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Effect of different pretreatment methods on structural carbohydrates of kenaf fiber

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Abstract. The application of pretreatment on lignocellulosic biomass is considered as a critical step to alter the recalcitrant structure of biomass especially in bioconversion process. Different pretreatment may result in different modification effects which is important to be rationally evaluated before adoption in order to achieve high yield of end products. Therefore, this study was done to evaluate the effect of different pretreatment methods on kenaf in terms of their chemical and physical properties. Kenaf was subjected to alkaline pretreatment using 4.42% sodium hydroxide operated at 100 °C for 1 hour and acid pretreatment using 0.1% nitric acid which was run at 60 °C for 12 hours. Through characterization study, higher structural carbohydrates were found in alkaline pretreated kenaf compared to acid pretreated kenaf with 73.4% and 70.3% respectively. The SEM, XRD and FTIR analysis showed the significant different of biomass structure before and after pretreatment. From this study, pretreatment process has been verified can improve the biomass compositions to be utilized for chemicals production by disrupt the structure and remove lignin which can be proven by the increasing in structural carbohydrates and decreasing in lignin yield from this characterization study.

1. Introduction

Lignocellulosic biomass is a plant dry matter that can be obtained from woody materials, agricultural residues and forestry wastes [1]. Biomass from these sources mainly comprised of three major components namely cellulose, hemicellulose and lignin, and the distribution of these components are different depending on the type of biomass [2]. Lignocellulosic biomass is readily available and cost-effective sources that can be converted into various types of value-added chemical including ethanol, acids, bio-oils and other fermentable sugars [3]. However, due to its well-known structural resistance, the production of these products from lignocellulosic biomass is limited. For this reason, the chemical pretreatment become necessary process to remove plant cell wall and simultaneously break the structure inside biomass before further process can be applied [2].

Different chemical pretreatment methods were identified from literature; alkali pretreatment, acid pretreatment, organosolv pretreatment and ionic liquid pretreatment [4–7]. Among these pretreatment methods, alkali and acid pretreatments are the most favourable pretreatment processes studied by researchers since these pretreatments can separate and remove lignin by destroying the lignin structure. When the study is involving the isolation of xylan, most of the studies preferred alkaline pretreatment because it can dissolve hemicellulose into the alkali liquid [5,8]. Only little discussion



was found regarding the use of acid pretreatment on lignocellulosic biomass due to the fact that this pretreatment will produce inhibitors such as acetic acid, hydroxyl methyl furfural and furfural in hydrolysate as their side products. However, the inhibitors production is believed can be controlled by controlling the acid pretreatment conditions.

Effect of pretreatment also different depending on the type of lignocellulosic biomass. For example, alkaline pretreatment works better on agricultural residues compared to wood lignocellulose [9,10]. Kenaf is classified under woody base lignocellulosic biomass. It is a commodity crop that usually grown in tropical areas including in Malaysia. Owing to the advantages in commercial and environmental, in 2009 kenaf was introduced as a new crop by National kenaf and tobacco board (NKTB) in Malaysia to replace the plantation of tobacco [11]. Kenaf plant consist of two parts; outer bast fiber and inner core fiber. Commonly, kenaf bast fiber is utilized for paper, textile and composites material, while kenaf core fibers is processed into absorbent materials. Furthermore, kenaf is identified as fascinating biomass to be utilized in bioconversion process due its fast-growing rate and high content of hemicellulose.

The effects of pretreatment on the chemical compositions and physical properties of kenaf is crucial to be evaluated especially in developing further process as it will influence the performance of the conversion process step later. Comparing and identifying the most suitable pretreatment method through the change of the structural carbohydrate compositions and properties may help in improving the yields. Thus, this study is aiming to investigate the effect of acid and alkaline pretreatment on kenaf fiber. In this study, kenaf was characterized using HPLC, SEM, XRD and FTIR in terms of its compositions and morphological properties before (native) and after pretreatments (pretreated) were applied.

2. Materials and methods

2.1. Raw material

In this study, kenaf fiber used was obtained from local kenaf plantation in Kelantan. Kenaf fiber was left dried under sun for up to 72 hours. Then, kenaf was sieved to make sure the particles size used was standardize at approximately 2 mm. The samples then packed and stored for further use.

2.2. Pretreatment

2.2.1. Acid pretreatment. The application of dilute acid pretreatment was based on the method in the study done by Yang et al. [12] with a little modification. Kenaf fiber was immersed in 0.1% (v/v) nitric acid (HNO_3) with solid to liquid ratio of 1:10. The schott bottle that contain sample was left soaked in water bath at 60 °C. After 12 hours, the sample was washed with tap water before dried in an oven at 60 °C for overnight.

