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Evaluation of morphological architecture of cellulose chains in grass during conversion from macro to nano dimensions

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Abstract: The cellulose nanowhiskers (CNW) are of imminent importance in the development of ecofriendly green material for environment. Morphological study of their structure was carried out after extraction from grass. The controlled alkali and acid hydrolysis after soxhlet extraction of bleached fiber in ethanol and water provided a mixture of micro/nano fiber which can be further converted into CNW by mechanical treatment. Width of obtained CNW were found to be ~10-65 nm with length of several nanometers as evidenced by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The changes in dimensions during alkali treatment, bleaching and acid hydrolysis of grass exhibited an interesting architecture and clarify the phenomenon of separation of nano level fibrils from the matrix of hemicellulose and lignin which starts after swelling of fibers and opening from middle followed by splitting from each other. The nanofibers were embedded in the matrix surrounded by amorphous layers. The size of fibers was directly associated with the extent of treatments. The crystalline part of fiber was intact during hydrolysis which must be attributed to easy removal of amorphous region by penetration of hydronum ions from acid. The results of SEM and TEM were correlated with the Scanning Ion Microscopy (SIM) which showed a direct evidence of breaking of thick fiber strands resulting in the formation of sharp edged crystalline entities composed of cellulose crystals.

Introduction

The numerous benefits of natural fiber reinforced polymer composites have increased a deliberate interest toward development of highly compatible biocomposites with fine tuned architecture. Other than being light weight and cheap, ubiquitous availability in a variety of forms, low density and comparable material properties with high molding flexibility, cellulose fiber reinforced composites have been accepted as nature friendly materials which make them a conceivable option in comparison of traditional fillers [1]**.**

Some time the increase in weight of this category of composites has been a very sensitive issue, limiting their viability to compete the cost performance ratio for higher constructive applications like automobile sector and building constructions [2]. The weight reduction may be assumed by application of nano size fiber as filler. The high surface area, i.e. long and thin fiber, may help to employ this fiber at comparatively lower concentration. The natural fibers may be processed to yield reinforcing elements of different mechanical properties, and cellulose fibers with moduli of 40 GPa can be separated by chemical pulping process. In addition, they may be subdivided through hydrolysis followed by mechanical disintegration into microfibriles [3].

Cellulose is the linear condensation polymer with D-anhydroglucopyranose units joined together by β-1,4 glycosidic bonds and the orientational arrangement of cellulose chains determines its many chemical and physical properties. The cellulose chains are biochemically synthesized in micro fibrils that constitute amorphous and crystalline domains. The amorphous region is susceptible to acid attack and can be removed by penetration of hydronium ions, hydrolytically cleaving glycoside bonds and releasing the crystallites [4, 5]. The morphological investigations provide direct information about structure of fibrilar bundles. These fibers may be in micro and nano dimension depending on the degree of treatments, and have a high 167.5 GPa Young's modulus along the chain axis as per theoretical [6] estimations. Thus, CNW is a significantly potential candidate for the material of future that can be used in number of applications such as thickeners, gel forming precursors [7]. CNW is highly applicable as nano filler for polymer matrixes such as poly(lactic acid) [7] silk fibroin [8], starch [9] and cellulose derivatives [10] which result in a hybrid of comparatively better mechanical properties with competitive environmental concerns. The larger surface area in combination of higher crystallinity additionally enhances its reinforcing capacity in tailored polymer composites. Nanosize (5-60 nm cross section with length 100 nm to several μm depending on source [11]) rod like cellulose crystallites particles, abbreviated as whiskers, can be extracted from laterally stabilized fibrils bundle by removing amorphous region through controlled chemical and physical treatments. Tunicate [12], bacteria [13], algae (valonia) [14], brown algae (oomycota) [15], commercially available microcrystalline cellulose [16], wheat straw [17], flax, hemp, kraft pulp, rutabaga [18] soybean stock [19], sugar beet [20], cotton [21] and wood straw [22] have emerged as popular sources for the extraction of CNW in recent years.

Nevertheless, such fibers have to conquer many obstacles before directing it towards industrial practices due to higher surface area with several hydroxyl groups on the surface which attract water as well as tend to form hydrogen bonding among themselves which result in the agglomeration and poor dispersion in polymer matrix. Time consuming preparation procedure with very low yield, commercial unavailability and comparative higher cost from a limited source further limit the use of CNW in many applications, particularly in the development of daily use disposable commodity products. Fiber architecture including, fiber geometry, fiber orientation, packing arrangement and fiber volume fraction, controls many composite properties, particularly mechanical.

The morphological studies have been helpful to understand the fashion of twisting and ordering of cellulose chains [23]. The geometry of most of vegetable fibers is ultimately controlled by the morphology and may be influenced by the way in which the fibers are extracted and processed. These processes may alter the microscopic defects in the fiber, which can lead to localized stress concentrations within the matrix. Since the architecture of fiber has direct influence on the properties of its composites, many attempts have been made to unfold the orientation of fibers, twisting of fibril strands ,formation of chiral helixes, shape and aggregation tendency [24-26]. The nano-dimension of cellulose is generally estimated by evidences through TEM, AFM, ^{13}C NMR, Cross polarization/magic angle spinning (CP/MAS) and XRD supported by polarized optical microscopy of colloidal aqueous suspensions after direct acid hydrolysis [27-28] without description of fiber structure during alkali and bleaching treatments at different levels. The present attempt is focused on the study of tentative morphological changes during conversion from macro to nano dimension of cellulose not only by acid hydrolysis but also at different stages of treatments sequentially from grass for the first time by monitoring through SEM, TEM and SIM. The effect of concentration of alkali and bleaching temperature was also investigated briefly.

Results and discussion

Alkali treatment

The SEM of washed and extracted samples (ethanol: water) after soaking at different concentrations are demonstrated in Figure 1a and b. It can clearly observed from the figure that fiber start swelling after soaking with base which causes softening of the cementing material on the upper surface of grass strands. The hemicellulose, mainly consisting of xylan, polyuronide and hexosan, has shown to be very sensitive toward hard base whereas it do not affect α-cellulose and lignin [5]. The sodium ions interact in the interfibriler regions and results in the increase of hydrodynamic volume by uncoiling and unfolding of the chains. Thus, this step may allow the solublization of hemicellulsoe and lignin by disrupting hydrogen bonding; these can be further removed by repeated filtration.

