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Research Article

Ethanol Production from Non-Food Tubers of Iles-iles (*Amorphophallus campanulatus*) by Using Separated Hydrolysis and Fermentation

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Abstract

The decrease in production and the raise in needs have led to the rise in oil prices. This work investigated the possibility of Iles-iles (*Amorphophallus campanulatus*) tuber flour, which is rich in carbohydrate content, as a raw material to produce bioethanol. To obtain the maximum ethanol concentration, several parameters had been studied, such as: the concentration of *α*-amylase and *β*-amylase in liquefaction and saccharification processes, respectively, the type of *S. cerevisiae* enzyme (pure, dry, wet and instant) and weight of Diammonium phosphate (DAP) as a nutrient for *S. cerevisiae* in fermentation. The result shows that the highest reducing sugar content (12.5%) was achieved when 3.2 ml *α*-amylase/kg flour and 6.4 ml *β*-amylase/kg flour were used during liquefaction and saccharification processes. Since the concentration of *α*- and *β*-amylase increased, the reducing sugar obtained also increased. The higher sugar content resulted the higher the ethanol concentration in the fermentation broth. Furthermore, the highest concentration of ethanol (9 %v/v) was obtained at 72 h fermentation using the dry *S. cerevisiae*, at 3.2 ml and 6.4 ml /kg flour of *α* amylase and *β*-amylase enzymes, respectively. From the study of the effect of *S. cerevisiae* type, it was shown that dry *S. cereviseae* produced the highest ethanol concentration 10.2% (v/v) at 72 h fermentation. The DAP was used as a nitrogen supply required by *S. cerevisiae* to growth and as a results can increase the ethanol concentration. The addition of DAP in the fermentation proved that 8.45% (v/v) of ethanol was obtained. This result shows that the proposed tuber flour has the potential a raw material for bioethanol production.

Keywords: Biofuel; Bioethanol; *iles-iles* tuber; *S. cerevisiae*; *α*-amylase; *β*-amylase

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1. Introduction

The decrease of oil domestic production caused the increase on the price of fossil fuels [1]. These facts catch the interest of many researchers to find the new technology to replace fuel oil with the environmentally-friendly fuel. One way to solve this problem is by using alternative energy such as bio-fuels. Biofuels must be technically feasible, economically competitive, environmentally acceptable, and readily available [2]. One of biofuels is bioethanol or C_2H_5OH, which is known as a type of bio-fuel that has the potential to replace fossil fuel [3].

Biofuels are made from bio-based materials through thermo chemical processes. The most

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famous and widely raw materials from agricultural product containing starch that have been used to produce bioethanol are cassava and corn, which also known as a main food in several countries, including Indonesia. However, the use of cassava for bioethanol production as energy source has affected to the availability of food because in some area, especially in Indonesia, cassava was used as the main food, as well rice [4]. Therefore, another non food raw materials which locally and abundantly available were still required to produce bioethanol.

Another sources such as agricultural wastes including wood, plants, switch grass, and cotton waste, which contain cellulose have been found as raw materials for bioethanol, in which the consumption of this waste for energy source to replace the starch will not interfere the availability of food [5]. However, Its have been reported that the technology to convert the agricultural waste was difficult and it required high cost process therefore it is still required more technological improvements, such as treatment, liquefaction and saccharification by special enzymes to hydrolyze cellulose into sugar [6]. Previous study found that wheat straw could be used as substrate for bioethanol however the conventional fermentation with *saccharomyces cerevisiae* could not ferment multiple sugar substrates of wheat straw to ethanol therefore it involved the recombinant microorganism *E. coli* [7].

