Biohydrogen Production through Separate Hydrolysis and Fermentation and Simultaneous Saccharification and Fermentation of Empty Fruit Bunch of Palm Oil

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Abstract

Indonesia produces 32 million tons of palm oil per year, the largest volume of yearly palm oil production. This massive amount of palm oil production has led to the accumulation of a large amount of empty fruit bunch (EFB) disposed as solid waste. EFB acts as a lignocellulosic biomass consisting of 43 % cellulose, 23% hemicellulose and 33% lignin. EFB can potentially be utilized as a raw material to produce biohydrogen, one of the various bio-energy forms. The EFB was initially subjected to delignification using Aspergillus fumigatus for seven days at 30°C in the pH range of 5-6. This biodelignification is intended to increase the accessibility of cellulase towards the EFB biomass before being converted into biohydrogen by Enterobacter aerogenes.

In this work, the delignified EFB was subjected to produce biohydrogen through SHF and SSF methods. The results demonstrate that the production of biohydrogen using EFB as raw material through the SHF method has many weaknesses. The main disadvantage was the product of reducing sugar (RS) inhibits cellulase activity. Cellulase was found to be inactive and sugar production stopped during the fermentation process. Furthermore, E. aerogenes underwent material insufficiency to be converted to hydrogen. The maximum product of biohydrogen was 15.5 ml/g EFB on 260 g EFB/L, with the cellulase concentration of 36 FPU/g EFB. Biohydrogen production using E. aerogenes was significantly affected by the cellulase concentration and the amount of EFB. The production of biohydrogen increased significantly using the SSF method. The highest production of hydrogen gas was 635.3 ml which was achieved at the EFB weight of 220 g/L with a cellulase concentration of 36 FPU/g EFB. The effectiveness of the SSF method for biohydrogen production is shown by the reduction of cellulose (28.70%) and hemicellulose (23.53%), compared to the SHF method.

Keywords: Biohydrogen, Saccharification, Fermentation, Empty Fruit Bunch of Palm Oil.

Introduction

The Indonesian Palm Oil Association states that Indonesia has a long-term target of producing 40 million tons of CPO per year by 2020. This means that the waste of oil palm in the form of empty fruit bunches will also be abundant. The significant quantity of this palm oil waste allows for an alternative source of renewable energy[1]. The EFB constitutes a lignocellulose material that consists of several chemical components, namely, 43% cellulose, 23% hemicellulose and 33% lignin. The trace element of sulphur and nitrogen indicates that the EFB is an environmentally-friendly biomass[2,3]. The EFB can potentially be used as raw material renewable energy because it is abundant, has high cellulose content and is a non-food source.

Hydrogen has been suggested as the ideal fuel of the future. It is considered among the cleanest energy carriers to be generated from renewable sources[4]. Interestingly, hydrogen has a potentially high efficiency of conversion to usable power, low generation of pollutants and high energy density[5]. The current global hydrogen production amounts to around 700 billion Nm^3 and is based almost exclusively on fossil fuels[6]. However, hydrogen could substitute fossil fuels if it is produced from renewable feedstock other than fossil fuels.[7]

Among the various renewable energy sources, biohydrogen is currently increasingly gaining traction, attributable to its high efficiency. Various technologies are readily available to produce biohydrogen from lignocellulosic biomass such as direct biophotolysis and dark fermentations. However, it has several drawbacks (low yield and slower production rate), limiting its practical application of conversion to usable power with less pollutant generation. Biohydrogen production from lignocellulosic biomass is particularly suitable for relatively small and decentralized systems and it can be considered an important sustainable and renewable energy source. The comprehensive life cycle assessment (LCA) of biohydrogen production from lignocellulosic biomass and its comparison with other biofuels can be employed as a tool for policy decisions.[1]

This study aims to investigate biohydrogen production from biomass through hydrolysis and fermentation. In order to improve the effectiveness of cellulase accessibility into cellulose and hemicelluloses of EFB, the EFB was treated by bio-delignification[8] through separate hydrolysis and

fermentation (SHF) and simultaneous saccharification and fermentation (SSF).