After removal of these materials from the surrounding matrix inter-fibrilar region become less dense which allow the rearrangement of fibers along the direction of tensile deformation [6]. The increases in separation intensity was observed by SEM in Figure 1b with the increased concentration of alkali where clearly detectable difference in the thickness and crowding density of fibers could be seen which was attributed to the penetration of more number of base molecules, causing a greater extent of individualization of cementing material at higher concentration. However, the size of fibers vary in a broad range may be attributed to the uneven distribution and density of surrounding less ordered amorphous chains. The hemicellulose material made up of carboxylic groups which converted into COONa by ionization and reduced the inter and intrarmolecular hydrogen bonding which further help to eliminate the hemicellulose material by dissolution. This phenomenon help to relax the crystallites that may be tailored in different dimensions under appropriate treatments conditions. This conversion phenomenon may be additionally accelerated at higher percentage of alkali causing more swelling resulting in higher separation.

The concentration of NaOH is very critical and has been found to cause the degradation in cellulose and to avoid this degradation an optimum concentration is always required [3]. Cellulose is a natural composite that is entirely surrounded by lignin and hemicellulose material and the higher concentration of NaOH for longer time may drastically alter the chain structure of cellulose by degradation due to reactions like peeling phenomenon [20]. The SEM of treated samples in Figure 1c. (1 hr. heating with 2% NaOH at 80 ^{0}C) shows a type of degradation in upper surface of fiber and consequently generates many individualized structures which confirm the indication of severe damage of outside layers by leaching upon heating treatments. A smooth strand could be detected with small kinks and white lines on the upper surface attributed to the complete removal of alkali prone material which was associated to form rough surface. The 2 % concentration of alkali is popularly used for fiber treatment [20] to make it compatible with thermoplastic polymers and a clear removal of surrounding layers could be evidenced as in present case. The application of high temperature during alkali treatment [21] to increase the exposure of cellulose chains may increase the chances of peeling. Thus, soaking methods appeared more sophisticated and effective due to less extent of fragmentation of fiber for removal of hemicellulosic material.

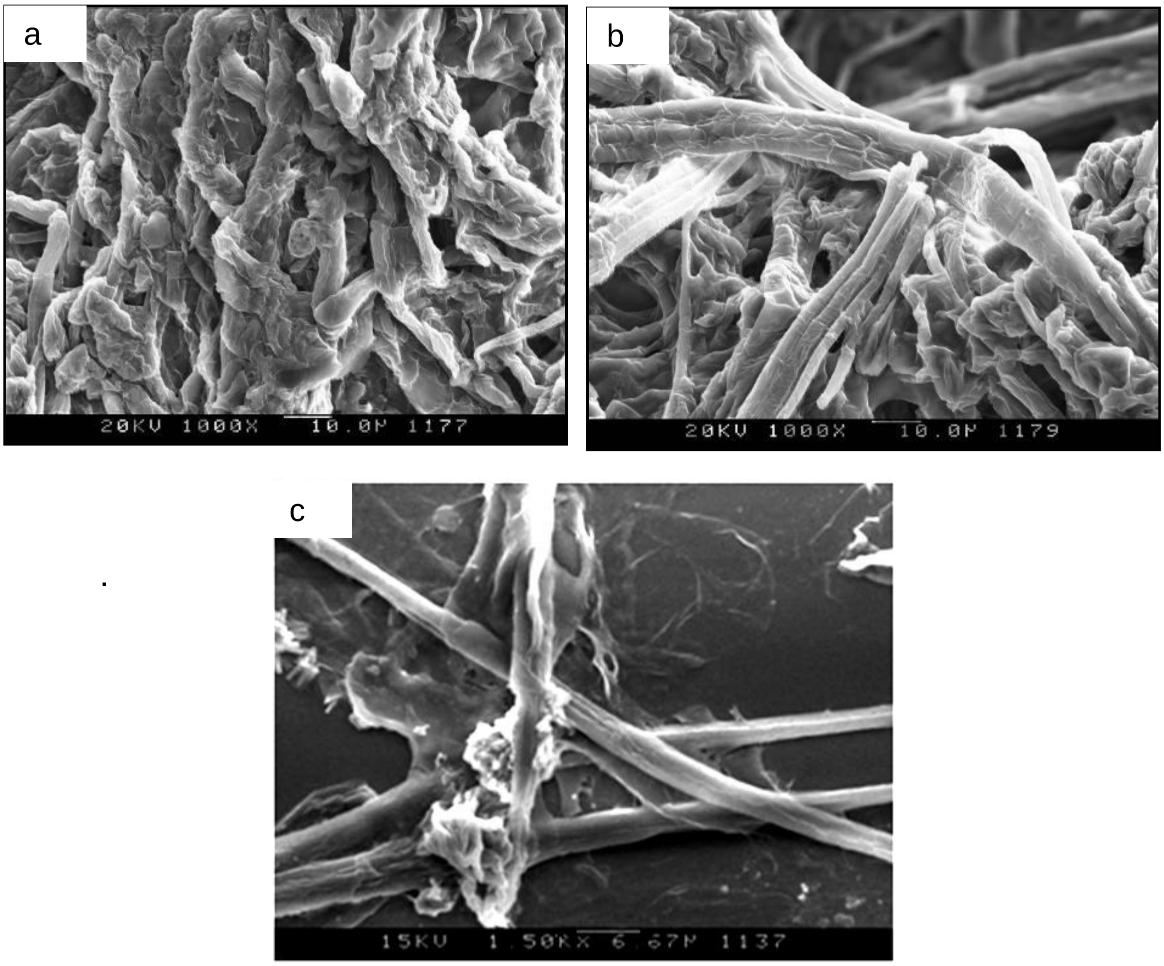

Fig. 1. SEM micrograph of (a) 2.5 % alkali soaked grass fiber, (b) 5 % alkali soaked grass fiber (c) alkali treated sample with 2% NaOH at 80 ^{0}C.

