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A facile approach for isolation of cellulose nanocrystals from banana fibres

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Nanocellulose, in form of cellulose nanocrystals (CNC-B) from banana fibres, has been isolated through steam explosion followed by chemical treatments and mechanical grinding. The nano size of isolated particles is confirmed by dynamic light scattering and transmission electron microscopy analysis and an aspect ratio up to 9.80 is reported. The improvement in crystallinity and removal of amorphous constituents are confirmed by X-ray diffraction and Fourier transform infrared analysis respectively. It is interesting to report that the present CNC-B is unable to show an antibacterial property, which might be due to complete removal of lignin.

Keywords: Antibacterial properties, Banana Fibres, Cellulose nanocrystals, Structural properties

1 Introduction

Nanocellulose is being extracted from various natural fibres and has been utilized in advanced applications (i.e. medicals, high strength bio-nanocomposites, electronic devices, flexible displays, transparent nanocellulose papers, anti-corrosion and self-cleaning surfaces, packaging, and so on) for the value addition of waste biomass [1-3]. Around 1.5 million tons of banana fibres are produced every year from waste pseudo stems of banana plants[4]. Utilization of cellulose-rich residue from banana cultivation wastes leads to reduction in environmental pollution and value addition to the cellulose byproduct. Banana fibres contain cellulose (60–65%), hemicelluloses (6–19 %), lignin (5–10 %), pectin (3–5%), ash (1–3%) and extractives (3–6%)[5]. The reported physical properties and mechanical properties of banana fibres are: diameter (80-250 µm), density (1350 kg/m^3), aspect ratio (150), elastic modulus (27-32 GPa), ultimate tensile strength (789 MPa) and percentage elongation (2.5-3.7)[5,6]. Two largest producers of banana fibres worldwide are India and Brazil[7].

Cellulose, chitosan, collagen, and soy protein isolates are some of the polymers which are biodegradable, environmentally safe, and nontoxic[8]. Cellulose is the most commonly used biopolymers because it is abundant, biodegradable and renewable[9]. Cellulose's worldwide annual production is approximately 1.5 × 10^{11} tons (refs 10,11). Several mechanical and chemical treatments have been applied on the plant fibres to remove hemicelluloses, lignin, waxes, and pectin to obtain nanocellulose. There are two forms of nanocellulose extracted from plant fibres namely cellulose nanocrystals and cellulose nanofibrils [12]. Nanocrystals are crystalline, whereas nanofibrils contain crystalline and amorphous regions with a diameter below 100 nm (ref. 13). Acid hydrolysis produces nanocrystals with a diameter of 20–60 nm and a length of 250–480 nm (refs 14,15). The conversion of micro-scale fibres into nano-scale fibres can also be achieved through mechanical treatments such as homogenization, microfluidization, and sonication. A ball milling process is used to prepare cellulose nanofibres with a diameter of 100 - 500 nm (ref. 16). Nanocellulose obtained from plant fibres presents their worthiness to be utilized as reinforcing materials to improve the properties of polymer matrix due to their abundant supply, sustainability, large surface-to-volume ratio, high stiffness, high flexibility, and good mechanical and thermal properties over other conventional fibres[17]. It has been used in various applications including polymer nanocomposite, protective coating, filtration system, transparent film, antimicrobial film, drug delivery, pharmaceutical, organic solar cell, super capacitor, flexible electronics, etc[18–20].

Steam explosion is a thermo mechanical process that provides fast processing as compared to other

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processes. In this process, bio-resources are heated under high pressure steam (20–50 bar, 160–270°C) for some minutes, followed by cooling at 32 °C. The steam explosion process can be divided into two stages, viz steam treatment at an elevated temperature and subsequent explosive defibration. Morjanoff *et al.* [21] studied the two stages separately to quantify each stage's relative benefits. Generally, the steam explosion process results in the hydrolysis of glycosidic bonds in hemicelluloses and cellulose to a lesser extent. It also leads to the cleavage of hemicellulose-lignin bonds. Deepa *et al*. [7] used steam explosion and found a high aspect ratio and high percentage yield of nanocellulose. Abraham *et al.* [22] presented a novel approach of steam explosion technique using mild concentration of chemicals to isolate the cellulose nano fibrils from banana, jute and pineapple fibres. Cherian *et al.* [23] found that after the steam explosion of pineapple fibres, cellulose content was increased from 81.27% to 93.45% , whereas hemicellulose decreased from 12.31% to 3.72%. They also reported a similar study for banana fibres and found that the cellulose content was increased from 64.04% to 82.37% and hemicellulose decreased from 18.6% to 13.97% (ref.24).

