

Synthesis and Characterization of Cellulose Nanofibers from Wood Waste

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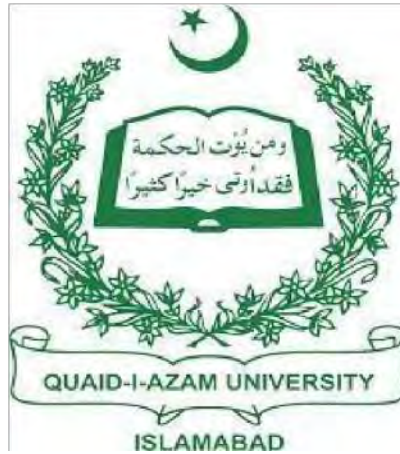
Quaid-I-Azam, University

Islamabad, Pakistan

2023

Synthesis and Characterization of Cellulose Nanofibers from Wood Waste

**A thesis submitted in the partial fulfillment of the requirements for the
degree of Master of Philosophy in biotechnology**



**BY
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2023



In the name of Allah, The Most Gracious, The Most Merciful.

DECLARATION

I hereby declare that the work presented in this thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of this thesis has been previously published or presented for any other degree or certificate.

Signature of Student

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Certificate of Approval

This is to certify that the research work presented in this thesis, entitled “**Synthesis and Characterization of Cellulose Nanofibers from Wood Waste**” was conducted by Miss Hifsa Zahid under the supervision of Dr. Faiza Rasheed.

No part of this thesis has been submitted anywhere else for any degree. This thesis is submitted to the **Department of Biotechnology, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad**, Pakistan in partial fulfillment of the requirements for the Degree of master’s in philosophy in the field of Biotechnology from the **Department of Biotechnology, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan**.

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Signature: _____

2. Supervisor

Signature: _____

3. Chairperson

Signature: _____

Dated:

DEDICATION

I dedicate my work to my supervisor and all my lab fellows and friends who helped me a lot and motivated me in the entire research work. Specifically, I dedicate this work to my loving and Respectable **Parents** because they supported me, motivated me, guided me, and remembered me always in their prayers.

Hifsa Zahid

ACKNOWLEDGEMENTS

Million gratitude to ALLAH Almighty, the most merciful and the most beneficial who granted us health and ability to seek knowledge and explore some of the many expect of His creation and bestowed us with the potential to bring this research work to its successful completion.

Countless mercy on the Holy Prophet Mohammad (S.A.W).

I am grateful to our Chairperson, Associate Professor Dr. Javeria Qazi for encouragement, cooperation, and affection for me during my studies. I am thankful to my supervisor Dr. Faiza Rasheed for her personal interest, valuable guidance, constructive criticism, encouragement, and continuous support during the whole span of my research work, her helping attitude, practical knowledge and patronizing concern provide me the facilities of center in proper, suitable, and helping manner.

I wish to express my sincere words of thanks to my beloved friends Afshan Abrar, Wajeeha abbasi, Iffat Irshad, Misbah Naeem, Maria Irshad, Yusra Azhar, Sidra, Maryam Mehmood, laiba Arshad, Anum Abbasi, Warda, shugufta abbasi for their attention, unceasing encouragement, friendly advice and continues support during my study. Additionally, I would love to express my deep feeling of my respect to my lab fellows Ibrahim khan, sara Fatima, Nashoba, Ayesha Asif, Sanam Gul Arshad, Muhammad Bilal Saeed, for their aspiring guidance and valuable suggestion during my lab experiments.

Hifsa Zahid

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LIST OF ABBREVIATION

Ab	Antibiotic
CMC	Cellulose microcrystal
CNC	Cellulose nanocrystal
CNF	Cellulose nanofibers
EU	Eucalyptus
FTIR	Fourier transform infrared
HNO ₃	Nitric acid
HCL	Hydrochloric acid
KBr	Potassium bromide
KOH	Potassium hydroxide
LCD	Liquid crystal displays
MFC	Microfibrilated cellulose
MFA	Microfibril angle
MS	Moderately sensitive
NaCl	Sodium chloride
PCFs	Purified cellulose fibers
PDI	Polydispersity index
PLA	Polylactic acid
PSD	Particle size distribution
SCB-I	Sugarcane bagasse (inner)
SCB-O	Sugarcane bagasse (outer)
Eu-CT	Chemical treated eucalyptus
ZS	Zeta sizer

ABSTRACT

Cellulose of wood waste i.e., Eucalyptus bark, and sugarcane bagasse were evaluated for the synthesis of cellulose nanofibers and the commercialization of many valuable products. The mechanical and chemical procedures for the conversion of cellulose to its nano sized forms were carried with specific protocols. The wood waste in the form of eucalyptus bark was put forwarded for the synthesis of nano cellulose fibers via chemical method by subjecting them with alkali treatment followed by oxidizing it with $\text{HNO}_3\text{-NaNO}_2$ mixture. The bark of eucalyptus converted to its powder form and further treated with strong alkali hydrolysis followed by strong acid treatment. Sugarcane bagasse was divided into two separate sections. i.e the outer and the inner part. Sugarcane bagasse and eucalyptus bark both were treated with benzene and ethanol with ratio of 2:1 respectively in Soxhlet apparatus to eliminate the existing wax content in the samples. Both were treated via these two methods for nanofibers extraction. Sodium chlorite and acetic acid were added in the dewaxed samples to leach out the lignin part of samples separately followed by thoroughly washing and filtration. Alkaline treatment of samples via the use of potassium hydroxide solutions to individualize hemicellulose, remaining starch content and pectin part. The resultant micro sized fibers were converted to nano sized by ultrasonication method and results were illustrated. The zeta sizer results of external part of sugarcane bagasse showed particle size distribution i.e., 151.1 ± 19.76 d.nm and 26.68 ± 2.33 d.nm and inner part showed 259.1 ± 68.4 d.nm. Particle size distribution 88.48 ± 5.20 d.nm and 132.5 ± 9.67 d.nm showed by bark sample via chemical and mechano-chemical method respectively. The zeta potential results of sugarcane bagasse reflected reliability and stability with values of -29.5 ± 7.31 mV and -15.3 ± 8.70 mV of outer and inner parts respectively. Eucalyptus bark zeta potential exhibited -29.3 ± 9.45 mV and -32.5 ± 9.56 mV via both methods. All the samples revealed antimicrobial activity against bacillus subtilis, pseudomonas aeruginosa and klebsiella pneumonia with chloramphenicol C30 being used as positive control. The nanofibers synthesized could be subjected for commercialization, synthesis of novel items and further implementation in pharmaceutical and food industries.

CHAPTER 1

INTRODUCTION

Cellulose, the most ubiquitous biopolymer as well as a major component of plant cell walls. Algae, fungus, bacteria, and some marine animals are some other living species that also contain it. Cellulose is a D-anhydro glucopyranose unit-based linear homopolymer connected by beta 14 glycosidic linkages. This natural polymer's repeating unit called cellobiose, is a dimer of glucose, unlike the glucose in other glucan polymers. These layered sequential glucose units accumulate to give rise the solid microfibrils. Intermolecular forces, especially the hydrogen forces are found in high number in cellulose because of the hydroxyl groups locating on the rings of the glucose. Cellulose can be treated chemically to obtain fibers on nanoscale. Various isolation techniques can be used to recover nanoscale cellulose fibers from a variety of cellulose materials, which can then be used to generate synthetic polymers or biomaterials.

All those fibers, where cross sectional structure or the diameter is less than 100 nm in width come into the category of nanofibers. Nanofiber research and development have been heavily supported in research field due to their extraordinarily properties like the surface area of nanofibers are much high and often drastically different properties from those of other bulk materials [1-4]. To thoroughly study how to utilize and the fundamental work on nanofibers in this decade has been pushed by the researchers for the development of reusable and sustainable products.

Amongst the crystalline structured polymers called cellulose builds up to become the most prevalent biopolymer found mostly in wood biomass. Hemicellulose and lignin are included in the architecture of the distinctive graded conformation of cellulose. Both the original cellulose fibers and chemically and mechanically altered celluloses have long been used in a variety of applications, from basic materials like as a raw material for paper and board and textile industries to more complexed products like in the production of liquid crystal displays (LCD) used in electronics and electrical devices, use in the dialysis machines, food industries and having medical applications.

Micro-fibrillated celluloses (MFC) and paper industry have both used partially or significantly fibrillated cellulose fibers as beaten pulps. MFC has been created at the industrial level from wood pulp/water slurries using a series of high-pressure homogenization treatments [5–6] and has been applied as a thickener and filter aid. The partial cleavage of numerous inter-fibrillar hydrogen bonds required for nanofibrillation of wood and other plant celluloses typically

results in high energy consumption, and complete individualization of wood cellulose fibers into 3–4 nm wide fibril elements without damage has not yet been accomplished. It has been discovered that mechanical fibrillation of wood cellulose/water slurries by grinder treatment is more successful in terms of nanofibrillation and energy consumption [7]. By using a grinder, wood cellulose fibers for papermaking have been reduced to 20–100 nm diameter nanofibers [8–9]. In addition to wood cellulose fibers, the usage of agricultural residues and its leftovers has also been investigated as a raw material and supplier for the purpose of doing nanofibrillation [10]. In depth laboratory experiments, the cellulose microfibrils were treated with some charged or ionic functional groups specifically to the surfaces to create high electrostatic repulsion between them in water. Treating the fibers with sulphuric acid at 45 °C for 1-4h is a historically noteworthy approach to add substituents to the exterior of the fibrils [11–12]. Cellulose nanocrystals are produced by successive mechanically disintegrating the leftovers which are treated with acids in addition to water using a device use for bringing uniformity in solution like sonicator or ultrasonic homogenizer. Wood celluloses can be converted into cellulose nanocrystals or nano whiskers that are 5–10 nm broad and 50–200 nm long, with a portion of them forming spindle-like bundles, by subjecting them to acid hydrolysis followed by the process of sonication. Weight recovery percentages, however, are poor, about 30–50% [12]. When applying high pressure homogenizer and refiner treatments to conduct nanofibrillation operations in water, the pre-treatment of cellulose using the enzyme i.e., cellulase and the process of carboxymethylation for treating wood celluloses, expenditure of energy consumption was decreased by many folds. [13–14]. The partly anionic groups grafted nanocellulose appears to disperse in water after grafting acrylonitrile onto wood celluloses and subsequent mechanical treatment [15]. Recent reviews on the subject have been published. Microfibrils treated with enzymes or the use of chemicals to modify the microfibrils of cellulose, and the resulting nanocrystals treated with different strong acids are investigated principally for recruiting as filler compounds/material in the synthesis of nanocomposites and other electro-optical films [16-17]. Plant cellulose microfibrils are bio-based nanofibers that were initially recognized as a major compound present in the cell walls (outermost layer of plant cell) of almost all plants. These microfibrils have consistent diameter and thickness, high crystalline structures. However, because of the presence of multiple hydrogen bonds/connections between the cellulose microfibrils conformations within the cellulose fibers, to separate individualized cellulose microfibrils included in plant cellulose fibers without any

damage to its structures become a challenging task as this is laborious and causes a significant production loss.

A contemporary global objective is the creation of more sustainable processes for a future that is greener and more biobased. To address environmental concerns and lessen the existing reliance on fossil fuels, research has been done to develop bio-based polymers. Petroleum-based polymers were produced in 299 million tons in 2013; the average annual growth rate is about 4%, and the demand is still rising [18].

LITERATURE REVIEW

Creating biopolymer-based materials from renewable resources is an emerging research field that is getting more and more interest from the scientific and business communities [19-20]. The annual share of bio-based materials in total polymer synthesis has increased more quickly than total output. The ability to produce bio-based polymers, which was 3.5 million tons in 2011, is predicted to increase to roughly 12 million tons by 2020 [21]. To create the initial generation of bio-based polymers, lignocellulosic biomass (starch and cellulose), fatty acids, and organic waste had to be used as building blocks (monomers). The production of lignocelluloses, the most plentiful source of monomers, accounts for 210.7 10⁶ tons of plant material annually [22] food supplies are not in conflict with it now. Along with lignin and hemicellulose, cellulose makes up 35–50% of the biomass that makes up lignocellulosic biomass [23]. Due to its availability and environmentally beneficial characteristics, including renewability, biocompatibility, and biodegradability, it offers the best option for replacing polymers made from petroleum.

1.1. Cellulose as basic feedstock for monomers production

Cellulose is a viable material for the manufacture of compounds and monomers. Through practical catalytic procedures, a large range of monomers are now produced from cellulose, and these have the potential to be used in the synthesis of biopolymers for use in a variety of sectors. In recent decades, the creation of organic, biodegradable polymeric materials has progressed a lot. [24-25]. Environmentally friendly composites and other biobased polymers or specifically cellulose-based materials has been subjected in the current world [26]. Since cellulose, nanoparticles synthesized from cellulose, and other compound products mainly derived from cellulose have some properties, like reinforcing abilities, light in weight, ability to degrade so imposing no environmental pressure, and are widely available everywhere, these are some phenomenal properties which make this biopolymer more suitable to be used in research. They can also be utilized in polymer bio (nano) composites as matrices or (nano) fillers. However,

the energy-intensive processing of lignocelluloses to remove them from lignin is necessary for the manufacture of biopolymers from cellulose, and the following production of biopolymers necessitates disintegration, chemical modification, or nanoscale synthesis.

1.2. Cellulose based biopolymers methods

There are now some basic methods for producing cellulose-based polymers. Depending on the type of cellulose being utilized, the best approach must be chosen. These strategies have been applied in a variety of biopolymeric material synthesis methods. Unaltered cellulose fibers chemically altered cellulose, such as cellulose derivatives, and nanocellulose have all undergone processing to produce a wide variety of biodegradable polymeric materials, including films, composites, nanocomposite systems, and composites films for use in a variety of applications. Sustainable biopolymers and useful polymeric materials have both been made using cellulose monomers. Reviews have occasionally discussed the usage of cellulose in biopolymeric applications. While purposefully avoiding a discussion of the classification and overview of all conceivable cellulose-based biopolymers currently. The advent of such sustainable projects would increase commercial and business interest in cellulose as a potential bio - based supplier and aid in the identification of the most suitable products in this domain. Such prospective approaches will promote the use of cellulosic functional polymeric materials for a variety of applications and the substitution of current petroleum-based polymers with sustainable biopolymers to satisfy the needs of a sustainable society.