Bleaching treatment

Bleaching was carried out after neutralizing and washing the alkali treated fiber, several times with distilled water till constant pH. It was performed by sodium chlorite in acetic buffer medium at 70 ^{0}C (BC1) and 85 ^{0}C (BC2) for 3 hrs. During these steps the color of grass fiber changes drastically to shining white by removal of tannins and lignin. Lignin is made of hydroxyl phenyl propane connected each other by partial carbon-carbon bonds in a helical structure.

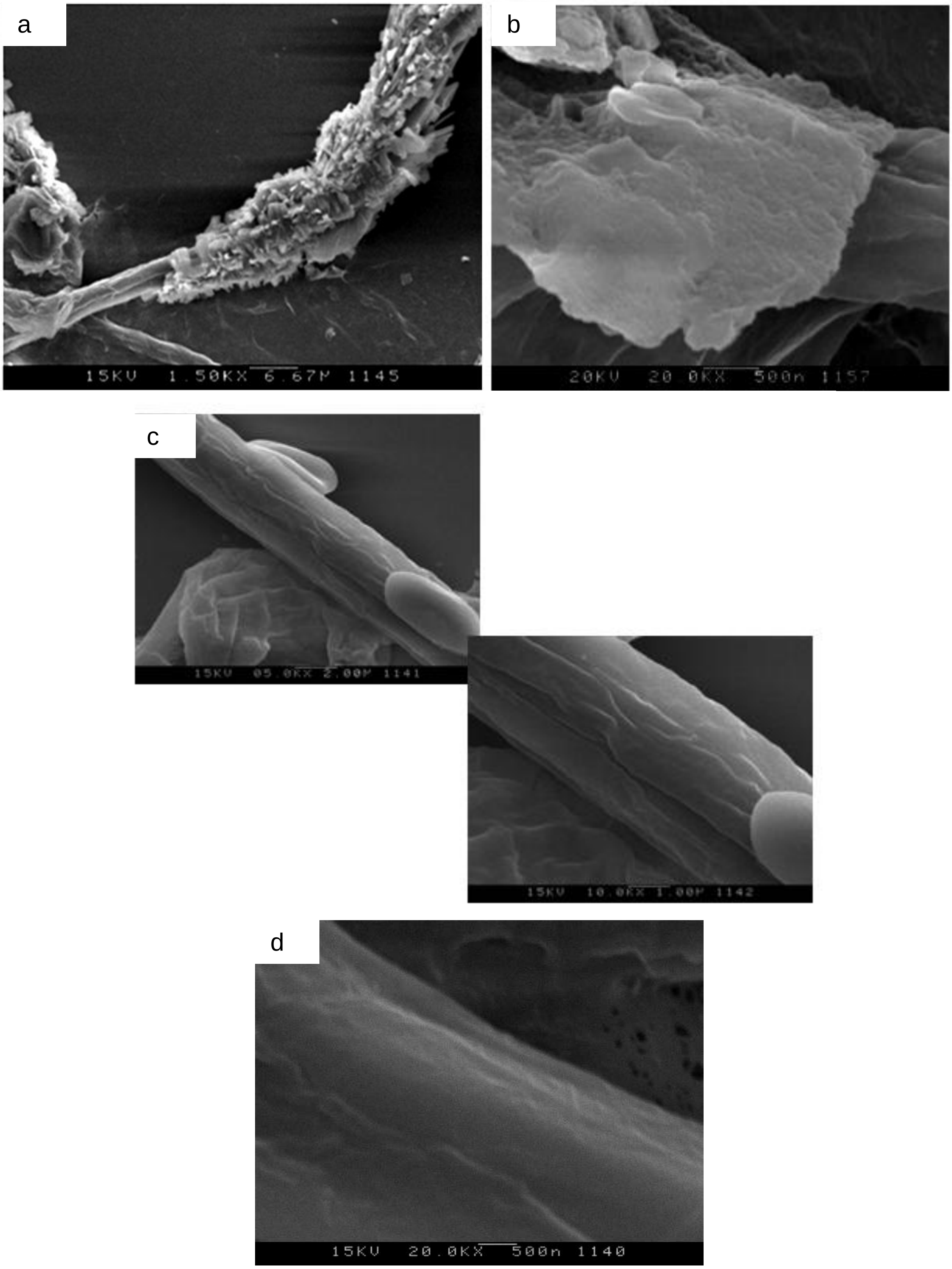

Fig. 2. The SEM micrographs of (a) BC1 showing the elimination of bulky surrounding surface, (b) BC1 exhibiting the removal of lumps from the upper surface of fiber, (c) BC2 with a smooth surface, (d) BC2 at higher magnification showing reduction in rough upper surface with some kinks.

The bleaching agent make it more hydrophilic by forming the carboxylic, carbonyl and hydroxyl groups that can be solubilized in alkaline medium leaving behind pure cellulose fibers.

Figure 2 a and b of BC 1 showed some rough surface than those of BC2 (Figure 2 c and d) confirming the effect of temperature conditions on the remaining part after alkali treatment. At high temperature most of the surrounding material is dissolved and a smooth surface comes out as is seen in Figure 2 c and d**,** most tentatively due to the penetration of bleaching agents inside cellulose bundles.

Acid treatment

Fiber structure was studied by drying it for 5 minutes immediately after acid hydrolysis under optical microscope at 50 X magnification as presented in Figure 3a.

