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Submission date: 07-Jan-2020 08:44AM (UTC+0700)
Submission ID: 1239660693
File name: C15.pdf (226.24K)
Word count: 3190
Character count: 17196

Journal of Energy and Power Engineering 11 (2017) 679-686
doi: 10.17265/1934-8975/2017.11.001

Effect of Glycerol Concentration and Heating Treatment on Delignification and Bioethanol Production of Sago Dregs

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Received: September 26, 2017 / Accepted: October 19, 2017 / Published: November 30, 2017

1. Introduction

The total production of Indonesian oil is decreasing, while the consumption is increasing. Issues of energy scarcity trigger a development of bioethanol renewable energy. The trend of an increased use of a renewable energy in 2007-2008 has triggered a crisis in the world food prices, which rise sharply, up to 75%, caused by the transfer of the use of food into biofuels [1]. Therefore, we need other non-food raw materials.

Lignocellulosic biomass is a potential source of raw material that is abundant, not including food, and that has not been widely used, and contains sugar structures that can be converted into bioethanol. The lignocellulosic biomass is a biomass coming from plants with the main components of lignin, cellulose, and hemicellulose that is not readily biodegradable [2]. One of the ingredients of lignocellulose is a sago dreg. Sago industry in the village of Plajan, Jepara produces a sago dreg waste that has not been utilized by the surrounding community and pollutes the environment. The lignocellulose material of the sago dreg is abundantly available to make this material potentially as one of the basic material of bioethanol manufacture.

The content of lignin in the sago dreg prevents an access to cellulose and hemicellulose, which will be the basic ingredient of bioethanol, so it needs to be done the delignification first. One of the delignification methods is organosolv delignification. The delignification organosolv is a pulping process with a solvent such as glycerol. The glycerol is a by-product of a biodiesel production. The rapid growth of a global biodiesel production indicates that a crude glycerol from biodiesel industry will be a cheap waste with a high cost to treat rather than a valuable by-product in the future. It also can be a

Corresponding author: Muhammad Luqman Hakim, master degree, research fields: bioethanol, renewable energy.

serious environmental problem. Therefore, the exploitation of the crude glycerol for pretreatment of biomass has a potency to be an attractive economic route for the utilization of the by-product directly, leading to commercial success of both bioethanol and biodiesel [3].

The previous research used surfactants to delignify the lignocelluloses and delignification by 20% [4], while the problems caused by the use of surfactant are an environmental pollution, especially by non-biodegradable petroleum-based surfactants.

In addition to being environmentally friendly, the use of the glycerol also has several advantages: (1) the low solvent cost as a raw glycerol is produced in the transesterification process for biodiesel production, (2) the pretreatment can be done under an atmospheric pressure so as to reduce the energy consumption, (3) the glycerol can easily penetrate the lignocellulosic biomass network and provide an effective reaction medium for the delignification in the high polar structure [5]. Meanwhile, according to Ref. [6], higher temperatures will simplify the decomposition of simple sugars and lignin compounds. Thus, the glycerol utilization and heating time are expected to delegate optimally the sago dreg. Ref. [5] reported that the use of 80% of glycerol at the heating time of 150 minutes at a temperature of 198.3 °C is able to produce pulp of 54.4%, lignin of 7.75%, deglinification of 81.4%, and hemicellulose content of 13.7%.

The above literature showed that the glycerol can be used to delignify the sago dregs with heating treatment. Therefore, the aim of this work was to find the suitable condition between the glycerol concentration and heating time in the process of delignification and bioethanol production.

2. Material and Methods

2.1 Materials

The material used is the sago dreg waste taken from one of sago flour producing industry in Plajan Village, Pakis Aji Sub-district, Jepara Regency, aquadest, glycerol, bread yeast (fermipan), urea, NPK.

2.2 Procedures

The sago dregs were taken from one of the sago industry in the village of Plajan Jepara. The process of delignification began by cutting the sago dreg approximately 1-2 cm with a pair of cuttings of 250 g of sago dregs inserted into the pan to be heated together with different glycerol formulas to boil with different heating time. During the heating process, the glycerol was stirred with the sago dregs. After the heating process was completed, allowed the sago dregs to cool to the temperature of 30 °C. The sago dregs were separated by glycerol using filters and then analyzed the content of the lignocelluloses (lignin, alpha-cellulose, and holocellulose) with Klason method [7].

