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ORIGINAL ARTICLE

Influence of Stirring Speed on Glucose and Ethanol Production in Simultaneous Saccharification and Fermentation Process

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ABSTRACT – Lignocellulosic biomass has a potential to be coverted to bioethanol which can be a new alternative for fossil fuel. Empty fruit bunches (EFB) is one of the most abundant lignocellulosic biomass in Malaysia, which has high content of cellulose and posses favorable physiochemical characteristics for bioethanol production via a process called simultaneous saccharification and fermentation (SSF). In SSF process, the reaction is initiated by diffusion and consolidation of the enzyme and its substrate. Thus, optimum stirring speed is crucial, as diffusion rate of substrates is influenced by the agitation of reaction mixture. The influence of stirring speed on the glucose and ethanol concentration in SSF process was investigated in the current study. Initially, 5 % (g/ml) of pretreated EFB in 1.5 liter of 0.05 M buffer citrate pH 4.8 were sterilized in autoclave at 121°C for 20 minutes. Then, enzyme Cellic Ctec-2 solution with concentration (1%) were added together with 1.5% (g/ml) Saccharomyces cerevisiae yeast in the bioreactor. The process was conducted in the bioreactor under temperature of 37°C with stirring speed of 100 rpm for 72 hours. SSF process experiments were repeated with the same setup except by varying the stirring speed (150 and 200 rpm) independently. From the results, the glucose concentration and ethanol yield of 200 rpm indicated less concentration in every 24 hours compared to 150 rpm and 100 rpm. The stirring speed of 150 rpm shows the highest glucose concentration (1.914 mg/ml) and ethanol yield (16%) obtained after 72 hours and determined as the best stirring speed for this experiment.

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INTRODUCTION

Exploration for alternative and cost-effective energy sources is emerging due to global warming, depletion of fossil fuels and rising prices of petroleum-based fuels. Production of biofuel from biomass is one of the alternatives and numerous new technologies focusing on improving yield, conversion efficiency and recovery of by-products with low investment cost and sustainable raw material have been explored to meet the continual global demand [1]. Biomass is the most abundant renewable source of energy which can be potetially used as raw material for biofuel production. It is plant-based materials consists of wide variety of agricultural residues, fruit and vegetable waste, pulp and paper waste and herbaceous energy crops. The abundance and potential transformation of these biomass into sugars, alternative fuels, and chemical feedstocks has increased research interest worldwide [2]. Biomass produced from the palm oil industries is getting more focus due to the sustainability, environmental concerns and abundant supply. Empty fruit bunch (EFB) is one of the oil palm residues that has favorable physiochemical characteristics and abundant supply to serve as potential feedstock to produce biofuel [3]. Biofuels are mainly classified into four types; bioethanol, biodiesel, biogas, and biohydrogen. Different sources are required to generate each type of these biofuels as edible and non-edible or food-based and waste-based. Three major steps in the conversion of lignocellulose to bioethanol include pretreatment, saccharification and fermentation. Pretreatment is performed before saccharification and fermentation to break the structure of lignocellulose for better access and reactivity towards cellulose and hemicellulose by microorganism [4]. There are several methods of pretreatment such as physical, chemical, physicochemical and enzymatic. Saccharification is the hydrolysis of cellulose and hemicellulose polymers to readily fermentable sugars using fungus or bacteria. Fermentation of these reduced sugars to bioethanol can be accomplished using fermenting strains.

Simultaneous saccharification and fermentation (SSF) is an idea of performing the enzymatic hydrolysis and fermentation simultaneously. Separated hydrolysis and fermentation (SHF) is a process of enzymatic hydrolysis followed by fermentation. The pretreated lignocellulosic biomass performed enzymatic hydrolysis, known as saccharification to convert cellulose into reducing sugars at the optimal condition of the saccharifying enzyme. As hydrolysis and fermentation processes carried out in two different reactors, the capital cost of SHF is higher than SSF. Another

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disadvantage in SHF is that the hydrolysis products mainly glucose and its corresponding disaccharide, cellobiose inhibit cellulase action. This will reduce the production yield of bioethanol. High capital cost, time taking and laborious drawbacks in SHF can overcome by SSF [5]. Gauss et al. [6] shows that the glucose yield in a traditional separate enzymatic hydrolysis was low due to product inhibition of the hydrolysis by glucose and cellobiose. The authors also demonstrated that higher overall ethanol yield was obtained by using SSF, which they attributed to the removal of glucose and cellobiose by the fermentation, which consequently reduce the product inhibition. Generally, the reaction initiated by diffusion and association of the enzyme and its substrate. Hence, optimum stirring speed is crucial to ensure good mixing and suitable diffusion rate of substrates [7]. Previous works has been done on the influence of stirring speed on enzymatic hydrolisis and fermentation processes for softwood, bagasse, spruce and wheat straw [8-12]. Studies have also been carried out on the influence of parameters such as solid loading, temperature and pH on SSF process. However, study on the effect of stirring speed on SSF has not been attempted particularly for raw material EFB. Thus, the present experimental study was conducted to study the influence of stirring speed on glucose and ethanol production in SSF process with EFB as the raw material.

