Research paper

Effects of organosolv pretreatment and acid hydrolysis on palm empty fruit bunch (PEFB) as bioethanol feedstock

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Abstract

Biomass synthesis of palm empty fruit bunch (PEFB) into sugars yield as raw material for bioethanol conversion has successfully been performed through organosolv pretreatment and acid hydrolysis processes. These synthesis processes were conducted to evaluate the effect of solvent concentration namely ethanol (C2H5OH) and sulfuric acid (H2SO4), reactions time and temperature against total sugars yield. It is optimized through pretreatment and hydrolysis processes, respectively. The optimum total sugars for pretreatment optimization was obtained 98.89 mg/L which correspond to 55% vol of C2H5OH with reaction time of 60 min at 120 °C. Hydrolysis process for pretreatment optimization was conducted at 1% vol H2SO4, reaction time of 30 min and temperature of 90 °C. The optimum pretreatment conditions were selected for further hydrolysis process with H2SO4 to optimize sugars yield amount in the slurries. The results of acid hydrolysis showed that the optimum amount of total sugars obtained was 133.17 mg/L at concentration H2SO4 0.5% vol with reaction time 30 min and temperature 100 °C. Therefore, it is concluded that organosolv pretreatment and acid hydrolysis can be used as a novel integrated method to optimize the total sugars production synthesized from PEFB to bioethanol.

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1. Introduction

The current statistic of world energy in 1973—2012 shows that the availability of fossil fuel sources present yearly regular reduction, compared to energy consumption and population growth. It brings the energy price higher [1]. The other issue in the use of fossil fuel is the increase of CO2 concentration content in the atmosphere that leads to climate change [2]. Therefore, alternative sources of energy such as solar, wind and biofuels have attracted attention of many researchers. It leads to development of alternative energy resources to replace the gap of reducing fossil fuel production [3–6].

Production of bioethanol from biomass is one of the alternatives to reduce both fossil fuel consumption and environmental pollution. Bioethanol is a gasoline additive/substitute for biofuel that widely used in transportation proposed [7]. Bioethanol can be synthesized from a few different kinds of materials which can be classified into three categories namely simple sugars, starch and lignocellulose [8]. Recently, the most common ethanol fuel originates from derivative of edible feedstock, such as corn grain (starch) and sugar cane (sucrose) [9]. Energy source from widely available and effective substrate such as lignocelluloses are potential for bioethanol production to make it more competitive in the future compared to fossil fuel [4,8,10,11].

Palm empty fruit bunches (PEFB) are the largest lignocellulosic biomass from waste of palm oil production in a few tropical countries such as Indonesia and Malaysia [12,13]. Lignocellulosic wastes from palm empty fruit bunches are a potential raw material for bioethanol production which consist of cellulose (44.2%), hemicellulose (33.5%) and lignin (20.4%) [14]. Sugars production from lignocellulosic biomass consists of two basic step namely pretreatment and hydrolysis, followed by fermentation and distillation for further ethanol production. Pretreatment is the first step to degrade biomass into sugars through hydrolysis step to enhance
the yield of sugars [15]. The major problem in sugars production from biomass is to break the cell wall, therefore it requires pretreatment process to facilitate the hydrolysis process [16].

A number of literature reported the different pretreatment methods to enhance the digestibility of lignocellulosic material, such as thermal pretreatment, steam pretreatment, alkaline pretreatment, organosolv pretreatment and dilute acid pretreatment. Each pretreatment method has its own effects on the cellulose, hemicellulose and lignin which are the three main components of lignocellulosic biomass [17]. Thermal and steam pretreatment were found as the most economical process when performed at the temperature range of 150–180 °C to avoid solubility of lignin which occurs at temperature higher than 180 °C and at the same time inhibit growth of bacteria [17–20]. It was also reported that alkaline pretreatment using sodium hydroxide and aqueous ammonia negligible effect through delignification process for softwood species which contain a large amount of lignin. These are more effective than using it in agricultural residues or hard wood materials [17,18,21,22]. Recently, many researchers used dilute acid pretreatment for bioethanol production [23–25]. However, the use of acids without separate lignin may generate more inhibitory substances to microorganisms [26].

