https://doi.org/10.17113/ftb.63.03.25.8746 review

Sustainability and Influencing Factors in Bacterial Cellulose Production: A Review of the Impact of Microorganisms, Culture Media, and Cultivation Methods

Running title: Advanced Bacterial Cellulose Production

Rebeca Priscila Flora Catarino*, Vinicius Avanzi Barbosa Mascareli, Viviane Lopes Leite da Costa, Ana Clara Longhi Pavanello and Wilma Aparecida Spinosa

State University of Londrina, Celso Garcia Cid Road, Km 380, 86051-970 Londrina, PR, Brazil

Received: 24 June 2024
Accepted: 6 March 2025

SUMMARY

This review offers a comprehensive analysis of bacterial cellulose (BC) production, with a focus on the key factors influencing the bioprocess, including microorganism selection, substrate optimization, and cultivation techniques. It addresses the challenges associated with BC production and proposes strategies for optimizing upstream processes, such as microorganism preservation, inoculum preparation, and culture medium formulation, which are critical for improving production efficiency. Additionally, the review explores the effects of fermentation parameters such as time, temperature, and oxygen availability on BC yield and quality. It also highlights the increasing interest in utilizing alternative substrates, particularly agro-industrial waste, to reduce production costs and enhance sustainability. By evaluating these factors, this review provides valuable insights for advancing BC production in both research and industrial applications.

*Corresponding author:
Phone: +55433371-4585
E-mail: rebeca.catarino@uel.br

Keywords: biocellulose production; oxidative fermentation parameters; microbial selection; bioprocess; agro-industrial waste

INTRODUCTION

The biotechnology industry produces numerous products derived from microorganism metabolism that benefit humans, animals, and the environment. Among these, bacterial cellulose (BC) has been gaining prominence in industrial applications due to its versatility. BC is a biodegradable, biocompatible biopolymer with high purity, and it is classified as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) (*1–3*).

Since its discovery in 1886 during vinegar production, BC has consistently attracted global research interest, with efforts focused on enhancing its production and improving its properties for high-value-added applications (*4,5*). Potential applications of BC include food additives and dietary fiber-enriched products (*6,7*), food packaging materials (*8*), and bioengineering uses such as cell and bioactive compound entrapment, biosensor development (*9–11*), cosmetics (*12*), wound dressings (*13*), and applications in electronics (*14*). Despite extensive research validating its potential applications, BC's commercial use remains limited due to high production costs. Advancing BC commercialization requires strategies to increase yield and reduce production costs, ultimately making this biopolymer economically viable for broader applications. To achieve this, optimizing microorganism activity, nutrient sources, and fermentation techniques is critical for overcoming these limitations.

From a metabolic perspective, bacterial cellulose is a product of oxidative fermentation by acetic acid bacteria. These microorganisms metabolize carbon sources, such as sugars, ethanol, and sugar alcohols, to produce energy through a series of enzymatic reactions (*15*). Understanding the mechanisms and factors involved in BC formation is crucial for controlling fermentation parameters and optimizing metabolite production. In general, a fermentation process includes three main stages: upstream steps (microorganism selection and preservation, inoculum preparation, and culture medium formulation), the fermentation phase, and downstream steps (product purification and waste management). Each stage directly or indirectly affects product yield, characteristics, bioprocess efficiency, and cost-effectiveness.

Currently, researchers are focusing on isolating new strains with high BC productivity, optimizing culture media, and improving fermentation methods to enhance the efficiency and feasibility of BC production (*16–18*).

This review provides a comprehensive analysis of the key factors influencing BC production,

with an emphasis on upstream processes such as microorganism selection and culture medium optimization. Unlike previous studies that focus on isolated aspects of the bioprocess, this review integrates these factors into a detailed discussion of fermentation methods and their critical role in enhancing production yield and cost-effectiveness. This review further highlights the importance of novel microbial strains and advanced fermentation techniques, providing a broader perspective on strategies for enhancing BC production scalability and commercial viability. Additionally, it incorporates recent advances in the use of agro-industrial waste as substrates, aligning with current sustainability trends in biopolymer production.

