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Extraction of Nanocellulose from Rice Straw and Its Applications

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Abstract

The research aims to extract nanocellulose from rice straw. The extraction involved multistep process; alkaline treatment, H_2O_2 bleaching and acidic hydrolysis. Further nanocellulose was used for antibacterial hydrogel design for wound healing. Characterization of nanocellulose was carried out using XRD. The average particle size was 3.5 nm. The antibacterial activity of nanocellulose were evaluated against E. coli and S. aureus strains. Nanocellulose showed promising antibacterial activity compared to standard drugs gentamicin and ampicillin.

Key Words:

Nanocellulose, Rice straw, XRD, Antibacterial activity, Alkali treatment.

1. Introduction:

The industry has been concentrating on the Synthesis and manufacturing of ecologically friendly materials as a result of the growing awareness of environmental protection in recent years. Bio-based plant materials made of cellulose, hemicellulose,and lignin have received a lot of attention (Mtibe et al., 2015, 1). These materials have drawn a lot of attention because of their superior qualities, which include biodegradability,renewability and biocompatibility (Brigham et al., 2018, 201801). Cellulose is the most prevalent polymer on earth. In addition to being created by particular bacteria, it is present in plants, algae, and certain marine species (Klemm et al., 2011, 434). Cellulose is distinguished by its renewable source, great abundance, non-toxicity, and hierarchical structure, which allows for the isolation of cellulose-based items with nanoscale dimensions. Materials that are mostly made of cellulose and range in size from 1 to 100 nanometers are known as cellulose nanomaterials. Both nanocrystals (CNC) and microfibrillated cellulose (CNF) are included in this definition . The nanocrystalline cellulose, known by its abbreviation CNC, is a rod-shaped material with a high degree of stiffness (Filson et al., 2009, 1808). This is because CNCs have a high degree of crystallinity, ranging from 54% to 88%. The diameter of cellulose nanocrystals ranges from 3 to 50 nm, and their length ranges from 50 to 500 nm. With lengthy, soft chains that range in diameter from 10 to 500 nanometers and micrometer-scale lengths that frequently produce network-like patterns, cellulose nanofibrils (CNF), also known as nanofibrillated cellulose, differ from CNCs in that they contain substantial amorphous regions. Bacterial cellulose, denoted by BC, is another kind of nanocellulose that fits this description. Instead of coming from plant sources, the gluconoacetobacter xylinus family synthesizes and ecretes the nanofibrils, (Klemm et al., 2011, 5438; Iguchi et al., 2004, 845). Bacteria grow for a few days in a liquid culture medium that contains glucose, phosphorus, carbon, and nitrogen to make bacterial nanocellulose.

1.1.The composition and characteristics of nanocellulose

1.1.1. The composition of nanocellulose :

Nanocellulose is a nanostructured material derived from cellulose, the most abundant biopolymer on earth. It is composed mainly of long-chain glucose molecules arranged in a hierarchical structure, which gives it exceptional mechanical, thermal, and chemical properties.

1.1.2. Molecular Structure of Cellulose Basic Unit: The fundamental building block of cellulose is D-glucose, which links together through β-(1→4) glycosidic bonds to form long, linear polymer chains.

Hydroxyl Groups (-OH): These groups enable strong hydrogen bonding between chains influencing cellulose's crystallinity and water interactions.

Degree of Polymerization (DP): refers to the number of glucose units in a cellulose molecule, typically ranging from a few hundred to several thousand, depending on the source (Moon et al., 2011, 3941).

1.1.3. Hierarchical Structure of Cellulose

Cellulose fibers are composed of highly ordered crystalline regions and less ordered amorphous regions. This structure determines the mechanical strength and flexibility of cellulose-based materials.

Levels of Cellulose Structure:

- Cellulose molecules: formed by repeating glucose units.
- Elementary fibrils: aggregations of cellulose molecules into tightly packed structures.
- Microfibrils : groups of elementary fibrils held together by hydrogen bonds and Van der Waals forces.
- Cellulose fibers: larger fiber assemblies found in plant cell walls (Fig. 2, and Fig. 3).

1.1.4.Chemical Composition and Functional Groups

While cellulose is primarily composed of carbon (C), hydrogen (H),and oxygen (O), its surface chemistry can be modified for different applications.

Primary Functional Groups: Hydroxyl (–OH) groups which enable hydrogen bonding and modification (Moon et al., 2011, 3941).

Carboxyl (–COOH) groups: Can be introduced for increased water dispersion.

Aldehyde (–CHO) groups: Useful in chemical crosslinking and functionalization.

1.2. Nanocellulose properties

Nanocellulose has several unique properties that make it valuable for various applications. Here are its key properties:

- High Strength & Stiffness

Nanocellulose has a high tensile strength, comparable to steel, making it useful in reinforcing materials.

- Lightweight

It has a low density, making it ideal for lightweight composites (Page& Elhosseiny, 1983, 99).

- High Surface Area

Due to its nanostructure, it has a large surface area, which enhances its reactivity and bonding capabilities.

- Biodegradability

It is fully biodegradable and environmentally friendly.

- High Water Retention

Nanocellulose can absorb and retain large amounts of water, making it useful in hydrogels and medical applications.

- Transparency

Certain types, like cellulose nanofibrils (CNF), can be highly transparent, making them useful in optical and electronic applications (Nogi et al., 2009, 1595).

- Potential for Electrical Conductivity

When chemically treated or combined with conductive materials, nanocellulose can be used in electronic and energy storage applications (Wang et al., 2017, 1702240).

1.3. Types of Nanocellulose :

● **Cellulose Nanocrystals CNCs appeared as short, rigid rod-like. Structures with high crystallinity. They are typically extracted via acid hydrolysis.**

● **Cellulose Nanofibers (CNFs) depicted as long, flexible fibrils containing both crystalline and amorphous regions. These are produced through mechanical fibrillation.**

● **Bacterial Nanocellulose (BNC) shown as an ultra-fine network synthesized by bacteria, known for its high purity and excellent water-holding capacity (Fig. 1) (Klemm et al., 2005 , 3358; Klemm et al., 2011, 5438; Eichhorn et al., 2010, 1)**

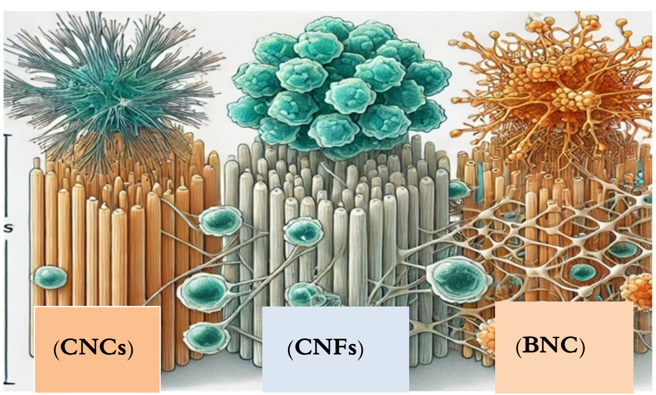


Figure 1: Types of nanocellulose

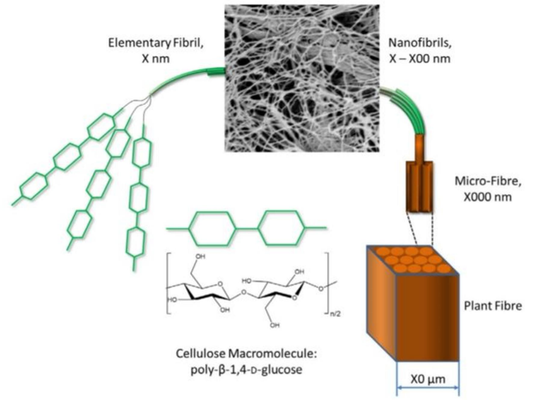


Figure 2. Hierarchical structure of plant fibres.