1.3. Different ways of forming cellulose-based polymers and their history

- Cellulose deconstruction monomer-based polymers
- Cellulose's fiber and its derivatives occurring naturally
- Nanocellulose-based polymers

Cellulose-based polymeric materials have been utilized for a very long period in many different industries. Cellulose was initially transformed into a regenerated form using the long-known viscose process for making cellophane and rayon, which involves derivatization without depolymerization. Derivatization was later abandoned in favour of the cuoxam and lyocell techniques for creating cellulose polymeric goods. As an alternative, cellulose can be depolymerized via catalysis to yield its monomeric units i.e., glucose sugar, which serves as the raw material for the manufacture of a large range of monomers through enzyme catalysis or converting into other products via biotechnological procedures. Many attempts were made to synthesize more sustainable other cellulose-based polymers from the processing of these monomers or their derivatives [27]. In the 19th century, thermoplastic bulk materials made from

cellulose derivatives had first been commercialized. Regenerated cellulose-based bioplastic sheets were created using the conventional viscose technique, which produces dangerous byproducts (CS₂ and H₂S). As a result of chemical modification of cellulose, derivative cellulose-based polymers have also been employed in a variety of applications (e.g., films synthesis and for production of bio coatings). The primary industrially used cellulose derivatives include cellulose derived acetate compounds and, cellulose derived esters for the processes like moulding, extrusion of materials, and films synthesis, regenerated cellulose for fibers, and cellulose ethers, which find extensive use in building materials, food and food additives, infrastructural materials like paints, and pharmaceutical and medical uses [28]. But there are very rare solvent systems which make the cellulose possible to dissolve homogeneously on commercial scale, the use of such polymer for biofilms synthesis which shows biodegradability is still challenging. [29–30]. Recently, functional bio composites derived from these biopolymers and composite films, gain much appreciation in industry, along with novel and improved polymeric thermoplastic film materials [31–32]. The third production technique gave rise to the subsequent synthesis of cellulose fibers, which are nanoscale in size. Nanocellulose was incorporated into several polymeric materials to create this generation of cellulosic biopolymeric products. The majority of the cellulosic biopolymeric materials in this generation are polymer composites and functional polymeric materials and composites. Due to their multiple uses in a variety of industries, these cellulosic biopolymeric materials are receiving more and more attention.

Since at least the middle of the 1980s [33] researchers from around the world have been studying the use of cellulose in bio-composite materials. However, their studies have only looked at the use of cellulose fibers and regenerated cellulose as reinforcing phases in polymer composites, such as polypropylene (PP) composites, where cellulose fibers have been used as the reinforcing phase [34]. Cellulose fibers, cellulose derivatives, and nanocellulose have recently been employed as matrix and fillers in composites and nanocomposites. Specific applications can be made for each of cellulose's functional roles in composites. The most significant and intriguing bio-composite materials are cellulose polymer composites, which have been developed because of research from the 1980s to the present. Furthermore, a great deal of cutting-edge functional polymeric materials based on cellulose have been produced. The addition of glycopolymers, obtained from either glucose monomer derived from cellulose or different cellulosic forms, which operate as matrices, has strengthened these polymer

composites. The synthesis of biopolymer-based materials derived from sustainable sources is an emerging field of study that is gaining more scientific and commercial interest [35-36].

1.4. Smart recycling/use of natural biopolymers

Cellulose and its reuse have been targeted in many publications currently as a major subject of study and research. The cellulose that is wasted worldwide in huge amount could be obtained and smartly used for the synthesis of many novel products like bio composites and as bio coating materials for the containers [37]. Among the wasted cellulose, about 80% of its part come from the household wastes, agricultural waste. In addition to this, the market trash and garden waste also contribute in to this 80% ratio [38]. Other cellulosic waste like the sawdust and other leftovers from wood making mills and factories, could be used as raw material for the processing of such biowaste and stands a principal resource and as an asset for bio growth and bio development. [39].

Most of the synthetic materials that human society used i.e., medicines, dyes, paints, and other industrial materials are all organic based i.e., large portion of these materials are derived from plants and trees. The biopolymer production worldwide is increasing and gaining more attention by the industrialists and researchers. By 2013 survey it was estimated that about 60 65% of the structure of total polymers, were biobased and in consistently elevating the graph [40]. Some reports presented the biobased synthetic materials reached to 12 million tons in 2020 which were about just 3.5 million tons in 2011 [40]. These numbers showed the exponential increase of biopolymers. Cellulose stands as a primary raw material from which many cellulose-based monomers and other chemically synthesized novel products could be obtained [41] [42]. Cellulose provides many exceptional characteristics like biodegradability, light in weight, enormously available in bulk amount everywhere and some other good mechanical

characteristics, making it more suitable candidate for the manufacturing of nano sized materials from it and other derivatives However, to obtain such products is not as easy as described some procedures and steps are so sophisticated and complexed that needs high practice and expertise to obtain better results. It's quite challenging for a researcher to optimize all the procedures and steps. As cellulose is a multilayered material so the laborious task amongst the whole procedure is to separate its layers i.e., separation of lignocellulose from lignin which needs chemical modification and treatment of the solution that contains cellulose.

1.5. Municipal solid waste with cellulosic waste as a main contributor

The cellulose in municipal solid waste is from (35-50%), with cellulose being its most important component structurally [43]. Hemicellulose [44] (20–35%) is the second largest structural fraction followed by lignin [45], which keeps third position in respect of ratio i.e.(15–25%) as main contributor in municipal waste that shown in Figure 1.1.

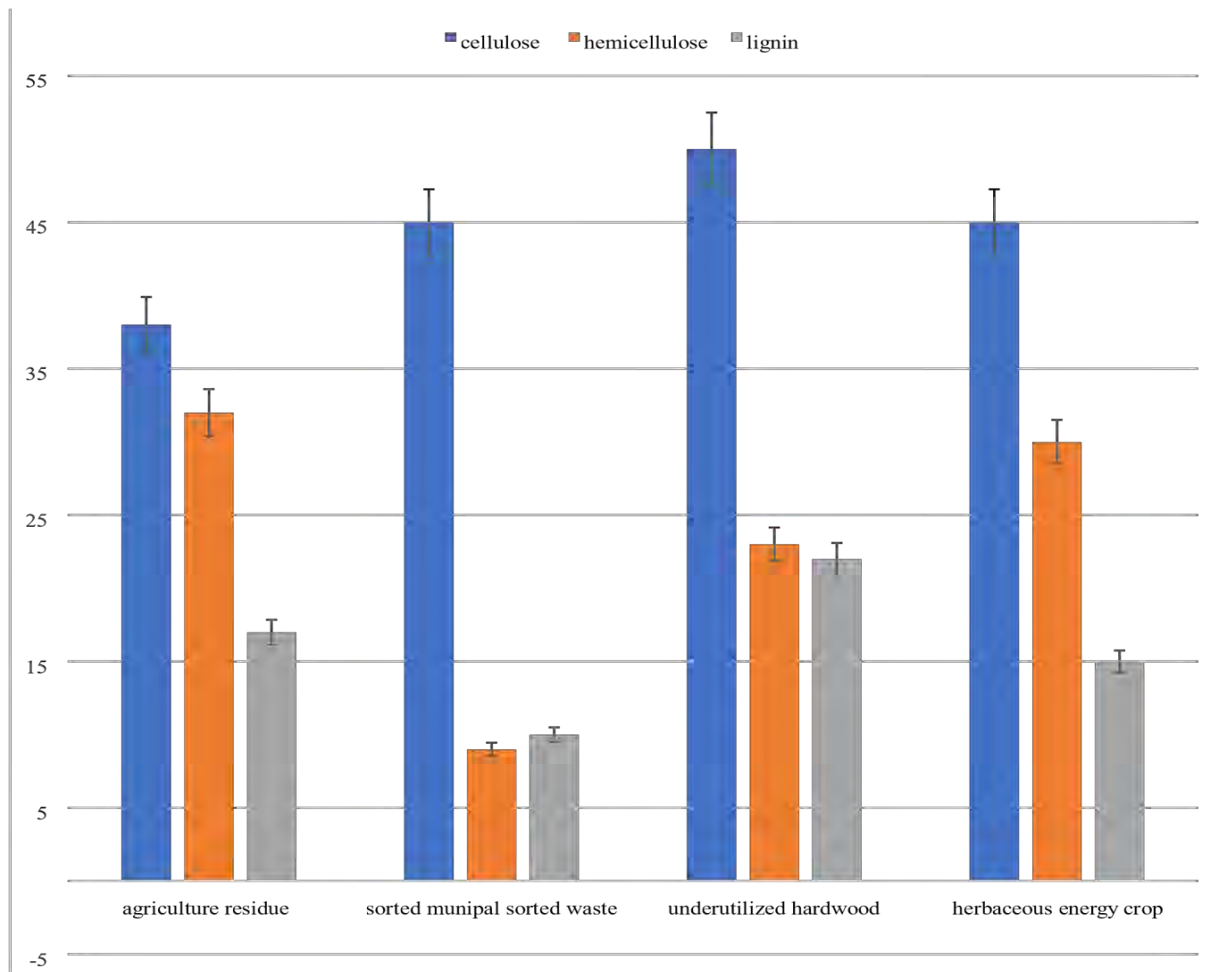


Figure 1.1. Relative content of biopolymer (%) in biowaste.

This graph show that the relative content of cellulose, hemicellulose, and lignin in biowaste of agriculture residue, sorted municipal sorted waste, underutilized wood and herbaceous energy crop. [43-45].

1.6. The hierarchical structure of Cellulose

There is a simple answer to the question that why nanofibers of cellulose and lignocellulosic biomass are showing extraordinary properties and characters. It is just because of the graded or hierarchical structure and conformation of the of lignocellulosic biomass and its fibers and the major constituents which made the all the lignocellulosic materials i.e the cellulose, hemicellulose, and lignin [110]. This hierarchical/ranked or graded structures of different materials varies from size in macro meters to nano meters and can be viewed in the Figure 1.2. Cellulose is best for its mechanical properties just because that its microstructure is ordered linear form. Hydrogen bonds present in cellulose structure are the result of multiple hydroxyl (-OH) groups in its repeating units. Wood pulp also called as bleached wood pulp or Kraft pulp is the most widely produced cellulosic form industrially which is generally used in paper industries [46]. The Figure 1.3 shows basic chemical structure of cellulose that is also called the repeating unit of cellulose structure.

The cellulose fibers vary in its diameter generally from 10-100nm depend which process has been done for its synthesis. The figure below shows a chain of variations in a material's diameter range which starts from a human and ends at a glucose molecule. Amongst them the place of cellulose could be placed in between antibody and the size of virus that are shown in the Figure 1.4 [46]. Cellulose could be extracted and purified via chemical modification and chemical fibrillation techniques. The bonds that are present between lignin and cellulose and between cellulose and hemicellulose should be exposed and tried to break them down as first step of the chemical modification technique. The second step after it is to extract the fibers. Kraft process as mentioned above is the most used method in chemical modification procedures that has been used for paper synthesis. For seeking the attention of researchers, a huge amount of the biomass generated from lignocellulose is available there in the form of agriculture residues that could be used in a creative way to make them valuable. [48].

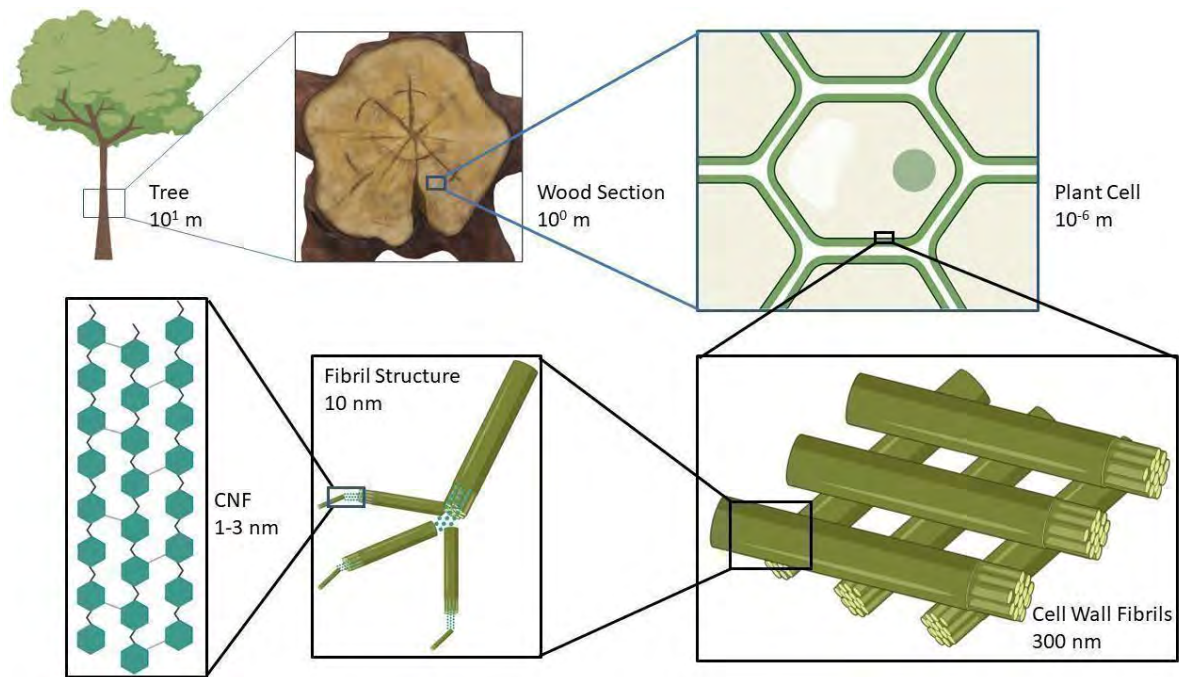


Figure 1.2. Scale of cellulose nanofiber.

Graded Structure of biomass originated from wood. Created in bio render [110].

