The Effect Of Temperature and Time on Hydrolisis of Palm Oil Empty Fruit Bunch and Its Enzymatically Biodegradation for Xylose Production

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Abstract

The acid hydrolysis and the heating of lignocellulosic waste from palm oil mills have caused irreversible damage to the natural environment and can create inhibitor compounds on hydrolysis, making the waste improper for fermentation media. In this study, an environmentally save hydrolysis was tested by heating it under high temperature which followed by enzymatic degradation. The objective of the present study was to determine the optimal reaction temperature and reaction time of hydrolysis for the production of xylose. Under the temperatures of 128 and 200°C with reactions time of 30, 45, and 60 min, the best result of hydrolysates were taken. Its hemicellulose, cellulose, lignin, water content (raw material), residual hemicellulose, reducing sugars, water content (hydrolysate) and xylose were analyzed. The results showed that the higher the temperature had resulted in the more reduction of sugar hydrolysis and the hemicellulose in the solid residue was slightly removed. Hydrolysis at the temperature of 200°C for 45 min had indicated a reduction of sugar yield of 17.71% (db). Biodegradation in enzymatic hydrolysis of xylose had increased by 113.79% at 24 -1.24 g xylose / 100 ml.

Key words: *Enzymatic, hemicellulose, palm oil empty fruit bunches, xylose*

1. INTRODUCTION

Palm oil is one of the non-oil commodity in Indonesia but up to 2010, 60 percent of the product was still in the form of Crude Palm Oil (CPO) (Jakarta Post, 2011).It was also recorded that there were 24 – 30 percent of empty fruit bunch. In 2002, Indonesia produced 5.0288 million tons of palm oil from 25 million tonnes of fresh Fruit Bunch (BPS, 2003). Of the amount available, there were 6-8 million tons of empty fruit bunch which had not been used optimally.

Oil Palm empty fruit bunch (OPEFB) has large amount of hemicellulose and a little lignin cellulose. Its chemical composition consists of 35.81 to 40% cellulose, hemicellulose 24 to 27.01%, from 17.7 to 21% lignin, and ash content of 6.04 to 15% (Pratiwi *et al.*, 1998; Azemi *et al.*, 1994 in Gumbira - Sa'id, 1996). The utilization of OPEFB so far had been focused more on the use its glucose instead of xylose found in its hemiselulose.

Actually, xylose can be converted into high-value products such as xylitol (approximately 10 times the price of sucrose) that may contribute equally with glucose (Dwivedi in Nabors and Gelardi, 1992).

Hemicellulose can be hydrolyzed into xylose product which is commonly accompanied by acid hydrolysis and high temperatures. This practice is responsible for irreversible damage to the natural environment and its hidrolysis inhibitor compounds such as acetic acid, furfural, and vanillin that made it improper for a fermentation medium, e.g for the production of xylitol. Therefore it is necessary to find an environmentally friendly hydrolysis which can minimize the formation of the inhibitor compounds.

Autohydrolysis experiment (without acid) on the hydrolysis of corn cob under high temperatures of 202 °C with a ratio of 1g of material: 8 g of water to produce 3.05 g xylose / l and high xylooligosaccharida by 25.4 g / l had been carried out by Rivas *et al.* (2002).

According to Nunes and Pourquie (1995), hydrolysis of Eucalyptus wood hydrolysates enzymatis on a rotary shaker with the temperature of 50 ° C for 24 h had yielded 11.67 g glucose / l, and xylose of 11.87 g / l but the results were not so high compared to the production of xylose of 13.71 g / l.

Therefore, a study to determine the optimal conditions for producing the enzymatic hydrolysis of xylose with variations of temperature and hydrolysis time should be carried out.

