KMITL Sci. Tech. J. Vol. 14 No. 2 Jul. - Dec. 2014

Ethanol Production from Sweet Potato by Enzymatic Hydrolyzation and *Saccharomyces cerevisiae* YRK 017 Fermentation

Duangjai Ochaikul[1,2*] and Amornrat Suwannaposri[1]

[1]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand
[2*]Bio energy Research, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

Abstract

The aim of this work was to utilize sweet potato for production of bioethanol. Optimal conditions of α-amylase and glucoamylase was carried on hydrolyzation of sweet potato and measurement of reducing sugars. The optimal α-amylase concentration and volume were 0.05% (w/v) and 5 ml, respectively at 90°C for 2 h. Using these conditions, the maximum concentration of reducing sugar was 16.43 g L^{-1}. The optimum glucoamylase concentration and volume were 0.015 (w/v) and 20 ml, respectively at 60°C for 4 h. The concentration of reducing sugar was 41.78 g L^{-1}. After hydrolyzation of sweet potato with these two enzymes at optimal condition and fermented using *Saccharomyces cerevisiae*YRK 017 (isolated from Loog-pang), the maximum ethanol concentration of 14.55 g L^{-1} was achieved after 72 h of separate hydrolysis and fermentation process (SHF). For simultaneous saccharification and fermentation process (SSF), the maximum concentration of ethanol was 12.62 g L^{-1}

Keywords: Sweet potato, *Saccharomyces cerevisiae*, Bioethanol, Simultaneous saccharification and fermentation

1. Introduction

The world – wide energy consumption has increased 17 – fold in the last century [1]. However, conventional energy resources, like fossil fuels, cannot meet the increasing energy demand. The quantities of conventional energy resources are limited and they have a considerable negative environment impact e.g. emission of CO_2 to the atmosphere and inducing climate change environmental pollution [2]. Therefore, the use of biofuels as alternative energy sources has many advantages. Bioethnol is one of the most promising biofuels from renewable resources. Fermentation derived ethanol can be produced from sugar, starch or lignocellulosic biomass. Sugar and starch based feedstocks are currently predominant at the industrial level and they are economically favorable. Sweet potato (*Ipomoea batatas*) is a cheap and available agriculture product, contained a large amount of starch. It is a suitable feedstock for industrial bioethanol production. It contains a considerable amount of highly active β – amylase [3]. Sankaranarayanan

*Corresponding author: E-mail: kodaungj@kmitl.ac.th

and Mukarukaka [4] reported that sweet potatoes contained about 22% starch and 5 – 6% sugar for a total of 27 – 28 fermentation material. The advantages of sweet potato are its easy growth, adaptation to many farming conditions and prices are more stable than other agricultural major energy crops [5].

The ethanol fermentation processes from starchy materials commonly involves two stages [6]; (i) liquefaction of starch by α-amylase and enzymatic saccharification of the low molecular weight liquefaction products such as dextrin to produce glucose; (ii) fermentation of glucose to ethanol. Many researchers have been attempted to combine the two-stage fermentation process in a single – step [7-8]. Simultaneous enzymatic saccharification and fermentation (SSF) process in terms of higher ethanol yield, lower energy consumption and shorter processing time. On the other hand, the critical problem with SSF is that it operated at non-optimal hydrolysis temperature since optimal temperatures for the yeast and the enzyme differ [9].

The aim of this work was to examinethe potential of sweet potato starch a source for ethanol production by enzymatic hydrolysis followed by*S.cerervisiae* fermentation via SHF and SSF process.

2. Materials and Methods

2.1 Sweet potato powder

Sweet potatoes were purchased from Ladkrabang Market, Bangkok, Thailand. They were sliced into a little piece and dried in hot air oven at 40°C for 2-3 days, milled into 0.2 mm of particle size. The milled sweet potato was stored in plastic bag at 4°C.

2.2 Enzymes andmicroorganisms.

Alpha-amylase from*Aspergillusoryzae* was used for sweet potato powder liquefaction. The enzyme activity was 37-20 U/mg. Amyloglucosidase from *Aspergillusniger*, activity was 25-9 U/mg solid was used for sweet potato powder saccharification. These enzymes were from Sigma, Aldrich Co, Ltd *Saccharomyces cerevisiae* YRK 017 was used for the fermentation of hydrolyzed sweet potato powder. The culture was isolated from Loog-pang [10] and was maintained on malt agar slant. The agar slant consisted of malt extract (3g L^{-1}), yeast extract (3 g L^{-1}), peptone (5 g L^{-1}), agar (20g L^{-1}) and distilled water (1 liter). Before used as an inoculum for the fermentation, the culture was aerobically propagated in 250 ml flask in a rotary shaker at 30°C for 16-18 h.

