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Optimization of Simultaneous Saccharification and Fermentation Incubation Time Using Cellulose Enzyme for Sugarcane Bagasse on The Second-Generation Bioethanol Production Technology

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Abstract

Bioethanol production using lignocellulosic materials (the second-generation technology) was consist of pretreatment and simultaneous saccharification and fermentation (SSF) process. This research optimize the SSF incubation time using cellulose enzyme and *Saccharomyces cerevisiae* combination for sugarcane bagasse. The bagasse was pre-treated by delignification with NaOH. The SSF was conducted by *Sacharomyches cereviceae*, cellulose enzymes (*Trichoderma reesei*) and nutrients combination during 3 d, 5 d and 7 d as incubation time variables. The optimum result was 0.748 5 % of ethanol concentration or dry weight conversion by 11.810 5 g · L^{-1} which obtained after 5 d incubation time based on ethanol conversion, microbiology test and sugar reduction concentration.

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Keywords: Bioethanol; cellulose enzyme; incubaton time; lignocellulose; simultaneous saccharification and fermentation; sugarcane bagasse

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Nomenclature

SSF	simultaneous saccharification and fermentation
TPC	total plate count
OD	optical density
GC	gas chromatography
min	minute
h	hour
L	liter
t	ton = 10^3 kg
yr	year
d	day = 24 h

1. Introduction

There are three kinds of materials which is used in the bioethanol production by fermentation, namely sugary materials (such as sugar cane, sugar beet, molasses and liquid fruit), starchy materials (such as grains and potatoes), and cellulosic materials (such as wood) [1,2]. Bioethanol production based on lignocellulosic materials was known as the second-generation technology such as sugarcane bagasse which is widely available as part of the sugar factory waste. The sugar factory, PT Madubaru-Madukismo, at Bantul – Yogyakarta was the biggest sugarcane industry around Yogyakarta district. It needs 350 000 t · y^{-1} to 400 000 t · y^{-1} of sugarcane to produce sugar regularly. The sugarcane production results 5 % sugar, 90 % bagasse cane waste, molase (sugar sludge waste) and water [3]. Sugarcane bagasse is one of the lignocellulosic materials potentials because of the compound composition of bagasse cane waste were cellulose (52 %), hemicellulose (20 %) and lignin (24 %) [4].

The second generation technology of bioethanol production consist of two main processes, which are hydrolysis of cellulosic and hemicellulosic to deliver reducing sugar and sugar fermentation into ethanol [5]. After pretreatment, which removing lignin and enhancing cellulose fraction on biomass, saccharification and fermentation are able to conduct at one flask that called simultaneous saccharification and fermentation (SSF) [6]. The advantage of this method is converting monosaccharide into ethanol simultaneously, reducing equipment cost and reducing contamination [7].

Based on previous research, SSF process of sugarcane bagasse resulted ethanol at 3.249 g · L^{-1} with xylane enzyme [8] and 21.372 4 g · L^{-1} with cellulose enzyme [9]. Some variables could be optimized in the SSF process, such as enzyme, temperature, yeast, nutrient, composition, incubation time, etc. One of them was incubation time with combination of SSF components which is conducted in this research. Based on previous results, the optimum incubation time of SSF are 3 d [8] and 7 d [4] using combination xylane enzyme and *Saccharomyces cerevisiae*. The different combination in the SSF components was able to decrease or increase optimum incubation time. The aim of this research was to optimize the incubation time of SSF using combination of cellulose enzyme and *Saccharomyces cerevisiae* for sugarcane bagasse. In this research, another variables such as nutrient, pH, temperature, etc were decided as fixed variables based on previous results [9].

2. Material and method

2.1. Material preparation

Fresh sugarcane bagasse were obtained from PT Madubaru-Madukismo, the sugarcane industry, Bantul, Yogyakarta. Sugarcane bagasse were delignificated by refluxing and heating on 1 N NaOH for 2 h.

2.2. Simultaneous saccharification and fermentation (SSF)

Medium for SSF as much as 20 mL consists of delignificated bagasse samples (1 g); nutrients $(NH_4)_2HPO_4$ (3.44 mL), $MgSO_4.7H2O$ (0.17 mL), yeast extract (6.88 mL); citrate buffer (pH 5.0); cellulose enzymes (20 fpu) and 25 % ($v \cdot v^{-1}$) of yeast *Saccharomyces cerevisiae*. SSF was conducted on ambient temperature and pressure with three variables incubation period of 3 d, 5 d and 7 d.