Moreover, organic waste has been found to be used as raw material for bioethanol however the production of bioethanol from organic waste involved the complicated process, such as the demolition waste, solid liquid separation process, continuous fermentation and anaerobic conditions [8]. Therefore, alternative processes to gain the maximum conversion with the simple treatment are still required. One of the materials which is found abundantly and have high cellulose content is *iles-iles* (*Amorphophallus campanulatus*) which can be used as a raw material to produce bioethanol. *Iles-iles* contains high of starch (77%), and another components including fiber (8.5%), crude protein (14%), sugar (3-5%), ash and vitamins (3.4-5.3%). The *iles-iles* has been widely planted as in Central Java and East Java. In remote area, villagers used the *iles-iles* tubers as food source, but now they have switched to cassava and rice for their primary food. The calcium oxalate content in the starch can cause itching, therefore utilization of the *iles-iles* tuber has been limited. This work investigated the possibility of the tuber as an alternative raw material for the production of bioethanol. To produce ethanol, in the first step, the flour was hydrolyzed which converted the carbohydrate containing in the flour into sugar by α-amylase and β-amylase enzymes then the sugar was subsequently fermented to ethanol by yeast *S. cerevisiae*. For that purpose, the main objectives are to determine the optimum hydrolysis parameters (α- and β- amylase enzymes) and fermentation parameters (*S. cerevisiae* type and DAP nutrient concentration) that resulted the highest sugar content as intermediate product and ethanol as the main product.

2. Materials and Methods

2.1. Starch Preparation

Iles-iles tubers were obtained from Wonogiri, Central Java, Indonesia. The tubers were washed with water thoroughly to remove all dirties particle. The tubers were chopped and dried (70 ^{o}C) until a constant weight and it was grounded to mesh-size. The *iles-iles* flour was prepared by washing the grounded *iles-iles* with deionised water which then subsequently dried (60 ^{o}C) until a constant weight. The flour was then stored at room temperature for further usage. The flour was used as substrate to produce bioethanol.

2.2. Starch Hydrolysis

Enzymatic hydrolysis including liquefaction by α-amylase and saccharification by β-amylase of the flour was carried out in a flask equipped with agitatation. The flour hydrolysis was performed at the flour: water ratio of 1: 4 in the flask agitated at 200 rpm. The first step, the flour slurry was added by α-amylase at different concentrations (0.8-6.4 ml/kg flour. The flour slurry was heated at 95-100 ^{o}C and pH 6 for 1 h. The second step was sacharification, β-amylase was added to the liquefied slurry and it was conditioned at pH 5, heated at 60 ^{o}C for 4 h. In saccharification process, the concentration of β-amylase at various concentrations (0.8, 1.6, 3.2 and 6.4 ml α-amylase/kg flour) were studied. The saccharified slurry was fermented with *S. cerevisiae*. The reducing sugar content of saccharified slurry was analyzed by spectrophotometry method.

2.3. Ethanol Fermentation

Anaerobic batch fermentations were performed in the flask at 30 ^{o}C for 72 h at pH 5.5. *S. cerevisiae* was used as fermentation organ-

ism. Four types of *S. cerevisiae* were used which include dry, instant, wet and pure. The concentration of *S. cerevisiae* enzyme in fermentation used was 10% (w/v). The samples during fermentation were withdrawn for 12, 24, 36 and 72 h to determine reducing sugars and alcohol concentration. To obtain the optimum ethanol yield, the effect of addition DAP with the range of 1-8 g/l at constant of 2 g/l urea to sample were studied.

2.4. Sample Analysis

Determination of reducing sugar was performed by the method of spectrophotometry. Samples as much as 0.01 ml (10 microns) in a test tube was added with 1000 microns/ml of glucose reagent, then it was incubated for 10 min in waterbath at temperature 37 ^{o}C. The reducing sugar was determined by readings the photometer (Boehringer 1040) at a wavelength of 546 nm. The ethanol content which obtained from the fermentation samples was analyzed using GC- Hewlett Packard Agilent 6890N equipped with a flame ionisation detector.

3. Results and Discussion

3.1. Chemical Composition of *Iles-iles*

The chemical composition of *iles-iles* tuber consists of starch, cellulose, hemicelluloses, lignin, ash content, and moisture content. The percentage of each composition can be seen in Table 1. As shown in Table 1 that tuber consisted of a high amount of starch. This characteristic is essential to convert raw material to produce bioethanol. The starch can be hydrolyzed into reducing sugar to form glucose which then the fermentation of glucose into ethanol.