In this study, organosolv pretreatment was used in optimize delignification process by separating a lignin residue. Organosolv pretreatment is a process to extract lignin from lignocellulosic feedstock using organic solvent or their aqueous solutions [27,28]. The organosolv pretreatment provides a pathway for biorefining of biomass due to its ability to produces some valuable byproduct for further synthesis of lignocellulosic feedstock [29]. Aqueous ethanol seems the most effective as organic solvent in organosolv pretreatment for lignocellulosic biomass. Xu and Pan et al. have found more than 90% sugars by organosolv pretreatment and enzymatic hydrolysis on softwoods and hardwoods [30–33]. Crude glycerol is also potential as solvent in organosolv pretreatment. However, delignification seems to be low (≤30%) due to lipophilic compounds from crude glycerol, which was inhibited the delignification [34]. Organosolv methods are commonly used to mitigate waste issues in pulp and paper industries due to its advantages of easy recovery solvent and reusability of chemical in pretreatment. It is also able to isolate the lignin waste as a solid and carbohydrates waste as syrup [35].

Hydrolysis process is required in the conversion of holocellulose into glucose and other monosaccharide. Acid hydrolysis has been used in this study to hydrolyze the feed stock after organosolv pretreatment process. Diluted acid hydrolysis has successfully been developed for bioethanol pretreatment process. Dilute sulfuric acid pretreatment can achieve high reaction rate and improve holocellulose hydrolysis [36]. Acid hydrolysis process is easier than enzymatic process because of the lower cost and it is simpler to use.

Furthermore, in this study we proposed biomass from palm empty fruit bunch (PEFB) as a feedstock for synthesis of total sugars using a combination method between organosolv pretreatment and acid hydrolysis. The objective is to obtain an optimum result of the total sugars, which is used for further conversion of bioethanol. The variation of solvent concentration, reaction temperature, and time were used as parameters to identify which combination of the variables that yield an optimum amount of sugars at pretreatment process and optimizing it at acid hydrolysis process, simultaneously.

2. Material and methods

2.1. Material preparation

Palm empty fruit bunches (PEFB) were collected from local plantations located in the city of Aceh, Indonesia, milled up into small pieces approximately 1 cm width and dried at 105 °C for 24 h. Dried pieces of PEFB were grinded to ensure a small size of feedstock material in the range of 500–710 μm before being stored in dessicator. C2H5OH (ethanol 99.5% ASC reagent absolute 200 proof, Across Organic), H2SO4 (Sulfuric Acid 95% solution in water, Across Organic) and NaOH (Sodium Hydroxyde pellet, Fisher Scientific) were supplied locally.

2.2. Organosolv pretreatment

Fig. 1 shows the pretreatment and hydrolysis process used to synthesis PEFB biomass into sugars. The organosolv pretreatment method was performed chemically as delignification process by dissolving dried PEFB with aqueous ethanol at solid-to-liquid ratio 1:10 (10 g in 100 mL) in erlenmeyer flask. The solutions were carried in variation of concentration (35, 55, and 75% vol), reaction temperature (80, 100, and 120 °C) and reaction time (30, 60, and 90 min). The residue obtained from the pretreatment process as sugars was separated from ethanol by filtration process and washed by hot distillate water simultaneously; it is indicated by clear color. These residues were then dried at 105 °C for 24 h to obtain a constant weight. The pretreated PEFB were carried out for lignin content analysis by Kappa number analysis and were hydrolyzed by acid hydrolysis at conditions of 1% vol H2SO4, reaction time 30 min and temperature of 90 °C.

2.3. Lignin analysis

Kappa number analysis was conducted to analyze the effect of pretreatment on lignin content. A 800 mL distilled water with 4 g of pretreated PEFB was stirred at 25 °C in a 1000 mL volumetric flask. Reagent of 100 mL Sodium permanganate (KMnO4) 0.1 N, 100 mL Sulfuric acid (H2SO4) 4.0 N was poured into the solution (final volume 1000 mL). Potassium iodide (KI) was added to the solution after 10 min reaction. The solution was titrated by sodium thiosulfate (Na2S2O3) 0.5 N and indicator starch 1 N. Blank (standard) was carried out by the same step without PEFB. Lignin content was obtained via the following formula [37,38]:

\[
p = \frac{(b - a) \times N}{0.1} \quad (1)
\]

\[
\log k = \log p/W + 0.00093(p - 12.5) \quad (2)
\]

\[
%\text{lignin} = 0.147 \times k \quad (3)
\]

where k is Kappa number (volume in milliliters of 0.1 N KMnO4 solution consumed by one gram PEFB), p is the volume of KMnO4 (mL), a is volume of titrant Na2S2O3 for PEFB titration, b is volume of titrant Na2S2O3 for blank titration, W is weight of sample and N is Normality of Na2S2O3 [37,38].

