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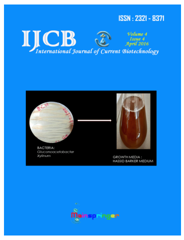

Cellulose Nanofibres: From Nature to Biotechnological Solution - Mini Review

Neha Nohwar, Sayali Upasham*, Shruthi Nair and Snehapriya Shyamprasad Vallabhajosyula

SIES Graduate School of Technology, Sri Chandrasekaendra Saraswathy Vidyapuram, Plot1-C D & E, Sector V, Nerul, Navi Mumbai.

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A B S T R A C T

Nanotechnology is the manipulation of matter at the nanoscale and thus imparting the molecules with unique properties restricted to the nanoscale. This paper discusses the various approaches of creating cellulose nanofibers namely a top-down and a bottom-up approach. Top down approach consists of several chemical or enzymatic methods. This employs chemical treatment using chemicals like KOH, $NAClO_2$, and H_2SO_4, followed by mechanical treatment using a homogenizer or using enzymes or enzymatic complexes, whereas a bottom up approach involves the utilization of a suitable substrate by a microorganism for the production of cellulose from the scratch and reducing its size to nanoscale to obtain nanofibres. Characterization of the samples with standard analytical biotechnological methods is also discussed like XRD, FTIR, SEM-TEM analysis. Lastly the paper deals with the application of the nanofibers in various fields. Environmental friendly source of aerogels, nano-paper, tissue engineering, uses in food packaging, polymer used as a filler to increase the strength of materials are some of the intriguing applications of nanofibers. Future prospects highlight the scope of further development in this field.

Introduction

Cellulose is considered to be the most abundant renewable polymer on Earth. This structural material is naturally organized as microfibrils linked together to form cellulose fibers. It is biosynthesized by a number of living organisms ranging from higher to lower plants, some amoebae, sea animals, bacteria and fungi. Cellulose consists of a linear homopolysaccharide composed of â-D-glucopyranose units linked together by â-1-4-linkages. Each monomer bears three hydroxyl groups. It is therefore obvious that these hydroxyl groups and their ability to form hydrogen bonds play a major role in directing the crystalline packing and also governing the physical properties of cellulose (Gilberto *et al.*, 2010). Both animate and inanimate matters contain nanostructures made of atoms, which - assembled and organised - are the building blocks of the environment. There are two basic approaches for creating nanostructures - bottom-up and top down. The bottom up method involves construction on a molecular scale from scratch using atoms, molecules and nanoparticles as building blocks. This method uses chemistry and physics derived technologies which are based on chemical synthesis or strictly controlled mineral growth. The top down method involves the disintegration of macroscopic material to a nanoscale by the following methods: mechanical (e.g. grinding), chemical (e.g., partial hydrolysis with acids or bases), enzymatic (e.g., treatment with enzymes hydrolyzing cellulose, hemicellulose, pectin and lignin) and physical (e.g. techniques using focused ion beams or high-power lasers)(Kazimierczak *et al*., 2012).

*Corresponding author.
Email address: upasham.sayali@gmail.com

Production of cellulose nanofibres

There are two approaches to produce cellulose nanofibres.

Top Down Approach

The top down approach deals mainly with the removal of non-cellulosic compounds to obtain pure cellulose and then mechanically treating it to get cellulose nanofibres. The treatment basically involves alkaline hydrolysis to remove pectin and lignin followed by bleaching to get rid of hemicellulose and lastly acid hydrolysis to remove mineral traces and to hydrolyze amorphous cellulose, providing the required nanofibers. Cellulose nanofibres obtained from organic waste or green waste exhibit great strength and have good optical properties. They can be obtained from a variety of substrates such as rice husk Fasce et al., 2011), sugarcane bagasse (Norrihan *et al.*, 2008, Ramezani *et al.*, 2012), corn cob (Norrihan *et al.*, 2008), banana peels Pelissari *et al.*, 2013), cassava bagasseWicaksono *et al*,2013), orange peel Hideno *et al.*, 2014), nypa fruit husk (Fauzee *et al.*, 2013), mandarin peels(Hiasa *et al.*, 2014), coconut coir fibres (Krishnan *et al.*, 2013) and many more.

