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Factorial Analysis on Nitric Acid Pretreatment of Oil Palm Frond Bagasse for Xylan Recovery

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Abstract

Hemicellulose in agricultural biomass are composed mainly of xylan which can be used for various application of valuable products including xylooligosaccharides. The aim of this work is to study the factors affecting acid pretreatment of oil palm frond bagasse for xylan recovery. The half fractional two-level factorial design with five factors was selected for the experimental design. The studied factors were nitric acid concentration (0.1 - 1.0%), temperature (37 - 90 °C), time (6 - 24 hours), solid loading (5 - 20%) and agitation (yes/no). The highest concentration of xylan at 0.5 g/L was obtained during pretreatment at 37 °C for 24 hours with 5% solid loading and 0.01% acid concentration without agitation. Among the factors involved, temperature was determined as the most significant factors in increasing xylan concentration followed by solid loading, time, acid concentration and agitation. The significant equation model represented in this study with the coefficient of determination (R^2) of 0.9909 demonstrated that the experiment was successful. High xylan concentration obtained in this study proved that the use of dilute nitric acid for OPFB pretreatment is significant to preserve and recover xylan in biomass.

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Keywords: Xylan; Acid pretreatment; Respond Surface Methodology

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1. Introduction

Lignocellulosic biomass, also known as lignocellulose is a plant dry matter. The renewable nature properties in lignocellulosic biomass make this matter become interests among the researchers as a source to produce value added products. Three main components were identified made up a major composition in lignocellulosic biomass; cellulose, hemicellulose, and lignin, and the distribution of these components varies depending on the type of plants [1]. Recently, wastes from oil palm industry especially oil palm frond (OPF) has gained much attention and become most lignocellulosic biomass studied due to the availability of this material. According to Awalludin et al. (2015), 44 million tonnes dry weight of OPF were obtained annually during pruning of OPF and on the replanting season, as much as 15 tonnes per hectare of dried OPF were produced. These OPF biomass if not being used will only been chipped, left rotten or burned in the plantation field which later will cause an environmental issue [3].

Xylan made up the major component in hemicellulose polymer and is the most abundant hemicellulose that occur in nature. Xylan is originated from β-D-xylopyranosyl (xylose) residues that are linked via β-1- 4 glycosidic bonds. Other groups are also identified attach to this backbone which known as glucuronic acid, arabinose, and acetic acid [4]. Xylan has been studied to be used in various application and one of it is in biocomposites [5,11]. Xylan also has been investigated in the conversions of chemicals since the monomer unit of xylan can be converted to other chemicals including xylitol [7], ethanol [6], and furfural [8] which are of commercial value. The problem arise as cellulose in biomass is coated by hemicellulose and enclosed with lignin forming cellulose-hemicellulose-lignin complex. This form works as a physical and chemical barrier which prevent the hydrolysis of biomass to occur under its natural condition [9]. Therefore, the pretreatment of biomass is necessary to break the cellulose-hemicellulose-lignin complexes and expose the polysaccharide components making it accessible for hydrolysis process.

Pretreatment technologies of lignocellulosic biomass can be classified into two categories; mechanical and chemical pretreatment. Commonly, pretreatment process will start with mechanical pretreatment by chipping, grinding, or milling to reduce the overall size and increase surface area of biomass [10]. Then, the disruption of chemical structure in biomass is achieved through chemical reaction mechanism using alkali pretreatment, acid pretreatment, organosolv process, or ionic liquids pretreatment [11]. Most of the previous studies preferred alkaline pretreatment to isolate xylan [12]. However according to Kumar et al. (2009), dilute acid pretreatment can be used to alter and expose the structure in biomass since it is low cost and economically practicable process. Besides, no report of factorial analysis with Response Surface Methodology (RSM) in the study of factors affecting acid pretreatment of biomass was identified so far. Golshani et al. (2013) suggested that the effect of interaction between factors and the determination of the most significant factor can be evaluated using experimental design developed by Design Expert Software.

Hence, the aim of this study is to investigate factors affecting dilute nitric acid (HNO_3) pretreatment of oil palm frond bagasse (OPFB) for xylan recovery using two level fractional factorial analysis and research surface

methodology (RSM). This research focus on five important parameters in pretreatment which are temperature, time, solid loading, acid concentration, and agitation.

2. Materials and Methods

2.1 Materials

Oil palm frond used in this study was collected from local oil palm plantation at Felda Lepar, Gambang, Pahang. The juice in OPF was removed by pressing the OPF using sugarcane pressed machine to obtain the OPFB. The OPFB was sundried for 2 to 3 days until the constant weight was obtained. The OPFB then grinded and sieved into fraction to make sure the particle size of materials less than 2 mm. The raw materials then sealed in plastic bag and stored for further use.

