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AT-303: COMPARISON OF SAGO AND SWEET SORGHUM FOR ETHANOL PRODUCTION USING *SACCHAROMYCES CEREVISIAE*

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Abstract

There is a growing interest in the application of bioethanol as biofuel since it has the possibility to be the potential substitute for fossil fuel. Selection of the best raw material for ethanol production is crucial for the substrate preparation. High amount of starch content is one of the important criteria in choosing the best suitable crop for bioethanol production. Two types of starchy crops, Sago and Sweet Sorghum are considered to have a high potential as an energy crop. The two-step enzymatic hydrolysis of sago and sweet sorghum were performed by commercially available α-amylase and glucoamylase enzyme. Further ethanol batch fermentation by *Saccharomyces cerevisiae* strain from the obtained hydrolysates of sago and sweet sorghum were studied. For both sago and sweet sorghum, the hydrolysis and fermentation were done in a 2 L stirred tank bioreactor, B-Braun fermenter, using the same process conditions. Each running was completed within 72 hours. The amount of glucose obtained after hydrolysis process was greater in sweet sorghum compared to sago, which are 50.07 and 48.7 g L^{-1}, respectively. The amount of ethanol concentration also higher for sweet sorghum compared to sago at the 72 h fermentation process, which are 40.11 and 26 g L^{-1}, respectively.

Key word: *Biofuel, Bioreactor, Fermentation, Hydrolysis, Substrate*

INTRODUCTION

Most of the natural resources such as petroleum and coal were heavily consumed at highest rate for the last past decades [1]. The heavy reliance on fossil fuel by modern economy nowadays might succumb to the fact that they will eventually be running out. Therefore, new development in biotechnology plays an important role in resolving part of the energy crisis that lie ahead. One that has stimulated worldwide interest is the utilization of renewable carbohydrate sources for the production of ethanol as a liquid fuels. Bioethanol as being called for ethanol that was made from biomass offers more advantages than fossil fuels since it is renewable and sustainable source of energy [2].

As the price of fossil fuel becomes higher, the implementation of bioethanol as an alternative fuel has become more appealing as bioethanol can provide the ability of being applied in current transportation and electricity generation without having a major modification to the existing system. Ethanol can either be used directly as fuel or being blended with gasoline to become gasoline oxygenate and producing gasohol [3]. Anhydrous ethanol is added to gasoline at a 20-26% proportion in volume [4]. Balat *et.al* [5] had indicated that appropriate blending of ethanol and gasoline has proven not just in promoting cleaner environment but also helps in balancing the economic value of fuel price.

The selection of best suitable crops for bioethanol production is one of the key factor to reduce the overall process cost and maintained the fuel market price. Ratnam *et.al* [6] had pointed out that substrate selection can be the main cost factor for ethanol industry. Sago (*Metroxylon sagu*) is a type of starchy crops that are gives promising future in bioethanol production. Starch from sago palm is the only commercial starch source that comes from the stem and contains bulky amount of starch in its trunk [7]. In Malaysia, sago palm is inexpensive and not nearly as agriculturally intensive as rice. The largest sago-growing in Malaysia are to be found outside the Peninsula, in the state of Sarawak, which is now the world's biggest exporter of sago, exporting annually about 25,000 to 40,000 ton of sago products to Peninsular Malaysia, Japan, Taiwan, Singapore, and other countries [8].

Alternatively, sweet sorghum is another starchy crop that can be used to produce bioenergy at practical scales for the industries [9] [10]. Many ethanol producers and as well as researchers have shown that grain sorghum is a reasonable raw material (technically acceptable, fits the infrastructure, and can be economically viable) for ethanol production and could make a huge contribution to the country's biofuel requirements [11]. The sweet sorghum grain can be hydrolyzed and fermented to produce bioethanol for use as a liquid fuel. Recently, Malaysia have shown high interest and planning to produce biofuel from sweet sorghum since ethanol produced from the plant can be utilized for biofuel to reduce fuel usage as well as expenses [12].

