JOBIMB, 2025, Vol 13, No 1, 70-76
https://doi.org/10.54987/jobimb.v13i1.1078

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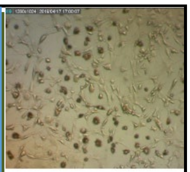

JOBIMB VOL 13 NO 1 2025
Lipid Accumulation Staining

Integrated Factors Influencing Bacterial Cellulose Production in Kombucha SCOBY: Bioprocess Strategies for Enhanced Yield

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HISTORY

Received: 19th May 2025
Received in revised form: 21st June 2025
Accepted: 28th July 2025

KEYWORDS

Bacterial cellulose
Kombucha
Bioprocess
Fermentation
Review

ABSTRACT

Bacterial cellulose (BC) is an eco-friendly biopolymer with unique properties, including high purity, mechanical strength, and biocompatibility. Its production is influenced by fermentation conditions such as carbon sources (e.g., sucrose, glucose), nutrient composition, temperature (25-32 °C), pH (4.0-7.5), and aeration. Static cultivation yields highly crystalline BC with layered structures, while agitated systems enhance productivity but reduce mechanical integrity. Process optimization using waste-derived substrates (e.g., molasses, fruit residues) and statistical modeling (e.g., RSM) improves cost efficiency and sustainability. BC's nanofibrillar structure provides exceptional tensile strength (200-300 MPa), high water retention (up to 98%), and thermal stability (decomposition at 300-350 °C). These properties make it valuable in biomedical applications (wound dressings, tissue engineering), food packaging (edible films), and industrial uses (nanocomposites, filtration membranes). However, scaling up production faces challenges, including genetic instability in continuous cultures, shear stress in bioreactors, and high downstream processing costs. Recent advancements focus on metabolic engineering, hybrid fermentation systems, and immobilized cell techniques to enhance yield and scalability. BC's potential as a sustainable alternative to synthetic materials is promising, particularly in medicine and green manufacturing. However, overcoming production cost and yield limitations remains critical for broader industrial adoption. Future research should optimize strain-specific fermentation, integrate circular bioeconomy principles, and refine functionalization techniques to expand BC's commercial applications.

INTRODUCTION

Process advancement strategies have increasingly focused on refining substrate matrices and environmental controls to enhance yield efficiency (**Fig. 1**). For example, empirical fermentation using tofu soy whey, when synergized with 8.5% (w/v) sucrose, 10% (v/v) kombucha microbial consortia, and thermophilic incubation at 32 °C, generated a BC yield of 4.20 ± 0.15 g/100 mL [1]. In kombucha-fermentative systems, key determinants such as black tea concentration (1.5-2.5% w/v), sucrose content (8-10% w/v), and fermentation duration (10-14 days) critically influence biopolymer output, with smaller symbiotic culture sizes improving mass transfer kinetics [2]. Substrate valorization from agricultural byproducts and underutilized *Camellia sinensis* cultivars is gaining traction as a viable strategy for cost-efficient and eco-compatible cellulose fabrication [3,4]. Experimental designs such as Plackett-Burman screening and Response Surface Methodology (RSM) have been instrumental in delineating multivariate influences on cellulose yield dynamics [5]. The trajectory of future bioprocess advancements is likely to converge on integrated strain-specific optimization, predictive nutrient modeling, and algorithm-guided bioreactor control to realize scalable, low-carbon BC production platforms [6].

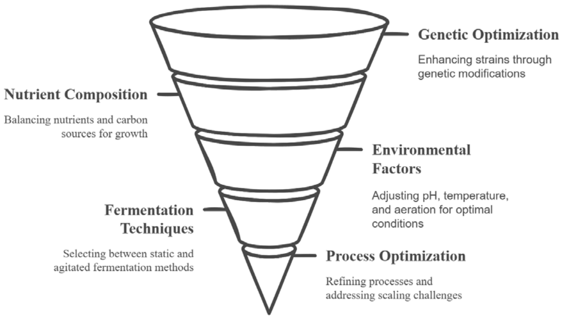

Fig. 1. Factors affecting bacterial cellulose production.