2.2.2. Alkaline pretreatment. Kenaf fiber was soaked in 4.42% (w/v) sodium hydroxide (NaOH) for 1 hour at 100 °C in water bath [8]. Then, the sample was washed with tap water until neutral and left dried in oven at 60 °C for overnight. The sample pretreated sample afterwards was stored for later use.

2.3. Composition of kenaf

The compositions in native and pretreated kenaf was identified based on the method provided by National Renewable Energy Laboratory (NREL). Native and pretreated kenaf was undergo soxhlet extraction to determine the total extractive in kenaf where in this experiment, the extractives such as non-structural carbohydrates were removed from biomass using water and ethanol as solvents. Prior to the extraction process, the sample was put through water extraction for 8 hours, followed by ethanol extraction for 24 hours. The removal of extractive in biomass is important to prevent any obstacle during later analytical steps [13]. The extracted samples afterward used in acid hydrolysis analysis using 72% sulfuric acid (H_2SO_4) to determine the total carbohydrates and lignin in kenaf fiber [14]. On

the other hand, the ash content in biomass was determined through ash analysis where the sample was put in muffle furnace for 24 hours with 575 ± 25 °C [15].

2.4. Surface morphology

The comparison of kenaf surface structure before and after pretreatment was done using scanning electron microscopy (SEM). Prior to the analysis, the samples were secured onto aluminium stub before coated with carbon layer. The sample images were captured with magnification of 500x until 2,000x.

2.5. Biomass crystallinity

The crystallinity of native and pretreated samples were identified using X-ray diffraction (XRD). The standard scattering angle (2θ) to find the crystallinity of biomass is ranging between 5° to 80° . The empirical method [30] as presented in equation (1) was used to calculate the crystallinity index (CrI) of native and pretreated kenaf fiber. In equation (1), I_{am} is the lowest intensity peak of amorphous region at $2\theta = 18^\circ$ whereas I_{002} is the highest peak intensity of crystalline fraction at $2\theta = 22^\circ$.

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

2.6. Spectroscopy characterization

Spectrum 100 FTIR Spectrometer was used to identify the functional groups presence in the native and pretreated kenaf. The infrared spectra of the sample were recorded over a wavelength ranging from 4000 to 400 cm^{-1} . Prior to the analysis, the samples were crushed to obtain smaller particles size and were blended with potassium bromide (KBr) with ratio 1:99.

2.7. Quantification of structural carbohydrates

Total structural carbohydrates in native and pretreated kenaf were identified using high performance liquid chromatography (HPLC) equipped with Rezex Phenomenex Monosaccharides column and refractive index (RI) detector. For HPLC analysis, 3 mL syringe equipped with 0.22 μm nylon syringe filter was used to draw the sample and injected into HPLC vial. Throughout the HPLC analysis, the injection volume for the samples was fixed 5 μL and the column temperature was maintained at 60 °C with mobile phase flowing at 0.4 mL/min. The structural carbohydrates were quantified based on the calibration curve of standard chemicals.

3. Results and discussions

3.1. Effect of pretreatment on lignocellulose composition

Table 1 shows the component of structural carbohydrates, lignin, ash, and extractive obtained from compositional analysis of native, acid and alkali pretreated kenaf fiber. From the results, structural carbohydrates form as the highest component in all samples with 72.76% in native kenaf, 70.35% in acid pretreated kenaf and 73.61% in alkali pretreated kenaf. This result reveal that there is 1.15% increment in structural carbohydrates after alkali pretreatment caused by the increasing in glucan content. Before pretreatment, the value of glucan was 47.35% and increase to 53.20% after alkali pretreatment. However, kenaf delignified by acid shows an opposite result where the value of glucan was decreased to 45.53% after pretreatment.

From literature, the overall composition of kenaf are glucan 31–70%, xylan 18–22% and lignin 3–19% [16–19]. The results obtained in this study still in range compared to the previous studies although the values of xylan and lignin obtained quite higher compared to them. The distribution of components in lignocellulosic biomass is varied depending on the climate changes, harvesting techniques, geographical location and area [8,20]. The reduction in xylan after acid and alkali pretreatment in kenaf substrate might due to the hydrolysis of kenaf into xylose when it is subjected to

acid pretreatment, while hemicellulose is known to dissolve in liquor when it is delignified by alkali solvent [21,22].

Table 1. Lignocellulosic composition of native and pretreated kenaf.