Material and Methods

Materials: The empty fruit bunch (EFB) was obtained from palm oil plantations in Bengkulu. It was milled and manually modified to a particle size of ± 4 mm. As the raw material of hydrogen production, EFB was delignified using *Aspergillus fumigatus*. The delignification increases the accessibility of cellulase on hydrolysis process of cellulose and hemicelluloses[8]. *Acidothermus cellulolyticus* cellulase was purchased from Meiji Seika Kaisha Ltd. (Tokyo). Cellulose was purchased from Wako Pure Chem. Co. Ltd. (Tokyo, Japan) and stored at room temperature.

Separate Hydrolysis and Fermentation (SHF): Saccharification of delignified EFB was conducted by cellulase. The reducing sugar (RS) was monitored during the saccharification process. The optimation of saccharification was performed by varying the amount of cellulase at 9; 18; 27 and 36 FPU/g EFB. The concentration of reducing sugar in the medium was determined by dinitrosalicylic acid (DNS) method.

Inhibitory Cellulase: The hydrolysis of 7 gr cellulose was performed in pure water as well as in 10 % phosphate buffer at a pH of 5.5-6.5. Cellulose was dissolved in a glucose solution of 0, 5, 10, 15 and 20 g /L. During saccharification, reducing sugar was measured by UV-Vis Spectrophotometry. The concentration of reducing sugar in the medium was determined by the DNS method. Samples were prepared in triplicates and the hydrolysis was carried out using an incubator at 37°C, with an orbital shaker set at 120 rpm. Hydrolysis was run for 96 h. Control samples (without enzymes) were prepared and analyzed for their sugar content at 15 min as well as at the end of hydrolysis to verify whether the sugars degraded over time.

Simultaneous Saccharification and Fermentation (SSF): The SSF method involves a simultaneous process of saccharification and fermentation. The enzymes and *E. aerogenes* were simultaneously introduced during the beginning of the process. The process was carried out in 100 mL vial bottles and placed in a shaking incubator (120 rpm, 37°C) for 96 h. The EFB as a substrate was prepared in 1 – 15 g/L, with a variation of loaded enzymes: 9, 18, 27, 36 FPU/g substrate of cellulase. Up to 10% of the phosphate buffer was added into the bottles, until the total volume of 50 mL (substrate, enzymes and buffer citrate) to keep the process conditions within the pH range of 5.5-6.5. Finally, the *E. aerogenes* was added to the SSF process.

Results and Discussion

Separate Hydrolysis and Fermentation (SHF): The separate hydrolysis and fermentation process is a hydrogen-making method with a hydrolysis step and a fermentation step taking place independently. The EFBs containing cellulose undergo a hydrolysis / saccharification process independently from the fermentation process. This is intended to facilitate control of each stage in order to achieve the desired results. Prior to the treatment of hydrolysis by enzymes, the biomass undergoes pretreatment to state the biomass with cellulase properties.

Figure 1 shows that more reducing sugar was formed while the amount of EFB and cellulase concentration increased. During the saccharification process, the highest reducing sugar observed was 14.8 g/L at 260 g/L TKKS and 36 FPU of cellulase. The EFB at 300 g /L decreased, thus reducing sugar production. This decrease was likely caused by less effectiveness of the mixture. In addition, the increase of reducing sugar is not linear which was probably caused by inhibiting sugar during the activity of cellulase.

