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Effect of Combining Electron Beam Irradiation and Alkaline Pretreatments of OPEFB for Enzymatic Hydrolysis and Fermentation of Ethanol

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Abstract

The effect of pretreatment process from the combination of electron beam irradiation and alkaline to Oil Palm Empty Fruit Bunch (OPEFB) was studied. The combination of pretreatment method was considered as an alternative way to increase glucose yield. In this study, OPEFB was pretreated using Electron Beam Irradiation (EBI) at 100 kGy and 300 kGy and followed by chemical pretreatment. In chemical pretreatment, irradiated OPEFB was reacted with sodium hydroxide 6% and 10% in stirred vessel at 4 bars and 150 ^{o}C for 30 min. The effectiveness of pretreatment was evaluated by calculating the composition of chemical component using National Renewable Energy Laboratory (NREL) Method. The samples which were hydrolyzed using enzymes with the addition of 30 FPU of Cellic®CTec2 per gram of pretreated biomass resulted high glucose in the amount of 9.86%. The fermentation process using *Saccharomyces cereviceae* obtained the highest ethanol concentration for 5.36% at 72h. The combination of the two pretreatment methods gave an effect on the weight loss, chemical composition, structure, and enzymatic hydrolysis product

1. INTRODUCTION

Utilization of fossil fuel should be minimized to reduce environmental problems such as global warming [1]. People have been trying to find an alternative energy that could be environmental friendly and biofuel was one of the solutions becoming attention nowadays. Currently, biofuel based on lignocellulose is considered as critical issue which can be used to solve the conflict between food and energy resources for fuel production [2].

Bioethanol, the product bioconversion of lignocellulose, could be used as substitution for gasoline [1]. Lignocelluloses which is produced by more than 60% of plant biomass, consist of cellulose, lignin and hemicelluloses [3,4]. Cellulose, the main important material for bioethanol, is a polysaccharide composed by D-glucose subunits, linked by β-1,4 glycosidic bonds [5]. Meanwhile, hemicellulose and lignin protect cellulose in the outside. Four main steps is carried out to produce bioethanol from lignocelluloses. First step is pretreatment which is delignification process for removing lignin component and for altering the structure of cellulosic biomass to be more accessible for saccharification process [6]. Several pretreatment methods were developed, including chemical pretreatment using alkaline [6-9], acid [10], microorganism [10-12] and irradiation [2,4,13]. Second step, namely saccharification, is an enzymatic hydrolysis process to hydrolyze cellulose into glucose. It required complex enzymes cellulase and β--glucosidase. Third step, fermentation, is process to convert saccharification product into ethanol using *Saccharomyces cereviceae*. The last process is distillation. The aim for this process is to

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separate ethanol from broth and purify it from water.

While conducting pretreatment, irradiation process will produce free radicals to modify lignin structure and breaks down the cellulose crystal regions. After the termination of irradiation, radicals produced in amorphous regions will disappear quickly, whereas those trapped in the crystalline and semi-crystalline regions of the cellulose structure decay and caused further degradation of the lignocellulosic biomass [4]. Meanwhile, alkaline pretreatment will cause swelling, leading to an increasing of internal surface area, decrease the degree of polymerization and crystallinity and also disrupt the lignin structure [9]. Shin et. al., showed that alkaline could dissolve more lignin and polysaccharides than hot water [14]. The combination of these pretreatments was expected to complement each other. Therefore, the efficiency of the process would increase. Pretreatment method exhibit a great effect in the structural modifications of lignocelluloses and enzymatic hydrolysis [15].

Previously, study regarding the combination of irradiation with various pretreatment of lignocelluloses to increase sugar yield has reported. Combining irradiation method with dilute acid could increase sugar yield until 85.1% [16], 90% [17]. While combination with hydrothermal treatment could increase sugar yield up to 20% [18]. Matsuhashi et al. also studied pretreatment process combining alkaline and irradiation on oil palm empty fruit bunch (OPEFB) which it was quite capable to enhance the enzymatic digestion, especially for xylan [19].

The objective of this study is to evaluate the combination of irradiation and alkaline pretreatment in order to increase ethanol yield. In this study, the pretreatment method of irradiation and alkaline combination will be compared with pretreatment using alkaline only.

