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Screening of factors influencing dilute nitric acid pretreatment for xylan recovery from oil palm frond bagasse

N A Mazlan[1], K A Samad[1], H W Yussof[1,*], R A Samah[1] and J Jahim[2]

[1]Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Kuantan, Pahang, Malaysia
[2]Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

*Corresponding author: hafizuddin@ump.edu.my

Abstract. Lignocellulosic biomass is the most abundant biodegradable material that has been used in obtaining various of valuable products. However, the conversion may hinder due to its solvent insoluble characteristic which can be treated by nitric acid pretreatment. Therefore, the present study aimed to investigate the effect of nitric acid pretreatment on oil palm frond bagasse (OPFB) for achieving high xylan recovery. A two-level factorial design was employed to determine the best condition for the process. The result revealed that the sequence of contribution effect toward the increment of xylan recovery was found to be temperature > solid loading > reaction time > acid concentration > agitation. Pretreatment of OPFB with nitric acid discovered the temperature of 37 °C with 5% solid loading at 0.01% acid concentration for 24 hours without agitation were determined as the best condition to recover up to 27.63% of xylan. The present results highlight that nitric acid pretreatment has the potential to improve xylan recovery by removing lignin to facilitate enzymatic hydrolysis for product recovery.

1. Introduction

The chemicals production via petrochemical process have created many environmental problems which urge the researchers to find alternative ways to replace this process. Hence, biochemical approach for chemical production has gain attention recently due to its environmentally friendly process to substitute the synthetic chemicals production, and this process is named as biorefinery. Biorefinery is the technique to convert biomass into valuable bio-based products where at the same time can minimize the carbon produced throughout the process [1]. The source of biomass used in biorefinery can be classified into several categories; household waste, animals and plants waste and agricultural and forestry residues [2].

One of the sources that widely studied currently in biorefinery field is lignocellulosic biomass due to its compositions that having a potential to produce numerous types of chemicals. This biomass comes from plant dry matter that build up by cellulose, hemicellulose and lignin as the major components besides other small amount elements such as protein and arabinose [3]. In lignocellulosic biomass, cellulose make a major structure with approximately 35–50% from the overall lignocellulosic biomass components and exist as a crystal in nature [4,5]. In contrast, hemicellulose has an amorphous structure and is a second major component in lignocellulosic biomass with 20–35% from the overall components [6]. The structure in hemicellulose mainly derived from xylan where the monomer unit of xylan was discovered by can be used as a resource to produce other products

including xylitol, glucose, xylooligosaccharides, ethanol and furfural [7–10]. However, lignin, which also made up a major component in lignocellulosic biomass, is well known as its complex molecule structure that resistant to chemical and enzyme attack. On account on this, it is necessary to break the structure that link cellulose, hemicellulose and lignin to make the chemical production process become possible [11].

Currently in tropical countries, oil palm biomass especially oil palm frond has turn up as the valuable materials that can be utilized in bioconversion due to its valuable content and readily available. There were approximately 44 million tonnes of OPF was attained during the replanting season and only left rotten or burned in the land field [12]. This action lead to the environmental degradation from the open burning and dumping of large amount of these agricultural residues. In 2010, OPF was identified inherent a potential to be used in biorefinery as a source for fermentable sugar production and since has been studied to produce many other products including glucose, bioethanol, succinic acid, quercetin and poly(3-hydroxybutyrate) [1,11,13–15].

Several processes were identified has been implemented in converting lignocellulosic biomass into value added products and one of it is the process that involve a pretreatment step namely physical, chemical or biological. However, combination of physical and chemical pretreatment is often used in most of the pretreatment process to improve downstream desired product yield [16–18]. Generally, the biomass was first subjected to mechanical pretreatment where in this step, the large size of biomass was grinded for the size reduction purpose [19]. After the desired size was obtained, the biomass then was subjected into the treatment using chemical to break the chemical structure in the biomass. The type of chemical pretreatments that notable in literature are alkali, acid and ionic liquid pretreatment [19]. For the xylan isolation process, most of the previous studies used alkali solvent to break the chemical structure and hence dissolve xylan into the alkaline liquid that was called as a black liquor [20]. However, Kumar et al. [21] has mentioned that the used of dilute acid as a pretreatment stimulant can alter the biomass structure besides is more economical compared to other chemical pretreatment processes.

