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CELLULOSE ISOLATION FROM *Leucaena leucocephala* SEED: EFFECT ON CONCENTRATION SODIUM HYDROXIDE

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Abstract

The isolation of cellulose fibres requires the removal of other components such as lignin, hemicellulose, and pectin from the biomass. In this study, the matured *Leucaena leucocephala* seeds (LLS) was applied as a raw material to extract cellulose. The influence of sodium hydroxide concentration (2% - 6%) on the structure of cellulose isolated LLS was studied. The highest yield 23.7% of cellulose was extracted using 4 wt% NaOH treatment. The chemical-physical properties of cellulose extracted were characterized using Fourier Transform Infrared (FTIR), Field Emission Scanning Electron Microscope (FESEM) and X-ray Diffraction (XRD). FTIR spectrum indicated that of all treated samples shows the peaks of cellulose structure. The highest crystallinity index of cellulose was obtained from 6% NaOH treatment which is 76.04%. Under FESEM images, the cellulose appeared in fibrils-like structure. The cellulose obtained can be further disintegrate to micro and nano cellulose to increase the chemical physical properties for various application such as in biocomposite, biomedicine and other value-added chemicals.

Keywords: *Leucaena leucocephala,* seed, cellulose, sodium hydroxide

Introduction

Lignocellulosic materials consist of cellulose, hemicellulose and lignin as major constituents (Ditzel et al., 2017; Loaiza et al., 2017). Cellulose was produced about 1.5 x 10^{12} tonnes every year that make it the most abundant polymer on earth (Grishkewich et al., 2017). Cellulose being applied in various industries such as foods, papers, cosmetics, pharmaceuticals and other industries due to its excellent properties and strength (Ding et al., 2018). However, cellulose commonly used as paper-based material. According to Yu et al. (2017), cellulosic paper has distinctive properties like flexibility, biodegradable and require low cost for production. This making it as a sustainable biomaterial in industries. The use of plant cellulose in this industry helps to maintain the market price and availability of cellulosic fibers for future, as many types of agricultural field are carrying cellulose extraction (Costa et al., 2013).

The Leucaena leucocephala seeds (LLS) contains a good quality fodder due to its high protein content. The usage of LLS as alternative protein in animal feed shows a positive result in growth rate, immune defense, nutrient digestibility and weight gain (Verma et al., 2008; Harun et al., 2017). In spite of excellent source of protein, it is not suitable to commercialize as animal feeding due to present of toxic compound known as mimosine. Due to abundant of Leucaena leucocephala tree in our country, research related to this plant is necessary. In recent years, extraction cellulose from fruits and vegetables have become a focus of many researchers and have been used for nanocomposite materials, binder and filler in food, fabric industry and medical tablet (Zhao et al., 2018). The demand of cellulosic fibre in the market expected to be continuously high; thus to extract cellulose in various plants to fulfil the market demand is necessary. In addition, the extraction of cellulose from LLS can contribute to the diversity of it uses. The used of LLS can reduce the number of trees to be cut down

due to focusing given to wood for cellulose production. In addition, the seeds of the plant can be reproduced without damaging the plants. Therefore, the use of *Leucaena leucocephala* seeds as the source for producing cellulose is promising.