Tibolla *et al*.[25] adopted chemical treatments followed by mechanical (high pressure homogenizer) treatments for the isolation of cellulose nanofibres from banana peel. Meng *et al*.[26] produced cellulose nanofibres and nanocrystals from liquefied banana pseudo-stem residue subsequently by high-intensity ultrasonic treatment and acid hydrolysis respectively. In another study, nanocellulose was also extracted from banana tree rachis fibre utilizing the process of, soap solution, benzene-alcohol, alkali and bleaching treatments[27]. Banana cellulose nanofibres were obtained by alkali treatment followed by oxidizing it with HNO_3-$NaNO_2$ mixture and also with HNO_3-$NaNO_2$ mixture [28].

From the detailed literature survey related to isolation of nanocellulose from banana fibres, it has been observed that several methods have been adopted by different research groups to isolate the nanocellulose in different forms from banana fibres. However, few studies have been reported in which steam explosion was used to isolate the nanocellulose from banana fibres. Some researchers have adopted steam explosion with mild chemical treatment for a long time. Research works are yet to be explored particularly on isolation of nanocellulose from banana fibres by steam explosion in order to reduce the treatment time. The present study is, therefore, undertaken primarily to overcome treatment time for the extraction of nanocellulose by chemical treatments assisted with steam explosion and to investigate its morphological, structural and antibacterial properties. In this work, banana cellulose nanocrystal (CNC-B) is extracted using steam explosion in an alkali medium for only one hour followed by bleaching of fibres, acid hydrolysis and mechanical grinding.

2 Materials and Methods

2.1 Materials

Long and cleaned banana fibres were collected from Women's Development Organization Dehradun, Uttarakhand, India. All the chemicals (sodium hydroxide with purity of 98%, sulfuric acid with purity of 98%, sodium hypochlorite with chlorine content of 10-12%) were purchased from Uma Scientific Corporation Limited Prayagraj, Uttar Pradesh, India.

2.2 Extraction of Nanocellulose from Banana Fibres

Nanocellulose was extracted from banana fibres using the various chemical treatment processes and mechanical grinding. The process details are provided hereunder.

2.2.1 Chemical Treatments

Initially, banana fibres were washed with distilled water and dried in an open atmosphere to remove the moisture. After that, fibres were chopped into small pieces of 2 cm. Subsequently, the chemical treatments were performed in the following three steps:

(i) Chopped banana fibres were first treated with 3% NaOH solution in an autoclave at 15Psi for one hour and performed under steam explosion.
(ii) Bleaching of fibres with NaOCl solution in the proportion of 1:3 at 50 °C for one hour. The same process was repeated twice.
(iii) Acid hydrolysis of fibres was performed with 10% H_2SO_4 in the proportion of 1:10 in an autoclave at 15psi for one hour and performed under steam explosion.

The fibres were repeatedly washed to achieve a neutral *p*H, and dried after each chemical treatment.

2.2.2 Mechanical Treatment

To convert the treated fibres into nanocellulose, fibres were grinded in many stages for several

minutes using a high speed Grinder (Sujata, 900 Watts). At each stage of grinding, a constant size of shortened fibres was obtained by a sieve shaker machine with a sieve number of 53 μm. A flow chart for the extraction of CNC-B is illustrated in Fig. 1.

2.3 Characterization

2.3.1 Yield Measurement

Yield of CNC-B was measured using the gravimetry method. The yield in terms of percentage was calculated using the following equation[25,29]:

$$\text{Yield (\%)} = W_2 / W_1 \times 100 \quad \text{... (1)}$$

where W_2 is the weight of the final dry nanocellulose; and W_1, the weight of the initial dry untreated banana fibres.

