



RESEARCH ARTICLE

NANOCELLULOSE FROM MICROBIAL FACTORIES: SUSTAINABLE PRODUCTION AND CHARACTERIZATION

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ABSTRACT

Nanocellulose, a versatile nanomaterial with remarkable properties, has gained significant attention for its potential in various industries, including healthcare, packaging, and materials science. In recent years, microbial factories have emerged as a sustainable and efficient source of nanocellulose. Nanobiocellulose refers to a nanoscale form of cellulose produced through biological processes, primarily by bacteria or other microorganisms. Nanobiocellulose is characterized by its nanoscale fibril structure, high aspect ratio, and unique physical and chemical properties, making it an increasingly valuable nanomaterial with diverse applications. This review explores the sustainable production through various microbial strains, cultivation methods, and bioprocess optimization strategies. It also explores advanced characterization techniques for nanocellulose, encompassing microscopy, spectroscopy, mechanical testing, thermal analysis, and barrier properties assessment. The applications of sustainable bacterial nanocellulose are also explored comprehensively along with challenges pertaining to sustainability, productivity, and other emerging trends.

1. INTRODUCTION

Nanobiocellulose, a nanoscale form of cellulose produced by microbial fermentation, has emerged as renewable and enduring nanomaterial with numerous practical uses across various fields. Cellulose, a natural polymer found abundantly in plant cell walls, has long been recognized for its sustainable and eco-friendly properties. The traditional source of cellulose, primarily derived from plant-based materials like wood and cotton, has limitations in terms of resource availability, energy consumption, and environmental impact. However, nanobiocellulose takes cellulose to the next level, offering unique nanoscale characteristics that make it an attractive material for innovative and environmentally conscious solutions. The interest in nanobiocellulose has surged in recent years due to its sustainable, biocompatible, biodegradable and remarkable mechanical strength (Klemm et al., 2001). As a sustainable material, nanobiocellulose is derived from renewable sources, often utilizing agricultural waste or low-cost carbon sources. Its production requires fewer resources and energy compared to traditional cellulose extraction methods, such as logging or cotton farming. This inherent sustainability aligns with the global push toward more environmentally friendly materials and practices.

Nanobiocellulose is primarily produced through the cultivation of cellulose-producing microorganisms, notably strains of bacteria like *Komagataeibacter xylinus*, which secrete cellulose nanofibrils during their growth and metabolic processes. These nanofibrils form an intricate network of nanocellulose, resulting in a material with exceptional properties at the nanoscale (Lin et al., 2013). Bacterial nanocellulose, also known as BNC or bacterial cellulose, is a distinctive type of exopolysaccharide produced by certain bacteria. It is composed solely of linked units of D-glucopyranose connected by 1,4-glycosidic bonds. This polymer's composition includes ultra-fine nanofibrils forming a 3D web-like structure, stabilized by both intermolecular and intramolecular

hydrogen bonds. The molecular formula for bacterial nanocellulose, $(C_6H_{10}O_5)_n$, matches that of plant-derived cellulose. However, there are notable differences in their physical and chemical properties. Unlike plant-based cellulose, bacterial nanocellulose does not contain lignin, hemicelluloses, or pectin. These components are typically present in cellulose obtained from plants. As a result, the purification process for bacterial nanocellulose is relatively straightforward and requires less energy compared to the purification of cellulose from plant sources (Huang et al., 2014).

Bacterial nanocellulose (BC) possesses distinctive characteristics that set it apart from plant-based cellulose. These distinct characteristics encompass an elevated degree of polymerization and exceptional tensile strength, primarily ascribed to its intricate and web-like network structure as documented by Iguchi et al. (2000), Tsouko et al. (2015). In contrast to cellulose obtained from plants, bacterial cellulose (BC) fibers showcase a larger specific surface area, an increased capacity to hold water, and a prolonged drying time (Sulaeva et al., 2015, Meftahi et al., 2009). Importantly, the production of BC is environmentally friendly since it doesn't require harsh chemical treatments for cellulose extraction and purification (Shi et al., 2014). Various factors, such as the specific bacterial strain, the composition of the culture medium, and the cultivation conditions, affect the yield and properties of BC. The culture medium's composition plays a pivotal role in shaping the material's morphology and physical characteristics, thereby influencing its potential applications.

Nanobiocellulose's remarkable properties, including its high surface area, flexibility, transparency, and capacity to hold water, have led to its adoption in a diverse range of applications. In the field of biomedicine, it finds applications in wound dressings, scaffolds for tissue engineering, and systems for delivering drugs, offering biocompatible and biodegradable alternatives (Jafari et al., 2017). In the food industry, nanobiocellulose is used for edible coatings and sustainable packaging materials, contributing

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to reduced food waste and extended shelf life. Its applications extend to environmental remediation, textiles, composites, electronics, and beyond, highlighting its versatility. In this era of sustainability and advanced materials, nanobiocellulose holds promise as an eco-friendly, innovative, and multifunctional nanomaterial. Its unique combination of properties and its environmentally responsible production make it a noteworthy candidate for addressing a variety of contemporary challenges. As research continues to advance in the field of nanobiocellulose, it is poised to play an increasingly significant role in shaping the future of sustainable materials and technologies. This review underscores the pivotal role of microbial factories and nanotechnology in sustainable nanocellulose production. It offers a comprehensive overview of sustainable practices, characterization techniques, and applications, thereby contributing to the broader understanding and utilization of nanocellulose in a sustainable and environmentally conscious manner.

2. SUSTAINABILITY SPOTLIGHT

Nanobiocellulose embodies sustainability through a range of compelling characteristics and applications. Derived from renewable sources and produced by microbial factories, it offers an eco-friendly alternative to traditional cellulose extraction methods, significantly reducing resource depletion and environmental impact. Its cost-effective production aligns with economic sustainability, making it accessible across industries. Nanobiocellulose is versatile, finding applications in areas like healthcare, packaging, and environmental remediation, where it contributes to waste reduction, improved resource efficiency, and lower energy consumption. Its biodegradability further ensures minimal long-term environmental impact. By fostering responsible resource management, economic viability, and environmental stewardship, nanobiocellulose plays a pivotal role in advancing sustainability in diverse sectors. This review underscores the pivotal role of microbial factories and nanotechnology in sustainable nanocellulose production. It offers a comprehensive overview of sustainable practices, characterization techniques, and applications, thereby contributing to the broader understanding and utilization of nanocellulose in a sustainable and environmentally conscious manner.