Conventionally, ethanol fermentation utilized sugar rich substrates such as sugar cane to produce the product although, sugar cane is costly and difficult to obtain since it is categorized under seasonal crops [13]. Therefore, it is a great economic advantage to expand the substrate choice to either starchy crops and cellulosic materials which are cheaper compared to sugar rich substrate [13]. Mojovic *et.al* [14] stated that USA and Brazil are the prominent producers of bioethanol in the world and mostly are made from corn and sugarcane. Therefore, studying sago and sweet sorghum as ethanol fermentable substrate will hopefully be able to break the monopoly of ethanol production and provides better opportunity for Malaysia in improving their economy.

This study involved two stages of bioconversion namely, hydrolysis and fermentation using sago starch and sweet sorghum as the raw material. The main aim of this study was to compare the amount of glucose obtained after liquefaction and saccharification processes of sago and sweet sorghum by commercially available α-amylase and glucoamylase and the amount of ethanol produced after the fermentation of glucose by *Saccharomyces cerevisiae* yeast. The conditions for starch hydrolysis and fermentation for both sago and sweet sorghum were fixed to be the same.

MATERIALS AND METHODS

Substrates

Sago starch was obtained from Riau Indonesia and sweet sorghum were ontained from Indonesian Bioenergy Foundation. The sago was believed to be a species of Angau Muda [15]. Both raw materials were readily processed and in the form of starch flour.

Microorganism

The dried form of industrial Saccharomyces cerevisiae was used in this research. Inoculum preparation was done by heating a 100 mL of distilled water to 40°C in a shake flask. 0.5% (w/w) of dried *Saccharomyces cerevisiae* yeast was added into the warm water to activate the yeast. The mixture was then left for 15 min at 150 rpm. The inoculum size was set to have the same initial concentration of cells per mL. Dilutions of the inoculums are done if the concentration of the cells is too high.

Enzymes

Both α-Amylase from *Bacillus subtilis* for liquefaction with an activity of 25,000 U/mL and glucoamylase from *Aspergillus niger* for saccharification with an activity of 130,000 U/mL was supplied by

Riau Enzyme Industry Indonesia. One unit of α-amylase equals to the amount of enzyme which liquefies soluble starch to get 1 mg dextrins at 70 ºC and pH 6.0 in one minute. One unit of glucoamylase equals to the amount of enzyme which hydrolyzes soluble starch to 1 mg glucose at 40 ºC and pH 4.5 in one hour.

Hydrolysis

The 2000 ml vessel was filled with 300 g of sago powder and 900 mL of distilled water. The resulting slurry was heated to 80ºC for 15 minutes for starch gelatinization [16]. After gelatinization, 0.1% (v/w) of α-amylase was added to the slurry and cooked at 80ºC with mixing for 1 hour using impeller at 500 rpm [17]. After one hour, the mixture was cooled down to 50°C for 3 min to make sure that the overall temperature has already drop in preparation for saccharification to take place and 0.1% (v/w) concentration of glucoamylase was added and the mixture was left for two hours at 250 rpm agitation. After the saccharification process completed, the remaining solid were removed and the mixture were cooled down to 35°C and the pH were adjusted to pH 5. Same procedure was used for sweet sorghum.

Fermentation

0.5% (w/w) of urea and 0.05% (w/w) of NPK (nitrogen, phosphorus and potassium) sources was added to the bioreactor with one liter working volume [17]. After 10 min, 100 mL or 10% (v/v) of the inoculated yeast was added into the medium of starch hydrolyzate. The mixture was mixed well for 5 min. Then, the agitation and the aeration were changed to 50 rpm and 0.1 vvm respectively. Fermentation process was carried for 72 h of incubation.

Sampling

During the fermentation, 15 mL of sample collected for every 12 h for the measurement of glucose and ethanol concentrations and was centrifuged at 5000 rpm for 15 min at 4ºC to remove the cell debris. The supernatants left were used to measure for ethanol and glucose concentrations. The pallets were used to determine the Cell Dry Weight (CDW).

Growth Determination

The cell growth was determined by VCN (viable cell number) analysis. VCN was performed by counting the viable cells in the 10 µL of sample after mixing it with 10 µL of tryphan blue solution. The cells were counted under the microscope with the aid of THOMA counting chamber. It has grids that contain 16 large squares, which again contain 16 small squares each. There viable cells were in shine color while the dead cells were in dark blue color.