2. EXPERIMENTAL SECTION

2.1. Biomass Feedstock

OPEFB was obtained from palm oil plantation in Palembang, South Sumatera, Indonesia. This biomass was air dried at ambient temperature for a day. Size reduction until 2 mm length of fiber was conducted using chipper and miller to maximize surface area of materials. The fiber then dried at 50 °C in the oven overnight. Moisture content of 6.58 % measured by Moisture Analyzer OHAUS MB 45. The dried OPEFB was stored in room temperature until used. Two drying methods were used to prepare sample to ensure constant weight. The material composition of OPEFB before and after pretreatment was analyzed using National Renewable Energy Laboratory (NREL) standard procedure [20]. Untreated OPEFB consist of lignin of 35.94 %, cellulose of 30.41%, and hemicellulose of 20.7 %.

2.2. Electron Beam Irradiation

The irradiation of the dried samples of OPEFB with electron beam was conducted at the Center for the Application of Isotopes and Radiation Technology, National Nuclear Energy Agency, Indonesia. The irradiation process was conducted using an electron accelerator GJ-2 (Shanghai Xiang-Feng Electric Manufacturing Works, China) at the total doses of 100 kGy (OPEFB contact with electron beam for 12 hours 20 minutes) and 300 kGy (37 hours contact time). Operating conditions of energy was 1.5 MeV, current 2 mA and conveyor speed 1.34 $m \cdot min^{-1}$[21].

2.3. Alkaline Pretreatment

Irradiated samples were pretreated using sodium hydroxide. 500 g of irradiated OPEFB was heated at 150 ^{o}C with 2500 ml of NaOH 6% and 10%, respectively. The process was carried out in a stirred vessel at 4 bars for 30 min, followed by neutralization and drying at 50 - 60 °C overnight. The composition of the component including cellulose, hemicellulose, and lignin was analyzed after pretreatment

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with duplo analysis.The average of value was shown in related figure.

The percentage of weight loss from each component after pretreatment was calculated using Eq.1 :

$$\%\ Weight\ loss\ of\ lignin = \frac{lignin\ untreated\ (g) - lignin\ treated\ (g)}{lignin\ untreated\ (g)} x\ 100\%$$

where :

lignin untreated = % lignin per gram sample x dry weight initial OPEFB (Eq.2)

lignin treated = % lignin per gram sample x dry weight OPEFB after pretreatment (Eq.3)

2.4. Enzyme

The combination of nzyme Cellic®CTec2 and Cellic®HTec2 from Novozyme were used for saccharification process of pretreated OPEFB. The activity of Cellic®CTec2 is 144 FPU·g^{-1} cellulose (measured by NREL method) [22], while the activity of Cellic®HTec2 is 240 CBU·g^{1} (reported by Novozyme). In this study, the saccharification applied Cellic®CTec2 of 30 FPU·g^{-1} dry biomass and one-fifth of Cellic® CTec2 (v/v) for Cellic®HTec2.

2.5. Saccharification

Buffer acetate pH 4.8 was added to 10 gram of pretreated OPEFB (dry weight), making this solution in 15% (w/v). The pH in the range of 4.8 – 5.2 was preferred to optimize the enzyme working. After that, the solution was sterilized at 121 ^{o}C for 15 min. After the temperature of the fermentation cooled down to room temperature, enzyme Cellic®CTec2 and Cellic®HTec2 added to the solution. Saccharification process was conducted in the shaking incubator at 50 ^{o}C for 72 h. Around 2 ml of sample was taken out from the flask for every 24 hour and duplo analysis were conducted.

2.5.1 Fermentation

One percent (w/v) of commercial dry yeast (*Saccharomyces cereviceae)* was added into each flask at the end of saccharification process. Fermentation process was conducted in the shaking incubator at 32 ^{o}C for 72 h. Agitation velocity was set to 150 rpm. In the end of fermentation process, the fermentation broth was distilled and alcohol content was measured by HPLC. As same as on saccharification process, samples were taken out after 72 hours fermentation process, and duplo analysis were carried out.