MATERIALS AND METHODS

Materials

The chemicals, D-(+)-Glucose, sulfuric acid, hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium citrate dihydrate, citric acid, 3,5 dinitrosalicylic acid, potassium sodium tartrate tertahydrate and alcohol were purchased from Sigma Aldrich. The enzyme, Cellic CTec-2 (Novozymes) and dry yeast (Saccharomyces cerevisiae) were used for hydrolisis and fermentation processes respectively. EFB was obtained from a local oil processing company in Malaysia.

Raw Material Processing and Pretreatment

EFB was washed with tap water and dried in an oven at 105°C for 8 hours to remove the moisture content. Then, the EFB was further processed by shredding, crushing, and grinding using grinder to the predetermined particle size to maximize the contact area of the substrate. Next, the EFB was sieved to 2 mm of particle sizes. The raw material, EFB were pretreated in 6 wt% of NaOH for 4 hours followed by washing with water. Then, the material was exploded in pressure cooker for 120 minutes. The exploded fiber was transferred to a mixing tank and water was added to achieve 10% consistency to remove the hemicellulose sugars component. This treatment was known as hydrothermal treatment. In this process, the mixture of exploded fiber and water was heated until temperature, 60 – 80°C ± 5°C for 1 hour. After that, the exploded fiber was sieved to separate with the water-soluble substances using vacuum filtration unit. After pretreatment, the solid fraction was dried at 50-60°C overnight in an oven.

Raw Material Characterization

Proximate analysis using Thermogravimetric Analysis was performed to obtain the cellulose and lignin content in the samples with chemical and hydrothermal treatment and sample with the chemical treatment only. Thermogravimetric Analysis (TGA) was conducted by using instrument (TGA Q500 V6.7 Build 203) with ramping rate in between 1 to 10 °C/min. Nitrogen gas was used at maximum temperature 700 °C to measure changes in weight in relation to changes in temperature.

Preparation of 0.05 M Citrate Buffer with pH 4.8

First, 1.2 liter of distilled water were prepared in a beaker. Then 11.657 g of sodium citrate dihydrate and 6.794 g of citric acid were added to the solution. Final desired pH was adjusted using HCl and NaOH. Distilled water was added until volume is 1.5 liter (Citrate Buffer (pH 3.0 to 6.2) [13].

Experiments in Bioreactor

Initially, 5 percent (g/ml) of pretreated EFB in 1.5 liter of 0.05 M buffer citrate pH 4.8 were sterilized by autoclave at 121°C for 20 minutes. Then, enzyme Cellic CTec-2 solution with concentration (1%) were added together with 1.5% (g/ml) Saccharomyces cerevisiae yeast in the bioreactor. The process was conducted in the bioreactor at 37°C with velocity agitation of100 rpm for 72 hours. SSF process experiments were repeated with the same setup except by varying the stirring speed to 150 rpm and 200 rpm independently.

Analysis of Glucose Concentration using Dinitrosalicylic acid (DNS) method

For each 1.5 ml sample, 3 ml of DNS reagent was added in tubes of 15 ml. The tubes were taken to a water bath at 100 °C for 5 minutes. Then, the tubes were cooled to room temperature. 10.5 ml of distilled water were added to complete the requiredvolume. After that, absorbance of each sample was taken using Thermo Scientific™ GENESYS™ 50 uv-visible spectrophotometers at 540 nm. Calibration curve for absorbance versus glucose concentration was constructed by

preparing glucose at different concentrations and taking their absorbance reading. Figure 1 shows the calibration curve of absorbance versus their glucose concentration. The equation obtained from the graph is

$$y = 0.6665x - 0.5418 \quad (1)$$

where y = absorbance (nm) and x = glucose concentration ($mgml^{-1}$). The glucose concentration for each sample was calculated by using Equation 1.