Component	Native kenaf (%)	Acid pretreated kenaf (%)	Alkali pretreated kenaf (%)
Total Structural Carbohydrates	72.76	70.35	73.61
1. Glucan	47.35	45.53	53.20
2. Xylan	25.41	24.83	20.41
Lignin	24.40	28.41	25.47
Ash	0.21	0.02	0.06
Total Extractives	2.63	1.22	0.86

3.2. Effect of pretreatment on surface morphology

Figure 1 represent micrographs obtained from SEM analysis for (a) native kenaf, (b) acid pretreated kenaf and (c) alkali pretreated kenaf. During the analysis, the images was captured with magnification ranging between 200x to 2,000x. The different of kenaf structure between native, acid pretreated and alkali pretreated can be distinguished from the images.

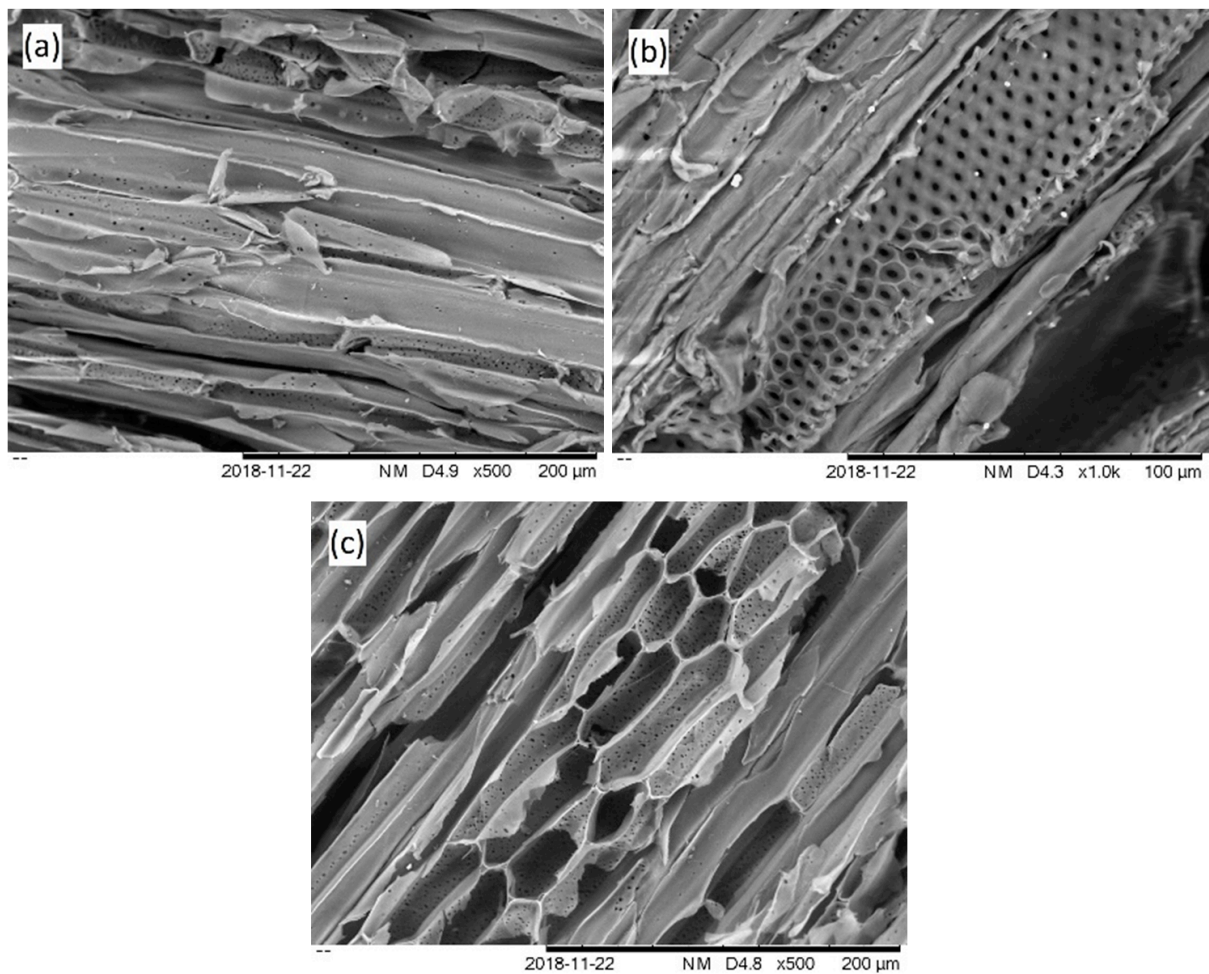


Figure 1. SEM images for (a) native, (b) acid pretreated and (c) alkali pretreated kenaf.