Thus, this study was done to evaluate the factors that understand give effect to dilute nitric acid (HNO_3) pretreatment of oil palm frond bagasse (OPFB) with the aim to get high xylan content. To achieve this, two level fractional factorial analysis and research surface methodology (RSM) was implemented with five factors were evaluated; temperature, reaction time, solid loading, acid concentration and agitation.

2. Materials and methods

2.1. Materials

This study used oil palm frond collected from local plantation in Kuantan, Pahang. To get the OPFB, the OPF was pressed using sugarcane pressed machine to remove juice in pressed OPF. The obtained bagasse then dried under sun for up to 3 days until the moisture content of OPFB less than 10%. After drying process, the OPFB was grinded to get OPFB in fiber form then was subjected to sieving to make sure the size of OPFB fiber was less than 2 mm. The sieved OPFB then stored in -20 freezer for further used.

2.2. Acid pretreatment

The raw OPFB fiber were soaked in dilute nitric acid at condition as per shown in table 1. The concentration of nitric acid added to the substrate was varied in the range 0.01 to 1.0% while the solid to liquid loading was assorted between 5 to 20%. The acid pretreatment of OPFB was conducted for 6 to 24 hours at 37 to 90 °C in water bath with (200 rpm) and without agitation. Once the pretreatment process was completed, the substrate then filtered and washed with tap water until neutral. The sample was dried in oven at 60 °C for overnight then stored prior further analysis.

2.3. Two-level factorial analysis experimental setup

For factorial analysis study, Design Expert 7.0 (Stat-Ease Inc., USA) software was implemented to conduct the experimental design. As displayed in table 1, five factors were believed will affected the pretreatment process; temperature (°C), reaction time (hours), solid loading (w/v %), acid concentration (v/v %), and agitation (rpm). The response of screening process using Response Surface Methodology (RSM) generated by the software as tabulated in table 2 where the coded values of -1 represent the low value while +1 is otherwise.

Table 1. Experimental design for factorial analysis with its response.

Factors	Coded	Type of factor	Low value (-1)	High value (+1)	Units
Temperature	A	Numerical	37	90	°C
Reaction time	B	Numerical	6	24	hours
Solid loading	C	Numerical	5	20	% (w/v)
Acid concentration	D	Numerical	0.01	1.00	% (v/v)
Agitation	E	Categorical	No	Yes	rpm

2.4. Determination of glucan, xylan and lignin composition

The composition of glucan, xylan and lignin in raw material was determined according to the National Renewable Energy Laboratory (NREL) method. The sample was first subjected to two step extraction process with 8 hours water extraction using water as a solvent in the first step followed by 24 hours extraction using ethanol in the second step. The extraction process was done to remove extractives in sample to prevent interference in analysis process later [22]. The extracted sample then put through acid hydrolysis to extract structural carbohydrates and determine the amount of lignin in biomass using 72% sulfuric acid (H2SO4) [23]. In this process, 0.3 g extractive free sample was added with 3mL 72% H_2SO_4 and incubated for 1 hour at 30 °C. After that, the sample was added with 84 mL distilled water and autoclaved at 121 °C for 1 hour to complete the cycle.

2.5. Quantification of structural carbohydrates

Structural carbohydrates in sample were determined using high performance liquid chromatography (HPLC) equipped with refractive index (RI) detector and Rezex RHM-Monosaccharide H+, 300 × 7.8 mm (Phenomenex) column. This column use required deionized water as mobile phase which flow at 0.4 mL/min with 5 µL injection volume at 60 °C column temperature. The glucan and xylan were quantified based on calibration curve obtained from the standard prepared within the range 0.5 to 10 g/L.

