

Available online at www.sciencedirect.com**ScienceDirect**

Energy Procedia 47 (2014) 268 – 272

Energy

Procedia

Conference and Exhibition Indonesia Renewable Energy & Energy Conservation
[Indonesia EBTKE CONEX 2013]

Optimization of Cellulose Enzyme in the Simultaneous Saccharification and Fermentation of Sugarcane Bagasse on the Second-Generation Bioethanol Production Technology

Satriyo Krido Wahono^{a,*}, Cici Darsih^a, Vita T. Rosyida^a, Roni Maryana^a, Diah Pratiwi^a

^aTechnical Implementation Unit for Development of Chemical Engineering Processes – Indonesian Institutes of Sciences
Jl. Jogja – Wonosari km 32, Gading, Playen, Gunungkidul, Yogyakarta, 55861, Indonesia

Abstract

Sugarcane bagasse as raw material were treated on chemical delignification with NaOH and used for Simultaneous Saccharification and Fermentation (SSF). SSF was incubated during 5 days by adding *Sacharomyces cereviceae*, cellulose enzymes (*Trichoderma reesei*) and nutrients. The variable of this research was the cellulose enzyme variation of 10-60 fpu with 10 fpu on intervals. Optimal results were obtained on 60 fpu which producing ethanol content of 1.3544% or dry weight conversion of 21.3724 g/L with potential prediction shaped on logarithmic equation. In the advanced prediction can be obtained approximate optimal value of enzyme contents on 96.6802 fpu.

© 2014 The Authors. Published by Elsevier Ltd.

Selection and peer-review under responsibility of the Scientific Committee of Indonesia EBTKE Conex 2013

Keywords: sugarcane bagasse; lignocellulose; simultaneous saccharification and fermentation; cellulose enzyme; bioethanol

1. Introduction

Population growth results increasing in energy consumption. For many years, people depend on fossil energy which is non-renewable resource and polluters. The increase in energy consumption is not proportional with supply in the world. Those problems encourage scientist to find out new energy resource to be an alternative energy, such as bioethanol for gasoline replacement.

* Corresponding author. Tel.: +62-274-392570; fax: +62-274-391168.

E-mail address: dna_tqim@yahoo.com, satriyo.krido.wahono@gmail.com, satr002@lipi.go.id

Bioethanol manufacturing technology develop more rapidly at this time. One of them is the second-generation bioethanol production technology based on lignocellulosic materials. Sugarcane bagasse is one of the lignocellulosic materials potential and widely available as part of the sugar factory waste. This technology was cheaply on production cost and safely on environment because using biomass waste such as bagasse sugarcane or palm oil empty fruit bunch [1]. The huge availability of biomass waste were new potential of energy resource using physical, chemical or biological conversion [2].

Sugar Factory, PT Madubaru, at Bantul – Yogyakarta was the biggest sugarcane industry around Yogyakarta district. They produce huge solid waste, one of them is bagasse cane. They need 350,000 – 400,000 tons per year sugarcane to produce sugar regularly. The sugarcane production results 5% sugar, 90% bagasse cane waste, molase (sugar sludge waste) and water. Sugarcane bagasse is one of lignocellulosic materials potentials due to the compound composition of bagasse cane waste were cellulose (52%), hemicellulose (20%) and lignin (24%) [3]. Lignocellulosic resources contain lignin, cellulose, and hemicellulose [4, 5, 6]. Lignin is complex molecule which constructed of phenylpropane and methoxy groups and a non-carbohydrate polyphenolic substance [5, 7]. Two main processes were involved in the production of bioethanol from lignocellulosic residues are the hydrolysis of cellulose and hemicellulosic to deliver reducing sugar and sugar fermentation into ethanol [8].

