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Comparison of Sweet Sorghum and Cassava for Ethanol Production by Using *Saccharomyces cerevisiae*

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Abstract: The two-step enzymatic hydrolysis of sweet sorghum and cassava were performed by commercially available α-amylase and glucoamylase and further ethanol fermentation of the obtained hydrolyzates by Saccharomyces cerevisiae were studied. For both sweet sorghum and cassava, the hydrolysis and fermentation were done in a 2 L stirred tank bioreactor, B-Braun fermenter, by using the same conditions. The amount of glucose obtained after hydrolysis process was greater in sweet sorghum compared to cassava, which are 50.07 and 40.00 g L^{-1}, respectively. Also, sweet sorghum gave higher ethanol concentration than cassava at the 64 h of fermentation process, which are 40.11 and 34.07 g L^{-1}, respectively.

Key words: Sweet sorghum, cassava, hydrolysis, fermentation, ethanol

INTRODUCTION

Nowadays, ethanol production from renewable resources has received great attention because of the increasing petroleum shortage (Amutha and Gunasekaran, 2001). Ethanol or ethyl alcohol produced by starch hydrolysis (liquefaction and saccharification) and sugar fermentation processes from biomass is called as bioethanol (Demirbas, 2008). The raw materials used in the ethanol production via fermentation can be classified into three main types of materials, which are sugars, starches and cellulose materials. Sugars can be converted into ethanol directly. Meanwhile, starches must be hydrolyzed to fermentable sugars by enzymes from malt or molds. On the other hand, cellulose materials must be hydrolyzed to sugars, generally by acids or alkali. For both the starches and cellulose materials, once simple sugars are formed, enzymes from microorganisms can readily ferment the sugars to ethanol (Lin and Tanaka, 2006).

Sweet sorghum (*Sorghum bicolor* L. Moench) is a C_4 plant characterized by a high biomass- and sugar-yielding and a high photosynthetic efficiency crop (Bryan, 1990; Billa *et al*., 1997). It also has a rapid growth rate as it has a shorter growing season than sugarcane and is therefore suitable to be grown in geographical areas because it is well adapted to temperate climate (Dolciotti *et al*., 1998) and resistance to drought (Kosaric and Vardar-Sukan, 2001). Meanwhile, it contains approximately equal quantities of soluble (glucose and sucrose) and insoluble carbohydrates (cellulose and hemicellulose) (Jasberg *et al*., 1983). Therefore, from many crops presently being investigated for energy and industry, sweet sorghum has been considered as one of the most promising crops for the production of ethanol (Gnansounoua *et al*., 2005).

Cassava (*Manihot esculenta*) is a perennial woody shrub with up to 32% (fresh) starch content (Dai *et al*., 2006). It is a cheap substrate that is easily available in tropical countries (Amuth and Gunasekaran, 2001;Kosaric and Vardar-Sukan, 2001). The benefits of using cassava as a raw material for ethanol production are, it can be planted on marginal lands where other agricultural crops such as sugarcane, rice, wheat and corn cannot be grown well (Zhang *et al*., 2003). Besides, it has high tolerance to drought because it can survive even during the dry season when soil moisture is low, but humidity is high. Also, it requires lower soil quality compared to sugarcane as it thrives better in poor soils than any other major food plant. Therefore, wide areas of little used land can be utilized for cultivation and fertilization is rarely necessary (Rice *et al*., 1986).

Starchy grains and effluent generated from starch processing units are the cheap feedstocks and could be used as potential raw materials for ethanol fermentation

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(Verma *et al*., 2000). Starch consists of two types of polysaccharides, the linear molecule, amylose and a highly branched molecule, amylopectin (Shariffa *et al*., 2009). Enzymatic hydrolysis is essential for the production of glucose syrups from starch because of the specificity of enzymes allows the sugar syrups production with well-defined physical and chemical properties and the milder enzymatic hydrolysis results in few side reactions and less browning (Lin and Tanaka, 2006).

The aims of this study were: (1) to observe the effect of liquefaction and saccharification processes on granular starch of sweet sorghum and cassava and (2) to compare the amount of glucose obtained after the liquefaction and saccharification processes of sweet sorghum and cassava by commercially available α-amylase and glucoamylase and the amount of ethanol produced after the fermentation of glucose by *Saccharomyces cerevisiae* yeast. The starch hydrolysis and fermentation for both sweet sorghum and cassava were fixed at same conditions.

MATERIALS AND METHODS

Substrates: Sweet sorghum grains were obtained from Indonesian Bioenergy Foundation and cassava tubers were obtained from the Selayang Wholesale Market, Selangor. Sweet sorghum and cassava were blended into small size of approximately 20 μm to enhance the hydrolysis process.