2.3.2 Dynamic Light Scattering (DLS)

DLS analysis was performed to estimate the size of CNC-B using the instrument DLS (model: Microtrac, Nanotrac wave at CIR Lab MNNIT Prayagraj, India) at temperature of 30 °C. The CNC-B was well dispersed in distilled water with ultra sonication before analysis.

2.3.3 Transmission Electron Microscopy (TEM)

TEM (Model: JEM-F200 at IISER Mohali, India) was used to measure the shape and size of CNC-B. Additionally, aspect ratio (length/diameter) of the CNC-B was also measured with the help of Image J software. Before starting of TEM analysis, the CNC-B solution was deposited on a 200-micron carbon grid and allowed to air dry for 24 h at 30 °C.

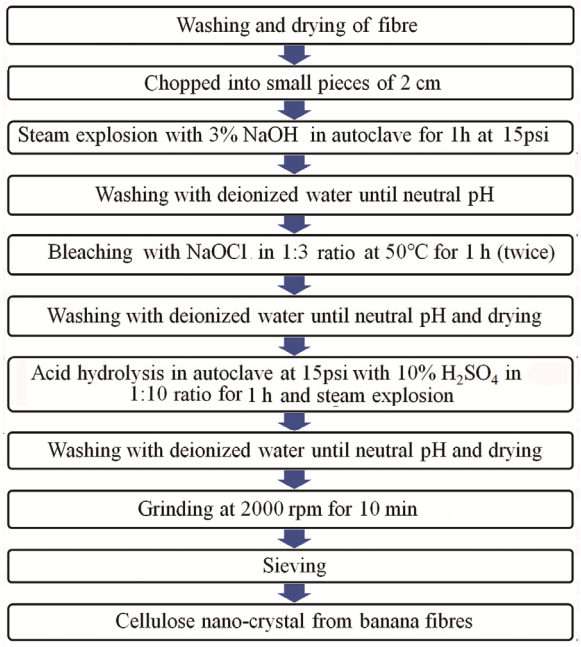

Fig. 1 — Extraction process of cellulose nano-crystal from banana fibres

2.3.4 Field Emission Scanning Electron Microscope (FESEM)

The morphology analysis of CNC-B and treated fibres was performed using FESEM (model: Neon-40) at CMTI Bangalore, India. The samples were made conductive by applying a very thin layer of gold on the surface of the samples.

2.3.5 X-ray Diffraction (XRD)

The crystallinity of CNC-B and treated fibres was determined using an X-ray diffractometer (Model: Smart lab 3KW at IIT Kanpur, India) with Cu Kα radiation at the 40 kV operating voltage. The analysis was performed between 4˚ and 70˚ of 2θ at a wavelength of 1.541Å. The crystallinity index of CNC-B and treated fibres was calculated according to Segal's method [30], as shown below:

$$\text{Crystallinity index, } I_{cr}\ (\%) = \frac{I_{200} - I_{am}}{I_{200}} \quad \text{... (2)}$$

$$\text{Degree of crystallinity} = \frac{I_{200}}{I_{200} + I_{am}} \quad \text{... (3)}$$

where I_{200} & I_{am} are the intensities at the crystallographic plane (200) and minimum intensity (which shows an amorphous region) respectively.

2.3.6 Fourier Transform Infrared Spectroscopy (FTIR)

The structural analysis in terms of change in chemical structure of CNC-B and treated fibres was performed by FTIR (model: Cary 660 at CMTI Bangalore, India) in infrared region 400- 4000 cm^{-1}. Peak heights were observed from the absorbance spectrum as a result of the presence of functional groups at different wavenumbers (cm^{-1}).

2.3.7 Antibacterial Analysis

The antibacterial analysis of CNC-B and treated fibres is performed against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) bacteria by the disc diffusion method. The zone of inhibition method is used to determine antibacterial activity.