2.5.2 Glucose Concentration Analysis

Glucose resulted from the saccharification process was measured by High Performance Liquid Chromatography (HPLC). The analysis used 5 mM H_2SO_4 as mobile phase at flow rate of 0.6 ml min^{-1} and oven temperature was maintained at 65 ^{o}C. The HPLC (waters, USA) system was equipped with AMINEX HPX 87H column and a guard column, an automated sampler, a gradient pump, and a refractive index detector. Prior to HPLC injection, all samples filtered through 0.2 mm syringe filters.

2.6. Alcohol Analysis

Alcohol, the fermentation product, was analyzed using Density/specific gravity meter DA-640 KEM. Amount of 10 ml of distillate placed into bottle and the concentration measured automatically.

2.7. Structure Analysis

Structure of OPEFB was analyzed using X-ray diffraction (XRD). The identification for crystallographic phase was conducted using Phillip PW 1710 diffractometer, with Cu Kα irradiation at 40 kV and 30 mA and a secondary graphite monochromator.

The crystallinity index both of untreated and treated OPEFB was calculated by the formula [25] :

$$C = [\frac{I_{002} - I_{am}}{I_{002}} \text{ x } 100\ ] \quad \text{(Eq.4)}$$

where C is crystalinity index; I_{002} is the overall intensity of the peak at 2θ angle, representing crystalline

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material, and I_{am} is the intensity of base line at 2θ angle, representing amorphous material in cellulose [26,27]. Crystallite sizes were calculated from the Scherrer equation.

$$L = \frac{K\lambda}{\beta \cos\theta} \qquad \text{(Eq.5)}$$

where L= average of particle size; K = 0.94 is a dimensionless shape factor [17]; λ = 1.54 Å is the X-raywavelength; and β = full width at half maximum.

3. RESULT AND DISCUSSION

3.1. Effect of irradiation and alkaline pretreatment on the weight loss

Table 1 shows the effect of the pretreatment combination to the weight of OPEFB. Weight loss after chemical pretreatment increased with increasing doses of irradiation either using the concentration of NaOH 10% or using 6%. Only 63.03 g or approximately 14% was recovered from 448.25 g OPEFB after pretreatment process 100 kGy and 10 % NaOH. It was observed that 90% of the raw material is lost from this process. If the irradiation doses were increased to 300 kGy, the recovered substrate was only about 6 % of the initial dry weight.

The increase in NaOH concentration will also enhance weight loss of the substrate. The highest weight lost was observed in pretreatment combination of irradiation at 300 kGy and 10% NaOH, that is 93.83 %. While, the lowest weight loss was observed in pretreatment combination of irradiation at 100 kGy and 6% NaOH, which is 346.72 grams, or about 77.35%. Coresponding to the data, a combination of irradiation and alkaline pretreatment on lignocelluloses materials gave a great effect on reducing the weight of the sample. Khan et. al. (2006) described the high - energy irradiation induces random chain scission in the biopolymers such as cellulose, hemicelluloses, and lignin, it is proportional with increasing of irradiation doses [14,23]. The treatment after irradiation with higher alkaline solution would cause degradation of cellulose and hemicelluloses to soluble materials [13]. In radiation exposure, cellulose macromolecule suffered cutting, while fragment content that have low polymerization increases gradually. The results are cellulose becomes fragile and easily soluble in aqueous media [24]. Therefore, the weight loss will be higher in this process.

Table 1. Effect of irradiation and alkaline pretreatment on the weight loss

Irradiation Pretreatment	**Chemical Pretreatment by 10% NaOH**		
	Dry Weight (g)		
	Before	**After**	**Weight Loss**
100 kGy	448.25	63.03	385.22
300 kGy	448.25	27.67	420.58
Irradiation Pretreatment	**Chemical Pretreatment by 6% NaOH**		
	Dry Weight (g)		
	Before	After	Weight Loss
100 kGy	448.25	101.53	346.72
300 kGy	448.25	68.24	380.01

3.2. Effect of irradiation and alkaline pretreatment on the chemical composition

Pretreatment of lignocellulosic materials is necessary to reduce some lignin by breaking-down lignin structure and disrupt the order of the crystalline cellulose regions, thus the enzyme could easily attack substrate. Partial and pretreatment combination (irradiation and alkaline) would be analyzed by comparing the component content.