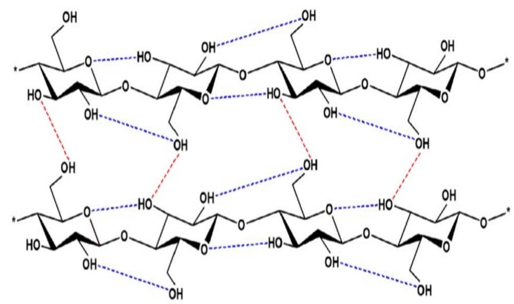


Fig. 3. Intramolecular (---) and intermolecular (---) hydrogen bonding networks in cellulose structure.

2. The theoretical framework

2.1. Methods of nanocellulose extraction

In general, synthetic routes for producing nanocellulose particle (NCP) can be classified into three board categories: physical, chemical, and biological methods .

2.1.1. Physical methods:

Nanocellulose can be synthesized through various physical methods such as evaporation/condensation, laser ablation, and arc discharge. The evaporation/condensation method typically uses a furnace tube at atmospheric pressure but faces challenges like slow thermal response and high energy consumption improved this method by utilizing a small ceramic heater with a localized heating area, which enabled better control over cooling and resulted in a high concentration of nanocellulose particles (NCPs) (Jung et al., 2006, 1662).

Laser synthesis involves laser ablation in a liquid medium without chemical reagents. The particle size and concentration are influenced by the laser fluence and number of pulses; higher fluence and longer exposure yield larger and more concentrated particles. This method allows for the production of pure nanocellulose colloids (Abid et al., 2002, 792).

The arc discharge technique proposed by Tien et al. creates cellulose suspensions in pure water without stabilizers or surfactants. In this method, cellulose wires submerged in water act as electrodes, and the arc discharge causes surface evaporation and condensation, forming stable nanocellulose particles ranging from 20–30 nm in size (Tien et al., 2008, 752).

2.1.2. Biological methods

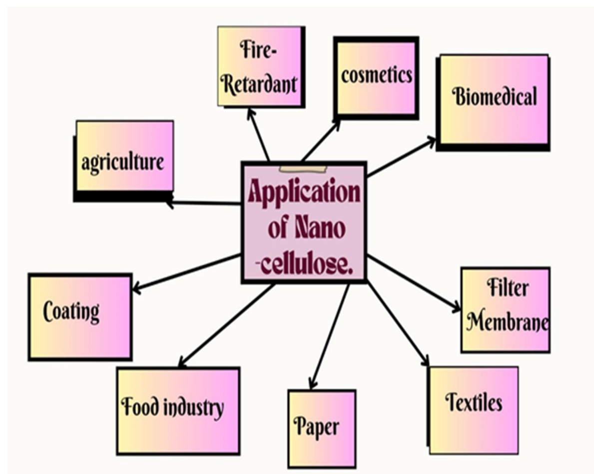
Enzymatic hydrolysis is a biological method used to convert cellulose fibers into washed cellulose by employing enzymes such as cellulase, cellobiohydrolase, and endoglucanase. These enzymes work by breaking the hydrogen bonds between cellulose fibers, allowing the process to proceed effectively despite its complex mechanism (Khalil et al., 2014, 649). During enzymatic activity, hemicellulose is removed, protecting cellulose from hydrolysis and yielding monosaccharides that can be fermented into bioethanol (Phanthong et al., 2018, 32). Celluloses and hemicelluloses act together to efficiently break down various lignocellulosic biomasses. Although the process is slower and conducted under milder conditions than acid hydrolysis, combining it with other techniques can reduce processing time (Wahlström et al., 2015, 694). A novel technique involved pretreating cellulose with an ionic solution, followed by laccase-enhanced enzymatic hydrolysis. This method resulted in nanocellulose with enhanced thermal properties, greater surface area, and higher crystallinity compared to conventional cellulose extraction methods (Moniruzzaman et al., 2013, 132).

2.1.3.Chemical methods:

The extraction of pure cellulose requires complex, multi-step procedures combining enzymatic, mechanical, and chemical treatments (Dufresne, 2013, 220). The main extraction methods for nanocellulose include acid hydrolysis (using sulphuric, acetic, or oxalic acid), bleaching (delignification using sodium chlorite or hydrogen peroxide), and alkali treatment (with sodium or potassium hydroxide).Cellulose nanocrystals (CNCs) are produced via direct acid hydrolysis, while cellulose nanofibers (CNFs) are obtained using mechanical methods such as grinding, cryocrushing, steam explosion, microfluidization, and high-pressure homogenization (Khalil et al., 2014, 649; Nechyporchuk et al., 2016, 2). Extraction techniques are categorized into four main types: pressure-induced, mechanical, enzyme-assisted, and green chemical methods. High-pressure homogenization (HPH) is particularly effective for CNC production on both industrial and laboratory scales. This method forces cellulose suspensions through a narrow nozzle at high pressure, creating nanoscale fibers through high shear force (Kargarzadeh et al., 2017, 1; Deepa et al., 2019, 41).Ionic liquids serve as effective solvents for CNC dissolution and further processing. Surface modification, such as TEMPO oxidation, converts primary hydroxyl groups into carboxyl groups, enhancing hydrophobicity (Saito et al., 2007, 2485; Fukuzumi et al., 2009, 162; Isogai et al., 2011, 71).Bacterial nanocellulose (BNC) can be produced using pretreatment, saccharification, and fermentation techniques. Recently, environmentally friendly methods like deep eutectic solvents, ammonium persulfate oxidation, and American value-added pulping have been explored for sustainable bioplastic production (Xia et al., 2014, 10586; Sirviö et al., 2015, 3401; Nelson et al., 2016, 267; Deepa et al., 2019, 41).

2.2. Application of Nanocellulose:

Nanocellulose have a lot of applications such as , paper, board, packing, food industry, coating, Textiles ,energy, sensors, inks, printing, construction, electronic, Adhesives, cosmetic, biomedical, Agriculture, detergent, hygiene …. Etc.