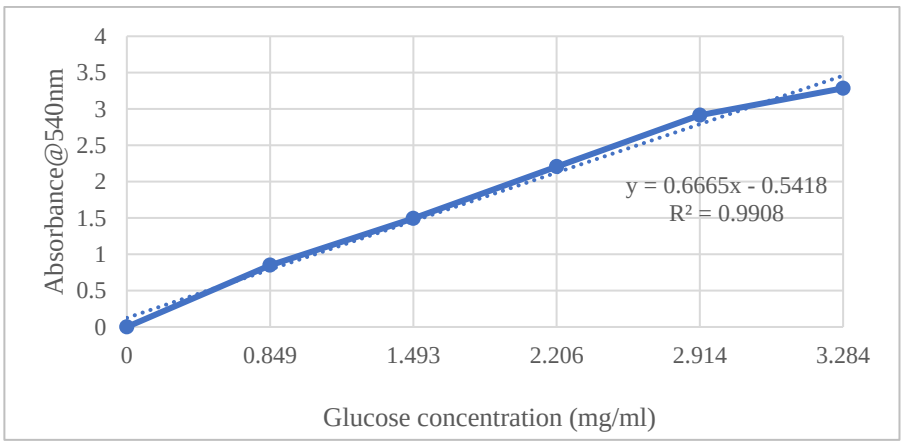

Figure 1. Calibration curve of absorbance versus glucose concentration.

Analysis of Ethanol Concentration by Using Refractometer

The retention time for complete fermentation process is 72 hours. For every 24 hours, the ethanol solution was taken and analyzed using refractometer. Calibration curve for refractive index versus ethanol percentage was prepared by preparing ethanol with different percentage (0–100)% and taking their refractive index reading. Figure 2 shows the calibration curve of refractive index versus their percentage of ethanol. The equation obtained from the graph is shown in Equation 2.

$$y = 0.0026x - 1.3337 \quad (2)$$

where y = refractive index and x = ethanol percentage (%). The ethanol percentage for each sample was calculated by using Equation 2.

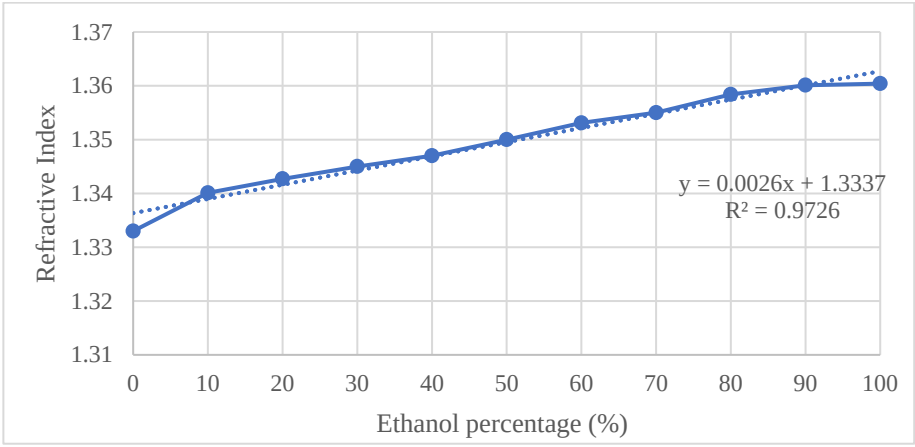

Figure 2. Calibration curve of refractive index versus ethanol percentage.

RESULT AND DISCUSSION

Effect of stirring speed on glucose concentration

SSF process was conducted at 100 rpm, 150rpm and 200 rpm to investigate the best stirring/agitation speed on glucose concentration and ethanol percentage. The results show that stirring speed had a negative effect in the glucose concentration and ethanol percentage indicating that both responses were increased if the stirring speed is decreased. Enzyme adsorption on cellulose is a complex process. It is also a vital requirement for a successful reaction of hydrolysis. To maintain enough contact among the substrate and the enzymes as well as to enhance heat and mass transport, adequate mixing is essential. On the other hand, the excessive mixing has been proven to deactivate enzymes and limit conversion yield [14].

From Figure 3, the highest glucose concentration for every 24 hours is shown by stirring speed of 150 rpm, followed by 100 rpm and 200 rpm. Thus, 150 rpm was defined as the best rotating speed for this experiment. For every 24 hours the glucose concentration was reduced for each rotating speed, because the glucose from the saccharification process converted into ethanol by fermentation process simultaneously. Saccharification rate and ethanol yields increases with higher agitation/shear rate but reduces with excessive mixing due to enzyme deactivation. In previous work by Mukata et al. [15], the results showed that the extent of cellulose conversion could be reduced by excessively high mixing speeds, more than 200 rpm while rapid hydrolysis rates at the initial stage and high conversion yields could be obtained by moderate mixing speeds between 100 and 200 rpm. Other studies by Ikwebe and Harvey [16] and Ado et al. [17] showed that the ethanol concentration reduced with higher shear rate. In Ado et al. [17], the ethanol concentration was higher as agitation increased from 200 rpm to 300 rpm, but the ethanol concentration decreased with rotational speed higher than 300 rpm.

