

# Pretreatment of Oil Palm Fronds for Improving Hemicelluloses Content for Higher Recovery of Xylose

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## Article history

Received :19 November 2012

Received in revised form :

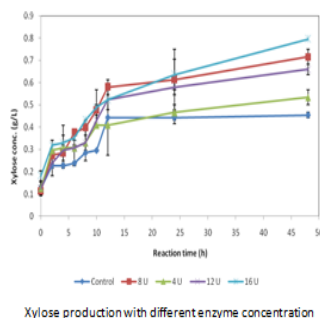
8 January 2013

Accepted :18 January 2013

## Graphical abstract

TABLE III  
LIGNOCELLULOSES CONTENT AFTER PRETREATMENT

Composition	Percentage (%)		
	Alkali	Dilute acid	Autohydrolysis
Extractives	5.87 ± 2.40	7.86 ± 3.02	7.97 ± 1.59
Holocelluloses	85.68 ± 2.99	82.30 ± 2.80	78.43 ± 1.59
α-cellulose	68.16 ± 2.38	54.93 ± 1.76	50.62 ± 0.63
Hemicellulose	17.51 ± 0.61	27.37 ± 1.89	27.80 ± 0.35
Lignin	2.28 ± 1.76	2.34 ± 0.41	5.98 ± 1.68
Ash	6.33 ± 1.52	7.69 ± 0.63	8.15 ± 0.20



## Abstract

Lignocelluloses material is known as a potential biomass for conversion into value-added product. Utilization of biomass for beneficial products particularly food and health-related, has gained increasing attention among researchers worldwide and the potential usage of the lignocelluloses biomass is much sought after nowadays. An oil palm frond (OPF) has a great potential to be used as a precursor for production of xylose. In order to increase the yield of xylose, pretreatment of lignocelluloses biomass is important as it will for example, enhance the accessibility of enzyme to convert hemicelluloses xylan into xylose. Therefore in this study, OPF was pre-treated using dilute acid hydrolysis (H<sub>2</sub>SO<sub>4</sub>), alkali (NaOH), and autohydrolysis methods. The result showed that autohydrolysis gave higher hemicelluloses content which was 27.80±0.35% as compared to alkali and dilute acid pretreatment with 17.51±0.61% and 27.37±1.89%, respectively. The autohydrolysis pretreated samples were then used for further enzymatic hydrolysis for xylan breakdown into xylose. Recovery of xylose was found to be higher at higher xylanase activity which was 16 U. Reaction time of 48 h with 1% substrate was able to produce up to 0.795 g/L xylose.

**Keywords:** Oil palm frond; xylose; pretreatment; xylanase activity

## Abstrak

Bahan lignoselulosa dikenali sebagai biojisim yang mempunyai potensi untuk proses penukaran kepada nilai tambah produk. Penggunaan biojisim sebagai produk bermanfaat khususnya dalam bidang makanan dan kesihatan telah menarik perhatian yang semakin meningkat di kalangan penyelidik di seluruh dunia dan kajian mengenai potensi penggunaan biojisim lignoselulosa telah giat dijalankan pada masa kini. Pelepah kelapa sawit (OPF) mempunyai potensi yang besar untuk digunakan sebagai substrat untuk pengeluaran xylosa. Dalam usaha untuk meningkatkan hasil xylosa, pra-rawatan biojisim lignoselulosa adalah penting kerana ia boleh meningkatkan akses enzim untuk menukarkan hemiselulosa xylan ke xylosa. Oleh itu, dalam kajian ini, OPF akan dirawat menggunakan hidrolisis asid cair (H<sub>2</sub>SO<sub>4</sub>), alkali (NaOH), dan kaedah autohidrolisis. Keputusan kajian tersebut menunjukkan kaedah autohidrolisis mengandungi kandungan hemiselulosa yang lebih tinggi iaitu sebanyak 27.80 ± 0.35% berbanding dengan kaedah pra-rawatan alkali dan asid cair dengan masing-masing menunjukkan hasil sebanyak 17.51 ± 0.61% dan 27.37 ± 1.89%. Sampel autohidrolisis yang telah dirawat kemudiannya digunakan untuk hidrolisis enzim bagi memecahkan xylan kepada xylosa. Penghasilan xylosa didapati lebih banyak pada aktiviti xylanase yang tinggi iaitu pada 16 U. Tindak balas selama 48 jam dengan substrat sebanyak 1% telah dapat menghasilkan sehingga 0.795 g / L xylosa.

**Kata kunci:** Pelepah kelapa sawit; xylosa; pra-rawatan; aktiviti xylanase

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## 1.0 INTRODUCTION

Lignocelluloses is an attractive renewable biomass for the production of various value-added products that comprise about half of the plant matter. Since lignocellulosic materials are highly abundant, plentiful and large availability in nature, it can be utilized to produce various value-added products such as

biofuels, animal feeds, enzyme and healthy food products. Xylan is known as the most common abundant hemicelluloses in lignocellulosic material and contributed around 20-40% of lignocellulosic material after cellulose.<sup>1</sup> Xylose is one of the beneficial products that can be produced from xylan.

