

# **Isolation of Nano Cellulose from Rubber Wood Fibre and Fibrillation Effects on Nano Cellulose Reinforced Poly (Ethylene Oxide)**

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*Abstract*—Wood and plants are cellular hierarchical bio-composites produced by nature, and are essentially semicrystalline cellulose microfibril-reinforced amorphous matrices made of hemicellulose, lignin, waxes, extractive and trace elements. Cellulose acts as a structural reinforcing agent that provides mechanical strength as well as chemical stability to plants. The development of low-cost, sustainable and renewable resources is critical to meet the growing environmental concerns and energy demands; the use of rubber wood is extremely beneficial to Malaysia as it is one of the main commodities. The current study aims to extract cellulose nanofibres from rubber wood (*Hevea brasiliensis*) fibers via high pressure homogenisation combined with enzymatic and chemical pretreatments. In this study the application of enzymes in fiber processing has been mainly directed towards the modification of hemicelluloses and lignin while preserving the cellulosic fraction. The diameter distributions of the resulting nanofibers were dependent on the number of times of cellulose solution is passing. The extent of dispersion improved significantly with increasing when number of passes through the high pressure homogenised. Fe-SEM study showed that the diameters of the nanofibers isolated ranged from 37 to 85 nanometer (nm) and estimated length is several micrometers ( $\mu\text{m}$ ). The nanocellulose fibres were studied further by examining with Fourier Transform Infrared Spectroscopy (FTIR). There was a disappearance of vibration peaks at 1730.28 and 1234  $\text{cm}^{-1}$  from spectra of treated fibres which shows the removal of hemicellulose and lignin components respectively. XRD results showed an increase in crystalline which resulted from the removal of lignin and hemicellulose. Moreover, the isolated cellulose nanofibres were used to reinforce poly(ethylene oxide) (PEO). PEO was dissolved in isolated nanocellulose liquid suspension followed by casting. The nanocomposites were characterized by using FTIR analysis and X-Ray diffractometry.

*Keywords:* Cellulose nanofibers, enzyme pre-treatment, Rubberwood, PEO, Nanocomposite

## 1. INTRODUCTION

In previous years, cellulose nanofibres (CNF) gathered high interest as one of the reinforcement of different kind of polymeric materials. This was due to CNF having unique and attractive properties such as, biocompatibility, biodegradability abundance, renewability, light weight and high-aspect-ratio [1]. All lignocellulosic bioresources like plants and wood are cellular hierarchical bio-composites produced by nature, and are essentially semicrystalline cellulose microfibril-reinforced amorphous matrices made of hemicellulose, lignin, waxes, extractive and trace elements [2]. Nanocellulose can be extracted from variety of ligno-cellulosic bio-resources like jute, flax, ramie, and hemp and by-products of agricultural crops via various methods. It is web-like network of cellulose fibrils composed of the amorphous and crystalline domains [3-4]. Various studies have been carried out to the synthesis of highly purified cellulose nanofibers from cellulosic materials via chemical treatments [5-6], enzymatic treatments [7-11], high pressure homogenizing [12-13], cryocrushing [14], grinding; [15-19], as well as a combination of two or several of the before mentioned methods [20]. Poly Ethylene Oxide (PEO) is the most used polymer that owing to its non-toxicity, biocompatibility, and biodegradability. Due to its versatile applications and easily solubility in water to aid in the dispersion of the nanocellulose synthesis from rubber wood fibres, PEO has been mostly used as material for coating to generate surfaces that resist non-specific protein adsorption and medical applications [21].

Hence, The objective of this work was to synthesize nanocellulose from rubber wood fibers by pretreatment of enzyme and chemical via high pressure homogenizer and its application in the preparation of poly(ethylene oxide)-based nanocomposites films.

## 2. MATERIALS AND METHODS

### A. Chemicals

Rubber wood fibers were supplied by Robin Resources Sdn. Bhd, Temerloh (Malaysia). Laccase enzyme with activity of 70 U/g was supplied by Novozyme, Malaysia. Sodium chlorite, acetic acid, potassium hydroxide and sodium acetate with a purity of 25%, 99.5%, 99.9% and 99.99%, respectively, were obtained from Sigma Aldrich (Malaysia). PEO in powder form with MW of 200000 kDa was also supplied by Sigma Aldrich, Malaysia.