3. ROLE OF MICROBIAL FACTORIES IN NANOCELLULOSE PRODUCTION

Nanocellulose, a nanoscale cellulose material with unique properties, has garnered significant interest as it has been drawn to because of its potential applications in numerous fields, including biomedicine, packaging, textiles, and advanced materials. In recent years, microbial factories, especially certain strains of bacteria, have emerged as promising and sustainable sources for nanocellulose production.

Microbial factories, such as bacteria belonging to the genera *Achromobacter*, *Azotobacter*, *Alcaligenes*, *Agrobacterium*, *Aerobacter*, *Gluconacetobacter*, *Pseudomonas*, *Dickeya*, *Rhizobium*, *Sarcina* and *Rhodobacter*, have demonstrated a remarkable ability to synthesize nanocellulose (Lin et al., 2013; Romling and Galperin, 2015). These microorganisms produce cellulose nanofibrils as a natural byproduct of their metabolism during the fermentation process. In particular, *Komagataeibacter xylinus*, formerly known as *Gluconacetobacter xylinus*, has been extensively studied for its nanocellulose-producing capabilities (Klemm et al., 2001). Other bacterial strains within the same species, such as *Komagataeibacter hansenii*, *Komagataeibacter nataicola*, *Komagataeibacter medellinensis*, *Komagataeibacter oboediensis*, *Komagataeibacter saccharivorans*, *Komagataeibacter pomaceti* and *Komagataeibacter rhaeticus* also hold significant potential for bacterial cellulose production (Castro et al., 2012; Li et al., 2019). These bacteria, known as acetic acid bacteria, show remarkable capability to transform a variety of carbon sources such as glucose, mannitol, glycerol, fructose, xylose, dicarboxylic acids or carbon sources like glucose, fructose, mannitol, xylose, glycerol, dicarboxylic acids or dihydroxyacetone into extended straight chains of β -D-(1 \rightarrow 4)-glucan. Subsequently, they expel these chains through multiple openings in their cytoplasmic membrane (Aswini et al., 2020).

Another promising avenue explored to improve the production and characteristics of bacterial nanocellulose (BC) involves the creation of genetically modified strains. Researchers have delved into the utilization of genetically engineered bacteria to enhance BC production and boost its overall yield. Furthermore, genetic engineering can be harnessed to

enhance the structural properties of BC, making it even more well-suited for specific applications as discussed by (Ul-Islam et al., 2017). The utilization of microbial factories for nanocellulose production offers several sustainability advantages. Unlike traditional cellulose extraction from plants, which involves resource-intensive processes, microbial synthesis produces cellulose with lower energy consumption and reduced environmental impact. Moreover, microbial factories can utilize renewable and inexpensive carbon sources, such as glucose or glycerol, further enhancing sustainability. Achieving high yields and desired properties of nanocellulose from microbial factories often involves bioprocess optimization. Factors like culture conditions (e.g., pH, temperature), carbon sources, oxygen supply, and agitation are carefully controlled to enhance nanocellulose production efficiency (Jozala et al., 2016).

Microbial nanocellulose offers the advantage of being produced in a controlled environment, allowing for the precise tailoring of its properties, such as crystallinity, porosity, and mechanical strength, to meet specific application requirements (Kralisch et al., 2015). While microbial factories are promising at the laboratory scale, scaling up nanocellulose production to meet industrial demands remains a challenge. Researchers are exploring various bioreactor designs and fermentation strategies to optimize large-scale production (Gao et al., 2019). Microbial cells represent a sustainable and efficient route for the production of nanocellulose. These microorganisms offer advantages in terms of resource utilization, environmental impact, and tunable nanocellulose properties, making them a promising source for the development of sustainable and eco-friendly nanomaterials. Further research and optimization are essential to realize the full potential of microbial factories in nanocellulose production.

4. BIOPROCESS OPTIMIZATION

The production of bionanocellulose hinges significantly on various parameters, including temperature, pH, incubation period, dissolved oxygen (DO) levels, composition of the growth media, methods of cultivation i.e. agitated or stationary and additional operational factors (Cielecka et al., 2020). Acetic acid bacteria produce bacterial nanocellulose through oxidative fermentation in a nutrient-rich culture medium like Schramm-Hestrin (SH). The thickness of the wet membranes can vary, reaching several centimeters, depending on factors such as the bacterial strain, growth duration, and environmental conditions. However, despite reaching relatively high levels of about 6–7 g/L in some cases (e.g., like 2.7–3.0 g/L for strain *Ga. hansenii* 53582 and around 1 g/L for reference strains), the actual process yield remains unsatisfactory when considering the operational costs at an industrial scale (Morgan et al., 2013).

The use of synthetic mediums for bacterial cellulose production is often considered costly. As a result, contemporary researchers are actively exploring alternative and more economical resources for this purpose. Some of these alternative resources include corn waste, tobacco waste extract, distillery effluents, spent materials from the wine industry, and various fruit by products, among others (Li et al., 2012; Matsuoka et al., 1996; García-Lomillo et al., 2017). Numerous studies are underway to improve the yield of BNC, with the aim of reducing operational expenses and increasing the yield of biocellulose synthesis. The successfully utilized economic by products from wine processing to produce Bacterial Nanocellulose (BNC) films (Zhao et al., 2018). Their results showed that these waste materials can effectively serve as substrates in fermentation media, offering abundant nutrients for the growth of *Ga. xylinus* BC-11.

In another study, production of BNC through *Ga. hansenii* UAC09 by utilizing extract of coffee cherry husk as carbon source was done by (Rani and Appaiah, 2013). This innovative approach resulted in a substantial increase in the yield of BNC as compared to culture media rich in glucose. Furthermore, fruit juices have emerged as viable culture mediums for bacterial cellulose production. Researchers have successfully employed fruit juices from pomegranate, pineapple, watermelon, muskmelon, orange, tomato as well as other sources like sugar molasses, sugarcane juice, starch hydrolysate, coconut water, and coconut milk as alternate carbon sources to foster the production of biocellulose (Hungund et al., 2013). These innovative approaches not only contribute to cost-effectiveness but also demonstrate the versatility of resources available for this biofabrication process.