3 Results and Discussion

3.1 Yield Analysis

The calculated yield percentage of the CNC-B is 38.4, considering an initial weight of banana fibres as 125 g and the final weight of CNC-B as 48 g. The alkali treatment is found efficient to eliminate most of

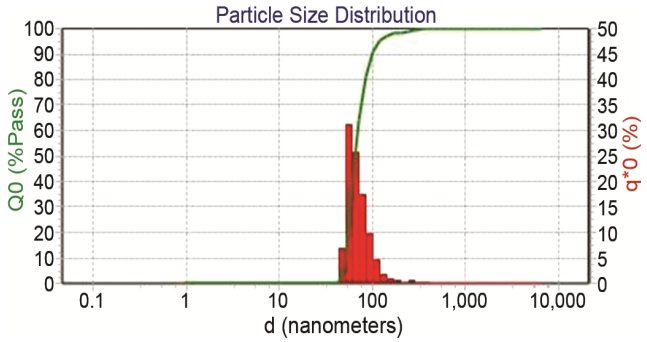

Fig. 2 — DLS analysis of banana cellulose nano-crystal

the hemicellulose from banana fibres, whereas bleaching removes lignin effectively. Finally, hemicelluloses and lignin are completely removed after acid hydrolysis and grinding. A lower value for yield percentage might be owing to the use of high concentration acid during hydrolysis, because higher acid concentration removes more amorphous compounds. Further, mechanical grinding may also be responsible for the low yield percentage of CNC-B[25]. The yield of cellulose nano fibrils extracted from banana peel was found to be decreased from 72 % to 27 %, when the acid concentration is increased [25]. In another study, yield is reported at about 71% and 52% for cellulose nanofibres and cellulose nanocrystals isolated from liquefied banana pseudo-stem residue respectively [26].

3.2 DLS Analysis

DLS techniques have been extensively used to determine the statistical distribution of nano sized particles. It provides information about variation in pass percentage (Q0) concerning variation in particle diameter (d). The particle size distribution of CNC-B is shown in Fig. 2. In this study, it can be clearly observed that almost all the particles are found in the nano range. The CNC-B with a size of around 70 nm has the maximum pass percentage (62), and most of the particles are found smaller than 100 nm. Further, the pass percentage of CNC-B is negligible for a size more than 100 nm. From this analysis, the calculated average size for CNC-B is reported as 65 nm. This method has already been employed to measure the nanosize of the particles by many researchers; for example, 50 nm for waste paper by Zhang *et al.*[31], 75 nm for rice husk by Varshney *et al.*[32], 60 nm for sugarcane bagasse by Gond *et al.*[33] and 68 nm for pine apple leaf fibres by Mahardika *et al.*[34].

3.3 TEM Analysis

A TEM analysis has been performed to estimate the shape, size and aspect ratio of CNC-B with the support of Image J software. Fig. 3 shows the TEM micrograph of treated fibres and CNC-B. The cylindrical/slender shape and average diameter of 14 nm for CNC-B are reported by this study. However, the length of CNC-B is ranged between 82 nm and 142 nm. Similar results for the range of diameter of nanocellulose are reported in past literature for rice straw (12-35 nm) and potato tuber (15-55 nm) [35]. The TEM results support the confirmation of the size of CNC-B with the results of DLS. The maximum aspect ratio is 9.80 and the average aspect ratio is 7. Varshney *et al.* [32] reported a range of the aspect ratios from 8.3 to 17 for rice husk nanocellulose extracted via microwave-assisted chemo-mechanical method. In another study [36], the range of aspect ratio is reported between 10.9 and 12.7 for CNC of kenaf extracted via acid hydrolysis.

3.4 FESEM Analysis

The morphology of CNC-B and treated fibres is analyzed by FESEM, which is presented in Fig. 4. The FESEM images of untreated banana fibres (UBF), alkali treated banana fibres (BFT1), bleached banana fibres (BFT2) and CNC-B are analyzed. A large diameter can be seen in Fig. 4(a), which may be due to the fibres consisting of many microfibrils. Meanwhile, the smooth surface of raw banana fibres indicates the presence of lignin, wax and oil [37-39]. A FESEM photograph of the banana fibres after steam explosion treatment is shown in Fig. 4(b). Steam explosions with alkali at high temperatures hydrolyze the hemicelluloses and make them water-soluble, and also depolymerize the lignin, which results in defibrillation of the fibres due to the removal of the cementing materials, as shown in Fig. 4(b) [19]. The FESEM micrograph shown in Fig. 4(c) is obtained after bleaching. Bleaching removes most of the lignin present in banana fibres, which improves further defibrillation. The removal of lignin is facilitated by sodium hypochlorite. The chlorine in the hypochlorite rapidly oxidizes the lignin. During lignin oxidation, hydroxyl, carbonyl, and carboxylic groups are formed, which facilitate lignin solubilization in an alkaline solution[23]. A high magnification image of CNC-B in a cylindrical/splendor shape with an improved surface can be viewed in Fig. 4(d).