After substrates were hydrolyzed to a monomer of sugar, they were fermented to biohydrogen. The fermentation process used *E. aerogenes* at a temperature of 37°C. The amount of reducing sugar was reduced and close to zero at the end of the fermentation process. The *E. aerogenes* consumed the sugars formed during the saccharification process. The product of biohydrogen was low during the fermentation process becassue there was no more sugar formed due to the termination of the cellulase activity. In addition, the yield of biohydrogen of the SHF process is 15.5 mL biohydrogen / g EFB. The primary advantage of this method is that it is possible to carry out the cellulose hydrolysis and fermentation on their own optimum conditions.[10]

Inhibitory cellulase assay: The cellulose hydrolysis step depends on the cellulose structure, the interaction between cellulase enzymes and cellulose fibers and the mechanism of hydrolysis of such enzymes in nature as well as inhibitors formation[11]. Inhibitors are chemical substances that are able to inhibit enzyme activity. The inhibitor works by attacking the enzyme's active site so that it is unable to bind to the substrate and the catalytic function of the enzyme is thus disturbed.

The following approach was taken to observe that the reducing sugar was inhibited by the the presence of pure cellulose. The EFB also contains minerals such as K and Ca. Other studies confirmed that some of these minerals do not act as inhibitors for cellulase. The observed results are shown in fig. 3. Cellulose was dissolved in a glucose solution of 0, 5, 10, 15, 20 g/L. During saccharification, a higher concentration of glucose solution tends to decrease the level of reducing sugar. In a solution without glucose, the resulting reducing sugar is 9.86 g /L which increases up to 13.36, 17.08, 21.25 and 24.593 g /L for glucose solutions of 5, 10, 15 and 20 g/L respectively. We suggest that monosaccharides such as glucose and xylose inhibit cellulase activity.

Simultaneous Saccharification and Fermentation (SSF): SHF is not an effective method to produce maximum biohydrogen. It is caused by the inhibition of sugar in

cellulase activity and the hydrolysis process does not occur during the fermentation step. On the other hand, some advantages of the SSF method are: (i) the product of sugar is directly consumed by *E. aerogenes* to produce biohydrogen and the formed reducing sugar does not inhibit the cellulase activity and (ii) during the fermentation process, cellulase still performs hydrolysis of EFB, hemicelluloses and cellulose to produce reducing sugar.

During this process, the glucose produced by the hydrolyzing enzymes is consumed immediately by the fermenting microorganism present in the culture. This is a significant advantage for SSF, compared to SHF; since the inhibition effects of cellobiose and glucose to the enzymes are minimized by keeping a low concentration of these sugars in the media[9]. Figure 4 shows that the highest production of bihydrogen on SSF method reaches 635.3 ml / gr EFB.

Cellulose, Hemicellulose and Lignin Content: The improved performance of biohydrogen production can be seen from the increase of lignin, as shown in figure 5. The percentage of lignin enhancement is shown by the increase of hemicelluloses and cellulose reduction during the SHF and SSF processes. In this study, lignin was employed as the basis for calculation because lignin does not undergo the hydrolysis process of cellulase.

The amount of lignin in the raw material (EFB) is used as the basis for each process performed. Table 2 shows that the amount of cellulose and hemicelluloses decreased after the hydrolysis step of SHF. This means that during the hydrolysis process, cellulose and hemicelluloses are decomposed by cellulase. After the fermentation process in SHF, the amounts of cellulose and hemicelluloses were not significantly different. This confirms that hydrolysis has stopped. *E. aerogenes* does not produce cellulose and no cellulose and hemicelluloses consumption occurs. The effective reduction value of cellulose and hemi cellulose increases up to 44.56% and 33.65% respectively.

In the SSF method, the reduction of cellulose and hemicelluloses decrease significantly. In accordance with the hypothesis during the production process of biohydrogen, cellulose and hemicellulose were hydrolyzed by cellulase to produce a reducing sugar. Subsequently, the reducing sugar was directly consumed by *E. aerogenes*. The effective reduction value of cellulose and hemicellulose increased up to 73.26% and 57.18% respectively.

