



**PRODUCTION OF CELLULOSE NANOFIBERS USING A NOVEL SUBSTRATE:
SOLANUM TUBEROSUM**

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Article Received on 14/12/2016

Article Revised on 05/01/2017

Article Accepted on 26/01/2017

ABSTRACT

Cellulose is the most abundant polysaccharide on earth and is an integral part of the cell wall of plants. In majority of plants this cellulose is present in a combination of hemicellulose, lignin and pectin. This property of plants can be exploited to gain a biotechnologically useful product which is Cellulose nanofibers. This paper mainly deals with the production of cellulose nanofibers from potato peels (*Solanum tuberosum*), using a chemical approach, where other non-cellulosic components are broken down to obtain pure cellulose, also their characterization using sophisticated technologies like FTIR, XRD, SEM and TEM analysis is discussed. Characterisation methods confirmed the formation of cellulose nanofibers in the range of 15 nm to 58 nm. Furthermore, this paper also discusses the future scope of Cellulose nanofibers obtained using potato peels and also suggests areas concerning applications which are yet to be touched upon.

KEYWORDS: *Cellulose nanofibers, FTIR, Biotechnology, Solanum tuberosum peels.*

[1] INTRODUCTION

Improper waste disposal is one of the many problem we face in our daily life. The vegetable markets contribute to the organic matter of the waste generated and dumped on the streets. There is a need to dispose this waste. Thus, instead of clearing the waste and dumping it for further treatment, we thought of an innovative method of converting vegetable waste into a useful application. Thus cellulose nanofibers come into the picture. As discussed earlier, cellulose is an integral part of the structure of plant cell. Exploiting this property will help us reduce the other components like hemicellulose and lignin to obtain pure cellulose and then subjecting it to mechanical treatment to reduce it to nanoscale. Further, the cellulose nanofibers can be formed into a sheet or fiber. These characteristics of cellulose nanofibers are expected to be of help in developing novel and highly functional products, highly functional packaging materials, transparent organic substrate materials, highly functional fibers, separation membranes, and regenerative biomaterials.

The field of nanotechnology is one of the upcoming areas of research in the modern field of material science. A lot of research has been done already for producing nanofibers but these nanofibers aren't eco-friendly and thus end up polluting the ecosystem much more than the original waste. Thus, cellulose nanofibers being water-dispersible and biodegradable, offer a better alternative to the previously formed nanofibers, while serving as a

unique and innovative method for efficient waste disposal technique.

Diana Fasce et al (2011) reported the use of multiple-step procedures to obtain valuable sub-products, including nanocellulose, from rice husk. Each sub-product was characterized after every step by analyzing the chemical composition. The results clearly showed that the selected procedure gave the possibility to separate silica in the first step and then to purify the resultant material, leading to nanocellulose production.^[1] Norrihan et al (2008) reported two different procedures for the isolation of cellulose from sugarcane bagasse and corn cob. These were the acetic acid - nitric acid mixture and the delignification with acidified sodium chlorite. The cellulose fibers and cellulose nanocrystals were characterized using FT-IR spectroscopy, CHN analyzer, Nikon optical microscope and Scanning Electron Microscope.^[2] Shinsuke Ifuku et al (2011) reported that cellulose nanofibers were isolated from parenchyma cells of pears and apples by one-time fibrillation using a grinding apparatus after the removal of matrix substances. The fibrillated cellulose samples were observed by field emission scanning electron microscopy, and were found to have a fine nano-structure. The fabricated nanofibers were characterized by FT-IR spectra and X-ray diffraction profiles, which showed that cellulose nanofibers were extracted.^[3] Omid Ramezani et al (2012) showed that bleached soda sugarcane bagasse pulp was used for nanocrystalline

cellulose production. Isolation and purification of cellulose was experimented by a post-bleaching stage under reflux using a mixture of Nitric acid and Ethanol. Cellulose crystallinity and degree of polymerization were measured by X-ray diffraction (XRD). Also, ultrasonication increased cellulose crystallinity value.^[4]