2.2.1. Biomedical applications

Cellulose nanoparticles are expected to find increasing use in biomedical applications. This is because of their biological qualities (low toxicity, biocompatibility, and biodegradability), enlarged surface area, and remarkable physical characteristics. Tissue skeleton for cell culture, excipient in pharmaceutical compositions, drug delivery, enzyme/protein immobilization and recognition, and antimicrobial materials are examples of molecule-level applications. Macroscopic biomaterials include replacements for blood vessels and soft tissues, skin, cartilage, and bone tissue, and antimicrobial materials (Lin & Dufresne, 2014, 302). The functional modification of cellulose nanoparticles can potentially be used to design potential biomedical applications. It is possible to take advantage of other characteristics such the ability to produce hydrogels, shear-thinning qualities, and spontaneous self-gelation. However, creating controlled properties, reliable and reproducible production techniques for biocompatible cellulose nanomaterials will be essential and beneficial to pave the way for greater acceptance of cellulose nanomaterials as a commer- cially available material in biomedical applications.

2.2.2. Nanocellulose in cosmetics

Nanocelluloses were first used as a film-forming ingredient in skincare and cosmetics formulations to provide a barrier that protected the skin from damaging UV rays (Mihranyan et al., 2012, 875).

Because of its many uses, nanocellulose can be used to enhance the texture, stability, and functionality of a variety of cosmetic products.

- Uses for skincare

In contemporary skincare formulas, nanocellulose is essential, especially in serums and moisturizers ,forms a protective layer over the skin to improve hydration (Dutta et al., 2022, 1).

Face masks offer a pliable, soft structure that fits the face and efficiently delivers active substances. Anti-aging creams increase the delivery of anti-aging ingredients, increase suppleness, and stop water loss.

- Uses for Haircare

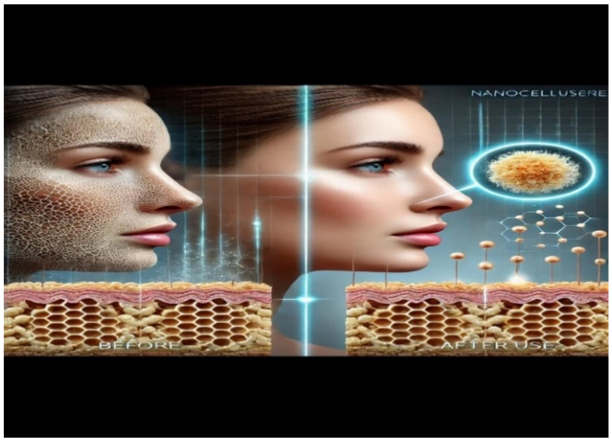
Nanocellulose utilized as a natural stabilizer and thickening in shampoos and conditioners and helps keep the scalp hydrated, which lessens dryness and irritation (Jorfi & Foster, 2015, 1).

Special characteristics of nanocellulose for skin application Some properties of nanocellulose are better than those of native cellulose, because of their high surface.

Reactivity and capacity to form a dense network structure with high intrinsic.

Strength and high stiffness along the single fibers improves (Blanco et al., 2018, 59; De Amorim et al., 2020, 851).

Effect of nanocellulose on the Skin, before and after use nanocellulose is a revolutionary ingredient in skincare, known for its exceptional ability to hydrate, smooth, and protect the skin. Before applying nanocellulose based skincare, the skin may appear dry, dull, and prone to fine lines. However, after use, nanocellulose penetrates deeply, improving moisture retention, enhancing elasticity, and promoting a radiant complexion. For example, in a clinical study, individuals who used a nanocellulose infused serum for four weeks reported a significant reduction in dryness and an improvement in skin texture (Bilal et al., 2020, 958).



(**Figure 4**) : **The figure demonstrates the skin before and after nanocellulose.**

2.2.3. Food industry

The toxicity of NFC and NCC before applying nano fibrillated cellulose (NFC) and nanocellulose crystals (NCC) in the food industry, it is essential to understand their toxicity. In this study, both were extracted from non-toxic or low-toxic raw materials (Lu et al., 2021, 101285).

There are only a few studies on the toxicity of NFC and NCC, and no evidence suggesting that they cause adverse effects on human health (Azeredo et al., 2017, 664; Vartiainen et al., 2011, 775).

Other safety studies on NFC have also demonstrated that NFC exhibited no sub lethal effects in vitro, as shown by the RNA inhibition test, no genetic toxicity in vitro, as confirmed by the ames test, and no cytotoxicity in vivo, as indicated by the nematode worm test (Alexandrescu et al., 2013, 1765; Pitk¨anen et al., 2014, 3871).

The applications of NFC and NCC in the food industry include three main categories: as food packaging materials, as food additives, and functional foods (Lu et al., 2021, 101285).

2.2.4. Coating

To enhance the coatings' mechanical qualities, CNC was added to the varnishes. The dispersion of the nanoparticles inside the matrix and their affinity for the matrix continue to be important factors in nano composites technology.

Cellulose nanoparticles can be added to water-based paints and varnishes to increase their durability. It is possible to improve the mechanical qualities, scratch resistance, and UV protection. Applications in this field may also benefit from cellulose nanoparticles' thickening, stabilizing, and iridescent qualities. A variety of coating applications benefit greatly from the shear-thinning action. Acryloyl chloride or alkyl quaternary ammonium bromides were used to modify CNC. The mechanical characteristics (hardness, adhesion, and resistance to abrasion and scratches) were examined and contrasted with the reference varnish devoid of nanoparticles. Wear resistance (abrasion and scratch) is increased by about 30–40% when modified CNC is added to UV-water-based coatings. Without sacrificing appearance. Water vapor transmission rate (WVTR) values were also shown to be limited by low concentrations of CNC to UV curable wood coating systems (Kaboorani et al., 2016, 17). Sag resistance is achievable due to the viscosity recovery effect (thixotropy) that cellulose nanoparticles produce, for certain uses, iridescent effects can also be profitably utilized, cellulose nanoparticles in

paints and coatings can also significantly reduce volatile organic compound (VOC) emissions (Dufresne et al., 2019, 76). Additionally, cellulose nanomaterial coatings can be utilized in bioengineering, cosmetics, biomedical, and medical applications (Dufresne et al., 2019, 76).

2.2.4.1. Paper

Use of nanocellulose fibers in Coating:

Nanocellulose fibers (CNF) are used in paper production to improve its properties, such as water resistance, surface strength, and smoothness. CNF enhances print resistance and increases the paper's resistance to environmental factors (Howard et al., 2015, 237; Sangl et al., 2013, 745; Tajvidi et al., 2016, 365). Improvement of physical and mechanical properties adding CNF to the coating helps improve the mechanical properties of the paper, such as air resistance, stiffness, and tensile strength. Increasing CNF concentration and the number of coating layers leads to significant improvements in these properties (Lavoine et al., 2014, 2879; Hubbe et al., 2017, 9556).