3. Results and discussions

3.1. Characterization of OPFB

Chemical composition of non-treated oil palm frond bagasse (OPFB) obtained in this study was 42.8% glucan, 20.9% xylan, 31.9% lignin, 0.8% ash and 3.6% extractives [24]. The results showed similarities for glucan and xylan described by Abdul Manaf [25] which recorded 41.7% and 18.5% of glucan and xylan respectively. However, lignin found in this study was much higher compared to theirs with difference as much as 11.4%. Therefore, it is important to remove lignin component to ensure high xylan content can be achieved. High lignin content may hinder the extraction of xylan due to formation of lignin-carbohydrate complexed that has formed between lignin and hemicellulose [26].

3.2. Factors affecting dilute nitric acid pretreatment

Screening the factors affecting dilute nitric acid pretreatment for high xylan recovery was performed and analyzed using 2^{5-1} fractional factorial design. High xylan recovery can be obtained by minimizing

lignin from OPFB. Meanwhile, glucan was preserved during acid hydrolysis since mild condition was subjected. Table 2 shows the experiment result of 16 runs for glucan, xylan and lignin. High xylan recovery was achieved at 37 °C with 5% solid loading and 0.01% acid concentration for 24 hours without agitation (Standard order 3). At these conditions, lignin was found to be 15.03% which was the lowest amount with glucan was 57.02%. The increment showed mild condition could improve xylan content while reducing 52.88% of lignin by converting it into phenolic compounds [27,28].

Table 2. A 2^{5-1} fractional factorial design at different pretreatment conditions with percentage of glucan, xylan and lignin.

Standard Order	Variables					Responses		
	A	B	C	D	E	Glucan (%)	Xylan (%)	Lignin (%)
1	37	6	5	0.01	Yes	51.39	23.64	17.58
2	90	6	5	0.01	No	47.06	22.18	26.95
3	37	24	5	0.01	No	57.02	27.63	15.03
4	90	24	5	0.01	Yes	49.83	22.78	21.07
5	37	6	20	0.01	No	53.06	23.80	22.53
6	90	6	20	0.01	Yes	56.75	25.85	17.29
7	37	24	20	0.01	Yes	52.73	23.25	23.76
8	90	24	20	0.01	No	73.33	0.00	26.66
9	37	6	5	1	No	54.50	25.43	18.95
10	90	6	5	1	Yes	62.51	17.00	20.40
11	37	24	5	1	Yes	50.56	22.46	21.53
12	90	24	5	1	No	60.15	16.56	19.95
13	37	6	20	1	Yes	49.32	21.08	19.11
14	90	6	20	1	No	58.41	14.8	23.68
15	37	24	20	1	No	49.01	21.03	26.41
16	90	24	20	1	Yes	64.06	0.00	31.33

A: Temperature (°C); B: Reaction time (hours); C: Solid loading (%); D: Acid concentration (%); E: Agitation (rpm).

3.3. Statistical modeling and ANOVA

Analysis of variance (ANOVA) for glucan, xylan and lignin are presented in table 3. The study found that the model developed by Design Expert software based on the analysis were significant for all the responses with F-value were 30.86, 230.47 and 19.05. In addition, the *p*-value of all the models were less than 0.05 which also indicating that the models were strongly significant. The determination of coefficient (R^2) found in this study showed 99.5, 99.89 and 98.7% of the data well-fit the model. The result indicated high agreement were observed between the predicted and actual values.

Table 3. Analysis of variance for glucan, xylan and lignin content.

Response	Glucan	Xylan	Lignin
Model	Significant	Significant	Significant
F-value	30.86	230.47	19.05
p-value	0.0318	0.0004	0.0166
R-Squared	0.995	0.9989	0.987
Adj R-Squared	0.9628	0.9946	0.9352

Table 4 presents the regression coefficient of linear regression equation for glucan, xylan and lignin. The relationship between the factors and response can be explained by the positive and

negative sign of the values. Positive sign indicates that the increase of the factor's value could increase the response. On the other hand, the negative sign implied the decrease of the response value with the increase of the value of factor. In order to improve xylan recovery in this study, low level value of the factors should be applied. While, high level value of agitation may improve the process. However, based on the coefficient value of 0.29 showed by the factors obviously indicated insignificant effects was expressed by the factor. Therefore, contribution of low- or high-level value of agitation was not important for the process which may be removed in further study.

