)

ABSTRACT

Indonesia has wide palm oil plantation which produce Empty Fruit Bunch (EFB) waste around 32 million tons per year. EFB is a potential material for bioethanol through pretreatment, saccharification, and fermentation. Fermentation has important role in bioethanol production because this process will convert glucose into ethanol. The most common microorganism used in fermentation process is *Saccharomyces cerevisiae*. But, the use of *S. cerevisiae* in bioethanol fermentation using lignocellulosic hydrolysate have a problem that microorganisms cannot grow well. This is due to the presence of inhibitor in the hydrolysate. Solution for this problem is using *S. cerevisiae* which cultivated on hydrolysate media that will be used in the fermentation (in this case EFB). This research will investigate cultivation of *S. cerevisiae* on EFB hydrolysate, to obtain the optimum operating conditions such as aeration and nutrients. Fed-batch system is used for cultivation. Optimum condition are determined after analyzing cell number and ethanol yield from dried *S. cerevisiae*. Optimum condition for cultivation are 1 v/v per min aeration and glucose 5 g/L which produce ethanol yield 24%. We also scale-up the dried yeast into 43.7 g and need a cost Rp 19,958/g which is more expensive than commercial yeast.

Keywords: Bioethanol; EFB; Hydrolysate; *Saccharomyces cerevisiae* cultivation

1. INTRODUCTION

Indonesia has wide palm oil plantation which produce Empty Fruit Bunch (EFB) waste around 32 million tons per year. Usually, EFB use for animal feed, while EFB is a potential material for bioethanol synthesis (Piarpuzan, 2011). The abundance of EFB in Indonesia and its potential as a material for bioethanol show that EFB should be investigated further as a medium for cultivation of *S. cerevisiae* to be used in the fermentation with EFB later. Hydrolysate of lignocellulose will be used for *S.cerevisiae* cultivation and bioethanol production.

Process of bioethanol production from lignocellulosic biomass includes three steps: there are pretreatment, saccharification, and fermentation. Fermentation is an important role in bioethanol production because this process will convert the glucose into ethanol. Historically, the most common type of microorganism used in fermentation process is *Saccharomyces cerevisiae*. This is because the ethanol production is quite high compared to other

* Corresponding author's email: heri.tkui@gmail.com, Tel. +62-21-7863515, Fax. +62-21-7863516
Permalink/DOI: http://dx.doi.org/10.14716/ijtech.v6i7.1225

microorganisms, reaching 18% of the fermentation broth (Lin, 2006). In addition, *S. cerevisiae* can grow in media containing simple sugars like glucose. The use of *S. cerevisiae* in bioethanol fermentation have a problem that microorganisms cannot grow well on the substrate when lignocellulosic hydrolysate is used. This is due to the presence of inhibitors in the substrate (Nilsson, 2001). A solution is found to this problem, namely by using *S. cerevisiae* which is cultivated on hydrolysate media that will be used in the fermentation. The use of *S. cerevisiae* that has been specially cultivated shows ethanol yield results better than when *S. cerevisiae* from the ordinary cultivation is used (Alkasrawi, 2006).

Cultivation of *S. cerevisiae* depends on the culture conditions, such as temperature, pH, aeration, agitation, and nutrients contained in the medium used. Temperature and pH conditions are generally the same for all strains of *S. cerevisiae*, which is at 30°C and pH 5 (Sherman, 2001). So, in this study we focus to investigate optimum condition of aeration and nutrients for *S.cerevisiae* cultivation.

Aeration is a factor that is particularly important because *S. cerevisiae* is a facultative anaerobic microorganism, which in the absence of oxygen will enter ethanol fermentation pathway. This condition is not desirable in cultivation because ethanol toxic to the microorganism. In this study, aeration rate 1 v/v per minute is used on the fed-batch cultivation of *S. cerevisiae* in a lignocellulosic hydrolyzate during 15 hours in which the biomass yield around 0.013 to 0.017 g/mL. In this study the rate of aeration also use 1 v/v per minute as well as the underlying value of 0.5 v/v per minute and 0 v/v per minute to determine their effect in batch cultivation and different medium (Petersson, 2007).

Nutrition is also a very important parameter because it is a food source for microorganisms. Without adequate nutrition, the growth of these microorganisms is inhibited and cannot produce a large number of cells in cultivation. Cultivation of *S. cerevisiae*, usually use glucose 20 g/L in liquid medium (Sherman, 2011). In this study we want to know whether adding amount of glucose to a much greater glucose concentration affects the growth of *S. cerevisiae*. Because of that, addition of glucose at 5 g/L and 10 g/L to the cultivation medium is being investigated.