Use of Modified Coating Materials: Although starch can be used as a coating agent, it is often modified due to its lack of fluidity and film-forming properties however, nanocellulose materials are used to improve performance (Afra et al., 2016, 455).

2.2.4.2. In Textiles

Nanocellulose, a nanomaterial obtained from natural cellulose fibers, possesses exceptional properties such as high strength, low weight, and biodegradability. Its application in the textile industry is growing, as it enhances fabric durability and optimizes functional performance (Jiang& Hsieh, 2015, 60).

- Enhancing the Mechanical Properties of Fabrics

Nanocellulose is incorporated into textile fibers to improve tensile strength, flexibility, and tear resistance. When added, Nano cellulose forms a nano network that enhances fiber bonding, increasing fabric strength without compromising its elasticity (Siqueira et al., 2010, 2665).

- Water and Stain Resistance

Nanocellulose is used to enhance fabric properties, making them more resistant to water and stains. When applied as a coating on fibers, it creates a nano barrier that prevents liquid and contaminant absorption, thereby extending the fabric's lifespan and reducing the need for frequent washing (Ferrer et al., 2017, 574). Nanocellulose enhances fabric dye absorption, minimizing water and chemical usage in conventional dyeing methods. Additionally, it improves color fastness, contributing to the longevity of textile products (Dikshit et al., 2021, 902).

- Antibacterial Fabrics

By incorporating nanocellulose with silver nanoparticles or zinc oxide, antibacterial fabrics can be developed. These textiles efficiently prevent the growth of bacteria and other germs, making them perfect for protective gear and medical apparel (Hubbe et al., 2017, 2142).

- Recycling of Textile Waste

Cotton fabric waste can be a valuable source for extracting nano-cellulose, which can be repurposed to create innovative materials like bio-textiles and high-quality paper. This approach promotes a circular economy and helps mitigate the environmental footprint of the textile industry (Jiang& Hsieh, 2015, 60).

2.2.5.Nano cellulose in agriculture

Nano cellulose, a natural material derived from cellulose, is gaining popularity in agriculture due to its eco-friendly, biodegradable, and versatile properties. It offers smart solutions to improve crop growth, soil health, and modern farming techniques, making agriculture more efficient and sustainable.

Uses of Nano cellulose in Farming

- Improving Soil and Water Retention

Nano cellulose hydrogels help the soil retain moisture, reducing water loss and making crops more resistant to drought. In arid areas, this is very helpful (Li et al., 2021, 202).

- Boosting Plant Growth

Especially treated nanocellulose can help plants absorb nutrients better and developed stronger roots, leading to healthier crops (González et al., 2019, 745).

- Eco-Friendly Packaging for Farm Products

Nanocellulose can replace plastic in agricultural packaging, reducing pollution and making packaging more sustainable (Singh et al., 2022, 299).

2.2.6. Application of Cellulose Filter Membrane in Industrial Wastewater Treatment :

Cellulose filter membrane is used in the treatment of industrial wastewater heavy metals, suspended particles, polycyclic aromatic hydrocarbons, and biomolecular contaminants (Ram et al., 2019, 115). Because of its exceptional mechanical strength and specific surface area, its hydrophilicity, its surface groups for cationic or anion selective grafting, and its tunable surface chemistry, NC offers significant benefits in wastewater treatment (Cao et al., 2013, 120). Oil and organic contaminants may be extracted from wastewater using functionalized CNF (Thakur et al., 2016, 148). On the surfaces, membrane fouling takes place. Biofilms typically grow on membrane surfaces as a result of non-specific interactions between contaminants and the membrane surface. The growth of biofilms or blockage of membrane holes lowers the permeability and selectivity of the membrane. Because of its high surface permeability, porosity, and hydrophilicity, the CNC-based nanocomposite membrane exhibits good antifouling properties (Bai et al., 2020, 122919). NC's surface modification can direct its selectivity and enhance its adsorption effectiveness for a range of contaminants in aqueous solution. One explanation could be that modification increases the number of active binding sites accessible, which enhances ion exchange properties and creates novel functional groups that facilitate metal absorption (Mautner et al., 2018 , 20170043).

2.2.7. Applications of Nanocellulose as a Fire-Retardant Material in the Military

Fire-retardant additives are crucial because polymeric materials, which are frequently employed in the military, present serious fire dangers. Materials with high mechanical strength and the ability to endure explosions are needed for military applications. Construction, clothing, helmets, guns, fighter jets, and other military vehicles all use these materials, because of its durability and light weight, rigid polyurethane (PU) is frequently employed in military insulation; nonetheless, it is extremely combustible (luo et al., 2017, 2762). PU is frequently used with flame-retardant chemicals to increase safety.

Composites made on nanocellulose are showing promise as fire-retardant materials. These composites improve fire safety by lowering petrol emissions and smoke toxins , mainly assisting in reducing fire-related deaths, and protecting a variety of innovative military goods (Mngomezulu et al., 2017, 235). Solution impregnation and surface treatments are used to incorporate flame-retardant additives such as minerals, phosphorus, boric acid, and halogens into nanocellulose. Nanocellulose is a useful material for military purposes since it may sometimes be combined with polyurethane to increase its fire resistance.

3. Methods of Research and the tools used

3.1. Materials

Following washing, rice straw (RS) was gathered and allowed to dry in the sun until its moisture content dropped. All of the chemicals used to extract nanocellulose, such as sulfuric acid (5%) and hydrogen peroxide (10%) and sodium hydroxide (5%, 1%, and 0.25%), were bought and employed straight away. Nanocellulose hydrogel was prepared using glycerol, citric acid, and chitosan.

3.2. Methods

Co-recovery of nanocellulose

The design for cellulose recovery and cellulose in nano size synthesis was developed based on previous researches With some adjustments where necessary (Do et al., 2020, 21263; Vo et al., 2024, 29901; Ly et al., 2024, 101804).

In general, rice straw cellulose extraction would traditionally follow 4 steps, specifically (1) the first alkali pretreatment, (2) the second alkali pretreatment and (3) the main bleaching stag and (4) Acid hydrolysis.

3.2.1. The first alkali pretreatment

10 g of dried rice straw after being initially ground was pretreated two times. The first was at 1 % NaOH solution with a solid-to-liquid ratio of 1 g: 20 mL at 70-90 °C in 120 min with stirring to extract the solid pulp.

3.2.2. The second alkali pretreatment

The second alkali treatment used NaOH 5% solution at a solid-to-liquid ratio of 1 g: 25 mL at 80 °C in 90 min under constant mixing. The yellow solid was the result of the process.

3.2.3. H_2O_2 Bleaching

The solid left would be bleached using 10 % of H_2O_2 solution with NaOH powder (0.25%)

with a solid–to–liquid ratio of 1 g: 20 mL at 70–80 °C in 45 min under continuous stirring to produce a yellowish white hue in the cellulose.