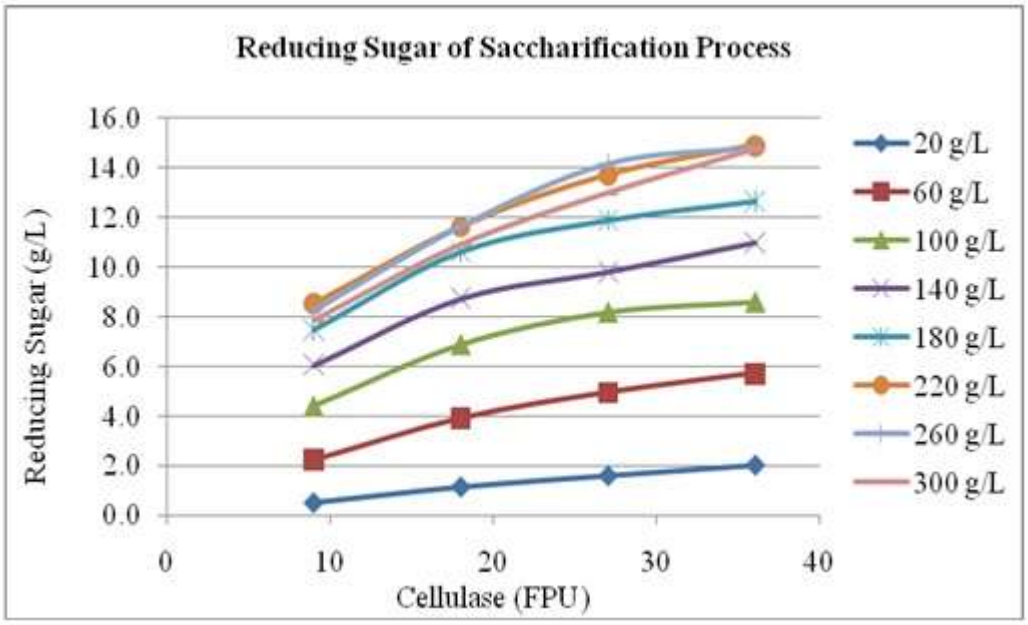

Figure 1: Reducing sugar of saccharification process

Table 1
The composition of lignin, cellulose and hemicelluloses among raw materials, hydrolysis of SHF, Fermentation of SHF and SSF

EFB	Hemicellulose	Cellulose	Lignin
Control	23.18%	43.44%	33.38%
SHF: Saccharification	17.13%	38.40%	44.48%
SHF: Fermentation	17.49%	38.09%	44.42%
SSF	10.66%	31.97%	57.38%