Nutrient Composition and Carbon Sources

Carbon substrate availability governs the metabolic trajectory of kombucha fermentation and dictates BC biosynthesis potential within the SCOBY (Symbiotic Culture of Bacteria and Yeast) consortium. Glucose, fructose and sucrose serve as primary carbon sources, with sucrose functioning as a hydrolysable disaccharide yielding equimolar monosaccharide fractions via invertase-expressing *Saccharomycetales*, thus enabling dual carbon channeling into yeast and bacterial metabolic networks [7,8]. Metabolic convergence of AAB and ethanol-producing yeasts sustains carbon flux equilibrium and optimizes redox balance, particularly under oxygen-limited static conditions. Strain-specific carbon substrate responsiveness produces divergent BC yields, *Komagataeibacter* spp. displays differential metabolic throughput based on hexose preference, with some isolates demonstrating higher cellulose titers under fructose assimilation [9].

Other microorganisms exhibit accelerated glucan assembly from glucose [10]. Such variability necessitates media customization calibrated to strain-specific enzymatic affinities and transport kinetics. Ethanol enrichment within a 0.5-2.0% v/v threshold modulates cellulose synthase (*bcs*) complex dynamics and augments acetic acid oxidation pathways, resulting in BC yield increases of up to 30% and concurrent alterations in nanofibrillar architecture and matrix density [11]. Process intensification strategies increasingly incorporate waste-derived substrates; molasses (12-18% fermentable sugars) and fruit processing residues (5-9% soluble carbohydrates) maintain 80-90% parity in BC yield relative to refined sugars, while conferring 30-40% reductions in input costs and aligning production pipelines with circular bioeconomy mandates [12,13].

Influences of Environmental Factors

The microbial activity within the SCOBY matrix is regulated by multiple interdependent environmental factors that collectively determine microbial growth, metabolic functions, and population dynamics. Temperature, pH, and aeration stand as crucial variables that directly influence both the biochemical transformations during fermentation and the overall stability of the microbial community. These factors work together to establish optimal conditions for the symbiotic relationships between bacteria and yeast, while simultaneously affecting the physical structure and metabolic efficiency of the SCOBY biofilm.

Temperature Regulation

The maintenance of optimal temperatures is critical for microbial activity within the SCOBY matrix [14]. The peak metabolic efficiency of *Komagataeibacter* species, as the primary BC-producing bacteria, occurred between 25 °C and 30 °C. A narrower range of 28 °C to 32 °C was associated with maximal growth and cellulose synthesis. Temperature deviations beyond these thresholds led to metabolic inhibition and reduced BC yield. Therefore, consistency in thermal conditions is necessary for reproducible pellicle formation and fermentation quality [13].

pH Influence on Microbial Activity

The acidity of the fermentation medium played a pivotal role in bacterial cellulose synthesis [11]. Typically, the optimal pH range for BC production spanned from 4.5 to 7.5, with the highest yields recorded between 4.5 and 6.0. Nonetheless, certain strains, such as *Komagataeibacter medellinensis*, demonstrated resilience under more acidic conditions [10]. The gradual pH declines due to acetic acid accumulation fostered a selective environment for acid-tolerant microorganisms. Process intensification via physicochemical tuning has demonstrated substantial improvements in BC biosynthesis under optimal acidic pH (4.0-5.0) achieved an 8.65-fold [15].

Aeration and Oxygen Dependence

The availability of oxygen is fundamental for bacterial cellulose polymerization. Static fermentation promoted the development of dense, layered BC membranes, whereas agitation resulted in fragmented cellulose structures. Excessive aeration is correlated with the emergence of non-cellulosic mutants in *Acetobacter xylinum* [11]. Precise oxygen regulation is essential to sustain high BC output without genetic instability. Jahan et al., [15] reported that substantial improvements in BC biosynthesis by *Gluconacetobacter* sp., also can be achieved under optimal agitation within120-150 rpm, resulting in an increase in BC yield, from 0.52 to 4.5 g/L.