The production of bacterial cellulose can be impacted by supplementary

by-products, like gluconic acid, as noted by (Zhang et al., 2016). To enhance the efficiency of the Hestrin Schramm medium, various carbon sources like mannitol, cellobiose, sucrose, xylose, and maltose can be used interchangeably with glucose. Although glucose is a preferred energy source for bacteria and a direct contributor to cellulose production, recent investigations have revealed that fructose exhibits significant potential for cellulose production compared to other carbon sources.

Carbon sources for production of bacterial cellulose can also be derived from spent materials and by products of beverage and sugar sector, offering a cost-effective approach. Furthermore, enhancing bacterial cellulose production can be achieved by incorporating additives into the culture medium. Common additives like agar, carboxymethyl cellulose, sodium alginate, xanthan, glycerol and ethanol have demonstrated substantial capability in promoting bacterial cellulose synthesis. Moreover, recent studies propose that incorporating buffers into the medium helps maintain the necessary pH level for bacterial cultivation, can significantly boost bacterial cellulose production, as demonstrated in the study by (Castro et al., 2012). Recent research indicates that lactate and methionine, when combined with a fructose-based medium, can increase cellulosic yield in *K. xylinus* subgroups. Additionally, corn steep liquor stands out with a remarkable 90% potential for bacterial cellulose production, as reported by (Cheng et al., 2017). These diverse strategies and resources contribute to the continuous improvement and cost-effectiveness of bacterial cellulose synthesis processes. Studies have demonstrated that augmenting conventional growth media with alternate additives, such as lactic acid, acetic acid, ethanol, sodium citrate, glycerol, vitamins, and polymers like agar or carboxymethylcellulose (CMC), can enhance the production of bacterial nanocellulose (Molina-Ramírez et al., 2018, Lu et al., 2015, and Cheng et al., 2011). To notably achieved a remarkable 279% increase in bacterial nanocellulose yield by adding alternative energy sources such as ethanol and acetic acid to the SH medium can impact the BNC production process (Molina-Ramírez et al., 2018). It's important to consider various factors like the medium volume, surface area, shape of the bioreactor, intensity of agitation, and other parameters, as they collectively influence the overall yield of biopolymer synthesis in bacterial nanocellulose (BNC) production and significantly impact the characteristics of the developed membranes (Jedrzejczak-Krzepkowska et al., 2016).

One promising avenue for boosting bacterial nanocellulose production and cost reduction involves the development of genetically engineered strains capable of producing BNC. Kuo et al. (2015) created a mutant of *Komagataeibacter xylinus* by intentionally disrupting the gene responsible for membrane pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ GDH) using homologous recombination. Similarly, detailed a fascinating genetic alteration of *K. xylinus* by introducing the gene that encodes *Vitreoscilla* hemoglobin (VHb) in a heterogeneous manner, resulting in a 25% increase in BNC yield under low-oxygen conditions (Liu et al., 2018). Another impressive example of the application of genetically engineered microorganisms comes from who achieved a 28-fold increase in biocellulose production from lactose in comparison to the parental strain (Battad-Bernardo et al., 2004).

Irrespective of whether static or agitated cultivation methods are employed, optimizing and controlling certain key process variables during the cultivation process are crucial for enhancing bacterial cellulose (BC) yield (Lee et al., 2014). One of these pivotal variables is pH. The ideal pH range for both cell growth as well as BC production varies depending on the microbial strain but typically falls within the range of 4.0 to 7.0 (Reiniati et al., 2017). It is advisable to continuously monitor and control pH levels to achieve maximum yield of bacterial cellulose. This is crucial because the pH of the culture medium may vary over time, primarily due to the buildup of secondary metabolites like acetic, gluconic or lactic acids. These compounds are produced as sugars and nitrogen sources are consumed (Lee et al., 2014). Aeration is another critical process variable that requires careful management during cultivation. Bacteria producing bio cellulose are highly aerobic, making the provision of suitable oxygen levels crucial, as emphasized by Wu and Li (2015). Inadequate content of dissolved oxygen can impede culture growth and biocellulose production, while excessive oxygen concentration may enhance the gluconic acid yield (Ul-Islam et al., 2017; Lee et al., 2014). Additionally, precise regulation of temperature during BC production is also essential, as it has a significant impact on BC yield and its properties. For instance, studies on bacterial cellulose (BC) production by *Komagataeibacter xylinus* B-12068 have

indicated that the optimal temperature range for both cell growth and BC production is relatively limited, typically between 28°C to 30°C. However, the physiological temperature range is broader, spanning from 20°C to 37°C (Volova et al., 2018).

The production process begins with the inoculation of a culture medium, often placed in shallow trays. This inoculation leads to the formation of a bacterial cellulose (BC) pellicle, that floats on the surface due to the generation of CO₂ bubbles by the bacteria. Cultivation typically lasts from 5 to 20 days, during which the biocellulose layer gradually fills the tray. The production of biocellulose depends on the surface area in contact at air and liquid interface. However, traditional static culture methods are time-consuming and have low productivity, which hampers their suitability for industrial applications (Lin et al., 2013). In static cultures, cellulose membrane developed in the medium tends to ensnare the bacteria, restricting their access to oxygen. Additionally, as nutrients are consistently utilized, their concentration diminishes over time, thereby constraining bacterial cellulose (BC) production (Esa et al., 2014). To address these limitations, recent advancements have led to the development of bioreactors which can produce high yields of BC under almost static conditions like the rotary biofilm contactor reported Horizontal Lift Reactor and aerosol bioreactor by (Hornung et al., 2007; Kralisch et al., 2009; Kim et al., 2007). Another innovative approach involves the use of airlift bioreactors, where oxygen is consistently delivered from the bottom of the reactor into the culture medium, ensuring a sufficient and continuous oxygen supply (Ul-Islam et al., 2015). This method is more energy-efficient and induces lower shear stress in comparison to stirred-tank reactors (Wu and Li, 2015). In contrast, agitated cultivation techniques involve constant mixing of oxygen into the medium, leading to higher bacterial cellulose (BC) yields compared to static cultures, thereby contributing to cost reduction (Ul-Islam et al., 2015). However, a notable drawback of agitation is the heightened risk of mutations in cellulose-producing cells due to the turbulence and shear stress (Park et al., 2004; Kim et al., in 2007). The bacterial cellulose (BC) produced, regardless of the cultivation method used, can be extracted from the culture medium through uncomplicated procedures. In agitated cultivation, BC can be isolated through filtration or centrifugation, whereas in static cultures, the BC film formed at the liquid-air interface is easily collectible. However, the BC obtained from the broth is not entirely pure, as it may contain impurities like residual cells and nutrients. Consequently, purification is essential before its intended application.

