B Manisha Reddy
P.G. Research Scholar,
Department of Renewable
Energy Engineering,
Tamil Nadu Agricultural
University, Coimbatore,
Tamil Nadu, India

P Subramanian
Professor and Head,
Department of Renewable
Energy Engineering,
Tamil Nadu Agricultural
University, Coimbatore,
Tamil Nadu, India

S Sriramajayam
Associate Professor,
Department of Renewable
Energy Engineering,
Tamil Nadu Agricultural
University, Coimbatore,
Tamil Nadu, India

P Vijayakumary
Assistant Professor,
Department of Renewable
Energy Engineering,
Tamil Nadu Agricultural
University, Coimbatore,
Tamil Nadu, India

K Raja
Assistant Professor,
Department of Nano Science &
Technology, Tamil Nadu
Agricultural University,
Tamil Nadu, Coimbatore,
Tamil Nadu, India

Corresponding Author
B Manisha Reddy
P.G. Research Scholar,
Department of Renewable
Energy Engineering,
Tamil Nadu Agricultural
University, Coimbatore,
Tamil Nadu, India

Extraction of cellulose from banana sheath and its characterization

B Manisha Reddy, P Subramanian, S Sriramajayam, P Vijayakumary and K Raja

Abstract

Cellulosic fiber finds an important role in modern science due to its biocompatibility, biodegradability and non-toxicity. Extracting cellulose from ligno-cellulosic biomass is one of the most important study fields nowadays. In this work, an attempt has been made to extract the cellulose from the banana sheath through steam explosion and chemical pretreatment methods. Banana sheath is an annually renewable agricultural by-product with high cellulose content. The main objective of this research is to study the physicochemical characterization of the raw banana sheath fibers and extracted cellulose. The X-ray diffraction studies revealed that the cellulose extracted through steam explosion is having more crystallinity index (65.7%) than chemically extracted cellulose (58.4%). Structural analysis by Fourier Transform Infrared (FTIR) Spectroscopy indicated that the lignin and hemicellulose were removed extensively through both methods. The morphology of the banana sheath fibers and extracted cellulose was investigated using scanning electron microscopy. In addition SEM showed that the microfibril surface diameter of the pretreated cellulose has been decreased.

Keywords: Cellulose, banana sheath, crystallinity, morphology

Introduction

Every day massive amounts of ligno-cellulosic wastes are produced by agricultural, food processing, alcoholic industries and the lumber industry. They are usually disposed of in an uncontrolled manner. As a result of their accumulation, several safety, health, aesthetic and environmental issues arise (Reshmy *et al.,* 2021) [12]. Ligno-cellulosic biomass is an abundant, renewable, easily available and economical raw material among natural sources of energy. These ligno-cellulosic biomass consists of wastes such as rice husk has 43.33% alpha cellulose content, maize cob 62.96%, banana stem 47.36% and groundnut shell 65.20% compared to the 63% alpha cellulose content of wood. By substituting such items for wood, deforestation and desertification, as well as its repercussions, could be avoided (Joshi *et al.,* 2018) [7]. Hemicellulose, lignin, and cellulose are the three polymeric components of ligno-cellulosic biomass. Flores-Velázquez *et al.,*(2020) [5] stated that cellulose makes up the majority of ligno-cellulosic material and cellulose is a polymer made up of repeating β-D glucose ($C_6H_{12}O_6$) units joined by covalent connections created by acetal functions between the equatorial OH groups of carbon atoms C4 and C1 through β- (1,4)-glycosidic bonds. Banana waste is one among these ligno-cellulosic biomass. Banana is a staple food grown in over 120 countries, with an output of more than 100 million tonnes in 2012 and a harvested area of almost 5 million hectares. Banana fruit consists of only 12% of total mass (Zuluaga *et al.,* 2007) [14]; the rest consists of peels, rachis, leaves, stalk, inflorescence, sheath, stem and pseudostem (Padam *et al.,* 2014) [11]. With a production of roughly 30 million tonnes in 2014, India is the world's biggest banana grower. Bananas are the second most widely grown fruits next to citrus accounting for around 16% of global fruit production and Tamil Nadu is India's biggest banana grower followed by Maharashtra (R. Kumar & Wyman 2009) [9]. For extracting cellulose from the ligno-cellulosic biomass, pretreatment techniques are required. Nieder-Heitmann *et al.,* (2020) [10] suggested physical pretreatment methods like thermal treatment, radiation and size reduction as well as biological pretreatment methods like white-rotFungi were not considered because they couldn't compete with other pretreatment methods in terms of process efficiency, time efficiency, selectivity and cost. Chemical (alkali, acid, ionic liquids, organosolvent and ozonolysis) and physicochemical (hot water, steam explosion, ammonia-based, wet oxidation and carbon dioxide) methods are commonly used. The cellulose obtained was characterized by the X-ray diffraction to identify the changes that occurred during the cellulose extraction