2.2 Acid pretreatment

Pretreatment process was carried out in 500 mL Schott bottle with fixed OPFB of 15 g at different condition according to Table 1. Nitric acid (Sigma, 70%) was added in the range of concentration between 0.01 to 1.0% and was added according to the solid loading of 5 to 20%. Pretreatment process was done in water bath at 37 to 90 °C for 6 to 24 hours. After pretreatment, the sample was washed with tap water until neutral and dried overnight in oven at 60 °C. The sample then stored for further analysis.

Table 1. Experimental design for factorial analysis

Factors	Coded	Units	Low value (-1)	High value (+1)
Temperature	A	℃	37	90
Time	B	hours	6	24
Solid loading	C	% (w/v)	5	20
Acid concentration	D	% (v/v)	0.01	1.00
Agitation	E	rpm	No	Yes

2.3 Two-level factorial analysis experimental setup

The experimental design for fractional factorial analysis in this study was done using Design Expert 7.0 (Stat-Ease Inc., USA) software. Five factors were investigated which are temperature (℃), time (hours), solid loading (w/v %), acid concentration (v/v %), and agitation (rpm), to analyze their effects on the xylan recovery using Response Surface Methodology (RSM). The response of screening process developed by the software as shown in Table 1.

2.4 Sample characterization

Xylan quantification was done according to the method proposed by National Renewable Energy Laboratory (NREL). The removal of non-structural materials from biomass was done using two step extraction process known as soxhlet extraction where ethanol (Sigma, 99%) and water were used as solvent. This step is important to remove water soluble materials such as inorganic materials, non-structural sugars, and nitrogenous material, and ethanol soluble materials like waxes and chlorophyll, to prevent interference in analysis process later. The extraction process was done according to the method propose by Sluiter et al. (2008) where the biomass was first subjected to water extraction for 8 hours before 24 hours ethanol extraction. In between of these two extractions, the biomass was dry in an oven for overnight at 60 °C.

After the extraction process, the extractive free sample was used to determine xylan content in biomass. The sample was hydrolyzed by adding 3 mL of 72% sulfuric acid (Sigma, 98%) in approximately 0.3 g sample. Each run was replicate three times. The sample was first incubated in water bath at 30 °C for 1 hour and stirred in interval of 15 min. The sample then added with 84 mL HPLC grade water and autoclaved for 1 hour.

2.5 Xylan quantification

The hydrolysate was determined using Agilent 1260 high performance liquid chromatography (HPLC) system equipped with refractive index (RI) detector. The separation column used was Rezex Phenomenex Monosaccharide column. Mobile phase used for this column was prepared using Milli-Q ultrapure water (Millipore, USA). the injection volume of sample was fixed for 5 µL with flow rate of 0.4 mL/min at a column temperature 60 °C. The calibration curve was prepared for xylan standard (Sigma) within the range 0.5 g/L to 10 g/L.

3. Results and discussions

3.1 Screening of acid pretreatment

The experimental design of half-level factorial analysis was performed to identify and evaluate the factors affecting the recovery of xylan during acid pretreatment of OPFB. Table 2 shows the screening table with different variables constructed by Design Expert software which represent different xylan concentration. The xylan concentration lies between 0 to 0.5 g/L was observed in this study. Run 13^{th} displayed the highest xylan recovery (0.5 g/L), equivalent to 27.63% xylan from total composition of OPFB which obtained during pretreatment at 37 °C for 24 hours pretreatment time with 5% solid loading and 0.01% acid concentration without agitation. The xylan content was found to be in line with Samanta et al. (2012) who reported 25.12% of xylan recovery. A small difference occur might be due to the raw material and pretreatment method used in both study is different. Besides, dissimilar location of the sample collection, climate, plant maturity and physical and chemical condition of soil could contribute to the varies of chemical substances in the lignocellulosic biomass and thus demonstrated differs content of xylan recovery [18].