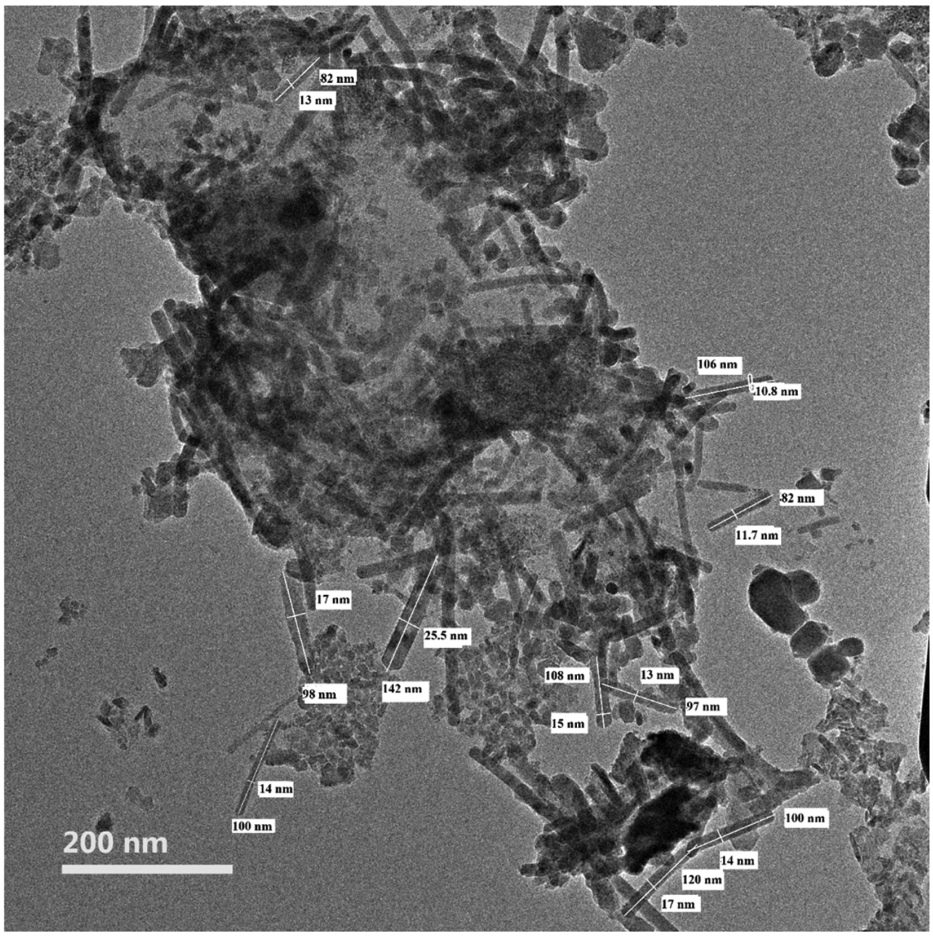

Fig. 3 — TEM image of banana cellulose nano-crystal

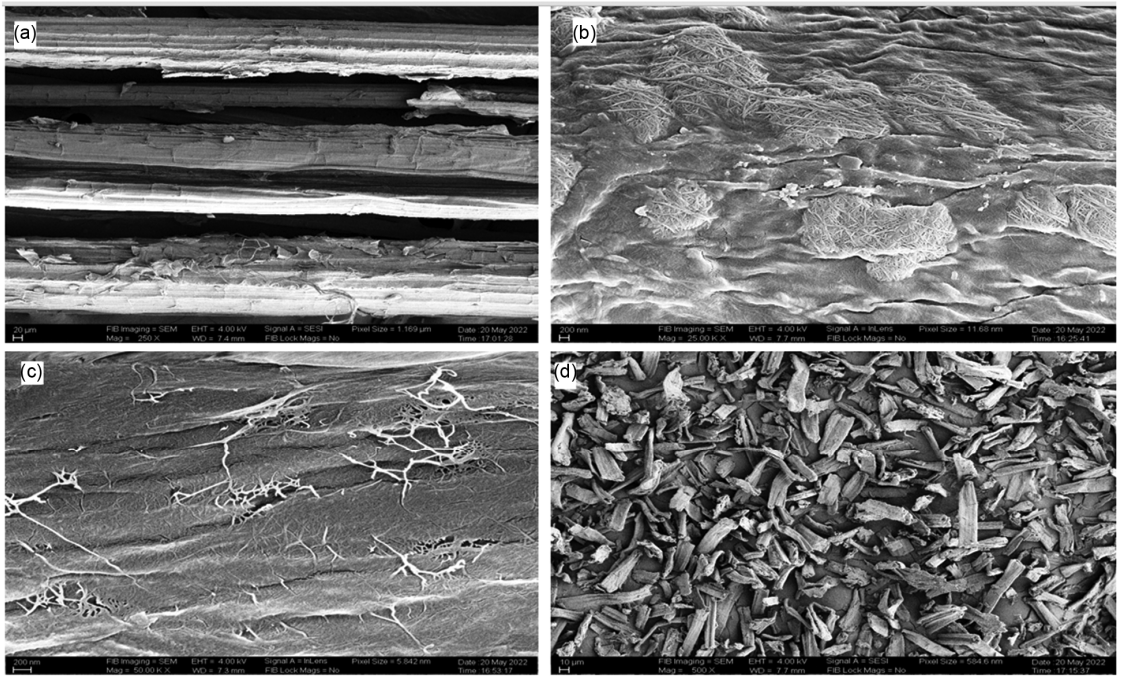

Fig. 4 — FESEM images of banana fibres (a) untreated, (b) alkali-treated, (c) bleached and (d) nano-crystal

3.5 XRD Analysis

This analysis is performed to see how the treatment processes affect the crystalline nature of CNC-B. Fig. 5 shows the XRD patterns of CNC-B and treated fibres. All the fibres and CNC-B presented almost similar diffraction patterns with two main reflection peaks at around 23° and 16 ° of 2θ. The highest values of intensities (I_{200}) for UBF, BFT1, BFT2, and CNC-B are observed at around 22.96°, 22.97 °, 22.71 ° and 22.89° respectively, while I_{am} is observed at around 19.35°, 19.58°, 18.94° and 18.96° respectively of 2θ. As a result of the processing, nanocellulose shows higher crystallinity than treated fibres. There are also shoulder peaks observed at around 16° of 2θ for all fibre types, indicating that cellulose structure has been improved after chemical processing and mechanical grinding.

The crystallinity index for UBF, BFT1, BFT2, and CNC-B is calculated to be 50.82, 58.93, 60.68 and 71.42% respectively. The CNC-B and UBF show the highest and lowest crystallinity index values respectively, while treated fibres have its intermediate values. After treatment, the crystallinity index is increased from 50.82% to 71.42%. The lowest crystallinity is observed in untreated fibres due to the presence of amorphous components. In CNC-B, the highest crystallinity is caused by the formation of H-bonds, which resist the free movement of cellulose chains concluding in the arrangement of disciplined crystals [39, 40]. The improved crystallinity index shows improvement in the rigidity, tensile strength, and stiffness of the materials [39]. After partial/complete removal of the hemicelluloses, lignin, and pectin, fibres crystallinity is found to be increased [39,41]. Acid treatment is used to remove the remaining randomly oriented amorphous materials.