process as well as changes in crystallinity index and crystallite size. The FTIR spectra of the biomass were acquired in order to determine the nature of the organic functional groups and key biomass constituents *viz.,* cellulose, hemicellulose and lignin that regulate thermal degradation behaviour. The alteration of fibre structure, as well as the morphology and size of the particles were studied using scanning electron microscopy (SEM).

Materials and Methods

Raw banana sheath, with its main chemical compositions of cellulose (39.10%), hemicellulose (12.30%), lignin (12.90%) and ash (8.83%), were obtained from the department of Horticulture, Tamil Nadu Agricultural University. The sheath was sun dried and size reduced to 1-4 cm. The sliced sheath was then pulverized using a CIT-FW-200 grinder and allowed to pass through 500µm sieve. A variety of chemicals are used to extract the cellulose from the banana sheath such as NaOH, $NaClO_2$, glacial acetic acid, oxalic acid, and citric acid, etc.

All of the reagents and solvents that were employed were of analytical quality.

Fig 1: Extraction of fibres from banana sheath

Physio-chemical properties of banana sheath

Banana sheath that had been obtained was subjected to proximate and ultimate examination. The moisture content of raw biomass was determined using a hot air oven (ASTM, E-871) at 105 °C for 24 hours. The sample was placed in a weighed crucible with a bent cover to allow the volatile matter to escape and stored in a muffle furnace (NREL/TP-510-42622) at 925±20 ℃. After heating for exactly 7 minutes, the crucible was removed and quickly cooled by laying it on a cold iron plate, before being transferred to a desiccator. The volatile matter was determined by weight lost. The ash content was evaluated in the muffle furnace at 550 ℃ for 1 hour. The procedure was repeated until no variation in the weight was observed. Fixed carbon of the biomass represents the unburnt carbon remains after volatile and moisture removal. It was calculated by difference, in the sum of ash content (%) and volatile matter (%) from 100. The 'Vario EL' elemental analyzer (ASTM E777) was used to perform the final analysis, which determined the carbon, hydrogen, and nitrogen contents of the feed materials.

Determination of Cellulose, hemicellulose and lignin

1 g of powdered sample has been taken in refluxing flasks and added 100ml of cold NDF and 100ml of ADF in another flask. Solution mixture was heated and refluxed for an hour. The contents were filtered to sintered glass crucibles by suction and washed with hot water followed by acetone. Residues were transferred into the Petri dish and dried at 100 ℃ for 8 hours. After cooling weight was taken. Acid detergent fibre was transferred in beaker and added 25-50 ml of 72% H_2SO_4. Solution was kept for 3 hours with intermittent stirring using glass rod and diluted with distilled water and filtered through Whatman no.1 filter paper. Sample was placed in oven for 8 hours at 80 ℃. Loss in weight was taken as weight of cellulose. Sample was then transferred to a silica crucible and kept in muffle furnace at 550 ℃ for 3 hours. Loss in weight is taken as lignin content.

Methods for the preparation of cellulose
Chemical treatment
Step 1: Alkali treatment of the fiber: It is a low-cost surface treatment that effectively modifies the surface of natural fibers.

It includes making a (1.5%, 2%, 5%, 7.5%) caustic soda (NaOH) solution and immersing the sheath fibers in it for 6 hours at room temperature. This is done to remove contaminants and ensure that the sheath can be used for a longer period of time.