Table 2. Experimental design for factorial analysis with its response

Run	Coded values of variables					Xylan concentration (g/L)
	A	B	C	D	E	
1	-1	-1	1	1	1	0.37
2	1	-1	-1	1	1	0.30
3	-1	1	-1	1	1	0.41
4	-1	1	1	-1	1	0.42
5	-1	1	1	1	-1	0.39
6	-1	-1	-1	-1	1	0.42
7	1	-1	-1	-1	-1	0.39
8	-1	-1	1	-1	-1	0.44
9	1	1	-1	-1	1	0.40
10	1	1	-1	1	-1	0.44
11	1	1	1	-1	-1	0.00
12	1	-1	1	1	-1	0.27
13	-1	1	-1	-1	-1	0.50
14	-1	-1	-1	1	-1	0.45
15	1	1	1	1	1	0.00
16	1	-1	1	-1	1	0.46

3.2 Analysis on the main and interaction effects

The result from the screening table was analysed by Design Expert software to assess the effects of the factors in influencing the xylan recovery. The percentage contribution of each factors is portrayed in Table 3. From the table, it is obviously displayed that the main effects of temperature (A) was introduced as the highest contribution for the acid pretreatment process with 24.32% contribution followed by solid loading, time, acid concentration and agitation with contribution percentage of 17.25%, 5.46%, 2.99% and 0.19% respectively. This contribution indicates that temperature plays a significant role in maximizing the xylan recovery compared to agitation which showed insignificant contribution percentage. In term of interaction, the effect of BC showed the most significant percentage in contributing to the xylan recovery with 15.84% contribution. While, the interaction of DE contributed at the second larger contribution percentage followed by AC and AB. Besides, the interaction effects of CE and BD gave only a small contribution percentage which indicate that these interactions do not much affecting the process.

In addition, the effect of the factors also can be visually evaluated using Pareto chart as shown in Fig. 1 where the bar length is proportional to the absolute value of the estimated effect. The analysis on significant effects of each factors at 95% confidence level were screened rapidly based on the two limits of value that shown in the figure. The effects of factors between the t-value limit (3.18245) and Bonferroni limit (8.57517) described that the factors are statistically significant at 95% level, which the factors above the Bonferroni limit demonstrated that factor is certainly significant to influence the response. In contrast, the factor with the value of t-value below the t-value limit is considered not significant at 95% confidence level and insignificantly influence to the response [18]. As can be

seen from the Pareto chart the main effect of temperature (A) was obviously significant since it exceeded the Bonferroni limit. The main effects of time (B) and acid concentration (D) and the interaction effects of BC, DE, AC, AB were considered significant at 95% confidence level. In the other hand, the main effects of C and E and the interaction effects of CE, BD and AE fall behind the t-value limit and considered insignificantly influence the pretreatment process for xylan recovery.

Table 3. Percentage contribution of each factor for xylan concentration recovery

Term	% Contribution
A-Temperature	24.32
B- Time	5.46
C- Solid loading	17.25
D- Acid concentration	2.99
E- Agitation	0.19
AB	7.19
AC	7.67
AE	0.91
BC	15.84
BD	1.08
CE	2.99
DE	13.20

Apart from that, the effects of each factor that influence the level of response also can be observed by the colour of the bar chart. The orange and blue bar chart indicate the positive and negative effect respectively in enhancing the xylan recovery. The positive effect reflecting that the higher value of effect could cause the increasing of xylan recovery, while the negative effect illustrated that by applying the lowest value of factor could increase the xylan recovery [19]. This study clearly showed that use of the lowest range value of temperature (37 °C), solid loading (5 w/v %), acid concentration (0.01 v/v %) and agitation (no) could increase the concentration of xylan. However, the longer time provided for the pretreatment could increase higher xylan concentration since pretreatment time were determined as positive effects for the process. This finding suggested that the complete reaction of hemicellulose could occurred during pretreatment when using lower value of temperature, solid loading and acid pretreatment without agitation at 24 hours for high xylan recovery. The full hydrolysis of hemicellulose will cause the formation of xylose as the product. To recover xylan from OPFB sample, partial hydrolysis need to take place using the condition as suggested in this study. The finding is consistent with findings of past studies by Chapla et al (2012) and Gowdhaman & Ponnusami (2015) which applied the same method of acid pretreatment for xylan recovery from different sample using 0.01 g/L H_2SO_4 and 0.001 g/L H_2SO_4 respectively with no agitation.

3.3 Statistical modelling and ANOVA

The validity of the model was statistically analyzed by ANOVA as presented in Table 4. The F-value were determined to check the statistical significance of a regression equation while the significance of each coefficient is

checked by the p-value. The model F-Value of 27.13 observed indicates the model was significant with only 1.00% chances the model could not be significant due to noise where the p-value of the model was 0.01. This result is positive since Qing et al. (2016) in their research mentioned that the factor is statistically significant when the p-value is below than 0.05. Besides, the ANOVA also revealed that the model term of A, B, C, AB, AC, BC and DE were significant. The p-value below 0.05 presented by temperature (A), time (B) and solid loading (C) show that the effect of the factors statistically significant and have strong effects on the pretreatment process for higher xylan recovery. On the other hand, the acid concentration (D) and agitation (E) are considered not significant since the p-value of the model term larger than 0.05.