After the delignification was completed, the bioethanol production process was started from hydrolysis, fermentation, and distillation. The process of hydrolysis began with cooking 1 liter of water with the sago dregs that had been delegated to boil. During the heating process, stirring was done with a stir bar. The heating was stopped after boiling. The levels of glucose were measured using a brix refractometer. After it cooled down, the liquid was transferred into the fermentor.

The fermentation process was done by adding yeast bread fermipan as much as 1.25 g into fermentor, which had been filled with a sago dreg liquid resulted from hydrolysis process. How to add the yeast bread fermipan into the fermentor was by putting and stirring the yeast into 100 cc warm water and then let it for 10 minutes. Added urea of 2.5 g and NPK of 0.625 g, then stirred them. The fermentation was carried out for 66 hours. The levels of glucose after fermentation were measured using a brix refractometer [8].

The distillation process began by preparing a distillator consisting of heating stove components, vacuum tubes, cooling tubes, hoses, reflux, vacuum pumps, and

Table 1 Research design.

Heating time	Glycerol concentration			
	G0	G1	G2	G3
T0	G0T0	G1T0	G2T0	G3T0
T1	G0T1	G1T1	G2T1	G3T1
T2	G0T2	G1T2	G2T2	G3T2

G0: glycerol 0%; G1: glycerol 30%; G2: glycerol 60%; G3: glycerol 90%; T0: 0 minute; T1: 15 minutes; T2: 30 minutes.

aquarium pumps. After the distillator was ready, the fermented liquid was fed into the vacuum tube. The liquid was heated to an evaporation temperature of 79-80 °C and the steam was fed to a cooling tube so that happened to a condensation process, which separated the bioethanol from the water. During the distillation process, the temperature was maintained at 79 °C. The bioethanol content was measured by alcohol refractometer or alcoholmeter.

2.3 Research Design

The research design used was completely randomized design of factorial pattern consisting of two factors 4 × 3. The first factor was the concentration of glycerol; the second factor was the duration of heating (as Table 1). Therefore, it was obtained 12 treatment combinations and each treatment was repeated 3 times replication.

3. Result and Discussion

3.1 Delignification Process

Delignification is one of the treatments that influence the bioconversion of lignocellulose biomass into bioethanol because the delignification has an effect on the hydrolysis and fermentation process. The data collection of lignocellulosic content was done at two times, before and after the treatment. The lignocellulosic levels (alpha-cellulose, holocellulose, and lignin) were analyzed by Klason method. The results of the lignocellulosic content analysis after the treatment can be seen in Fig. 1.

Fig. 1 shows irregular ups and downs. This is due to the mutual interaction between G and T treatments, also due to a pretreatment process with dynamic and complex organosolv method. So do not be surprised if the treatment in this study experience up and down irregularly. According to Ref. [9], a pretreatment process is a multi-scale and non-uniform structure interaction system. The complex and dynamic heterogeneous structure is the key factor influencing the transport and reaction processes, which result in the large of different pretreatments results.

The levels of alpha-cellulose, and holocellulose before treatment amounted to 37.6% and 39.51% and then increased afterward. While the lignin levels before the treatment amounted to 36.56% and decreased afterward. This shows that the treatment has successfully delignificated the sago dregs so that the access to the alpha-cellulose and holocellulose is achieved.

3.1.1 α-Cellulose

α-Cellulose is a linear chain of β-1,4 linked D-glucose units, has a large number of hydroxyl groups along its backbone and forms well-ordered hydrogen and van der Waals bonding networks that are responsible for its compact crystalline structure [10].

α-Cellulose on the G3 treatment showed the most optimal result than other treatments, i.e. on the G3T1 and G3T2 treatment, which respectively resulted in 39.64% and 39.37% of α-cellulose content. The G3T1 and G3T2 treatment also showed no significant differences. The high concentration of glycerol on the G3 treatment caused the sago dregs were optimal delignification so that it did not need to take longer heating time, the delignification was optimal by the use of T1, without having to use T2 heating time. Ultimately, the most effective treatment to delegate the sago dregs was the G3T1 treatment.