3.2. The Effect of *α*-amylase Concentration

Compared to acid hydrolyzed, enzymes hydrolyzed use inherent mild reaction conditions which led to the use of *α*-amylase enzyme in the most starches hydrolysis [9]. *α*-amylase is thermoresistant bacteria which obtained from *Bacillus licheniformis* or a strains of *Escherichia coli* or *Bacillus subtilis*. To increase the rate of hydrolysis by α-amylase enzyme, the substrate suspensions should be brought to high temperatures (90-110 ^{o}C) for the breakdown of starch kernels [10]. The usage of *α*-amylase in hydrolysis of starch is to crack the *α*-1,4-glucoside into glucose monomers which is used for fermentation [11]. Hydrolysis of the flour was investigated at various concentration of *α*-amylase enzyme (0.8, 1.6, 3.2 and 6.4 ml/kg flour) with the process conditions at 95-100 ^{o}C and pH 6 for 1 h. The liquified flour slurry was saccharified with 3.2 ml *β*-amylase/kg flour at 60-65 °C for 4 h at pH 5.

Table 1. Dry - *iles-iles* flour composition

Parameter	Composition (%)
Starch	71.25
Hemi-cellulose	3.30
Cellulose	8.54
Water	8.50
Raw fiber	5.85

Figure 1 shows the effect of various concentration of *α*-amylase in liquefaction with a constant of *β*-amylase concentration (6.4 ml/kg flour) of saccharification on reducing sugar from the flour substrate water ratio of *iles-iles*:H_2O = 1:4 (w/v). Liquefaction of the flour was performed with the conditions at concentration of flour substrate of 25% w/v, temperature of 95-100 °C, time of 1 hour, pH 6, agitation of 200 rpm. Saccharification of liquefied *iles-iles* flour was performed at *β*-amylase concentration of 6.4 ml/kg flour, pH 5, 60 °C, 4 hours. As shown in Figure 1, at saccharification time increased caused the higher the reducing sugar. The highest reducing sugar concentration (14 %v/v) was achieved at 3.2 ml *α*-amylase/kg flour (%v/w). This result indicates that an increased in reducing sugar concentration was influenced by the *α*-amylase concentration. The higher the *α*-amylase was added, caused the increased of glucose which was produced from the cracking of *α*-1.4 glucosidal molecule containing in starch. According to Whitaker, the addition of enzymes concentration caused an increase the hydrolysis reaction [12]. However, when the reaction was reached an equilibrium, the additional of enzymes was not effective.

The reducing sugar produced from saccharified *iles-iles* starch was fermented using dry *S. cerevisiae* at 30 ^{o}C and pH 4.5 for 72 h. A 200 mL of the saccarified slurry was added with 0.2 g DAP, 0.4 g urea, and 10% (w/v) of dry *S. cerevisiae*. The correlation between the fermentation time towards ethanol and reducing sugar for various concentration of α-amylase during fermentation process can be seen in Figure 2.

With increasing of fermentation time had affected the decrease of reducing sugar. This result showed that the reducing sugar was used for ethanol production [13]. During fermentation, the sugar obtained from the saccharifica-

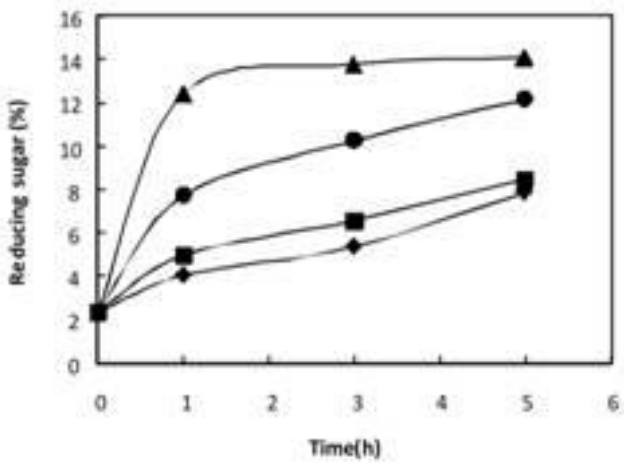

Figure 1. The effect of *α*-amylase concentration on reducing sugar of saccharified slurry. *α*-amylase concentration (ml/kg flour): = 0.8; = 1.6; = 3.2; = 6.4