Interconnected Environmental Factors

Generally, the interdependence of temperature, pH, and aeration dictated fermentation dynamics. Elevated temperatures accelerated metabolic rates, hastening acid production and pH decline [12]. Variations in aeration influenced both thermal distribution and volatile compound evaporation. Uncontrolled parameter fluctuations disrupted bacterial metabolism, leading to inconsistent BC yield and structural integrity.

SCOBY Fermentation Techniques: Static vs. Agitated Systems

Static fermentation is characterized as the traditional method for BC production in kombucha SCOBY cultivation. Undisturbed culture conditions are maintained in this approach, with a distinct cellulose pellicle formed at the air-liquid interface [16]. Higher crystallinity and mechanical properties, such as an enhanced Young's modulus, are observed in BC produced under static conditions due to the sequential deposition of cellulose layers [11]. The layered architecture of the pellicle is attributed to bacterial migration toward oxygen, which results in stratified cellulose structures. Despite these structural advantages, static systems are limited by low productivity and extended cultivation periods, often requiring weeks to achieve substantial yields [16]. Constraints in oxygen availability at the air-liquid interface and nutrient diffusion barriers caused by pellicle thickening are identified as primary factors for these limitations.

Agitated fermentation systems are implemented to overcome the challenges of static cultivation. Mechanical mixing is employed in bioreactor designs such as stirred tanks, airlift reactors, and rotating disc configurations to enhance oxygen transfer and nutrient homogeneity [12,14]. Increased BC production rates are achieved through the elimination of oxygen gradients and uniform bacterial growth. However, morphological alterations in BC are induced by agitation, with fragmented fibers, irregular pellets, or suspended particles produced instead of cohesive pellicles [16]. Reduced crystallinity and mechanical strength are correlated with these morphological changes. A critical issue in agitated systems is the emergence of non-cellulose-producing bacterial mutants, which is linked to shear stress-induced genetic mutations during prolonged cultivation. Hybrid fermentation systems are developed to combine the benefits of static and agitated methods. Horizontal fermenters equipped with rotating discs are designed to optimize oxygen transfer while minimizing shear forces, thereby preserving bacterial cellulose synthesis [11].

Modified airlift and stirred-tank reactors are engineered to improve oxygen distribution and reduce mechanical stress on bacterial cells, enhancing scalability and yield consistency [7]. In general, the selection of fermentation methodology is determined by application requirements and production scale. Static systems are preferred for artisanal kombucha production, where traditional textural and structural properties are prioritized. Meanwhile, agitated or hybrid systems are favored in industrial applications, such as materials science or biomedical fields, where high throughput and efficiency are prioritized over material properties, provided downstream adjustments are feasible [17].

Process Optimization and Scaling Up Challenges

The industrial-scale biosynthesis of BC via *Komagataeibacter* spp. fermentation faces structural limitations stemming from intrinsic biological constraints and engineering inefficiencies, hindering commercialization despite its material advantages over plant-derived cellulose [12]. *Komagataeibacter* spp., particularly under heterotrophic conditions, demonstrates reproducibly high BC productivity, positioning them as primary candidates for industrial deployment [9]. Quantitative inter-strain comparisons under controlled environmental constraints revealed superior performance by *Komagataeibacter hansenii* and *Gluconacetobacter rhaeticus*, contingent on optimized nutrient load, oxygen availability, and pH buffering [13].