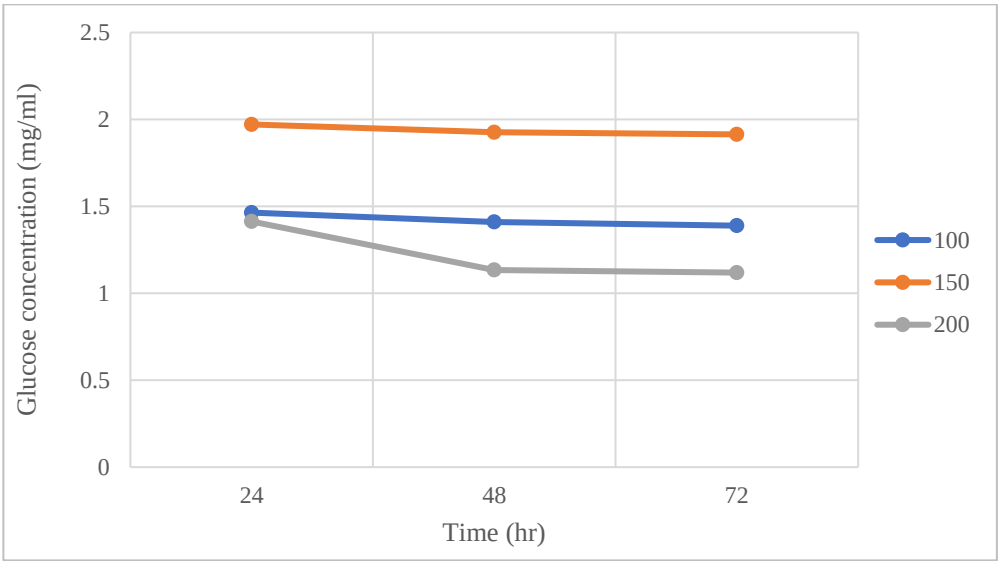

Figure 3. Graph of glucose concentration at various stirring speed.

Effect of stirring speed on ethanol concentration

Cellulase inhibition by the generated sugars is significantly reduced because of the product in SSF process. This is an appealing technique for increasing saccharification rate to produce glucose which eventually increases the ethanol generation by fermentation. This was achieved by using the glucose produced during the saccharification process directly to generate ethanol. Therefore, excess glucose concentration does not block or slow down the saccharification process. Reaction of an immobilized enzyme with a substrate on a solid surface forms a depletion zone when the rate of substrate consumption surpasses the rate of substrate supply by diffusion. As a result, agitation of the reaction mixture speeds up the reaction by increasing the apparent diffusion rate of the substrate [7]. As discussed earlier, the saccharification process is important for a successful SSF process. From the Figure 4, 150 rpm contribute the highest ethanol percentage compared to 100 rpm and 200 rpm. This is due to the higher efficiency of saccharification process, which supports the high production of ethanol. The ethanol percentage also escalates every 24 hours due to the increase in fermentation process by saccharomyces cerevisiae which converts glucose into ethanol.

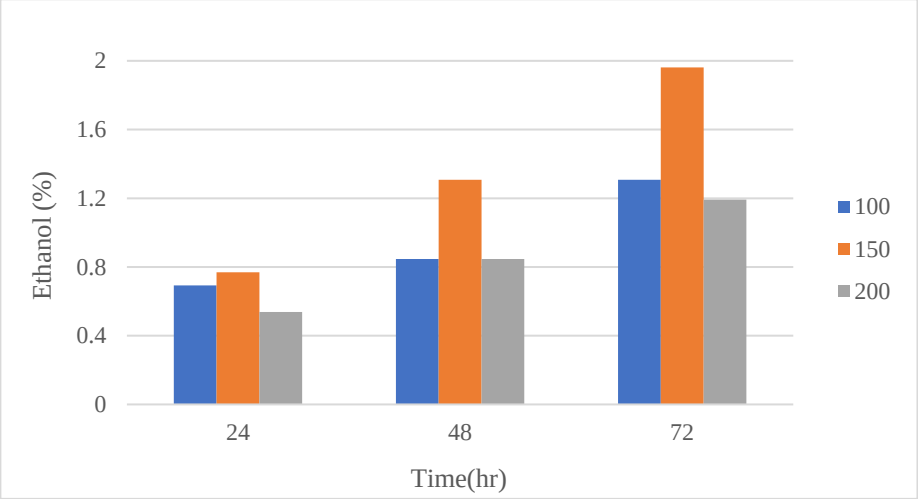

Figure 4. Graph of ethanol percentage at various stirring speed.