2.3 Liquefaction

Ten g of sweet potato powder was mixed with water at the weight ratio of 1:15. The liquefaction was carried out at 90°C, pH 5.0 for 2 h by adding 0.05, 0.10, 0.15 and 0.2 % (w/v) enzyme α-amylase and the amount of this enzyme was 5, 10, 15 and 20 ml. After centrifugation of the culture broth at 3,500 rpm for 15 min (Refrigerated Centrifuge, Z383K, HERMLE, Germany) to remove solid contents, the supernatant obtained was analyzed for reducing sugars by using Somogyi-Nelson method [11].

2.4 Saccharification

The liquefied mash was performed by adding 0.005, 0.010, 0.015 and 0.020 % (w/v) enzyme glucoamylase and the amount of this enzyme was 5, 10, 15 and 20 ml. The saccharification was carried out at 60°C for 4 h. The samples were analyzed as described above.

2.5 Ethanol fermentation

2.5.1 Separated hydrolysis and fermentation process (SHF)

The SHF process was performed by adding appropriate concentrations and amount of α-amylase and glucoamylase enzymes and adding 10% (v/v) of *Saccharomyces cerevisiae* YRK 017. The fermentation was carried out at 30°C in static flask for 72 h. After centrifugation of the culture broth at 3,500 rpm for 15 min to remove yeast cells and total solids, the supernatant obtained was measured by gas chromatography (GC-17 A, Shimadzu) with a flame ionization detector and the column temperature was 150°C.

2.5.2 Simultaneous enzymatic saccharification and fermentation process (SSF)

The SSF process was performed by adding appropriate concentration and amount of α-amylase enzyme at 90°C , pH 5.0 for 2 h. After that adding appropriate concentration and amount of glucoamylase enzyme and *Saccharomyces cerevisiae* YRK 017 at 30°C in static flask for 72 h. Reducing sugars and ethanol were analyzed.

2.6 Statistical analysis

Data was reported as mean $\pm$ standard deviation from triplicate determination. Analysis of varience (ANOVA) accompanied with DMRT test (SPSS for window) were conducted to identify the significant difference between samples ($p<0.05$)

3. Results and Discussion

3.1 Liquefaction

The effects of the concentrations and amount of α-amylase enzyme were investigated. The higher enzyme concentration resulted in increasing maximum reducing sugar (Figure 1a). Alpha amylase concentration of 0.2% gave the highest reducing sugar (19.60 g L^{-1}), but it was in significantly different with 0.05, 0.1 and 0.15 %α-amylase. For reducing the cost, the authors used 0.05% α-amylase to hydrolyze the starch. The variousa mount of α - amylase for hydrolysis was studied. The result found that 5, 10, 15 and 20 ml of α - amylase gave high reducing sugar (16.43–16.47 g L^{-1}) and were insignificantly different. (Figure 1b).Then, the optimal conditions for liquefaction of sweet potato powder by α-amylase were enzyme concentration of 0.05% (w/v) and amount of 5 ml at 90°C for 2 h. These conditions produced 16.43 g L^{-1} reducing sugar.