Velayudham Navaneetha Krishnan *et al* (2013) demonstrated that cellulose nanofibers can be isolated from coconut coir fibers by chemical treatment using alkaline and mineral acids and inorganic salts, followed by mechanical treatment and disintegration methods like sonication, cryo-crushing and dissolution. The size and morphology of cellulose nanofibers were investigated by using the Field Emission Scanning Electron Microscope (FESEM). FT-IR spectra confirmed the presence of hydroxyl groups, C-H bond and the C-O-C groups in the synthesized cellulose nanofibers.^[5]

Franciele Maria Pelissari *et al* (2013) showed that cellulose nanofibers were isolated from banana peel using a combination of chemical treatments, such as alkaline treatment, bleaching, and acid hydrolysis. Fourier transform infrared spectroscopy confirmed that alkaline treatment and bleaching removed most of the hemicellulose and lignin components present in the banana fibers.^[6] Rumpoko Wicaksono *et al* (2013) reported that cellulose nanofibers were made from cassava baggase, which is a solid by-product of cassava starch, by following chemical treatment to obtain only cellulose followed by mechanical treatment to obtain its nanofibers. The characterization of cellulose nanofibers was done by methods such as Scanning electron microscopy (SEM), Zeta potential measurement, Fourier transform infrared (FTIR) spectroscopy, X-ray Diffraction (XRD).^[7]

Akihiro Hiden *et al* (2014) reported the preparation and characterization of nanofibers consisting mainly of cellulose microfibrils from orange peel (OP), which is a significant byproduct of orange juice production. Three treatments (boiling, alkaline, and pectinase) were investigated with and without subsequent grinding treatment. It was possible to prepare the cellulose nanofibers (CNFs) using these methods, except for the boiling treatment with grinding. Interestingly, only pectinase and a mild-physical blender treatment without grinding produced nanofibers.^[8]

Siti Norfatimah Fauzee *et al* (2013) showed that cellulose was extracted by alkali and acid protocols from nypa fruit husk, the agricultural waste in order to produce nanofiber. In acid protocol, the pretreated sample was hydrolyzed with 10 % and 17% sodium hydroxide (NaOH), and then refluxed using a mixture of sodium chlorite (NaClO₂) and acetic acid (CH₃COOH). However, since the cellulose from the alkali protocol is pure, fibrillated and with low crystallinity, it was chosen for dissolution to be electrospun into nanofiber.^[9]

Shou Hiasa *et al* (2014) demonstrated that cellulose obtained from mandarin (Citrus unshiu) peel waste was purified by the removal of oil, coloring substances, and pectin. Two pectin-removing methods, namely multistep and hydrothermal treatments, were investigated to compare their purification efficiencies. The multistep treatment consisted of three steps: removal of metal in pectin, depolymerization, and dissolution of pectin. After the pectin removal, the purified cellulose from the mandarin peel waste was fibrillated by sonication to obtain cellulose nanofibrils, yielding cellulose fibers with widths of 2–3 nm, as observed by atomic force microscopy.^[10]

Table 1. Different vegetable/fruit waste used for synthesis of cellulose nanofibers

VEGETABLE/FRUIT WASTE	REFERENCES
Rice Husk	[1]
Sugarcane Baggase	[2][4][19]
Corn Cob	[2]
Coconut coir fibres	[5]
Banana peels	[6]
Cassava Baggase	[7]
Orange peel	[8]
Nypa fruit husk	[9]
Mandarin peel	[10]

[2] MATERIALS AND METHODS

2.1. Materials: The potato peels which were the substrate used for production of cellulose nanofibers were obtained from a local market in Navi Mumbai, India and was a waste residue of the commercial product. The chemicals used namely Potassium hydroxide, sodium hypochlorite, sulphuric acid and potassium metabisulphite were ordered from Chemco, India. The centrifugation machine, Incubators and homogenizer were present in the lab of our college in Navi Mumbai. FTIR analysis was carried out at the SIES Institute of

Chromatography, Navi Mumbai. SEM and TEM analysis was carried out at S.A.I.F, IIT Bombay, Mumbai.