2.3. Analysis of the SSF results

Total Plate Count (TPC), Optical Density (OD) and sugar reduction concentration analysis were conducted on pre and post incubation. TPC was determined using the facilities of microbiology laboratory at UPT BPPTK LIPI. OD determined using UV-VIS spectrophotometer at UPT BPPTK LIPI on 660 nm wavelength. Sugar reduction concentration was taken with Nelson Methods using UV-Visible spectrophotometer on 540 nm wavelength [4]. Ethanol concentration was determined using Gas Chromatography (GC) type HP 5890 at Mathematics-Chemistry Laboratory-UGM Yogyakarta.

3. Result and discussion

Sugarcane bagasse as raw material of bioethanol production has been calculated sugar reduction concentration of 0.003 mg · mL^{-1}, so the sugar fermentation process which converted into alcohol can be ignored because it is very small [4]. Before the SSF, sugarcane bagasse pre-treatment with chemical method delignification using NaOH was conducted. It resulted the material for SSF with lignin content on 6.940 %, hemicellulose content on 9.246 % and cellulose content on 78.184 % [9]. The SSF process with incubation time variables were shown on Figure 1 and the results of this process were shown on Table 1.

(a) (b)

Fig 1. (a) The SSF process of sugarcane; (b) The ethanol of SSF results

Table 1. The results of SSF with incubation time variables

No	Incubation time	Ethanol content (%)	Ethanol dry weight conversion (g · L^{-1})	TPC (10^6)	OD on 660 nm	Sugar reduction concentration (mg · mL^{-1})
1	0 d	0.000 0	0.000 0	5	1.592 0	0.253 9
2	3 d	0.533 6	8.420 2	840	1.682 0	0.376 3
3	5 d	0.748 5	11.810 5	3 710	1.715 5	0.441 8
4	7 d	0.253 5	4.000 2	1 750	2.387 0	0.373 2

In the SSF process, cellulose of sugarcane bagasse was converted into glucose by cellulose enzymes then glucose was converted into ethanol by *Saccharomyces cerevisiae* simultaneously. The GC charts of ethanol content as the SSF results was shown in Figure 2. It can be seen that the ethanol peak appears at about 2.5 min to 2.7 min. The peak appears at about 2.7 min to 2.9 min is propanol peak, which is used as control in the analysis. The ethanol dry weight conversion results of three incubation time variables was described in a graph, which is shown in Figure 3. Figure 3 shows that the incubation time variables have maximum ethanol content between 4 d to 5 d incubation time.

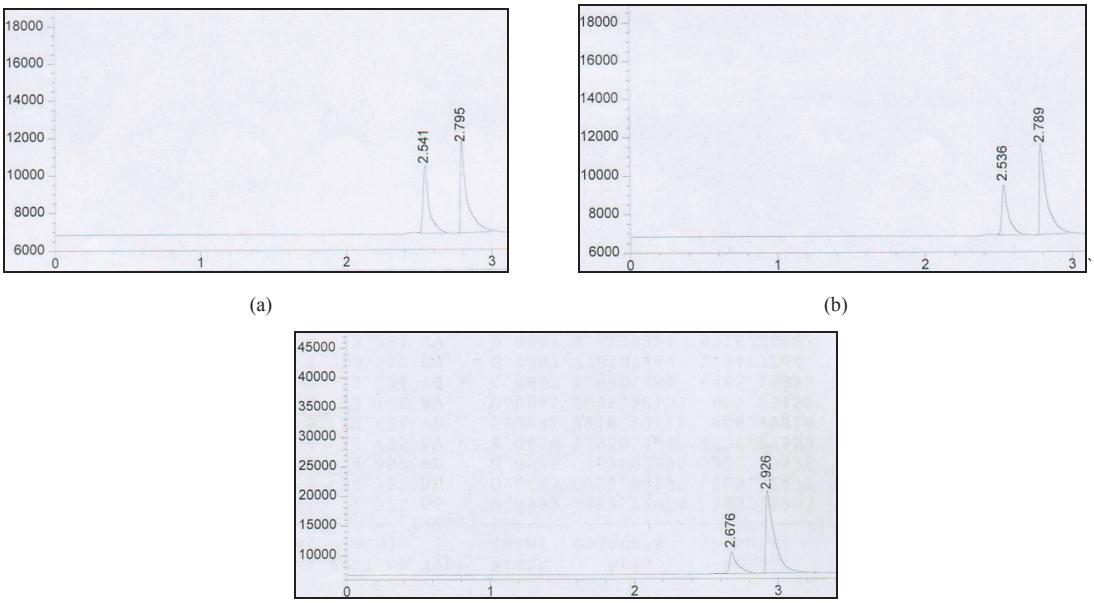


Fig 2. GC analysis result of SSF with incubation time variables : (a) 3 d; (b) 5 d; (c) 7 d