Step 2: Autoclave process: Steam pretreatment is accomplished by directly inserting NaOH-treated fibers into the autoclave at high steam pressure. For 1.5 hours, the mercerized sheath fibers were subjected to a pressure of 103 Pa. After the steam explosion, the fibers were extracted and thoroughly cleaned with distilled water. These cleaned fibers were then dried for 4 hours at 60 °C in a hot air oven.

Step 3: Bleaching of fiber: The fibers were then treated with a solution containing 5% sodium chlorite ($NaClO_2$) in HCl with a pH of 2.3. $NaClO_2$ is an inorganic sodium salt that is utilized as bleach. Fibers were immersed in the solution for 1 hour on a magnetic heater at 50 °C. Essentially, the bleaching process is used to eliminate any leftover lignin.

Step 4: Treatment of bleached fibers with acid: Following the bleaching treatment, the fiber is mildly acid treated with (2%, 5%, 7.5% and 10%) oxalic acid for acid hydrolysis. For 1.5 hours, fibers were immersed in an oxalic acid solution. To remove any traces of acid, the fibers were thoroughly rinsed with water.
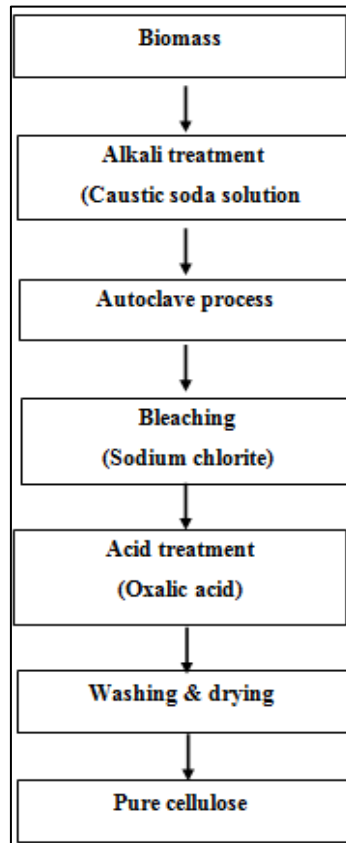
Fig 2: Flowchart for chemical treatment

Steam explosion treatment

The biomass was steamed at high pressures and temperatures between 13, 15 and 17 bar and 192, 200 and 205 °C respectively for 5 to10 min residence time in a reactor during the steam explosion pre-treatment with (citric acid, NaOH, and water) and without the addition of catalysts. The cellulose bundles are defibrillated by the abrupt pressure release, resulting in enhanced cellulose accessibility for enzymatic hydrolysis and fermentation. The fibres were discharged via a connecting pipe and gathered in a collection bin after being steam-exploded. The outcome was sludge, and the fibres were dried in an oven at 100 °C for 8 hours.
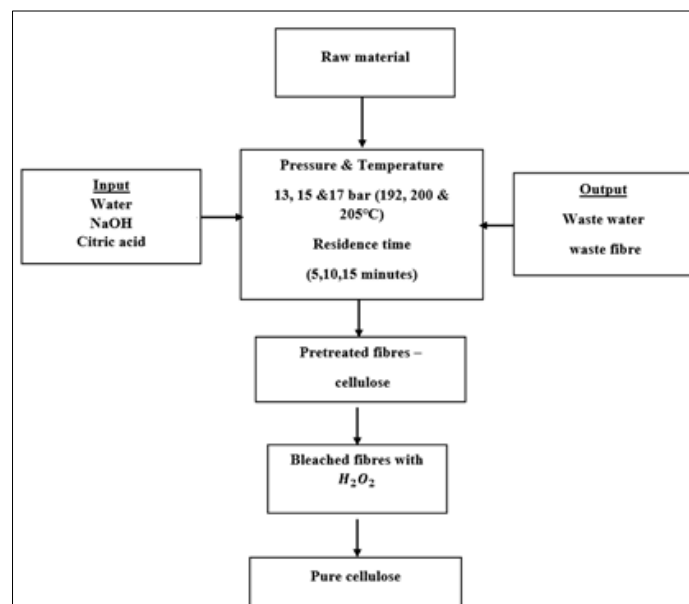
Fig 3: Flowchart for steam explosion treatment