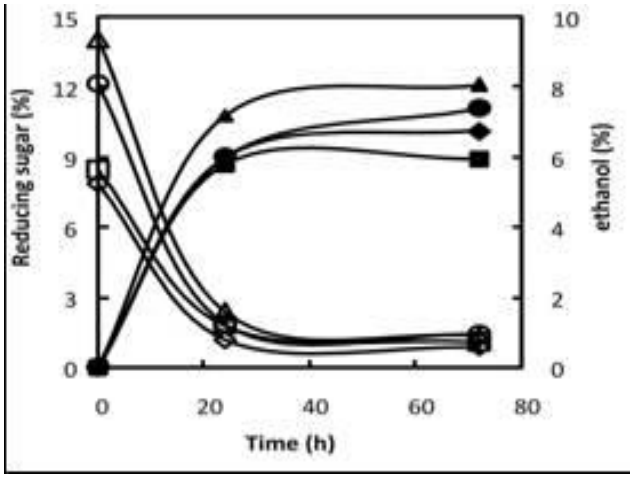

Figure 2. Various effect concentration of *α*-amylase on reducing sugar and ethanol concentration during fermentation process. *α*-amylase concentration (ml/kg flour): =0.8; =1.6; =3.2; =6.4. Ethanol concentration (% v/v) at various *α*-amylase (ml/kg flour): = 0.8; =1.6; =3.2; =6.4

tion was converted to ethanol by *S. cerevisiae* therefore the reducing sugar content decreased with increasing time. The highest ethanol concentration (8.1% v/v) was obtained at 3.2 ml *α*-amylase/kg flour. This result was similar to Silverstein *et al*. which reported that an increasing of *α*-amylase concentration, the ethanol product was increased [14].

3.3. The Effect of *β*-amylase Concentration

There are two steps of hydrolysis process of *iles-iles* flour to be sugar, liquefaction and saccharification using *α*- and *β*-amylase enzymes, respectively. According to Balat, in saccharification the oligosaccharides which resulted of liquefaction by a single or mixture enzymes is hydrolyzed to form reducing sugar [15]. *β*-amylase has been found played a significant role for widely hydrolysis, therefore in this work, various concentration of *β*-amylase of 0.8, 1.6, 3.2, 6.4 ml were studied at conditions of 60-65 °C and pH 5 for 4 hours.

Figure 3 shows the effect of *β*-amylase enzyme concentration on reducing sugar during saccarification of *iles-iles* flour. The saccharified of the liquefied flour slurry was carried out in *iles-iles*:H_2O ratio of 1:4 (w/v), with conditions of liquefaction was kept constant at 95-100 °C, time of 1 hour, pH 6, *α*-amylase concentration of 3.2 ml/kg flour. Saccharification condition was at temperature of 60-65 °C, 4 hour, pH 5. The results show that the highest of reducing sugar concentration (12.5% v/v) was obtained at 6.4 ml of *β*-amylase/kg flour. This result shows that with an increased of *β*-amylase concentration, the reducing sugar formed was also increased. This results was due to the *β*-amylase was hydrolyzed the amylase and amylopectin into *D*-glucose by cracking the *α-D*-(1,4), *α-D*-(1.6) and *α-D*-(1,3). In addition, reducing sugar content was also influenced by the time of hydrolysis, where with an increase of saccharification time the reducing sugar content also increased. However, if the saccharification time was occurred in an excess time, it would cause the polymerization of reducing sugar [16].

After the saccharification of *iles-iles* flour slurry completed, glucose was converted into ethanol through microbial fermentation using dry *S. Cerevisiae* at 30 °C and pH 4.5 for 72 h. The highest ethanol concentration of 8.6 % (v/v) was obtained at *β*-amylase of 6.4 ml/kg flour. An increased of reducing sugar resulted in increased of ethanol. Fermentation time also had affects the concentration of ethanol, with increasing fermentation time, the ethanol was increased and resulted in decreasing on reducing sugar until it was reached an equilibrium between substrate and enzyme. This was due to the *S. ceresiviae* has reacted to convert the reducing sugar into ethanol as shown in Figure 4.