Saccharomyces cerevisiae is generally use directly in wet conditions for bioethanol fermentation, but there are some shortcomings in its use, especially in terms of storage time and practicality. When *S. cerevisiae* is stored in a petri dish and put in fridge it only lasted for one year and its activity was reduced (Tanguay, 1974). As a result, rejuvenation must be done. Dry yeast have a longer shelf life and more practical to use, but most commercial dry yeast are intended for food and drink. Until now there has not been dried yeast specifically manufactured for bioethanol fermentation, primarily from lignocellulosic biomass.

Based on the background, we will investigate the effect of aeration and nutrients on *S. cerevisiae* cultivation using lignocellulosic hydrolysate from EFB to produce dry yeast. Cultivation is carried out using a batch system on fixed temperature and pH conditions. The dry yeast that we produce will be used in fermentation process of EFB hydrolysate to produce ethanol. From this research, we can get optimum operating conditions of aeration and nutrients, cultivation time, *S. cerevisiae* activity and fermentation performance.

2. EXPERIMENTAL

2.1. Materials

The main raw material in this study is the EFB that has been pre-treatment and obtained from LIPI Chemistry Serpong. *Saccharomyces cerevisiae* UICC Y-17 was obtained from the Department of Biological Science, Universitas Indonesia. The enzyme cellulase and β-glucosidase used for hydrolysis is Cellic Tac (Novozyme) and Onozuka RS (Yakult). Other

supporting materials, such as sodium hydroxide, citric acid, glucose, and potato dextrose agar are purchased from Sigma Aldrich, whereas skim milk was purchased at a local supermarket.

2.2. Inoculation Media Preparation

Preparation of inoculation media uses hydrolyzed EFB that have been pre-treated and hydrolysis using cellulase and β-glucosidase at 50°C and pH 5 with 100 rpm agitation for 72 h. Acidity is controlled using citric acid buffer. EFB consist 10% of the total solution from dry weight. Requirement of cellulase enzyme in cultivation calculated using Equation 1, while the ratio of the enzyme cellulase and β-glucosidase was 4:1. The results of hydrolysis then filtered using filter paper and heated to 90°C for 15 min before autoclaving at 121°C and pressure of 1 atm.

$$\text{Cellulase [mL]} = \text{Total EFB mass [g]} \times (20 / \text{FPU enzyme}) \quad (1)$$

2.3. Cultivation dan Parameter Optimization

Cultivation of *Saccharomyces cerevisiae* is performed in a 250-ml Erlenmeyer at 30°C for 24 h with additional variations of the glucose 0, 5, and 10 g/L; and aeration 0, 0.5, and 1 v/v per min. Variations can be seen in Table 1.

Table 1 Parameter Variations

Code	Aeration (v/v per min)	Glucose addition (g/L)	Yeast
A	1.0	0	Cultivation
B		5	
C		10	
D	0.5	0	Cultivation
E		5	
F	0.0	0	Cultivation
F*	-	-	Commercial

The addition of glucose before cultivation is done. Aeration rate settings using a flowmeter which connected to the pump so air enter the flask which containing the culture. A total of six experiments were performed. Every one hour 10 µL sample was taken to see the number of cells, using a Neubauer Improved counting chamber and calculation shown in Equation 2.

$$\text{Cell concentration} = \text{number of cells} / \text{volume [mL]} \quad (2)$$

2.4. *Saccharomyces cerevisiae* Separation and Drying

Separation is done by centrifugation at a speed of 5000 rpm for 20 min to separate the *Saccharomyces cerevisiae* after cultivation. It is then soaked in skim milk for 2 h and then dried using a spray dryer with an inlet temperature of 130°C. Drying is done in LIPI Biology Cibinong.

2.5. Bioethanol Production Trial

Dried *Saccharomyces cerevisiae* is used for bioethanol production trials. A total of 0.01 g of dried *Saccharomyces cerevisiae* are put in 30 mL of EFB hydrolysate and fermented at 37°C and pH 4 for 24 h with agitation rates of 100 rpm. Commercial dry yeast fermentation is

conducted for comparison. Fermentation results are then analyzed using HPLC with Aminex HPX-87P column at LIPI Chemistry Serpong.

2.6. Production Scale-up of Dried *Saccharomyces cerevisiae*

Using the best result from previous steps, a total volume of 10 L culture is obtained. Separation and drying of Saccharomyces uses the same methods as previous steps.