Lignin protect the cellulose and hemicellulose from enzymatic hidrolysis [9], so the pretreatment on lignocellulosic is important. Pretreatment will remove lignin and enhance cellulose fraction on biomass. There are many methods such as chemical, physical, and biological to pre-treat lignocellulosic. Furthermore, saccharification and fermentation are conducted at one flask that called simultaneous saccharification and fermentation (SSF). The advantage of this method are monosaccharida can convert to be ethanol simultaneously and reduce equipment cost. Idea of SSF declared by Gauss et al. in the patent at 1976 [10]. Based on previous research, SSF process of sugarcane bagasse resulted the highest ethanol at 3,249 g/L or 5.6 % per mass of bagasse with 1% (v/v) addition of hydrochloric acid [1]. The purpose of this research was to determine optimum value of cellulose enzyme in the simultaneous saccharification and fermentation of sugarcane bagasse.

2. Material and method

2.1. Material preparation

Fresh sugarcane bagasse was got from PT Madubaru, a sugar industry, Bantul, Yogyakarta. Sugarcane bagasse were delignificated chemically with 1N NaOH on 2 hours reflux and heating

2.2. Simultaneous saccharification and fermentation (SSF)

Medium for SSF as much as 20 ml consisting of delignificated bagasse samples (1 gram); nutrients $(\text{NH}_4)_2\text{HPO}_4$ (3.44 ml), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.17 ml), yeast extract (6.88 ml); citrate buffer (pH 5.0); cellulose enzymes (10-60 fpu) and 25% (v/v) of yeast *Saccharomyces cerevisiae*. SSF conducted with an incubation period of 5 days.

2.3. Analysis of ethanol concentration

Ethanol content was determined using Gas Chromatography (GC) type HP 5890 at Mathematics-Chemistry Laboratory-Gadjah Mada University Yogyakarta

3. Result and discussion

Sugarcane bagasse as raw material of bioethanol production has been calculated sugar reduction concentration of 0.003 mg/ml, so the sugar fermentation process which converted into alcohol can be ignored due to its very small. Before the SSF, sugarcane bagasse pre-treatment with chemical method delignification using NaOH was conducted. Contents of cellulose, hemicellulose and lignin were determined by the Chesson method [11]. Delignification results of sugarcane bagasse with chemical methods was shown in Table 1.

Table 1. Delignification results of sugarcane bagasse.

Type of sugarcane bagasse	Lignin (%)	Cellulose (%)	Hemicellulose (%)
Raw material	24.778	46.893	18.468
Chemical delignification	6.940	78.184	9.246

Based on Table 1, it was known that lignin content reduction of 17.838% and hemicellulose content of 9.222%. It was happened due to lignin and hemicellulose were dissolved in NaOH solution. On the other hand, cellulose content increases on 31.291%. This escalation occurs due to cellulose undissolved in the NaOH solution, so that the fiber component of the total mass bagasse is dominated by cellulose.

Bagasse delignification results were used as raw materials of the SSF process. SSF process was conducted in 20 ml tube. Each tube contains 1 gram of sugarcane bagasse, yeast (*Sacharomyces cereviceae*), variation of cellulosic enzyme based on *Tricoderma riisei* (10, 20, 30, 40, 50, 60 fpu) and nutrients. SSF process was conducted for 5 days and the results was shown in Figure 1 and 2. The harvest contents of ethanol were determined using Gas Chromatography (GC). Chart of the highest yield analysis (60 fpu) is shown in Figure 2.

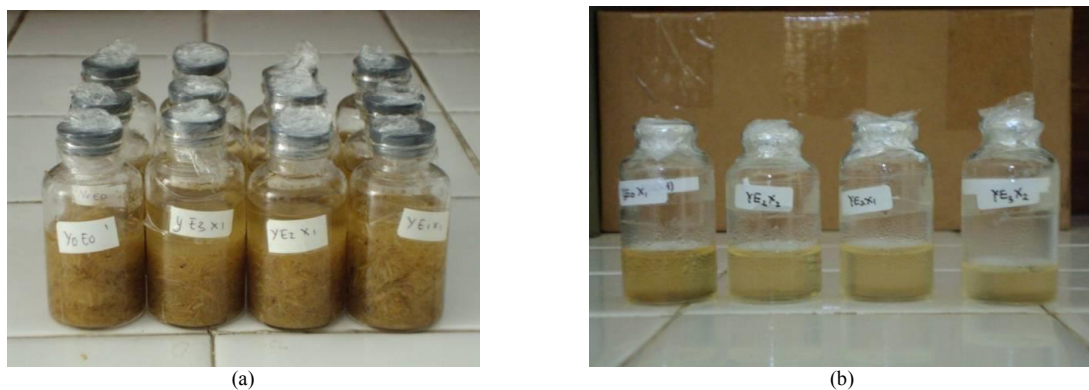


Fig. 1. (a) The SSF process of sugarcane; (b) The ethanol of SSF process results.