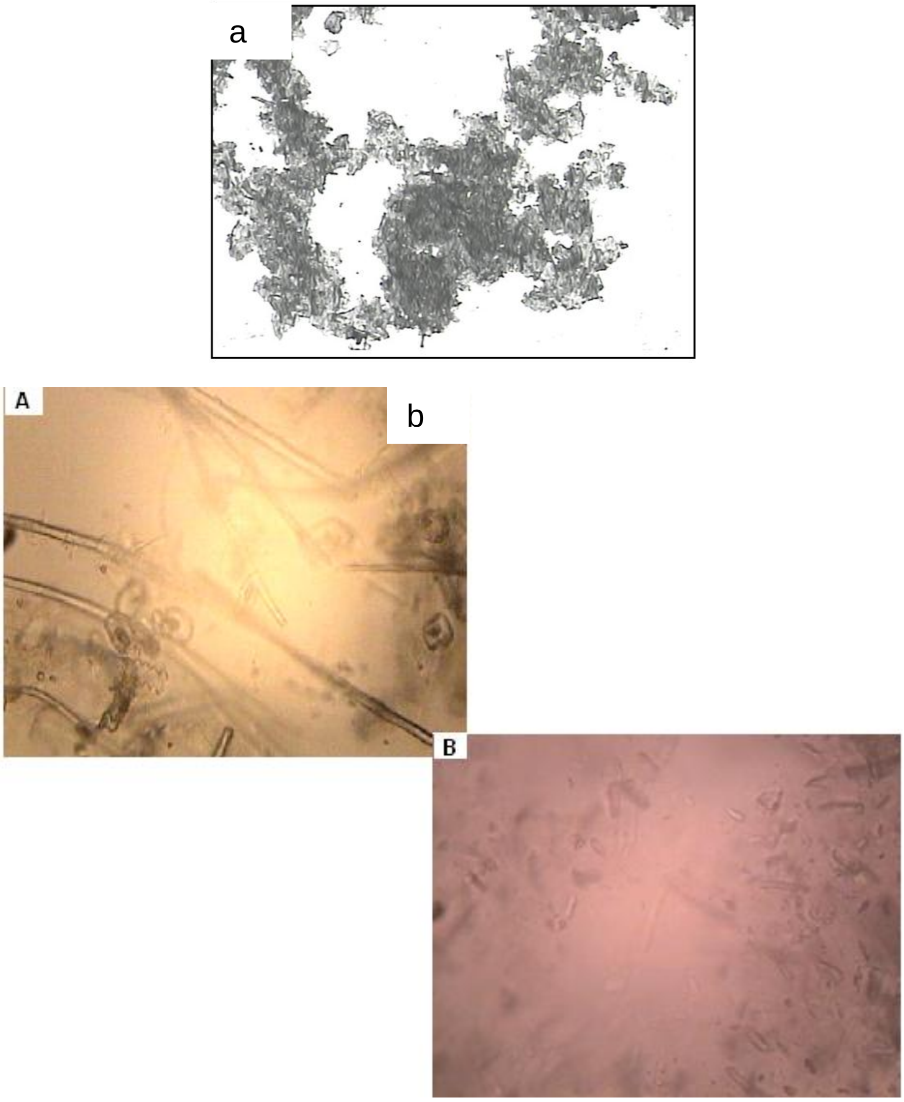

Fig. 3. (a) Optical micrographs of acid treated fiber (50 X). (b) The digital picture of specimen showing long round ended fibers with extent of fragmentation (A, alkali treated and B, acid treated).

It was seen that agglomeration increased with drying and fibers come close to each other in the form of lumps. The observable color changes in fibers toward black at this stage provide the information of severe degradation by acid in the present system. It was confirmed that water help to keep them away from each other by establishing the hydrogen bonding between water molecules which was destroyed after further drying through faster evaporation.

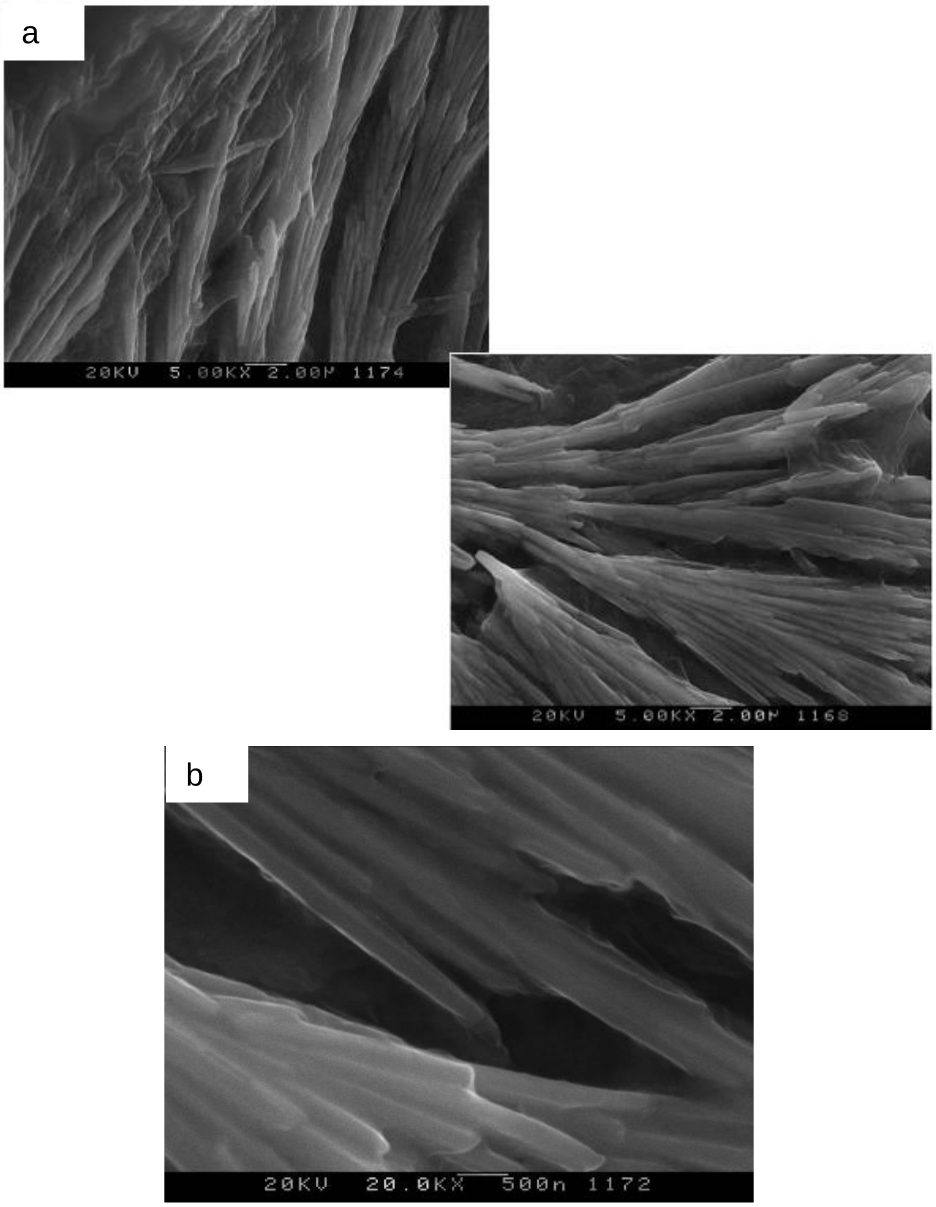

Fig. 4. The SEM micrograph of (a) acid treated samples, (b) acid treated samples at higher magnification.