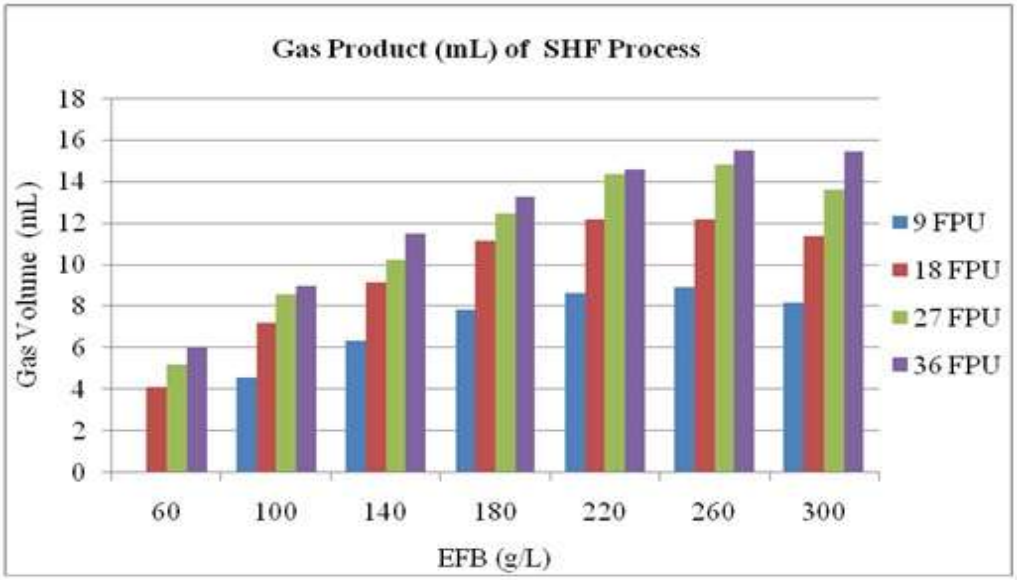

Figure 2: Biohydrogen production of fermentation on SHF process

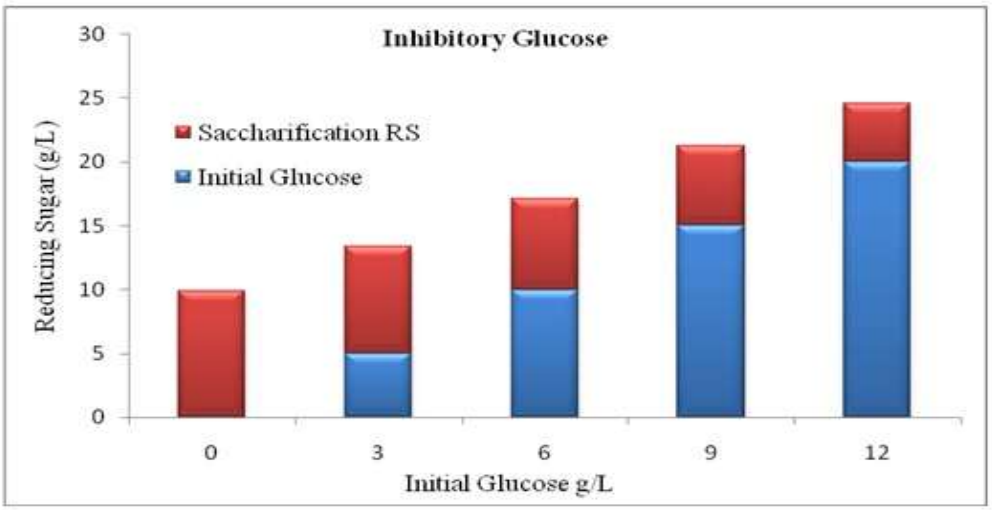

Figure 3: Inhibition of glucose on saccharification process

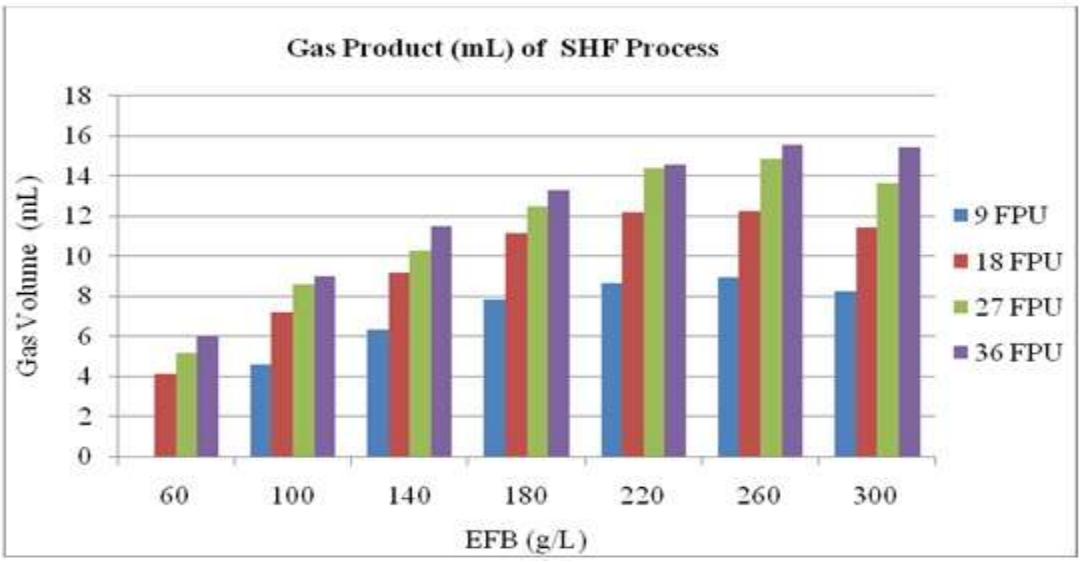

Figure 4: Product of biohydrogen in SSF method

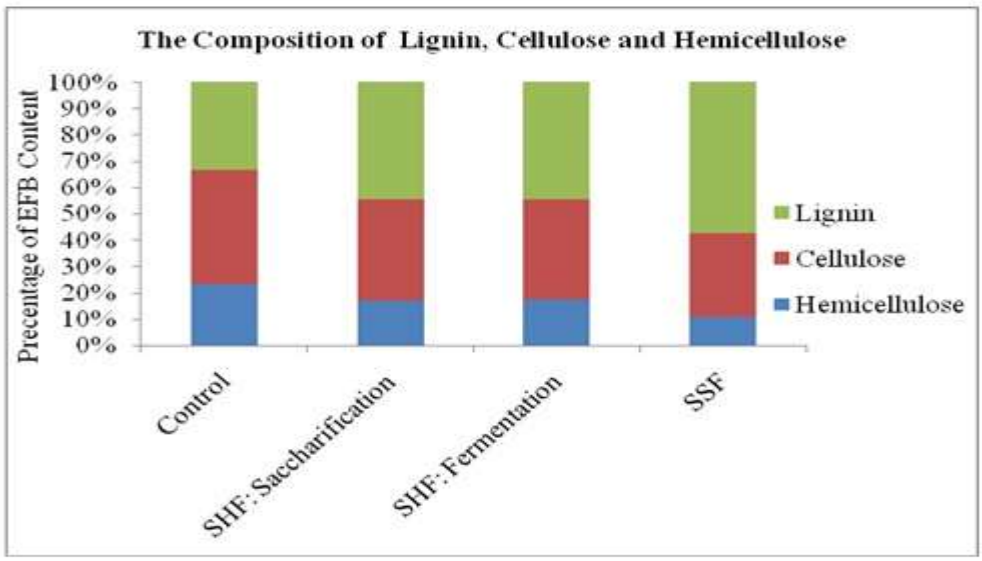

Figure 5: The Composition of lignin, cellulose and hemicelluloses among raw material, hydrolysis of SHF, fermentation of SHF and SSF

Conclusion

1. The EFB can be used as a raw material for the production of biohydrogen.
2. The characterization of the biohydrogen production process using EFB as a raw material can be performed through the SHF method. This process obtained the following results:

a. Reducing sugar resulted from the cellulosic hydrolysis process inhibiting cellulase activity.
b. The hydrolysis process of cellulose was not performed in the fermentation stage.
c. *E. aerogenes* did not produce cellulases or consume celluloses and hemicelluloses.
d. The amount of reducing sugar formed during the saccharification process determines the amount of biohydrogen production.
e. The maximum production of biohydrogen was achieved on 260 gr EFB/L with cellulase 36 FPU / g EFB with the volume of biohydrogen, 15.5 ml biohydrogen / g EFB.

3. The SSF method has been shown to sifnificantly increase biohydrogen production.

a. Maximum biohydrogen is achieved at 220 gr EFB / L with cellulase 360 FPU / gr EFB, with the number of biohydrogen 635.3 ml biohydrogen / gr EFB.
b. The effectiveness of biohydrogen production was shown by increasing cellulose to 28.70% and hemicellulose to 23.53%, compared to the SHF method.

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