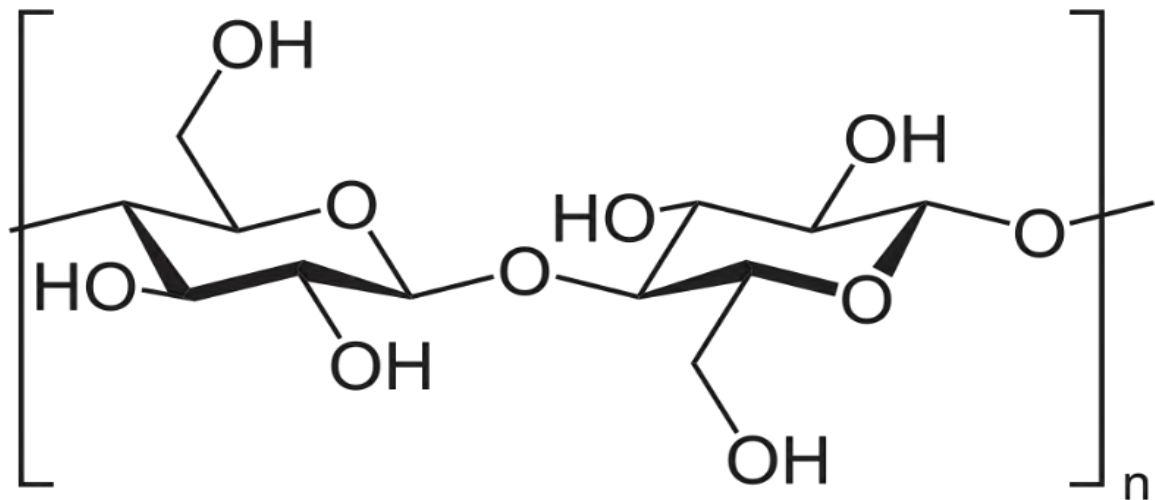


Figure 1.3. Structure of cellulose

The representation of cellulose and its chemically defined structure. Adopted from [46]

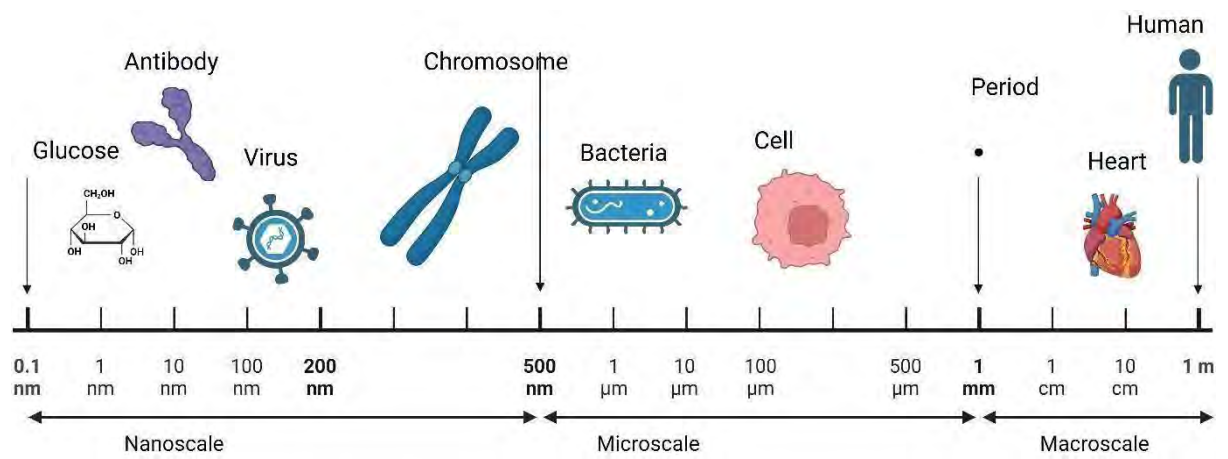


Figure 1.4. Size and dimension analysis

Size & dimensions of different materials for demonstrating the size of CNFs. Created in bio render [46].

1.7. Cellulose as a sustainable biomaterial

As a material, cellulose Materials made from biobased sources that are sustainable and environmentally friendly offer a novel answer to the growing world environmental pollution [49]. Recently, the use of biobased engineering polymers like polylactic acid has been encouraged by targeted reductions in petroleum-based goods and materials (PLA) [50]. The use of potentially renewable and/or biobased resources can also aid in resolving several global problems, including dependence on foreign oil, decreasing air and water quality, and climate change. Furthermore, the widespread use of biobased resources as engineering materials offers the possibility of new revenue streams that can be achieved locally [51].

A viable alternative for usage as an engineering material, cellulose is a biobased resource. The most prevalent naturally occurring polymer on Earth, cellulose has very peculiar mechanical properties, particularly at the nanoscale [52]. Lignocellulosic biomass mainly includes cellulose, the prime component of all cells of plant materials.

Plants' shape and structure are provided by a naturally occurring bio-composite made up of cellulose, hemicellulose, lignin, and pectin. Wood, cotton, flax, and other naturally occurring composite materials have been utilized as building and engineering materials for thousands of years, and they are still widely employed today across a wide range of industries [53]. These lignocellulosic materials (wood, cotton, flax, etc.) owe their everlasting effect to their abundance, superior mechanical qualities, and adaptability in use.

1.8. Nomenclature of nanocellulose

As was already discussed, cellulose is a biopolymer with a hierarchically organized structure that is made up of primary cellulose chains that are then arranged and grouped to form microfibrils, these fibrils further form the cellulose bundles. All these fibers originated from the cellulose, their morphologies and conformation define the intensity and nanocellulose processing because of this hierarchical organization. Aspect ratio (length to diameter ratio), average fiber diameter and length, diameter and length distribution, and crystallinity are all components of fiber morphology are shown in figure 1.5[54-56]. Short, inflexible, pleomorphic particles have been referred to as cellulose nanocrystals (CNC), also called as cellulose nano whiskers. They are typically formed because of strong acid hydrolysis, which separates the stiff crystalline sections from the amorphous phases of cellulose strands. Standard terminology recommends that CNC have an aspect ratio of 5–50 and a diameter of 3–10 nm [56]. Traditionally, CNC has been used to reinforce polymer matrices. The term “Cellulose nanofibers” (CNF) refers to long, flexible fibers that resemble ropes and have both crystalline

and amorphous sections. CNF should have an aspect ratio of greater than 50 and a diameter of roughly 5 to 30 nm [56]. Due to a high level of intermolecular hydrogen bonding, CNF create a strongly linked network when dried. It might be challenging to distinguish between cellulose nanofibers (CNF) and cellulose microfibrils (CMF), or between CNF and CNC, when lignocellulosic biomass is subjected to gentler chemical and mechanical treatments. Researchers have employed varying nomenclature in reference to samples with nano-scale cellulose dimensions, which has led to uncertainty in the literature because the product's fiber morphological distribution may span micro- and nanoscales [56].

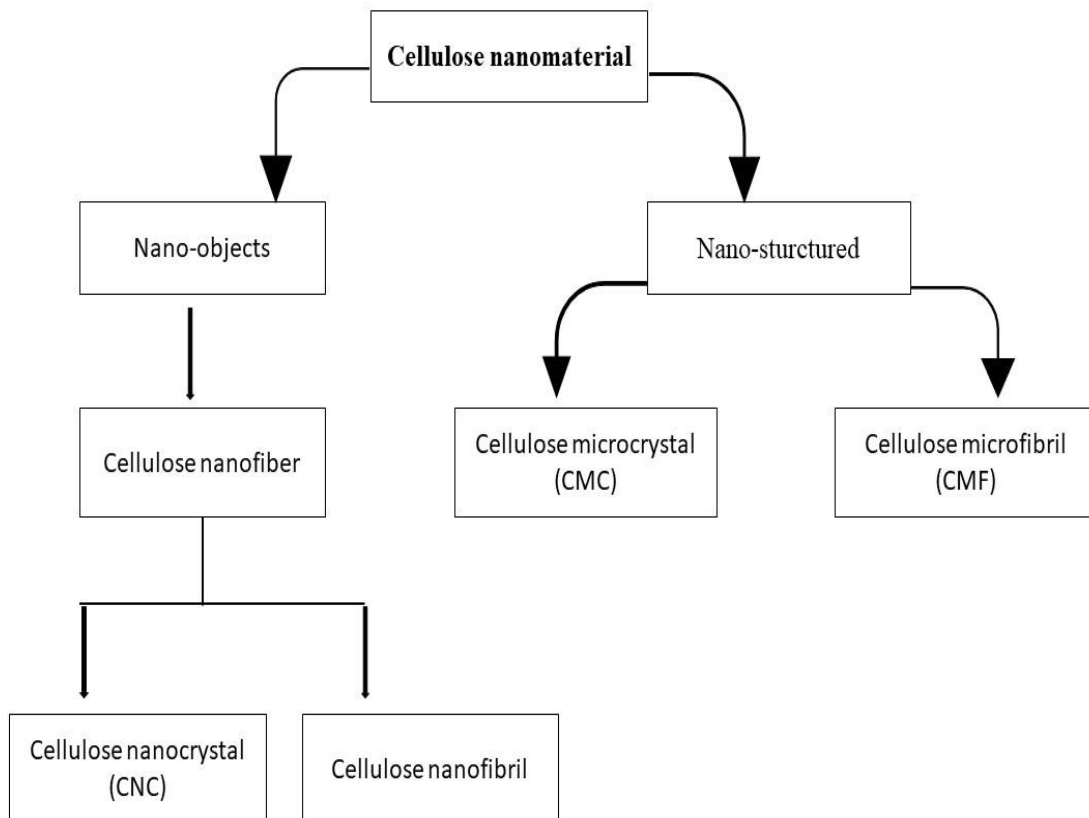


Figure 1.5. Nomenclature for cellulose nanomaterials

Proposed standard nomenclature for cellulose nsanomaterials. modified from [56].

1.9. Processing CNF sustainably

Fibrillation, or less frequently defibrillation, is the breakdown of native cellulose bundles into CNF. The most frequent methods for achieving the fibrillation of biomass into CNF include a variety of enzymatic, chemical, and mechanical treatments, or a combination of these, all of which have been extensively discussed elsewhere [57] [58]. Pre-treatment, which occurs before primary mechanical fibrillation, is essential for efficient and sustainable CNF synthesis because it alters the surface functionality of cellulose microfibrils and disrupts the composite biochemical matrix of the plant cell wall. By lowering operational problems and energy consumption during mechanical fibrillation and enhancing final nanofiber characteristics, pretreatment increases process efficiency.

The papermaking industry, which produces the most cellulosic material, uses standard kraft pulping. This method uses the steps cooking followed by bleaching process for the purpose to dissolve most of the lignin and the hemicellulose content and convert them into the black liquor waste stream [59]. The most recent techniques for sustainability are, however, heading in one of two directions. Researchers are starting to develop biomass fractionation technologies to separate these components from cellulose before fibrillation, acknowledging the potential usefulness of pure forms of hemicellulose and lignin as precursors for high value biochemical synthesis [60]. Instead, scientists are using softer chemical pre-treatments to keep lignin and hemicellulose residues in the cellulose pulp, which has been suggested to increase fibrillation effectiveness both during and after mechanical treatment [61] [62].

1.10. Agricultural waste as an offer for CNFs

Renewable, biodegradable, and inexpensive raw materials are characteristics of biomass from both the forestry and agricultural sectors. Wood sources have the advantage of producing biomass throughout a wide growth range reasonably quickly and with high density [63]. CNF was initially made from wood-based biomass, in part because of its easy integration into the current forestry and papermaking sectors. However, over the past 15 years, the need for sustainability has prompted academics to investigate non-wood lignocellulosic biomass sources. Compared to wood, agricultural leftovers provide several agronomic advantages, including shorter growth cycles, higher biomass production, higher carbohydrate content, yearly renewability, and reduced water and fertilizer needs for some species [63]. Such elements support agricultural leftovers' availability, renewability, rapid biomass generation, processing sustainability, and latent economic potential [64]. Additionally, some non-wood species, particularly C4 grass variants, can thrive in hot, humid, and semi-arid climates with

only minimal irrigation needs [65]. Additionally, in areas with poor agronomic quality of the land, cultivating certain non-wood species might be the only practical alternative. To minimize competition for land, water, and nutrients with other food or energy crops, the ideal biomass feedstock in this situation would be a fast-growing, drought-tolerant crop that is adapted to a wide range of agro-ecologies, including marginal land [66]. Considering the material characteristics of CNF products, agricultural-based CNF offers performance comparable to that of wood-based CNF while also enhancing process sustainability in several ways. Technically speaking, agricultural biomass can increase the efficiency of CNF processing since it contains more hemicellulose and less lignin than wood sources, which results in less chemical, and energy being used during pulping and fibrillation [67]. Additionally, agricultural wastes are primarily used today as animal feed, soil fertilizer, or as a low-grade energy source when burned [68]. There is a substantial possibility to valorize these biomass resources due to their low initial cost, the low economic value of existing uses, and the high value of prospective CNF products. Similar situations of the valorization of agricultural residues have been studied for their conversion to bioethanol and biogas [69] and for the creation of bionanomaterials [70]. The last benefit of creating CNF from agricultural wastes is that it addresses environmental problems brought on by their existing low-value applications, such combustion. Additionally, the ability to obtain environmentally sustainable biomass is made possible by the high biomass production from short growth cycles with low resource input (productivity), especially for agricultural crops that can withstand drought [71].

By utilizing leftover materials or by cultivating biomass on marginal land that is unsuitable for typical food or fuel crops, sustainable biomass procurement also tries to avoid the food vs fuel dilemma. Wood is the main source of cellulose, although agricultural crops that are grown specifically for food or energy produce lignocellulosic biomass as a secondary consequence. An efficient and sustainable strategy for managing crops and allocating resources is to employ the biorefinery idea, where the primary product is used for high value uses and the secondary waste materials go through valorization. This system generates goods through a variety of value streams, including as biofuel, biochemical, and biomaterial uses, to get the most out of a given piece of land.

Agricultural residues include the leftovers after crop cultivation and industrial wood wastes as sources of CNF have recently been the subject of several publications. Moreover, several outstanding reviews have been written about the production of different types of nanocellulose,

the mechanical characteristics of CNFs [72], and their various uses in polymer nanocomposites, packaging [73], and papermaking [74].

1.11. Wood based CNFs vs and non-wood- based CNFs

1.11.1. Variations in the biochemical makeup

The original biochemical makeup of CNF derived from wood versus non-wood sources is a crucial determinant of how those materials differ in terms of their characteristics. 90% of the dry weight of both wood and non-wood biomass is made up of cellulose, hemicellulose, lignin, and pectin [75]. However, because various species have varied physiologies and evolutionary histories, the proportional makeup of these factors varies among species.

Additionally, biochemical composition differs between various plant parts due to an unequal distribution of organic and inorganic substances during plant development and differs across species based on the maturity plants, cycle of reproduction, climate, and management strategies of the plant [54].

Despite diversity between each species, the metabolic makeup of wood and non-wood biomass differs significantly. Woody biomass has a higher lignin content than herbaceous and agricultural biomass, with hardwood stems typically containing 18–25% and softwood stems typically containing 25–35% [76]. Comparatively, an average agricultural residue's biochemical makeup is made up of 35–50% cellulose, 23–35% hemicellulose, and 14–20% lignin [77].