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

Where AV is the average viable cell count, DF is dilution factor and Vis the volume of chamber (mL).

CDW Determination

Empty Aluminum boat was dried in an oven at 80°C for 24 h and the weight was recorded (weight A). Then, the pallet taken from the centrifuged sample was added with 10 mL of distilled water and suspended by vortex inside a falcon tube. The mixture was poured inside the Aluminum boat and was placed in oven at 80°C for 24 h. The drying process was repeated until constant weight (weight B).

$$CDW = (\text{weight B} - \text{weight A}) / \text{Volume of sample} \quad (2)$$

Glucose and Ethanol Determinations

Supernatant collected from the centrifuged sample 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 flow rate of 0.5 mL min^{-1} [17].

Calculation of Kinetic and Yield Parameters

The maximum specific growth rate (μ_{max}) was calculated during exponential phase from the slope of the graph of ln TCN vs. time. Doubling time (t_d) was calculated by incorporating μ_{max} into the formula $t_d = \ln 2/\mu_{max}$. Yield of ethanol based on cell growth, $Y_{p/x}$ was calculated during exponential phase. Biomass yield coefficient, $Y_{x/s}$ and ethanol yield coefficient, $Y_{p/s}$ were calculated during exponential growth from the slope of CDW vs. glucose concentration and ethanol concentration vs. glucose concentration graphs, respectively [17].

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 Ref. [18].

RESULT AND DISCUSSION

Fermentation

From the graph of growth profile shown in Figure 1, the growth of *S. cerevisiae* for both sago and sweet sorghum can be seen to be quite similar. However the viable cells are more in sweet sorghum compared to sago. It can be said that maybe the cause is due to the high concentration of glucose in the sweet sorghum compared to sago. It occurs that high glucose concentration can really improve the microbial performance. In alcoholic fermentation, the growth of *S. cerevisiae* is highly dependent on the initial concentration of glucose. The growth of *S. cerevisiae* could be easily inhibited by high concentration of ethanol and glucose and currently this inhibition problem is overcome by diluting the starting sugar solutions and by adding water during fermentation to dilute the ethanol concentration in the fermentation broth [19]. It was also showed that there is no lag phase during the growth of *S. cerevisiae* in both substrates. It could be considered that by the time of the first sampling, the yeast were already ended the lag phase and going through the exponential phase. As been mentioned by Sener *et.al* [20], the exponential phase of *S. cerevisiae* (Uvaferm CM) at 25°C was ranging from 0-108 h. Figure 1 also showed that the stationary phase for both substrates were about the same which was starting at 32 h. On the other hand, Ref. [20] also mentioned that the stationary phase of *S. cerevisiae* (Uvaferm CM) was until 120 h and been followed by the death phase. The death phase of *S. cerevisiae* in sweet sorghum was much faster than sago. The death phase in sweet sorghum happened at the 48 h of fermentation time while as for sago the death phase happened at 56 h.

Glucose consumption and ethanol production by *S. cerevisiae* yeast can be seen in Figure 2. As the fermentation started, the glucose concentration was higher for sweet sorghum compared to sago. However, as the time of fermentation increased, the glucose concentration decreased rapidly for both substrates until 16 h. There were some differences of glucose consumption between sago starch and sweet sorghum after the 16 h fermentation. The glucose concentration in sweet sorghum appeared to be lower compared to sago at the end of the 72 h fermentation. Aggarwal *et.al* [21] had mentioned that glucose was the main sugar in the enzymatic hydrolysate of sweet sorghum starch. Thus, glucose was considered to be the only substrate during the fermentation. Nevertheless, Suyandra [22] had stated that from the analyses of the sago starch hydrolysate, the hydrolyzed starch contains high amount of glucose and maltose. Thus, the unutilized maltose could be the explanation on why the sugar concentration was slightly higher for sago compared to sweet sorghum at the end of the fermentation. Furthermore, the starch content in sweet sorghum is quite high in about 69-71% starch compared to sago (Angau Muda species) which contains 39-41% starch [21] [15].