BACTERIAL CELLULOSE

Bacterial cellulose (BC) is an extracellular polysaccharide produced by microorganisms. Unlike plant cellulose, it is a highly pure form of cellulose with a higher degree of crystallinity, which makes it mechanically stronger and more suitable for specialized applications. While plant cellulose is a component of plant cell walls and typically contains impurities such as hemicelluloses and lignin, BC is synthesized in a controlled environment by microorganisms, ensuring superior purity and uniformity (*4,19,20*).

The first observation of BC dates back to 1886, when Adrian Brown reported the formation of a white, gelatinous film on the surface of the medium during acetic fermentation for vinegar production. At that time, BC was referred to as vinegar-plant or vinegar-mother. Subsequent chemical and structural analyses confirmed its similarity to plant-derived cellulose (*4,19,20*). Today, BC is also known as biocellulose, microbial cellulose, or bacterial nanocellulose.

The main BC-producing strains belong to acetic acid bacteria (AAB). *Komagataeibacter* genus, due to the higher yields compared to another AAB genera (*21,22*). For microorganisms, BC is a metabolite produced during acetic acid fermentation (oxidative fermentation) that assists in flotation by acting as a cell support at the air-liquid interface. This mechanism, linked to aerobic metabolism, ensures cell survival under stress conditions. In addition, BC helps in cell protection against dehydration, ultraviolet radiation, and acetic acid diffusion to the cytoplasmic membrane (*23–26*). In vinegar production, large volumes of BC might become an issue as they require extra cleaning steps in the fermenters. Also, the presence of AAB in organic vinegar or remaining cells in conventional vinegar (not completely removed by filtration) can affect the visual appearance of the final product (*27*).

However, investigation on chemical composition and structural properties suggested that after purification steps the biopolymer has the potential to produce biotechnological products. Since

then, researchers have focused on understanding the mechanisms of BC synthesis, isolating BC-producing microorganisms, and optimizing production for controlled processes at both laboratory and industrial scales.

Bacterial cellulose-producing microorganisms

Several microorganisms have been identified as producers of BC, such as *Aerobacter*, *Acetobacter*, *Komagataeibacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Pseudomonas*, *Rhizobium* and *Sarcina* (*21,22*). Among the AAB species, *Komagataeibacter xylinus* is considered a model for obtaining this biopolymer from different carbon and nitrogen sources due to the high BC yield. However, new species of *Komagataeibacter* have been continuously isolated for this purpose, including *Komagataeibacter medellinensis*, *Komagataeibacter intermedius*, *Komagataeibacter hansenii*, *Komagataeibacter europaeus*, and *Komagataeibacter rhaeticus* (*28–31*). These microorganisms are usually isolated from Kombucha, fruits, vegetables, and vinegar (*32–36*). Apart from AAB, new genera and species of microorganisms have been reported as BC producers, for example *Bacillus licheniformis* (*37*), *Enterobacter* sp. FY-07 (*38*), and *Lactobacillus hilgardii* IITRKH159 (*39*).

Bacterial cellulose production is mainly carried out by Gram-negative bacteria, particularly from the genus *Komagataeibacter*, such as *Komagataeibacter xylinus*. These bacteria are efficient BC producers due to their specialized outer membrane and secretion systems, which facilitate the release of cellulose into the extracellular space. Strains like *Komagataeibacter rhaeticus* K3 and *Gluconacetobacter xylinus* have shown high BC yields, often utilizing simple sugars, such as glucose and sucrose. However, the production process requires optimized media and strict environmental control, such as pH and temperature, for maximum efficiency. While Gram-negative bacteria tend to outperform Gram-positive species in BC productivity, the potential of Gram-positive bacteria has recently gained more attention (*40*).

As reported recently by Saleh *et al.* (*40*), Gram-positive bacteria, including *Lactiplantibacillus plantarum* AS.6, *Lactobacillus hilgardii,* and *Bacillus velezensis*, have also demonstrated BC production, though at lower yields compared to Gram-negative strains. The study identified *Lactiplantibacillus plantarum* AS.6 as a promising BC producer with a productivity rate of 56 %, higher than that of other Gram-positive species. When optimized, *L. plantarum* AS.6 can produce 4.51 g/L of BC, doubling the yield compared to the basal medium. This suggests that, with proper optimization of the growth medium, Gram-positive bacteria can be competitive in BC production. Additionally, *L. plantarum* AS.6 produced composites with strong antibacterial activity, indicating its potential for

biomedical applications such as wound dressings and drug delivery.