At the macroscale, bioreactor configurations introduce mechanical stressors, where shear forces from agitation disrupt bacterial pellicle formation while remaining essential for oxygen diffusion, creating an unresolved trade-off between aeration and cellular integrity [11]. Microbial population dynamics further exacerbate productivity losses, as genetic drift in suspended cultures favors non-cellulolytic mutants during extended batch processes. Recent advances target these structural bottlenecks through metabolic engineering interventions that amplify precursor allocation toward BC biosynthesis pathways, alongside immobilized cell systems that suppress mutant dominance by spatially restricting phenotypic diversification [13]. Krystynowicz et al., [11] reported that horizontal rotating-bed bioreactors mitigate shear damage while maintaining high surface-to-volume ratios for bacterial adhesion, though scalability remains constrained by biofilm heterogeneity. Substrate diversification utilizing lignocellulosic waste streams demonstrates cost-reduction potential but requires strain-specific enzymatic pretreatment to overcome catabolite repression [14]. Process optimization has benefited from multivariate statistical modeling, particularly RSM, which refines parameter interdependencies in complex fermentation systems [18]. Non-invasive monitoring techniques, such as optical coherence tomography for real-time biofilm thickness mapping, enhance process control but lack standardization across production scales [19]. Furthermore, downstream processing persists as a critical cost barrier, necessitating innovations in dewatering and purification to align with biorefinery frameworks that valorize metabolic byproducts [16]. Future structural improvements must therefore address genetic instability in continuous culture systems, develop shear-resistant reactor geometries, and optimize *in situ* functionalization of BC nanofibers to expand industrial applicability.

Characteristics and Applications of Bacterial Cellulose

Generally, BC is defined as a helpful biopolymer due to its exceptional properties and applications in food, agriculture, medicine and various other spheres [12, 20]. The non-disease-causing bacteria of the *Komagataeibacter* genus carried out most of the BC production through extracellular biosynthesis. BC is purer than the plant-derived cellulose that contained impurities such as lignin and hemicellulose [21]. The general BC characteristics and applications are depicted in **Fig. 2**.

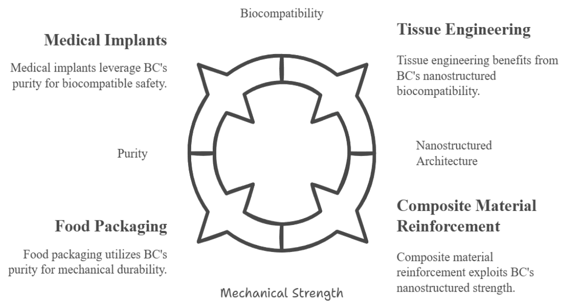

Fig. 2. Bacterial cellulose (BC) characteristics and applications.

Characteristics of Bacterial Cellulose

Bacterial cellulose (BC) is distinguished by its intrinsic compositional purity, alongside its highly ordered nanofibrillar architecture, characterized by a crystallinity index ranging from 84% to 89% and remarkable mechanical metrics, including tensile strengths between 200-300 MPa and Young's moduli of 15-18 GPa [12,16]. These physicochemical features render BC a structurally robust biopolymer with broad functionality across biomedical domains such as dermal scaffolds and wound matrices through its role in edible barrier films and rheological stabilizers, and industrial sectors including nanocomposite reinforcement and membrane filtration technologies. The high water-binding capacity of its interlaced microfibrils underpins this versatility. Characterization of the resulting polymer confirmed preservation of the cellulose Iα allomorph, corroborated by FTIR spectral peaks at 3340 cm^{-1} (O–H stretch), 2890 cm^{-1} (C–H stretch), and 1640 cm^{-1} (H–O–H bending), and XRD diffraction maxima at 2θ = 14.6°, 16.8°, and 22.7°.