2.2. Methods

Vegetable substrate of Potato (*Solanum tuberosum*) peels which were cleaned with pure distilled water were utilized for the production of cellulose nanofibers. These potato peels were then divided into two batches, one which was subjected to pre-treatment and the latter one without any pre-treatment. The first batch included peels which were treated with an antioxidant potassium

metabisulphite, $K_2S_2O_5$ solution (1% w/v), this concentration was just enough to prevent the oxidation of the peels without affecting the process of cellulose nanofiber formation. The concentration was decided after standardisation procedures.^[6] This treatment lasted for 24 hours and then the peels were dried in a hot air oven overnight at 90°C. This was then grinded in a mixer and was sieved using a 200 US mesh screen and then stored at 4°C.

2.2.1. Chemical Treatment: Both the batches of the potato peels were subjected to a chemical treatment which was a combination of alkaline hydrolysis, bleaching and acid hydrolysis. Initially both the batches were subjected to hydrolysis of KOH of concentration varying from 4% to 5%^{[11], [12], [7] and [6]} for 1 hour at a temperature of 80°C in the hot air oven. This alkaline hydrolysis helps in the removal of hemicellulose and lignin present in the sample. This was followed by a bleaching treatment using sodium hypochlorite ($NaClO_2$) (1%-5% w/v) whose pH was adjusted to 5.0 using a solution of 10% acetic acid. It was carried out at a temperature of 70°C for an hour and facilitated the removal of lignin from the peels. Another round of alkaline hydrolysis was carried out to remove the non-cellulosic components completely using KOH (same conditions as above). Finally it was subjected to acid hydrolysis using H_2SO_4 at 80°C for an hour in the hot air oven. This was carried out in order to remove the mineral traces from the sample and to obtain amorphous cellulose.^{[7][20]}

2.2.2. Mechanical treatment: After carrying out the chemical treatment, the residue was subjected to centrifugation at room temperature at 10000 rpm for 5 minutes. Formation of a clear pellet was observed which was then subjected to homogenization. The significance of mechanical treatment is that it is used to downsize the cellulose microfibrils obtained to a Nanoscale. Thus, this step is crucial for obtaining a fixed diameter of

nanofibers. After carrying out homogenization for 20 minutes, the sample was dried overnight in the hot air oven at a temperature of 100°C. At the end of this step, we obtained cellulose nanofibers with diameters ranging from 15 nm to 58 nm.

2.2.3. Standardisation: In order to determine the maximum cellulose concentration, i.e. the most effective method of producing cellulose nanofibers, Anthrone test was carried out on the sample.^[13] The concentration of KOH was varied from 4% to 5% and also that of $NaClO_2$ from 1% to 5%. The standard graph was plotted *After carrying out the Anthrone test according to the protocol mentioned by Hedge and Hofreiter.^[13]

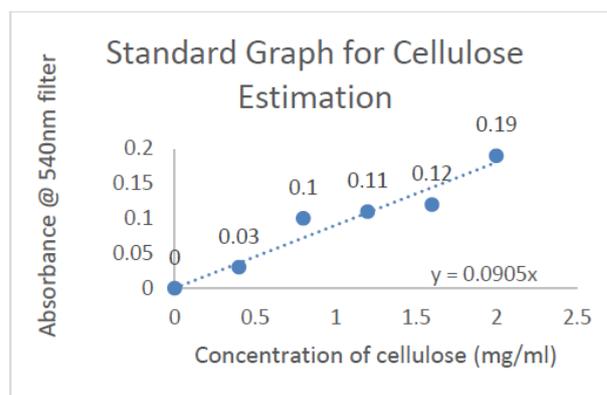


Fig.1. Standard graph for cellulose estimation.

[3.] RESULTS AND DISCUSSION

3.1 Anthrone test

Anthrone test was carried out by the protocol given by Hedge and Hofreiter^[13] and the results are given in Fig 2 and 3 resp. for without pre-treatment and with pre-treatment resp. As discussed earlier, it was concluded that there was maximum concentration of Cellulose at 4% of KOH and 5% of $NaClO_2$ (with pre-treatment).