Cellulose was extracted from various sources, rice husk (Collazo-Bigliardi et al., 2018; Johar et al., 2012), coffee husk (Collazo-Bigliardi et al., 2018), elephant grass (Nascimento and Rezende, 2018), corn (Smyth *et al*., 2017), sugarcane bagasse (Feng et al., 2018), pamelo peel (Liu et al., 2018), mango seed (Henrique et al., 2013) and tea waste (Zhao et al., 2018). Different plant will have different amount of cellulose present. Wood is the primary source of cellulose and used commercially. Alkaline treatment process is the common method applied for cellulose extraction; which is the biomasses mixed with alkaline at a hot temperature for a specific time. The extraction parameters such as concentration, temperature and time of reaction will affect the cellulose recovery and properties in terms of degree crystallinity, functional group and surface morphology. Sodium hydroxide (NaOH) has been studied by many researchers. In their researched, oven-dried banana fibers were treated with different concentrations of NaOH (2% - 5%) at 80°C -100°C for 2-4 hr (Elanthikkal et al., 2010; Henrique et al., 2013). Nevertheless, the extraction process varies depending on sources due to the interaction of polysaccharides bond, lignocellulosic composition and ratio of amorphous region (Szymańska-Chargot et al., 2017). The aim of this study was to extract high yield of cellulose fibres from the *Leucaena leucocephala* seed using alkali and bleaching treatments. In this studied 2%, 4% and 6% sodium hydroxide concentration was selected to compare the effect of cellulose isolated in term of yield and quality. Although, there are a few studied cellulose isolations from LLS are documented in the literature, however, extraction of these seed using alkaline method have not been studied. So, further investigations on various cellulose extraction method from LLS are necessary for better understanding the material properties.

Methods

Sample preparation

The seed of *Leucaena leucocephala* (LLS) was collected from Kepong, Malaysia. The seed was separated from the pods manually and was placed in an oven at temperature 65 ºC for 24 hours for drying purpose. The dried LLS was grounded into powder using waring blender and kept in seal container. The chemicals were used in this experiment are sodium hydroxide, sodium chlorite, acetic acid, sulfuric acid and hydrogen peroxide. All chemicals used in this study were reagent grade chemicals and were used as received without further purification.

Characterization of LLS

Holocellulose content

The holocellulose content was determined using method described from Yeh et al. (2004). At first, 3.0 g of LLS powder was placed into 250 mL beaker and mixed with 20 mL distilled water. The solution was heated to 90 ºC in continuous stirring. Then, 15 mL of 20.0 wt% $NaClO_2$ and 6.0 mL acetic acid was added into the solution for every 30 minutes within 2 hours. After 2 hours of reaction, the solution was cooled down to room temperature. Then, the solution was filtered and washed by using distilled water and acetone. Lastly, the product was oven dried at 90 ºC for 6 hours and the final product was weighed. This test was conducted in triplicates. The product formed is holocellulose. The formula to calculate the holocellulose content is as follows;

$$Holocellulose\ (\%) = \frac{mass\ of\ the\ product\ formed}{mass\ of\ initial\ sample} x\ 100 \qquad \text{equation (1)}$$

Cellulose and hemicellulose content

The holocellulose consist of alpha cellulose and hemicellulose. Hence, to determine the content of alpha cellulose and hemicellulose, the holocellulose are further synthesized. The quantification method was followed based on the procedures described by Smyth et.al. (2017). First, about 1.0 g of

holocellulose obtained from previous reaction was placed into a 200.0 mL beaker and was treated with 40.0 mL of 17.5 wt% NaOH for 30 min before adding 40.0 mL of distilled water. The solution was stirred and the reaction was occurred for 30 minutes. After that, the solution was filtered and washed with distilled water. The residue obtained was mixed with 1M of acetic acid for 5 minutes before being filter and washed with distilled water. The product (cellulose) was dried at temperature 90 ºC for 2 hours. The hemicellulose content can be known by subtracting the weight of holocellulose and the alpha cellulose.

Lignin content

To identify the lignin content of LLS, TAPPI method T222om – 11 and method described by Agustin-salazar (2018) were used. A 5.0 g of LLS powder was mixed with 50.0 mL of 72 wt% H_2SO_4 for 2 hours under stirring and terminated by adding 1.2 L of distilled water. Then, the solution was filtered by using filter paper and the dried product obtain was weighed to determine the amount of insoluble lignin present in the LLS.