Panaitescu et al., [22] reported the polymerization process of β-1,4-linked glucopyranose units in BC can develop a three-dimensional nanofibrillar network to obtain a crystallinity of 60% to 85%. BC has a water retention capacity of up to 98% of its total weight, and this property is a unique BC nanostructure [23,24]. The crystalline nanofibrillar structure of BC provides good tensile strength of the BC (200-300 MPa). BC decomposition temperature was found to be between 300-350 °C, and the possibilities of its chemical modification were studied through the application of grafting and cross-linking [25]. The impact of culture conditions on the properties of BC was studied by Kosseva et al., [24], and the pH was reported within the range of 4.0-6.0 and the temperature at 25-30 °C. BC applications in medical spheres increased due to its safety and ability to keep the wound wet [20]. The key issues of producing BC on a large scale

https://doi.org/10.54987/jobimb.v13i1.1078

were its standardization and quality assurance under the conditions of ramping up production [21]. The research into alternative raw materials was conducted to reduce the cost of production, and the properties of the material were controlled with the help of chemicals [22]. Nano-architecture of BC can be modified through post-synthesis methods to enhance performance in specific applications [20,23].

Applications of Bacterial Cellulose

Bacterial cellulose (BC) produced by *Komagataeibacter* spp. is a crystalline and mechanically strong biocompatible material that might be considered a promising sustainable and functional versatile biomaterial. These properties are due to its strength, water absorption, and biodegradability level [1,13,16]. The chemical structure of BC can be modified to meet the demand of various industries. Based on environmental concerns and the use of sustainable materials instead of plastic, BC as a by-product of kombucha tea is emerging as a possible contender for new materials discovery [3,26]. The biosynthesis of BC in kombucha fermentation is governed by several parameters such as carbon source, nitrogen content and pH, microbial strain and time [16,25,27]. Previous reports suggested the use of alternative, cheaper, and less ecologically harmful carbon and nitrogen sources to modify the characteristics of the material [28,29]. There are specific advantages and challenges in every field BC application [3,13,26].

The structure of this material gives it a large area for chemical changes and makes it stronger [30]. The skin-like properties of BC suggest that it can help with skin regeneration and wound care since it maintains moisture and speeds up the healing process [31]. Its use in drug delivery systems is being improved by making changes to its properties [32]. Besides, BC is used in dental implants, tissue engineering, and cell cultures [33]. In other places, BC can be used as an additive in manufacturing processes [34]. The industry considers it sustainable, yet it has some issues, such as using a lot of water and becoming less flexible after drying [35]. BC is also being applied in electronics, paper, and cosmetic products [36,37]. BC is expected to have a significant impact, particularly in medicine and the production of sustainable goods. Owing to its unique properties, it offers a promising alternative to conventional materials, especially from an environmental perspective. Nonetheless, challenges such as high production cost and low yield must be addressed to enable broader adoption, including its potential use in biodiesel applications [35].

Network Visualization: Structural Landscape

The network visualization from the VOSviewer analysis shows how complex is the research landscape is around in the production of bacterial cellulose (BC), especially when we look at it in the larger context of kombucha SCOBY fermentation systems (**Fig. 3**). The visualization shows a series of tightly linked clusters that are held together by high-degree nodes like review, nanocellulose, and acetic acid bacterium. These nodes are thematic hubs in the literature that serve as foci points that bring together different research pathways. The prominence of review signifies that systematic reviews and meta-analyses play a pivotal role in the integration of findings from microbial biotechnology, materials science, and process engineering to guide emerging research priorities especially in future trends [38,39]. The nanocellulose cluster is very important because it links research on biomaterial development, composite fabrication, as well as surface modification to improve functions, sorption or strength. This shows how important it is to optimize functional performance for biomedical and packaging applications [40-42].