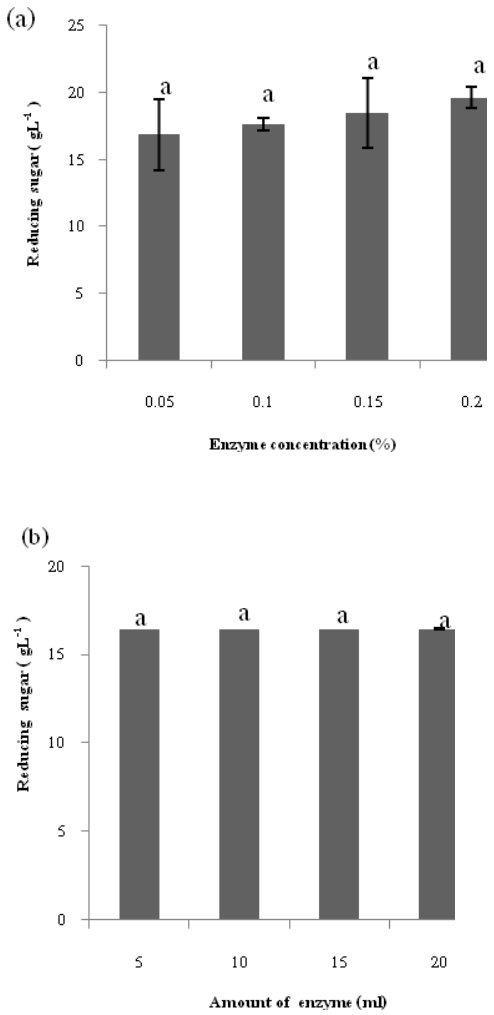

Figure 1 Effect of α-amylaseon the enzymatic hydrolysis; (a) Plot of reducing sugar as a function of concentration of enzyme and (b) amount of enzyme.

3.2 Saccharification

The effect of concentrations and amount of glucoamylase enzyme were investigated. The higher enzyme resulted in increasing in maximum reducing sugar (Figure 2). The highest yield of reducing sugar achived using 0.02% glucoamylase concentration, but not significantly from using 0.015% of glucoamylase. The optimal condition for saccharification byglucoamylase enzyme concentration was 0.015% (w/v) and amount of this enzyme 20 ml at 60°C for 4 h. These conditions produced 41.78 g L^{-1} reducing sugar (Figure 2a, 2b).

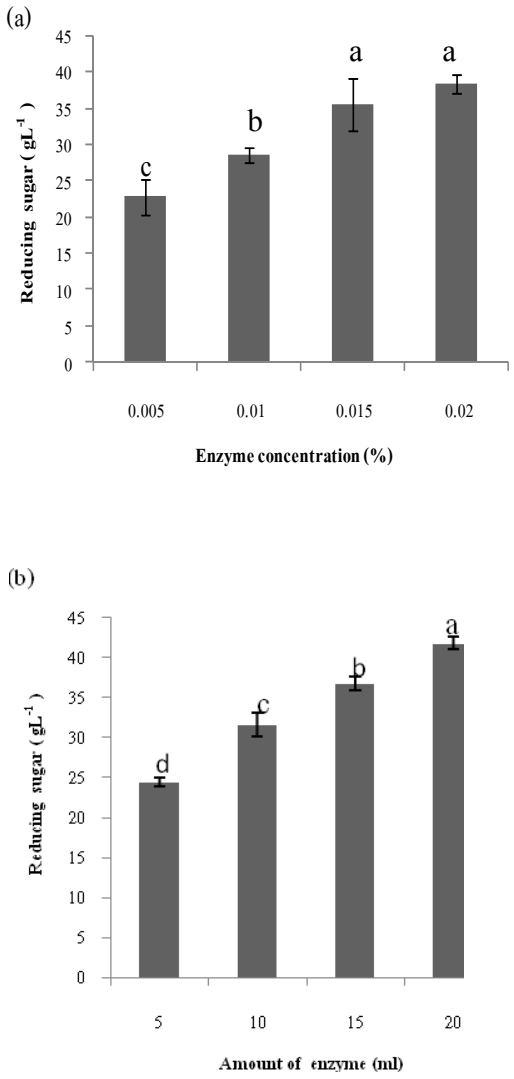

Figure 2 Effect of amyloglucosidase on the enzymatic hydrolysis; (a) Plot of reducing sugar as a function of concentration of enzyme and (b) amount of enzyme.

3.3 Ethanol production

Separate hydrolysis and fermentation process (SHF) of sweet potato powder was examined, compared with simultaneous saccharificationand fermentation process (SSF). The results are summarized in Table 1.

Table 1 Ethanol yield obtained during the SHF and SSF process using sweet potato powder and *S. cerevisiae* YRK 017

Type of the process	Reducing sugar (gL^{-1})		Ethanol Concentration (gL^{-1})
	Before fermentation	After fermentation	
SHF	41.78 ± 0.65	0.83 ± 0.06	14.55 ± 0.21
SSF	16.43 ± 0.01	0.56 ± 0.03	12.62 ± 0.17