3. RESULTS AND DISCUSSION

3.1. Empty Fruit Bunch Hydrolysis

Product of EFB hydrolysis is hydrolysate EFB which will be used for *S. cerevisiae* cultivation and fermentation process. Glucose concentration in hydrolysate of EFB was calculated using DNS method. From this method, we get absorbance unit (AU) from spectrophotometer, then we convert AU into glucose concentration (g/mL) based on calibration curve (Figure 1). From calibration curve, we get glucose concentration of hydrolysate from EFB cultivation process is approximately 40 g/mL. In addition, we also analysis glucose concentration of hydrolysate from the EFB during fermentation process using DNS method and glucose concentration is 14.7 g/mL.

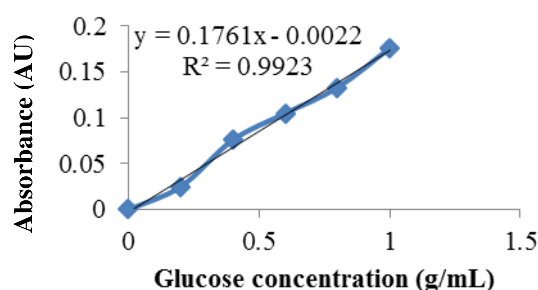

Figure 1 Callibration Curve for DNS Test

3.2. Cultivation Parameter Optimization

There are six variations (Tables 2 and 3) that we investigated. Table 2 shows the effect of nutrients on *S.cerevisiae* cultivation with constant aeration (1 v/v per min). Aeration rate 1 v/v per min is constant variable because previous study (Petterson, 2007) shows that optimum aeration rate on the fed-batch cultivation of *S. cerevisiae* in a lignocellulosic hydrolysate is 1 v/v per min. Besides, Table 3 shows the efect of aeration on *S. cerevisiae* cultivation with constant nutrients (0 g/mL).

Table 2 Cultivation comparison with glucose addition variation

Glucose Addition (g/L)	Cell Concentration after 24 hour (10^7 cell/mL)	Time to reach Peak (h)
0	4.89	19
5	1.91	15
10	2.52	20

Table 3 Cultivation comparison with aeration variation

Aeration (v/v per minute)	Cell Concentration after 24 hour (cell/mL)	Time to reach Peak (h)
0	3.66×10^{6}	21
0.5	1.95×10^{8}	21
1	4.89×10^{7}	19

Figure 2 shows that the addition of glucose actually cause a decrease in the number of cells generated from cultivation. High concentration of glucose contributes to the osmotic pressure of the cell. Similar results are found by Logothetis et al. in 2007 that high concentration glucose in cultivation can cause osmotic pressure and make cell lysis. In addition, cultivation without addition of glucose can reach peak at 15 hours, this is faster than high concentration glucose. High concentration glucose will make *S. cerevisiae* cell need a long time to adapt with hydrolysate and high glucose concentration also contributes to the osmotic pressure of the cell. Then, with the addition of 10 g/L glucose can reach peak about 20 hours. The end result is fewer cells with increasing glucose is presumably because time to reach peak is too long, so *S. cerevisiae* cells not reach maximum growth after 24 h.

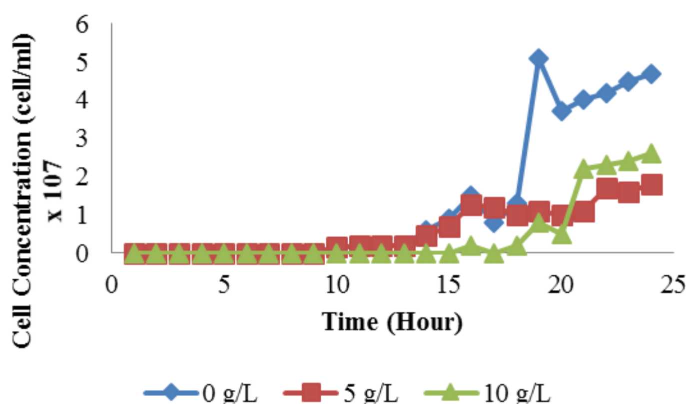

Figure 2 Effect of glucose addition on growth of *S. cerevisiae* on aeration rate of 1 v/v per Minute

Effect of aeration on the cultivation can be seen in the curves in Figure 3. In this experiment, nutrient is constant variable. We don't add glucose in hydrolysate EFB (without glucose addition = 0 g/L). Figure 3 show that aeration at 0.5 v/v per min gives better results in terms of the number of cells produced after 24 h than 1 v/v per min. While, the aeration 1 v/v per min give faster peak than others because higher aeration rate will give faster peak, but also faster stationary phase too. Then all of cultivation results will go to the next steps, separation and drying process to produce dried yeast. After that, we can investigate yeast activity in fermentation process to get the optimum condition.