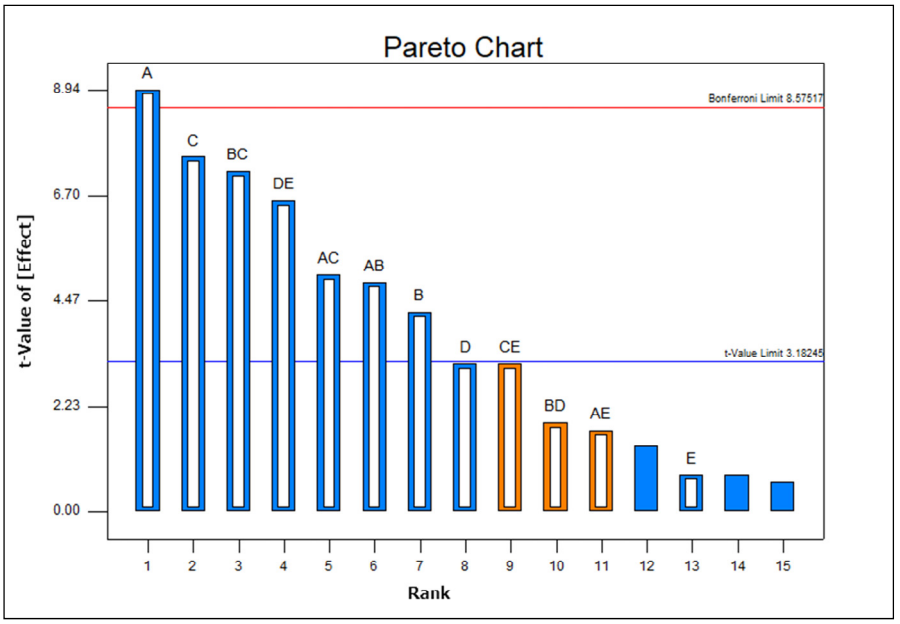

Fig. 1. Pareto chart for each factor with their value effect to the process

The accuracy of the experimental data to fit the predicted data is illustrated by the R^2 value. The R^2 value obtained from ANOVA table is 0.9909 which indicates that only 0.91% of the data not fit the model. which indicates that only 0.91% of the data not fit the model. This value is robust, and it shows that this model is a good fit and can well explained the variation. The final model equation in term of coded factors for xylan recovery from OPFB through acid pretreatment process can be expressed as follows:

$$y = 0.35 - 0.071A - 0.034B - 0.06C - 0.025D - 6.25\times10^{-3}E - 0.039AB - 0.04AC + 0.014AE - 0.058BC + 0.015BD + 0.025CE - 0.053DE \quad (1)$$

where Y is the concentration of xylan (g/L), A is the temperature, B is the time, C is the solid loading, D is the acid concentration and E is the agitation. The unknowns A, B, C, D and E are represented as the main effects while AB, AC, AE, BC, BD, CE and DE are the interaction involved in the pretreatment process.

Residual analysis was tested using normal probability plot to check the adequacy of the model as presented in Fig. 2(a). The plot shows that the residuals follow a normal distribution along the straight line thus conforming the statistical analysis. This finding is consistent with the study done by Ding (2015) where the author examined that if the residuals follow a normal distribution without any obvious pattern, all the points will follow a straight line. Furthermore, the predicted values versus actual values plot as represented in Fig. 2(b) is to check the difference between both values. The plot shows the actual data are equally scattered on the straight line representing that both actual and predicted values are in reasonable agreement.