The positive interaction demonstrated by G3T1 treatment, will at least benefit the second-generation of bietanol producers, especially those using sago dregs to save the cost of bioethanol production and increase their profits. This is also the goal of

researchers such as Refs. [11-14] to obtain an effective and efficient delignification treatment. Wang [15] hopes that with the discovery of an effective and efficient method of delignifying lignocellulose, may save 35-45% production cost.

3.1.2 Holocellulose

Holocellulose is a total fraction of carbohydrates consisting of cellulose and hemicellulose [16]. The hemicelluloses are heteropolymers (xylan, mannan, galactan, and arabinan, glucuronic acid, acetyl groups and uronic acids) and contain ˜D-xylopyranosyl residues linked by 1,4 glycosidic bonds [17].

Fig. 1 shows that the treatment of glycerol concentration and heating time did not give a significant difference between control (G0T0) and other treatments. Even the controls show the results are quite optimal that produces holocellulose content of 66.32%, although it was not the best results. G3T0 shows the most optimum results among other treatments, which yields a holocellulose content of 67.37%.

Fig. 1 also shows that in the treatment of G0, G1, G2 and G3 the most efficient and effective in delignifying the sago dreg is on the T0 heating time. The results of this study can be used for second-generation bioethanol producers because it can save the cost of bioethanol production. Therefore, a high concentration of glycerol and a long heating not always will produce a lot of holocellulose. Precisely by happening like this, proving that between the treatment G and T occurs interaction.

The resulting holocellulse content is influenced by internal and external factors. According to Ref. [18] internal factors include sago dreg, time of harvesting sago, and how sago tree is cultivated. According to Ref. [19] external factors that affect many holocellulose content are the pretreatment method used, because each method of pretreatment has its own advantages and disadvantages. Chen [9] added that different pretreatment methods have different key points. Soevaluating various pretreatment methods directly through thetest data is not accurate. Therefore, a scientific, economically feasible, and highly productive pretreatment method should be developed on the basis of the evaluation standard.

3.1.3 Lignin

Lignin is a complex hydrophobic, cross-linked aromatic polymer that interferes with the hydrolysis process. It has a three-dimensional heterogeneous polycrystalline reticulated polymer, which belongs to polyphenolic compounds. Such polymeris formed by phenyl propane structural units via ether linkages and carbon—carbon bond connection, and it lacks regularity and order liness of the repeating units [20].

Fig. 1 showed the treatment of giving the glycerol concentration and heating time did not show the significant differences between (G0T0) control and other treatments. The differences in results that occurred in G0, G1, G2, and G3 were interesting to discuss as it happened a good interaction.

The G3 treatment showed the optimal results than others, i.e. the G3T0 and the G3T2 respectively produced 11.31% and 12.65% of delignification. The most efficient and effective treatment between those two, however, was the G3T0 as it only needed T0 to make the delgnification ran optimally. The G0 treatment also showed the same as the G3 that it did not need a long heating time to make delignification ran optimally. The G0T0 treatment was more efficient and effective compared to the G0T1 and G0T2 treatments as it could cut the expense of the second generation of bioethanol production from the lignocellulose biomass material.

However, it was different from the G1 and G2 treatments. In order to degrade the lignin structure on the G1 and G2 treatments, a long heating time (T2) was needed. The G1T2 treatment showed the more optimal result compared to the G1T0 and G1T1 treatments. The G2T2 treatment also showed the more optimal result compared to the G2T0 and G2T1 treatments. This showed that the more a heating time given, the more the delignification ran optimally. This

is in accordance with the research of some experts, Ref. [5] reported that the optimal conditions for delignification by manipulating the physical factor are at the temperatures of 141.7-198.3 °C for 23-277 minutes; Ref. [21] reported that the best delignification pretreatment on straw was by heating at the temperature of 217.7 °C for 42.2 minutes producing 69% of glucose levels. Zhang [22], did a heating at the temperature of 200-240 °C for 4-12 minutes to break the cell wall of biomass.