Acetic fermentation and bacterial cellulose synthesis

Bacterial cellulose is an oxidative metabolism product. Acetic acid bacteria obtain energy through the oxidative fermentation (acetic fermentation) pathway to compensate for the low energy yield from aerobic respiration and improve biomass formation through incomplete substrate oxidation (*41,42*). During acetic fermentation, organic substrates such as ethanol, glucose, organic acids, and polyols, are incompletely oxidized to CO_2 and H_2O. The residual products of this metabolism are utilized in the biotechnology industry to produce high-value-added products (*e.g.* ketones, organic acids, and exopolysaccharides, such as bacterial cellulose) (*43*).

BC biosynthesis is a highly precise and specific process controlled by catalytic and regulatory enzymatic complexes using uridine diphosphate glucose (UDP-Glucose) as a precursor (*44*). In this mechanism, the initial stage is the phosphorylation of glucose to glucose-6-phosphate by the enzyme glucokinase. Then, the phosphoglucomutase promotes the isomerization of glucose-6-phosphate to glucose-1-phosphate, which will be converted to UDP-glucose by uridine diphosphate pyrophosphorylase. The polymerization of glucose into β-glucan chains occurs from UDP-glucose by cellulase synthase, a complex of four subunits, namely bacterial cellulose synthase subunits A; B; C and D, (*BcsA*, *BcsB*, *BcsC*, and *BcsD)*, coded by three (*bcsAB, bcsC*, and *bcsD*) or four (*bcsA*, *bcsB*, *bcsC*, and *bcsD*) genes. Finally, β-glucan chains are crystallized into cellulose (*22,45,46*).

The synthesized chains are excreted into the medium through pores in the microorganism's cell allowing the elongation and association of the chains in the extracellular medium resulting in BC sub-fibrils (1.5 nm wide). These sub-fibrils give rise to nanofibrils (3–4 nm thick), which ultimately form cellulose ribbons (40–60 nm wide and 3–8 nm thick). The random arrangement of the BC ribbons results in a three-dimensional, porous, and highly crystalline network (*3,4,21,47,48*). The material observed in the culture medium could have different shapes depending on the cultivation method, the strain, and nutrient sources (Fig. 1).

Structure, function, and application

As previously mentioned, BC has a structure similar to that of plant-derived cellulose. Cellulose is formed by β-glucopyranosyl units linked by glycosidic bonds β- (1→4) resulting in a long-chain polymer with a degree of polymerization greater than 20.000 (*49*). The association between β-glucopyranosyl units originates a planar structure forming a ribbon. This planar and linear structure allows the formation of fibrous and polycrystalline bundles along extensive zones due to the

association between cellulose molecules by hydrogen bonds. These structures contain both amorphous and crystalline zones (*49–51*).

In recent years, the production of cellulose from microorganisms has been the subject of research in several countries since the fermentation process originates a material with superior properties and high purity, allowing its application in products such as food, biomedicine, and pharmaceuticals (*16,52,53*). The BC structure formed by the three-dimensional network ensures remarkable properties, namely high mechanical strength, crystallinity, stability to chemical agents and high temperature, high water retention capacity, and resistance to degradability. BC is lignin and hemicellulose-free, nor requiring intense purification, and is also a biocompatible and biodegradable biopolymer (*21,54,55*).

High crystallinity is one of the main characteristics of BC, and the degree of crystallinity ranging from approximately 60 to 90 % depending on the cultivation conditions and strain ability to convert the substrate and adaptation to the fermentation system. Crystallinity influences other characteristics of the biopolymer such as mechanical properties and thermal stability (*34,56,57*). Concerning the crystalline structure, cellulose I (Iα and Iβ) and cellulose II forms are frequently obtained under fermentation culture. The Iα (triclinic) and Iβ (monoclinic) forms correspond to crystalline structures and differ by the distribution of intra and interunit hydrogen bonds. In cellulose II, the random arrangement of chains results in highly amorphous regions, which also differs by its high thermodynamic stability. In most cases, higher crystallinity is observed in static culture, while amorphous content is more prominent in agitated cultivation (*21,58,59*).