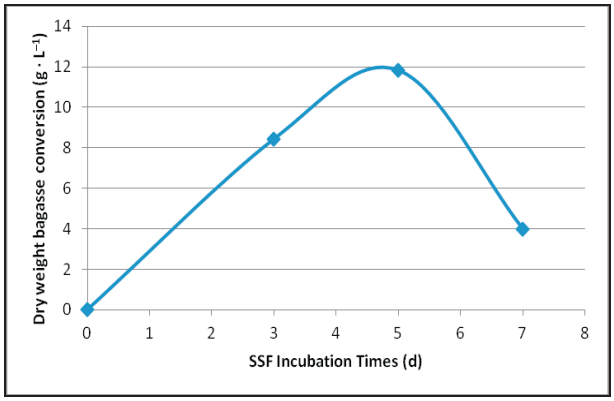
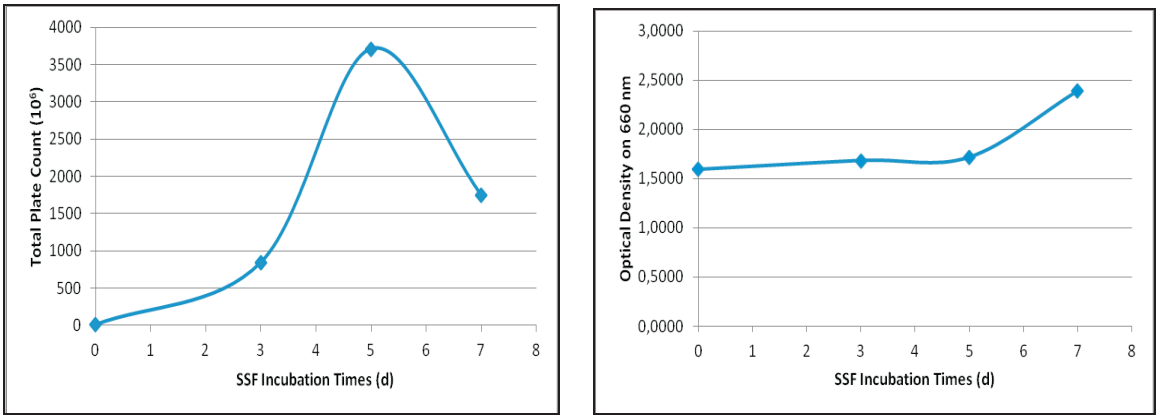


Fig. 3. Graphic of SSF results with incubation time variable

The optimum ethanol content results were supported by the data of yeast activity (TPC and OD) and sugar reduction concentration which conducted on pre and post SSF incubation. In this process, yeast activity was

determined with TPC and OD which showed on Figure 4. The TPC graphic has similarity with standar graphic of yeast activity [10,11] consisting of log phase, stationary phase and lag phase. Based on the TPC graphic, the log phase was estimated on 3 d to 5 d incubation time, the lag phase on 5 d to 7 d and the stationary phase on 4 d to 6 d incubation time. The OD graphic showed that the number of yeast increases with incubation time. Although on 5 d to 7 d incubation time showed an increased yeast number, but did not show increased yeast activity because the inactive yeast was also calculated in the OD graphic. Based on the combination of TPC and OD results, the optimum results of yeast activity was obtained on 4 d to 6 d incubation time.



(a) (b)

Fig 4. Graphic of *Saccharomyces cerevisiae* activity : (a) Total plate count; (b) Optical density

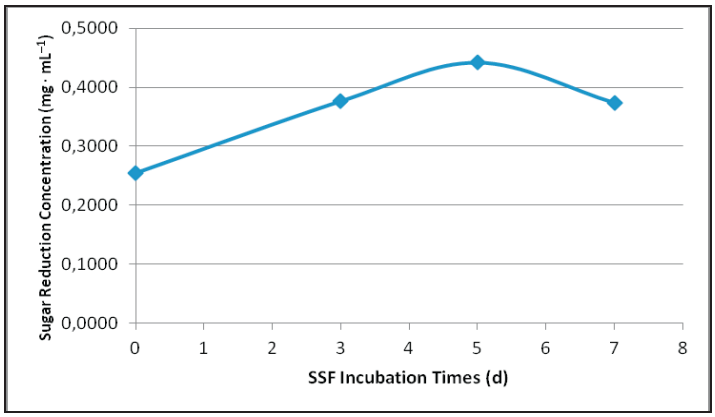


Fig 5. Graphic of sugar reduction concentration

The results of sugar reduction concentration was shown in Figure 5. Sugar reduction concentration increased with incubation time until 5 d incubation time then it decreased. The maximum sugar reduction concentration was obtained on 5 d incubation time. It means that before 5 d incubation time, cellulose enzyme converted into glucose and balanced with the yeast performance in converting glucose into alcohol. However, after 5 d incubation time, cellulose enzyme was not working optimally which is showed by the decrease in the sugar reduction concentration. Whereas, at the same time the number of active yeast decreased which is shown by the graph of TPC and OD. It