Fig. 1 also shows the differences in the results of alpha-cellulose, holocellulose and lignin analysis. The holocellulose shows the highest results compared to the alpha-cellulose and lignin. The holocellulose is the term used to refer to the cellulose and hemicellulose. The hemicellulose is more easily degraded than lignin or cellulose. This is because the degree of olimerization or hemicellulosic molecular weight is lower when compared to the lignin and cellulose and the structures of hemicelluloses, which are random, amorphous, and branched [2]. The hemicellulose begins to degrade at the temperature of 180-340 °C [23]. The cellulose will be degraded at the higher temperatures between 315-380 °C due to the crystalline and amorphous cellulose compositions. While the lignin becomes the most difficult part to decompose with decomposition temperature reaches 900 °C due to the structure of lignin, which consists of aromatic rings with various branches [24].

3.2 Production of Bioethanol

The production of bioethanol consists of 3 main stages, namely hydrolysis, fermentation, and distillation. The parameters observed in the process of bioethanol production were the large decrease of glucose level between after and before the fermentation and bioethanol content. The results of the analysis of glucose levels are presented in Fig. 2 and the levels of bioethanol are presented in Fig. 3.

Fig. 2 showed the treatment of glycerol concentration and different heating time showed no significant difference between control (G0T0) and other treatments. Although not significantly different, some treatments showed optimal results, i.e. in the treatment of G1T2, G2T2, and G3T2 a large decrease in glucose levels by 3%. This becomes a successful parameter of fermentation.

Cellulose and holocellulose delignification results will be continued to the hydrolysis process resulting in simple sugars (glucose). Glucose will be used by bread yeast to grow and develop. The higher the glucose content after treatment is, then the bread yeast growth is more optimal. The more optimal the growth of yeast bread is, the more glucose consumed so that glucose levels after fermentation will decrease. The lower the glucose level is after fermentation, the higher the expectation of the higher the bioethanol produced.

This decrease in glucose occurs due to the use of glucose by bread yeast to sustain life. Bread yeast requires energy such as ATP (adenosine triphosphate) and to get it then the yeast microbial bread consumes sugar that can be glucose and other simple sugars. Glucose is used yeast for two things that are to grow and multiply, some will be converted into metabolite products such as alcohol [8].

The next stage is the distillation that will produce bioethanol levels. The most optimal treatment in the bioethanol production process is shown in the G3 treatment which produces the highest bioethanol content of 25% (Fig. 3). This is not surprising because from the beginning of the treatment of delignification, hydrolysis, and fermentation, the G3 treatment does indicate that it will produce the highest bioethanol levels. In general, the end result of bioethanol production is determined by the pretreatment process (delignification) and three main processes namely hydrolysis, fermentation, and distillation. In particular, the end result of bioethanol production is influenced by many visible things (pH, temperature, other physical conditions) and unvisible (yeast conditions) [25-27].

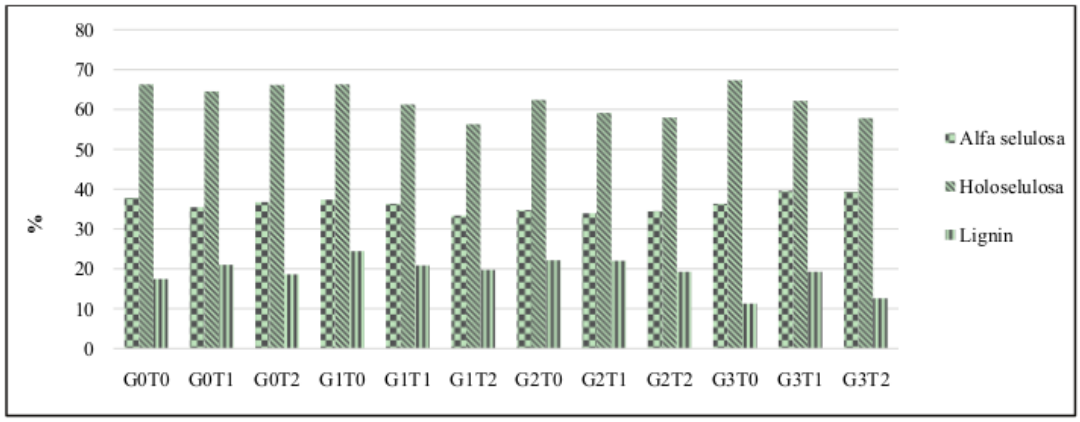

Fig. 1 The average data of the lignocellulose level analysis after the treatment.