The extent of purification needed depends on the specific use. For medical purposes, a more rigorous purification process is essential to eliminate impurities compared to applications in the food industry and food packaging sector. Several drying methods can be used for BC, including ambient temperature drying, freeze drying, oven drying and supercritical drying. The choice of method should align with the desired properties and characteristics for the final utilization of the biocellulose. This selection is crucial, as the drying process can significantly influence the properties of BC (Vasconcellos and Farinas, 2018; Zeng et al., 2014).

5. NANOTECHNOLOGY IN SUSTAINABLE PRODUCTION OF BIOCELLULOSE

Nanobiotechnology, the convergence of nanotechnology and biotechnology, holds immense promise in transforming traditional production processes into sustainable and eco-friendly practices. By leveraging the unique properties of nanoscale materials and biological systems, nanobiotechnology offers innovative solutions for resource-efficient and environmentally conscious production. This interdisciplinary field combines the precision of nanotechnology with the versatility of biotechnology to address complex challenges in various industries (Cui and Lieber, 2001). One of the significant contributions of nanobiotechnology is its role in enabling green synthesis processes. By utilizing biological entities, such as enzymes, microorganisms, or plant extracts, in conjunction with nanomaterials as catalysts or templates, nanobiotechnology promotes cleaner and more sustainable chemical reactions (Puri et al., 2012). For instance, bio-nanocatalysts can enhance the selectivity and efficiency of reactions, reducing the need for hazardous chemicals and minimizing waste generation (Kah et al., 2018).

Biocellulose is a natural, highly pure form of cellulose produced by certain bacterial strains. It has gained significant attention in various sectors, including pharmaceuticals, food, cosmetics, and biomedicine, due to its

remarkable properties like high water retention capacity, high purity, biocompatibility and biodegradability. To further enhance its properties, nanoparticles and additives can be incorporated into biocellulose materials. Nanocellulose exhibits distinct features that set it apart from conventional materials. These characteristics include unique morphology, crystalline structure, geometric dimensions, high specific surface area, liquid crystalline behavior, alignment and orientation, rheological properties, barrier properties, surface chemical reactivity, biodegradability, biocompatibility, mechanical reinforcement and non toxicity. Leveraging these exceptional properties, the envisioning of both "nano-enhanced" and entirely novel "nano-enabled" products has emerged. These products span a spectrum from common applications like composite reinforcement, rheological modifiers, or paper additives to advanced applications such as tissue engineering, drug delivery, and functional materials (Osterberg and Cranston, 2014). The fibrillated network of bacterial nanocellulose (BNC) yields excellent mechanical properties, including remarkable elasticity (with a Young modulus ranging from 15 to 18 GPa) and tensile strength. These qualities position BNC at a significantly high level compared to other natural polymers (Gorgieva and Trcek, 2019). BNC possesses impressive characteristics, including its potential to absorb and retain large quantity of water for extended periods, release substances gradually over time, maintain its structural integrity under various conditions, and have a porous structure. It also exhibits a partially crystalline nature, which gives it both mechanical strength and resistance to heat. BNC is highly transparent, and it can be shaped into three-dimensional structures. Additionally, it forms a thin, fibrous network, further enhancing its versatility and utility (Ye and Kalendar, 2023; Ullah et al., 2022). Nanoparticles such as cellulose nanocrystals (CNCs) have been widely used to strengthen biocellulose materials. A study demonstrated the successful incorporation of CNCs into bacterial cellulose, resulting in improved tensile strength, including increased mechanical properties by (Moon et al., 2011). Silver nanoparticles have shown promise in conferring antimicrobial properties to biocellulose. Research investigated the incorporation of silver nanoparticles in bacterial cellulose for potential applications in wound dressing by (Cacicedo et al., 2016). They found that the incorporation of silver nanoparticles increased the antibacterial potential of the biocellulose material. Biocellulose-based materials are investigated for applications in delivering pharmaceuticals. Researchers like have investigated the potential of biocellulose as a drug carrier (Iguchi et al., 2000). While this study did not specifically use nanoparticles, it highlights the versatility of biocellulose as a drug delivery platform. Has utilized montmorillonite nanoparticles to enhance the barrier properties of biocellulose films (Montesano et al., 2017). Their study showed that the addition of nanoparticles led to enhanced water vapor barrier properties in the biocellulose-based material.

While nanobiotechnology holds great promise for sustainable production, it also raises ethical and safety concerns. The potential environmental and health impacts of engineered nanoparticles, as well as issues related to responsible innovation, must be carefully considered. Effective risk assessment, regulation, and public engagement are essential to ensure that nanobiotechnology contributes positively to sustainability without unintended consequences (Lin and Lin, 2009). Nanobiotechnology represents a transformative force in achieving sustainability across various industries. By harnessing the unique capabilities of nanomaterials and biological systems, nanobiotechnology enables greener synthesis processes, environmental remediation, sustainable agriculture, and advanced biopharmaceuticals. However, it is crucial to navigate the challenges and ethical considerations to fully unlock the potential of this interdisciplinary field for sustainable production.

6. CHARACTERIZATION TECHNIQUES FOR BACTERIAL NANOCELLULOSE

To utilize the full potential of microbial nanocellulose, the knowledge of its structure at the nanoscale is necessary. Following are some of the techniques used for the characterization of microbial nanocellulose.

6.1 Particle Size

The apparent particle size of nanocellulose, particularly the hydrodynamic diameters can be measured using Dynamic light scattering (DLS) (Beyene et al., 2018). When nanocellulose samples undergo laser scanning, microscopic particles within colloids exhibit Brownian motion, leading to fluctuating intensities of light scattering over time. Particle-by-particle light scattering is used in nanoparticle tracking analysis to offer detailed particle size data with high resolution and the Stoke-Einstein relation may

be used to determine hydrodynamic diameter (d_H) (Foster et al., 2018). High-resolution microscopy and light scattering may be used to determine particle size and size distribution (Singh et al., 2021).

6.2 Turbidity

The haziness of nanocellulose suspensions can be employed to assess the evenness of nanoparticle distribution. Less turbidity suggests more fibrillated nanocellulose or less aggregated nanoparticles. The presence of particles scatters light, generating turbidity. With a UV-Vis spectrophotometer, one can utilize turbidity measurements to quantify the variation in light scattered, transmitted, or absorbed over time (Foster et al., 2018).