Preparation of cellulose from LLS

Sodium hydroxide treatment

A 10.0 g of LLS powder was mixed with 200 mL of 2 wt% NaOH solution. The reaction was done in a water bath at 80 ºC with continuous stirring for 2 hours. After that, the solution was cooled at room temperature and followed by filtration. The residue was washed several times with distilled water until become neutral and was proceed to bleaching process. The above steps were repeated with different concentration of NaOH which are 4wt% and 6wt%.

Bleaching

The treated sample was mixed with 200 mL of 2 wt% of H_2O_2. The mixture was stirred for 2 hours at temperature 80 – 100 ºC. Then, the solution was filtered and washed with distilled water until become neutral. This bleaching process was repeated 3 times. Then, the sample was dried in an oven at 90 ºC for 6 hours before been weighed.

Characterisation of cellulose

Fourier Transform Infrared (FTIR)

All samples (LLS and cellulose) were grounded and mixed with potassium bromide (KBr) powder with ratio 1:100 in order to produce a pellet. Perkin Elmer Spectrum 100 IR spectrophotometer was used to obtain the spectra under frequency range 400-4000 cm^{-1} with 24 scans and $4cm^{-1}$ resolution for each sample. The procedure was repeated for all cellulose samples.

Field Emission Scanning Electron Microscopy (FESEM)

FESEM (JEOL JSM-7600F) was used to analyse the microstructure of the LLS and cellulose samples. A small amount of the samples was coated on carbon tape and observed at 15kV of voltage.

X-ray Diffraction (XRD)

The diffraction pattern of the LLS and cellulose samples was analysed by PANanalytical X'Pert HighScore Plus' device. The samples were compressed to form a disk with 10 mm in diameter. This XRD instrument using CuK α radiation as a beam source and recorded in 2 theta range. The operating voltage and current used was 45 V and 40 mA respectively. The crystallinity index of each of sample were calculated by using Segal equation which is;

$$CI = \frac{(I_{002} - I_{am})}{I_{002}} x\ 100\% \qquad \text{equation (2)}$$

where CI is crystallinity index, $I002$ is the highest intensity at 2θ of 22 and Iam is the intensity of the amorphous peak at 2θ of 18.0 (Kouadri & Satha, 2018).

Result and Discussion

Characterization of seed

Lignocellulosic compound mainly contains holocellulose and lignin. Holocellulose is an insoluble polysaccharide fraction that consist of cellulose and hemicellulose. Isolation of holocellulose using acid method generally resulted in the loss of some carbohydrates and a possible retention of lignin (Carrier et al., 2010). The yield of holocellulose from LLS is found to be 37. 28 ± 7.38 %. The amount is less compared to other parts of L*eucaena leucocephala* tree such as wood, branches and twigs (Lopez et al. 2008). However, the amount of lignin is less in seed which gave an advantageous during the pre-treatment method. While, in this study the amount of cellulose obtained by alkaline treatment method around 28.29 ± 3.07%. While, Husin *et al.*, (2017) extracted the cellulose by hot water treatment and followed by treating the samples with 80% of acetic acid and 65% of nitric acid solution. They obtained percent yield of cellulose was 33% which is slightly higher than this research paper. As compared to acid method, the application of alkaline method involved multiple stage of treatment which contributed the loss of cellulose. Therefore, it can be concluded that selection extraction method will influenced cellulose yield (Sun et al., 2004).

Table 1**.** The yield of holocellulose, cellulose, hemicellulose and lignin.

Compound	Percent yield (%)
Holocellulose	37.29 ± 7.38
Cellulose	28.29 ± 3.07
Hemicellulose	9.00 ± 0.505
Insoluble lignin	8.30 ± 0.67

Effect of concentration sodium hydroxide

The average yield of cellulose from 2wt%, 4wt% and 6wt% of NaOH are 22.62%, 23.70% and 22.44% respectively. The difference between all three treatments are less than 1.3%. Hence, the different NaOH concentration do not influenced the amount of cellulose extracted.

Table 2. The amount of cellulose from different treated NaOH.