Figure 1.6 illustrates that 19 wood species and their biomass having lignin content higher and that of hemicellulose content lower than the composition of CHL of the other 30 herbaceous as well as agricultural biomass types, The CHL content was thoroughly reviewed and compared amongst the biomasses of wood and non-wood biowaste materials, there was no discernible variation in the amount of cellulose present. It is generally known that woody biomass has a larger lignin content than agricultural biomass, which increases its resistance to chemical and enzymatic pre-treatment [78] [79]. Nevertheless, lignin has been found to improve CNF fibrillation and nanomaterial properties through the following mechanisms: (1) its radical scavenging ability, which stabilizes cellulosic mechano-radicals generated during fibrillation; (2) its bulky conformation, which sterically hinders CNF packing during filtration to improve dewatering efficiency; and (3) its amorphous and hydrophobic nature, which produces a smoky appearance [80] [81]. Therefore, depending on the intended use, lignin can have either negative or positive impacts on the formation of nanocellulose.

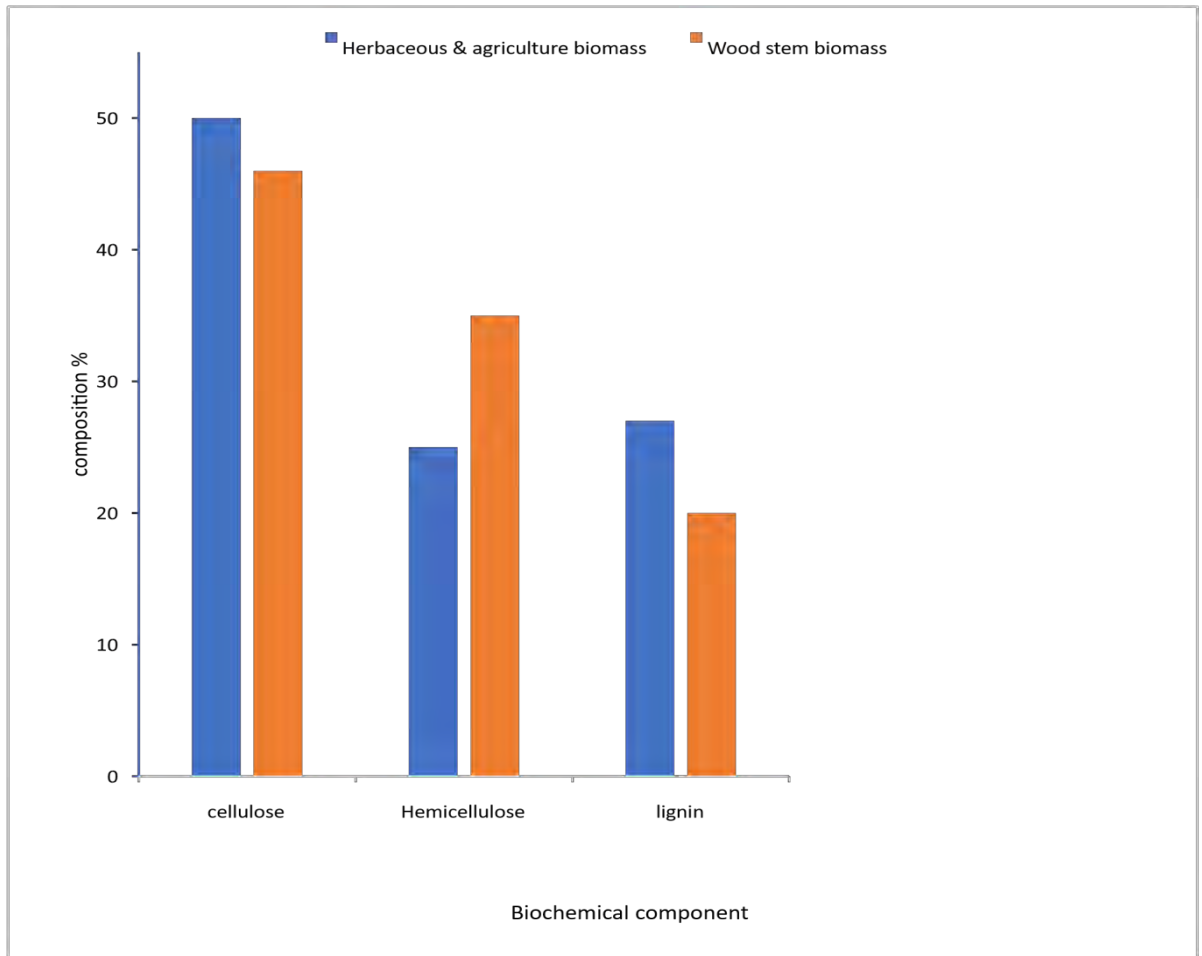


Figure 1.6. CHL composition

Cellulose©, Hemicellulose(H) and Lignin(L) composition of wood stem and herbaceous & agricultural biomass. Created in excel [82].

1.11.2. Variation in ash content

In addition to having a larger ash percentage than wood, non-wood biomass, especially wood stem biomass, has a higher CHL composition [83]. Ash content in non-wood biomass is primarily in the form of silica (SiO₂), which assembles into phytolith structures with a dumbbell shape [64]. In the Poaceae family of grasses, where it is common, high ash content is known to have a protective effect against plant wilting during drought and to dissuade plant feeding animals including herbivores, insects, and fungus [84]. Additionally, non-wood sources with low water usage efficiency, including wetland plants like rice, have particularly high ash contents that can make up as much as 25% of the dry weight of the biomass [85].

1.11.3. Morphological difference

There are different scales because of the hierarchy of biomasses of plants and variations in plant morphology might affect the effectiveness of CNF processing and the characteristics of nanofibers. Plant tissues are composed of groups of cells that serve the main tasks for the plants for their survival like water transport supported by xylem tissues and food transport by phloem tissues, metabolic functions by parenchymal cells, or tissues for giving support to the plant, depending on the kind and part of the plant (sclerenchyma). In plants, the cell wall normally has a thickness of 0.1-3 μm and surrounds an empty cell centre [86]. The porosity present in the wood stems ranges from 47-62% for hardwood species and 38-56% for species having softwood [87], whereas agricultural leftovers have a porosity of 59 to 84% [88].

To maintain process efficiency and for the processing of biomass (raw material), all the pathways and steps including hydrolysis with acids, fermenting the material, gasification, pyrolysis and cracking, and the final step of nanofibrillation, the materials are required in particle form [89]. Over a variety of milling screen aperture diameters, ground willow chips consistently had a higher bulk density than ground *Miscanthus* and switchgrass biomass, which is reciprocally related to the average particle size of the ground biomass [90]. Moreover, the internal cell wall structure is made more accessible through the deconstruction of the cell wall structure during biomass comminution, enhancing chemical penetration during pre-treatment and enhancing the material characteristics of CNF products [86]. The native fiber shape of various biomass types is a significant element impacting the mechanical characteristics of the final CNF product. The ranges of cellulose fiber crystallinity between wood based and non-wood originated fibers—55 to 70% and 90–95%, respectively— are noticeably different [91]. It has been discovered that cellulose fibers' strength and stiffness are increased by crystallinity [92]. According to the fibril type, location, and plant age, MFA in wood varies from 3 to 50

degrees [93], whereas the MFA in some non-wood biomass normally ranges from 6 to 25 degrees [94]. Thus the, choosing the right biomass feedstock is crucial to maximizing material performance, process sustainability, and financial gain in the production of CNF because different biomass feedstocks differ in their biochemical composition.

1.12. Applications and industrial relevance of CNFs

There are wide applications of cellulose nanofibers i.e., the CNFs could be implemented for the synthesis of biocatalysts, electro-optical films, nanofiber-reinforced composites, in the field of physics they can be targeted for making microelectronics, gas-barrier films, flame resistant materials, and for the synthesis of qualitative soundproof materials and other high-tech and high-performance materials are examples of potential uses for nanofibers. The nanofibers have a diverse set of industrial applications. CNFs produced via different process.

For example electrospinning, thermal treatment, and via chemical modification have many medical applications especially for wound healing materials synthesis and in the tools of dentistry [95]. Specifically in the field of biotechnology the CNFs can be implemented in drug delivery for the treatment of many fatal diseases and in tissue engineering which could be adopted in gene therapy cases, a laborious and expensive form of treatment. In the field of industrial biotechnology, the synthesized nanofibers from cellulose can be utilized as biosensors in making the filtration media. Moreover, one of the industrial applications of CNFs is also the enzyme immobilization especially in food industries [96]. In such process the enzymes used for making foods industrially and commercially, are held in place via the use of nanofibers so that to perform the entire process of catalysis in a controlled way. By the end of the reaction the enzyme easily detaches, and no further catalysis happens. The use of CNFs in paper making industries has been highlighted. Normally during the synthesis of paper, some other synthetic additives are always added to improve the strength of the paper as a demand by the customers [97]. Thus, the CNFs with their promising advantageous mechanical properties can highly improve and enhance the quality of paper if used in paper industries as an additive. Multiple companies including Innventia, and Holmen paper (Companies in Sweden for CNFs synthesis), Borregaard (based in Norway), American process (present in United States of America) and some others are now currently synthesizing and commercializing CNFs following their own respective processes [98]. The target of all these companies is to create more industrial applications of CNFs.

1.13. Aims and objectives

This study's goal is to investigate how to utilize a trash. i.e., wood waste in a smart and scientific way. The samples in the current study are experimented and processed for the formation of cellulose-based nanofibers. Also, attempts were made to find out the optimum and easy methods to obtain and separate the cellulose from waste of samples taken for research.

- The isolation of cellulose-based nanofibers from wood waste through different chemicals including alkali and acids along with some mechanical processes were tried to be examined and obtaining the cellulose-based nanofiber using chemical method by (if possible and available).
- The target of this study was to make CNFs from the wood waste and to introduce these CNFs to the fields of applied sciences along with some advances to the CNFs as well if possible.
- The evaluation of antimicrobial potential of CNFs was also one of the objectives of this research as the product may get implementation in pharmaceutical industries. The specific strains of bacteria were applied to analyse the antibacterial effect of CNFs.
- Because that cellulose nanofibers could have many applications in industries, medical fields and in environmental sciences thus all efforts were done for obtaining better results in respect of good quality CNFs synthesis.

CHAPTER 2

MATERIALS AND METHODS

2.1. Chemicals

Chemicals that were used in all these experimentations, including sodium hydroxide (NaOH), nitric acid (HNO₃), sodium nitrite (NaNO₂), absolute ethanol (commonly used solvent for many solutions), sulphuric acid (strong acid), benzene, urea, hydrochloric acid (HCl), potassium hydroxide (KOH), glacial acetic acid, sodium chloride (NaCl) used in the research were purchased and supplied by Sigma company also some were purchased from VWR.

2.2. Reagents preparation

2.2.1. Acidified sodium chlorite

3% Stock solution of acidified sodium chlorite was prepared by suspending 2.5g of sodium chlorite into 14ml acetic acid and raised the volume up to 100ml with deionized water. The different concentration of acidified sodium chlorite (0.5% and 1%) was prepared from 3% stock solution of acidified sodium chlorite.

2.2.2. 3% potassium hydroxide

Potassium hydroxide stock solution prepared by taking 0.38g of potassium hydroxide and dissolving it into 12.5ml of water.

2.2.3. 6% potassium hydroxide

stock solution of potassium hydroxide was prepared by dissolving 0.75g of potassium hydroxide into 12.5ml of water with the help of stirrer

2.2.4. 0.2M nitric acid solution

Dissolving 1.2 ml of nitric acid into 100ml dis.H₂O to form 0.2M of nitric acid solution.

2.2.5. 0.02M sodium nitrite solution

Stock solution of sodium nitrite prepared by taking 0.138g of sodium nitrite and dissolve it into 100ml dis.H₂O.2.3.

Sample Preparation

Bark of eucalyptus and sugarcane bagasse (SCB) were obtained from the wood waste of eucalyptus tree present in Quaid I Azam University Islamabad and the fruit stalls that present near Khanna pull at Dr Qadeer Road Rawalpindi, respectively. Stem barks of eucalyptus and SCB were collected via razor bladder. The collected bark of eucalyptus and SCB were inspected for any infected areas and were converted into pieces, placed in an aluminium foil, and kept in oven at 37°C for 5 days to

remove the moisture and then grinded with the help of Kitchen grinder till the size of powder particles become too fine as shown in the figure 2.1.

-



Figure 2.1. Moisture removal of sample

(A) raw of sugarcane bagasse (SCB) in drying oven (B) dried material of sugarcane bagasse (C) drying sample of eucalyptus bark in oven (D) powdered of eucalyptus bark.

2.4. Chemical purification of cellulose

Two methods were used for the chemical modification for the synthesis of CNFs. The purification of cellulose nano fibers was carried out according to [99,100].

2.5. Method 1

Extraction of CNFs from the bark of eucalyptus and SCB followed by method [99]. Firstly, the dried bark of eucalyptus and SCB were washed with deionized water. 10g dry material (dried bark of eucalyptus and SCB) was added in deionized water (300mL) at 70 ° C. The mixtures were kept under constant stirring for 1h and cooled at room temperature. To remove the wax, 8g of each sample was refluxed with solution o 2:1 of benzene and ethanol respectively at 80 ° C for 6 h. The procedure was performed in in Soxhlet apparatus as shown in Figure 2.2.