Table 4. Regression coefficient of experimental model for glucan, xylan and lignin.

	Coefficient Estimate		
Factor	Glucan	Xylan	Lignin
Intercept	55.61	19.22	22.01
A-Temperature	3.41	-4.32	1.40
B-Reaction time	1.48	-2.51	1.20
C-Solid loading	1.48	-2.99	1.83
D-Acid concentration	0.46	-1.92	0.66
E-Agitation	-0.96	0.29	-0.51
AB	1.35	-2.56	N/A
AC	2.65	-1.74	-0.51
AD	1.81	-0.88	N/A
AE	N/A	1.22	N/A
BC	1.22	-2.65	1.99
BD	-1.60	N/A	0.93
BE	-1.83	N/A	1.71
CD	-2.34	N/A	0.63
CE	N/A	1.03	-0.47
DE	1.51	-2.45	0.93

Model equation for glucan, xylan and lignin through dilute nitric acid pretreatment was generated using the regression coefficient value from table 5. The equations for the response of glucan, xylan and lignin are presented as in equation (1), (2) and (3), respectively below:

$$Glucan\ (\%) = 55.61 + 3.41A + 1.48B + 1.48C + 0.46D - 0.96E + 1.35AB + 2.65AC + 1.81AD + 1.22BC - 1.6BD - 1.83BE - 2.34CD + 1.51DE \quad (1)$$

$$Xylan\ (\%) = 19.22 - 4.32A - 2.51B - 2.99C - 1.92D + 0.29E - 2.56AB - 1.74AC - 0.88AD + 1.22AE - 2.65BC + 1.03CE - 2.45DE \quad (2)$$

$$Lignin\ (\%) = 22.01 + 1.4A + 1.2B + 1.83C + 0.66D - 0.51E - 0.51AC + 1.99BC + 0.93BD + 1.71BE + 0.63CD - 0.47CE + 0.93DE \quad (3)$$

where A, B, C, D and E are referred as the main effects of temperature, reaction time, solid loading, acid concentration and agitation. Meanwhile, AB, AC, AD, AE, BC, BD, BE, CD, CE and DE are the interaction effects.

Figure 1 demonstrates the predicted versus actual yield of glucan, xylan and lignin at various pretreatment conditions. The actual values showed a scatter distribution alongside the predicted values (straight lines) which indicating high correlation observed between predicted and experimental data.