2. RESEARCH METHOD

2.1. Raw Material

Raw material used in this study was Oil Palm Empty Fruit Bunches (OPEFB) obtained from PT. Plantation VII archipelago, Mount Sugih Central Lampung. Peptone (PA), KH_2PO_4 (PA), $MgS0_4$ (PA), $(NH_4)\ 2SO_4$ (PA), acetic acid (PA), Na acetate (PA), reagent Nelson A, Nelson B, arsenomolybdat (PA), and distilled water were obtained from the Laboratory of Analysis of Agricultural University of Yogyakarta and the xylan was obtained from Inter-University Center (PAU) Microbiology Laboratory, Department of Food and Nutrition, Gadjah Mada University, Yogyakarta. Pure cultures of *Aspergillus niger* was obtained from the Laboratory of Biotechnology Faculty of Agricultural Technology, Gadjah Mada University.

2.2. Equipments

The equipments used in this study consisted of a set of glassware (pyrex) for analysis, UV spectrophotometer (Milton Roy Spectronic, 20D), waterbath (Kottermann, Germany), pH Meter (pH 620 meter Methrom, Swiss), digital balance brand Sartorius (BL 210S), reactor (Stainless Steel, with fuel nikelin), centrifuge (Hettich EBA 8 S), autoclave (All American Pressure Sterilizer Model No. X 1941), heater (Rinai), cabinet dryer (Wangdi), blender (National), HPLC (refractive index detector, column Bio_Rad HPX-87 H, 0.01 NH2SO4 eluent, flow rate 0.5 ml / min, and column temperature of 50 ° C).

2.3 Research site

The research was conducted at the Laboratory of Agricultural Product Processing, Mercu Buana University Yogyakarta and Laboratory of Chemistry, Inter-University Center (PAU) Food and Nutrition, Gadjah Mada University, Yogyakarta.

2.4. Research Procedures

2.4.1. Preparation of OPEFB powder

OPEFB (1.2 kg) was cut into 2-3 cm, washed and dried at 70 ° C in a cabinet dryer until the moisture content reaches 10%. Dried material is then milled using a blender at a speed number 3 for 10 minutes and sieved by 35 mesh sieve to produce a powder of 1.08 kg. OPEFB powder which passes the filter was then used for preparation of hydrolysates.

2.4.2. Preparation of OPEFB hydrolysates

A 25 grams of OPEFB powder were put into 500 ml erlenmeyer filled by 125 ml of distilled water (1 gram of material: 5 ml distilled water). This products were made in 2 tests. It were then heated to a various temperature of 128 ° C (autoclave) and 200 °C (reactor) for 30, 45, 60 minutes. After the hydrolysis, the hydrolyzate, and the mixture were filtered and the residue (solids) were analyzed. The flowchart of preparation of OPEFB was showed in Fig. 1.