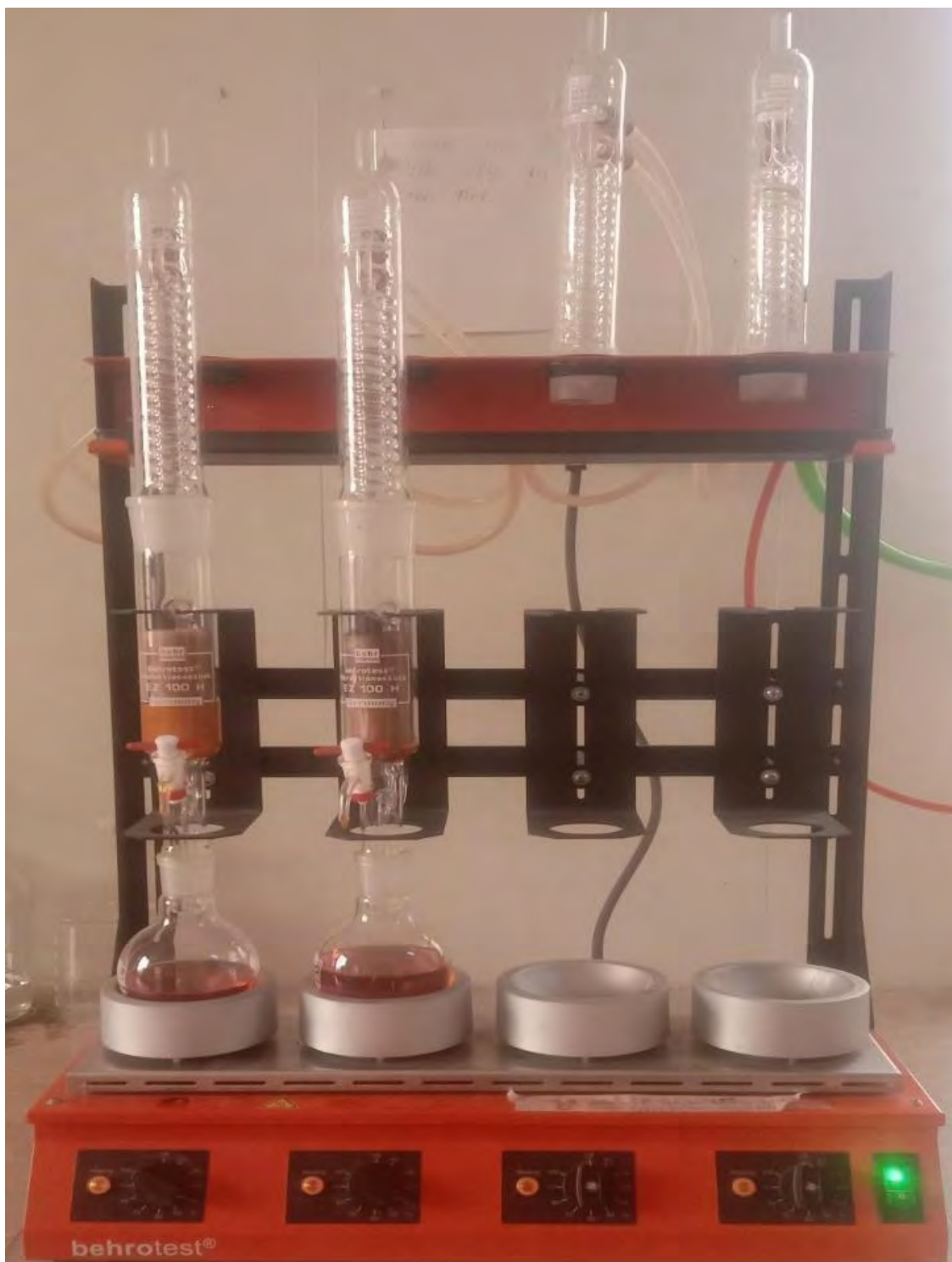


Figure 2.2. Soxhlet apparatus setup used for the dewaxing

Dewaxing the sugarcane bagasse and eucalyptus bark via Soxhlet treatment with benzene to ethanol (2:1).

2.5.1. Acid hydrolysis

Secondly, lignin present in the sample was extracted by dissolving the sample with acidified sodium chlorite solution at 60 ° at 320rpm with continuously stirring with the help of magnetic stirrer for an hour. For this purpose, take 1g biomass per sample, 2.5g of sodium chlorite and add it into acetic acid and then add deionized water to make an acidified sodium chlorite solution to leach out the lignin from the sample that show in figure 2.3. This step is repeated five times followed by extensive washing with deionized water at each turn. Filter the fiber via Whatman filter paper and kept overnight at 25°C.

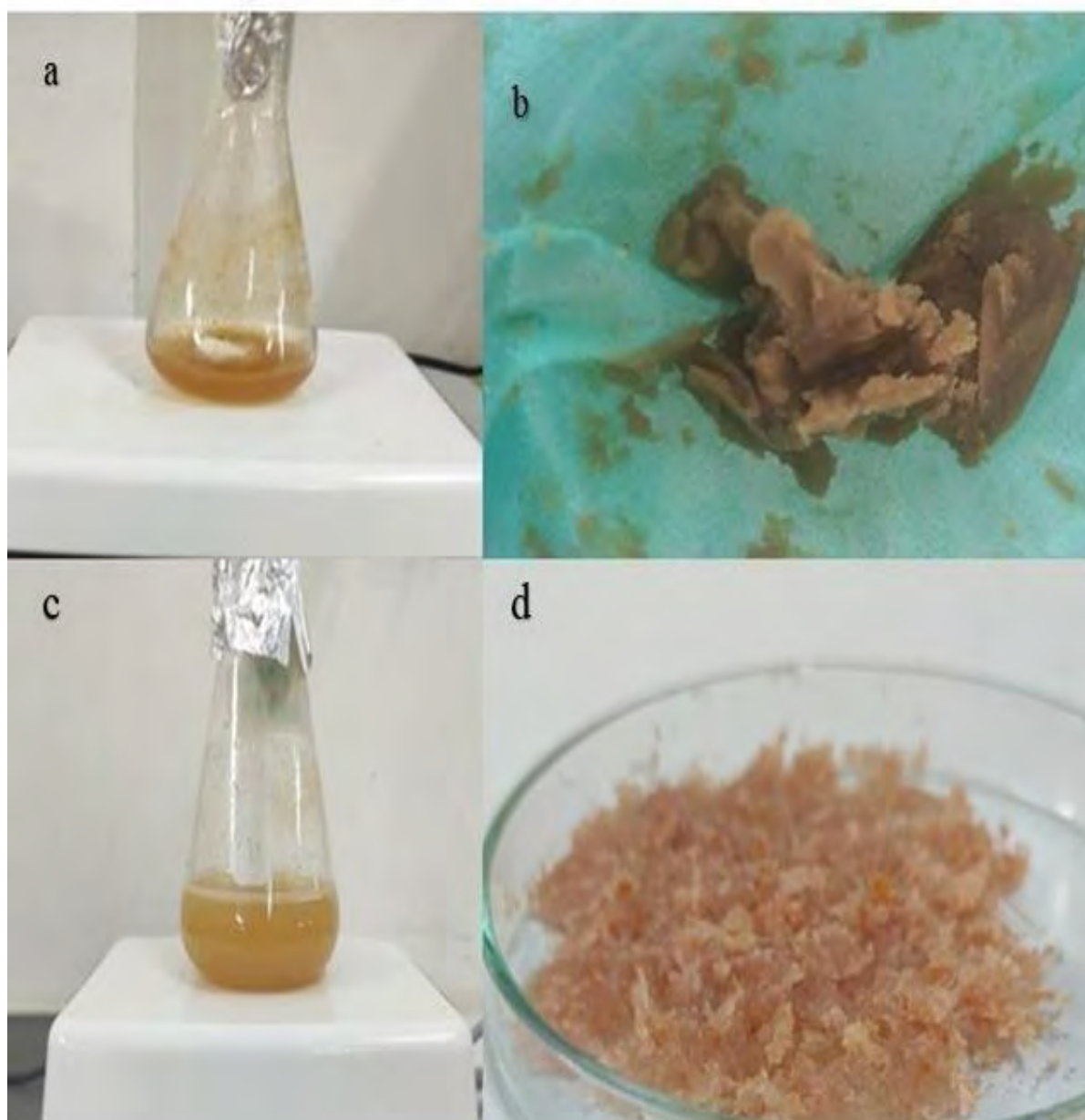


Figure 2.3. Bleaching treatment of sample

Bleaching the samples: a” and” b” is bleaching of sample eucalyptus ‘s bark and “c” and “d” representing the bleaching of SCB.

2.5.2. Alkaline treatment

In the next step, the samples were treated and mixed with 3% KOH solution at 80 °C for 2h, and then moved to next solution of 6% potassium hydroxide at 80 °C for 2h. This two-phase treatment of sample was to remove and leach out all the existing hemicellulose, residual starch in sample, and pectin (normally present in hard wood). After the completion of leaching process and removal of these materials chemically, the purified samples were filtered to get the residue and rinsed thoroughly with distilled water to bring the solution pH to normal.

2.5.3. Micro to nanocellulose fiber

Sonication process is carried out for obtaining the nanocellulose fiber. So, the chemically purified fibers of cellulose (PCFs) which were extracted from the two different types of plant fibers were soaked in container of distilled water (120ml), and the cellulose suspensions obtained were then brought for sonication, done at 60°C for 30 min using a sonicator machine to isolate and extract the nanosized fibers of cellulose. The whole process for the extraction of CNFs treatment was done inside the ice bath; the presence of the ice was mandatory for the process till the sonication done so that to lower the exothermic reaction. After sonication treatment, centrifugation was done at 13000rpm for 20min, and the supernatant was separated. After that, the cellulose suspension is poured into petri dish and kept into hot plate to completely remove moisture from the samples that shown in figure 2.4. Weight the samples one by one and stored it at room temperature for further analysis.

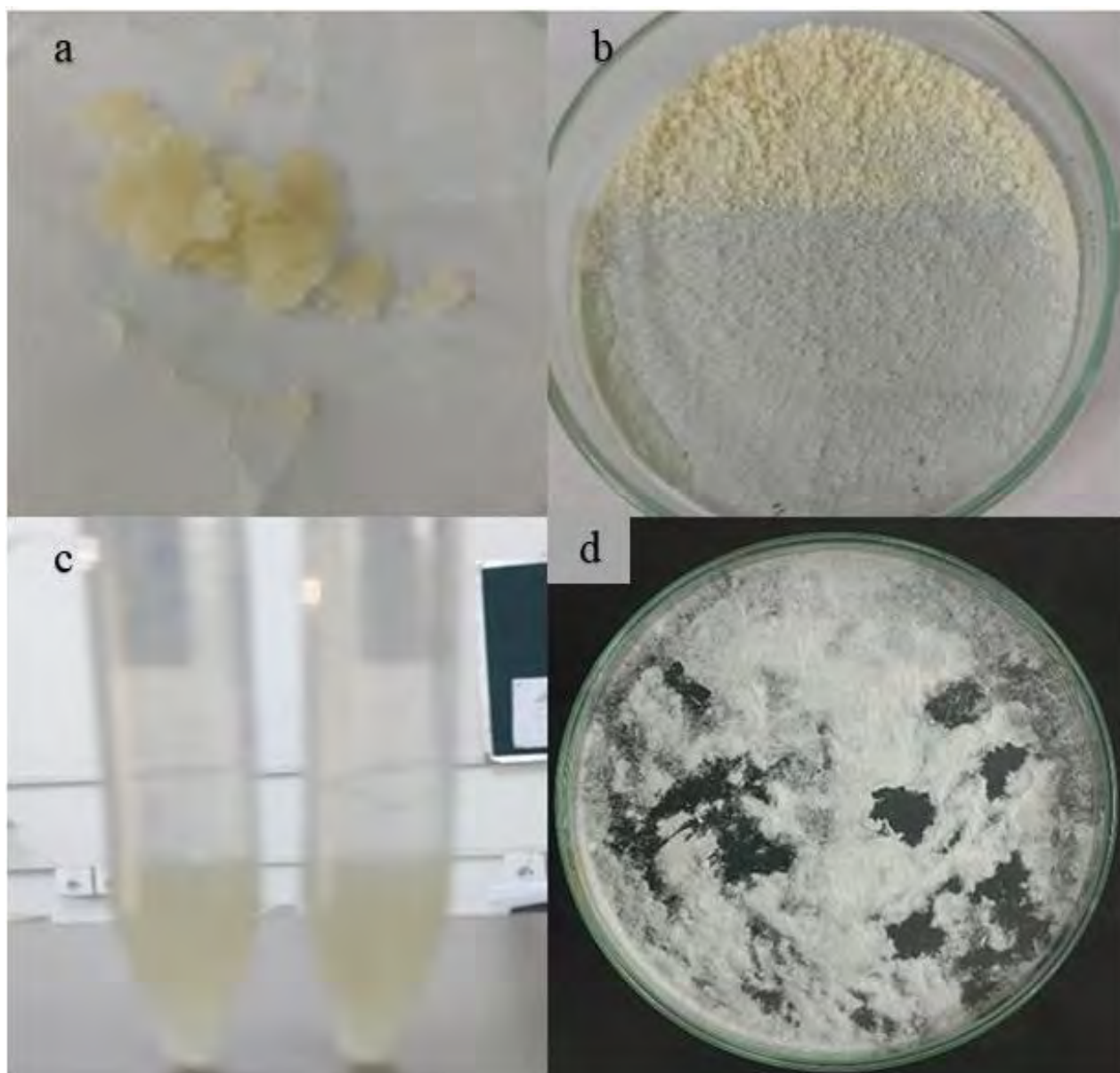


Figure 2.4. Formation of cellulose

Formation of cellulose nanofibers: “a” and “b” CNFs obtained from eucalyptus bark. While “c” and “d” are CNFs from SCB.

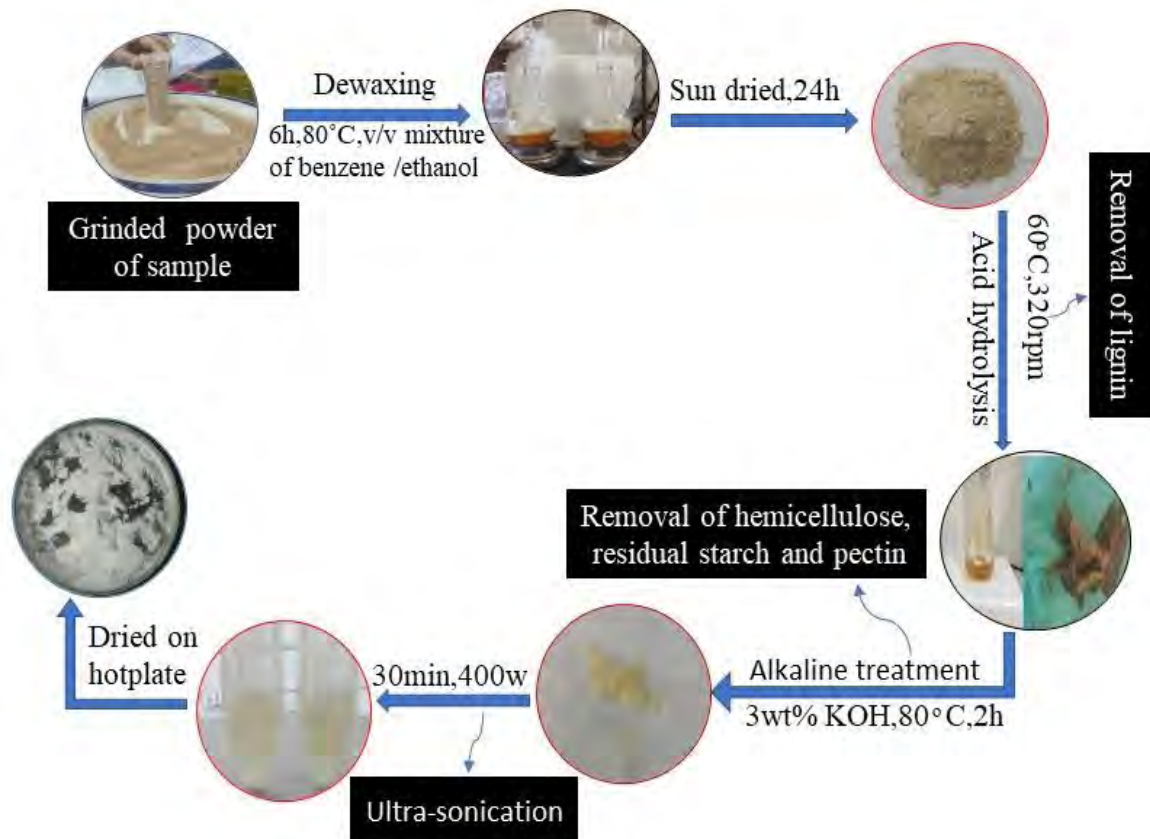


Figure 2.5. Schematic illustration of CNFs extraction

An illustration diagram of cellulose nanofiber extraction from wood waste (SCB, Bark of eucalyptus). Created in word.