The lignin percentage in the pretreated sample shows in Figure 1a. It illustrates that the pretreatment was successful to decrease the lignin content in untreated OPEFB, 35.94%. Using only alkaline pretreatment, the lignin concentration can be reduced to 10.84% and 15.74% over 10% and 6% NaOH respectively. For instance, in 100 gram of untreated OPEFB, 35.94 gram represents lignin, then after alkaline preatreatment the amount of lignin decreased to be 10.84 gram by 10% NaOH. Irradiation of 100 and 300 kGy, followed by alkaline pretreatment reduced the lignin content of untreated OPEFB in less

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amount compare to that of using alkaline pretreatment. This may be due to the effect of cellulose dissolution in alkaline after irradiation process. For irradiated lignocellulose, alkaline solution dissolves cellulose in greater amount if it compared to natural lignocellulose. The consequence of this process is that the percentage of lignin after combine pretreatment will be higher. High cellulose will be resulted from this pretreatment since lignin has been reduced.

Figure 1b shows cellulose concentration after irradiation and alkaline pretreatment using NaOH. The cellulose content of untreated OPEFB increased from 30.41% to 65.98% by alkaline process only. The best combination of pretreatment occcured using 100 kGy + 6% NaOH. Cellulose was obtained higher around 58% by these method. In the case of utilization of 10% NaOH, the addition of irradiation method was not prefered because it reduces the percentage of cellulose.

The composition of hemicellulose shows in figure 1c. Using 6% NaOH was not really effective to remove hemicellulose from sample even with the addition of irradiation before chemical pretreatment. However, the applied irradiation could decrease percentage of hemicellulose as well as using 10% NaOH.

The combination of irradiation and chemical which irradiated 100 kGy followed by alkaline pretreatment of 6% NaOH was effective to the substrate. Applying the right dose of irradiation could reduce the utilization of high concentration of alkaline in chemical pretreatment. Although the results were not as good as using 10% NaOH. However, it could minimize waste from the process. The was produced from alkaline pretreatment can pollute the environment.