6.3 Crystallinity Index

Cellulosic materials are divided into two types: crystalline or ordered and amorphous or disordered. The crystallinity index (CrI) or degree of crystallinity is characterized as the proportion of crystalline regions to amorphous regions, influences the characteristics of microbial nanocellulose. Many methods, including X-ray diffraction (XRD), nuclear magnetic resonance (NMR), Raman spectroscopy and FTIR, may be used to quantify CrI , although XRD is the most commonly used. Many researchers employ the Segal approach to analyze the X-ray diffractogram. CrI may be computed using this approach by:

$$CrI\% = \frac{(I_{002} - I_{am})100}{I_{200}}$$

where I_{002} is the highest diffraction intensity of the crystalline material's (002) reflection and I_{am} depicts the height of the minimum between the 002 and 101 peaks. The approach, however, can only be used to offer an approximate measure of crystallinity degree (Park et al., 2010). Ruland's technique is a more stringent methodology for estimating the crystallinity, and it is used by XRD software such as Topas. Presuming that the complete diffracted intensity arises from both amorphous and crystalline areas, the cellulosic nanomaterials' XC (degree of crystallinity) may be calculated using the following formula.

$$X_c\% = \frac{A_c}{A_c + A_a} \times 100$$

where A_c is the area of crystalline region and A_a is the area of amorphous region of the deconvoluted XRD pattern after background spectrum elimination. This deconvolution technique has been employed to examine the structural characteristics of various polymeric materials. used XRD to estimate the crystallinity of bacterial cellulose and bacterial cellulose nanocrystals, used Scherrer's equation to compute crystallite size (CS) normal to the (1200) planes (Vasconcelos et al., 2017).

6.4 Young's Modulus

Young's modulus serves as a measure of a material's stiffness or its ability to resist elastic deformation under a load, calculated from the slope of the initial linear region on the stress-strain curve. The Young's modulus of microbial nanocellulose filaments has been approximated. Initially, the mechanical properties of microbial cellulose were mainly explored in sheet form, revealing moduli ranging between 30 and 40 GPa in early studies (Tajima et al., 1995). Subsequently, the Young's modulus of a single microbial cellulose filament was determined using a Raman spectroscopic technique. This method involved measuring the shift in the central position of the 1095 cm^{-1} Raman band, corresponding to the stretching of the glycosidic bond in the cellulosic backbone (Hsieh et al., 2008). The outcome of this approach indicated a Young's modulus of 114 GPa for a single filament of microbial cellulose.

6.5 Degree Of Polymerization

Cellulose polymer is a straight-chain homopolysaccharide composed of anhydrous D-glucose units linked by -1,4-glycosidic linkages. The degree of polymerization (DP), representing the number of anhydrous glucose units in the polymer chain, is a crucial structural characteristic with a substantial impact on mechanical properties. DP can be expressed as a weighted average, numerical average, or viscosity average (Hu et al., 2015). In the case of *Acetobacter xylinum* bacterial cellulose, DP was assessed viscometrically in cadoxen (Okajima et al., 1991). The investigation revealed a rapid increase in the polymerization degree of bacterial cellulose during the culture stage. Under synchronous culture conditions, the maximum polymerization value for bacterial cellulose reached 1500. Remarkably, bacterial nanocellulose demonstrated an exceptionally high DP, with values reaching up to 8000 (Börjesson and Westman, 2015).

6.6 Atomic Force Microscopy

It is utilized for investigating topography and three-dimensional surface morphology (Vahabi et al., 2013). The method can also be applied to study the mechanical properties and interactions of nanocellulose in diverse solutions. This includes examining the reactions between cationic and anionic functionalized cellulose nanocrystals and colloid probes. Additionally, the approach assesses the elastic modulus and provides insights into the adhesive responsiveness of cellulose microcrystals in biological systems, composites, gels, and emulsions. Furthermore, it offers information about the thickness of particles deposited on surfaces, but with low lateral resolution.

6.7 Transmission Electron Microscopy

TEM is used as one of the most useful technique for determining the shape and size of biocellulose nanofibrils (CNF) and biocellulose nanocrystals (CNC). In this approach, electrons traverse through the sample, providing a better nanometer and sub-nanometer lateral resolution for as low as 0.2 nanometer. There is no sampling issue in TEM as it can measure large amounts of particles. TEM gives information about the structure and morphology of the sample. Moreover, TEM can be used to study the degradation and biosynthesis mechanism of nanocellulose (Kaushik et al., 2015).

6.8 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is a surface imaging technique that generates secondary electrons from the sample surface through inelastic scattering. This method is versatile and can be employed to evaluate the size, shape, dispersion, and aggregation of nanoparticles. Field emission SEM is particularly capable of resolving nanoparticle sizes as small as 1 nm. Additionally, SEM can be utilized to examine the impact of various treatments on the morphology of a sample (Chieng et al., 2017). The choice between SEM and Transmission Electron Microscopy (TEM) for characterizing cellulose nanoparticles depends on the specific parameters that need measurement. SEM is a preferred option for confirming the presence of cellulose nanoparticles, assessing their quality, analyzing dispersion, and conducting low-resolution qualitative analysis.

6.9 Fourier Transform Infrared Spectroscopy (FTIR)

Functional groups within nanoparticles can be identified through Fourier Transform Infrared Spectroscopy (FTIR). In FTIR spectroscopy, the sample is exposed to infrared light, causing molecular vibrations in the sample when the frequency matches the natural frequency of vibration. Each functional group exhibits a specific absorption frequency, resulting in distinct peaks for each functional group. Therefore, different molecular configurations produce unique infrared spectra. The instrument measures the amount of light absorbed or transmitted and presents it as a function of wave number. In a study comparing bacterial cellulose generated by *Gluconoacetobacter xylinus* in the Hestrin and Schramm (HS) medium with regular cellulose spectra using FTIR, bands in the ranges of 3700-3000, 2970-2800, 1100-1073, and 1034-1023 cm^{-1} were found to be similar to ordinary cellulose. The only notable difference observed was the displacement of FTIR bands in the range of 1620-1380 cm^{-1} due to the bacterial cellulose being treated with NaOH (Auta et al., 2017).