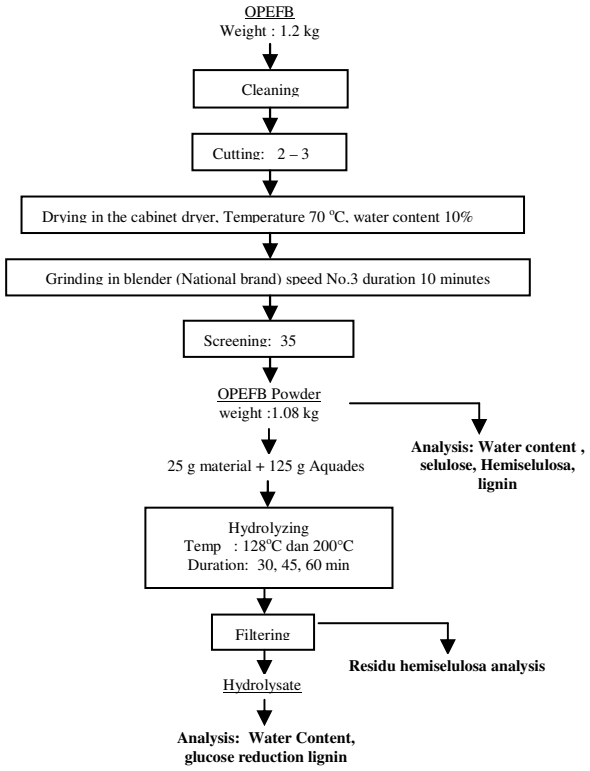

Figure 1. Preparation of OPEFB Hydrolysate

2.4.3. Xylanase enzyme production (crude enzyme)

Xylanase enzyme production was conducted by using liquid media according to Zhao, *et al.* (2002) with the following composition: 0.05% xylan, 0.1% peptone, 0.3% KH_2PO_4, 0.05% $MgSO_4$, 1% $(NH_4)2SO_4$. It was then put into 50 ml of hydrolysate (hydrolysate was elected) that had been prepared. Mushrooms were grown on media production of xylanase at 30° C immersion 72 hours while shaken at 150 rpm and then separated between liquids and solids (biomass) by centrifuged at a speed of 5000 x g. Supernatant of the liquid medium was used as a crude enzyme. The flowchart of xylanase enzyme production was depicted in Fig. 2.

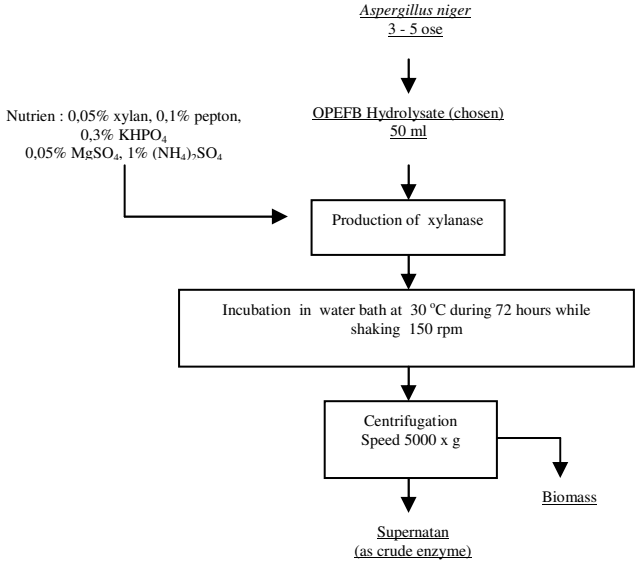

Figure 2. Production of Xilanase Enzyme (Crude Enzyme)

2.4.4. The enzymatic biodegradation of OPEFB hemicellulose

Hydrolysate was obtained from hydrolysis of OPEFB powder (best results) with pH adjusted using acetate buffer (200 mM) and then mixed with the supernatant (with a ratio of 1:1) and degraded at 50 ° C while shaken for 24 hours. The sampling with volume 3 ml was taken every 6 hours and then centrifuged at 5000 U/min to separate the liquids and solids. Supernatant was then analyzed using HPLC and the observed parameter was the change in xylose. The flowchart of enzymatic biodegradation was showed in Fig. 3.