The top down method also constitutes the enzymatic degradation for producing nanofibers. Kazimierczak *et al.*, 2012 worked on hemp (*Cannabis sativa* L.) using cellulolytic complex of â-glucanase, which was then subjected to mechanical disintegration using a homogenizer for reducing it to nano-scale Szczêsna-Antczak *et al.*, 2012 reported that there can be two ways in which enzymes could be incorporated in-order to produce nanofibers. The first approach deals with the usage of enzymes like pectinases, hemicellulose and ligninase to facilitate the removal of non-cellulosic materials. The second approach is with using industrial cellulases, which can breakdown cellulose into smaller structures.

There are several benefits in resorting to enzymatic hydrolysis than chemical treatment as longer nanofibrils with increased internal connections are obtained via the former method which increases its desirability to be used as a reinforcing agent in composites. If enzymatic treatment is used prior to homogenization, the energy spent on mechanical processes is much lesser which then reduces the production cost of nano-fiber

Bottom Up Approach

Some specific strains of bacteria have the ability to produce cellulose and it is called bacterial cellulose. Among the bacteria, one of the most advanced types of purple bacteria is the common vinegar bacterium, *Acetobacter*. This non-photosynthetic organism can procure glucose, sugar, glycerol or other organic substrates and convert them into pure cellulose. Unlike the cellulose from wood pulp, cellulose produced by an *Acetobacter* strain is devoid of other contaminating polysaccharides (Keshk,2014).

The strains of *Acetobacter*, to obtain pure cellulose used are listed in the table – 1. The consumption of various minerals were studied for the production of bacterial cellulose by *Acetobacter xylinum*. The treatments maintained under high agitation showed high consumption of minerals (Almeida *et al.,* 2013). Acetic acid, one of the common by-products during hydrolysis was shown to be beneficial for bacterial cellulose production and was found to be a promising substrate to improve xylose utilization (*Yang et al.,* 2014). Phenolic compounds, if present in the medium, should be non-inhibitory to the growth of micro-organism, however it was found that coniferyl aldehyde was the most potential inhibitor for the growth of *Gluconacetobacter xylinus* (Zhang *et al., 2*014). Also, the combination of production of bacterial cellulose and hydrolytic enzymes from fiber sludge was proven to be successful (Cavka *et al.*, 2013).

Characterization of cellulose nanofibres

Cellulose nanofibres can be identified using various approaches. A number of techniques are used for this purpose, including Scanning Electron Microscopy (SEM), TEM, Fourier Transmission Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD).

Scanning Electron Microscope

Scanning electron microscope (SEM) analysis is employed for characterization of size, shape & morphologies of the formed nanoparticle. SEM gives high-resolution images of the surface of a sample.

Fourier Transmission infrared spectroscopy-FTIR

FTIR is a chemical analytical method which measures infrared intensity versus wavelength or wave number of light. It is used to analyze the biomolecule and also the bonding interaction between them. IR spectroscopy also detects the vibrational characteristics of chemical functional groups of the sample.

X-Ray Diffraction

XRD is a technique used to study the phase composition, crystal structure, texture or orientation of the sample. The principle of XRD is that the X-rays are passed through a material and the pattern produced gives information on the size and shape of the unit cell.

Transmission Electron Microscope

The morphology and diameter of cellulose nanofibers can be investigated by Transmission Electron Microscopy.

Applications of cellulose nanofibres

Unique properties like very fine diameter, high strength and relative ease of production have enabled the use of nanofibers in fields ranging from biomedicine to food packaging.

Nanopaper

Ultralong cellulose nanofibers with extremely high aspect ratio manufactured from waste corrugated paper was used to produce nanopaper by filtration and oven drying the cellulose nanofiber suspension. The nanopaper presented high tensile properties, with a tensile strength of 135 MPa and a tensile modulus of 6.67 GPa. The obtained nanopaper also exhibited high transmittance of 85.2% at 600nm wavelength. The high performance nanopaper can be used as a strong sheet-like material or as a lightweight reinforcement phase in biocomposites. It appears to be an ideal candidate for substrates for transparent conductive films, metal writing, e-papers, solar cells and gas barrier films (Wang *et al.,* 2014).