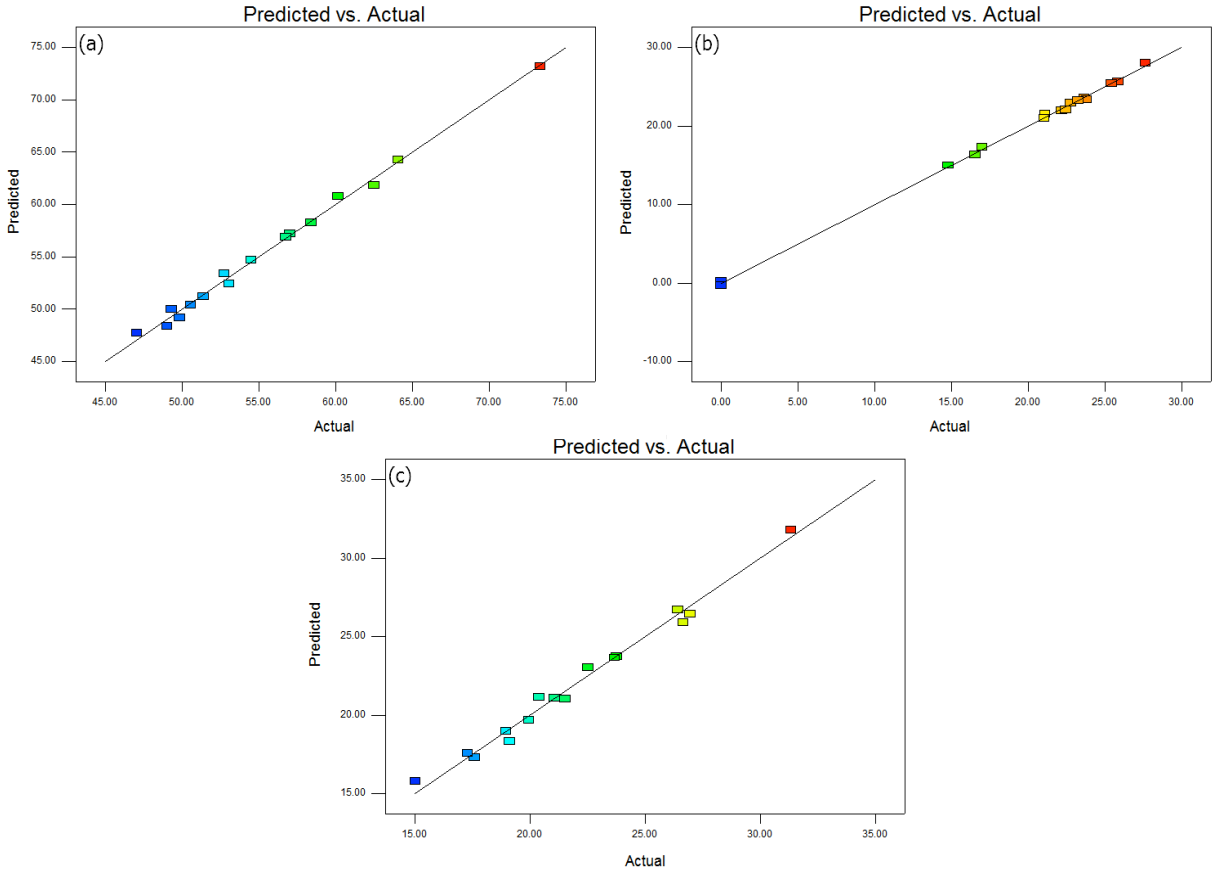

Figure 1. Predicted versus actual yield for (a) glucan (b) xylan and (c) lignin.

3.4. Analysis the main effects for xylan recovery

Analysis of the main effects is important to be evaluated to study the role of each factors in contributing xylan recovery. Table 5 presents the percentage contribution of each factors towards the pretreatment process. Temperature was found to be the most contributable factors at 29.34%. Solid loading stood as the second important factors at 14.05% followed by reaction time and acid concentration at 9.85 and 5.81%. Among the factor, agitation was insignificant in contributing to the process with value less than 1%.

Table 5. Percentage contribution of factors affecting xylan content.

Term	% Contribution
A-Temperature	29.34
B-Reaction time	9.85
C-Solid loading	14.05
D-Acid concentration	5.81
E-Agitation	0.13

The effects of the factors also can be expressed by half-normal plot as shown in Figure 2. The farthest to the right point indicated the higher effect presented by the factor. The figure illustrated similarities between table 5 in term of degree of significance which A (temperature) showed as the strongest effect on dilute nitric acid pretreatment for xylan recovery since it was far away from the point of zero value followed by C (solid loading), B (reaction time), D (acid concentration) and E (agitation). The factor falls along a relatively the straight line is considered insignificant to the process which showed by E (agitation). Furthermore, the positive and negative effects given by each factor

could also be studied by the plot. As previously mention in regression coefficient values on the positive and negative sign, the effects illustrated by half-normal plot are shown by its colour, where positive and negative effects are stated by orange and blue colour, respectively. Based on the result, temperature was significant in influencing xylan recovery at low level value. Theoretically, temperature of 37 °C may promote higher xylan recovery but started to decrease as the temperature increases to 90 °C. Temperature increment cause the degradation of hemicellulose (xylan) into xylose. Moreover, conversion of xylose into furfural may also arise together with degradation of cellulose (glucan) when temperature is excessively increase [29].