3.4. The Effect of Different Types of *S. Ceresiviae*

The first step to produce ethanol from

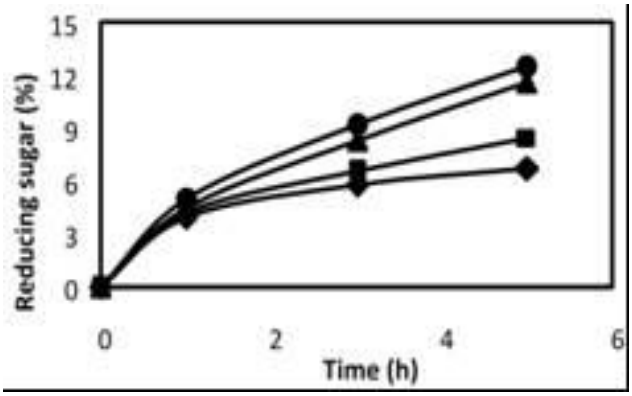

Figure 3. The effect of β-amylase concentration on reducing sugar of saccharified slurry. Reducing sugar at various β-amylase concentration (ml/kg flour): = 0.8; = 1.6; = 3.2; = 6.4

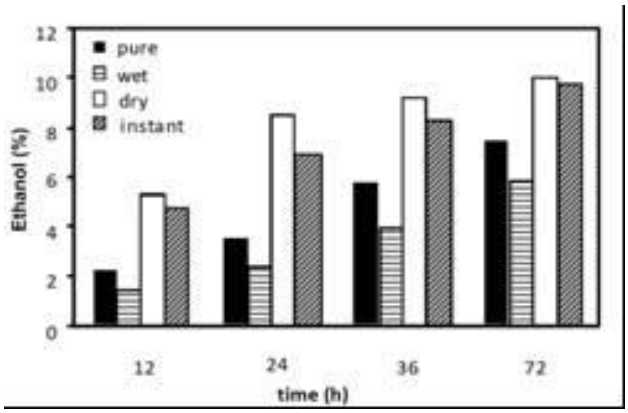

Figure 5. The effect of different types of *S. cerevisiae* (dry, wet, pure, instant) on ethanol (% v/v) for flour/H_2O = 1:4 (w/v).

starch is by hydrolysis of starch into reducing sugar and followed by fermentation of reducing sugar using *S. Ceresiviae*. Hydrolysis is a reaction of starch with water, which is employed to break down the starch into fermentable sugar. The supernatant from enzymatic hydrolysis of starch contains glucose. *S. cerevisiae* and *Zymomonas mobilis* are microorganism which are capable to efficiently ferment the glucose into ethanol, but they are unable to ferment xylose [17]. *S. Ceresiviae* has been generally selected in widely ethanol production due to its potentiality, its tolerant to high ethanol content, its ability to live at high temperature, its stability in the fermentation condition at low pH [18].

Figure 5 shows that ethanol concentration was increased with increasing of fermentation time for different type of *S. cerevisiae* (pure, wet, dry, instant). As the time increased, the activity of *S. cerevisiae* was also increased in producing ethanol. The results of this study

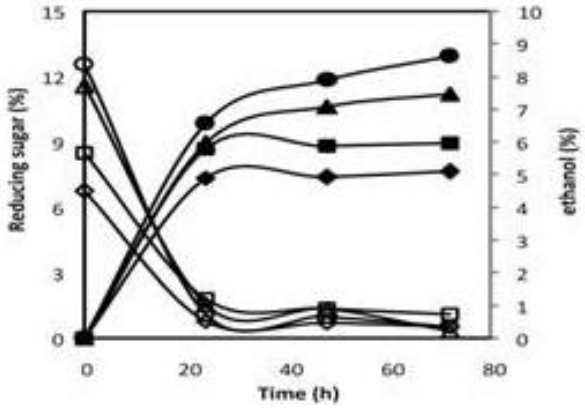

Figure 4. The effect various concentration of β–amylase on reducing sugar on reducing sugar and ethanol concentration in fermentation process. Reducing sugar at various β-amylase (ml/kg flour): =0.8; =1.6; =3.2; =6.4
Ethanol concentration (% v/v) at various β-amylase (ml/kg flour): = 0.8; =1.6; =3.2; =6.4