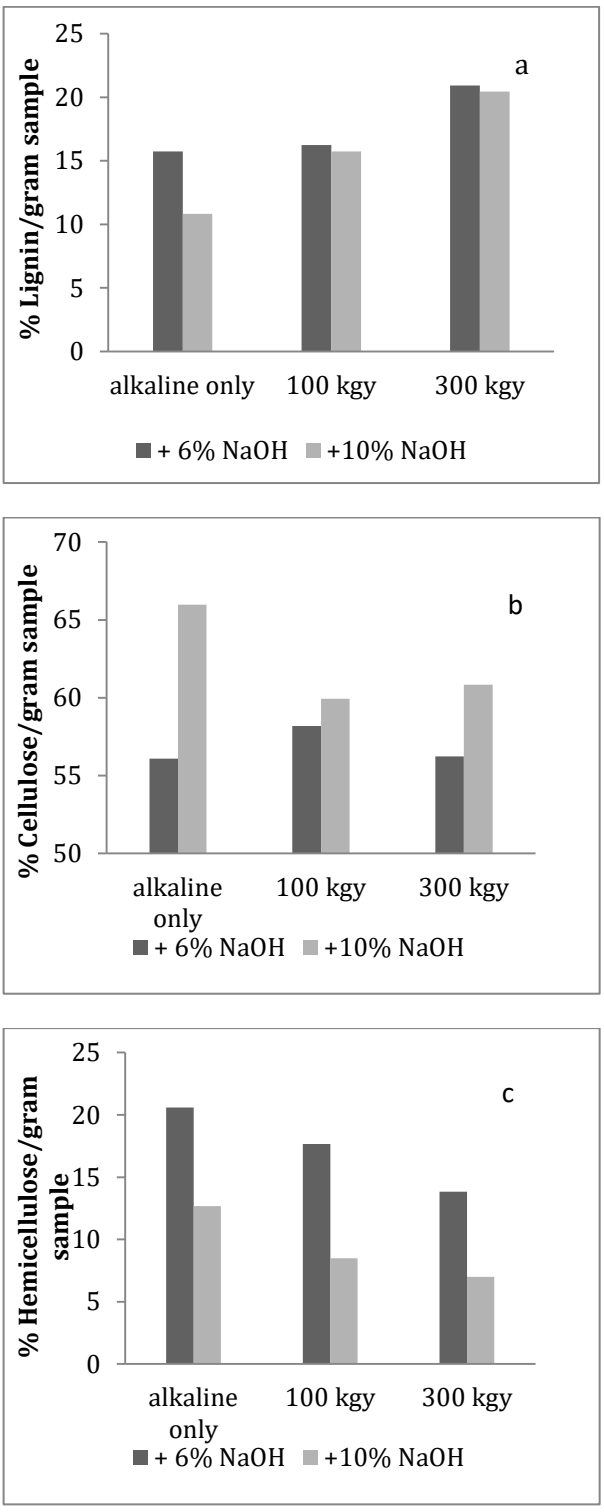

Fig. 1. Lignin content (a), cellulose (b), hemicellulose (c) contentof OPEFB after pretreatment per gram of sample. Irradiation was followed by chemical pretreatment using (■) 6% and (■) 10% NaOH

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Table 2. Percentage of weight loss each component by pretreatment process

	% lignin	% cellulose	% hemicellulose
Irradiation + Alkaline Pretreatment			
100 kGy + NaOH 6%	89.76	56.67	80.67
300 kGy + NaOH 6%	91.13	71.84	89.83
100 kGy + NaOH 10%	93.84	72.29	94.23
300 kGy + NaOH 10%	96.49	87.65	97.91

Based on Tabel 2, both alkaline pretreatment and combine pretreatment had the ability to reduce lignin dan hemicellulose component. Using 300 kGy+NaOH 10% almost all of lignin and hemicellulose removed from OPEFB. In the case of cellulose, the combine irradiation and alkaline pretreatment could decrease the amount of cellulose to 50%. The irradiation applied could make cellulose dissolve in the alkaline.

3.3. Effect of irradiation and alkaline pretreatment on the structure

Figure 2. shows the XRD pattern of untreated and treated OPEFB. It can be seen that the crystallity of the material was increased after pretreatment. The peaks observed at 2θ = 13.3° – 17.3°; 18.4° – 25.6°; 32.06 – 36.26° are indicating crystalline region of cellulose. This was supported by Wu et. al which stated that microcrystalline cellulose showed around 16.5, 22.5, and 34.5◦ [28]. Treated sample with NaOH 10% increased the crystallinity index from 38.33% to 59.48% (Table. 3), showed that the NaOH pretreatment successfully removed the amorphous fraction (lignin and hemicellulose) in OPEFB. This was also illustrated in Figure 1 which describes the increasing of around 65% after pretreatment with NaOH 10%.

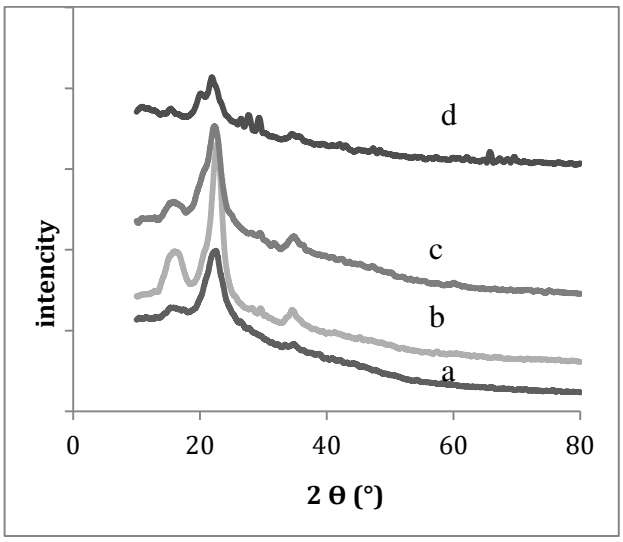

Fig. 2. XRD Pattern of Untreated and Treated OPEFB
Legend: (a) OPEFB untreated (b) OPEFB NaOH 10% (c) OPEFB irradiated 100 kGy + NaOH 10% (d) OPEFB irradiated 300 kGy + NaOH 10%