The large surface area, the high number of hydroxyl groups, and its porosity enable BC to interact with water and polymers allowing the application as a support material for enzymes, cell, and nanoparticle immobilization. BC has a high water-holding capacity (WHC), retaining approximately 90% of its weight. This property is due to the strength of the hydrogen bonds involved in the adsorption of water molecules on the surface of the fibers and the density of the bond between the crosslinked fibers. The presence of thin and long ribbons in the BC structure also explains the greater water retention capacity, the moldability and the high tensile strength (*30,60–62*).

Regarding these characteristics, bacterial cellulose showed potential for food, bioengineering, cosmetics, biomedical, and electronic fields (Table 1 (*7,19,46,63–82*)).

The material can be used in different shapes, for example, nanofibers, nanocrystals, dried or wet pellicles and spheres. In food applications BC acts as a multifunctional ingredient and its addition in food products does not affect sensory characteristics since it can be colored and flavored (*69,83*). From the nutritional aspect, cellulose has a health-promotion function and can be used as a

dietary fiber source, and low calorie or gluten-free products. As food additives these biopolymers have been suggested as a stabilizer, thickener agent and texture modifier (*1,3*).

The three-dimensional, porous, and crystalline structure added to the presence of hydroxyl groups in the surface area allows BC association to other polysaccharides and proteins by hydrogen, van der Waals, and hydrophilic bonds improving their functionalities. Otherwise, the crystalline structure facilitates hydrophobic interactions, ensuring BC's amphiphilic properties (*63,70*). The formation of nanocomposites is useful to biodegradable food packaging and edible film development, in this case BC could be associated to other polymers, proteins, bioactive composites, and inorganic nanoparticles to enhance mechanical and thermal properties, barrier performance, and antimicrobial properties (*84–86*). Some research reports the use of chemical, mechanical, and enzymatic modification to improve or develop specific properties allowing more applications (*63–65,87*). Besides the several applications fields, the low yield and high production costs are a limiting factor. In this context, it is crucial to understand and optimize the production parameters to improve the cost-effective bioprocess.

Briefly, BC production comprises three stages, (1) upstream, (2) fermentation and (3) downstream. Upstream includes strain selection and conservation, inoculum and medium preparation, and definition of cultivation conditions. During fermentation several parameters can affect the yield and product characteristics, therefore they should be controlled, for example, pH, oxygen, temperature, agitation. Otherwise, downstream steps require the product purification, neutralization, characterization, and effluent treatment. A summary scheme of BC production is shown in Fig. 2.

Various strategies can be employed to overcome or minimize limiting factors at each step of the process, thereby optimizing BC production. The following sections discuss the features of the central factors of BC production (microorganism, substrate, and cultivation methods), including some strategies to improve this bioprocess.

ACETIC ACID BACTERIA FOR BACTERIAL CELLULOSE PRODUCTION

The cultivation of BC producers is a crucial stage, considering the nutritional requirements for cell metabolism and the microorganism's adaptation to fermentation conditions. In this context, the researchers are constantly trying to find new strains with high capacity to produce BC. AAB strains are fastidious microorganisms to cultivate and isolate, posing challenges for researchers to find alternatives that optimize cell growth and BC production (*88–90*). *Komagataeibacter* species have been previously described as the most efficient in BC production due to their ability to grow and produce BC from different carbon and nitrogen sources (*91,92*). *K. intermediuns, K.rhaeticus, K.*

hansenii and K. medellinensis are frequently referred to highly BC producers, similar to the *K. xylinus* strain, the role-model on BC production. The main sources of BC producers are vinegar (*33,93*), kombucha (*94*), fermented juices (*29*), and fruits and vegetables (*15*).