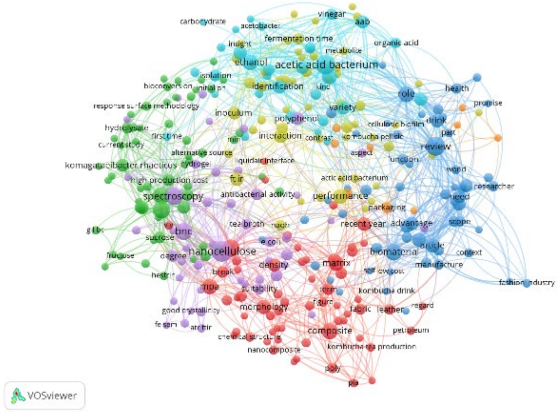

Fig. 3. A keyword co-occurrence network map made with VOSviewer from a Scopus bibliometric search on making bacterial cellulose in kombucha SCOBY systems. The map shows high-frequency keywords and how they relate to each other. These keywords are grouped into different thematic clusters that show the main research areas in the field. Core clusters emphasize subjects such as microbial consortia dynamics (e.g., acetic acid bacteria, lactic acid bacteria), material innovation (nanocellulose, composite reinforcement), and process optimization (e.g., fermentation kinetics, waste-derived substrates). The size of each node shows how often a keyword appears, and the lines that connect them show how often two keywords appear together, showing how they are related and how they overlap thematically. This picture shows how the research field is currently organized, making it easier to find mature knowledge areas, new topics, and possible ways to improve BC yield and scalability in line with the principles of the circular bioeconomy.

Another important structural motif revolves around the acetic acid bacteria, which are the main type of bacteria found in kombucha SCOBY consortia. Its centrality in the network shows that it plays a role in both cellulose biosynthesis and the production of secondary metabolites. This connects the studies on process optimization with studies on biofilm morphology and fermentation kinetics [43,44]. The co-location of this node with biofilm, bioconversion, and antimicrobial signifies interdisciplinary research endeavors at the nexus of microbial ecology and functional material synthesis [45,46]. Weighted centrality measures further show that there are strong links between keywords related to nanocellulose research and sustainability, which suggests that bioresource use, circular economy strategies, and nanostructure functionalization are all coming together in the same way [47].

The network's topology suggests that research in this field is organized around three main pillars: (i) the consolidation of existing knowledge through integrative reviews; (ii) the advancement of nanocellulose material science; and (iii) the mechanistic clarification of microbial fermentation systems, especially the metabolism of acetic acid bacteria in mixed cultures. Peripheral nodes, while less interconnected, demonstrate potential as emergent thematic niches-illustrated by research on composite reinforcement, enzymatic modification, and the utilization of agro-industrial waste as a carbon substrate [48,49]. These peripheral domains, frequently situated at the peripheries of the primary network clusters, may signify innovation frontiers where novel methodologies and applications are in their nascent stages. The network analysis shows that methodological rigor, cross-disciplinary integration, and application-oriented innovation all work together to make BC research part of a strong but flexible scientific ecosystem.

Overlay Visualization: Temporal and Citation Dynamics

Overlay visualization offers temporal and bibliometric insights into the progression of BC research within kombucha SCOBY systems (**Fig. 4**). The colors of the nodes and the overlay gradients show how recent the publications are and how much they have been cited, which shows that different research foci are at different stages of development. For instance, nanocellulose has a lot of citations and was published recently (2022-2024), which shows that it is a rapidly growing subfield with a lot of potential for biomedical and environmental applications [50,51]. On the other hand, nodes like acetic acid bacterium and lactic acid bacterium have a moderate recentness but a strong historical citation performance, which means that they are well-known for their role in understanding and improving the fermentation process [52,53].