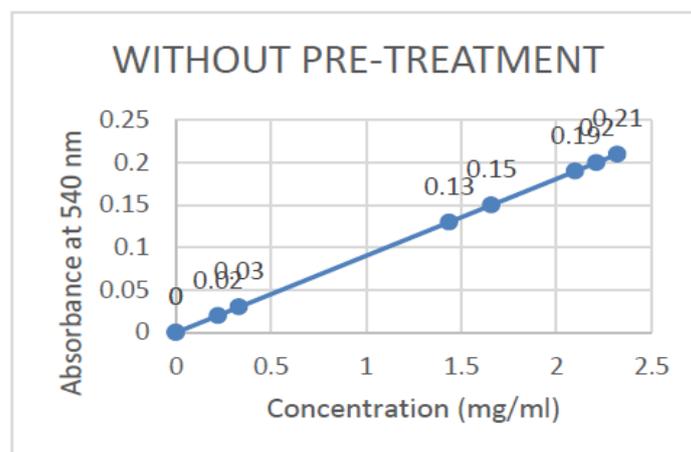


FIG.2. Readings for cellulose content without pre-treatment.

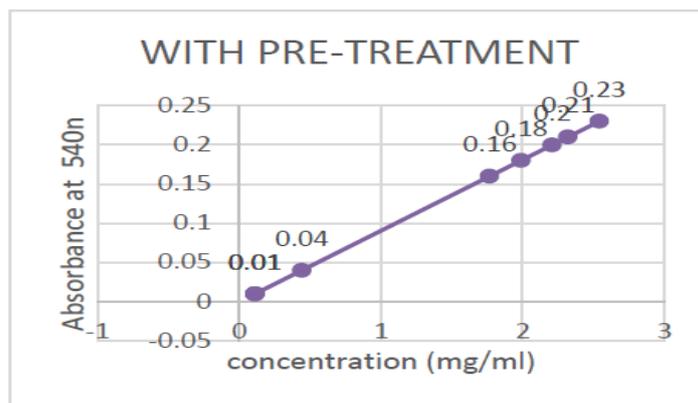


FIG.3.: Readings for chemical treatment with pre-treatment.

3.2. FTIR ANALYSIS

On comparing the sample of potato peels with the standard cellulose FTIR results, we found many similar peaks. Also sharper peaks were observed in the region of 500 cm^{-1} to 1500 cm^{-1} wavenumber range i.e. our region of interest. Sharper Peak at 900 cm^{-1} suggests increase in cellulosic content after treatment. Peak at 1370 cm^{-1} confirms that it was cellulose as it represents the $\text{CH}_2\text{-CH}$ groups of cellulose.^[7] Absence of peaks at

1740 cm^{-1} and 1246 cm^{-1} suggest removal of hemicellulose and lignin as the peak at 1740 cm^{-1} correspond to the C=O stretching of the acetyl and uronic ester groups of hemicellulose, also the carbonyl ester linkage of the carboxylic groups of lignin and the latter peak is attributed to a C-O-C bond of the aryl-alkyl ether and the C=C stretching from the aromatic ring of lignin.^[14]

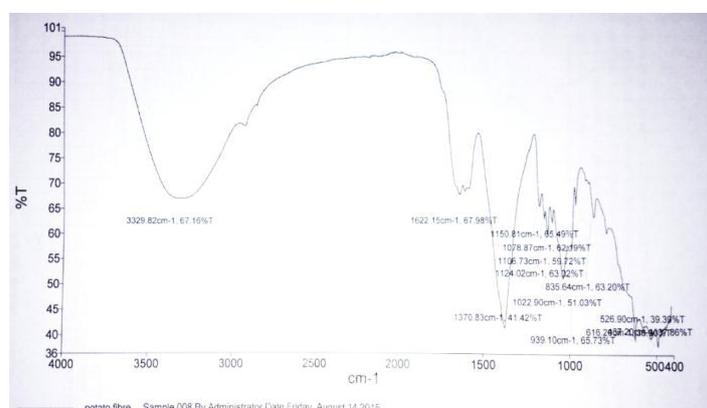


FIG.4. FTIR results of the sample.

3.3.X-RAY DIFFRACTION ANALYSIS.