One process of direct acid treatment was also carried out parallely in this batch of reaction after alkali treatment by escaping bleaching step. Figure 5 showed the single strand of fiber whose surface was smooth after alkali treatment has many exposed micro/ nano-size fibers after acid hydrolysis. The SEM of this specimen confirmed the degradation of amorphous region from the surface which may not contribute to alter the crystallinity of remaining embedded cellulose chains due to very small quantity.

Thus the direct acid hydrolysis has the capacity to produce fibrils or to split out the more crystalline nanosize cellulose in specimens after washing the acid sensitive amorphous part. The direct acid hydrolysis of grass fiber did not give well separated CNW as in other reported cases from microcrystalline cellulose [16]. The acid hydrolysis is found to remove amorphous part (region of less ordered loosely packed chains) more quickly from the surface without altering the bulk due to difference in activity of both crystalline and amorphous regions which finally lead to the exposure of reaming more crystalline part. While studying the fiber structure of cotton linter after acid hydrolysis [26], the obtained results were concluded about cellulose fibers; those have relatively slow hydrolysis rate, as water molecules and acid are unable to penetrate these ordered crystalline regions and the amorphous cellulose leach out from the microfibrils during hydrolysis.

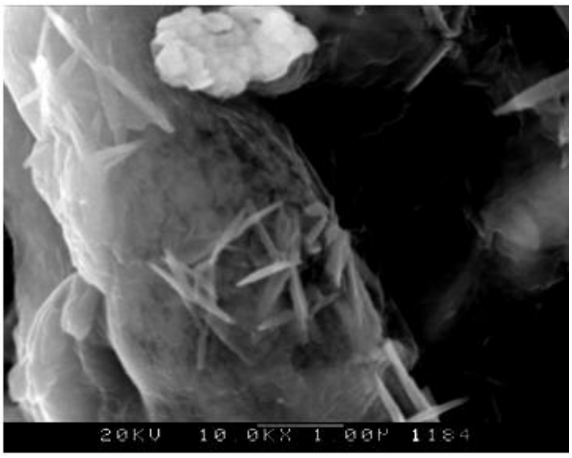

Fig. 5. SEM micrograph of directly acid treated samples.

Mechanical treatment

The effect of mechanical treatment through sonicaton on the extent of separation was studied by differing the total time. The SEM of 5 minute sonicated samples displayed the nano-sized (~45-50 nm thickness) agglomerated tightly bounded moieties (Figure 6a). The TEM in Figure 6b exhibited a high agglomeration for 5 minute sonication. The naofibers were found to be well separated after 15 minute sonication time as in Figure 6 c. This increased time, produces the CNW of ~ 10 nm thickness with several nm lengths. The degree of agglomeration increased with time which must be due to water evaporation from the sample as nanofibers tend to reach at the corners of specimen.

Thus, the complete separation could be possible only by mechanical treatment that provides high shear and turbulence to reduce the size of overall fiber [18]. This treatment puts a high mechanical impact and ruptures the cell wall by high energy transfer. In a comparative attempt the images of samples were investigated through SIM for 1.8 min after alkali treatments and an interesting phenomenon was detected. The objective was to study the mechanical treatments and the effect of high energy radiation on the cellulose bundles. The clear picture of big cellulose (Figure 7a, b) strands was captured showing the degradation of surrounding material and exposure of pointed rods like structures from the thick strand of more than 5 µm and a bunch of microfibrils of ~ 160 nm was observable which seems to be exposed out from strands.