3.2.4. Acid Hydrolysis

The preparation of nanocellulose was conducted using diluted H_2SO_4 (5%) with a solid–to–liquid ratio of 1 g: 20 mL for one h under tightly controlled temperature range of 40–42 °C (Vo et al., 2024, 29901). The mixture after the reaction was diluted 10 times with distilled water. The solid phase was separated from the acidic liquid by dialysis and consequently, freeze–dried and ground to yield nanocellulose (NC) in powder form (Fig. 5).

3.2.5. Preparation of nanocellulose Hydrogel

Disperse nanocellulose (1 g) in 20 ml of distilled water, then add 4 ml of the nanocellulose solution to 10 ml of 6% citric acid to increase cohesion, followed by adding 4ml of glycerol to enhance flexibility and durability with heating at 70 °C for 40 minutes, then add 0.1 g of chitosan as an antimicrobial agent, with good stirring to ensure uniform dispersion and crosslinking, and finally pour the solution into a glass plate and place it in the freezer at –50 °C for 24–48 hours to obtain a porous structure that helps absorb liquids (Fig. 6).

3.2.6. Characterization techniques

X–ray diffraction analysis was done using a XRD equipment model D2 phaser 2nd gen (Brucker, Germany).

3.2.7. Antibacterial activity of nanocellulose

Test Method:

The agar well diffusion method was used to evaluate the antimicrobial activity of the tested compounds.

Tested Microorganisms:

Staphylococcus aureus was used as gram–positive bacteria, and Escherichia coli as gram–negative bacteria:

Culture Media Used:

Nutrient agar for bacterial strains

Standard Drugs:

Ampicillin for Gram–positive bacteria

Gentamicin for Gram–negative bacteria

DMSO used as the negative control (solvent)

Testing Procedure (Scott, 1989,161).

Sterile media were poured into Petri dishes (20–25 ml) and allowed to solidify.

Bacterial suspensions were prepared at a concentration of 1.5×10^8 CFU/mL (McFarland 0.5).

Turbidity was adjusted to OD = 0.1 at 625 nm.

A sterile cotton swab was used to spread the suspension on agar.

Wells (6 mm in diameter) were made and filled with 100 µL of the test solution.

Plates were incubated at 37°C for 24 hours.

Statistical Analysis:

One–way ANOVA followed by Duncan's multiple range test was used to analyze differences.

Results were expressed as Mean ± Standard Error (SE).

Significance levels:

$P < 0.05$: statistically significant

P < 0.01: highly significant

P < 0.001: very highly significant

Methodology of MIC

For each strain, three to five isolated colonies were selected from the fresh agar plate and were transferred into a tube containing 3–4 ml of sterile broth medium. The bacterial suspension was mixed well and incubated at 35–37°C for 2–6 h. The turbidity of the bacterial suspension should be equal to or greater than the turbidity of a McFarland Standard 0.5. After that, 1 mg of the tested compound (antimicrobial agent) was dissolved in 1 ml DMSO and two-fold serial dilution was done using broth medium. A fixed volume of the prepared bacterial inoculum was added to each tube and incubated for at 37 °C 16–20 h. The MIC is defined as the lowest concentration of the antimicrobial agent that inhibits visible growth of the tested isolate as observed with the unaided eye (Chudáčková et al., 2010, 62).

4. Results of Research

4.1. Characterizations

The XRD analysis revealed the characteristics of amorphous nanocellulose. Further, XRD patterns of nanocellulose (Fig. 7) showed three peaks at 2 theta angles of 15.9 °, 22.5 °, and 33°, which correspond to crystal planes (110), (200), and (004), respectively (Baati et al., 2017, 2350). The average crystal size of nanocellulose is 3.5 nm as recorded by XRD.

Figure(5): rice straw, celluolose, and nanocellulose

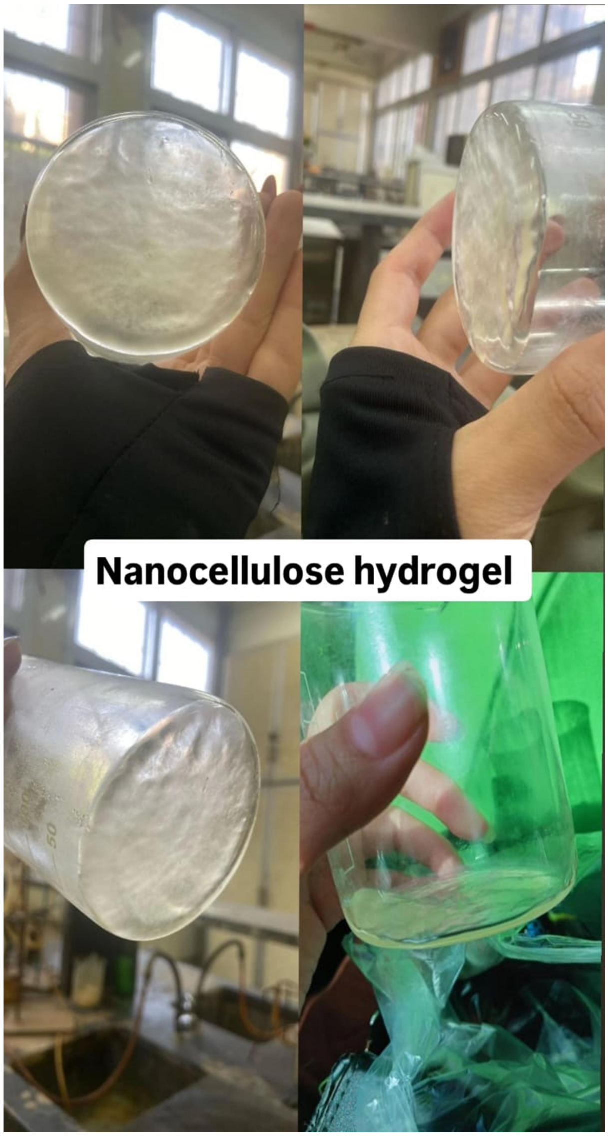


Figure (6): nanocellulose hydrogel.

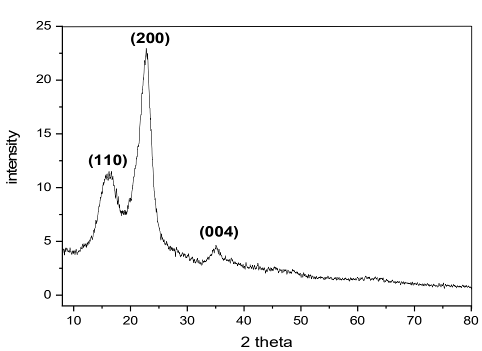


Figure (7): XRD pattern of nanocellulose

4.2. Antibacterial activity

Table 1 Antibacterial activity of nanocellulose

Sample / Microorganism	Nanocellulose	Standard
Gram negative bacteria		**Gentamicin**
***Escherichia coli* (ATCC: *10536*)**	**5**	**31.25**
Gram positive bacteria		**Ampicillin**
***Staphylococcus aureus* (ATCC:13565)**	**2.5**	**62.5**

Nanocellulose was screened for antibacterial potential and exhibited promising broad spectrum antibacterial activity against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive). The antibacterial activity is presented in terms of minimum inhibitory concentration in Table 1. From the MIC values, it is observed that, compared with standard antibiotics gentamicin (MIC 31.25 µg/mL), nanocellulose showed promising antibacterial activity against E. Coli test strain with MIC 5 µg/mL. In the case of S. Aureus strain, the minimum inhibitory concentration (MIC) of nanocellulose 2.5 µg/mL is much lower than that of ampicillin (62.5 µg/mL), indicating higher potency. This suggests that nanocellulose is significantly more effective than standard drugs gentamicin and ampicillin.