### B. Preparation of cellulose fiber from rubber wood fibres

This process consists of two distinct stages, namely enzymatic pre-treatment and chemical pre-treatment. In the enzymatic pre-treatment stage Well-dried 15 gm fibres were suspended in 485 grams of deionized water to prepare a solution of 3% w/w consistency (mass pulp/mass suspension). A buffer solution of sodium acetate and acetic acid was added to maintain the solution at pH 5. Laccase enzyme 6 U/g was added to obtain a desired lignin oxidation as per the method suggested by Lei, Lin, and Li (2008) with 40-55°C temperature for the best enzymatic reaction, the reaction was kept at uniform rotational speed of 160 rpm for

8 hours. Once the enzymatic pre-treatment has been completed, the suspension was filtered out and the fibers were kept in oven at 60°C overnight to stop the enzymatic reaction. This was done to stop reaction of enzyme with the fiber which might deteriorate the desired results for the subsequent treatments.

In the chemical pre-treatment stage, the wood fibers from the previous step were purified to prepare the cellulose fibers. Initially, the remaining % of lignin from the after effect of enzymatic pre-treatment was removed from the samples using acidified sodium chlorite solution at 75 °C for a 45 mins. Next, the samples were treated in 3% w/w potassium hydroxide at 80 °C for two hours, and then in 6% w/w potassium hydroxide at 90°C for two hours in order to dissolve hemicellulose and residual starch. After a series of chemical treatments, the samples were filtered and rinsed with distilled water until the residues were neutralized. The process was followed by oven-drying at 55 °C for 24 h and dried cellulose fibers were obtained.

### C. Preparation of cellulose nanofibers

From the previous step the dried cellulose fibres were soaked in distilled water for 8hours. A mechanical stirrer used for cellulose fibres dispersion at 1500 rpm for an hour. The fibres were then put in a ultrasonicator bath for 15 minutes at 65 °C and again for 90 minutes at 45 °C for better dispersion. Fibres were then passed through a homogenizer (MODEL: ARIETE NS10011) minimum 10 time sto break down fibres into nono particles. Homogenizer pump was operated at a constant pressure of 500 kPa.

### D. Preparation of nano cellulose Reinforced Poly (Ethylene Oxide) composites

The composites of PEO and nano cellulose fibres were prepared by adding nano cellulose fibres (5 wt%) solution suspensions to a PEO solution dissolved in distilled water under vigorous mechanical stirring at room temperature for 4 hours and subsequent sonication for 15 min for better dispersion. The mixture of nano cellulose and PEO was then poured in a petridish and dried in a desiccator for a week for complete water evaporation.

### E. Scanning Electron Microscopy (SEM)

The micrographs of the rubber wood fibres and PEO composites were taken by a Field Emission Scanning Electron Microscope (FE-SEM, JSM-7800F). All samples were sputter-coated with platinum to avoid charging.

### F. X-Ray Diffraction Technique (XRD)

The X-ray diffraction (XRD) was performed in an XRD analyzer. The samples were scanned in 2θ ranges 3-80° at a rate of 1deg/min. The generator was operated at Cu/30 kV/15 mA. The inter layer spacing (d002) of carbonaceous materials was calculated in accordance with Bragg equation:  $2d \sin\theta = \lambda$  and The crystallinity index of cellulose,  $C_{I_r}$ , was calculated (1) based on the reflected intensity data following the method of Segal et al. (1959) :

$$C_{I_r}(\%) = [(I_{200} - I_{am}) / I_{200}] \times 100 \quad (1)$$

where  $I_{200}$  is the peak intensity corresponding to both the amorphous and crystalline fractions of cellulose I and  $I_{am}$  is the peak intensity of the amorphous fraction.

### G. Fourier Transform-Infrared Spectroscopy (FTIR)

SB fibres, and PEO composite films was obtained using the FTIR spectroscopy in transmittance mode was used for the characterization of the functional group. For the solid samples, KBr pellets with 1 mass% of the powdered material were produced. The FTIR transmittance spectra were obtained with a Spectrum 1000 Perkin-Elmer spectrometer in the spectral area of 500–4000  $\text{cm}^{-1}$ , with a resolution 4  $\text{cm}^{-1}$  and 50 scans. Background spectra were collected before every sample

### H. Thermo gravimetric Analysis (TGA)

Thermo gravimetric Analysis (TGA) measures the amount and rate of change in the weight of a material as a function of temperature or time in a controlled atmosphere. Thermo gravimetric analysis was carried out to examine the extent of thermal decomposition of the fibers after each treatment. The thermal stability data of each sample were obtained using a thermo gravimetric analyzer (TA Universal Q500) under linear temperature conditions. The temperature was set from 100 to 800 °C at a heating rate of 10 °C/min in a nitrogen atmosphere.