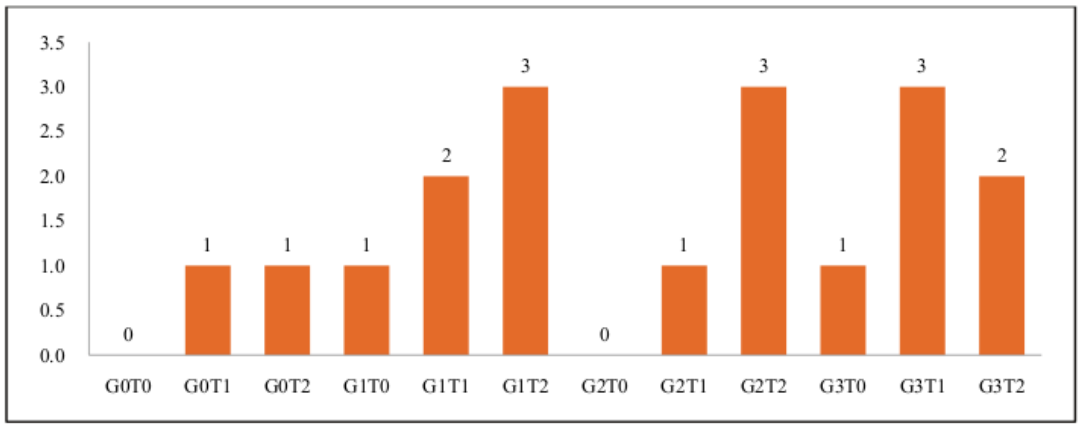

Fig. 2 The large decrease glucose levels after fermentation with before fermentation.

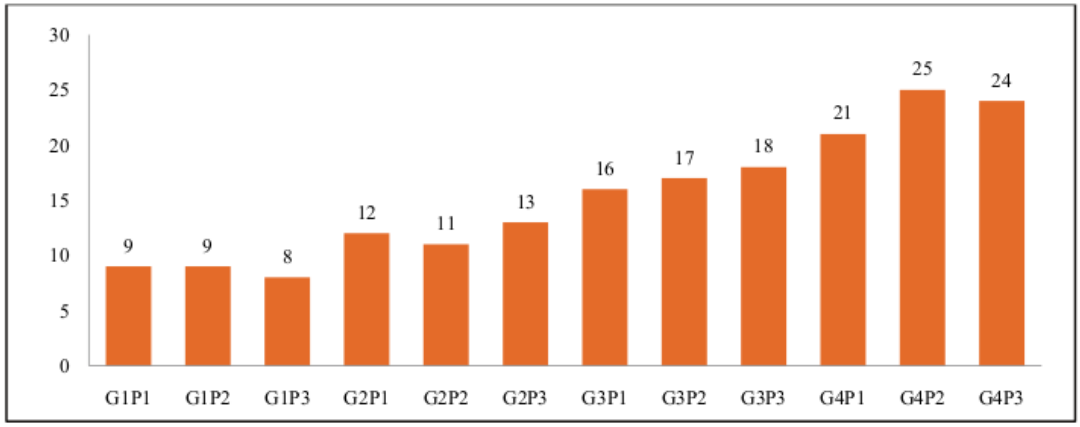

Fig. 3 The avarage data of the bioethanol content.

In contrast, the control treatment (G0T0) looks surprising. Its delignification looks optimal, but its bioethanol content is the lowest. This is because the fermentation process is not optimal. The lessons that can be learned from this study is not necessarily the one that looks good in the beginning, it will be good also at the end. In addition, it does not necessarily look good at the beginning, will still be not good at the end. Hopefully, we can keep having a positive thinking to whatever it is.

4. Conclusions

The most optimal treatment in the delignification of the sago dregs was the treatment of 90% of glycerol concentration with a heating time of 15 minutes resulting 19.3% of lignin content, 39.64% of alpha-cellulose content and 62.18% of holocellulose levels. Then, it was followed by the bioethanol production and produced 25% of bioethanol.

Acknowledgements

This work was supported by project funds from the Ide Gila Energy Competition PT PERTAMINA Indonesia.

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