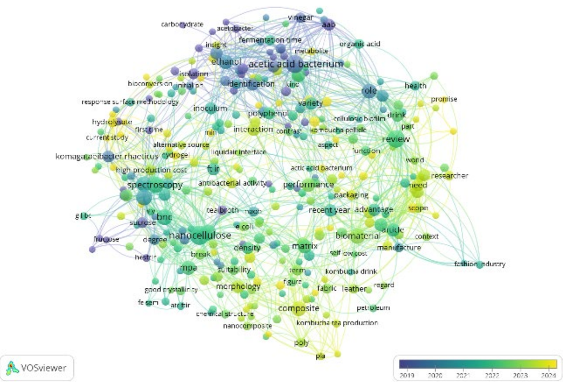

Fig. 4. An overlay visualization of keyword co-occurrence made with VOSviewer from a Scopus bibliometric search on how to make bacterial cellulose in kombucha SCOBY systems. The size of the nodes shows how often the keywords appear, and the color gradient from blue to yellow shows the average year of publication. Blue means older studies, and yellow means newer research activity. This temporal mapping shows how the focus of research has changed over time. For example, it has gone from basic studies on microbial metabolism and static culture methods to more recent work on nanocellulose functionalization, composite development, sustainability integration, and scale-up strategies. This shows both established areas of research and new areas of research that are starting to emerge.

Emergent specializations aimed at improving BC's structural integrity and functional performance for specific industrial applications are indicated by smaller, more recent nodes with high overlay intensity, such as composite reinforcement or mechanical property tuning [54,55]. These changes fit in with bigger trends toward eco-friendly material engineering and replacing plastics with bio-based polymers [56]. In contrast, large nodes with cooler overlay tones often correspond to mature methodological domains, like static versus agitated culture systems. In these domains, innovation has slowed down, but the knowledge base is still important for scaling up processes [57].

Citation overlay patterns also display that the focus of research methods has changed over the past ten years. Earlier studies primarily focused on microbial growth kinetics, yield optimization, and carbon source utilization [58]. In contrast, recent publications increasingly emphasize sustainability metrics, life cycle assessment, and functionalization strategies tailored to specific application requirements [59,60]. The simultaneous presence of elevated recency scores alongside sustainability-related terminology indicates that environmental imperatives are influencing research priorities, especially regarding the integration of a circular bioeconomy [61]. The overlay analysis shows that BC research is moving in two directions at the same time: foundational work that has been cited a lot is still important, and a lot of new, application-driven studies are shaping the field's near-term future. For kombucha SCOBY-derived BC, this trajectory illustrates the stability of fundamental microbial and biochemical knowledge alongside the dynamism of interdisciplinary innovation focused on industrial scalability, product diversification, and alignment with global sustainability objectives [62,63].

SUMMARY

Bacterial cellulose is a sustainable biomaterial with unique properties including high purity, mechanical strength, and biocompatibility, making it valuable for medical, food, and industrial applications. Its production depends on carefully controlled fermentation conditions involving specific carbon sources, optimal temperature ranges, proper acidity levels, and regulated oxygen supply. Traditional static methods produce high-quality cellulose with excellent structure, while agitated systems increase output but affect material properties. Researchers are improving production efficiency by using agricultural byproducts and advanced process optimization techniques. The material's outstanding strength, exceptional water retention, and thermal stability enable diverse uses from medical dressings to advanced composites. However, large-scale manufacturing faces several challenges including microbial instability, equipment-related stress factors, and expensive processing requirements. Current developments focus on genetic modifications, combined fermentation approaches, and specialized culture systems to enhance production capacity. As an environmentally friendly alternative to conventional materials, bacterial cellulose shows significant potential across multiple industries. The key to wider commercial use lies in overcoming current limitations in manufacturing costs and production yields while maintaining the material's superior qualities. Future advancements will likely focus on refining production techniques, such as metabolic engineering of high-yield strains, AI-driven bioreactor optimization, and sustainable waste-derived substrates to enhance scalability and cost-efficiency. Concurrently, research will expand practical applications in biomedical engineering, eco-friendly packaging, flexible electronics, and nanocomposites, further integrating bacterial cellulose into circular economy frameworks.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support provided by Universiti Putra Malaysia through the Fundamental Research Grant Scheme (FRGS/1/2020/STG01/UPM/02/14; Vote: 5540357; Code: 01-01-20-2225FR), as well as the essential facilities made available for the successful conduct of this research.

CONFLICT OF INTEREST

The author declares that there is no conflicts of interest related to this publication.

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