6.10 Raman Spectroscopy

Raman spectroscopy is a robust technique for studying cellulose materials, offering capabilities in the identification of cellulose nanomaterials and the assessment of cellulose crystallinity. It is particularly useful for investigating nanocellulose composites, such as examining the dispersion of cellulose nanocrystals (CNCs) in polymers and evaluating the interactions between CNCs and the matrix (Agarwal, 2017). Raman spectroscopy, complementary to IR spectroscopy, probes the vibrational characteristics of molecules. In a study conducted by Hsieh et al. (2008) on bacterial cellulose samples from *Gluconoacetobacter xylinum*, Raman spectroscopy was employed. Peaks observed with an 830 nm IR diode laser resembled the cellulose-I structure, featuring dominant bands at 1095 cm^{-1} attributed to the glycosidic stretching mode in the cellulose structure, or to coupling between C-O and C-C bonds within pyranose rings and glycosidic linkages. Shifts in these bands under applied stress provided insights into BNC (Bacterial Nanocellulose) molecule deformation, revealing connections to stress in cellulose polymer chains. Remarkably, CNC derived from bacterial cellulose exhibited spectra

comparable to CNC derived from wood pulp (Hsieh et al., 2008).

6.11 Nuclear Magnetic Resonance (NMR) Spectroscopy

The mechanism of NMR spectroscopy is spinning shifts in atom nuclei in the presence of an external magnetic field at radio frequencies. Because this effect is limited to nuclei with spins other than zero, only nuclei having an odd mass number, such as ^1H , ^{35}Cl , produce NMR spectra. For example, ^{12}C and ^{16}O nuclei are unable to produce NMR spectra, but nuclei with half spin are preferred, such as ^{13}C , ^1H , and ^{15}N . ^{13}C NMR was performed on both untreated bacterial nanocellulose and enzyme-treated bacterial nanocellulose. Bacterial cellulose was discovered to be extremely crystalline based on the strength of peaks at 89-84 ppm. When enzymes were applied to bacterial cellulose, the resulting spectrum diverged from that of untreated bacterial cellulose. It was characterized by predominant polysaccharide signals within the 55 to 110 ppm range, accompanied by less distinct signals (Auta et al., 2017).

6.12 Thermogravimetric analysis (TGA)

Thermogravimetric analysis is a method that monitors the variation in mass of a material as affected temperature while using a controlled temperature program (Ioelovich, 2016). TGA was used to investigate the thermal stability of bacterial cellulose both before and after enzymatic hydrolysis (Auta et al., 2017). After 200°C, the sample degradation began for untreated BC. The enzymatically treated sample exhibited stability up to approximately 150°C before undergoing disintegration, and the mass loss for cellulase-treated bacterial cellulose was higher compared to untreated bacterial cellulose.

6.13 Differential Scanning Calorimetry (DSC)

DSC is utilized to evaluate changes in enthalpy caused by variation in physical and chemical characteristics of a material as a function of temperature or time at a constant applied temperature (Marway, 2017). DSC was performed on biocellulose both before and after enzymatic hydrolysis in a work by (Auta et al., 2017). The glass transition temperature (T_g) and melting temperature (T_m) of untreated and treated cellulose were measured to describe their thermal transitions. T_g and T_m values for untreated bacterial nanocellulose were about 38°C and 117°C, respectively. The bacterial cellulose showed stability up to about 200°C, after which it began to decompose, with disintegration happening around 338°C. The glass transition temperature (T_g) of the cellulase treated samples was determined as 115°C, with no evidence of a melting transition. Disintegration commenced above 150°C for the enzyme-treated samples, with substantial breakdown at 170°C. BC's reduced thermostability might be attributed to enzyme hydrolysis, which produces lower molecular weight oligosaccharides.

7. APPLICATIONS OF SUSTAINABLE BACTERIAL NANOCELLULOSE

Though the chemical structure of bacterial nanocellulose is similar to plant cellulose, its biological, mechanical, and physical features are different and much better than plant cellulose. Because of these extraordinary properties, bacterial nanocellulose has found its applications in many fields such as drug delivery, biomedicine, cosmetics, food, etc. (Lin et al., 2013). The purest form of bacterial nanocellulose is obtained through the process of biosynthesis which does not contain lignin, pectin, and hemicellulose (Chawla et al., 2009). Therefore, it is easy to refine bacterial nanocellulose as compared to plant cellulose (Shi et al., 2014). The aforementioned features made bacterial nanocellulose an interesting material to be applied in various sectors. These applications are summarized in figure 1 and discussed with detail in the subsequent sections.

7.1 Food Applications

In 1992, the Food and Drug Administration (FDA) has regarded bacterial nanocellulose as "generally recognized as safe" (GRAS) dietary fibre (Shi et al., 2014; Park et al., 2010). Bacterial nanocellulose has numerous applications in the food industry due to its pure nature, wide range of shapes and textures (e.g., whiskers, films, multi-shaped pulps, filaments, spheres, and particles), ability to adapt in situ modifications, like colour and flavour of culture media, and simple manufacturing procedure (Shi et al., 2014). Considering the features listed above, bacterial nanocellulose could be utilized as an adjunct in eatables and the food business, such as classic desserts, reduced cholesterol diets, vegan substitute for meat, additive in beverages or food, and as a food packaging material, among other things (Azeredo et al., 2019). The shapes and purposes of bacterial nanocellulose in commercial applications vary, depending on the

fermentation processes used. A jelly-like bacterial nanocellulose pellicle is formed when it is produced through static fermentation, which is used as a raw material for food ingredients and desserts. On the other hand, the agitated fermentation produces a hydrocolloidal bacterial nanocellulose which is being utilized as suspenders in beverages and thickeners (Zhong, 2020). The human body cannot synthesize the enzyme cellulase, as a result, the digestion and absorption of bacterial nanocellulose does not occur in the intestine and is excreted in the feces (Fontana et al., 2017).

In 1960s and 1970s, bacterial nanocellulose was first used in foods in Philippines as a dessert called 'nata de coco' (Iguchi et al., 2000). The dessert Nata de coco is rich in fiber, and low in calorie and cholesterol (Ullah et al., 2016). Just like the dietary fiber obtained from everyday foods, it can also provide benefits to the health of human beings by lowering the occurrence of chronic illnesses such as CVD, obesity, and diabetes (Anderson et al., 2009). It is commonly consumed in the form of a dessert because of its smooth and cold sensation, crisp texture, and juicy flavor. It's also used as an ingredient to impart different texture and flavors in meals (Shi et al., 2014). In the present time, nata de coco is abundantly present in the markets all over the globe in various flavors and textures.