Nano-reinforcements

Cellulose nano-reinforcements can be used to improve mechanical and barrier properties of biopolymers, whose performance is usually poor when compared to those of synthetic polymers. Nanocomposite edible films have been developed by adding cellulose nanofibers (CNF) in different concentrations (up to 36 g/100 g) as in the case of nano-reinforcement of mango puree based edible films. The addition of CNF was also an effective way to improve the water vapour barrier of the films. Its influence on glass transition temperature (t_g) was small but significant. The previous study further demonstrated that the properties of mango puree edible films can be significantly improved through CNF reinforcement (McHugh *et al.,* 2009).

Nanocomposites

Nanocomposites developed from cellulose nanofibers fabricated from softwood pulp can be used for biomedical applications. The nanomaterials were used as a substitute for ligament or tendon which could withstand the same or nearby stresses and had a higher mechanical strength as compared to synthetic polymers having the same function. Two types of composites were prepared: - 1) from cellulose nanofiber suspensions and 2) from cellulose nanofibers suspensions mixed with collagen fibres obtained from horse tendons. The characterization of the composites was done by Scanning Electron Microscopy (Mathew *et al.*, 2011).

Table - 1: Bacterial strains for the production of cellulose nanofibers.

MICRO-ORGANISM	REFERENCES
A. xylinum BRC 5	Hideno *et al., 2*014
G. hansenii PJK (KCTC 10505 BP)	Fauzee *et al*., 2013. Hiasa *et al*., 2014
Acetobacter sp. V6	Hwang *et al*.,1999
Acetobacter sp. A9	Jung *et al., 2*005
A. xylinum BPR2001	Park *et al., 2*003 Son *et al., 2*003
Acetobacter xylinum ssp. *sucrofermentans* BPR2001	Son *et al., 2*001
G. xylinus strain (K3)	Bae *et al., 2*004
Acetobacter xylinum NUST4.1	Chao *et al., 2000*
Gluconacetobacter xylinus IFO 13773	Bae *et al., 2*004