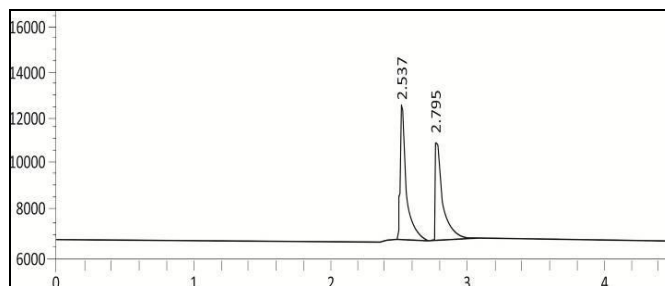


Fig. 2. GC analysis result chart of 60 fpu enzyme.

In Figure 2, it can be seen that the ethanol peak appears at 2.537 minutes, while the peak appearing at 2.795 minutes is propanol peak which used as control in the analysis. It was also conducted control variable without enzyme (0 fpu) with similar with the standards ingredients. The control was conducted to determine the amount of ethanol which converted from glucose which added in yeast nutrient. The result is equal to alcohol content of 0.1372% or dry weight conversion of 2.1650 g/L. This suggests that the actual conversion of cellulose to ethanol is difference of sugarcane bagasse existing value in the Table 2 with the control variable. The complete SSF results of ethanol content was shown in Table 2.

Table 2. The SSF result of enzyme optimization.

No	Enzyme Content variables (fpu)	Ethanol Content (%)	Dry Weight Conversion (g/L)	Differences of Dry Weight Conversion (g/L)
n			DWC	$DWC_n - DWC_{n-1}$
1	0 fpu	0.1372	2.1650	-
2	10 fpu	0.3336	5.2642	3.0992
3	20 fpu	0.7485	11.8113	6.5463
4	30 fpu	0.8652	13.6529	1.8423
5	40 fpu	1.1054	17.4432	3.7903
6	50 fpu	1.2519	19.7550	2.3118
7	60 fpu	1.3544	21.3724	1.6174

Based on Table 2, it can be seen that the higher enzymes contents have comparison with the higher ethanol conversion results. Trend of increasing ethanol contents product is shown in Figure 3(a). Based on Figure 3(a), the trend of increased contents of ethanol can be made two types of equations. They are linear equation with values : $y = 0.309x + 4.0666$ and logarithmic equations with values : $y = 8.8985 \ln(x) - 15.364$. From both equations, the better accuracy rate is logarithmic equation. This suggests that the increased contents of ethanol yield will reach the optimum value. This condition also can be seen through the difference of dry weight conversion in comparison with previous/lower variable of enzyme contents ($DWC_n - DWC_{n-1}$). This trend is downward trend as shown in Figure 3(b). The linear equation for difference of dry weight conversion was obtained with value : $y = -0.0519x + 5.0177$, so in the condition no additional ethanol/no dry weight conversion ($y = 0$) can be estimated at contents of enzymes 96.6802 fpu.