Bacterial nanocellulose is used as an operational addition in the food-related industries. Several drinks and soupy meals containing particle components like oatmeal, soybean milk, coffee, milk beverages etc. needs suspension. To suspend particles, surfactant agents and thickeners such as pectin, xanthan gum, soybean polysaccharide, and carboxymethyl cellulose are commonly mixed in the broth (Dourado et al., 2016). But these preparations are not stable and frequently exhibit insignificant suspension stability. They are frequently plagued by phase separation and transparency interferences. Furthermore, the excessive viscosity gives clients an unpleasant taste. As a result, novel agents as a suspension are needed which have low viscosity and high dispersion ability. Under these conditions, bacterial nanocellulose pellets generated by agitated fermentation are discovered to have a high capacity to suspend particles (Zhong, 2020).

Bacterial nanocellulose is used in food packaging to ensure product safety and extend shelf life. In active bacterial nanocellulose-based packaging systems, antimicrobial agents, taint and moisture removers, oxygen and ethylene scavengers are all employed (Tome' et al., 2010). Furthermore, controlled heterogeneous esterification using hexanoyl chloride was used to create modified bacterial nanocellulose with customized surface and barrier characteristics (Tome' et al., 2010). After the process of esterification, the bacterial nanocellulose membrane demonstrated improved hydrophobicity while retaining the virgin bacterial nanocellulose's bulk structure. At different RH values, the permeability for moisture was measured. The calculation of humidified carbon dioxide, oxygen and nitrogen was also done. The modified bacterial nanocellulose showed a 50% reduction in all the gases as well as water vapor permeability (Tome' et al., 2010).

7.2 Acoustic Transducer Diaphragm

Yamanaka et al. (1989) for the first time presented the non-food, highly significant potential and prospective application of bacterial nanocellulose along with its composite materials in the field of acoustic transducers. The extraordinary property of bacterial nanocellulose for retaining its shape, as evaluated by Young's Modulus, combined with the material's significant core loss makes it perfect for speaker diaphragms. Sony Corp. sold the innovative diaphragms as loudspeakers and headphones because they displayed two distinguishing properties: high sonic velocity and minimal dynamic loss (Iguchi et al., 2000).

7.3 Personal Care Products

The products used for the personal hygiene/care and home are the second largest area for the exploitation of bacterial nanocellulose after food. These products should have non-toxic and biocompatible ingredients. Customers like naturally-occurring materials that are highly pure and safe. Bacterial nanocellulose is a natural material produced by the process of fermentation through some specific microbes, which is an excellent product in terms of biocompatibility (Roman, 2015). The process of static fermentation synthesizes bacterial nanocellulose pellicles that have been employed as a raw material for face masks (Hainan Yeguo Foods Co., Ltd.). As compared to silk or non-woven cellulose face masks, masks made up of bacterial nanocellulose offer superior water retention and provide a pleasant sense of cooling and softness due to their reticulate 3D network structure at nanoscale (Amnuait et al., 2011; Pacheco et al., 2018). Furthermore, the very spongy architecture of bacterial nanocellulose allows it to carry diverse nutrients and even medicinal substances (Chantereau et al., 2020). The porosity also provides a role to the bacterial

nanocellulose pellicle in the controlled-release of the trapped substances (Numata et al., 2015; Perugini et al., 2018). Additionally, on the basis on this function, these face masks can be employed in treating some moderate skin problems and cosmeceuticals (Almeida et al., 2014; Morais et al., 2019).

7.4 Biomedical Applications

Bacterial nanocellulose has enormous significance in the field of biomedicine such as tissue engineering scaffolds, wound dressing, dental implants, artificial skin, biosensors, vascular grafts, hemostatic substances, and drug delivery (Carvalho et al., 2019; Anton-Sales et al., 2019; Rajwade et al., 2015). Bacterial nanocellulose's excellent purity and biocompatibility are required for every application in the field of biomedicine. The concentration of endotoxin in bacterial nanocellulose are well regulated at 20 units of endotoxin per device, which meet the terms of Food and Drug Administration (FDA) requirements for in vivo use (Petersen and Gatenholm, 2011). Furthermore, bacterial nanocellulose has a distinct reticulate 3D network structure, which confers a number of benefits such as huge surface area, outstanding moisture-holding capacity, high permeability for gas or liquid, amazing mechanical capabilities, and transparency (Sulaeva et al., 2015; Thomas, 2008). Because of these distinguishing properties, bacterial nanocellulose is a particularly unusual material that showcases its superior nature in the field of biomedicine. The wound dressing materials synthesized by using bacterial nanocellulose are regarded as a successful product in the market. Many other items related to medication administration, tympanic membrane replacement, vascular grafts, and contact lenses are also in the row (Coelho et al., 2019).

Bacterial nanocellulose is first used in dressing the wounds because of its excellent biocompatibility, semi-transparency, flexibility, high permeability, and extraordinary tensile strength (Curvello et al., 2019). Following extensive testing, it was discovered that bacterial nanocellulose has a number of extra benefits, such as the elimination of exudes while permitting secretion and exchange of gases, the reduction of pain, electrolytes and protein loss, the prevention of infections, and the acceleration of wound healing (Abeer et al., 2014). These distinct benefits give rise to bacterial nanocellulose in the wound dressing device industry. Bacterial nanocellulose-based wound dressings outperform gauze or synthetic materials in terms of efficacy and are utilized for treating abrasions, skin graft sites, skin grafts, post-operative surgical wounds, burns, diabetic ulcers, pressure ulcers, arterial and venous ulcers, lacerations, and other conditions (Portela et al., 2019). High wearing comfort, reduced discomfort, exudate control, quicker wound healing, superior aesthetic result, and cost-effective usage because of lengthy change interval for the change of dressing material are all advantages.

7.5 Bacterial Nanocellulose In Textiles

Bacterial nanocellulose is also commercially used as a source of raw material for fabrics free from plants such as rayon (Huang et al., 2014). Because petrochemically-manufactured fibers like polypropylene, terylene, acrylon, and nylon are non-degradable, their widespread usage has resulted in significant environmental contamination (Wei and Zimmermann, 2017). Reinforced fibers based on plants like rayon and cuprammonium are often made from cotton pulp and wood. Even though they are biodegradable, the process of making pulp often utilizes a lot of energy and pollutes the environment since a lot of chemicals are used. In comparison to plant cellulose, bacterial nanocellulose is easily purified, thus, lowering environmental consequences. Bacterial nanocellulose has been effectively transformed into viscose-rayon fibers, giving a plant-based fiber alternative (Nanollose Ltd.). The technique of producing rayon from bacterial nanocellulose fermentation uses little energy and water. Taken as a whole, it appears to be a viable approach for bacterial nanocellulose use, offering a sustainable alternative to plant-based rayon fibers.