At present, oil palm biomass is known as the most potentially lignocelluloses biomass that can be utilized for

xylose production. Commonly, wastes from oil palm crops are used as animal feed but this is not the optimal economically beneficial way of manipulating the wastes. The wastes are usually left to rot or burned in the field after harvesting. Malaysia is known as one of the countries with luxury amount and diversity of renewable resources including oil palm waste, sugarcane bagasse and rice straw. Malaysia is known as the world's leading palm oil producer and exporter, accounting for about 47% of global palm oil production and 89% of exports.<sup>2</sup> The production of palm oil has increased from 8.5 million tons in 2000 to 10.5 million tons by 2010. In 2007 alone, Malaysia is reported to produce approximately 38,256 dry kton of oil palm lignocellulosic biomass, which comprised 44% of OPF.

The idea of bioconverting OPF into xylose has gained interest since OPF is abundant waste with a potential to be converted into valuable products. In order to enhance the breakdown of xylan into xylose, pretreatment of OPF is needed to increase the accessibility of enzyme hydrolysis for optimum hydrolysis process.

Different types of pretreatment including alkali dilute acid and autohydrolysis pretreatment was studied to determine which method is able to extract higher hemicelluloses for xylose production. The main purpose of the study is to investigate the best pretreatment method in order to obtain higher hemicelluloses to be further converted into xylose.

## 2.0 EXPERIMENTAL

### 2.1 Materials

Oil palm fronds (OPF) were obtained from oil palm plantation in Universiti Teknologi Malaysia (UTM). The sample was grounded and sieved to a particle size smaller than 1 mm and dried in an oven at temperature 60°C for 3 days. The dried sample was stored at room temperature in plastic bag before used for further analysis. The lignocelluloses content (cellulose, hemicelluloses, lignin and ash) of raw oil palm frond were analyzed according to Ehman<sup>3</sup> while for extractives content and other composition by Teramoto *et al.*<sup>4</sup> A commercial enzyme, xylanase was used for enzymatic hydrolysis process. All other chemicals used were of reagent grade.

### 2.2 Alkali Pretreatment

OPF sample was soaked in NaOH solutions with concentration 1.25 M. The sample was treated at 85-90°C for 1 h. The treated samples were then washed thoroughly with de-ionized water until neutrality, air dried and stored. The dried sample was used for lignocelluloses content determination after alkali pretreatment.

### 2.3 Dilute Acid Pretreatment

Fresh sample was soaked in dilute acid solution with concentration of 0.01M H<sub>2</sub>SO<sub>4</sub> and incubated in oven at 60°C for 12 h. The OPF was then filtered and washed thoroughly with de-ionized water until all the acid is washed out before the sample was dried in the oven. Distilled water was later added to the OPF sample in a ratio 1:3 (w/w) and autoclaved it at 121°C, 15 psi for 1h. The autoclaved sample was filtered through Whatman filter paper, dried and mashed to obtain xylan powder.

### 2.4 Autohydrolysis

OPF sample with dry weight 20g was placed in 500ml of Schott bottle. Then, distilled water was added in a ratio 1:10 and the mixture were autoclaved at 121°C, 15psi for 1 hour. The autoclaved sample was centrifuged at 2380g for 15 min. The supernatant of samples were filtered with Whatman filter paper. The filtrate was freeze-dried at -40°C and a vacuum of 1.93 × 10<sup>-3</sup> psi before further analyses and enzymatic treatment.

### 2.5 Enzymatic Hydrolysis

The pretreated sample with higher hemicelluloses content was used for further enzymatic hydrolysis. The sample was dissolved in sodium acetate buffer 0.05 M, pH 5.0 in 250 mL Erlenmeyer flask to a concentration of 1% (w/v). Crude enzyme was added at varying concentrations of 4 U, 8 U, 12 U and 16 U. The enzymatic hydrolysis was carried out and incubated in a rotary shaker at 150 rpm for 48 h. Samples were analyzed periodically with 2 h intervals for 12 h and proceeded with 12 hour interval until reached 48 h reaction time. 5 mL of samples was taken out and the enzyme reaction was stopped by boiling it for 10 min before subjected to analyses. Duplicate sample for enzyme hydrolysis were set up for each of the experiment.

### 2.6 Xylose determination

The amount of reducing sugar was determined by using dinitrosalicylic acid (DNS) method with xylose as a standard. Every sample was analyzed for xylose concentration at different xylanases activities.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Lignocelluloses Characterization of OPF

Lignocellulose content of fresh OPF was analyzed in order to determine the hemicelluloses content in fresh OPF without any pretreatment. The amount of hemicelluloses and other composition in OPF are shown in Table 1. Comparison of main lignocelluloses content of different biomass is shown in Table 2.