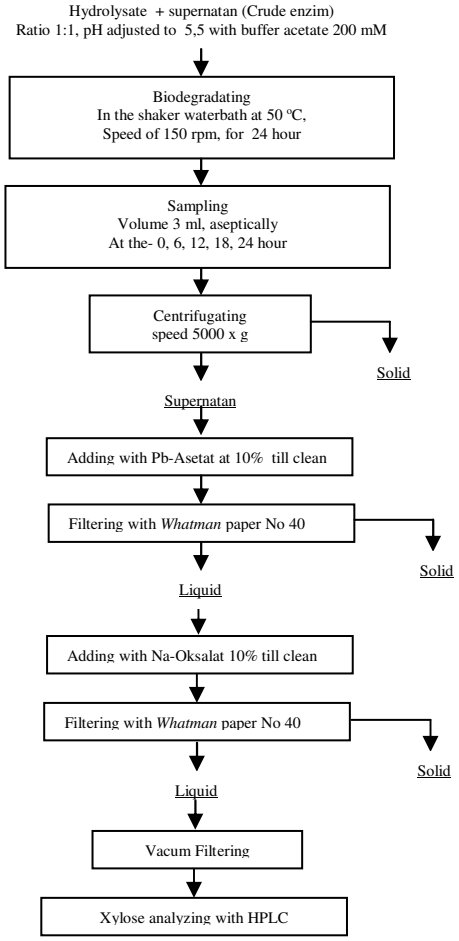

Figure 3. Enzymatic Biodegradation

2.5. Analysis of Research

The analysis includes the analysis of basic materials (OPEFB powder), the water content (AOAC, 1995), cellulose, hemicellulose, and lignin (Datta, 1981), whereas the hydrolysate analysis includes the determination of water content (AOAC, 1995), and sugar reduction Nelson-Somogy method (AOAC, 1995), hemicellulose residue (Datta, 1981), as well as the determination of xylose by HPLC, using a refractive index detector, column Bio-Rad HPX-87 H, 0.01 N H_2SO_4 eluent, flow rate 0 , 5 ml / min and a temperature of 50°C.

2.6. Statistical analysis

The design of experiments used in this study was Complete Randomized Design factorial arranged with the treatment consists of two factors, namely: the temperature (T): 128 and 200°C and the hydrolysis time (t): 30, 45, 60 minutes. The observation results were

analyzed by variance (F test) on the real level of 0.05 and a real different test followed by DMRT (Duncan Multiple Range Test).

3. RESULTS AND DISCUSSION

3.1. Raw Materials

Table 1. Chemical Composition of OPEFB

Component	Contain (%, db)
Hemiselulosa	22,11
Selulosa	42,86
Lignin	17,58
Water	10,35

The research components of lignocellulose OPEFB had shown to contain of hemicellulose, cellulose, and lignin at 22.11, 42.86, and 17.58%, respectively. These were consistent with previous research conducted by Darnoko, *et al.* (1991) which reported that the content of hemicellulose, cellulose and lignin of OPEFB were 22.84, 45.95, and 16.49%, respectively. Slight differences were probably due to the oil palm varieties and different environmental conditions. According to Chandrakant and Bisaria (1998), the hemicellulose content of the annual crop is about 15-30%. When compared to different materials such hemicellulose levels, such figure are not much different, but in Indonesia the OPEFB is very potensial waste to be utilized as it is very abundant in number that is expected to reach 6-8 million tons (BPS, 2003).

Hemicellulose is composed of heteropolimer, mainly by xylose, and it is easier to hydrolyze than the crystalline cellulose and lignin in the form of crystalline. Based on Table 1, hemicellulosa content is higher than the lignin, so it can be expected that harsh treatment (high temperature) would be more effective to eliminate its hemicellulose compared to the elimination of its lignin. It also reveals that these wastes have more potential to be used as feedstock then to be media in the production of xylose compared to glucose utilization in cellulose which has been widely applied. Lignin can hinder the effectiveness of the hydrolysis of hemicellulose and cellulose. The water content in the study was 10.35% (wb). It was adjusted into 10% in order to facilitate the preparation of media for xylitol production. If the moisture content is more than 10% , the mass of solid material will be less and thus the result will also be small.

3.2. Hydrolysis results of OPEFB

The results of hydrolysis of Oil Palm Empty Fruit Bunches (OPEFB) was presented in Table 2.

Table 2. Hydrolysis Component Results

Components	Temperature Treatment (^{o}C) / duration (minute)					
	128 ^{o}C			200 ^{o}C		
	30	45	60	30	45	60
Hemicelluloses Residue (%db)*	16.38^{e}	15.90^{d}	15.43^{c}	13.19^{b}	9.95^{a}	9.87^{a}
Reduction Glucose (%db)**	12.34^{a}	14.79^{b}	15.53^{c}	16.17^{d}	17.71^{e}	17.76^{e}

Note : the number after different letter indicating significant differences
* on OPEFB waste ** on OPEFB hydrolysate

From Table 2, it can be determined the higher the temperature and time increase, the more likely hemicellulosa residue levels down. At the treatment temperature of 200°C for 45 and 60 min, there was no difference which means that the time had no significant effect at all. The hydrolysis temperature of 200°C treatment for 45 min was at the optimal condition.