Figure - 1: Flowchart of Chemical Treatment

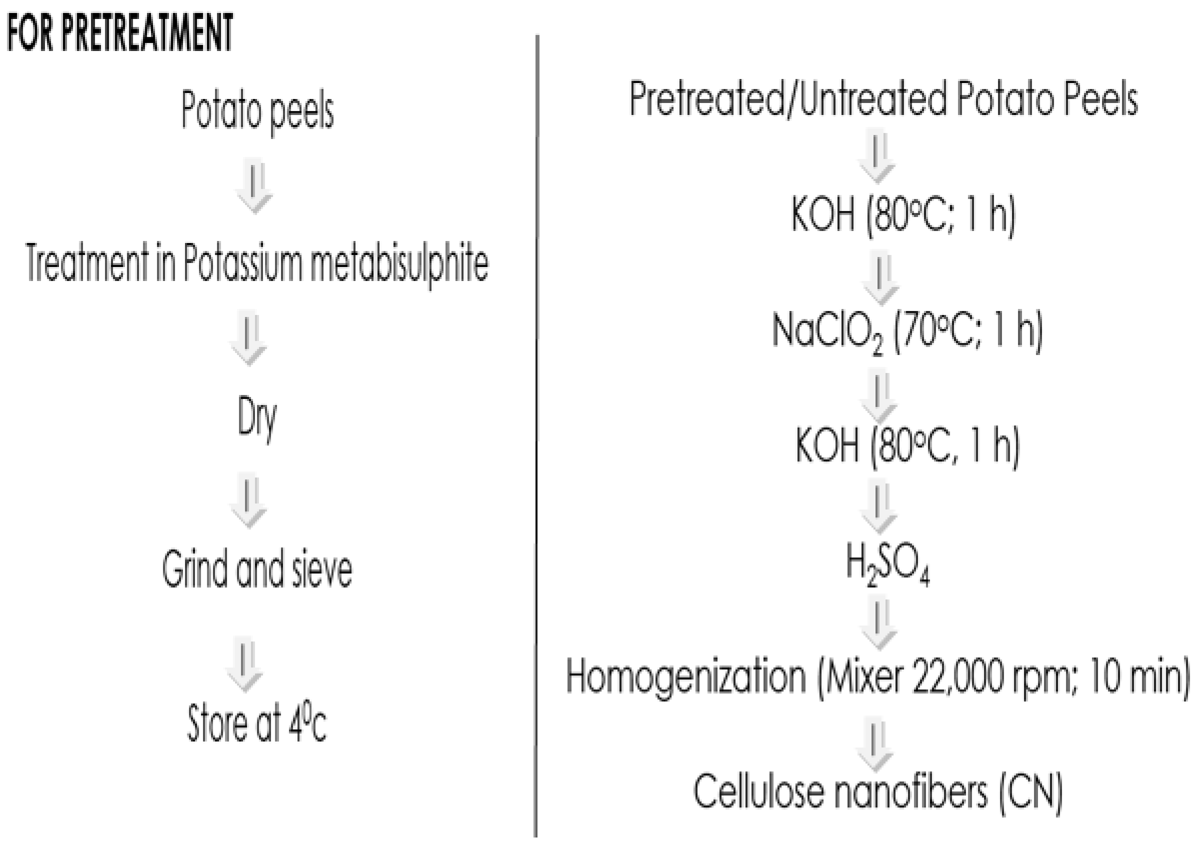


Figure - *2*: Synthesizing Bacterial Cellulose

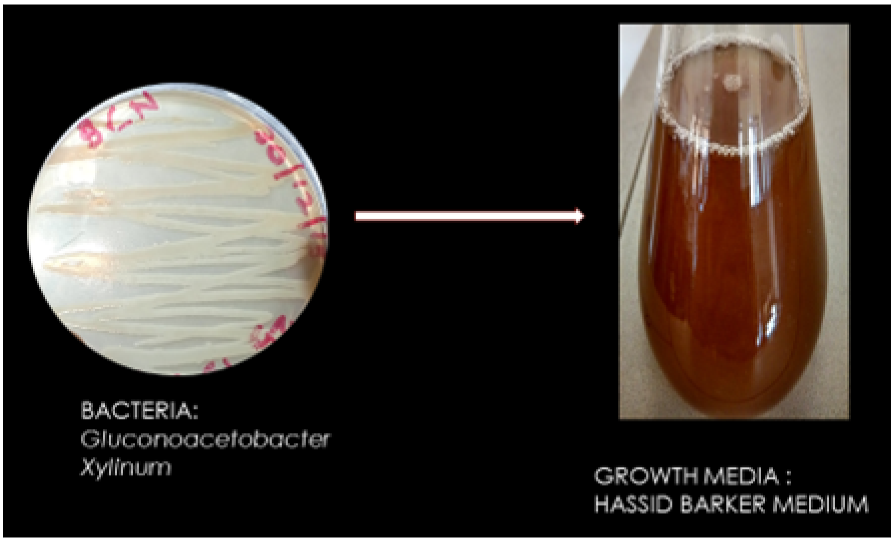
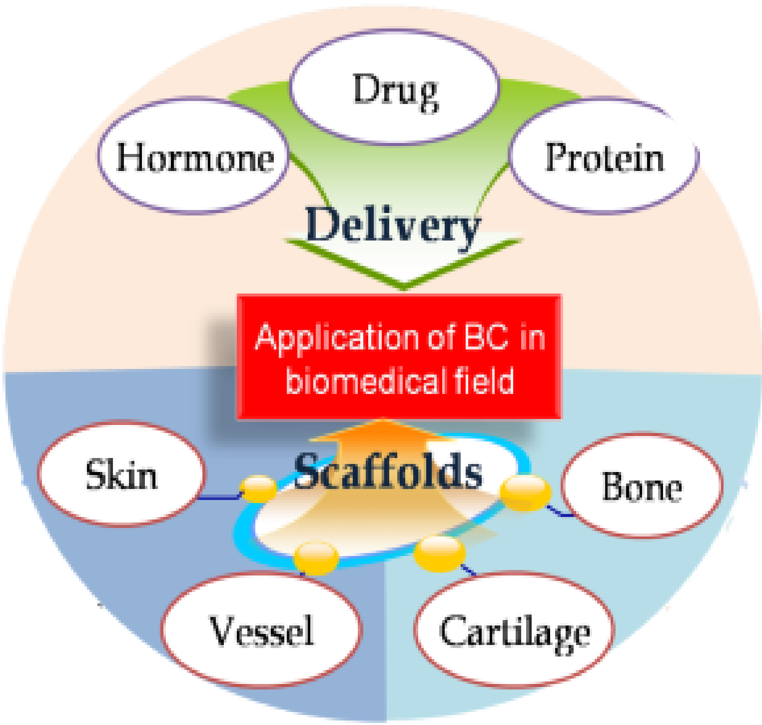