**Table 1** Lignocelluloses of fresh OPF (before pretreatment)

Composition	Percentage (%)
Extractives	8.33
Holocelluloses	82.12
α-cellulose	58.27
Hemicellulose	23.85
Lignin	5.25
Ash	4.56

**Table 2** Main composition of lignocelluloses biomass<sup>5</sup>

Biomass	Composition (%)		
	Cellulose	Hemicellulose	Lignin
Sugarcane bagasse	40.0	24.0	25.0
Rice straw	35.0	25.0	17.0
Wheat straw	30.0	50.0	20.0
Corn cob	45.0	35.0	15.0
Oil palm fronds (OPF) <sup>a</sup>	44.0	30.4	15.4

<sup>a</sup> data from Saleh *et al.*<sup>6</sup>

From the result presented in Table 1, the percentage of hemicelluloses in OPF was compared to content of the material in other biomasses including OPF, from previous studies (see Table 2). The content of hemicelluloses obtained in this study (23.85%) is slightly lower than that obtained by Saleh *et al.*,<sup>6</sup> which is 30.4%. This is due to the different sources of OPF used in the study and might be due to the different maturity level of the OPF plant. According to Hodson *et al.*,<sup>7</sup> the concentration of lignin and lignocellulose concentration varies with plant height-form, age and anatomical structure. The maturation of living plant material does increase lignin and lignocelluloses. It is also slightly lower than the other biomass materials which are sugarcane bagasse (24.0%), rice straw (25.0%), and significantly lower than wheat straw (50.0%) and corncob (35.0%). Even though the hemicelluloses content in OPF is lower than the other biomass materials, the breakdown of xylan into xylose as a product still can be maximized by pretreatment in order to increase the accessibility of xylanases in xylose production.

With pretreatment, the whole xylan in hemicelluloses can be further converted into desired products through enzymatic hydrolysis. The efficiency of enzymatic hydrolysis is dependent on the biomass digestibility. Moreover, the lignin content and crystallinity play an important role in biomass digestibility. The lignin content can be reduced and at the same time the crystallinity structure of biomass can be disintegrated by pretreatment. In addition, pretreatment is able to disrupt the internal structure of holocelluloses, increase the reaction surface and increase the porosity of biomass.<sup>8</sup>

Different pretreatment methods including alkali, dilute acid and autohydrolysis were conducted and the lignocelluloses content of each sample were analyzed in order to determine the hemicelluloses content after pretreatment process. The contents are shown in Table 3.

**Table 3** Lignocelluloses content after pretreatment

Composition	Percentage (%)		
	Alkali	Dilute acid	Autohydrolysis
Extractives	5.87 ± 2.40	7.86 ± 3.02	7.97 ± 1.59
Holocelluloses	85.68 ± 2.99	82.30 ± 2.80	78.43 ± 1.59
$\alpha$ -cellulose	68.16 ± 2.38	54.93 ± 1.76	50.62 ± 0.63
Hemicellulose	17.51 ± 0.61	27.37 ± 1.89	27.80 ± 0.35
Lignin	2.28 ± 1.76	2.34 ± 0.41	5.98 ± 1.68
Ash	6.33 ± 1.52	7.69 ± 0.63	8.15 ± 0.20

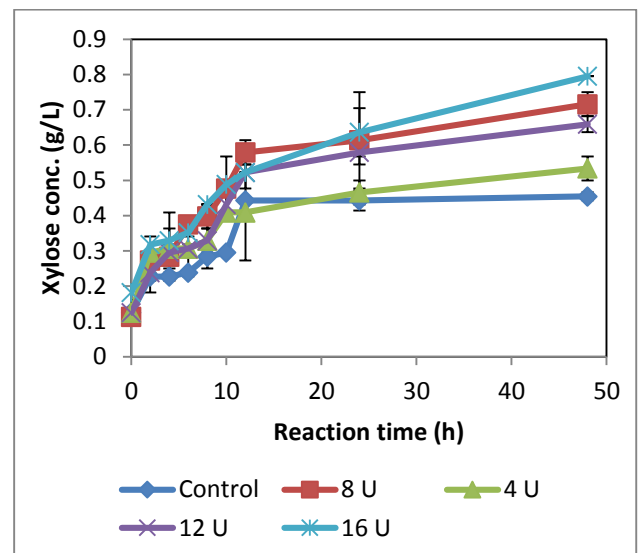
From Table 3, sample pretreated with autohydrolysis contains higher hemicelluloses which is  $27.80 \pm 0.35\%$  compared to dilute acid and alkali pretreatment with  $27.37 \pm 1.89\%$  and  $17.51 \pm 0.61\%$ , respectively. The percentage of hemicelluloses content after pretreated with dilute acid is actually just slightly lower than the autohydrolysis pretreated sample. However, dilute acid requires high-cost corrosion resistant material in large scale production.<sup>4</sup> Besides neutralization process is needed for dilute acid as well as alkali pretreatment.