7.6 Paper Manufacturing

According to reticulate and highly branched bacterial nanocellulose obtained during agitation culture condition are ideal for the manufacturing of finest paper (Johnson and Neogi, 1989). They also created glass fiber, copper powder and calcium carbonate composites. It has demonstrated that adding disintegrated bacterial nanocellulose to paper pulp allows for the production of a paper with improved mechanical properties (Yamanaka and Watanabe, 1989). Small pieces of bacterial cellulose were also discovered to be beneficial for improving folding durability and strengthening pulp sheets (Iguchi et al. 2000). When 15% bacterial nanocellulose was added to the composite paper, it had nearly four times the folding durability of pure pulp paper. Additionally, the inclusion of bacterial nanocellulose improved the Young's modulus (GPa)

from two to three and a half. When compared to conventional paper, the bacterial nanocellulose integrated paper sheets displayed better mechanical properties such as Young's modulus and tensile strength (Cheng et al. 2011).

7.7 Filtration

That investigated the specialized application of bacterial nanocellulose as a filter medium (Takai, 1994). The integration of different polymers, for example, carboxymethyl chitin, carboxymethyl cellulose, polyethylene glycol etc. into the cellulose can simply be done by mixing these substances into the starter culture medium. These polymers demonstrated extremely strong rejection for solute, thus making them suitable for pervaporation and ultrafiltration (Lin et al., 2013).

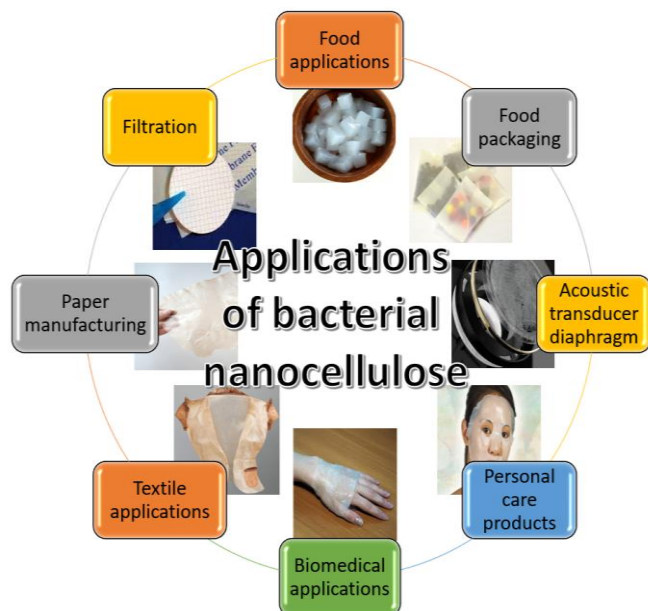


Figure 1: Applications of bacterial nanocellulose.

8. CHALLENGES AND FUTURE DIRECTIONS

Nanobiocellulose (BNC) has emerged as a promising biomaterial owing to its exceptional characteristics such as biocompatibility, higher surface area and mechanical strength. Despite its potential, several challenges hinder its widespread application. The cost-intensive production process and difficulties in scaling up production while maintaining consistent quality pose significant barriers to large-scale commercialization (Czaja et al., 2006). Conversely, various researches are aiming to reduce economical production of BC by suggesting more cost-effective alternate fermentation media for replacing the conventional and expensive Hestrin and Schramm (HS) medium. Various research endeavors are exploring the use of alternate cost-effective media, like by-products from the food industry as well as cashew tree gum (Pacheco et al., 2017; Fan et al., 2016; Revin et al., 2018; Molina-Ramirez et al., 2018). These substitute sources present a promising option for producing BC tailored for applications in food and food packaging industries, where the stringent purity requirements of biomedical applications might not be necessary. Ensuring the purity and consistency of BNC remains a challenge, as variations in the production process can lead to inconsistent material properties, limiting its utility in various industries (Gama et al., 2016). Additionally, the limited productivity and yield of BNC produced by the microbial cell pose another challenge. To overcome this challenge, extensive attempts have been dedicated for the development of advanced bioreactors, cell factories, and the engineering of genetically modified microbes. While the cell-free system comes with various benefits, including reduced production expenses, increased yields, and a regulated production setting for BC with improved properties, it requires further exploration in terms of better molecular and biochemical regulation, stability of enzyme, batch synthesis and other related factors.

Additionally, enhancing the mechanical strength and flexibility of BNC while retaining its biocompatibility presents a complex engineering challenge, particularly in the context of biomedical applications (Gama et al., 2016). Functionalizing BNC for specific applications and ensuring its compatibility with other materials also demand further research and development efforts (Bae et al., 2019). Additionally, preserving its biodegradability while ensuring a prolonged shelf life is essential for

sustainable utilization across diverse fields, including environmental and biomedical applications. To extend the practical applications of bacterial cellulose (BC) across sectors like food and food packaging, a thorough optimization of the entire production process is required. This optimization encompasses the assessment of various factors such as culture media composition, fermentation systems, genetic engineering methods, and post-production modifications (Gama and Dourado, 2018). Overcoming these challenges through advanced manufacturing techniques and innovative research will be pivotal in unlocking the full potential of nanobiocellulose for diverse applications.

9. CONCLUSION

Nanobiocellulose (BNC) stands as a promising biomaterial with diverse potential applications across various fields, including biomedicine, environmental sustainability, and industrial sectors. Despite the existing challenges in its production, scalability, and functionalization, ongoing research and development efforts are continually addressing these obstacles. As BNC continues to garner attention for its biocompatibility, mechanical strength, and biodegradability, it holds the promise of revolutionizing industries and technologies, paving the way for the development of sustainable and innovative solutions in biomedicine, tissue engineering, and beyond. Further exploration and advancements in manufacturing techniques and material science will undoubtedly lead to the broader adoption of nanobiocellulose, thereby unlocking its full potential and maximizing its impact in various applications.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare

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