To increase the activity of nanocellulose as inhibiting agent by adding partials of nano silver or nano zinc oxide or nano copper oxide or chitosan

5. Interpretation of Results

In the present work, we successfully extracted nanocellulose from rice straw biomass using alkaline pretreatment, H_2O_2 and acidic hydrolysis. The formation of nanocellulose was confirmed by XRD pattern. Additionally, three peaks at two theta angles of 15.9 °, 22.5 °, and 33 ° were visible in the XRD patterns of nanocellulose (Fig. 3). These peaks correspond to crystal planes (110), (200), and (004), respectively (Baati et al., 2017, 2350; El-Sheekh et al., 2023, 10188). According to XRD, nanocellulose has an average crystal size of 3.5 nm.

Nanocellulose was screened for antibacterial potential and exhibited promising broad spectrum antibacterial activity against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive). The antibacterial activity is presented in terms of minimum inhibitory concentration in Table 1. From the MIC values, it is observed that, compared with standard antibiotics gentamicin (MIC 31.25 μg/mL), nanocellulose showed promising antibacterial activity against E. coli test strain with MIC 5 μg/mL. In the case of S. aureus strain, the minimum inhibitory concentration (MIC) of nanocellulose 2.5 μg/mL is much lower than that of ampicillin (62.5 μg/mL), indicating higher potency. This suggests that nanocellulose is significantly more effective than standard drugs gentamicin and ampicillin

The above results offer evidence on the use of the antibacterial nanocellulose in dressings, wound bandage and home cleaning.

Nanocellulose-based hydrogels are promising in tissue engineering, drug delivery, and wound healing due to their high surface area, biocompatibility, and mechanical strength. They support cell growth, enable controlled drug release, and enhance wound healing by retaining moisture and mimicking natural tissue structure (De France et al., 2017, 4609; Temo et al., 2024, 7692; He et al., 2022, 830).

So, we design antibacterial nanocellulose hydrogel that helps in wound healing beacause of its good liquid absorbtion ability, moisturizing ability and non-toxic properties. Further, chitosan as a natural polymer with inherent antibacterial properties, it can be blended with nanocellulose to improve its stability and enhance antimicrobial performance.

6. Conclusion

Cellulose was extracted from rice straw using a multistep extraction and pretreatments with alkali, and H_2O_2. Acid hydrolysis was carried out to obtain nanocellulose. Screening the antibacterial properties of nanocellulose was also, carried out. The result showed promising antibacterial activity against E. coli and S. aureus strains. So, we designed antibacterial nanocellulose hydrogel.

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References and Sources

Abid, J. P., Wark, A. W., Brevet, P. F., & Girault, H. H. (2002). Preparation of silver nanoparticles in solution from a silver salt by laser irradiation. Chemical Communications, (7), 792–793.

Afra, E., Mohammadnejad, S., & Saraeyan, A. (2016). The use of cellulose nanofibrils as a coating material and their effects on paper properties. Progress in Organic Coatings, 101, 455–460.

Alexandrescu, L., Syverud, K., Gatti, A., & Chinga-Carrasco, G. (2013). Cytotoxicity tests of cellulose nanofibril-based structures. Cellulose, 20, 1765–1775.

Azeredo, H. M., Rosa, M. F., & Mattoso, L. H. C. (2017). Nanocellulose in bio-based food packaging applications. Industrial Crops and Products, 97, 664–671.

Baati, R., Magnin, A., Boufi, S. (2017). High solid content production of nanofibrillar cellulose via continuous extrusion. *ACS Sustain. Chem. Eng., 5 (3)*, 2350–2359.

Bai, L., Wu, H., Ding, J., Ding, A., Zhang, X., Ren, N., ... & Liang, H. (2020). Cellulose nanocrystal-blended polyethersulfone membranes for enhanced removal of natural organic matter and alleviation of membrane fouling. *Chemical Engineering Journal*, *382*, 122919–122930.

Bilal, M., Iqbal, H. M. N., Hu, H., Wang, W., & Zhang, X. (2020). State-of-the-art advancements in nanocellulose-based materials for cosmetic and biomedical applications. International Journal of Biological Macromolecules, 162, 958–975.

Blanco, A., Monte, M. C., Campano, C., Balea, A., Merayo, N., & Negro, C. (2018). Nanocellulose for industrial use: Cellulose nanofibers (CNF), cellulose nanocrystals (CNC), and bacterial cellulose (BC). In Handbook of Nanomaterials for Industrial Applications, 50, 59-64

Brigham, T., Barden, C., Dopp, A. L., Hengerer, A., Kaplan, J., Malone, B., ... & Nora, L. M. (2018). A journey to construct an all-encompassing conceptual model of factors affecting clinician well-being and resilience. NAM perspectives, 8(1), 201801b

Cao, X.; Huang, M.; Ding, B.; Yu, J.; Sun, G. Robust polyacrylonitrile nanofibrous membrane reinforced with jute cellulose nanowhiskers for water purification. Desalination 2013, 316, 120–126.

Chudáčková, E., Bergerová, T., Fajfrlík, K., Červená, D., Urbášková, P., Empel, J., ... & Hrabák, J. (2010). Carbapenem-nonsusceptible strains of Klebsiella pneumoniae producing SHV-5 and/or DHA-1 β-lactamases in a Czech hospital. FEMS microbiology letters, 309(1), 62–70.

De Amorim, J. D. P., de Souza, K. C., Duarte, C. R., da Silva Duarte, I., de Assis Sales Ribeiro, FFf., Silva, G.S.,& Sarubbo, L. A. (2020). Plant and bacterial nanocellulose: production, properties and applications in medicine, food, cosmetics, electronics and engineering. Environmental chemistry Letters, 18, 851–869.

De France, K. J., Hoare, T., & Cranston, E. D. (2017). Review of hydrogels and aerogels containing nanocellulose. Chemistry of Materials, 29(11), 4609–4631.

Deepa, B., Chirayil, C. J., Pothan, L. A., & Thomas, S. (2019). Lignocellulose-based nanoparticles and nanocomposites: preparation, properties, and applications. In Lignocellulose for future bioeconomy, 41–69.

Dikshit, P. K., Kumar, J., Das, A. K., Sadhu, S., Sharma, S., Singh, S., ... & Kim, B. S. (2021). Green synthesis of metallic nanoparticles: Applications and limitations. Catalysts, 11(8), 902–937.