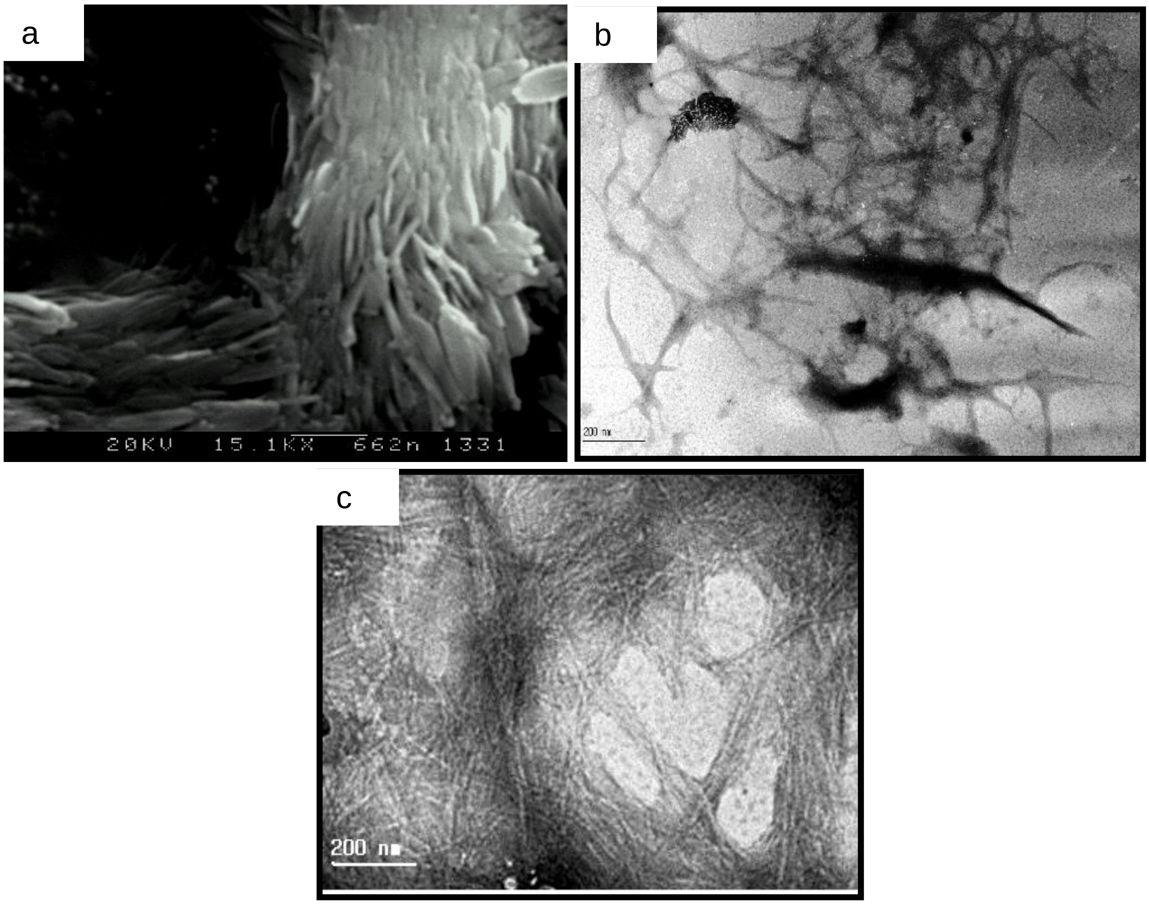

Fig. 6. (a) The SEM of 5 min sonicated sample, (b) TEM of 5 min sonicated samples, (c) TEM of 15 min sonicated samples.

In the image of Figure 7c the sharp edges needle shaped crystals appeared separated out completely from each other. Since the size of these fibrils did not change significantly in these images, indicating that they have freed from large strands and may consist of several nano fibers similar to SEM after acid hydrolysis. With the same specimen, after 1.8 min scanning, we did not observe anything confirmatory except for some dark degraded samples. Thus fine edge particles underwent complete degradation before the nano-level separation. Thus, SIM provides direct evidence of opening of more crystalline cellulose after easy degradation of surrounded part and supported the fact that the nanosized strands were embedded in the amorphous matrix.

The changes in functionality, thermal stability, and crystallinity

The crystals were found embedded in the deep, surrounded by less ordered matrix which was removable after different treatment. To fuel the architectural conclusions made by SEM, TEM, and SIM, the samples were examined to study the functional groups changes, crystalline and thermal nature by FT-IR, XRD and TGA respectively. FT-IR analysis was performed to monitor carboxyl and hydroxyl region (1800 -1600 cm^{-1} for the C=O and 3600–3000 cm^{-1} for the OH vibrations) as these groups undergoes variations during conversion of macro to nano fiber [30].

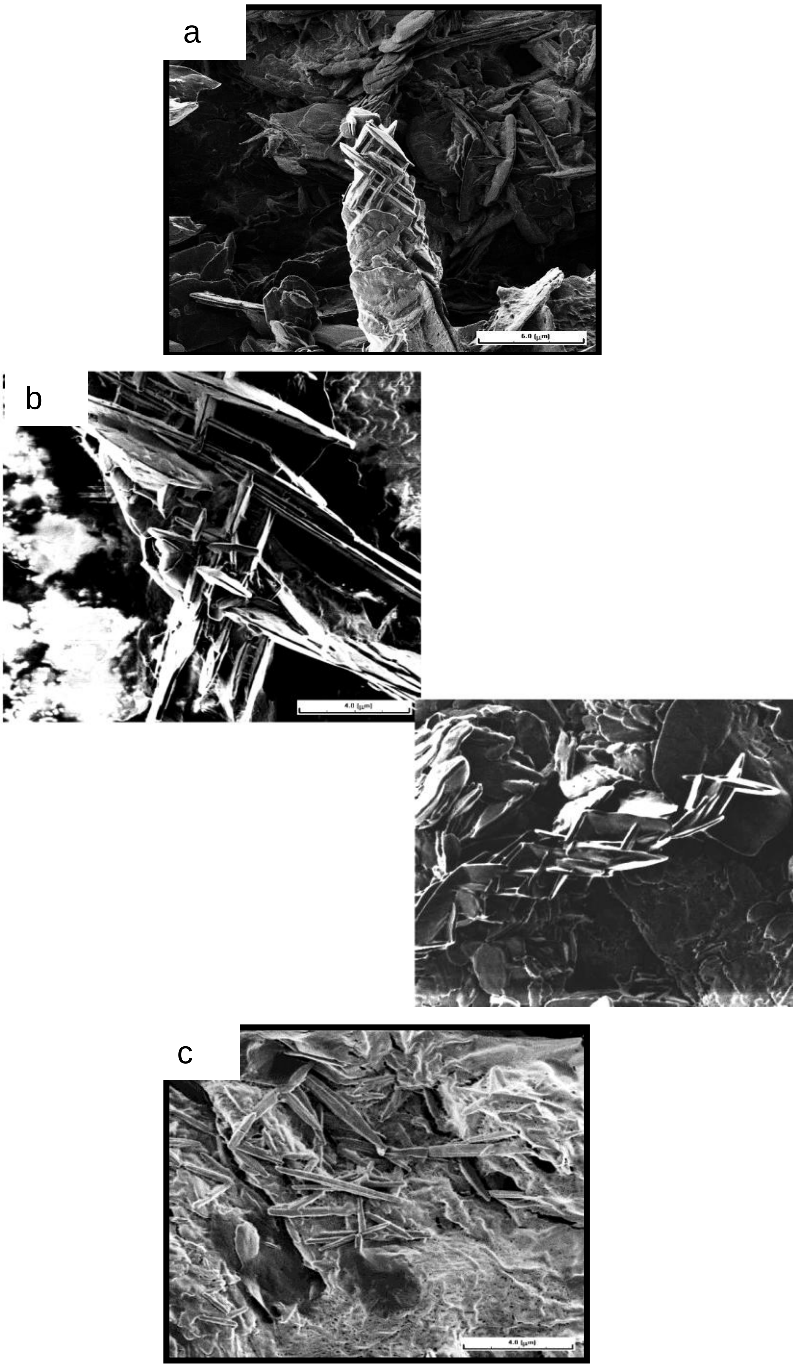

Fig. 7. (a) SIM micrograph of grass fiber after starting the scanning, (b) SIM micrographs of grass fiber after 45 s. Scanning, (c) SIM micrograph of grass fiber after 1.1 min scanning.