	Yield (%)
2% NaOH treated	22.62%
4% NaOH treated	23.70%
6% NaOH treated	22.44%

Although, the different on concentration's do not play huge difference on the amount of cellulose isolated but it affects in term of colour of the product. Figure 1 showed the effect of treatments to the colour of cellulose obtained. After the alkaline treatment the dark brown color of LLS turn to yellowish and white product depends on concentration of NaOH used. Hence, a higher NaOH concentration would require less repeating bleaching process in order to lighten the end product.

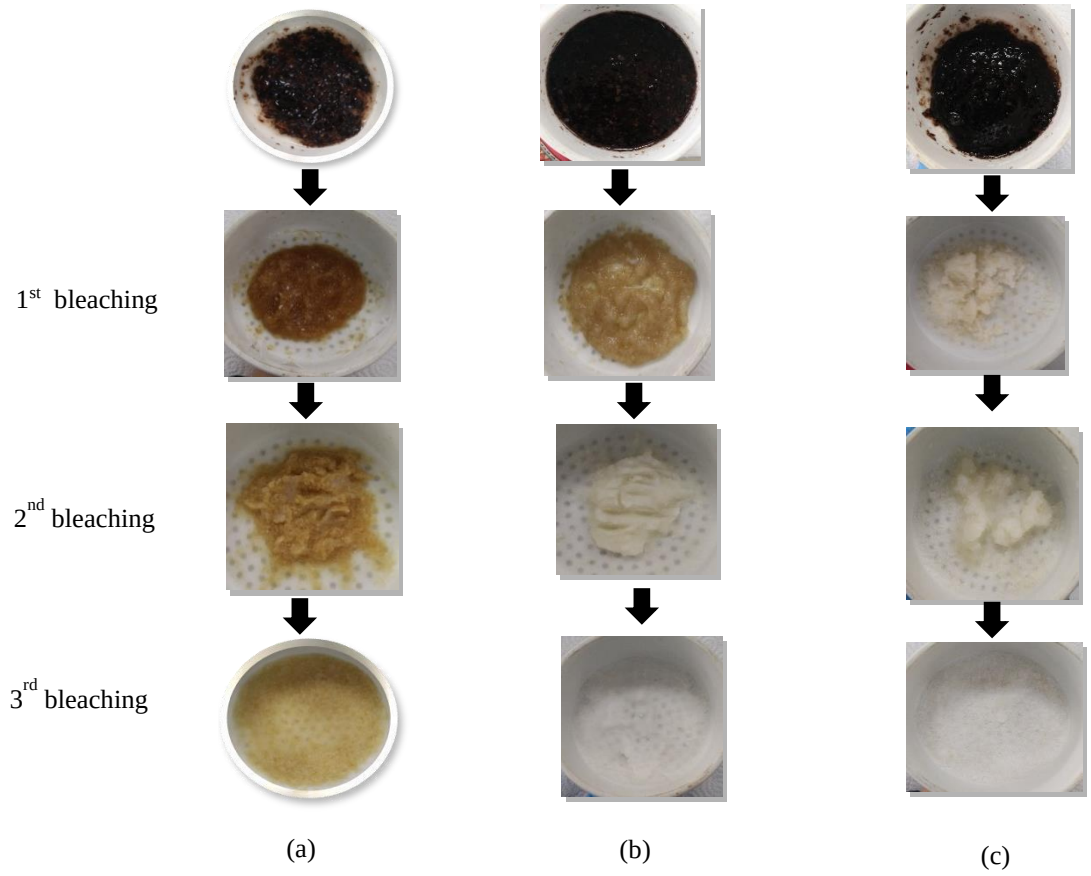

Figure 1. The changes in colour during the treatments (a) 2% (b) 4% c) 6% of NaOH followed by bleaching.