Do, N. H., Pham, H. H., Le, T. M., Lauwaert, J., Diels, L., Verberckmoes, A., ... & Le, P. K. (2020). The novel method to reduce the silica content in lignin recovered from black liquor originating from rice straw. Scientific reports, 10(1), 21263–21273.

Dufresne, A. (2013). Nanocellulose: a new ageless bionanomaterial. Materials today, 16(6), 220–

Dufresne, A. (2019). Nanocellulose processing properties and potential applications. *Current Forestry Reports*, *5*, 76–89.

Dutta, S. D., Patel, D. K., & Lim, K. T. (2019). Functional cellulose-based hydrogels as extracellular matrices for tissue engineering. Journal of Biological Engineering, 13(1), 55–74.

Eichhorn, S. J., Dufresne, A., Aranguren, M., Marcovich, N. E., Capadona, J. R., Rowan, S. J., ... & Peijs, T. (2010). Current international research into cellulose nanofibres and nanocomposites. *Journal of materials science*, *45*, 1–33.

El-Sheekh, M. M., Yousuf, W. E., Kenawy, E. R., & Mohamed, T. M. (2023). Biosynthesis of cellulose from Ulva lactuca, manufacture of nanocellulose and its application as antimicrobial polymer. *Scientific Reports*, *13 (1)*, 10188–10200.

Ferrer, A., Pal, L., & Hubbe, M. (2017). Nanocellulose in packaging:

Advances in barrier layer technologies. Industrial Crops and Products, 95, 574–582.

Filson, P. B., Dawson–Andoh, B. E., & Schwegler–Berry, D. (2009). Enzymatic–mediated production of cellulose nanocrystals from recycled pulp. *Green Chemistry*, *11*(11), 1808–1814.

Fukuzumi, H., Saito, T., Iwata, T., Kumamoto, Y., & Isogai, A. (2009). Transparent and high gas barrier films of cellulose nanofibers prepared by TEMPO–mediated oxidation. Biomacromolecules, 10(1), 162–165.

González, L., Sánchez, J., & García, M. (2019). Enhancement of plant growth using nanocellulose: Improved nutrient absorption and root development. Journal of Plant Nutrition, 42(5), 745–753

He, P., Dai, L., Wei, J., Zhu, X., Li, J., Chen, Z., & Ni, Y. (2022). Nanocellulose–based hydrogels as versatile drug delivery vehicles: A review. International Journal of Biological Macromolecules, 222, 830–843.

Howard, K. W., & Hodgson, K. T. (2015). Journal of Coatings Technology and Research, 12, 237–245.

Hubbe, M. A., Rojas, O. J., Lucia, L. A., & Sain, M. (2017). Cellulosic nanocomposites: A review. BioResources, 12(1), 2142–2233.

Hubbe, M. A., Tayeb, P., Joyce, M., Tyagi, P., & Pal, L. (2017). Rheology of nanocellulose–rich aqueous suspensions: A review. Bioresources, 12(4), 9556–9661.

Iguchi, K. I., Matsuura, K., McNyset, K. M., Peterson, A. T., Scachetti-Pereira, R., Powers, K. A., ... & Yodo, T. (2004). Predicting invasions of North American basses in Japan using native range data and a genetic algorithm. *Transactions of the American Fisheries Society*, *133*(4), 845–854.

Isogai, A., Saito, T., & Fukuzumi, H. (2011). TEMPO–oxidized cellulose nanofibers. nanoscale, 3(1), 71–85.

Jiang, F., & Hsieh, Y. L. (2015). Cellulose nanocrystal isolation from tomato peels and assembled nanofibers. Carbohydrate polymers, 122, 60–68.

Jorfi, M., & Foster, E. J. (2015). Recent advances in nanocellulose for biomedical applications. Journal of Applied Polymer Science, 132(14), 1–19.

Jung, J. H., Oh, H. C., Noh, H. S., Ji, J. H., & Kim, S. S. (2006). Metal nanoparticle generation using a small ceramic heater with a local heating area. Journal of Aerosol Science, 37(12), 1662–1670.

Kaboorani A, Auclair N, Riedl B, Landry V. (2016). Physical and morphological properties of UV–cured cellulose nanocrystal (CNC) based nanocomposite coatings for wood furniture. Prog Org Coat, 93, 17–22.

Kaboorani, A., Riedl, B., Blanchet, P., Fellin, M., Hosseinaei, O., & Wang, S. (2012). Nanocrystalline cellulose (NCC): A renewable nano-material for polyvinyl acetate (PVA) adhesive. European Polymer Journal, 48(11), 1829-1837.

Kargarzadeh, H., Ioelovich, M., Ahmad, I., Thomas, S., & Dufresne, A. (2017). Methods for extraction of nanocellulose from various sources. Handbook of nanocellulose and cellulose nanocomposites, 1, 1-49.

Khalil, H. A., Davoudpour, Y., Islam, M. N., Mustapha, A., Sudesh, K., Dungani, R., & Jawaid, M. (2014). Production and modification of nanofibrillated cellulose using various mechanical processes: a review. Carbohydrate polymers, 99, 649-665.

Klemm, D., Heublein, B., Fink, H. P., & Bohn, A. (2005). Cellulose: fascinating biopolymer and sustainable raw material. *Angewandte chemie international edition*, *44*(22), 3358-3393.

Klemm, D., Kramer, F., Moritz, S., Lindström, T., Ankerfors, M., Gray, D., & Dorris, A. (2011). Nanocelluloses: a new family of nature-based materials. *Angewandte Chemie International Edition*, *50*(24), 5438-5466.

Klemm, F., Bleckmann, A., Siam, L., Chuang, H. N., Rietkoetter, E., Behme, D., ... & Pukrop, T. (2011). β-catenin-independent WNT signaling in basal-like breast cancer and brain metastasis. *Carcinogenesis*, *32*(3), 434-442.

Lavoine, N., Desloges, I., Khelifi, B., & Bras, J. (2014). Impact of different coating processes of microfibrillated cellulose on the mechanical and barrier properties of paper. Journal of Materials Science, 49, 2879-2893.

Li, X., Zhang, L., & Li, Z. (2021). Hydrogels based on nanocellulose for improving soil water retention and crop resistance to drought. Journal of Agricultural Science, 13(3), 202-210.

Lin, N., & Dufresne, A. (2014). Nanocellulose in biomedicine: Current status and future prospect. *European Polymer Journal*, *59*, 302-325.

Lu, Q., Yu, X., Yagoub, A. E. A., Wahia, H., & Zhou, C. (2021). Application and challenge of nanocellulose in the food industry. Food Bioscience, 43, 101285.

Luo, F., Wu, K., Li, D., Zheng, J., Guo, H., Zhao, Q., & Lu, M. (2017). A novel intumescent flame retardant with nanocellulose as charring agent and its flame retardancy in polyurethane foam. Polymer Composites, 38(12), 2762-2770.