Commonly, native biomass will show smooth surface structure when it is analysed using SEM. However, this study gives non-uniform surface structure of native kenaf maybe caused by the mechanical pretreatment that applied on kenaf during the size reduction process. On the other hand, the fractured surface can be seen clearly on the kenaf fiber where small pores appeared in both acid and alkali pretreated kenaf. Meanwhile, rectangular ray patterns are spotted on alkali pretreated kenaf which proved the removal of outer layer of kenaf cell wall. The structural pattern of native and pretreated kenaf surface structure acquired from SEM analysis in this study are comparable with structure of untreated, acid and alkali pretreated Napier grass fiber obtained in the study done by Kamarullah et al. [23]. From this result, pretreatment process is proved can remove the outer layer of plant cell wall and consequently disrupt the structure of the plant which lead to the exposure of cellulose and hemicellulose [24].

3.3. Effect of pretreatment on crystallinity

Like other lignocellulosic biomass, kenaf consist of cellulose, hemicellulose and lignin as their major components. Between these three components, only cellulose having a crystallinity property [25]. Figure 2 illustrates the XRD profiles for untreated, acid and alkali pretreated kenaf fiber. The XRD patterns obtained in this study is typical XRD pattern for lignocellulosic biomass and comparable with some other previous studies where the major crystalline peak appears at around $2\theta = 22^\circ$ which believed represents cellulose structure [26–28]. Cellulose structure consists of multiple hydroxyl groups which made up from the intramolecular and intermolecular of hydrogen bonds. The arrangement of these bonds accountable to the crystalline order in lignocellulosic biomass [29]. The crystallinity index for untreated, acid and alkali pretreated kenaf fiber calculated using empirical method suggested by Segal et al. [30] are 49.3, 51.1 and 61.3% respectively. From this result, the CI value for untreated kenaf is found lower than both pretreated kenaf. The increasing of CI value after pretreatment process might due to the increasing in cellulose structure in biomass. This result is in line with the study done by Rahnama et al. [5] where they found that the CI value for untreated rice straw was lower than the pretreated one with 50.81% for untreated rice straw and 62.41% after alkaline pretreatment was applied.

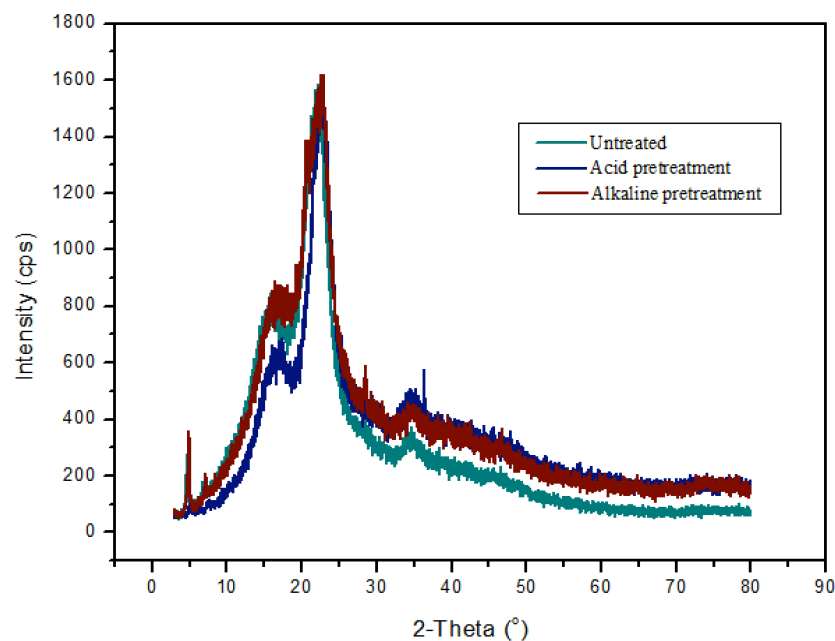


Figure 2. XRD pattern for untreated, acid pretreatment and alkali pretreatment kenaf fiber.

3.4. Effect of pretreatment on chemical structures

In this study, the analysis using FTIR was done to identify the changes of chemical compositions in kenaf fiber before and after pretreatment was carried out. Overall, the spectrum obtained before and after pretreatment process are comparable and no chemical changes are identified. This result exhibit that pretreatment process does not cause any conversion to the chemical structure inside the biomass. The FTIR spectroscopy of untreated, acid pretreated and alkaline pretreated kenaf fiber are depicted in figure 3, while the correlated infrared positions with their functional groups are listed in table 2.