2.6. Method 2

Followed by Kumar *et al.*, 2019, extraction of CNFs from the bark of eucalyptus was performed the following procedure [99]. Good quality grinder was used to chop and refine bark of eucalyptus till the size of sample reduced to ~1 mm that shown in Figure 2.6. Then, quantity of 1g of fine chopped bark sample was soaked in 12wt% NaOH solution and kept on a magnetic stirrer at 25°C for 24 h for hydrolysis. After treating the sample with NaOH the sample was rinsed and washed thoroughly with distilled water and the pH was brought to neutral that was shown in Figure 2.7.



Figure 2.6. Grinded sample of eucalyptus's bark

Bark of eucalyptus were grinded with the help of grinder and weight the grinded sample.



Figure 2.7. Measuring the pH of sample

Add small amount of d. water and measure the pH of sample. The pH of sample was neutral.

For the synthesis of cellulose nanofibers, 0.5g of alkali i.e., NaOH treated fibers of the sample were taken in 100ml round bottom flask. The flask was poured with 14ml Nitric acid (0.2 mol). The nitric acid was added to the solution gradually for proper soaking of the sample in acid. At final step a 0.38g (0.02 mol) of sodium nitrite was added into flask and properly Shaked. The whole reaction was carried and performed at 70°C on magnetic stirring at 500rpm for 10h as shown in Figure 2.8.

When the reaction started, the formation of red fumes occurred in the flask. To prevent these red fumes from escaping, the mouth of round bottom flask was covered with a glass stopper. After the fixed duration the reaction was stopped by the gradual addition of distilled water in the flask. After that the solution was centrifugated at 3000 rpm with a ratio 2:1 between ethanol: water respectively for adjusting pH of solution and the supernatant and pellets were separated successfully. Then sediments formed in falcon tubes were taken, rinsed and washed thoroughly with dH₂O and kept in refrigerator in resuspended form with dH₂O at 4 °C for onward characterization analysis of nanofibers (Figure 2.9).



Figure 2.8. Magnetic stirring of sample
Treating the fibers with sodium hydroxide and nitric acid at 70 °C on magnetic stirrer for 10h.

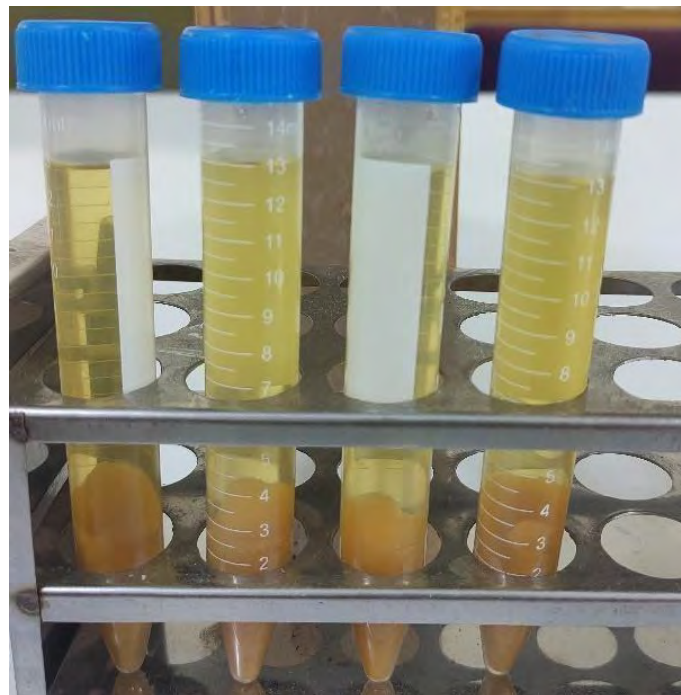


Figure 2.9. Cellulose nanofiber purification from eucalyptus bark
Cellulose nanofiber purification from eucalyptus bark by subjecting them to acid hydrolysis along with some other chemical treatment followed by the process of centrifugation.

2.7. Characterization

2.7.1. Zeta-potential and particle size analysis

The size of the cellulose nano fiber that obtained from bark of eucalyptus and sugarcane bagasse were examined and characterized in a laser diffraction apparatus (Malvern Instruments Ltd., Mastersizer 2000—model APA2000, UK). The machine was equipped with a dispersion unit (Hydro 2000S, model AWA2001) to improve and enhance sample dispersibility for better results. Water being a good solvent used for immersing sample. The samples were injected into the sides of each zeta sizer cell with the help of syringe in a fixed and tight conformation to avoid the formation of bubbles inside the cell. After pouring all the samples, the cells were placed inside the holder of zeta sizer equipment one by one. The electrodes in zeta sizer cell were properly placed towards the gold plate of the instrument for passing of electric current from the instrument to the sample for the measurement of zeta potential. The temperature was set automatically in the instrument also the size option was set too before operating the machine. Measurements of the result were done in triplicate as usual, at room temperature. The apparatus named light scattering (Malvern Instruments Ltd., Zeta sizer Nano Series—model Nano ZS, UK), was used to examine the dimensions of CNFs i.e., length and the surface charge and surface area. This measurement was made in addition to water as solvent. Four samples were tested in zeta sizer for CNFs length and its potential too the results were promising for all the four samples.

2.7.2. FTIR spectroscopy

In cellulose sample, FTIR spectroscopy was used to identify the various functional groups their vibration properties. The apparatus was used to record the FTIR spectra of the sample. The range of this FTIR instrument was $400\text{-}4000\text{cm}^{-1}$. The samples used for the analysis were in powder form and then converted to finer powder via fiber microtome. The chemical KBr (Potassium bromide) was used in sample grinding to obtain full transmittance of the spectra because KBr shows 100% transmittance in the range of $400\text{-}4000\text{cm}^{-1}$.

2.8. Antimicrobial Assay

2.8.1. Material

CNFs were prepared by mechanochemical, and TEMPO oxidized method. The extracted CNFs were dissolved in solution (urea +NaOH). *K. pneumonia*, *P. aeruginosa*, and *Bacillus subtilis* were tested for antibacterial activity. These were obtained from Microbiology Lab of QAU.

2.8.2. Method

The commonly used Agar method, a simple technique to measure the antibacterial activity of a sample was used to measure the sample's extract i.e., cellulose nanofiber' antibacterial potential. There are two methods to measure antibacterial characteristic of a sample i.e., disc method and well diffusion method but the technique which was more suited to the sample was well diffusion as in disc method, only a small amount of the drug/chemical to be loaded gets absorbs in it and make the method less effective.

CHAPTER 3

RESULTS and DISCUSSION

3.1. Particle size distribution and Zeta potential analysis

Results of particle size distribution and potential are shown the figures below.

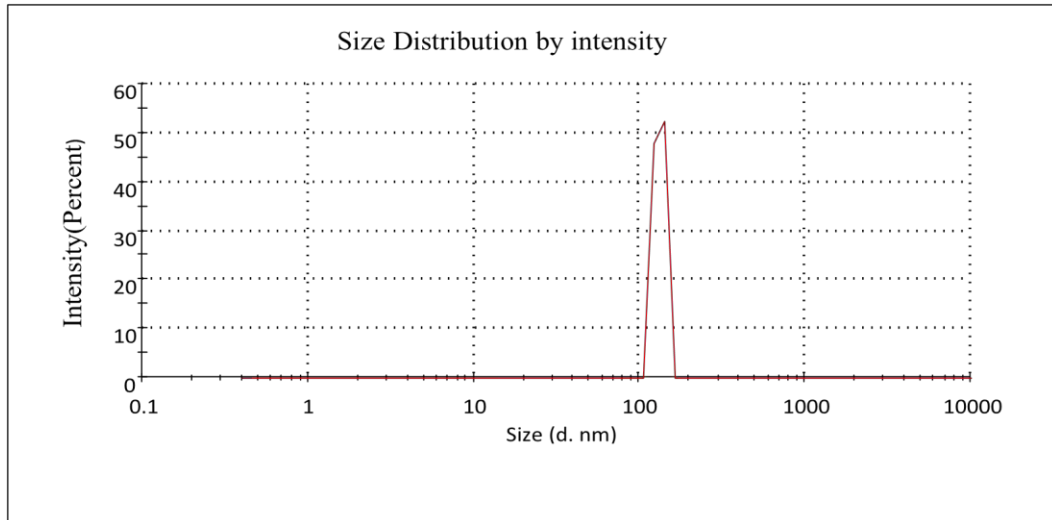


Figure 3.1. PSD of CNFs obtained from EU-1

This graph show that the particle size distribution (PSD) of CNFs that are obtained from eucalyptus's bark via chemo-mechanical treatment is 132nm.

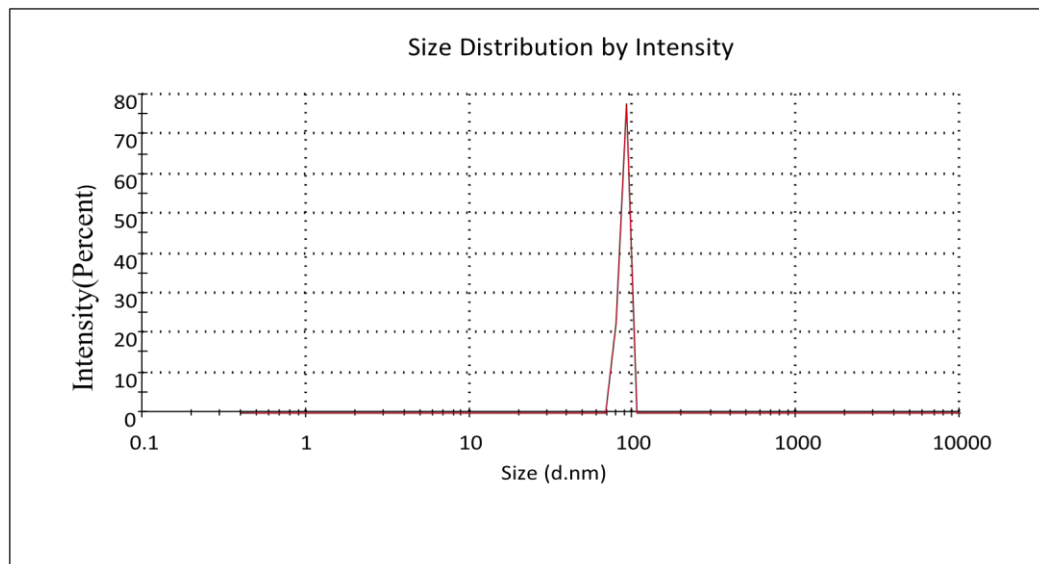


Figure 3.2. PDS of CNFs obtained from EU-CT

This graph show that the particle size distribution (PSD) of CNFs that are obtained from eucalyptus's bark via chemical treatment is 88nm.

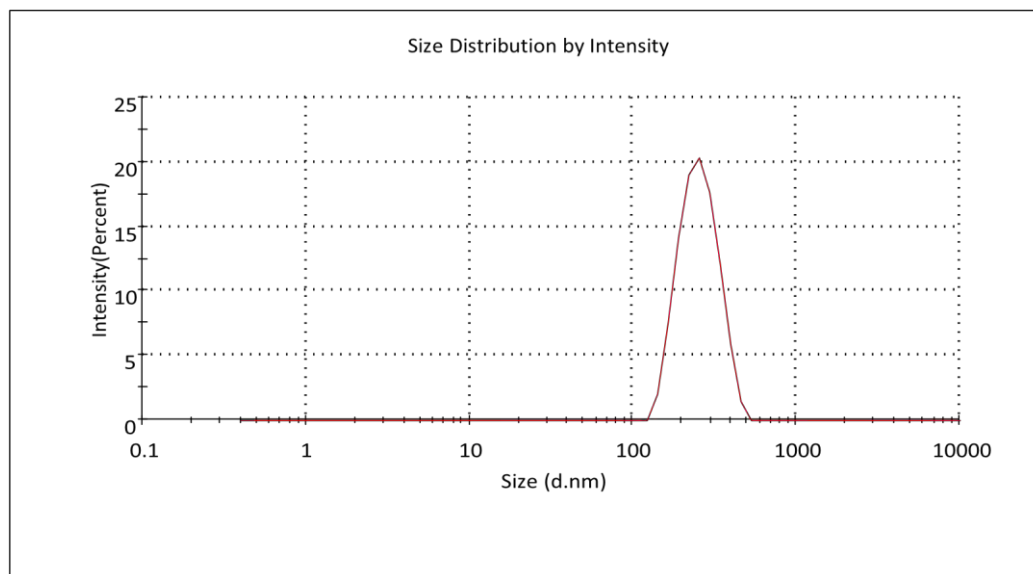


Figure 3.3. PSD of CNFs obtained from SCB-I

This graph show that the particle size distribution (PSD) of CNFs that are obtained from inner part of sugarcane bagasse (SCB-I) via chemo-mechanical treatment is 256nm.