In the present case a complication by the moisture absorption was removed by calibration with vacuum dried specimens. The significant shift in 3500-3000 cm-^{1} toward higher frequencies in case of treated fiber must be due to the presence of more number of free exposed OH groups at the surface. Figure 8 illustrates the variations under carbonyl region where the peak at 1245 cm^{-1} assigned to methyl ester groups was vanished and establish the removal of hemicellulose [30]. The vibration peak at 1257 cm^{-1} and 1510 cm^{-1} for benzene ring and -C-O stretching of lignin was disappeared due to the elimination of lignin from the treated fiber [18].

The cellulose microfibrils have the existence of both crystalline and amorphous regions with amorphous part of lower density in the nanocrystalline areas. Typical X-ray diffraction in Figure 9 for CNW and vacuum dried grass after passing through sieves and refluxing in ethanol and water solution, exhibited the changes of crystallinity in Figure 9 as evidenced by the pattern and shape of diffraction peaks. In native grass two well defined peaks were present at 2θ =22.3 and 2θ =34.5 degrees with broader shoulder at 2θ =16.4 degrees, those can be correlated with (200) and (023) or (004) crystallographic planes respectively whereas broader shoulder at 2θ =16.4 may be due to the (110) or ($\overline{110}$) . CNW presented two peaks at 2θ =15.5 and 2θ =16.2 degree for the (110) and ($\overline{110}$) plane and sharp peaks reflect the presence of high crystallinity which is confirmation of presence of cellulose I.

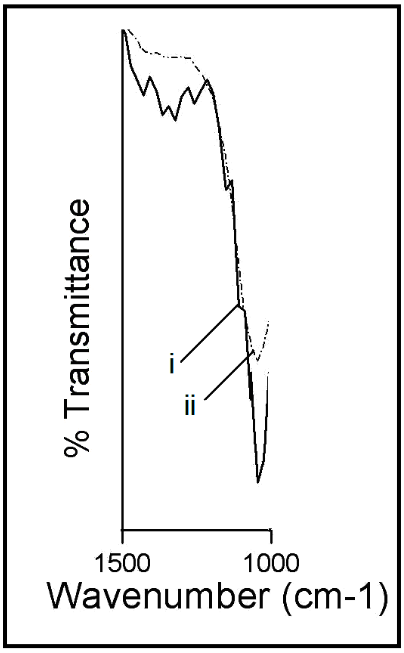

Fig. 8. Variation in carbonyl region (i before treatment, ii. after treatment).

Figure 10 explains the tentative graphical presentation of cellulose nanocrystals embedded in the matrix. As soon as the cleavage of amorphous chains propagates, individual crystallites come out due to higher freedom of motion and make microsize crystals after aggregation [15]. The overall study gave the credit to conclude that CNW has higher crystallinity and this evidence was confirmed by FT-IR spectra where the peak at 712 cm^{-1} was visible corresponding to cellulose I allomorphs. During thermal analysis CNW showed two well separated pyrolysis processes [18] and degradation occurred to a wider temperature range starting from 220 ^{0}C to 500 ^{0}C with initial moisture loss at 120 ^{0}C in form of absorbed moisture. Untreated fiber exhibited many steps of degradation including at 110 ^{0}C for water and 280 ^{0}C for hemicellulose decomposition. The decomposition after 280 ^{0}C was not observed for nano whiskers which may further support the removal of hemicellulose during alkali treatment. Nano crystals start decomposition at lower temperature than microcrystals due to the increasing number of free end chains at the surface area, being liable to earlier decomposition whereas the thermal resistance can be increased by neutralizing the sulphate groups [32]. The increase in first degradation temperature, corresponds to moisture in whiskers, this must be attributable to the strong adhesion of moisture molecules in highly exposed fibers of more surface area.

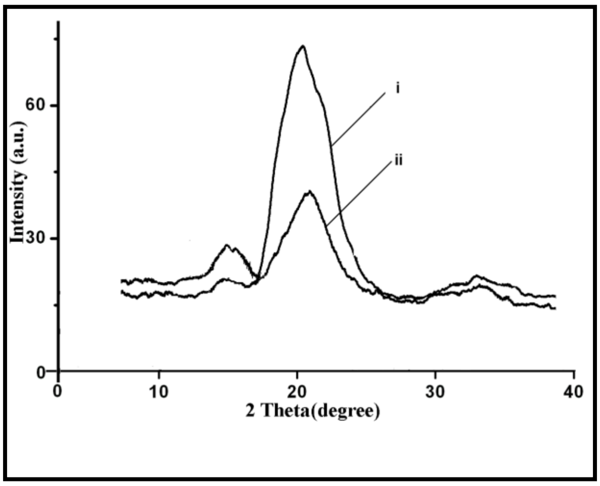

Fig. 9. Variation in XRD of fibers (i after and ii before treatment).