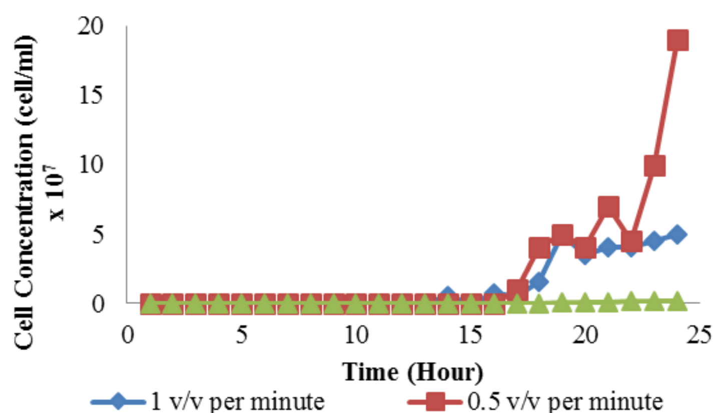

Figure 3 Effect of aeration rate on growth of *S. cerevisiae* without addition of glucose

3.3. Purification of *Saccharomyces cerevisiae*

Wet weight of *S. cerevisiae* from each experiment are listed in Table 4. Experiments without aeration (Sample F) did not have good results because it is difficult to separate so this sample is eliminated in the next stage. Then, *S. cerevisiae* are dried with a skim milk additive. The results can be seen in Table 5.

Table 4 *S. cerevisiae* wet weight

Aeration (v/v per min)	Glucose Addition (g/L)	*S. cerevisiae* wet weight (g)	Code
1	0	1,0381	A
	5	0,0463	B
	10	0,1346	C
0,5	0	0,4051	D
	5	0,5777	E

Table 5 Spray drying results

Aeration (v/v per min)	Glucose Addition (g/L)	Dried yeast (g)	Code
1	0	0.2225	A
	5	0.0398	B
	10	0.1145	C
0,5	0	0.1948	D
	5	0.1144	E

3.4. Bioethanol Production

The test results of fermentation using dried *S. cerevisiae* are compared with commercial dry yeast which can be seen in Table 6.

Table 6 Consumed glucose and ethanol yield

Code	Glucose consumed (g/mL)	Ethanol concentration (g/mL)	Ethanol Yield** (%)
A	6.52	0.3824	4
B	1.58	0.2462	24
C	1.08	0.4124	38
D	2.78	0.3369	12
E	5.05	0.2203	4
F*	4.88	0.2847	6

*Commercial dry yeast
** Ethanol Yield is the ratio of the amount of ethanol formed per amount of sugar consumed

From these data it can be seen that the dried yeast was able to ferment ethanol with a pretty good result. This suggests that the drying process managed to maintain the viability of yeast. Sample A and E both shows great sugar consumption but very little ethanol yield. This is presumably because the cells in the sample consume glucose to reproduce themselves within 24 h. The use of commercial yeast (Sample F*) to produce ethanol has lower yield than samples B, C, D. This is consistent with the theory that the yeast grown on lignocellulosic hydrolysate will give better result on the fermentation process using lignocellulosic hydrolysate. It also prove that commercial yeast growth was inhibited by inhibitors found in lignocellulose hydrolysates that they can not ferment ethanol well. Comparison for all results can be seen in Table 7.

Table 7 Overall study result

Code	Variation		Cell concentration (cell/mL)	Time to reach peak (h)	Dried yeast (g)	Ethanol yield ** (%)
	Aeration (v/v per min)	Glucose addition (g/mL)				
A	1	0	4.89×10^{7}	19	0.22	4
B		5	1.91×10^{7}	15	0.04	24
C		10	2.52×10^{7}	20	0.12	38
D	0.5	0	1.95×10^{8}	21	0.19	12
E		5	2.66×10^{7}	19	0.11	4
F	0	0	3.66×10^{6}	21	-	-
F*	-	-	-	-	-	6

*Commercial dry yeast
** Ethanol Yield is ratio of the amount of ethanol formed per amount of sugar consumed

From overall data, can be seen that average sample C, which cultivated with aeration 1 v/v per minute and the addition of glucose as much as 10 g/L, looks superior, but the yield of ethanol produced compared to the number of cells are fewer than sample that were cultivated with aeration 1 v/v per min and extra glucose 5 g/L. Seen in terms of the number of cells produced, sample B has a smaller number but can produce ethanol yield large enough to make sample sample B is most superior of all samples.