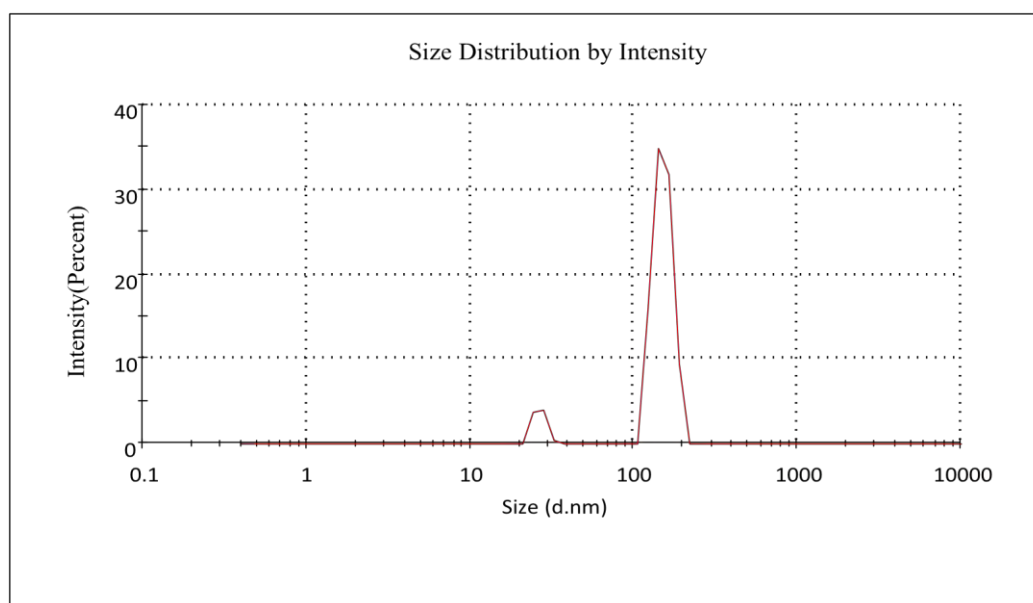


Figure 3.4. PSD of CNFs obtained from SCB-O

This graph show that the particle size distribution (PSD) of CNFs that are obtained from Outer part of sugarcane bagasse (SCB-O) via chemo-mechanical treatment is 256nm.

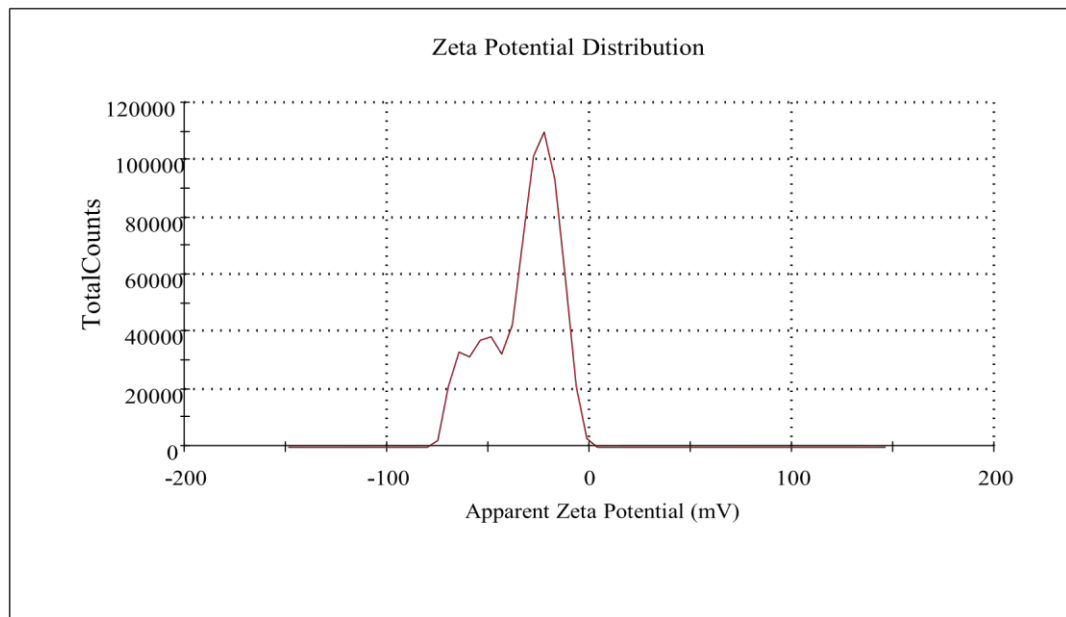


Figure 3.5. Zeta-potential of CNFs from EU-1

This graph show that the zeta potential of CNFs that are obtained from eucalyptus's bark via chemo-mechanical treatment is -32mV.

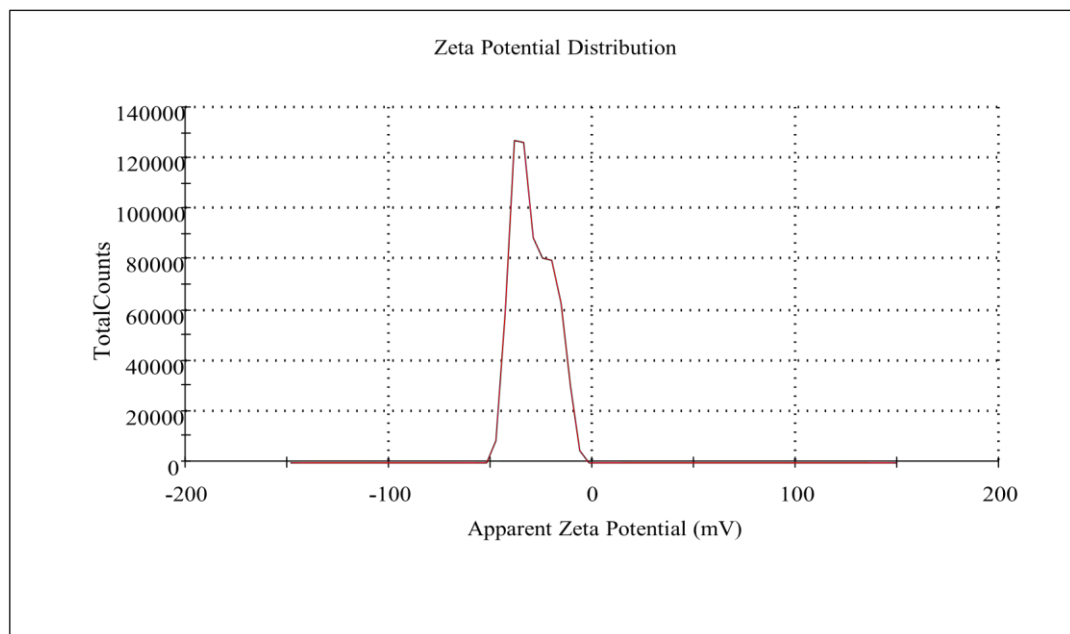


Figure 3.6. Zeta-potential of CNFs obtained from EU-CT

This graph show that the zeta potential of CNFs that are obtained from eucalyptus's bark via chemical treatment is -31.4mV.

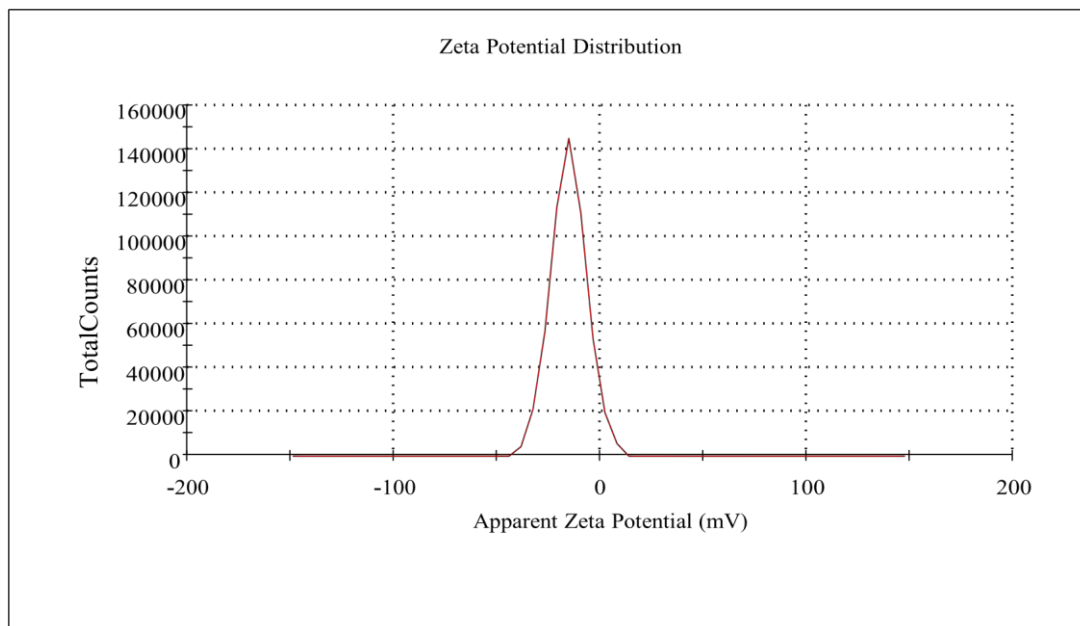


Figure3.7. Zeta-potential of CNFs from SCB-I

This graph show that the zeta potential of CNFs that are obtained from inner part of sugarcane bagasse (SCB-I) via chemo-mechanical treatment is -15.3mV.

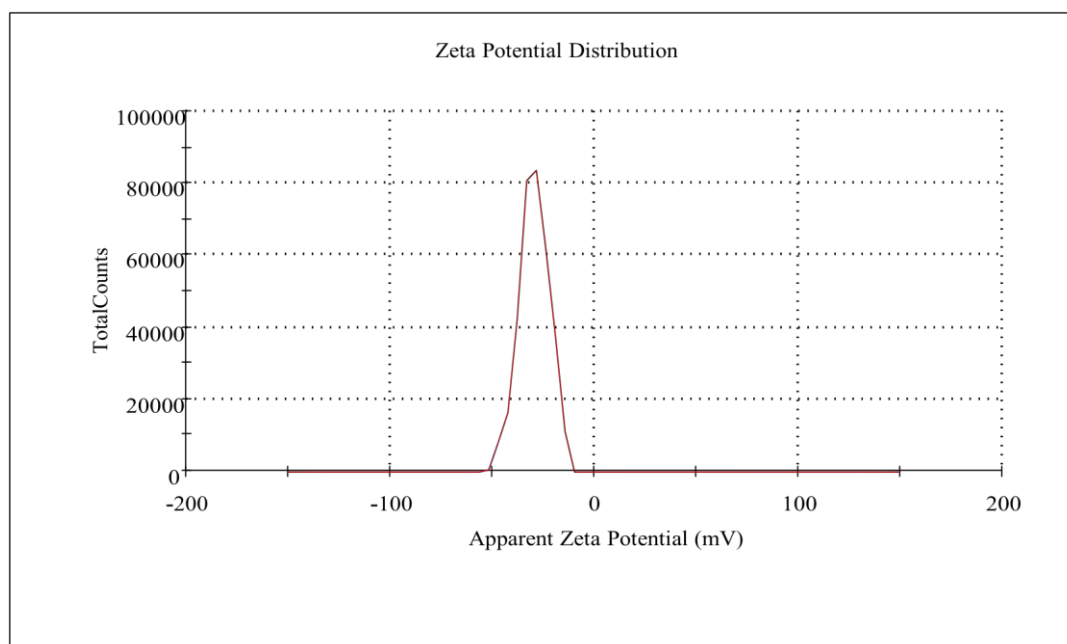


Figure 3.8. Zeta-potential of CNFs from SCB-O

This graph show that the zeta potential of CNFs that are obtained from outer part of sugarcane bagasse (SCB-O) via chemo-mechanical treatment is -29.3mV.

The PSD and average particle sizes of CNFs collected from various sources were calculated using the Malvern Zeta Sizer, a particle size analyser. According to Figure 3.2, the EU-CT sample (88 nm) had the smallest particle size, followed by the EU-1 (132 nm) as shown in figure 3.1. Furthermore, the SC-O particle size was 151 nm reported in figure 3.4 and the sample SC-I come with particle size of 259 nm which is the largest particle size amongst all samples as illustrated in Figure 3.3. The most effective and finer particle size was reported by EU-CT. The zeta potential and particle size distribution of each CNF are shown in Table 3.1. The zeta potential for extracted CNFs is shown in Figure 3.5-3.8. The CNFs produced by SCB-O have a bimodal distribution with two distinct length sizes. Bimodal peaks demonstrate the higher aggregation susceptibility of CNFs produced from SCBO. When compared to the other extracted CNFs, the peak position of the length distributions of CNFs from EU-CT was lower. This indicated that EU-CT was more aggressive; to produce shorter nanofibers, it deteriorated the amorphous zones and partially damaged the crystalline zones of the fibers.

According to Tholstrup Sejersen (2007), various properties of the nanofibers, such as surface charge (zeta potential), can affect how efficient they are as a reinforcing agent. Zeta potential values of ± 30 mV are considered stable dispersion, which explains why the Zeta potential is an important metric for examining the dispersion stability of CNFs in aqueous suspension. To maximize their degree of dispersion in the composite, nanosized particles should have a high zeta potential for the colloidal suspension to be able to resist aggregation [101]. All four CNFs samples in this study had a zeta potential that was measured to be between 15 and 37 mV. Contrarily, SCI isolated CNFs with the highest zeta potential (-15.3 mV), followed by EU-CT (-29.3 mV), SCB-O (-29.5 mV), and EU-1 (-32.5 mV), indicating that the resulting nanofibers were more electrically stable. The fibers were more electrostatically attracted to one another. In this situation, and there were enough surface charges to maintain the suspension. Overall, we showed that it is possible to transform waste into functionally and environmentally significant micro- and nanocellulose fibers that are stable in aqueous solutions for longer periods of time. Such reliable technologies enable novel applications in numerous sectors, including the food, pharmaceutical, packaging, and cosmetics industries.

Table 3.1. Particle size distribution and Zeta-potential of CNFs

Sample	Zeta-potential (mV)	Particle-size distribution (d.nm)
Mechano-chemical treatment		
Eucalyptus's bark (Eu-1)	-32.5	132.5
Inner Sugarcane bagasse (SCB-I)	-15.3	259.1
Outer sugarcane bagasse (SCB-O)	-29.3	151,26.68
Chemical treatment		
Eucalyptus's bark-CT (Eu-CT)	-31.4	88.48

3.2. Fourier transformed infrared spectroscopy (FTIR)

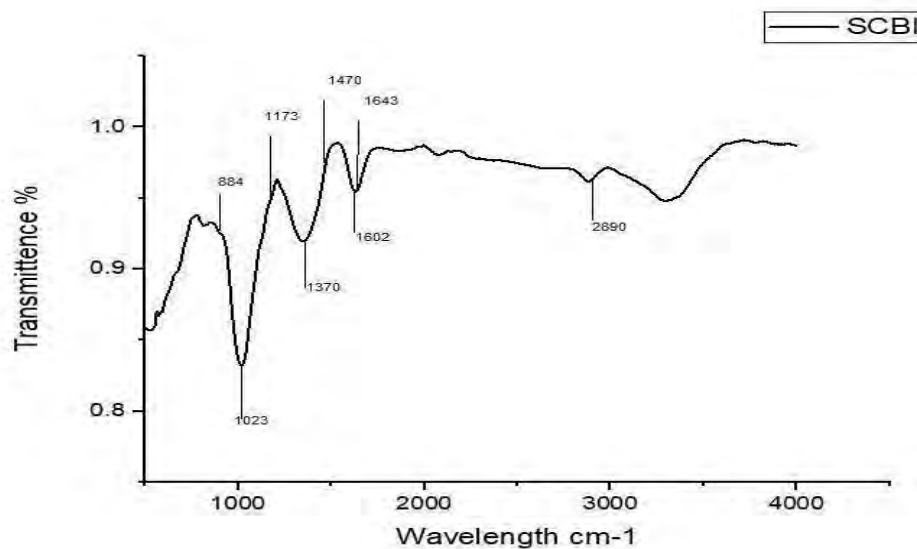


Figure 3.9.