### 3. RESULTS AND DISCUSSION

#### A. Field Emission Scanning Electron Microscopy (FE-SEM) analyses

FESEM is an effective media to investigate the morphologies of fiber. It is believed that the enzyme-chemo-mechanical treatment employed will alter the morphology of the fibers in terms of size. The variations in the fiber morphology are evident from the FESEM images (Figure 1). Figure 1a and b correspond to surface of raw cellulose fibers and surface of oflaccase and chemical treated fibers; which suggests that clusters of individualized fibers can be seen on the surface and the reduction of fibers diameters are more pronounced. Here, individualized nanofibers can be obtained by sonication and high pressure homogenization treatment (Figure 1c and d). FE-SEM study showed that the diameters of the nanofibers isolated ranged from 37 to 85 nanometer(nm) and estimated length is several micrometers ( $\mu\text{m}$ ). FE-SEM images in Figure 2(a) shows nanocellulose Reinforced Poly (Ethylene Oxide) composites and fig 2(a) shows the FE-SEM micrographs of pure PEO and PEO and nanocellulose.

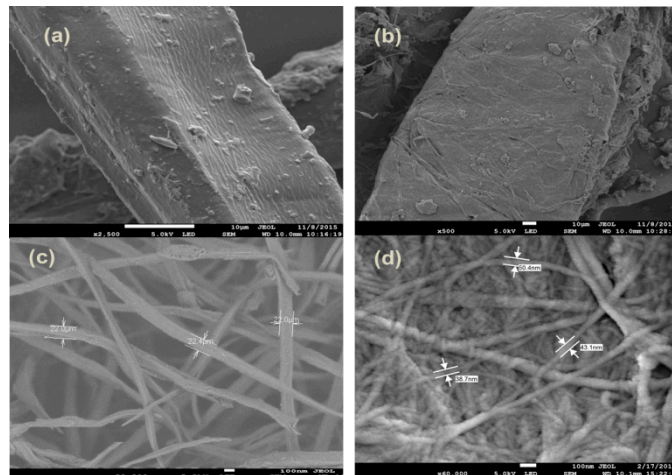


Figure 1: FE-SEM micrographs of (a) : Surface of raw cellulose fibers and (b) Surface of oflaccase and chemical treated fibers; (c) pure cellulose fibers after sonication and (d) nanofibers after chemical treatment, sonification and homogenization.