means the amount of glucose converting into alcohol was reduced. Therefore, the SSF process would be more effective if carried out up to 5 d incubation time when yeast and cellulose enzyme in optimum condition.

4. Conclusion

Optimal results for incubation time of SSF using combination of cellulose enzyme and *Saccharomyces cerevisiae* for sugarcane bagasse were obtained on 5 d by 0.748 5 % ethanol concentration or conversion of dry weight by 11.810 5 g · L^{-1}.

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References

[1] Sastramihardja U. Pengantar mikrobiologi umum [Introduction of general microbiology]. Bandung : ITB-Press; 1985 [Bahasa Indonesia]

[2] Maryana R. Kajian : Pengembangan bioetanol dari starchy materials dan lignoselulosa sebagai salah satu energi alternatif [Review: Bioethanol development from starchy material and lignocellulosic material as alternative energy]. In : Proceeding of Chemistry and Chemistry Education Seminar at Semarang State University. Semarang. November 11th, 2006 [Bahasa Indonesia]

[3] Wahono SK, Kismurtono M, Rosyida VT, et al. Pengembangan produksi bioetanol berbahan baku biomassa [Bioethanol production development based on biomass]. Technical Research Report of UPT BPPTK LIPI. Yogyakarta; 2012 [Bahasa Indonesia]

[4] Wahono SK, Rosyida VT, Afrizal A, Kismurtono M. Residual sugar reduction concentration parameter at the product of simultaneous saccharification and fermentation process of second-generation bioethanol from bagasse cane. In : Proceeding of International Conference on Sustainable Engineering and Aplication. Yogyakarta. November 6th-8th, 2012. p. 27-30.

[5] Sarrouh BF, Silva SS, Santos DT. Technical/economical evaluation of sugarcane bagasse hydrolysis for bioethanol production. Chem Eng Technol. vol. 30. no. 2; 2007: 270-275

[6] Gauss, WE, Suzuki S, Takagi M. Manufacture of alcohol from cellulosic material using plural fermenters. U.S. Pat. 3,990,94 gradation of lignin 4.USA. 1976.

[7] Darsih C, Rosyida VT, Wahono SK. Bagasse delignification effect on reducing sugar processes using simultoneous saccharification and fermentation. In : Proceeding of 2nd Basic Science International Conference at Brawijaya University. Malang. February 2012; 2012: Ch1-Ch5

[8] Samsuri M, Gozan M, Mardias R, et al. Pemanfaataan selulosa bagas untuk produksi ethanol melalui sakarifikasi dan fermentasi serentak dengan enzim xylanase [Utilization of cellulosic bagasse for the production of ethanol via simultaneous saccharification and fermentation with xylane enzyme]. Makara, Technology Journal. Vol. 11. No. 1. April 2007; 2007: 17-24 [Bahasa Indonesia]

[9] Wahono SK, Darsih C, Rosyida VT, Maryana R, Pratiwi D. Optimization of cellulose enzyme in the simultaneous saccharification and fermentation of sugarcane bagasse on the second-generation bioethanol production technology. In : Praptiningsih GA, Anggi N, Agus SY, Andi S, editors. Conf. and Exhibition Indonesia Renewable Energy & Energy Conservation 2013. Energy Procedia – Elsevier. Vol 47. 2014 : 268-272

[10] Wahono SK, Damayanti E, Rosyida VT, Sadyastuti EI. Laju pertumbuhan *Saccharomyces cerevisiae* pada proses fermentasi pembentukan bioetanol dari biji sorgum (*Sorghum bicolor* L.) [Growth rate of *Saccharomyces cerevisiae* at bioethanol production from fermentation process of seed sorghum (*Sorghum bicolor* L.)]. In : Proceeding of National Seminar on Chemical Engineering and Process at Diponegoro University. Semarang. July 26th, 2011. p. D04 1-D04 6 [Bahasa Indonesia]

[11] Fardias S. Fisiologi fermentasi [Phisiology of fermentation]. Bogor : Lembaga Sumber Daya Informasi – IPB; 1988 [Bahasa Indonesia]