FTIR-spectrum of CNFs obtained from SCB-I

FTIR-spectra of CNFs obtained from SCB-I identify the chemical bonds in a molecule by producing an infrared absorption spectrum. The main spectral bands at 1023 are produced by stretching vibrations of the C-O ether. The additional peaks that are more clearly visible in the 890-895 cm^{-1} range are related to the cellulosic glycosidic linkages.

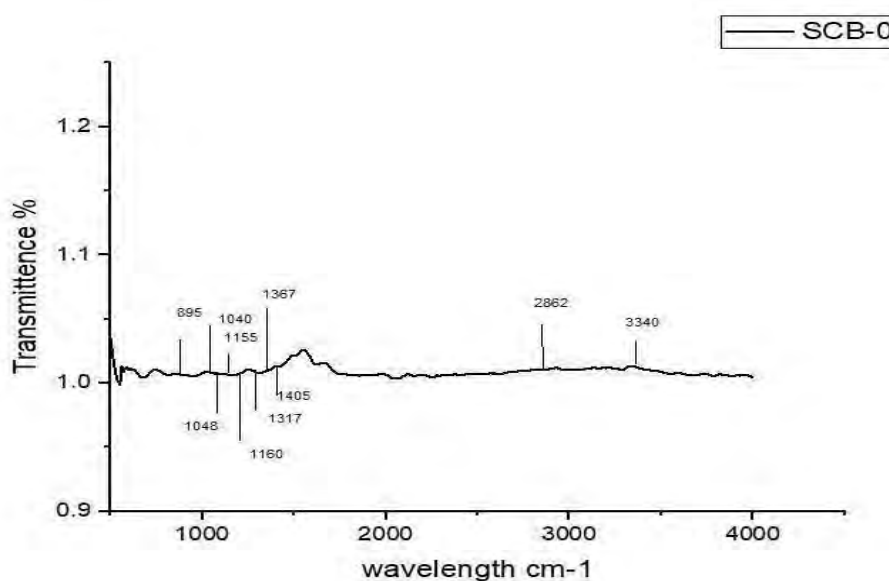


Figure 3.10. **FTIR spectrum of CNFs obtained from SCB-O**

The chemical bonds of a molecule are identified by FTIR-spectra of CNFs produced from SCB-O by creating an infrared absorption spectrum. Peak at 1048 cm^{-1} corresponds to the stretching vibration of the C-O-C pyranose ring in cellulose. CNF exhibits CH₂ wagging, C-H bending, and CH₂ scissoring motions at 1317 cm^{-1} , 1367 cm^{-1} , and 1405 cm^{-1} respectively.

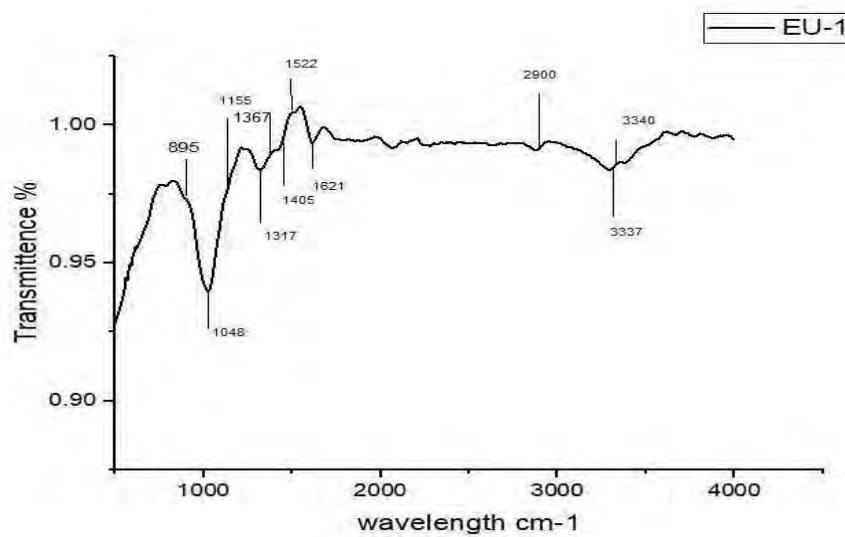


Figure 3.11. FTIR-spectrum of CNFs obtained from EU-1

The chemical bonds of a molecule are identified by FTIR-spectra of CNFs acquired from EU-1 by creating an infrared absorption spectrum. A stretching band for the CC ring is visible at the peaks at 1155cm^{-1} . The peak at 3337cm^{-1} was due to the OH stretching.

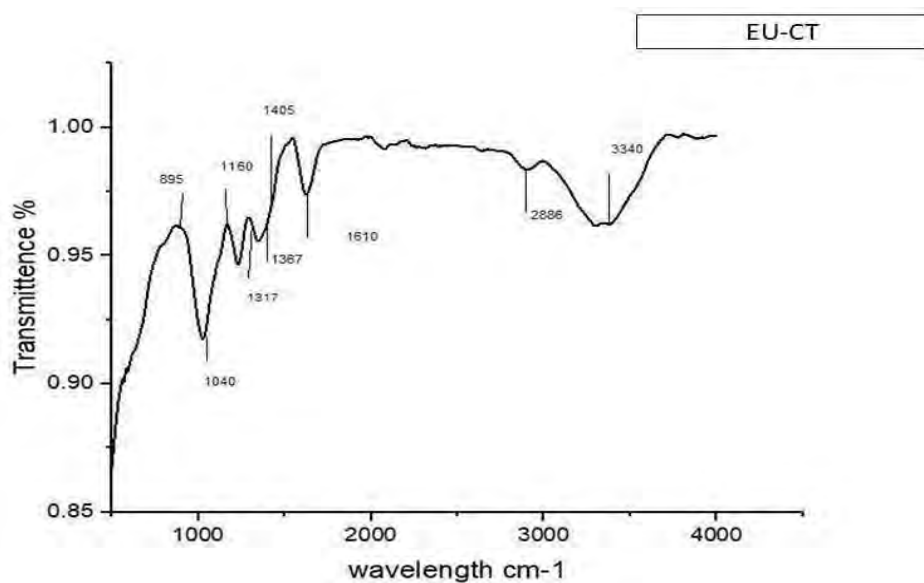


Figure 3.12. FTIR-spectrum of CNFs obtained from EU-CT

FTIR-spectra of CNFs obtained from EU-CT identify the chemical bonds in a molecule by producing an infrared absorption spectrum. Peak at 1040cm^{-1} is associated with the stretching vibration of cellulose's C- O ether ring. Asymmetric and symmetric CH₂ group vibrations are represented by the peaks between 2820cm^{-1} and 2900cm^{-1} . The peak at 3340cm^{-1} was due to the OH stretching.

Table 3.2. FTIR spectral peak values and functional groups of CNFs obtained from Different woody sources.

Wavelength cm^{-1}	Vibration modes	References
890-895 cm^{-1}	Vibration of Cellulosic glycosidic linkages	[102]
1040 cm^{-1}	C-O vibration of ether group	[108]
3340 cm^{-1}	O-H stretching	[107]
2820-2900 cm^{-1}	CH ₂ vibrations	[100]
3337 cm^{-1}	O-H stretching	[99]
1048 cm^{-1}	Stretching of C-O-C pyranose Ring	[105]
1317 cm^{-1}	CH ₂ wagging	[103]
1367 cm^{-1}	C-H bending	[106]
1405 cm^{-1}	CH ₂ scissoring vibration	[103]
1155 cm^{-1}	Stretching vibration of C-C ring	[104]
1160 cm^{-1}	C=O Vibration of Carbonyl group	[104]
1154 cm^{-1} - 1159 cm^{-1}	C-C Ring stretching	[103]

The structural changes brought on by chemical treatments are frequently studied using FTIR spectroscopy. The FTIR spectra of the four distinct cellulose nanofibers were displayed in figure 21-24. The main spectral bands at 1040 and 3340 cm^{-1} are produced by stretching vibrations of the C-O ether and OH groups respectively. Asymmetric and symmetric CH_2 group vibrations are represented by the peaks between 2820 cm^{-1} and 2900 cm^{-1} . The peak at 3337 cm^{-1} was due to the OH stretching; this band was connected to intermolecular hydrogen bonds in cellulose [105]. The additional peaks that are more clearly visible in the 890-895 cm^{-1} range are related to the cellulosic-glycosidic linkages [100]. Peak at 1048 cm^{-1} corresponds to the stretching vibration of the C-O-C pyranose ring in cellulose. CNF exhibits CH_2 wagging, C-H bending, and CH_2 scissoring motions at 1317 cm^{-1} , 1367 cm^{-1} , and 1405 cm^{-1} respectively because of polysaccharide. A stretching band for the C-C ring is visible at the peaks at 1155 and 1160 cm^{-1} . Additionally, the peaks at 1154 cm^{-1} and 1159 cm^{-1} exhibit the C-C ring stretching band. The shoulder in 1731 cm^{-1} in the spectrum of, SCB1, and SCBO corresponded to vibrations of the acetyl and uronic ester groups of hemicelluloses or to the ester linkage of carboxylic groups of the ferulic and p-coumaric acids of lignin, respectively [107]. The substantial peak at 1738 cm^{-1} totally disappeared in CNFs that are obtained from EU-CT source, showing that the majority of the lignin and hemicelluloses were eliminated by the subsequent chemical and ultrasonic treatments. When compared to other CNFs that are extracted by mechano-chemical processing, CNFs that are obtained from EU-CT source display a strong peak at 1610 cm^{-1} , which corresponds to carbonyl groups [99].

According to these data, hemicelluloses and lignin were mostly eliminated during the chemical processes, leaving cellulose as the primary constituent CNFs extracted from EU-CT source, however the other sources showed the incomplete removal.

3.3. Antibacterial analysis of CNFs

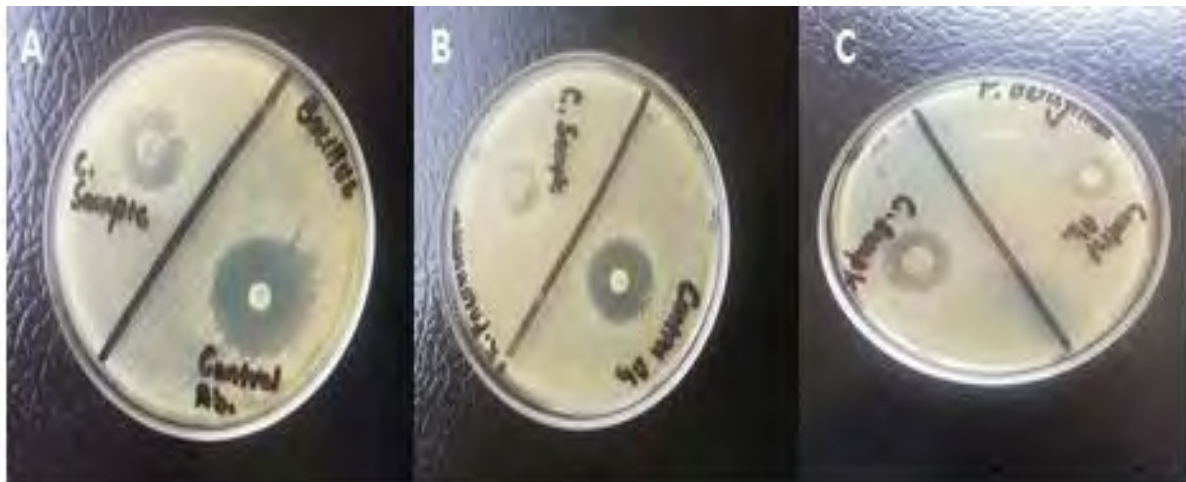
The agar well diffusion method was used to find out the anti-bacterial activity of the extracted cellulose nanofiber. The chloramphenicol c30 was used as a control. Table 3.3 shows sensitive (S) inhibitory zone diameter for the C30 is $\geq 18\text{mm}$, moderately sensitive (MS) zone is 13-17mm and Resistant (R) zone diameter is $\leq 12\text{mm}$ in accordance to [109]. *Klebsiella pneumonia* shows 16mm diameter against Sample and control antibiotic C30 shows 23mm diameter zone of inhibition. Sample shows the 22mm diameter zone of inhibition against *Bacillus subtilis*, but the control Ab shows the diameter of 30mm. Similarly, *Pseudomonas aeruginosa* shows 20mm against the sample and control antibiotic shows 16mm diameter zone of inhibition. so according to the disk diffusion zone diameter chart the bacterial strains *Klebsiella pneumonia* is MS, *Pseudomonas aeruginosa* is S and *Bacillus subtilis* is S and these bacterial strains are susceptible to the CNFs sample as shown in Table 3.4.

Table 3.3. Zone size interpretation of chloramphenicol

Control antibiotic (Ab)	Disc content	Diameter of zone of inhibition(mm)		
		Resistance	Moderate	Sensitive
Chloramphenicol©	30mcg	12	13-17	≥18

Table 3.4. Zone of inhibition's measurement

Bacterial strains	C-30	Sample	Result
<i>Klebsiella pneumonia</i>	23mm	16mm	MS
<i>Pseudomonas aeruginosa</i>	16mm	20mm	S
<i>Bacillus subtilis</i>	30mm	22mm	S

**Figure 3.13. Antibacterial activity of CNFs**

Growth inhibition caused by CNFs A) *Bacillus subtilis* plate with control and CNFs B) *K. Pneumonia* plate with control and CNFs C) *P. aeruginosa* plate with control (Ab) and CNFs.

CONCLUSION

The nanofibers synthesized from all the samples tested showed promising results and thus can hold a good potential for further usage by the industries. The cellulose in waste form if assessed and taken with such creative and smart way could aid commercially as well as minimizes the trash quantity globally. The work done here is a clear indication that waste form of cellulose holds good potential to bring novelties by chemical, biological and mechanical treatment.

Disclaimer

All the pictures that are included in this thesis are added solely by the author.

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