At the end of the fermentation, ethanol concentration was higher for sweet sorghum compared to sago which was 40.11 and 26 g L^{-1}, respectively. The major differences on the ethanol concentration between the two substrates may due to the higher amount of glucose in sweet sorghum compared to sago. Both of the substrates produced the high amount of ethanol at the end of fermentation which is at 72 h. It has been observed from the product formation that, the ethanol production inside the bioreactor did not inhibit the growth of *S. cerevisiae.* As stated by Meintjes [19], a major problem associated with ethanol production through fermentation remains the inhibition of the yeast *Saccharomyces cerevisiae* by the ethanol produced. The graph also showed that, as the growth of *S. cerevisiae* yeast stopped for both sago and sweet sorghum at 32 h, the production of ethanol was still increasing until the 72 h of fermentation time. This happened due to the fact that *S. cerevisiae* yeast cell growth in ethanol fermentation is considered to be a non-associated growth since the ethanol was excreted extracellularly by the *S. cerevisiae* yeast [23].

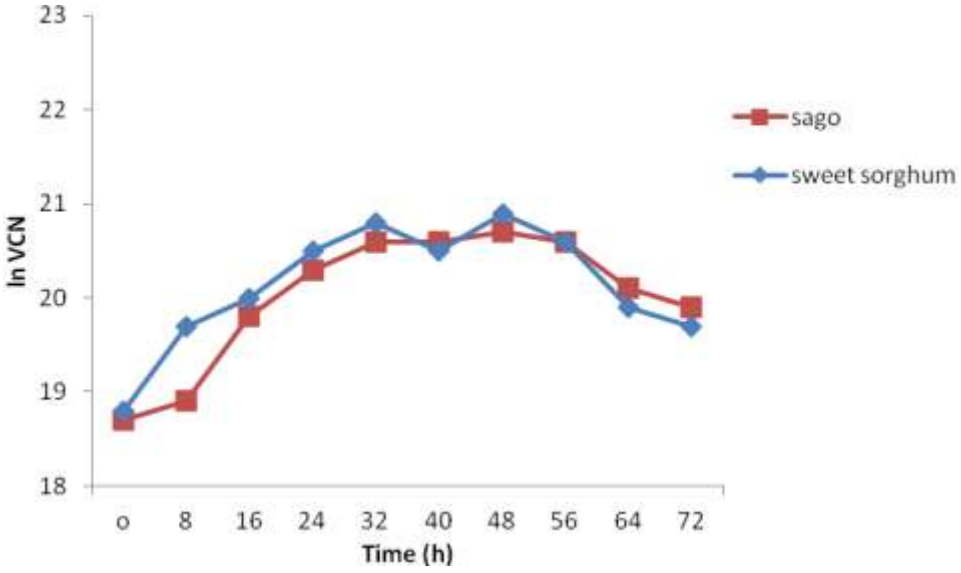

Figure 1: Growth profile of *S. cerevisiae*

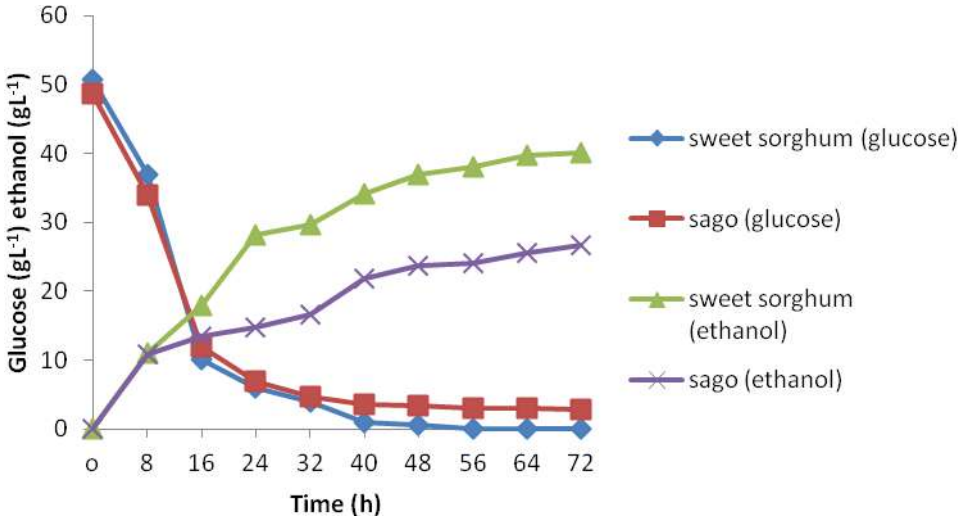

Figure 2: Glucose consumption and ethanol formation by *S. cerevisiae*

Kinetics and Yield Parameters

Table 1 summarizes all the important kinetics parameters in the ethanol production from fermentation of sago and sweet sorghum by *Saccharomyces cerevisiae.* All parameters showed different values for different substrates. Between sago and sweet sorghum, sweet sorghum gives a higher value than sago in terms of $\mu_{max,}$ td, $Y_{P/X,}$ $Y_{X/S,}$ $Y_{P/S,}$ P, Q_P and $E_{y..}$ *S. cerevisiae* growth rate (μ_{max}) was higher which is 0.03 h^{-1} for sweet sorghum. The conversion of carbon source to biomass ($Y_{x/s}$) was 0.067 g g^{-1} and the conversion of carbon source to ethanol ($Y_{p/s}$) is 0.8338 g g^{-1}. Also the yield efficiency (E_y) was greater for sweet sorghum at 292.75% as it depends or linearly relates on $Y_{p/s}$ parameter [17]. Meanwhile, the yield of ethanol base on