Table 4. Analysis of variance (ANOVA) table for factorial analysis process

Source	Sum of squares	df	Mean Square	F-value	P-value	
Model	0.33	12	0.028	27.13	0.0100	Significant
A-Temperature	0.081	1	0.081	79.89	0.0030	
B-Time	0.018	1	0.018	17.93	0.0241	
C-Solid loading	0.058	1	0.058	56.66	0.0049	
D-Acid Conc.	0.010	1	0.010	9.84	0.0518	
E-Agitation	6.250E-004	1	6.250E-004	0.61	0.4902	
AB	0.024	1	0.024	23.63	0.0166	
AC	0.026	1	0.026	25.18	0.0152	
AE	3.025E-003	1	3.025E-003	2.98	0.1830	
BC	0.053	1	0.053	52.03	0.0055	
BD	3.600E-003	1	3.600E-003	3.54	0.1564	
CE	0.010	1	0.010	9.84	0.0518	
DE	0.044	1	0.044	43.38	0.0071	
R^2	0.9909					

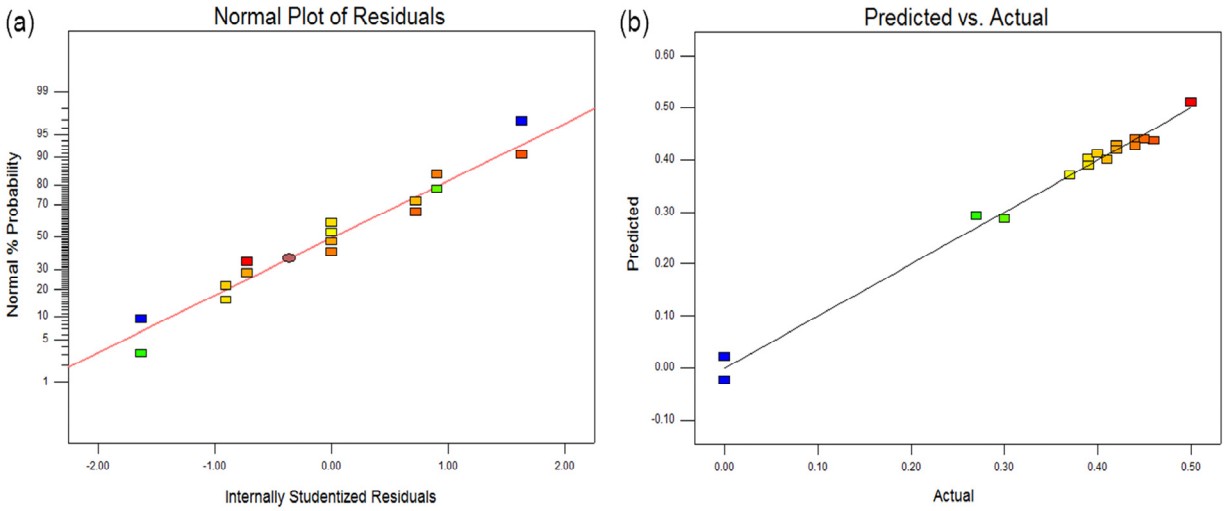

Figure 2. Plot of (a) normal probability of residuals and (b) predicted versus actual values of xylan concentration

3.4 Validation for factorial analysis

Validation of the model was performed to validate the suitability of the model equation in predicting the xylan concentration. Two points of condition used in this validation experiment were obtained from the screening table and design expert software. Validation experiment using Run 13 from experimental table condition where the highest xylan concentration produces (Point A) and the best condition suggested by Design Expert software (Point B) were conducted in triplicate. Percentage error were measured based on the difference between predicted and experimental value as presented in Table 5. The percentage error of 7.69% and 9.78% for Point A and Point B respectively showed the reasonable close of experimental value to the predicted value. Low percentage error (< 10%) obtained in this study proved the validity and adequacy of the model and thus confirmed that the validation experiment is satisfied and successful.

Table 5. Validation runs for factorial analysis

Description	Point	
	A	B
Conditions		
Temperature (°C)	37	37
Time (hours)	24	6
Solid loading (w/v %)	5	20
Acid concentration (v/v %)	0.01	0.03
Agitation (rpm)	No	Yes
Predicted value (%)	28.04	29.38
Experimental value (%)	25.89±0.73	26.51±0.46
Error (%)	7.69	9.79

4. Conclusions

Nitric acid pretreatment for xylan recovery from OPFB has been carried out to evaluate the factors affecting the process through half fractional two-level factorial design. The result of factorial analysis from Design Expert software recommended that the use of lower condition with the increase of time incubation contributed to the improvement of xylan recovery. The highest amount of xylan 0.5 g/L was obtained during pretreatment without agitation at 37°C with 20% solid loading and 0.01% acid concentration for 24 hours pretreatment time. It was found that the contribution sequence of the factors affecting the xylan recovery to be temperature > solid loading > pretreatment time > acid concentration > agitation. The result of factorial analysis from Design Expert software obtained in this study can provide suggestion for further experiment especially in optimization of the nitric acid pretreatment condition. With a suitable condition obtained, recovered xylan can later be utilized to produce other valuable products such as xylooligosaccharides.

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