Ethanol yield

The ethanol yield for each stirring speed for SSF is determined using the Equation 3 by [18].

$$\text{Ethanol yield (\%)} = \frac{[EtOH]_F - [EtOH]_0}{0.511 \times f \times [Biomass]_0 \times 1.111} \times 100\% \quad (3)$$

where the term $[EtOH]_f$ is final ethanol concentation, $[EtOH]_0$ is the intial ethanol concentration, $[Biomass]_0$ is the initial dry biomass concentration (g/L, w/v), f is the cellulose fraction (g/g, w/w), 0.511 is the conversion factor for glucose to ethanol, 1.111 is the conversion factor for cellulose to equivalent glucose [18].

The samples from the EFB pretreatment were analyzed using TGA and the cellulose fraction, f that obtained from the TGA analysis is 42.8%. Figure 5 shows the result of ethanol yield at 100 rpm, 150 rpm and 200 rpm for every 24 hours. Based on Figure 5, it shows increasing trend for ethanol yield for every 24 hours due to the production of ethanol through fermentation. Ethanol yield for 150 rpm is the highest for every 24 hours, followed by 100 rpm and 200 rpm. As stirring speed increases, the mixing of material and the solution is improved, hence boosting the ethanol production. However, the ethanol yield for stirring speed of 200 rpm is the lowest may be due to limited extent of cellulose conversion by the high mixing speed and at such a high speed, there is also a possibility of enzyme being denatured. Thus, the production of ethanol reduced. It can be seen that the trend of ethanol yield in Figure 5 is similar to ethanol concentration in Figure 4, as the ethanol yield from the process depends on the final concentration of ethanol from SSF process.

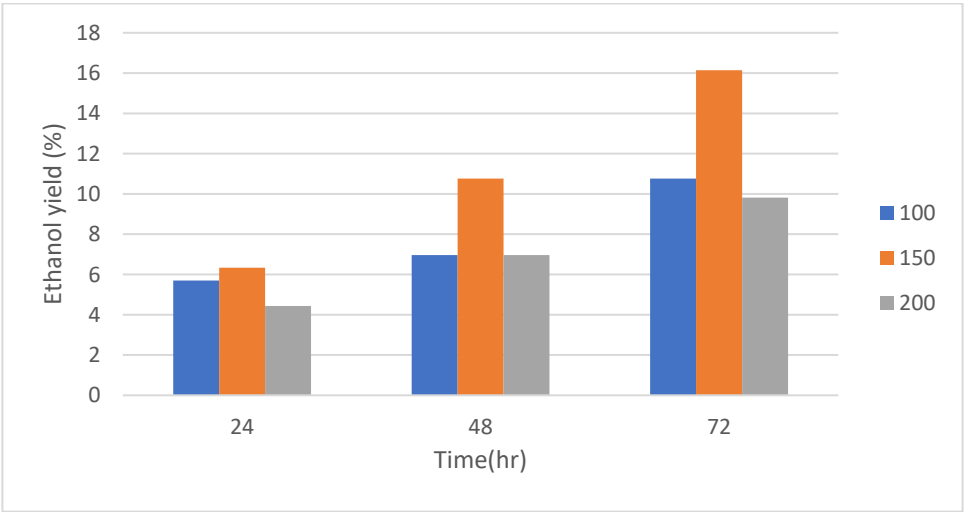

Figure 5. Graph of ethanol yield at various stirring speed.

CONCLUSION

This study was mainly focused on the effect of stirring speed towards glucose concentration and ethanol yield from the conversion of EFB via SSF process. The influence of stirring speed in SSF proved that 150 rpm is the most suitable stirring speed to be used in SSF compared to 100 rpm and 200 rpm based on the glucose concentration and ethanol yields obtained. Glucose concentration and ethanol yield of 100 rpm and 200 rpm in SSF for stirring speed parameter happened to be less than 150 rpm for every 24 hours of analysis. For optimal heat and mass transfer requirements without harming or denaturing the enzymes or fermenting microorganisms, precise mixing of the mixture is critical [19]. Combined intervals of no mixing with short periods of high or low speed mixing, called intermittent mixing regimes may help the saccharification process by reducing energy consumption and limiting enzyme inactivation while still providing reasonable conversion yields [19].

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