Conducting irradiation pretreatment before alkaline pretreatment has an effect in decreasing the crystallinity index. Crystallinity index of OPEFB irradiated 100 kGy + NaOH 10% which was 47.53% (Table 3) and the peak around 2θ = 13.3° – 17.3°; 18.4° – 25.6° in Figure 2 was not as higher as OPEFB NaOH 10%. More reduction also occurred when 300 kGy of irradiation was applied. It might be due to the degradation of cellulose by combination of both pretreatments. It is also described in Table 2 that the amount of cellulose was decrease in the combine pretreatment. Irradiation will make cellulose into amorphous region. Therefore, it could be dissolved in the alkaline pretreatment [13]. Takacs *et al.* confirmed this with SEM analysis that the combination of gamma iradiation and alkaline pretreatment would make the degradation of some fibrils [29]. The decreasing of cellulose content by only with gamma-irradiation was also observed by Wang et.al.. He found that cellulose was decreased until 14% from raw material 400 kGy [30].

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Table 3. Crystallinity Index of OPEFB Untreated and Treated

Sample	Sample Crystallinity Index (%)	Amorph quantity (%)	Crystal lite Size (nm)
OPEFB untreated	38.33	61.67	5.68
OPEFB NaOH10%	59.48	40.52	8.93
OPEFB Irradiated 100 kGy + NaOH 10%	47.53	52.47	7.44
OPEFB Irradiated300 kGy + NaOH 10%	12.85	87.15	7.83

Table 3 shows the amorphous phase quantity of OPEFB treated by NaOH 10% has lower value than OPEFB treated by combined-pretreatment (irradiated 100 kGy or 300 kGy-NaOH 10%). Amorphous quantity of OPEFB treated tended to be higher compared than raw material. For pretreatment method with the combination of irradiation and NaOH 10%, the crystallinity index decreased after irradiation doses above 300 kGy. While, the crystallite sizes of OPEFB treated by NaOH 10% also increased and the value was higher than OPEFB untreated and OPEFB combined irradiated-NaOH 10%.

3.4. Effect of irradiation and alkaline pretreatment on the enzymatic hydrolysis

Lignin is one of the components which restricts the access of hydrolytic enzymes to attack cellulose [31]. After reducing some lignin in the pretreatment process, enzymatic hydrolysis or saccharification also become the focus in this study. Figure 3 shows glucose obtained from HPLC analysis. Glucose increased significantly with the increasing of reaction time in saccharification.

As shown in Figure 3, the lowest glucose concentration was from OPEFB pretreated by 6% NaOH, followed by combination pretreatment of 100 kGy and 6% NaOH. Applying 6% NaOH gave lower sugar concentration result than using 10% NaOH which also occurred with the combination of irradiation pretreatment. The figure showed an increasing of glucose concentration in 24 h, 48 h, 72 h. In 48 hours, OPEFB which pretreated by 100 kGy and 10% NaOH gave higher value than single 10% NaOH pretreatment. However, in 72 hours the opposite occured. There is only slightly difference of glucose concentration of irradiation and no irradiation pretreatment.

In 72 hours, it was predicted that cellulose (maybe hemicelluloses) had been converted to sugar. The maximum glucose of 10.29 % was obtained when the hydrolysis was conducted by using OPEFB pretreated with NaOH 10%. It might be caused by the high of cellulose content in pretreated OPEFB that influenced the conversion of glucose and xylose.

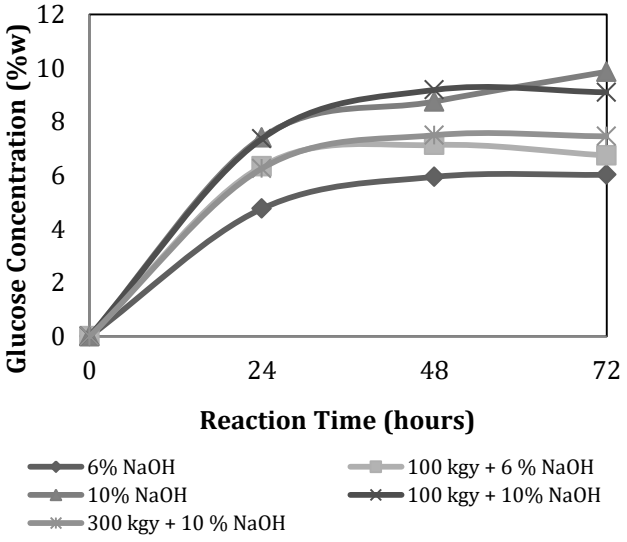

Fig. 3. Glucose Concentration of Pretreated Empty Fruit Bunch during Saccharification Process [32]