X-ray diffraction (XRD) is an analytical technique used for phase identification of any crystalline substance. It provides information on unit cell dimensions, atomic and molecular crystal structure. The final sample obtained after homogenization was subjected to X-ray Diffraction. Instrumentation and parameters used for analysis of sample are given below On comparing XRD pattern of

sample with standard cellulose XRD pattern, we found many similarities. A sharp peak was obtained at $2\theta = 220$ which indicates presence of cellulose. Presence of narrow peak is indicative of crystalline nature of cellulose. Many peaks were obtained in the range of $2\theta = 300$ to $2\theta = 330$ which further confirm the presence of cellulose nanofibers.

Fig.5. Instrumentation and parameters details

Goniometer	TTRAX3 theta-theta gonio.(manual Ts)
Attachment	Standard sample holder
Monochromater	Fixed Monochromator
ScanningMode	2Theta/Theta
ScanningType	Continuos Scanning
X-Ray	40kV/200mA
DivSlit	2/3 deg.
DivH.L.Slit	10mm
SctSlit	2/3 deg.
RecSlit	0.15mm

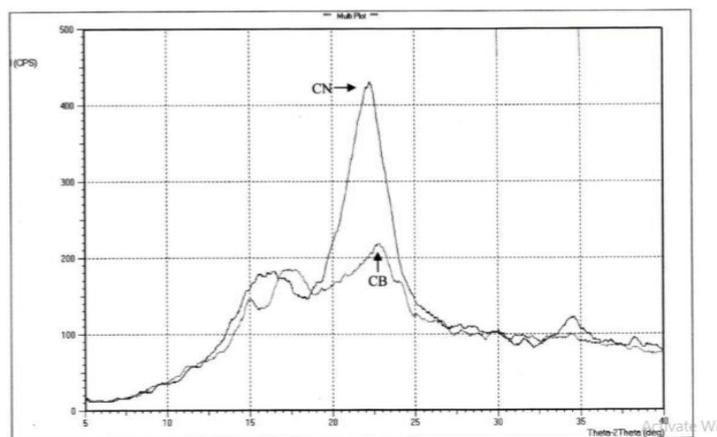


Fig 6. Standard results for XRD^[7]

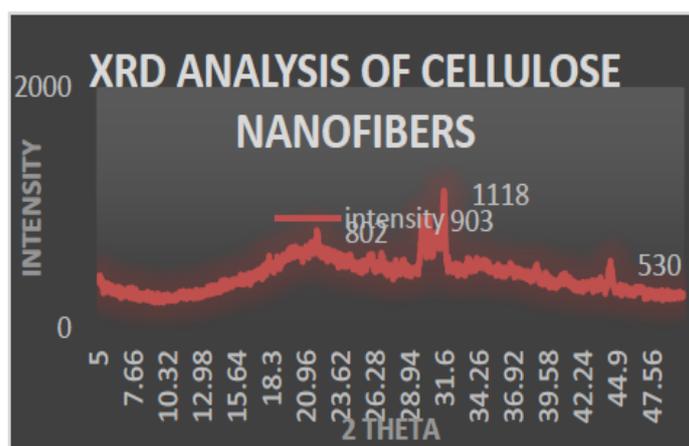


Fig.7. XRD analysis of the sample.

3.4. MICROSCOPIC ANALYSIS

The final powdered flakes obtained were then subjected to SEM and TEM analysis by dispersing it in distilled water followed by sonication for 20 minutes. For SEM analysis the nanofibre solution was fixed on an aluminium stub and coated with platinum layer to improve the conductivity and was visualized under a Field emission gun - scanning electron microscope (model no. JSM-7600F).

The morphology of the nanofibers and their range of diameter was investigated by TEM analysis, where the sonicated and dispersed sample was placed on a microgrid covered with a thin copper layer and dried for 30 minutes and then viewed under a transmission electron microscope (model no. FEI). The SEM analysis confirmed the formation of Cellulose nanofibers and the diameters were in the range of 16nm to 18nm. Micrographs from SEM analysis also displayed many clusters of nanofibers in the sample with small traces of salt particles.