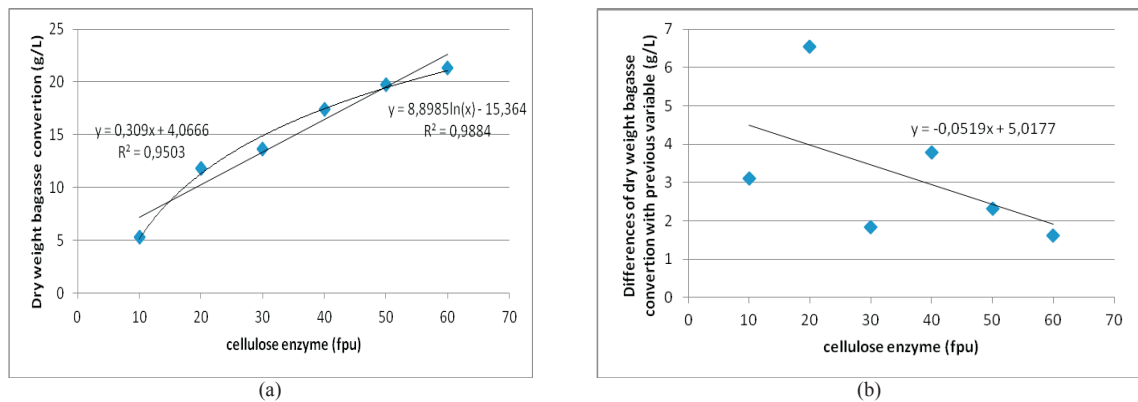


Fig. 3. (a) Dry weight bagasse conversion chart of cellulose enzymes (*Trichoderma reisei*);
(b) Differences of dryweight bagasse conversion chart with previous variable of cellulose enzyme contents.

4. Conclusion

Optimal results were obtained by the cellulose enzyme on 60 fpu by 1.3544% ethanol concentration or conversion of dry weight by 21.3724 g/L with continued optimization potential prediction shaped logarithmic equation. In the advanced prediction can be obtained approximate optimal value enzyme contents on 96.6802 fpu. On further research, it is recommended to conduct further variables of cellulose enzyme as verification of predictions which have been made in this research.

Acknowledgements

The authors are thankful to UPT BPPTK LIPI Yogyakarta especially for Alternative Energy Team and Chemical and Environmental Technology Team for support of facilities in this research.

References

- [1] Samsuri M, Gozan M, Mardias R, Baiquni M, Hermansyah A, Wijanarko A, Prasetya B, Nasikin M. Utilization of cellulosic bagasse for the production of ethanol via simultaneous saccharification and fermentation with xylanase enzyme. *Makara, Technology Journal* 2007; 1(1):17-24.
- [2] Hermiati E, Mangunwidjaja D, Sunarti TC, Suparno O, Prasetya B. Utilization of bagasse lignocellulosic biomass for the production of bioethanol. *Litbang Pertanian Journal* 2010; p. 121-130.
- [3] 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 Application*. Yogyakarta. November 6th-8th, 2012. ISBN 978-602-18167-0-7; 2012. p. 27-30
- [4] Kuila A, Mukhopadhyay M, Tuli DK, Banerjee R. Production of ethanol from lignocellulosics: a enzymatic venture. *EXCLI Journal* 2011; 10:85-96.
- [5] Hamelinck CN, Hooijdonk GV, Faaij APC. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle-, and long term. *Biomass & Bioenergy* 2005; 28:384-410.
- [6] Tjokrokusomo SW. The power of fungi bioremediation and agricultural waste bioconversion use fungi culture technology. *Tek. Ling Journal* 2007; 8:54-60.
- [7] Taherzadeh MJ, Karimi K. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *Int. J. Mol. Sci* 2008; 9:1621-1651.
- [8] Sarrouh BF, Silva SS, Santos DT. Technical/ economical evaluation of sugarcane bagasse hydrolysis for bioethanol production. *Chem Eng Technol* 2007; 30(2): 270-275.
- [9] Sasikumar E, Viruthagiri T. Simultaneous saccharification and fermentation (SSF) of sugarcane bagasse-kinetics and modelling. *International Journal of Chemical and Biological Engineering* 2010; 3:57-64.
- [10] Olofsson K, Bertilsson M, Liden G. A short review on SSF – An interesting process option for ethanol production from lignocellulosic feedstocks. *Biotechnology for Biofuels Journal* 2008; 1:1-14.
- [11] Datta R. Acidogenic fermentation of lignocellulose-acid yield and conversion of component. *Biotechnology and Bio engineering* 1981; 23(9): 2167-2170.