Microorganisms: Dried-form industrial *Saccharomyces cerevisiae* yeast was used in this research. For inoculum, 100 mL of distilled water was heated to 40°C in a shake flask. After that, 0.5% (w/w) of *S. cerevisiae* yeast was added into the warmed water to activate the yeast. The mixture was left for 15 min at 150 rpm.

Enzymes: Both α-amylase from *Bacillus subtilis* and glucoamylase from *Aspergillus niger* were obtained from local market. The activities of the two enzymes were 90% each.

Hydrolysis: The two liters B-Braun bioreactor was filled with 300 g of sweet sorghum and 900 mL of distilled water. Then, 0.1% (v/w) of α-amylase (from the amount of sorghum) was added and the mixture was cooked at 90°C and mixed at 500 rpm for 1 h. After 1 h, the mixture was cooled down to 50°C and 0.1% (v/w) of glucoamylase was added and the mixture was left for 2 h with 250 rpm agitation. Next, the remaining solids of the sorghum were removed from the solution and were cooled down to 35°C and the pH was adjusted to pH 5. The same procedures were repeated for cassava.

Fermentation: In the meantime, 0.5% (w/w) of urea and 0.05% (w/w) of NPK (nitrogen, phosphorus and potassium) were added to the bioreactor. After 10 min, the activated yeast solution was added to the two liters bioreactor. The mixture was mixed well for 5 min and left for 8 h without agitation. Then, the agitation was changed to 50 rpm until 72 h of incubation.

Analyses: During the fermentation, data was collected for every 8 h for the measurement of Viable Cell Number (VCN), Cell Dry Weight (CDW) and glucose and ethanol concentrations.

Scanning electron microscopy: The microstructure of the starch granules were viewed with a Field Emission Scanning Electron Microscope (FESEM). The starch samples were collected after liquefaction and saccharification processes separately. The samples were centrifuged at 5000 rpm for 30 min. The supernatant was transferred into centrifuge tube and kept in -20°C for glucose determination. The pellet which contains the starch granules was washed with distilled water and dried at 30°C.

Growth determination: For measurement of cell growth, VCN analysis was performed by mixing 10 μL of the sample with 10 μL of tryphan blue. The mixture was mixed well and 10 μL of the mixture was put into THOMA counting chamber. Then, the cells were counted under the microscope. The viable cells were shine in color while the dead cells were blue in color.

$$VCN = AV \times DF/V \quad (1)$$

where, AV is the average viable cell count (cells), DF is the dilution factor, V is the volume of chamber (mL).

CDW determination: For the measurement of CDW, empty falcon tube was dried in an oven at 80°C for 24 h (A). Then, 7 mL of the sample was centrifuged at 5000 rpm for 30 min. The supernatant was transferred into centrifuge tube and kept in -20°C for glucose and ethanol determinations. The pellet was added with 10 mL of distilled water and vortexed. Then, the tube was centrifuged again at 5000 rpm for 30 min and the supernatant was discarded. Next, the tube was placed in oven at 80°C for 24 h. The drying process was repeated until constant weight (B):

$$CDW = (B-A)/Volume\ of\ sample \quad (2)$$

Glucose and ethanol determinations: Samples for glucose and ethanol determination were centrifuged at 5000 rpm for 30 min to remove cells. The supernatant was filtered through a 0.45 μm membrane and analyzed by High Performance Liquid Chromatography (HPLC) equipped with a refractive index detector. The column used for separation was a SUPELCOGEL C-610H column. 10 μL of sample was injected into HPLC and separation was performed at 30°C with 0.1% H_3PO_4 as the mobile phase at a flow rate of 0.5 mL min^{-1}.

Calculation of kinetic and yield parameters: The maximum specific growth rate (μ_{max}) was calculated during the exponential phase from the slope of the graph of ln VCN vs. time. Doubling time (t_d) was calculated by incorporating μ_{max} into the following formula $t_d = \ln 2/\mu_{max}$. Yield of ethanol based on cell growth ($Y_{P/X}$) was calculated during the exponential phase from the slope of ethanol concentration vs. CDW graphs. Biomass yield coefficients ($Y_{X/S}$) and ethanol yield coefficients ($Y_{P/S}$) were calculated during exponential growth from the slope of CDW vs. glucose concentration and ethanol concentration vs. glucose concentration graphs, respectively (Shuler and Kargi, 1992). Ethanol concentration (P) was analyzed by HPLC. Meanwhile, the volumetric ethanol productivity (Q_p) and the percentage of conversion efficiency or yield efficiency (E_y) were calculated as shown by Laopaiboon *et al.* (2007).