Characterization of cellulose

Fourier Transform Infrared (FTIR)

Based on Figure 2, the peak at 2903 cm^{-1} was represent the C-H stretching of aromatic functional group (Ditzel et al., 2017). The intensity of this peak indicates the crystallinity of the cellulose where the higher the intensity of the absorption band, the higher the crystallinity of the cellulose (Husin et al., 2017). For 2% NaOH and 4% NaOH treated samples, it shows a higher intensity of crystallinity compared to the 6% NaOH treated sample at both peaks.

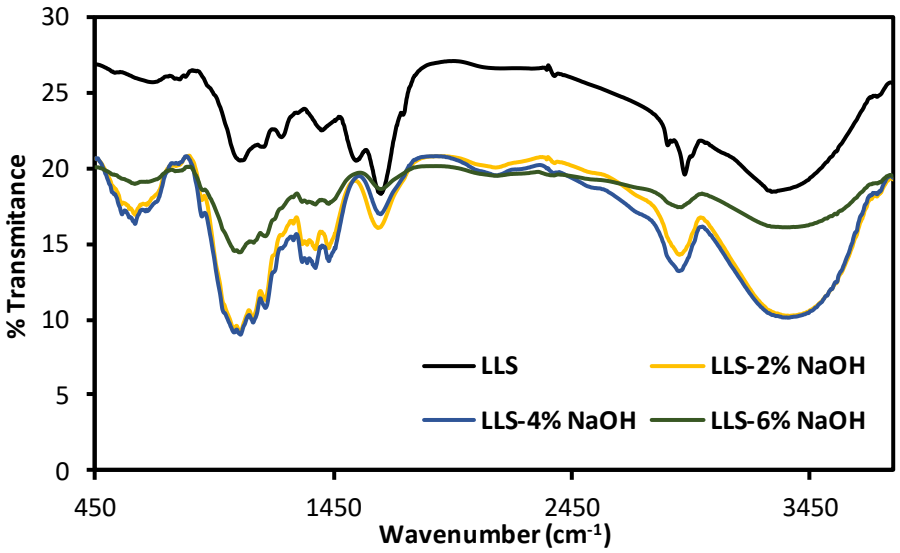

Figure 2. The FTIR spectra of LLS and different treated samples.

Moreover, the peaks present at range 1641.53 to 1649.58 cm^{-1} involved the O-H bond of water absorption (Johar et al., 2012; Liu et al., 2018; Reddy et al., 2018). In the spectra of the LLS, a small peak at 1746.32 cm^{-1} was obtained. This peak corresponding to the C=O stretching of acetyl and ester group of hemicellulose or carboxylic acid in the lignin group (Adewuyi et al., 2017; Liu et al., 2018; Frone et al., 2017). Since this peak were unseen in all three alkaline treated samples, we can conclude the hemicellulose and lignin are removed after the treatment. The peaks at 1165.88 cm^{-1} and 1059.20 cm^{-1} are representing C-C stretching band and C-O-C vibrational pyranose ring band respectively (Ditzel et al., 2017). Lastly, at all alkaline treated sample spectra's the peak that indicate the cellulosic β-glycosidic linkage also can be identified at range 897.15 to 899.30 cm^{-1} (Johar et al., 2012; Ditzel et al., 2017). The absorption band obtained from the different NaOH treatment was summarize in Table 3.

Table 3. The absorption band of LLS and different treated samples.

Functional group	**Absorption band (cm^{-1})**			
	LLS	**LLS - 2% NaOH**	**LLS-4% NaOH**	**LLS-6% NaOH**
O-H Alcohol	3292	3366	3360	3367
C-H Aromatic	2926	2904	2903	2900
C-H Alkane	2857	-	-	-
C=O Ester	1746	-	-	-
C-H Alkane	-	1430	1430	1430
C-C Pyranose ring	-	1165	1165	1163
C-O-C Pyranose ring	-	1059	1059	1053

X-ray Diffraction (XRD)