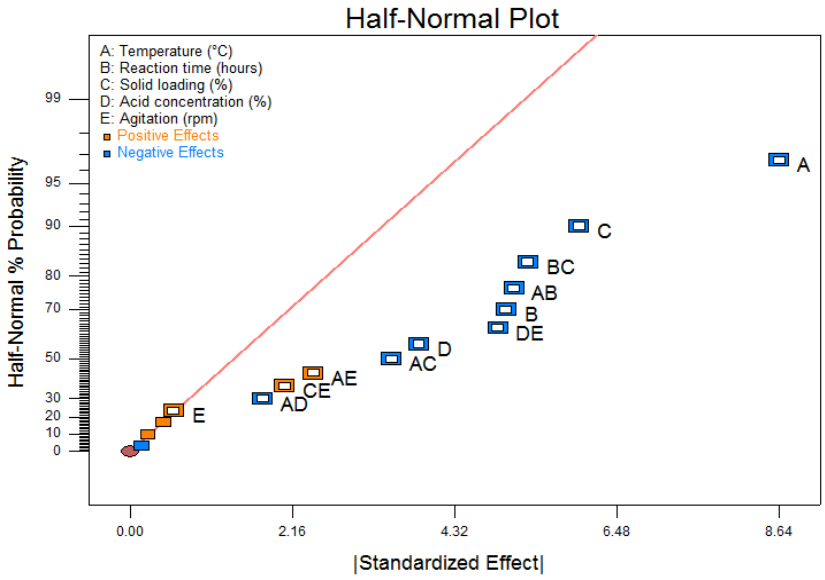

Figure 2. Half-normal plot of effect for xylan content.

3.5. Validation experiment

Table 6 shows the result of validation experiments which were performed in triplicate based on condition suggested by Design Expert software to confirm the suitability of model obtained for xylan recovery. In this experiment, glucan at in range value with xylan at maximum value and lignin at minimum value were appointed. Based on the software setting, temperature of 37 °C, reaction time of 24 hours, solid loading of 5% and acid concentration at 0.01% without agitation speed were proposed as the best conditions for higher xylan recovery at desirability of 0.977. Predicted values for glucan, xylan and lignin at these best conditions were 57.19, 28.04 and 15.78% respectively.

Table 6. Validation of theoretical value suggested by Design Expert.

Description	Response (%)		
	Glucan	Xylan	Lignin
Predicted value	57.19	28.04	15.78
Experimental value	54.53 ± 0.07	25.89 ± 0.73	16.48 ± 0.18
Error	4.43	7.69	4.43

The result presented that glucan, xylan and lignin content found in this study were 54.53 ± 0.07, 25.89 ± 0.73 and 16.48 ± 0.18% with percentage errors of 4.43, 7.69 and 4.43% respectively. Percentage error is obtained by subtracting predicted value to experimental value, then, the absolute value is divided by the predicted value and multiplied by 100. Low percentage error observed in this study implied that the model attained were adequate and repeatable to determine the responses in future studies thus improve high xylan recovery by removing high lignin content.

4. Conclusion

Study on several factors influencing nitric acid pretreatment for xylan recovery from oil palm frond bagasse (OPFB) was performed via factorial design. The study found that temperature was the strongest factor could affect the process in determining xylan content. Significant experimental models were obtained for glucan, xylan and lignin with determination of coefficient of 0.9950, 0.9989 and 0.987, respectively. Validation experiment with small percentage errors proved the suitability of the model for repeatable experiment. High xylan content was successfully recovered by lowering the operating conditions for 24 hours while rising the conditions resulted in xylan removal entirely from the plant material. The study demonstrates that studying the factors affecting dilute nitric acid pretreatment may enhance xylan recovery at the best conditions.

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