Fig 4: Cellulose e

Characterization of the extracted cellulose
X-ray diffraction (XRD)
In a Carl Zeiss Jena URD - 6 diffractometer, monochromatic Cu-K$_\alpha$ radiation (λ=1.5406 A$^\circ$) was generated at a voltage of 40 kV and a current of 20 mA for X-ray diffraction investigation. At a scan rate 2°/min, the Bragg angle was adjusted from 3° to 50°. The peak profiles were resolved using Microcal origin^{TM} 8.5 algorithms, using Lorentz and Pearson VII functions for crystal peaks and a fifth-degree polynomial function for the background profile.

Fourier Transform Infra-red (FTIR) Spectroscopy
FTIR is an intriguing tool for assessing structural changes in samples caused by chemical treatments. This analysis was used to explore the structural changes from banana sheath to cellulose using a Nexus Thermo FTIR (Thermo Nicolet, USA) spectrophotometer. The samples were oven dried for 4-5 hours at 105 °C, then mixed with KBr in a 1:200 (w/w) ratio and pressed into pellets under vacuum. In the transmittance mode, the FTIR spectrum of the samples was obtained in the range of 4000-500 cm^{-1}.

Scanning electron microscopy (SEM)
External morphology (texture), chemical composition, crystalline structure and orientation of the materials that make up the sample are all revealed by scanning electron microscopy. On a graphite ribbon fastened to an aluminium sample holder, dry powder samples were disseminated. In a modular high-vacuum coating system, the powder was sputter-coated with gold and analysed using a Quanta 250 scanning electron microscope at a filament voltage of 20 kV.

Results and Discussion
The physiochemical parameters of banana sheath fibres employed in this work are listed in the Table 1. According to (Jayaprabha *et al.,* 2011) [6] banana sheaths have a high moisture content and very little dry matter, with water accounting for 97.14% or more. Although there are lot of ash and volatile materials in the mixture, it has no effect in synthesis of cellulose. The particle size distribution of biomass material is critical in the physicochemical conversion process, because cellulose, hemicellulose, and lignin are present in lignocellulosic biomass; the size of the biomass is highly divided. The bulk density of biomass is a key factor in this because it contributes to increase the yields by increasing the surface area of the biomass. Ultimate analysis is useful during cellulose extraction analysis because it allows us to see

what changes have occurred in the functional groups. The chemical compositions of banana sheath fibres were determined at various phases of processing. The results revealed that untreated banana fibres have a high cellulose concentration of 39.10%, as well as hemicellulose and lignin values of 12.30% and 12.90%, respectively. Untreated banana sheath fibres have a high cellulose concentration, indicating that they are ideal for use as cellulose sources. The higher the cellulosic content of agricultural waste biomass, the faster it biodegrades and thermally degrades, resulting in larger cellulose yields. When analysing biomass as a potential energy fuel, the contents of cellulose, hemicellulose, and lignin are useful. The higher the cellulosic content of agricultural waste biomass, the faster it biodegrades and thermally degrades, resulting in larger cellulose yields.

Sulfuric acid is the most commonly used acid in chemical treatments (H$_2$SO$_4$). It is frequently utilised, however it has several drawbacks, such as the generation of inhibitory chemicals and reaction vessel corrosion. As a result, researchers have pretreated lignocellulosic biomass with various acids such as oxalic acid and maleic acid, among others. Alkali reagents cause lignin structural change, cellulose swelling, cellulose decrystallization and hemicellulose solvation by degrading the side chains of esters and glycosides. The alkali-treated fibres were snapped by the rapid fall in pressure. During the bleaching process, the percentage of lignin and other cementing components in the raw fibre decrease, resulting in bleached fibre. (Waghmare and Khan 2021) [13] reported that the fibres are then acid hydrolyzed using Oxalic acid, which reacts with the sodium derivate of the fibre to generate pure cellulose.