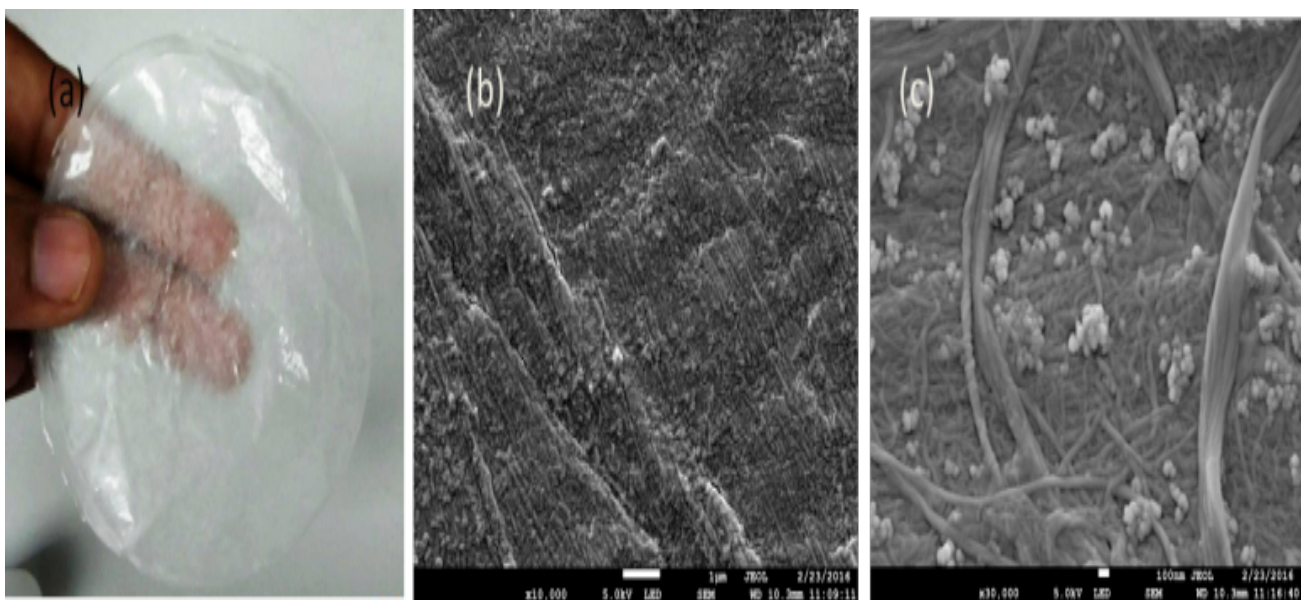


Figure 2: (a) nano cellulose Reinforced Poly (Ethylene Oxide) composites and FE-SEM micrographs of (b) pure PEO (c) PEO and nano cellulose

### B. Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR spectroscopy is an indispensable technique in establishing variations in the chemical structures of the isolated samples introduced by different treatments. Figure 3 compares the FTIR spectra of the untreated and chemo-mechanically treated fibers in combination with laccase enzyme. The results indicated the occurrence of chemical reactions during chemical treatments of the fibers. The dominant peaks observed in all samples in the regions 3335  $\text{cm}^{-1}$  and 2902  $\text{cm}^{-1}$  are due to stretching of the hydroxyl group and C-H groups of cellulose respectively [22-23]. When the spectra of the untreated rubber wood fibers are compared with those of the chemo-mechanically treated ones, the most evident difference is the disappearance of the vibration peak formerly observed at 1732  $\text{cm}^{-1}$  from the spectra of the treated fibers. The disappearance of this prominent peak in the spectra of the is attributed to the C=O stretching in the acetyl and uronic ester groups of the hemicelluloses or the ester carbonyl groups in the p-coumaric units of the lignin [24-26]. The disappearance of this peak from the spectra of the treated fibers can be ascribed to treatment of the raw fibers with NaOH followed by bleaching as these treatments are known to remove most of the hemicelluloses and lignin from the rubber wood fibers [25,26]

On the other hand, the peak detected at 1593  $\text{cm}^{-1}$  in the spectra of the untreated fibers is attributed to the C=C stretching of the lignin carboxyl groups while the peak at 1237  $\text{cm}^{-1}$  in the same spectra corresponds to the C-O-C stretching of the aryl-alkyl ether linkage in lignin [27]. Disappearance of these two peaks from the spectra of the bleached pulp fibers and nanofibers is believed to be due to removal of lignin by the chemical treatments. Absence of this peak from the spectra of the bleached pulp fibers and nanofibers confirms that lignin was successfully removed from the fibers with the combination of enzyme and chemical treatment.

### C. X-Ray Diffraction (XRD)

Figure 4 portrays the equatorial X-ray diffraction patterns of cellulose crystallinity of both the untreated (a) and treated (b) Rubberwood fibers.