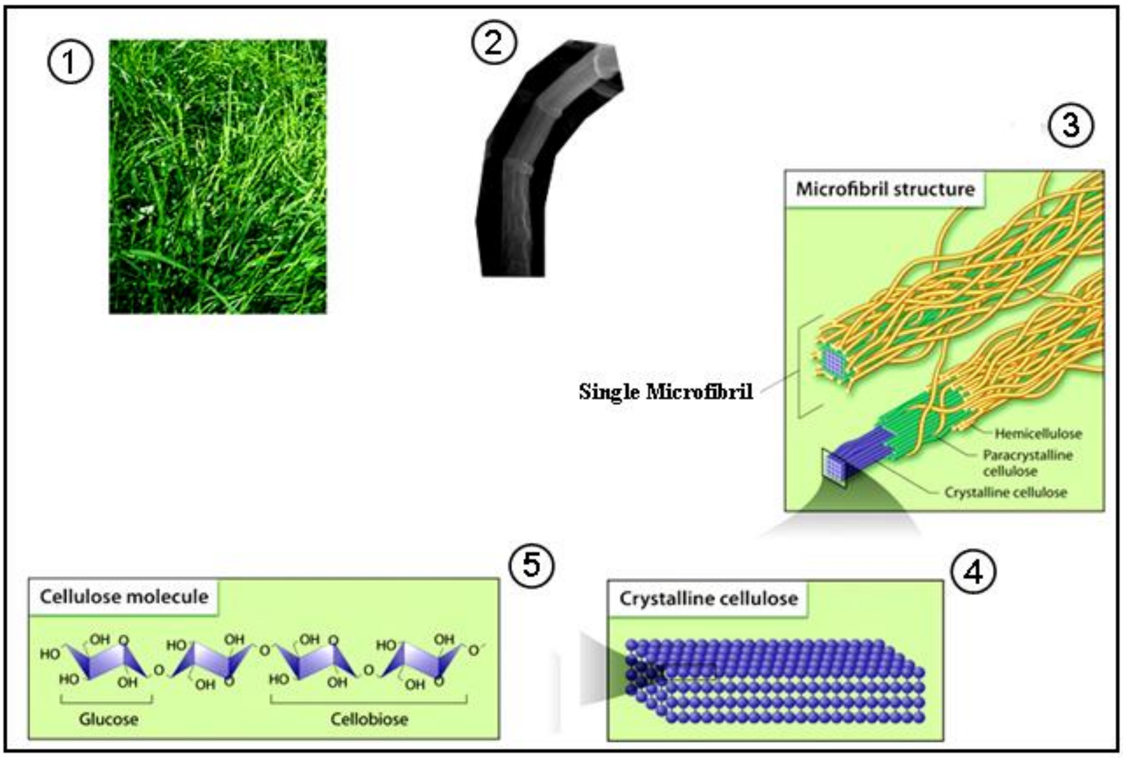

Fig. 10. Presentation of cellulose nanocrystals inside the matrix of hemicellulose, lignin, wax etc. 1. Grass in ground, 2. SEM of treated strand 3, 4, 5 are bundles of microfibrils, layers of cellulose crystals and chain of cellulose respectively [31].

Conclusions

This study was focused on qualitatively understanding how cellulose crystals come out in different size and shape from the grass at different stages of treatments which has a certain scope in order to enhance the potential of grass fiber as reinforcing material for constructive applications. Acid hydrolysis yields fibrous whiskers by selective degradation of easily assessable amorphous region. The agglomeration was destroyed up to higher strength by mechanical treatment and nanocrystals forms aqueous suspension. Treatment time seems a critical parameter to extract the cellulose nanowhiskers from grass fibers and in general, increase in treatment time of every step alters the nature of size and shape of fibers. Soaking of fiber was helpful to swell the inter-fibrillar region reasonably well which further help to dissolve the surrounded cementing material for removing the embedded cellulose nanocrystals. Although the precise and quantitative information could not come out with the SIM, still confirmatory information of exposure of nano-sized fibrils was directly evidenced from the micrographs. The direct acid treatment can also produce some nanofibers on the surface of cellulose bundles in the grass but the amount and size was directly influenced by bleaching treatment and temperature.

Experimental part

Materials

All the chemicals were from Dae Jung Chemicals and Metals Co. Ltd, Gyonggi-do, Korea and used as received. Sodium chlorite was obtained from Kanto Chemical Co. Inc., Tokyo, Japan and used as received. Grass fiber (Zoysa japonica and tenuifolia) was collected from ground of Seoul National University.

Extraction of CNW

The grass was treated with physical and chemical methods in order to remove the cementing material to obtain the CNW [29]. The fibers were soaked with 2.5 and 5 % NaOH for 2 days at room temperature and 1 hr heating at 80 °C followed by washing for several hrs with distilled water. The acid treatment of bleached grass fibers was carried out by 65 %, sulphuric acid, for 15 minute) with bleached grass which provides the mixture of nano fiber. The bleaching [20] was carried out at 70 and 85 °C for 3 hrs. and all treatments were conducted in inert atmosphere by mechanical stirring in two necked flask .The effect of sonication was also monitored and two different time intervals were taken at 5 and 15 minutes for study.

Scanning Electron Microscopy (SEM)

The stained samples were dried under vacuum for 24 hrs at 50 °C. These gold-coated specimens were examined under electron microscope (DS-130C; Topcon) for monitoring the morphological changes. The samples were dried under vacuum before coating.

Optical Microscopy (OM)

OM was performed at 50 X with an Olympus optical microscope by putting the sample on glass plate after drying.

Transmission Electron Microscopy (TEM)

TEM was applied to monitor the extent of modification by treatment on the fiber and to measure the shape and size. One drop of the aqueous suspension was put on carbon grid and allowed to stand under vacuum for overnight.

Scanning Ion Microscopy (SIM)

The SMI3050 (SII technology) of SIM (through focused ion beam) system was used to investigate the degradation pattern of cellulose fiber after chemical treatment. This system enable to image by scanning the ion beam at the place electron beam as in SEM. When imaging, 4 nm in diameter of the ion beam was used with 10 pA of the ion current and micrographs were collected from the same samples at different time intervals.

Fourier Transform Infrared Spectroscopy (FT-IR)

Attenuated Total Reflectance Infrared Spectroscopy (FT-IR) spectra of samples were recorded using the FT-IR 300E, Jasco spectrometer. The incident beam angle was 458 degree. At each measurement, 60 scans were acquired with a spectral resolution of 4 cm^{-1}. The Win First computer program, version 3.57 (Mattson Instruments), was used for acquisition and elaboration of IR spectra.

X-Ray Diffraction (XRD)

The XRD pattern of samples was obtained by a Rigaku (Japan) diffractometer with Cu- Kα radiation at 50 kV at the scan rate of 1° /minute.

Thermogravimetric Analysis (TGA)

Thermo- gravimetric analysis (TGA) was performed under nitrogen atmosphere from 50-500 ^{0}C at a heating rate of 10 ^{0}C / min.

Acknowledgements

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