Table 1: Physiochemical properties of raw banana sheath fibers

Analysis	Value
Proximate analysis	
Moisture content (%)	97.14
Volatile matter (%)	75.51
Ash content (%)	8.83
Fixed carbon (%)	15.67
Ultimate analysis	
Carbon (%)	35.23
Hydrogen (%)	3.47
Nitrogen (%)	1.06
Sulphur (%)	-
Oxygen (%)	62.21
Bulk density (kg/m^3)	318.28
Compositional analysis	
Cellulose (%)	39.10
Hemicellulose (%)	12.30
Lignin (%)	12.90

In steam explosion pretreatment, different catalysts of NaOH, water and citric acid are used for significant improvement in the efficiency. The fibres were treated with these catalysts at varying pressures, temperatures, and residence times to remove the cellulose in the steam explosion procedure. Agbor *et al.,* (2011) [1] reported that among water, citric acid and NaOH catalyst, NaOH has been determined to be the most successful catalyst. Steam condenses and permeates the biomass during the process at such high pressures, triggering an autohydrolysis reaction due to the organic acids created by the acetyl groups in hemicelluloses. The breaking of glycosidic linkages occurs, resulting in the solubilization of hemicellulose reported by Duque *et al.,* (2016) [4]. Chemical

changes generated by SE pretreatment on biomass include partial hydrolysis and solubilization of hemicellulose to the liquid fraction, depending on operation conditions, as well as redistribution and removal of lignin to an extent. From Table 2, we can observe that steam explosion pretreatment gave the best results in which biomass is impregnated with the appropriate catalyst before being introduced into the SE reactor in acid catalyzed SE. Acid catalysts have various advantages including increased hydrolysis rate, full removal of hemicelluloses, and the ability to operate at lower pretreatment temperatures and residence times.

Table 2: Compositional analysis of banana sheath after pretreatment methods

Sample	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Cellulose yield (%)
Chemically treated fiber	46.50	9.34	11.62	28.40
Steam exploded fiber	50.34	7.63	8.34	42.50

The structural examination of plant fibres prior to and after pretreatment has been thoroughly investigated using FTIR spectroscopy. Fig. 3 shows the FTIR spectra of materials obtained after various phases of treatment. The stretching vibrations of the –OH and –CH groups were responsible for the prominent peaks in all spectra, which were found between 3600 and 2800 cm^{-1} which was reported by Sain & Alemdar

(2008) [2].

The acetyl and uronic ester groups of hemicelluloses, as well as the ester linkage of carboxylic groups of the ferulic and p-coumaric acids of lignin, were responsible for the peak at 1369 cm^{-1} in the spectra of raw sheath fibres. In the spectra of chemically treated cellulose samples, this peak was notably weaker and relocated to a lower wave number (1368 cm^{-1}). This was due to the autoclave stage partially hydrolyzing hemicellulose and pectin. In the spectra of steam treated cellulose samples, the peak at 1365 cm^{-1} was sharper. This was due to bleaching with hydrogen peroxide convert cellulose to oxycellulose, and certain hydroxyl groups in cellulose were replaced with ketone groups as a result. The reaction of acetic acid with cellulose, on the other hand, can yield ester groups. Furthermore, providing greater steric hindrance ester groups to the cellulose surface aided future fibrillation. The aromatic skeletal vibrations of lignin were represented by the peak at 1032 cm^{-1}. Due to an increase in relative lignin concentration, this peak got sharper in the spectra of raw sheath fibres.

The lack of a peak at 1032 cm^{-1} in the spectra of steam treated cellulose samples suggested that the lignin was eliminated during the bleaching process. The crystalline band of cellulose coincided to the peak at 527 cm^{-1}.

The peak in the spectra increased sharper when more treatments were conducted.