RESULTS AND DISCUSSION

Liquefaction and saccharification: Figure 1 shows the FESEM images of the starch granules. For both sweet sorghum and cassava, the surfaces of granules were quite smooth, but there is slight evidence of fissures, indentations or pores (Fig. 1a, d). After the liquefaction process, the pinholes and equatorial grooves or furrows were exist (Fig. 1b, e). At the end of saccharification process, the pinholes and equatorial grooves or furrows were became obvious (Fig. 1c, f). At the end of liquefaction process, sweet sorghum gave lower amount of glucose compared to cassava, which are 0.60 and 1.54 g L^{-1}, respectively. Meanwhile, after saccharification process, the concentration of glucose was higher in sweet sorghum than cassava, which are 52.63 and 43.57 g L^{-1}, respectively.

Fermentation: Figure 2 indicates that the growth profiles of *S. cerevisiae* yeast for both sweet sorghum and cassava are quite similar. However, the number of viable yeast cells is higher in cassava than sweet sorghum. According to Fig. 2, it can cautiously be stated that there is no lag phase observed during the growth of *S. cerevisiae* for either substrates. Also, there is no obvious different between the exponential, stationary and death phases. The number of viable yeast cells is increasing with the time until 32 h of fermentation time, which is considered as the exponential phase. The stationary phase for cassava is longer than sweet sorghum. The death phase of sweet sorghum happened at 56 h while cassava at 64 h.

Glucose consumption and ethanol formation by *S. cerevisiae* are shown in Fig. 3. At the beginning of the fermentation, the glucose concentration was greater in sweet sorghum compared to cassava, which are 50.07 and 40.00 g L^{-1}, respectively. As the fermentation time increased, the glucose concentration for both sweet sorghum and cassava decreases rapidly until 20 h and reduced slowly until at the end of fermentation. At the 72 h of fermentation time, the ethanol concentration was higher in sweet sorghum compared to cassava, which are 37.24 and 33.32 g L^{-1}, respectively. However, both sweet sorghum and cassava produced the highest amount of ethanol at 64 h, which are 40.11 and 34.07 g L^{-1}, respectively. At 32 h, the growth of *S. cerevisiae* stopped and the stationary phase started when almost all glucose was used up and ethanol production became slower.

Kinetic and yield parameters: Table 1 summarizes the important kinetic parameters of the ethanol fermentation from sweet sorghum and cassava. All parameter values were dependent on the type of feedstock. When cassava was used as the raw material, the results gave higher value for μ_{max}, $Y_{X/S}$, $Y_{P/S}$ and E_y, while lower value for t_d, $Y_{P/X}$, P and Q_p.

Liquefaction and saccharification: The susceptibility of starch granules can be classified by the intensity and the

Table 1: Kinetic and yield parameters of *S. cerevisiae*

	Parameters								
			$Y_{P/X}$	$Y_{X/S}$	$Y_{P/S}$				
Substrates	μ_{max} (h^{-1})	t_d (h)	----------($g\ g^{-1}$)----------			P (g L^{-1})	Q_p (g L^{-1} h)	E_y (%)	t (h)
Sweet sorghum	0.0333	20.8	14.241	0.04	0.6445	40.11	0.63	126.37	64
Cassava	0.0506	13.7	10.958	0.0658	0.8339	34.07	0.53	163.51	64