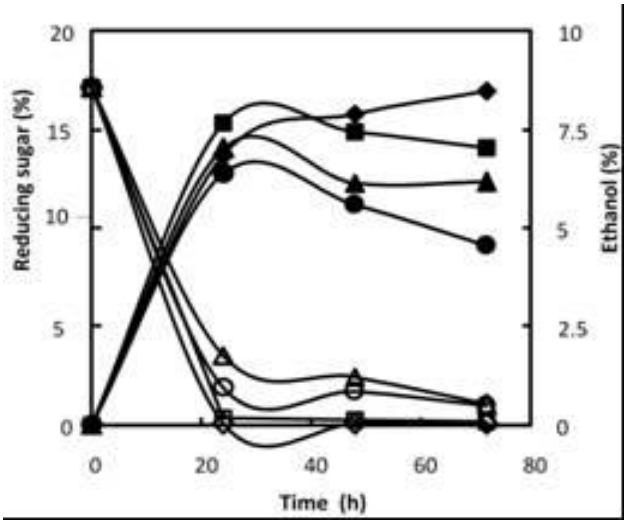

Figure 6. Effect of various concentration of DAP on reducing sugar and ethanol (% v/v). Reducing sugar (%v/v) at various concentration of DAP (g): =2; =1; =0.5; =0.25
Ethanol concentration (%v/v) at various conc of DAP (g): = 2; =1; =0.5; =0.25

shows that the fermentation using dry *S. cerevisiae* at 72 h was the highest compared with pure, wet, and instant yeast. A similar result was reported for the fermentation using *S. cerevisiae* [18].

3.5. Effect of DAP Concentration

DAP was used as a nutrient to supply nitrogen which was required to support *S. Ceresiviae* growth and to increase the fermentation rate which led to increase ethanol yield. Previous study reported by Bafrncov *et al*. found that nitrogen was necessary for the growth and multiplication of yeasts and it also influenced the ethanol tolerance of yeasts and ethanol productivity. However, in excess of DAP could cause the decreasing of ethanol produced [18].

The effect of DAP concentration was also studied at 0.25-2 g loaded on fermentation substrate with concentration of *iles-iles* flour in slurry of 25% w/v and the use of the dry *S. ceresiviae*. Prior to fermentation, the liquefaction was carried out using *α*–amylase at concentration of 1.6 ml/kg flour at conditions of temperature at 95-100 °C, for 1 hour, pH 6, 200 rpm, and pH 6. Meanwhile, saccharification was carried out using *β*-amylase at 3.2 ml/kg flour at 60 °C, 4 hours, pH 5. Fermentation condition was at temperature of 30 °C, pH 4.5 and 72 hours. The results show that 2 g DAP loaded could produce the highest ethanol concentration (8.4%). The result shows that fermentation time also affected the ethanol yield as indicated by an increase in ethanol produced (Figure 6) when fermentation time increased, and consequently a decreased in reducing sugar.

4. Conclusion

It can be concluded that *iles-iles* tuber is an attractive raw material for the production of bioethanol. The highest concentration of ethanol obtained from the tuber flour fermentation was achieved at 72 hours using the dry *S. Ceresiviae*. Prior fermentation, the liquefaction was obtained at optimum conditions with 3.2 ml *α*-amylase/kg flour, 6.4 ml *β*-amylase/kg flour and 2 g/l of DAP. It can be concluded that iles-iles tuber flour is proven to be an attractive raw material for the production of bioethanol. For industrial application however, an efficient collection of the tubers from the remote area are required. Further research is still required to gain the maximum benefit of iles-iles tuber plant, especially for sustainable energy supply projects.

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References

[1] Koike, Y., Tang, Y.Q., Syo, T., Osaka, N., Morimura, S., and Kida, K. (2009). Production of Fuel Ethanol and Methane from Garbage by High Efficiency Two Stage Fermentation Process. *Journal of Bioscience and Bioengineering*. 108: 508–512

[2] Meher, L.C., Sagar, D.V., Naik, S.N. (2006). Technical Aspects of Biodiesel Production Transesterification - A Review. *Renewable and Sustainable Energy*. 10: 248–68.