Higher hemicelluloses content will ensure higher xylose production in enzymatic hydrolysis. Autohydrolysis is one of the effective and more advantages methods in pretreatment for degradation of hemicelluloses from lignocelluloses biomass. Autohydrolysis provides more environmentally friendly method compared to the other chemical methods because it does not use corrosive chemical in the treatment. Autohydrolysis also poses

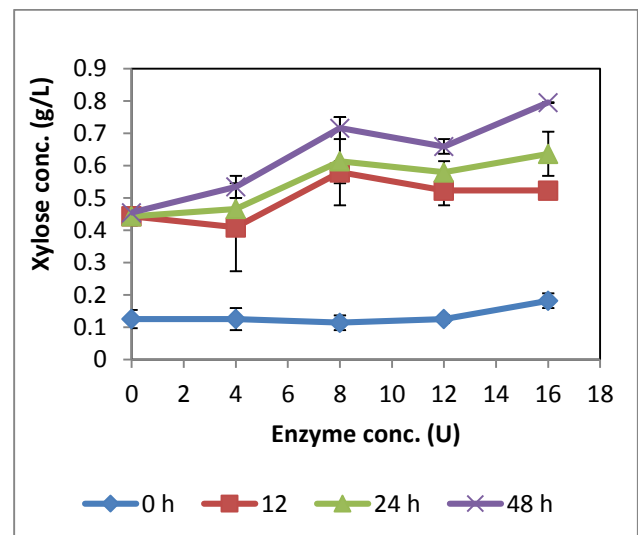
many advantages such as eliminating corrosion problem, no neutralization process is needed, excellent selectivity towards cellulose degradation even in mild condition, reduce unwanted sugar-degradation found in the media and improve the susceptibility of solid residues for further hydrolysis.<sup>9</sup> By using autohydrolysis, the hydronium ion from autohydrolysate causes catalytic depolymerisation of hemicelluloses to xylose and xylooligomers and cleavage of acetyl group to acetic acid that enhance the increasing of hydronium ion in the reaction media.<sup>10</sup>

### 3.2 Production of Xylose from Autohydrolysis Pretreated OPF

Figure 1 (a) and (b) show the effects of reaction time and xylanase concentration on treated OPF in the production of xylose during enzymatic hydrolysis.



(a)



(b)

**Figure 1** Enzymatic hydrolysis of oil palm frond pretreated using autohydrolysis as functions of reaction time (a) and enzyme concentration (b)

From Figure 1 (a), a higher xylose production can be seen at higher xylanase activity which is 16 U. Increasing the xylanase activity from 4 U to 8, 12, and 16 U increases xylose production from 0.534091 g/L to 0.715909, 0.659091 and 0.795455 g/L, respectively after 48 hour reaction time. The increasing trend of each enzyme concentration was significantly increase from 0 to 12 h; however after 12 h to 48 hour, the xylose production keeps on increasing but not as significant as during the reaction time less than 12 h. As shown in Figure 1 (b), the increasing of xylanase concentration or activity from 4 U to 8 U does significantly affect the increasing of xylose production, but not from 8 U to 16 U. This is due to enzyme activity, which is limited by the substrate availability in the hydrolysis process. For control or without xylanase in the reaction, the degradation process to produce xylose does still occur, but at a slower rate compared to the reaction with the presence of xylanase. Xylanase is capable of breaking down the xylan in OPF sample to produce xylose and other reducing sugars. Increasing the enzyme activity with longer hydrolysis time does not give a substantial xylose yield and was not advantageous. Furthermore, Yang *et al.*<sup>11</sup> have reported that the increasing of xylanase activity from 5 to 10 U increased the reducing sugar only up to 12 g/L from 11 g/L after 24 h of hydrolysis time.

#### ■4.0 CONCLUSION

From this study, it was shown that pretreatment method is important in order to extract higher amounts of hemicelluloses from OPF biomass and increase the accessibility of lignocelluloses biomass for enzymatic hydrolysis. Autohydrolysis pretreatment was proven to be an effective method and more environmentally friendly for hemicelluloses degradation. In addition, pretreated sample with higher

hemicelluloses was able to produce higher amount of xylose as a result of the increased xylanase activity at longer hydrolysis time.

#### Acknowledgements

The authors are grateful and would like to express their sincere appreciation to the Ministry of Higher Education (MOHE) and Department of Bioprocess Engineering, Universiti Teknologi Malaysia (UTM) for their kind support and research grant GUP (Q.J130000.7125.00J23) throughout this study.

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