3.6 FTIR Analysis

The purpose of this analysis is to compare the changes in the chemical structure of fibres after being treated by recognizing the functional groups present. Figure 6 shows the FTIR spectra of UBF, BFT1, BFT2 and CNC-B. A broad absorption band in the range of 4000–2990 cm^{-1} is observed for all the fibres and CNC-B which represents the OH stretching vibration in cellulose, hemicelluloses, and lignin [25]. The absorption band in the range of 2880-2900 cm^{-1} is also seen for all types of fibres and CNC-B which shows the H-C-H group of cellulose, hemicellulose, and lignin. It is noticed that the peaks at 1732 cm^{-1} due to C=O stretching vibration of acetyl of lignin and hemicellulose are seen in untreated fibre and disappeared in treated fibres and CNC-B. The peaks at 1506 cm^{-1} due to C=O stretching of aromatic ring of lignin become slightly less intense after alkali treatment and bleaching and completely disappeared in CNC-B [42,43]. The peaks at 1640 cm^{-1} in untreated fibres reflecting the bending vibration of absorbed moisture becomes less intense after each treatment indicating that water uptake is reduced [42]. The most significant absorption band at 1035 cm^{-1} is found to be continuously increased from untreated fibres to CNC-B, indicative of an improvement in cellulose and surface area [33].