The maximum ethanol concentration of 14.55 g $L^{-1}$was achieved after 72 h of separate hydrolysate and fermentation process, while simultaneous saccharification and fermentation process gave ethanol concentration of 12.62 g L^{-1}. Saha and Cotta [12] demonstrated that the separated hydrolysis and fermentation (SHF) approach worked better than the simultaneous saccharification and fermentation (SSF) method, with respect to ethanol yield. The maximum concentration of ethanol from wheat straw hydrolysate and recombinant *E. coli* strain FBR 5 was 18.9 $\pm$ 0.9 g L^{-1} with a yield of 0.29 g perg of straw by SHF. For SSF, the maximum concentration of ethanol was 15.1 $\pm$ 0.1 g L^{-1}which gives a yield of 0.23 g per g of straw. Ohgren *et al.* [13] compared two different process configuration, simultaneous and fermentation (SSF) and separate hydrolysis and fermentation (SHF), regarding ethanol production from steam-pretreated corn stover. The enzymatic loading in these experiments was 10 FPU/g of water-insoluble solids and the yeast concentration was 1 g L^{-1} (dry weight) of a *Saccharomyces cerevisiae* strain. SSF gave a 13% higher overall ethanol yield than SHF (72.4% and 59.1% of the theoretical).

4. Conclusions

From this investigation, SHF and SSF process of sweet potato powder to ethanol could be achieved using *S.cerevisiae* YRK 017. SHF process gave higher ethanol yields (14.55 g L^{-1}) than SSF process (12.62 g L^{-1}).

5. Acknowledgement

The authors are grateful to Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Thailand for partial financial support to this study.

References

[1] Demirbas, A. **2007**. *Progress and recent trends in biofuels. Prog. Energ. Combust.,* 33, 1-18.
[2] He, MX., Feng, H., Bai, F., Li, Y., Liu, X., Zhang, Y. **2009**. Direct production of ethanol from raw sweet potato starch using genetically engineered *Zymomonas mobilis*, *Afr. J. Microbiol. Res.* 3, 721-726.
[3] Shengjun, W., Zhengyu, J., Qunyi, T and Hanging, C. **2009**. Sweet potato : A novel substrate for pollulan production by *Aureobasidium pullulans*. *Carbohydrate polymer*, 76, 645-649.

[4] Sankaranarayanam, M. and Mukarukaka, P. **2007**. Study on alcohol production from sweet potato. *Enzyme and Microbial Technology*, 27, 370-380.
[5] Tsai, W.T., Lan, H.F., Lin, D.T. **2008**. An analysis of bioetanol utilized as renewable energy in the transportation sector in Taiwan. *Revew Sustain Energy Rev*., 12, 1364-1382.
[6] Thomsen, A.B., Medina, C., Ahring, B.K. **2003**. Biotechnology in ethanol production. **In :** Larsen, H., Kossmann, J., Petersen, I.S. editors. Risoenergy report 2. Riso National Laboratory, 40-44.
[7] Kurosawa, H., Nomura, N., Tanaka, H. **1988**. Ethanol Production from starch by a co-immobilized mixed culture system of *Aspergillus awamori* and *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.*, 33, 716-723.
[8] Hwang, W.S., Luen, G.G., Chen, W.H., Men, L.C., Wang, J.B and Kuo, MC. **2007**. The current status of the technology development for the cellulosic ethanol at INER. **In:** Yang, S.S., Sayigh, A.A.M., Lai, C.M.,Chen, S. editors. Development and applications of renewable energy. Taiwan:National Taiwan University, p.35.
[9] Nikolic, S., Mojovic, L., Rakin, M. and Pejin, D. **2009**. Bioethanol production from corn meal by simultaneous enzymatic saccharification and fermentation with immobilized cell of *Saccharomyces cerevisiae* var. *ellipsoideus*. *Fuel*. 88, 1602-1607.
[10] Rattanapreedakul, W. **2006**. Study on fungi and yeast isolated from Look-pang in comparison with pure culture for sato production. *KMITL Science and Technology Journal*, 10, 12-18.
[11] Nelson, N. **1944**. A photometric adaptation of the somogyi method for the determination of glucose. *Journal of biological Chemistry*. 153, 375-380.
[12] Saha, B.C. and Cotta, M.A. **2006**. Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw.*Biotechnol. Prog*., 22, 449-453.
[13] Ohgren, K., Bura, R., Lesnicki, G., Saddler, J. and Zacchi, G. **2007.** A comparison between simultaneous saccharidication and fermentation and separated hydrolysis and fermentation using steam-pretreated corn stover. *Process Biochemistry*, 42, 834-839.