cell growth ($Y_{p/x}$) was higher for sweet sorghum at 10.957 g g^{-1} due the fact that the cells are able to consume glucose more and convert it to ethanol. Moreover, the ethanol concentration (P) was higher in sweet sorghum fermentation which is 40.11 g L^{-1} due to high glucose concentration at the beginning of fermentation.

Adapted from Ref. [20], for ethanol fermentation by *S. cerevisiae* (Zymaflore VL1) and *S. cerevisiae* (Uvaferm CM), the $\mu_{max,}$ $t_{d,}$ $Y_{p/x,}$ $Y_{x/s,}$ and $Y_{p/s}$ were in the range of 0.0205-0.0350 h^{-1}, 19.8–33.8 h, 8.10-9.40 gg^{-1}, 0.0525-0.0580 g/g and 0.455-0.499 g/g, respectively. Furthermore, according to Ref. [24] the kinetic values for the ethanol fermentation by *S. cerevisiae* (indigenous AR5) in terms of $\mu_{max,}$ $Y_{x/s,}$ and $Y_{p/s}$ were given as 0.37 h^{-1}, 0.05 gg^{-1} and 0.44 gg^{-1}, respectively. Moreover, Ahmad *et.al* [23] stated that the kinetic parameters of ethanol fermentation by *S. cerevisiae* for $\mu_{max,}$ $Y_{x/s,}$ $Y_{p/x}$ and $Y_{p/s}$ were given as 0.084 h^{-1}, 0.136 gg^{-1}, 4.913 gg^{-1} and 0.6682 gg^{-1}, respectively.

Table 1: Kinetic and Yield parameters

Substrate	μ_{max} (h^{-1})	td (h)	$Y_{P/X}$	$Y_{X/S}$	$Y_{P/S}$	P (gL^{-1})	Q_P ($gL^{-1}h$)	E_y (%)	t(h)
			(gg^{-1})						
Sago	0.0303	12.8	7.561	0.0549	0.4425	26.01	0.42	143.92	72
Sweet sorghum	0.0505	13.6	10.957	0.0657	0.8338	40.11	0.52	163.50	72

CONCLUSION

From the results shown, it can be concluded that the glucose concentration was higher in sweet sorghum compared to sago. Due to the high concentration of glucose, sweet sorghum also gives higher ethanol concentration from the fermentation process by using S*accharomyces cerevisiae*. The highest ethanol production is at the end of the fermentation time which was at 72 h of fermentation. The growth of *S. cerevisiae* was a non-associated growth since the ethanol concentration was still increased after the cells stop growing. Further research could be done by combining the two substrates of sweet sorghum and sago starch for the usage in ethanol fermentation by using S*accharomyces cerevisiae.*

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