Overall, hydrolysis temperature had greater influence than the hydrolysis time. It has been proved previously by Darnoko *et al.* (1991) by using three factors: the acid concentration, temperature, and the hemicellulose hydrolysis of oil palm empty fruit bunches. Of the three factors studied, it seemed that the acid concentration gave the greatest influence, followed by hydrolysis temperature and hydrolysis time of the residue hemicellulosa. Proficiency level of the data of the least possible residual hemicellulose fraction of dissolved hemicellulose was significantly increased.

The increasing hemicellulose fractions were also shown with increasing sugar content reduction. The higher the temperature and the longer the hydrolysis time had increased sugar reduction, besides the residual factor of its hemicellulose temperature and time had no longer significant effect on the reduction of sugar in the treatment of 200°C for 45 min. As a comparative material, Rivas *et al.* (2002) reported that the hydrolysis of corn on the cob with a ratio of 1 g of material: 8 g of water produces xylooligosaccharida by 25.4 g / l, 3.05 g xylose / l, arabinose 1.75 g / l, glucose , 0.75 g / l, 0.55 g furfural / l acetic acid and 1.75 g / l. The increasing temperature can help the amount of fraction.

3.3. Enzymatic biodegradation

The results of acid hydrolysis without (autohydrolysis) which is conducted on the success of the previous process with parameter increasing temperature and increasing time hemicellulose had created less residue and higher of sugar reduction. At the treatment temperature of 200°C for 45 and 60 min, it was recorded that no difference, which means that the temperature and time had no significant effect on enzymatic biodegradation so that the best selected hydrolysate hydrolysis results in a shorter treatment time was on the treatment temperature of 200°C for 45 min. The process was then continued by using a xylanase enzymatic biodegradation to break xylooligosaccharida (dissolved in reducing sugar) into xylose. The changes of resulting xylose was presented in Table 3.

Table 3. Production of Xylosa by *Aspergillus niger*

Medium	Product	Biodegradation time (hour)				
		0	6	12	18	24
OPEFB Hydrolysate	Xylose (g/100 ml)	0.58	0.78	1.01	1.08	1.24

From Table 3, it reveals that the xylose generated at the six level had increased from 0.58 to 0.78%. This suggests that the enzyme was active and can be cut into xylooligosaccharida xylose. In the 12th hour, xylose increased to 1.01%, as well as at the 18th and the 24th hour to 1.08% and 1.23%. At 24 hours biodegradation time, there was a large enough increasing of xylose which reached 113.79%. It shows that the longer time the more xylose produced. However, the results of biodegradation at 24 hours have not been up since biodegradation is still on going after 24 hours and likely to generate more xylose. Previous research had undertaken by Yulianto *et al.* (2002) in which hydrolysis of OPEFB using 2.5% sulfuric acid at a temperature of 95°C for 10 h (reflux) of 0.24% xylose yield, had produced greater enzymatic hydrolysis.

CONCLUSION

The effect of temperature and time on the hydrolysis of oil palm empty fruit bunches had indicated that the higher of temperature and the longer of hydrolysis, the sugar produced was higher. Hydrolysis at a temperature of 200°C for 45 min had been reducing sugar yield of 17.71% (db). The yield of biodegradation in enzymatic hydrolysis of xylose increased by 113.79% at 24 hours to reach 1.24 g xylose / 100 ml. Based on these results, however, there is still an ample opportunity for further research on the optimal time of enzymatic biodegradation to produce xylose as much as possible.

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