Table 3: FTIR analysis of the banana sheath fibers in different stages

FTIR (cm^{-1}) spectra (%T)						
Sample	–OH stretching	C–H vibration	C–O stretching	Absorbed water	C–H stretching	Aromatic ring vibration of lignin	C–C stretching
Raw fibre	3023	2142	1740	1659	1369	1215	1032
CT fibre	3017	2124	1739	1621	1368	1213	1025
SE fibre	3015	2120	1738	1608	1367	-	1023

CT- Chemically treated; SE – Steam exploded
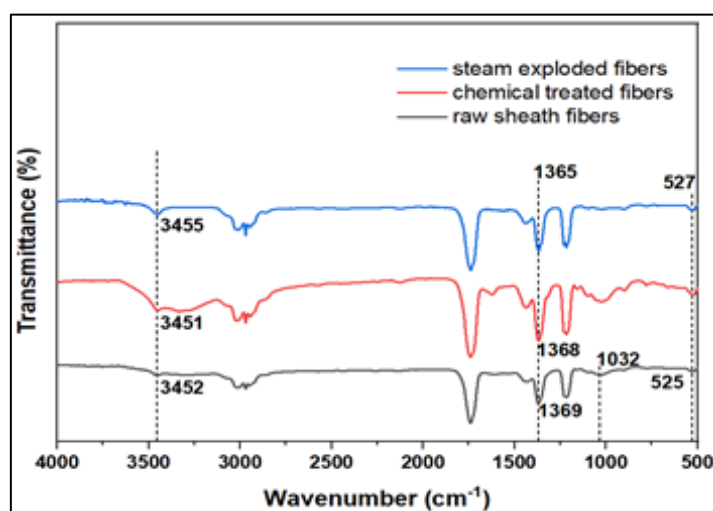
**Fig 5:** FTIR spectra of the raw sheath fibres, chemical and steam treated cellulose samples

X-ray diffraction gives a qualitative and semi-quantitative evaluation of the amorphous and crystalline cellulosic components in a sample, it has been frequently employed to analyse the crystalline structure of cellulose. Figure 4 shows the XRD patterns of raw sheath fibres, chemically treated cellulose samples and the steam treated cellulose samples. Besbes *et al.,* 2011 [3] reported that the major diffraction intensities at 22.6° and 18.5° were found in

all samples, showing that they were all cellulose types. Table 4 shows the results of XRD analysis at various stages. The crystallinity index increased from 33.20 to 65.70% when non-cellulosic components were removed. Table 6 also shows the crystallite size of the samples at various phases. The Scherrer analysis was used to determine the crystallite size of the samples. Compared with the untreated samples, the crystallite size of the sheath cellulose samples shrinks after chemical and

steam explosion treatments. Due to the exposure of the fibres to high pressures, temperatures and reaction periods followed by the bleaching procedure, the crystallite size of steam exploded cellulose samples reduced as compared to chemically treated cellulose samples.

Table 4: XRD analysis of extracted cellulose samples

Sample	2θ (Main reflection)	Crystallinity index (%)	Crystallite size (nm)
Raw banana sheath	22.93	33.2	4.63
CT cellulose	22.62	58.4	3.21
SE cellulose	22.72	65.7	2.80
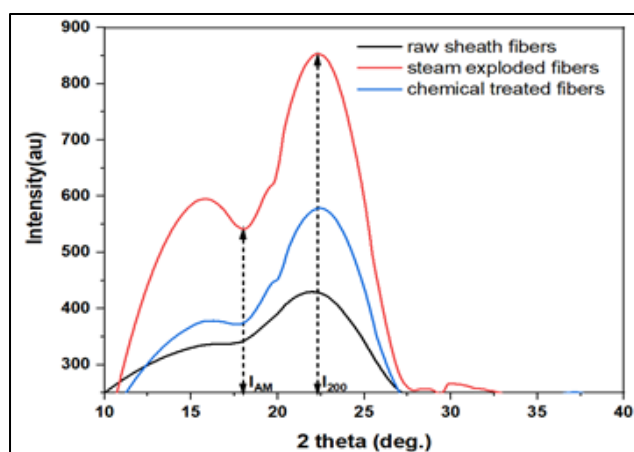
Fig 6: XRD analysis of banana sheath fibers in different stages

The following observations are obtained after studying the SEM micrographs. The surface of raw banana sheath fibre is made up of fibre and pith, with a high percentage of extractives layers, as shown in (Fig. 7aand 7b) (waxes, pectin, oil, etc.). Parallel stripes form the fibre surface, which has a high aspect ratio.