3.5. Production Scale-up of Dried *Sacchromyces cerevisiae*

A total volume of 10 L culture is used for this scale up. After processing, around 43.7 g of dried *Saccharomyces cerevisiae* is obtained. Production cost analysis are explained in sub chapter below.

3.5.1. Comparison of production cost

The cost for produce dried *Saccharomyces cerevisiae* in this study are compared with commercial dry yeast that can be found in the market. This commercial yeast was produced for the purpose of food (bread) and manufactured using molasses and dried using a fluidized bed dryer (Bekatorou, 2006). Price for per gram of dry yeast is commercially Rp 35,000 per 44 g, or roughly Rp 795 per g. Production cost of dried *Saccharomyces cerevisiae* made in this study is of Rp 19,958 per g (Table 8).

From the Table 8, production cost of dried *Saccharomyces cerevisiae* in this study is much more expensive than commercial yeast. The price is much higher due to the raw materials derived from lignocellulosic materials media which must pre-treatment prior to use with enzymes. In this study, enzyme activity is fairly high at around 16,000 U/g so it has a expensive price. Using a crude enzyme may suppress production costs. One of the other factors that influences production costs is the cost for rent a spray dryer. it would be better to buy equipment (spray dryer) as an investment, so that future production costs can be reduced.

Table 8 Production cost

Cost Component	Name	Mass (g)	Cost (IDR)	Cost per Batch (IDR)
Raw Materials for Cultivation Media	Glucose	100	800	80,000
	Citric acid	2.7	50	135
	Cellulase	0.43	425,000	182,750
	β-glucosidase	0.1	250,000	25,000
	Aquades	10000	5	50.000
Spray drying addictive	Skim Milk	500	20	10.000
Equipment	Plastic Wrap	1	25,000	25,000
	Alumunium Foil	1	30,000	30,000
	Erlenmeyer Flask	3	85,000	255,000
	Tube	2	5,000	10,000
Equipment Rent	Spray drying	1	300,000	300,000
Transportation	LIPI Cibinong	2	15,000	30,000
Production cost of 50 gram dried *S. cerevisiae*				997,885
Cost per gram				19,958

4. CONCLUSION

Optimum operating conditions for cultivation are aeration rate 1 v/v per min and glucose concentration is 5 g/L, which produce ethanol yield 24% from 0.04 g dried yeast. Concentration of glucose in the hydrolyzate for cultivation process reached 40 g/mL. Under this conditions, large aeration rate is favorable for cell growth and lower glucose concentration do not actually cause inhibition of cell growth. But, production costs of dry *Saccharomyces cerevisiae* is Rp 19,958/g which is more exspensive than commercial yeast.

5. ACKNOWLEDGEMENT

This research was supported by grants from Direktorat Jenderal DIKTI and DRPM Universitas Indonesia.

6. REFERENCES

Alkasrawi, M., Rudolf, A., Liden, G., Zacchi, G., 2006. Influence of Strain and Cultivation Procedure on the Performance of Simultaneous Saccharification and Fermentation of Steam Pretreated Spruce. *Enzyme Microbial Technol.*, Volume 38, pp. 279–286

Lin, Y., Tanaka, S., 2006. Ethanol Fermentation from Biomass Resources: Current State and Prospects. *Applied Microbiol Biotechnol.*, Volume 68, pp. 627−642

Logothetis, S., Walker, G., Nerantzis, E.T., 2007. Effect of Salt Hyperosmotic Stress on Yeast Cell Viability. *Natural and Science*, Volume 113, pp. 271−284

Nilsson, A., Taherzadeh, M.J., Liden, G., 2001. Use of Dynamic Step Response for Control of Fed-batch Conversion of Lignocellulosic Hydrolyzates to Ethanol. *J Biotechnol.*, Volume 89, pp. 41−53

Piarpuzan, D., Quintero, J.A., Cardona, C.A., 2011. Empty Fruit Bunches from Oil Palm as Potential Raw Material for Fuel Ethanol Production. *Biomass and Bioenergy*, Volume 35, pp. 1130−1137

Petersson, A., Liden, G., 2007. Fed-Batch Cultivation of *Saccharomyces cerevisiae* on Lignocellulosic Hydrolyzate. *Biotechnol Lett.*, Volume 29, pp. 219–225

Sherman, F., 2001. Getting Started with Yeast. *Methods Enzymol*, Volume 350, pp. 3–41

Tanguay, A.E., Bogert, A.B., 1974. Survival of *Saccharomyces cerevisiae* and *Sarcina lutea* at Refrigerator Temperatures. *Applied Microbiology*, Volume 27(6), pp. 1175−1176