BC producers have developed mechanisms to survive in stressful conditions, usually like the isolation environment, for example, resistance to high acid and ethanol concentration. All these mechanisms make AAB attractive to industrial processes and are useful to improve the biopolymer formation, extending the rate for bioprocess design (*92*,*95*). Also, some *Komagataeibacter* strains presented a remarkable ability to produce BC under alkaline conditions, which could be associated with the protective nature of the material (*29,96*). Additionally, resistance mechanisms have been investigated at different cultivation, such as static and agitated, and the adaptation to highest rotational speed is referred as a strain-dependent characteristic (*91*). The cultivation under laboratory conditions and synthetic media can reduce the microorganism resistance suggesting that these mechanisms are inducible or transient (*97,98*). However, the product formation must be evaluated considering the interaction between strain, culture medium and cultivation method once the performance of the microorganisms is affected by the cell adaptation to the cultivation conditions. In this case, the same strain cultured in different bioprocesses does not always reproduce the same yield and productivity (Table 2 (*17,29–30,56,93,99–102*)).

Stress conditions can adversely affect cell metabolism and BC production. These factors may induce spontaneous mutations in cells, resulting in atypical cell morphology and growth, and may also inactivate essential enzymes for polymer synthesis, thus reducing yield and material characteristics (*24,102*). Besides reducing BC production, the effect of cellulose non-producing cells can be investigated by the polymer structure, and crystallinity is the most affected (*103*). Additionally, the presence of mutant cells influences the fiber assembly process due to the formation of soluble polysaccharides, such as acetan, as both use the same starter molecule, UDP-glucose (*104*). Regarding microorganism activity, it is also important to consider the effects of the cultivation parameters on the emergence of mutant cells. Changes in process parameters, pH, temperature, culture medium volume and oxygen availability affect the cell metabolism and product formation (*105*).

Similarly, stress conditions, such as high sugar concentration, anaerobic conditions, and high temperatures, can induce the viable but non-culturable (VBNC) state, which influences cell monitoring and product formation. Briefly, in VBNC state even if the cells are alive, they do not grow on conventional media (nonselective) used to form colonies. The main affected metabolic and morphologic characteristics are the modification of wall cell components, reduction in respiration rate, nutrient transport, macromolecular synthesis. Nevertheless, cells in VBNC state are more resistant to

physical-chemical stress and antibiotics. Modification in the environmental conditions and the media composition could favor the cell growth and reverse this cell state (*41,106,107*).

To improve material formation, BC-producing strains can also be obtained using genetic engineering techniques. Key strategies include modifying the *acs* operon, responsible for cellulose synthesis, and genes like *pgi* and *zwf*, which enhance carbon metabolism to generate BC precursors. Additionally, disrupting competing pathways, such as polyhydroxybutyrate (PHB) synthesis, and regulating cyclic di-GMP levels have shown significant improvements in production efficiency. These modifications leverage advanced synthetic biology techniques to optimize bacterial strains for industrial applications (*108–110*). Yang *et al*. (*111*) developed a recombinant strain for BC production in mannose-rich media by introducing genes from the *Escherichia coli* K-12 strain, which increased BC production on 84 % compared with the wild-type strain. Jacek *et al*. (*112*) modified *K. hansenii* motility and cell size, which are suggested to influence the yield and network organization in BC structure. The use of genetic engineering resulted in thicker ribbons of cellulose arranged in looser networks, and the biopolymer are suggested for scaffold production.

Monitoring and control of cell growth

The isolation, cultivation, and cell preservation are crucial steps in bioprocess due to their influence on biopolymer formation. The success of AAB cultivation for bacterial cellulose production has been associated with the nutritional requirements of the microorganism and the cell growth control (*27,113*).

Culture media for microorganisms are classified based on their composition, such as chemically defined (synthetic), complex, selective, differential, and enrichment media. Using complex medium (composition not exactly known) is a strategy to evaluate the growth characteristics of unknown strains or to create an environment able to supply the complex nutritional requirements of some microorganisms (*114*).