[3] Li, H., Kim, N.G., Jiang, M., Kan, J.W., Chang, H.N. (2009). Simultaneous Saccharification and Fermentation of Lignocellulosic Residues Pretreated with Phosphoric Acid–Acetone for Bioethanol Production. *Bioresource Technology*. 100: 3245–3251.

[4] Darmawan, E. (2010). Singkong Sambung sebagai Bahan Bakar Alternatif Masa Depan, http://ladangsawah.blogspot.com/2010/04/judul-singkong-sambung-sebagai-bahan.html (in Indonesian)

[5] Schell, D.J., Riley, C.J., Dowe, N., Farmer, J., Ibsen, K.N., Ruth, M.F., Toon, S.T., Lumpkin, R.E. (2004). A Bioethanol Process Development Unit: Initial Operating Experiences and Results with a Corn Fiber Feedstock. *Bioresource Technology*. 91: 179–188.

[6] Yu, Z., Zhang, H. (2003). Ethanol Fermentation of Acid-Hydrolyzed Cellulosic Pyrolysate with *Saccharomyces cerevisiae*. *Bioresource Technology*. 90: 95–100.

[7] Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V. (2005). Dilute Acid Pretreatment, Enzymatic Saccharification and Fermentation of Wheat Straw to Ethanol. *Process Biochemistry*. 40: 3693–3700.

[8] Koike, Y., Tang, Y.Q., Syo, T., Osaka, N., Morimura, S., Kida, K. (2009). Production of Fuel Ethanol and Methane from Garbage by High Efficiency Two Stage Fermentation Process. *Journal of Bioscience and Bioengineering*. 108: 508–512.

[9] Shavanas, S., Padmaja, G., Moorthy, S.N., Sajeev, M.S., Sheriff, M.S. (2011). Process Optimization for Bioethanol Production from Cassava Starch using Novel Eco-Friendly Enzymes. *Biomass and Bioenergy*. 35: 901-909.

[10] Oscar, J.S., Carlos, A.C. (2008). Trends in Biotechnological Production of Fuel Ethanol from Different Feedstocks. *Bioresource Technology*. 99: 5270-5295.

[11] Siqueira, P.F., Karp, G.P., Carvalho, J.C., Sturm, W., José, A., León, R., Tholozan, J.L., Singhania, R., Pandey, A., Soccol, C.R. (2008). Production of Bio-Ethanol from Soybean Molasses By *Saccharomyces cerevisiae* at Labora-

tory, Pilot and Industrial Scales. *Bioresource Technology*. 99: 8156–8163.

[12] Whitaker, J.R. (1996). *Enymes In: Food Chemistry*. O.R. Fennema. M. Dekker, New York

[13] Wu, X., Staggenborg, S., Propheter, J.L., Rooney, W.L., Yu, J., Wang, D. (2009). Features of Sweet Sorghum Juice and Their Performance in Ethanol Fermentation. *Industrial Crops and Products*. 31: 164–170

[14] Silverstein, R.A., Ye, C., Ratna, R.M., Sharma, S., Boyette, M.D., Jason, O. (2007). A comparison of Chemical Pretreatment Methods for Improving Saccharification of Cotton Stalks. *Bioresource Technology*. 98: 3000-3011

[15] Balat, M. (2011). Production of Bioethanol from Lignocellulosic Materials via The Biochemical Pathway: A review. *Energy Conversion and Management*. 52: 858–875.

[16] Sharma, S.K., Kalra, K.L., Grewal, H.S. (2002). Fermentation of Enzymatically Saccharified Sunflower Stalks for Ethanol Production and Its Scale Up. *Bioresource Technology*. 85: 31–33.

[17] Shavanas, S., Padmaja, G., Moorthy, S.N., Sajeev, M.S., Sheriff, M.S. (2011). Process Optimization for Bioethanol Production from Cassava Starch using Novel Eco-Friendly Enzymes. *Biomass and Bioenergy* 35: 901-909

[18] Bafrncova, P., Smogrovicova, D., Slavikova, I., Patpova, J., Domeny, Z. (1999). Improvement of Very High Gravity Ethanol Fermentation by Media Supplementation Using Saccharomyces Cerevisiae. *Biotechnology Letters* 21: 337-441.