2.4. Acid hydrolysis

The optimum organosolv pretreatment condition was selected based on amount of sugars yield at chosen hydrolysis condition. The pretreated PEFB were hydrolyzed by sulfuric acid (H2SO4) solvent at solid-to-liquid ratio of 2:5 in a 100 mL volume erlenmeyer flask. The erlenmeyer containing pretreated PEFB and sulfuric acid was loaded to water bath shaker (WNB14, Memmert). The condition of hydrolysis was varied in order to observe the effect of hydrolysis condition through total sugars yield. The condition chosen for this study are sulfuric acid concentration (0.5% vol, 1%
vol and 2% vol), reaction time (60 min, 70 min, 80 min, 90 min and 100 min), and temperature (30 °C, 45 °C, 60 °C and 70 °C). The hydrolysate was then separated from solid residue in order to evaluate the total sugars yield.

2.5. Total sugars yield analysis

The hydrolysates were carried out to measure total sugars yield by phenol-sulfuric acid method. A glucose standard solution of 2 mL was prepared in various concentrations of 0, 10, 20, 30 and 60 ppm mixed with 1 mL phenol solution (concentration of 5% vol) in a test tube, respectively. Furthermore, 5 mL of concentrated sulfuric acid was dropped to the surface of the solution vertically and the mixture leaved for 10 min before the measurement of spectrophotometer (HACH DR/2010) at wavelength 490 nm. Every measurement was conducted twice. The result of the measurement is known as standard curve. Moreover, the sugars solution was prepared by mixing hydrolysate and distillate water at a composition solid-liquid ratio 1:250 as a sample solution. A 1 mL sample solution was mixed with 1 mL distilled water in a test tube. H2SO4 (purity 98%) and phenol solution (concentration 5% vol) was added simultaneously to the test tube with composition liquid-liquid ratio 5:1 prior to their spectrophotometer measurement [39].

3. Results and discussion

3.1. Effect organosolv pretreatment on PEFB

Pretreatment process was conducted to break lignin bond with hemicellulose by unbinding the ether chain of molecule lignin, it is also break microphibril crystal chains of molecule cellulose to elevate the degradation into its monomers during hydrolysis process. Organosolv pretreatment process was performed due to the advantageous of easy process, possibility of solvent recovery and the effectiveness of organic solvent to break the internal bonding of lignin with hemicellulose [2]. In addition, ethanol solution was chosen as a solvent since it is considered as low cost organic solvent and is easy to recover [35]. The study about pretreatment on PEFB by various process has been conducted by a number of researchers [16,21,40,41]. Organosolv pretreatment has also studied on softwoods and hardwoods to enhance enzymatic hydrolysis yield in several studies [27,28,31]. However, there is no study conducted organosolv pretreatment to acid hydrolysis. In this study, the effects of organosolv pretreatment on lignin content are presented as Kappa Number. Kappa number estimates the number of lignin left in the sample, which is carried out by titration using potassium permanganate. The volume (mL) of 0.1N potassium permanganate consumed by 1 g sample of PEFB is defined as Kappa number [37]. Lignin content is apparently brown during the heating process, which the clearer solution estimates the less lignin content in the sample. The effect of ethanol concentration on Kappa number is presented in Fig. 5. Kappa number decreased significantly after 35% vol of ethanol concentration. It shows that the number of degraded lignin has increased by the increasing of ethanol concentration. Thus, cellulose and hemicellulose are able to convert into sugars by acid hydrolysis at chosen condition (1% vol H2SO4, reaction time

![Fig. 1. Pretreatment and hydrolysis process of biomass to produce sugars yield.](image)

![Fig. 2. Effect of temperature on sugars yield during pretreatment process at 35% vol ethanol concentration (Hydrolysis condition of 1% vol H2SO4, 30 min reaction time and temperature of 90 °C).](image)
30 min and temperature of 90 °C) in order to investigate the effect of organosolv pretreatment on sugars yield.

The total sugars yield is effected by ethanol solvent concentration in certain range temperature and reaction time. Figs. 2 to 4 present the yield of total sugars obtained at each variation parameter (temperature and C₂H₅OH concentration) and different reaction time of pretreatment. Fig. 3 presents the optimum yield of total sugars, which is obtained 98.89 mg/L at condition of 55% vol ethanol concentration, temperature of 120 °C and reaction time of 60 min.