Figure 3 shows the spectrum of LLS as well as different treated samples obtained from XRD instrument. Based on Figure 3, the peak for raw sample (LLS) do not shows a strong peak and indicate that the raw sample having an amorphous feature. This is due to the present of hemicellulose and lignin which is amorphous in nature (Liu et al., 2018; Feng et al., 2018). Meanwhile, the peaks of treated samples showing the property of cellulose itself which is semi-crystalline. The intense peaks at 2θ value of 22.6º could be assigned for the crystallinity while the peaks with low intensity at 15.0º verified for amorphous region of the cellulose (Tan et al., 2015). These observable peaks indicate the cellulose present are cellulose type I (Frone et al., 2017; Feng et al., 2018) and not a type II cellulose because there is no doublet peak at 22.6º (Naduparambath et al., 2018). Crystalline form of cellulose type I is common found in natural sources such as plants and bacteria.

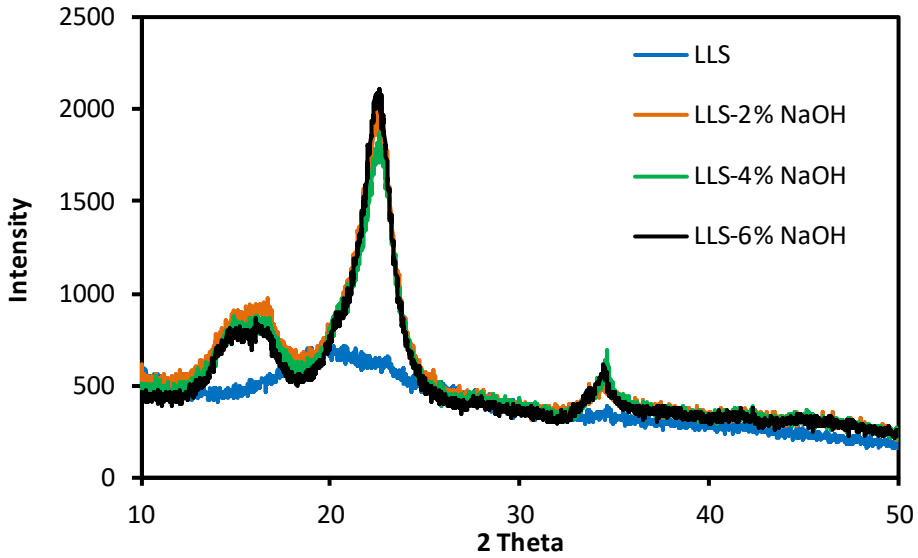

Figure 3**.** The XRD spectrum of LLS and different treated samples.

The crystallinity index was calculated and summarize in Table 4. LLS treated with 2%, 4% and 6% NaOH gives value of 71.14%, 70.50% and 76.40% respectively. 6% NaOH treatment cellulose give the highest CI followed by 2% then 4% NaOH treatment product. However, the difference was less significant as the CI obtained were close to each other. Therefore, it can be deduced that all three concentrations able to remove the amorphous region equally. The high CI of the cellulose obtained indicating the method used were good enough to be applied although the degree of crystallinity of natural fiber can be increased to >90 (Feng et al., 2018).

Table 4. The crystallinity index (CI).

Sample	Degree I_{002}	I_{002}	Degree I_{am}	I_{am}	CI (%)
2% NaOH treated	22.62	2003	18.94	578	71.14
4% NaOH treated	22.61	1878	18.83	554	70.50
6% NaOH treated	22.56	2093	18.22	494	76.40

Field Emission Scanning Electron Microscope (FESEM)

The morphology of the LLS and cellulose treated from different NaOH concentration were studied by using FESEM. The LLS morphology was studied to identify the changes occur after the alkaline treatment and bleaching procedure. Figure 4 shows the resulted FESEM image of all four type of samples.