Ly, T. B., Tran, N. T., Pham, C. D., Nguyen, D. D., Mai, P. T., & Le, P. K. (2024). Innovative method for rice straw valorization into nanocellulose, lignin and silica. *Bioresource*

Technology Reports, *25*, 101804–101812.

Mautner, A., Mayer, F., Hervy, M., Lee, K. Y., & Bismarck, A. (2018). Better together: synergy in nanocellulose blends. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 376(2112), 20170043–20170061.

Mihranyan, A., Ferraz, N., & Strømme, M. (2012). Current status and future prospects of nanotechnology in cosmetics. Progress in materials science, 57(5), 875–910

Mngomezulu, M. E., & John, M. J. (2017). Thermoset cellulose nanocomposites: flammability characteristics. Handbook of Nanocellulose and Cellulose Nanocomposites, 1, 235–272.

Moniruzzaman, M., & Ono, T. (2013). Separation and characterization of cellulose fibers from cypress wood treated with ionic liquid prior to laccase treatment. Bioresource technology, 127, 132–137.

Moon, R. J., Martini, A., Nairn, J., Simonsen, J., & Youngblood, J. (2011). Cellulose nanomaterials review: structure, properties and nanocomposites. *Chemical Society Reviews*, *40*(7), 3941–3994.

Mtibe, A., Linganiso, L. Z., Mathew, A. P., Oksman, K., John, M. J., & Anandjiwala, R. D. (2015). A comparative study on properties of micro and nanopapers produced from cellulose and cellulose nanofibres. *Carbohydrate Polymers*, *118*, 1–8.

Nechyporchuk, O., Belgacem, M. N., & Bras, J. (2016). Production of cellulose nanofibrils: A review of recent advances. Industrial Crops and Products, 93, 2–25.

Nelson, K., Retsina, T., Iakovlev, M., van Heiningen, A., Deng, Y., Shatkin, J. A., & Mulyadi, A. (2016). American process: production of low cost nanocellulose for renewable, advanced materials applications. Materials research for manufacturing: An industrial perspective of turning materials into new products, 267–302.

Nogi, M., Iwamoto, S., Nakagaito, A. N., & Yano, H. (2009). Optically transparent nanofiber paper. *Advanced materials*, *21*(16), 1595–1598.

Page, D. H., & Elhosseiny, F. (1983). The mechanical–properties of single wood pulp fibers. 6. Fibril angle and the shape of the stress–strain curve. *Pulp & Paper–Canada*, *84*(9), TR99–T100

Phanthong, P., Reubroycharoen, P., Hao, X., Xu, G., Abudula, A., & Guan, G. (2018). Nanocellulose: Extraction and application. Carbon Resources Conversion, 1(1), 32–43.

Pitk¨anen, M., Kangas, H., Laitinen, O., Laitinen, O., Sneck, A., Lahtinen, P., Peresin,M. S.,& Niinim¨ aki, J.(2014).Characteristics and safety of nano-sized cellulose fibrils.Cellulose, 21, 3871–3886.

Ram, C., Zaman, B., & Dhir, A. (2019). Study on corrosion investigations in industrial effluents: a review. Corrosion Reviews, 37(2), 115-130.

Saito, T., Kimura, S., Nishiyama, Y., & Isogai, A. (2007). Cellulose nanofibers prepared by TEMPO-mediated oxidation of native cellulose. Biomacromolecules, 8(8), 2485-2491.

Sangl, R., Auhorn, W., Kogler, W., & Tietz, M. (2013). Surface sizing and coating. Handbook of Paper and Board, 745-784.

Scott, A. C. 1989. Laboratory control of antimicrobial therapy, p. 161-181. In J. G. Collee, J. P. Duguid, A. G. Fraser, and B. P. Marmion (ed.), Mackie & McCartney-practical medical microbiology, 13th ed. Churchill Livingstone.

Scott, D., Barber, J. B. P., Levine, E. L., Burrill, W., & Roberts, S. A. (1998). Radiation-induced micronucleus induction in lymphocytes identifies a high frequency of radiosensitive cases among breast cancer patients: a test for predisposition?. British journal of cancer, 77(4), 614-620.

Singh, P., Kumar, S., & Shah, R. (2022). Eco-friendly nanocellulose-based packaging materials in agriculture: A sustainable approach. Journal of Agricultural Packaging Technology, 11(4), 299-307.

Siqueira, G., Bras, J., & Dufresne, A. (2010). Cellulose whiskers versus microfibrils: Influence of the nature of the nanoparticle and its surface functionalization on the thermal and mechanical properties of nanocomposites. Biomacromolecules, 11(10), 2665–2673.

Sirviö, J. A., Visanko, M., & Liimatainen, H. (2015). Deep eutectic solvent system based on choline chloride-urea as a pre-treatment for nanofibrillation of wood cellulose. Green Chemistry, 17(6), 3401-3406.

Tajvidi, M., Gardner, D. J., & Bousfield, D. W. (2016). Cellulose nanomaterials as binders: Laminate and particulate systems. Journal of Renewable Materials, 4(5), 365–376.

Tamo, A. K. (2024). Nanocellulose-based hydrogels as versatile materials with interesting functional properties for tissue engineering applications. Journal of Materials Chemistry B, 12(32), 7692-7759.

Thakur, V. K., & Voicu, S. I. (2016). Recent advances in cellulose and chitosan based membranes for water purification: A concise review. Carbohydrate polymers, 146, 148-165.

Tien, D. C., Liao, C. Y., Huang, J. C., Tseng, K. H., Lung, J. K., Tsung, T. T., ... & Stobinski, L. (2008). Novel technique for preparing a nano-silver water suspension by the arc-discharge method. Reviews on Advanced Materials Science, 18(8), 752–758.

Vartiainen, J., Pöhler, T., Sirola, K., Pylkkänen, L., Alenius, H., Hokkinen, J., ... & Laukkanen, A. (2011). Health and environmental safety aspects of friction grinding and spray drying of microfibrillated cellulose. Cellulose, 18, 775–786.

Vo, N. T., Pham, C. D., Ly, T. B., Dang, M. D., Do, N. H., & Le, P. K. (2024). Valorization of rice straw for valuable materials: towards a zero–waste recovery process. Biomass Conversion and Biorefinery, 14(23), 29901–29910.

Wahlström, R. M., & Suurnäkki, A. (2015). Enzymatic hydrolysis of lignocellulosic polysaccharides in the presence of ionic liquids. Green Chemistry, 17(2), 694–714.

Wang, X., Yao, C., Wang, F., & Li, Z. (2017). Cellulose-based nanomaterials for energy applications. Small, 13(42), 1702240–1702274.

Xia, S., Baker, G. A., Li, H., Ravula, S., & Zhao, H. (2014). Aqueous ionic liquids and deep eutectic solvents for cellulosic biomass pretreatment and saccharification. RSC advances, 4(21), 10586–10596.