The original sheath fibre had a significantly larger diameter and each fibre appeared to be made up of many microfibrils. The surface is dense and interconnected with the pith by thick-walled fibre cells. Parallel stripes make up the fibres, which are covered in extractives on the outside. After removing hemicellulose and other residual extractives, the chemically treated defibrillate fibres are shown in (Fig. 7c and 7d). Hemicelluloses and lignin are non-cellulosic components that form a network that links the fibre bundles together in a composite-like structure. This network is weakened by the loss of hemicelluloses during the alkali treatment, causing some lignin to become loose and fall out. After the delignification process with acidified sodium chlorite, certain other extractives will be used to validate the removal of lignin. Acids hydrolyze hemicellulose, resulting in a liquid phase high in xylose and low in lignin. As a result of the elimination of non-cellulosic elements, the diameter of the fibrils is reduced to some amount as seen in Fig.

The cell wall structure is loosening and hemicellulose and pectin are partially hydrolyzed as a result of the steam explosion. In raw sheath fibre, there were bundles of fibres that formed a tight web-like structure, as seen in (Figure 7a and 7b). This occurred because the H_2O_2 bleaching eliminated lignin, resulting in less binding components.

The SEM images of one individual microfibril at higher magnification (Fig. 7b, 7d, 7f 1000x) showed many terraces, steps, and kinks formed after the purification treatment, and a close look at the microfibril surface at a higher magnification

showed that the surface of cellulose is almost free of trenches, but there are visible boundary edges in different regions.
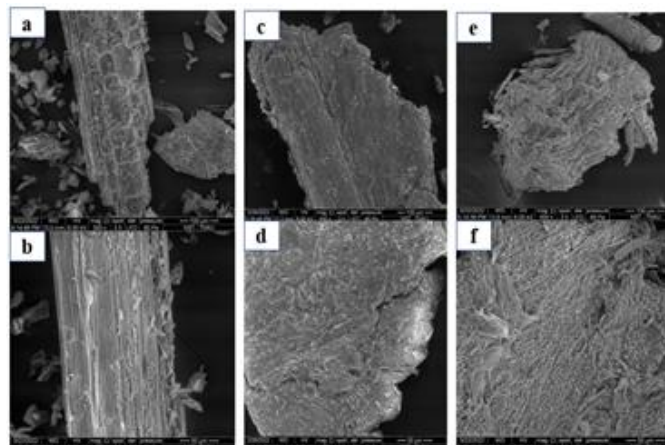
7a and 7b: Raw sheath fibre at 500x & 1000x; 7c and 7d: Chemically treated cellulose at 500x & 1000x; 7e and 7f: steam exploded cellulose at 500x & 1000x

Fig 7: SEM images at various magnifications

Conclusion
To extract cellulose from banana sheath fibres, two procedures were used: chemical and steam explosion. Chemical treatment using 2% NaOH and 5% oxalic acid resulted in the maximum cellulose recovery of 46.50%. Steam treatment at 13 bar pressure, 192 °C temperature and 5-minute residence period with a particle size 3 cm and 0.1M NaOH catalyst resulted in a maximum cellulose recovery of 50.34% in steam explosion method. During the bleaching process, the lignin and hemicellulose components of raw fibre are depleted. FTIR spectroscopy revealed that steam explosion was more effective at removing hemicellulose and pectin from sheath fibres, whereas H_2O_2 bleaching was more effective at removing lignin. As non-cellulosic components were removed, XRD revealed an increase in crystallinity, with the steam exploded cellulose fibres attained the maximum crystallinity index of 65.7%. There has been a decrease in crystallite size from 4.63 nm (raw fibre) to 2.80 nm (steam exploded cellulose). Steam explosion treatment resulted in the highest cellulose yield of 42.5%, compared to chemical treatment of 28.4%. Among the two treatment methods, the steam explosion treatment method has produced the best results, as it uses less chemicals, takes less time to extract cellulose, and is an environmentally benign process for preparing cellulose.

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