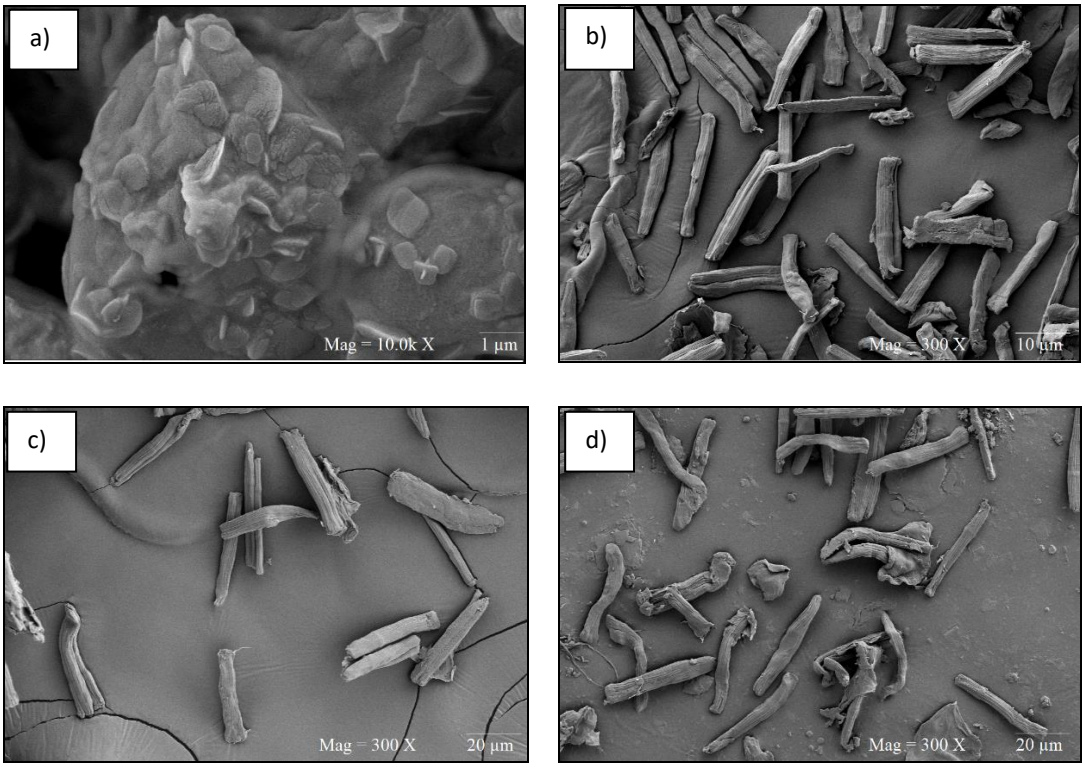

Figure 4**.** FESEM image of (a) LLS (b) LLS-2% NaOH (c) LLS-4% NaOH (d) LLS- 6% NaOH

By referring to figure 4 (a), the FESEM image of LLS shows a globular shape with flakes present on the surfaces. In comparison with treated samples (b), (c) and (d), the features appear to be fibril like structure. Some of the fibrils appear individually and there were also some fibrils intact together. Similar finding also reported from previous studies conducted from different sources of cellulose (Zhao et al., 2018; Johar et al., 2012). The difference in morphology between LLS and cellulose formed from different treated samples are due to the removal of hemicellulose, lignin and others non-cellulosic compounds (Senthamaraikannan and Kathiresan, 2018).

Conclusions

Treatment with 4% NaOH solution obtained the highest amount of the cellulose which is 23.70%. On the other hand, treatment with 6% NaOH coupled with bleaching treatment produced the whitest strand of cellulose fiber. FTIR results for all treated LLS shows a significant peak of cellulose. The highest crystallinity index calculated based on the XRD data is 76.04% for cellulose treated with 6% NaOH. The morphology of cellulose studied under FESEM image shows a fibril-like structure with diameter less than 9.0 µm. This study suggested that the use of seed of *Leucaena leucocephala* seeds might be a feasible option as a feed material for the production of cellulose. Further in can be disintegrate to micro and nano cellulose to increase the chemical-physical properties for various application such as in biocomposite, biomedicine and other value-added chemicals.

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