Figure – 3: Biomedical Applications of Bacterial Cellulose-based Biomaterials (Lina Fu *et al., 2011*)



Nanofilms

Cellulose nanofibers can be made from cassava bagasse, which is a solid by-product of cassava starch by following chemical treatment to obtain only cellulose and mechanical treatment to obtain its nanofibers. Tapioca starch films were prepared using a film solution, cellulose nanofibers suspension and sorbitol as a plasticizer (Wicaksono *et al.*, 2013).

Bacterial Cellulose Gelatin Film

Nanocellulose-gelatin (bacterial cellulose gelatin (BCG)) film was produced by a supplement of gelatin, at a concentration of 1%–10% w/v, in a coconut-water medium under the static cultivation of *Acetobacter xylinum*. The two polymers exhibited a certain degree of miscibility. The BCG film displayed dense and uniform homogeneous structures. Incorporation of gelatin into a cellulose nanofiber network resulted in significantly improved optical transparency and water absorption capacity of the films. A significant drop in the mechanical strengths and a decrease in the porosity of the film were observed when the supplement of gelatin was more than 3% (w/v) (Taokaew *et al.*, 2013).

Conclusion and Future Prospects

Major breakthroughs have been made in the field of nanotechnology with the advent of cellulose nanofibers, which certainly need special attention as the future scope for them is endless. Nanofibers can be generated using chemical methods and enzymatic methods where the substrates are broken down into smaller fragments of pure cellulose while removing other non-cellulosic components. It can also be biosynthesized by *Gluconoacetobacter xylinum*, using a substrate which consists of high cellulose and low lignin content and which reduces the load of carrying out pre-treatment as it favours efficient bioconversion. The bacteria is provided a medium rich in glucose under both shaking followed by static fermentation.

The theory that *G. xylinum* cannot hydrolyse xylose is refuted by the research conducted by Cavka et al., 2013, furthermore they suggest that conditioning of hydrolysates and optimization of the cultivation conditions are likely to result in higher volumetric yields of nanocellulose. Another method to improve the amount of nanofiber generated is reduction in the concentration of phenolic aldehydes during pre-treatment, or employing detoxification for their removal. As lignocellulosic mass used as a substrate can contribute to increase in phenolics, it calls for an in-depth study in the removal of these compounds which play an inhibitory role on the growth of the bacteria, so that this economical and abundantly found substrate can be used to produce cellulose nanofibers. Ben-Hayyim and Ohad, 1965, displayed that the rate of cellulose synthesis was slightly increased by addition of Soluble Na-carboxymethylcellulose (CMC) to the system.

Findings in this paper suggested that cellulose aggregates formed during shaking or pellicles formed on the quiet surface of suspensions of cells comprised mainly of intertwined, randomly oriented fibrils forming a matrix in which trapped the bacterial cells. Characterization procedures followed comprised of field-emission scanning electron microscopy and X-ray diffraction analysis. Study for phenolics involved utilization of sophisticated and high end instruments like HPLC-UV-DAD (high-performance liquid chromatography equipped with a UV detector and a diode array and multiple wavelength detector) and fluorescence staining.

Nanotechnology holds major importance for developing high end biosensors, in tissue engineering as surgical tendon or ligament replacements, improved food packaging material, reinforcement agent or fillers for increasing the strength of materials. With a wide scope for applications and being derived from natural sources in a cost effective manner, Nanofibers offer to be a great biotechnological solution.

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