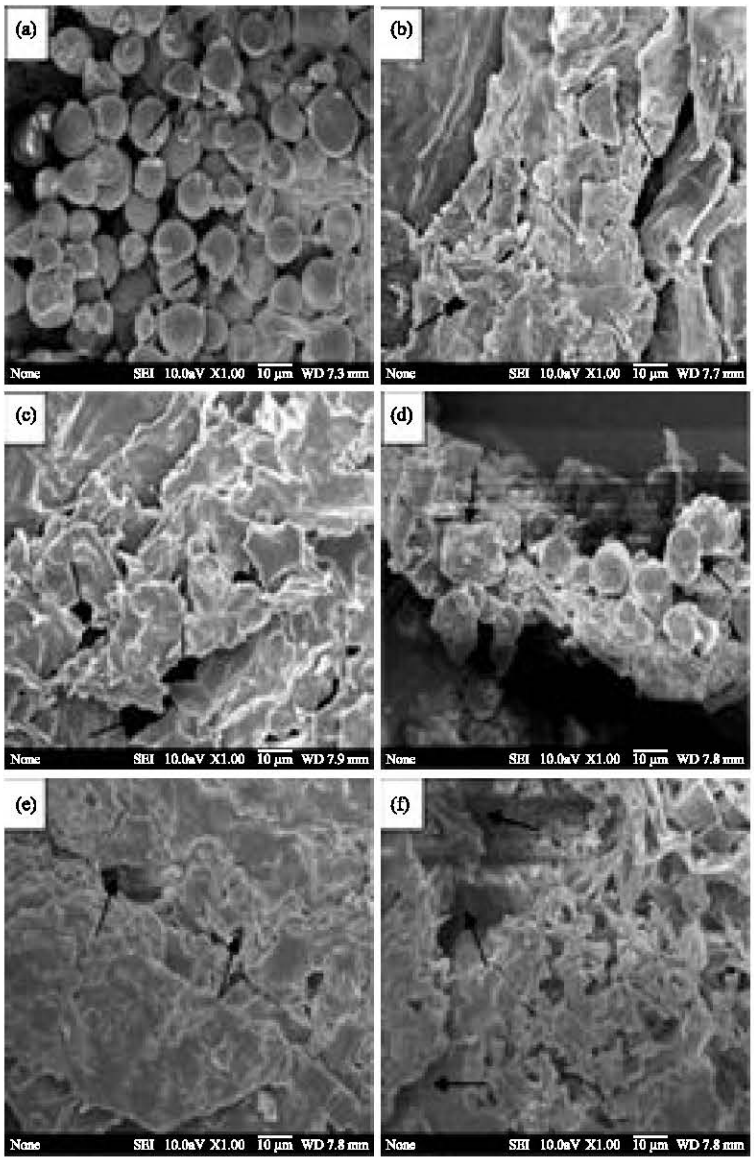

Fig. 1: FESEM images (1000x) for (a) control cassava, (b) liquefied cassava, (c) saccharified cassava, (d) control sweet sorghum, (e) liquefied sweet sorghum and (f) saccharified sweet sorghum (scale bar = 10 µm)

manner by which the granules are eroded and corroded (Gallant *et al.*, 1982). According to Shariffa *et al.* (2009), the enzymatic erosion happened mainly at the surface of starch granules. However, during the liquefaction process, it is possible that the small swelling of the granules can cause the naturally present pinholes and internal cavities in starch granules to expand and become bigger and thus allowing α-amylase enzyme to penetrate easily into the granules. Juszczak *et al.* (2003) found that any pores present on the surface of the granules could become the centers of attraction, which made the granules more susceptible to enzymatic attack. In addition, the pinholes and equatorial grooves or furrows were exist after liquefaction process due to the reason that heating of starch in water causes disruption of hydrogen bond between the polymer chain inside the granule, which can weaken the granule, so that the enzyme can penetrate and degrade the starch easily. Moreover, Kurakake *et al.* (1996) discovered that heat-moisture treatment, which involves heating of starch at a relative humidity of 100%, is effective in enhancing the adsorption of α-amylase. The pinholes and equatorial grooves or furrows were become obvious after saccharificaton process. The number of holes was increased and the size of the holes was larger.

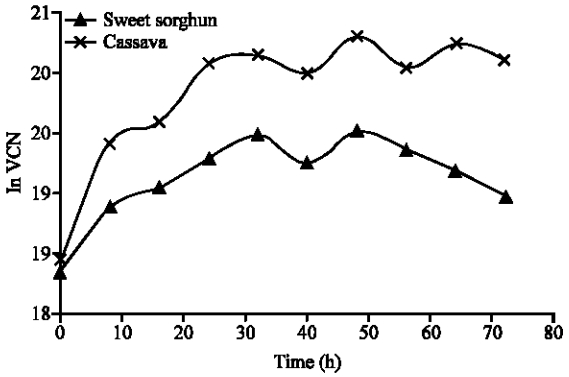

Fig. 2: The growth profile of *S. cerevisiae*

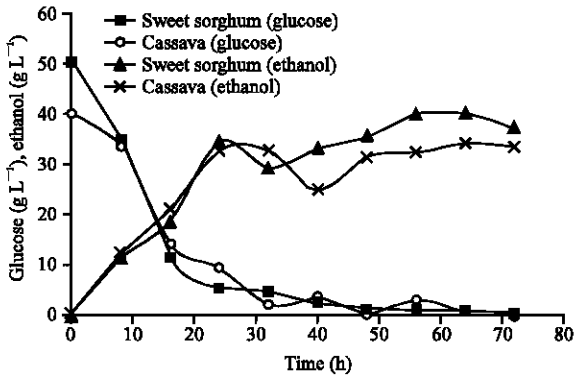

Fig. 3: The glucose consumption and ethanol fermentation by *S. cerevisiae*

This is because the condition of the starch granules after the liquefaction permits glucoamylase enzyme to penetrate into the granules more extensively and forms pores and channels in the granules.