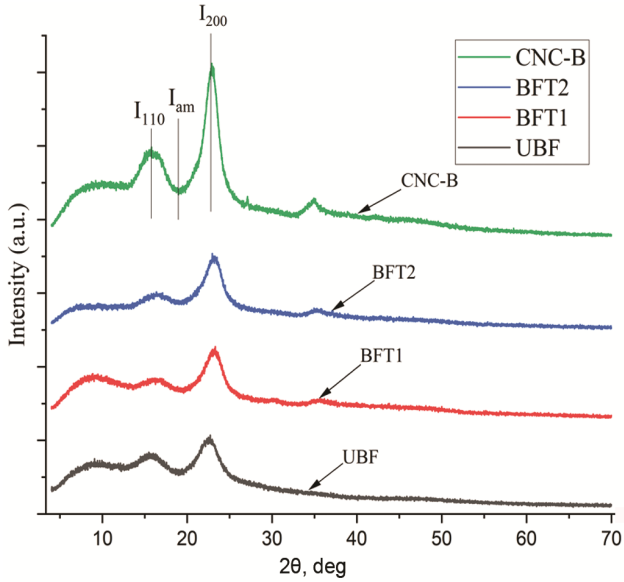

Fig. 5 — XRD results of banana fibres and cellulose nano-crystal

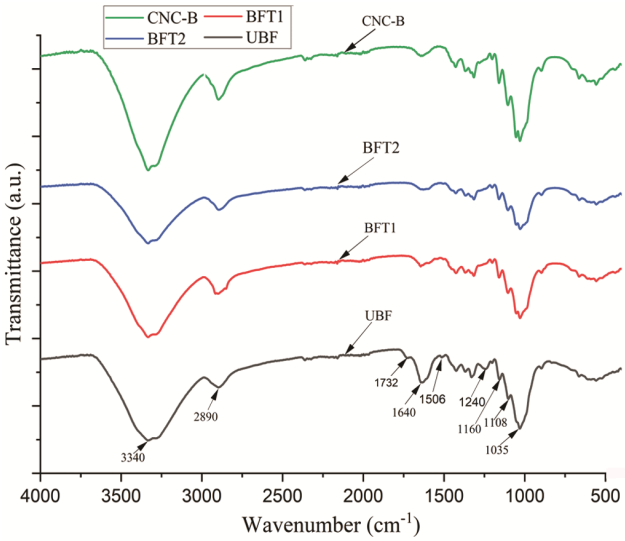

Fig. 6 — FTIR spectra of banana fibres and cellulose nano-crystal

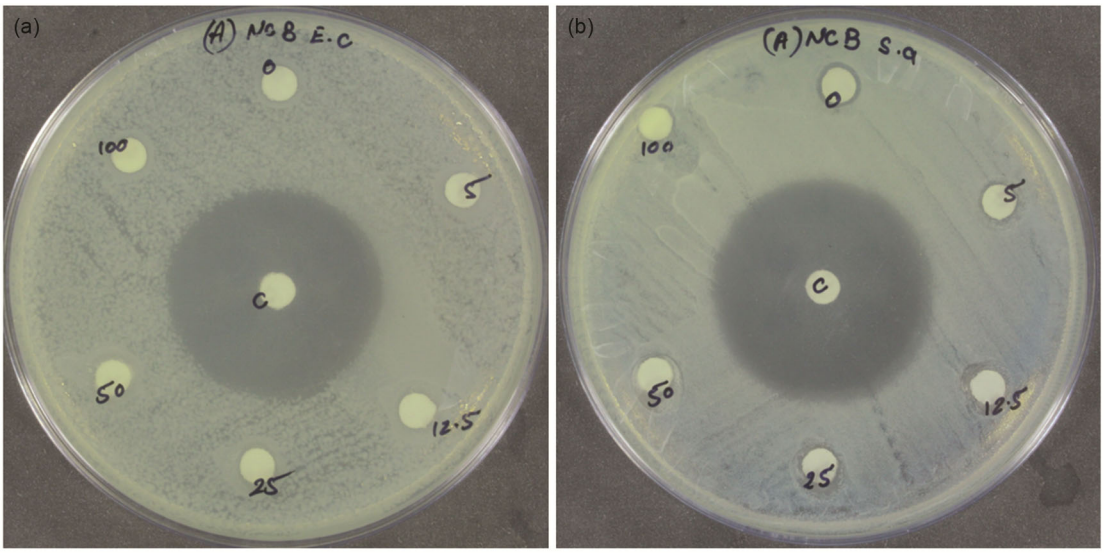

Fig. 7 — Antibacterial analysis of banana cellulose nano-crystal against bacteria (a) *E. coli* and (b) *S. aureus*

3.7 Antibacterial Analysis

For antibacterial analysis, two different Mueller Hinton Agar (MHA) plates are spread with 100 µL of log cultures of *E.coli* and *S.aureus* (adjusted to 0.5 McFarland Unit), and the MHA plate is spread with culture plate bacteria followed by placing the discs containing 10 µL of different dilutions concentration of CNC-B (0 - 100 mg/mL). Each disc is loaded with 10 µL of solvent so that extract could diffuse to the medium. One disc is loaded with solvent alone, which serves as vehicle control, and the ciprofloxacin disc (20 µg) is taken as a positive control. The plates of *E.coli* and *S.aureus* are then incubated at 37°C for 24 h, and clear zones created around the discs are measured and recorded. In Figs 7(a) and (b), vehicle control is shown with 0, 5, 12.5,25, 50 and 100, which represent the concentration of CNC-B in mg/mL on ciprofloxacin disc, and C shows the positive control.

The antibacterial response induced by nanocellulose against *E. coli* and *S. aureus* is investigated through the disk diffusion method, in which if an antibacterial agent can diffuse into agar, the inhibition zone becomes evident as a halo around the impregnated disc. The antibacterial activity is investigated by studying the bacterial growth on the agar plates in the presence of CNC-B. It is investigated after 24 h so that the paper disc impregnated with CNC-B does not reduce the growth of the bacteria, as demonstrated by the absence of a halo of growth inhibition around the paper disc. The CNC-B does not show any antibacterial properties because of the complete removal of lignin. The phenolic structure and biocidal activity of lignin are mainly responsible to reduce antibacterial properties of nanocellulose. A similar antibacterial result is reported by Valencia *et al.* [43], that without residual lignin, nanocellulose could not decrease the growth of any bacterial strains.

4 Conclusion

Nanocellulose has been successfully extracted from banana fibres by chemical treatments assisted by the steam explosion followed by chemical treatments and mechanical grinding. The present facile approach for the isolation of nanocellulose is found to be suitable to reduce the extraction process time and chemical requirements considerably. A lower cellulose yield of around 39 % is reported in the present study. TEM and DLS results confirm the diameter of nanocellulose in the nano range. The FESEM micrographs show the changes in the surface after the process of treatment. FTIR results reveal a significant change in chemical structure, and elimination of hemicellulose and lignin in treated fibres and CNC-B. The crystallinity index of nanocellulose reaches 71.42% from 50.82% after treatment, which is confirmed by XRD analysis. CNC-B does not show any antibacterial response because of the complete removal of lignin.

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