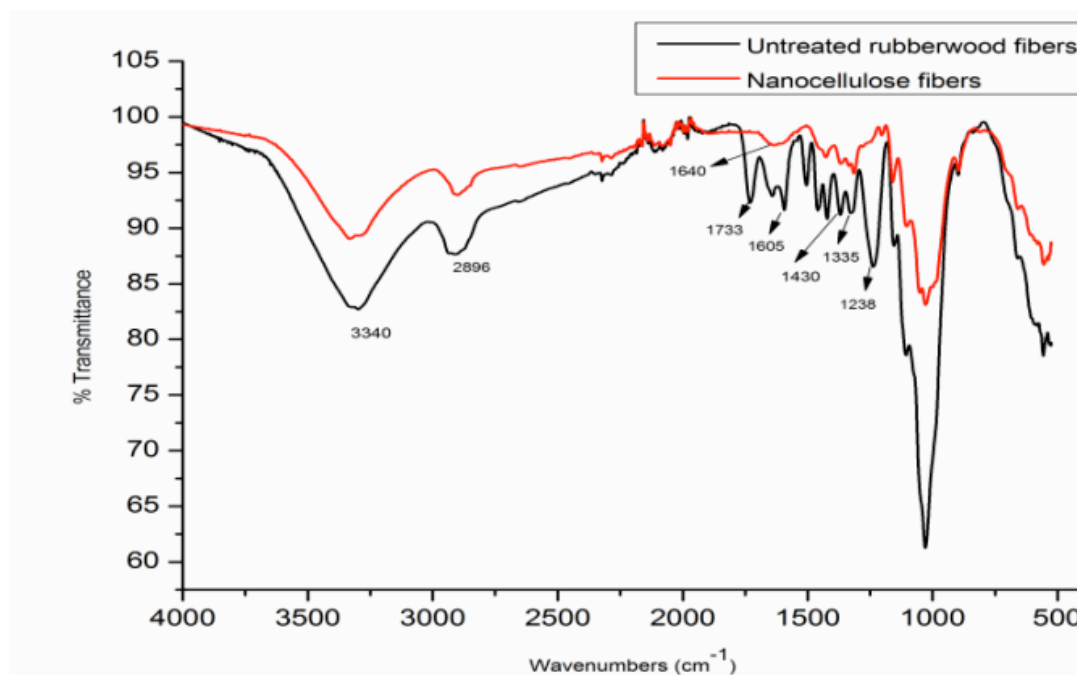


Figure 3: FTIR spectra of untreated rubber wood fibers and nanocellulose fibers

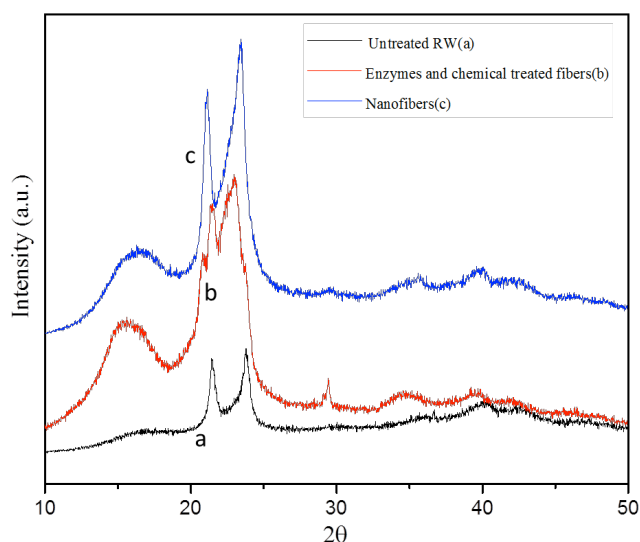


Figure 4: X-Ray Diffraction of treated and untreated fibers

The results showed that the X-ray diffraction patterns of the Rubber wood fibers have changed after the enzymes and chemicals treatments. These points to that the chemicals employed did not spread beyond the surfaces of the amorphous and crystalline parts of the fibers and that swelling took place only between the crystalline areas. Hence, crystalline and non-crystalline components still coexisted and there was some obvious change in the crystallinities of the enzyme-chemo-mechanically treated fibers from their original states.

When compared with the untreated Rubber wood fibers, the enzyme-chemo-mechanically treated fibers exhibited a distinguished higher crystallinity. The crystallinity values for the untreated rubberwood fibers, the rubber wood enzyme chemically treated fibers, and the RW nanofibers were 45, 65, and 70%, respectively. Due to removal of lignin and hemicellulose during the enzyme-chemical treatments, it was much expected that the crystallinity would increase. Another potential explanation for this increase in crystallinity, besides the removal of lignin and hemicellulose, is that the greater hydrolysis of the amorphous areas over the crystalline ones and the incidence of peeling reactions in the amorphous areas increased the crystallinity after the chemical treatments [28].

#### 4. CONCLUSION

Synthesis of cellulose nano fibers from rubber wood fibers was achieved via enzymatic and chemical treatments in combination with sonication and high pressure homogenization treatment in this research. The extent of dispersion improved significantly with increasing the number of passes by homogenization treatment. Microscopy study showed that the diameters of the nanofibers isolated ranged from 37 to 85 nanometer (nm) and estimated length is several micrometers ( $\mu\text{m}$ ). The chemical structures of the fibers before and after enzyme-chemo-mechanical treatments were monitored by FTIR spectroscopy. The FTIR spectra indicated that the removal of the lignin and most of the hemicellulose from rubber wood fibers after the enzyme and chemical treatments. The rubber wood fibres extracted through different treatments were successfully incorporated in PEO via solution casting. These films may be useful and can be potentially applied in various medical fields or such as bio-nanocomposites and so on.

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