TEM micrograms confirmed the formation of cellulose nanofibers from potato peels. The sample had a cluster of nanofiber fibrils with a wide range of diameters like 15.10 nm, 16.58 nm, 53.89 nm etc. These diameters confirmed that the chemical treatment successfully removed lignin and hemicellulose and other bonding non-cellulosic material. The diameter of the nanofibers obtained display a huge variation which might be due to the lack of a high pressure homogenization step. Nevertheless the diameter when compared to other sources is relatable, for e.g. Banana peels (10.9-22.6 nm)^[6], rice straw (12-35 nm)^[15] and wheat straw (15-35 nm)^[16], sugarcane bagasse (30 nm)^[17] and sugar beet (30-100 nm).^[18]

TEM micrographs of the suspension revealed that it was a cross network of many nano-sized fibrils. Certain places indicated a thick bundle of fibers which might be in the microscale, this might be due to the lack of high pressure during homogenisation.



Fig.8.SEM micrographs of cellulose nanofibers

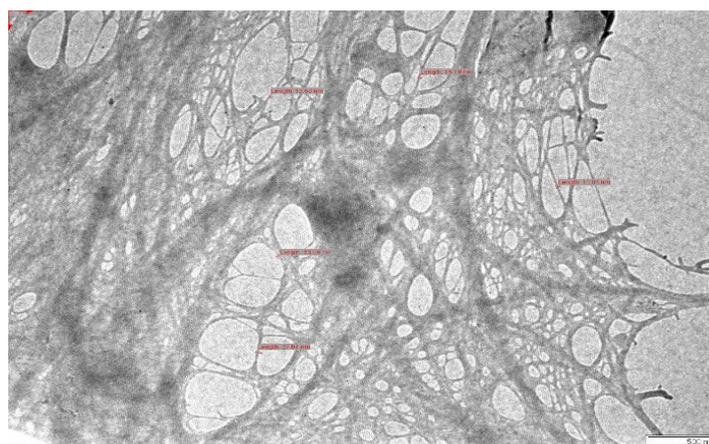


Fig.9.TEM micrograph of cellulose nanofiber.

[4] CONCLUSION AND FUTURE PROSPECTS

TEM and SEM micrographs confirmed the formation of cellulose nanofibers from potato peels. Through these experiments it was established that by pre-treating the substrate with potassium metabisulphite, there is a high concentration of cellulose obtained. The concentration of potassium hydroxide and sodium chlorite were optimized to get the maximum concentration of cellulose which was determined by Anthrone test and the concentration of cellulose was found to be maximum at 4% of potassium hydroxide and 3% of sodium chlorite. These samples were then subjected to different methods of characterization which were Fourier Transform Infrared Microscopy, X-ray Diffraction, Transmission Electron Microscopy and Scanning Electron Microscopy. FTIR results summed up that there was successful removal of other non-cellulosic components and that the sample contained pure cellulose albeit some salts. The diameters obtained from this treatment are in the range of 15 nm to 55 nm which might be in a slightly wide range. However this can call for an improvement in these steps and proper variation of the dependent variables in the experiment in order to obtain a diameter in a narrow range.

Cellulose nanofibers are widely used in biomedical applications. However, the purity of the cellulose

nanofiber obtained from potato waste is questionable if it is to be used for sensitive biomedical applications. The compatibility of these applications with living systems, also needs to be analysed in the form of lab tests, clinical trials etc. By further reducing the size of nanofibers, we can exploit its unique nanoscale properties.

ACKNOWLEDGEMENTS

We would like to express our greatest gratitude to Mrs. Neha Nohwar, our Project Guide, Assistant Professor, Department of Biotechnology, SIES Graduate School of Technology, Nerul for her inspiring, constructive and valuable suggestions throughout this work. Her able knowledge and expert supervision with unwavering patience fathered our work at every stage, without her warm affection and encouragement; the fulfilment of the task would have been very difficult. We express our sincere thanks to Mrs. Katyayini Tembadmani, (Head, Department of Biotechnology, SIES Graduate School of Technology, Nerul) for showing sustained interest and providing help throughout the period of work and for her active co-operation and sincere help.

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