The use of irradiation seems to be more effective on pretreatment with NaOH 6%. The results from the glucose concentration analysis in 72 hours recorded to reach 6.7% when using irradiation 100 kGy before alkaline pretreatment. The substrate which pretreated only by using NaOH 6% was able to produce 6% glucose. According to this, it can be seen that using irradiation will increase glucose production in saccharification process. The

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enzyme can easily access the cellulose because substrate has irradiated.

The addition of irradiation to the substrate which has been pretreated NaOH 6% can increase the total reduction of sugar in the saccharification process. However, an increase in irradiation dose of 100 kGy to 300 kGy did not give a significant effect of the addition of sugar. Adjacent graph between two variables in Figure 1 showed that excessive doses will decrease cellulose content existing on the substrate so that the sugar produced will be lower.

In contrast to 6% NaOH, the combining irradiation and NaOH pretreatment of 10% gave different results. The use of irradiation at 100 kGy and 300 kGy before NaOH 10% gave lower results compare than using only alkaline. The use of 300 kGy irradiation before alkaline pretreatment only produced glucose by 7.45% (w/w), whereas 9.86% glucose was produced without irradiation. Ribeiro et.al mentioned that the biggest obstacle in irradiation is the destruction of the product. To avoid the destruction of the product such as loss of sugar due to uncontrolled degradation of cellulose and hemicellulose, the doses used should be as low as possible but still considering lignin removal[32]. Figure 3 also shows that substrates pretreated by only 10% NaOH gives the best result.

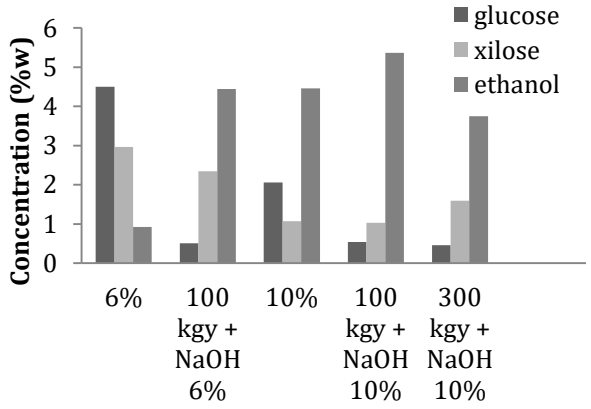

Fig. 4. Ethanol Yield of Substrate Variable [32]

Figure 4 shows ethanol obtained from fermentation process using *Saccharomyces cereviceae*. The efficiency of fermentation efficiency varied eventhough the same variety and weight of yeast was used. Lowest efficiency was found in the OPEFB which was pretreated by NaOH 6%. Although glucose concentrations were still high, the ethanol was low. The same result was also observed in the pretreated substrate that using only 10% NaOH. Glucose produced in the saccharification process was not maximally converted into ethanol by yeast. Meanwhile, the OPEFB treated with the combination of irradiation and alkaline, alcohol produced 4.45, 5.36, 3.75% for the substrate 100 kGy + 6% NaOH, 100 kGy + NaOH 10%, and 300 kGy + NaOH 10% respectively.

4. CONCLUSION

The application of the combination between electron beam irradiation and alkaline for pretreatment of OPEFB affected the weight loss, chemical composition, structure, and enzymatic hydrolysis. After pretreatment, weight of substrate decreased to 93.83%. The chemical composition also affected with the decreasing lignin in the substrate. The structure of pretreated cellulose tended to change to amorphous cellulose which was indicated from the decrease of cellulose crystalinity index in the pretreated OPEFB. The combination of 100 kGy irradiation and 10% NaOH gave the highest ethanol concentration (5.36%). However, it caused high weight loss. Therefore, economic consideration was needed to select the pretreatment method. The combination of 100 kGy irradiation and 6% NaOH could be considered for reducing utilization of NaOH solution. Alkaline pretreatment using 10% NaOH also could be an alternative to skip the irradiation process. Both of the process produced the similar ethanol concentration (4.45%).

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