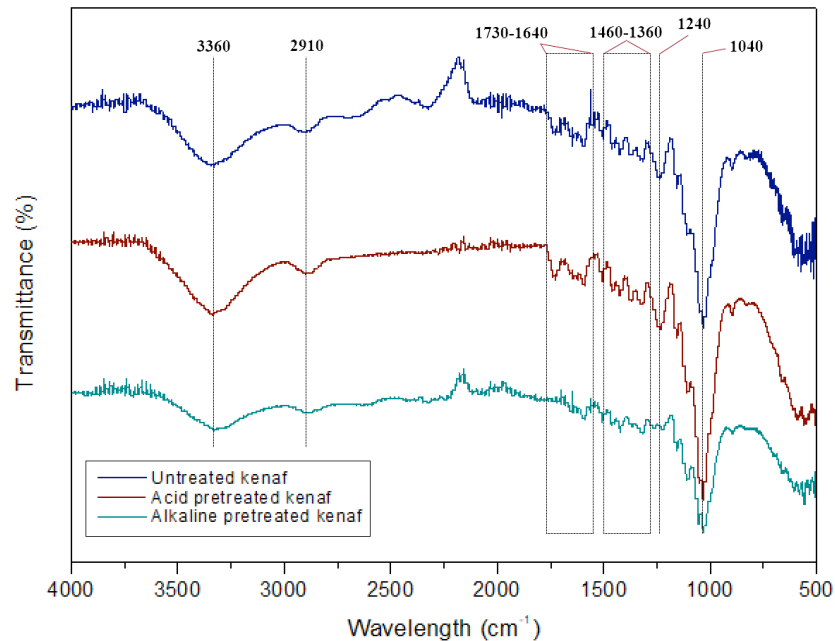


Figure 3. FTIR spectra for untreated, acid pretreated and alkaline pretreated kenaf fiber.

Table 2. Functional group absorbance for main components in kenaf fiber.

Component	Wavenumber (cm ⁻¹)	Functional group
Cellulose	3360	O-H stretching
		N-H stretching
	2910	C-H stretching
	1420	-CH ₂ scissoring
		C-H deformation
	1240	C-H bending
Hemicellulose	1040	C-OH stretching
	1750-1650	C=O Stretching
	1240	C-H bending
	1040	C-OH stretching
Lignin	1370	C-H deformation
	1520	Phenyl ring stretching
	1460-1420	-CH ₃ deformation
	1237	C-O stretching

Associated to the infrared spectra, the first peak can be spotted at wavelength 3360 cm^{-1} , where according to Hsu et al. [31], the broad bend ranging between $3400\text{--}3200\text{ cm}^{-1}$ corresponds to the stretching of O-H and N-H that recognized as the hydrogen bonds in cellulose. Another peak that represents cellulose in biomass is the peak located at wavelength 2910 cm^{-1} that exhibits the C-H stretching of CH₂-OH in cellulose [32]. The sharp absorption bends that labelled between $1730\text{--}1640\text{ cm}^{-1}$ are correspond to the C=O stretching of hemicellulose ester [33]. Other important peaks that represent cellulose and hemicellulose are 1240 and 1040 cm^{-1} which represent the C-H bending and C-OH stretching respectively. For lignin, the building block of lignin in biomass is derived from one or two methoxy groups. This methoxy groups can be found within the peaks ranging between $1500\text{--}1420\text{ cm}^{-1}$ and in this study, these peaks are located at $1460\text{--}1420\text{ cm}^{-1}$. The FTIR spectrums for untreated and pretreated kenaf fiber obtained in this study showed the standard FTIR spectrum of biomass that identical with other previous studies [23,34,35].

4. Conclusion

This study was conducted to characterize and compare kenaf fiber before and after pretreatment process. Overall, results obtained in characterization section, SEM, and XRD are in parallel. After biomass was subjected to pretreatment, the number of structural carbohydrates was increasing from 72.76% for non-treated kenaf to 73.61% for alkaline pretreated kenaf and decreasing for acid pretreated kenaf to 70.35%. Through SEM images, the pretreatment process has proved can remove the outer layer of plant cell wall thus expose composition in the biomass. XRD spectrometry also proved the existence of cellulose at approximately $2\theta = 21$ and $2\theta = 23$ in samples. In addition, an FTIR spectrums show there are no changes in chemical structure before and after pretreatment. This study has demonstrated that alkaline pretreatment is the most suitable pretreatment method for kenaf fiber in exposing glucan for further research. In contrast, acid pretreatment was proven can preserve more xylan compared to glucan. The information regarding physicochemical and surface characteristics obtained in this study very useful to choose the best pretreatment methods to be utilized in bioconversion depending on the desired end products.

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