Esat Gumuskaya [42] investigated the chemical content of pulp at various heating temperature between 120 °C—200 °C and reported that the sugars content in pulp slurries increased with the heating temperature. On the other hand, lignin content seemed decrease with heating temperature. Thus, it is good to set the heating temperature higher than 120 °C in delignification process in order to obtain high sugars yield. The high temperature reaction promote higher reaction rate, which elevated separated lignin from biomass. However, the reported thermal stability of hemicellulose was between 180 and 340 °C and will be degraded above 340 °C. Hence, the heating temperature higher than 180 °C might cause the decreasing of sugars yield due to the degradation of hemicellulose [20,43]. Figs. 2 to 4 clearly show the comparison of total sugars yield at 30 min, 60 and 90 min, respectively. The highest and the lowest yield of total sugars were obtained at 60 min and 90 min, respectively. It implies that the long duration of heating process of biomass by ethanol solution seems to dissolve some amount of hemicellulose and at the same time increase the solubility of lignin in pulp slurries [20,44].

Figs. 2 to 4 also show that the increasing of ethanol concentration in the solution effects the increasing of total sugars yield. The yield of sugars tends to increase with ethanol concentration of 35% vol and 55% vol. It’s due to the ether chain breaking of lignin and hemicellulose molecules is optimized by the increasing of ethanol concentration, which is allows more hemicellulose and cellulose to be converted into sugars [35]. However, it seems to decrease at ethanol concentration of 75% vol due to the effect of heavy concentration of ethanol that causes a decomposition of some hemicellulose content above 60% vol of ethanol solution addition [31].

3.2. Effect of hydrolysis on sugars yield

The optimum yield (at condition of 55% v ethanol, temperature of 120 °C and reaction time 60 min) of total sugars yield was carried out from pretreatment process for further hydrolysis to obtained more total sugars yield. Hydrolysis process is basically break polymer chain of cellulose and hemicellulose into its monomers. Acid hydrolysis seems to break the glycoside bonding through three main steps which are interaction H₂SO₄ as proton with oxygen glycosides which is bonded with sugar unit, breakdown process of C—O bond and formed cation intermediates carbanium, and finally the interaction of H₂O molecules form into a stable sugars to release proton [45]. Furthermore, hydrolysis also degrades hemicellulose into simple sugars such as glucose, arabinose, mannose and dextrose to enhance total sugars yield [46].

The total sugars obtained after hydrolysis by variation of concentration H₂SO₄, heating temperature and reaction time was presented in Figs. 6–8. It shows that the yield of sugars tend to decrease with the increasing of H₂SO₄ concentration. Orchidea [46] in his work also reported the reduction of sugars yield with the acid concentration. High acid concentration causes the degradation of cellulose and hemicellulose and also produced formic acid and furfural that decreased the total sugars yield [46]. Hence, the low acid concentration solution seems to work better and avoid furfural byproduct which could inhibit fermentation process for further
bioethanol production process. Furthermore, the reaction time was not linearly affected against sugars yield. The highest yield is at 45 min and started to decrease in reaction time above 45 min. Palmqvist and Hahn-Hagerdal [47] in their work of fermentation of lignocelluloses reported that a long duration of hydrolysis reaction will formed a toxic inhibitor that becomes hydroxymethyl-furfural to produce formic acid. While the degradation of lignin formed phenol compounds that can inhibit microorganism growth in the fermentation of bioethanol production [48]. Inversely, the increase of heating temperature shows a significant effect on it. According to Humprey [49], temperature range 80–120 °C will degrade cellulose into its monomers for reaction time of 30–240 min. Fig. 4 shows the optimum condition of the hydrolysis process performed at 0.5% vol H2SO4 concentration, 45 min reaction time and heating temperature 100 °C. The corresponding total sugars yielded at these condition was 133.17 mg/L. This shows that the increasing of temperature seems to optimize the breaking of cellulose and hemicellulose polymer chains into sugars. Thus, the sugars yield is increase by the increasing of temperature reaction.

4. Conclusions

The effect of organosolv pretreatment and acid hydrolysis processes using ethanol solvent and sulfuric acid, respectively to convert PEFB into sugars for bioethanol feedstock were successfully evaluated in this study. The lignification of biomass with ethanol 55% vol (C2H5OH), at 120 °C for 60 min seems to produce an optimum yield of total sugars of 98.89 mg/L. Moreover, acid hydrolysis process which involves the hollocellulose into its monomers is also able to optimize the total sugars yield using sulfuric acid (H2SO4) at 0.5% vol and 100 °C for 45 min. These results suggest that palm empty fruit bunch (biomass) have a good potential to be used as a feedstock for further bioethanol conversion. However, a study of pretreatment to obtain the highest sugars yield to generate better ethanol content is necessary.

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References

[10] S. Chiesa, E. Gnansounou, Use of Empty Fruit Bunches from the Oil Palm for