As can be seen in Fig. 1a and d, the surface of cassava's granules are smoother than sweet sorghum. Also, the pitting occurred on the edges of the cassava granules are more severe compared to sweet sorghum. Thus, cassava is more susceptible to enzymatic attack and gave higher amount of glucose after liquefaction compared to a sweet sorghum. Meanwhile, the concentration of glucose was higher in sweet sorghum than cassava because the starch content in sweet sorghum is about 69-71% (Aggarwal *et al*., 2001) while the starch content in cassava is about 32% (Dai *et al*., 2006).

Fermentation: From the growth profile, it can cautiously be stated that there is no lag phase observed during the growth of *S. cerevisiae* for either substrates. But, it should be considered that the samples in this study were withdrawn at 8 h intervals and the lag phase could have already taken place before the first sampling. According to Şener *et al.* (2007), for fermentation of *S. cerevisiae* (Uvaferm CM) at 25°C, the range of exponential phase is from 0-108 h, which is very long. On the other hand, the stationary phase is only until 120 h, followed by death phase. Aggarwal *et al.* (2001) mentioned that glucose was the main sugar in the enzymatic hydrolysate of sweet sorghum starch. Thus, glucose was considered as the substrate in this study. As stated previously, the glucose concentration was greater in sweet sorghum compared to cassava is because the starch content in sweet sorghum is about 69-71% (Aggarwal *et al*., 2001) while the starch content in cassava is about 32% (Dai *et al*., 2006). Furthermore, since the starch particles in cassava are bigger and there are some branched structures, more glucoamylase enzyme has to be added (Lin and Tanaka, 2006). Moreover, the growth of *S. cerevisiae* stopped and the stationary phase started when almost all glucose was used up and ethanol production became slower. Özilgen *et al.* (1991) reported that ethanol accumulation can inhibit specific growth rate, specific ethanol production rate, cell viability and substrate consumption.

Kinetic and yield parameters: It can be observed from the results that cassava provides better conditions for *S. cerevisiae* as the yeast growth rate (μ_{max}), conversion of carbon source to biomass ($Y_{X/S}$) and conversion of carbon source to ethanol ($Y_{P/S}$) is greater compared to sweet sorghum. Also, the yield efficiency (E_y) was greater in cassava as it depends or linearly relates on $Y_{P/S}$ parameter. On the other hand, the doubling time (t_d) was inversely proportional to μ_{max}. The *S. cerevisiae* in sweet sorghum consumes more time in order to increase the population. Meanwhile, the yield of ethanol based on cell growth ($Y_{P/X}$) was higher in sweet sorghum because even though the number of yeast cells is lower, the cells have more ability to consume glucose and convert to ethanol. Moreover, the ethanol concentration (P) was higher in sweet sorghum due to higher glucose concentration at the beginning of fermentation. Also, the volumetric ethanol productivity (Q_p) was linearly proportional to P.

According to Laopaiboon *et al.* (2007), for ethanol production from sweet sorghum juice in batch fermentation by *S. cerevisiae*, the $Y_{P/S}$, P, Q_p and E_y were between 0.39-0.42 g/g, 73.57-100.37 g L^{-1}, 1.68-2.04 g L^{-1} h and 76.47-82.35%, respectively. Meanwhile, for ethanol fermentation by *S. cerevisiae* (Zymaflore VL1) and *S. cerevisiae* (Uvaferm CM), the μ_{max}, t_d, $Y_{P/X}$, $Y_{X/S}$, $Y_{P/S}$ were in the range of 0.0205-0.0350 h^{-1}, 19.8-33.8 h, 8.10-9.40 g g^{-1}, 0.0525-0.0580 g/g and 0.455-0.499 g g^{-1}, respectively (Şener *et al*., 2007). On the other hand, for the batch fermentation of *S. cerevisiae* on hydrolysed waste starch using 80-100 g L^{-1} glucose, the μ_{max}, $Y_{X/S}$, $Y_{P/S}$ and Q_p were between 0.27-0.35 h^{-1}, 0.061-0.55 g g^{-1}, 0.46 g g^{-1} and 2.9-3.0 g L^{-1} h, respectively (Davis *et al*., 2006).

CONCLUSION

From the FESEM images, in a granular state, sweet sorghum starch was observed to be less susceptible to enzyme attack compared to cassava starch. The glucose concentration was higher in sweet sorghum as it contains greater percentage of starch. The highest ethanol was produced from sweet sorghum at 64 h of fermentation time. The growth rate of *S. cerevisiae* was faster in cassava due to the shorter doubling time.

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