Culture media for AAB isolation, pre-activation, and inoculum propagation are formulated to satisfy nutritional demands, providing components that simulate the characteristics of the isolation environment, such as high-sugar content, acetic acid, and ethanol, which are found in fermentation bioreactors, fruits, vinegar, or fermented beverages—common sources for AAB isolation. The main elements to cell formation are Carbon (C), Nitrogen (N), Hydrogen (H), Oxygen (O), Sulfur (S), And Phosphorus (P) since these components are used in proteins, nucleic acids, carbohydrates, and lipids synthesis (*114,115*).

Carbon and nitrogen are the most significant nutrients due to their structural role in various

cellular components (*17*). Carbon is an essential element to the synthesis of organic components in cell metabolism and energy production. In the culture medium this macronutrient is provided through sugar which represents a large percentage in the formulation. Although AAB can metabolize several carbon sources, ethanol, glucose, mannitol, and glycerol are the most common substrates to these metabolic pathways since they are oxidized by the membrane-bound (periplasmic) dehydrogenases, not requiring previous hydrolysis reaction which would mean an extra cell work. For instance, key dehydrogenases in the oxidative fermentation of carbon sources include pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) and aldehyde dehydrogenase (ALDH), which oxidize ethanol to acetaldehyde and further to acetic acid, respectively. Additionally, PQQ-dependent glucose dehydrogenase (PQQ-GDH) oxidizes glucose to D-glucono-δ-lactone, while PQQ-glycerol dehydrogenase (GLDH) oxidizes polyols to ketones (*41,42,116*).

Nitrogen is another essential element in the culture medium, required for the production of proteins, enzymes, nucleic acids, and biomass formation. To AAB cultivation and BC production this nutrient may be supplied by organic (yeast extract, peptone, malt extract and amino acids) and inorganic sources, for example, ammonium sulfate ($(NH_4)_2SO_4$) and ammonium nitrate (NH_4NO_3) (*17,100,117*).

Also, minerals and vitamins play essential roles in cell growth. Mineral components influence enzyme activity, nitrogen fixation, and electron transfer from substrate to oxygen. Some essential minerals for AAB metabolism are molybdenum (Mo), boron (B) and manganese (Mn). In addition, vitamins such as p-aminobenzoic acid, pyridoxine (B_6), cyanocobalamin (B_{12}), nicotinamide (B_3), and ascorbic acid have shown significant effect on cell growth and BC production (*115,118,119*).

Table 3 (*18,33,97,116,120–124)* shows the culture medium most used to AAB and its composition. These culture media are mainly formulated by sugar (carbon source) and yeast extract or peptone (nitrogen source). Frequently, additives have been incorporated into culture media to supply the microorganism requirement for nutrients. For example, ethanol has been suggested as an alternative energy source to microorganism growth, also supporting cell recovery from viable but non culturable (VBNC) state and inhibiting the non-producing cells (*34,125*). Similarly, organic acids such as acetic, citric, malic, lactic, pyruvic, and succinic acid could be metabolized by AAB and used as intermediate metabolites to energy production (*116,126*).

In addition to media composition, alternative methods can be applied during isolation or enrichment stages in solid media preparation, such as double-layer agar. For AAB strains from industrial vinegar production this method simulates the growth conditions in fermentation tanks (*116*). This technique was described by Entani *et al*. (*120*) and consists in creating an inferior layer with broth

with 0.5 % agar. Further, the surface will be coated by the broth with 1.0 % agar. The use of double-layer agar plate supplies a high humidity environment, for the cells favoring the growing of high-acidity colonies (*116,120*).

Alternatives to cell enumeration

Estimating the cell population is essential for understanding the microorganism's growth profile, metabolic aspects related to BC production, and fermentation control. The cell growth profile typically consists of four stages: lag, exponential (log), stationary, and death. In the lag phase, cell concentration increases slowly as the microorganism adapts to the cultivation conditions. The exponential growth phase reflects the most intense cell activity and substrate consumption. During the stationary phase, nutrient availability is reduced, and the growth rate equals the death rate. However, cells remain active and continue producing metabolites, such as bacterial cellulose. Finally, the nutrient limitation results in cell death (*127,128*). Traditionally, the AAB population is determined by cell enumeration in plating or microscopy, and turbidimetric and gravimetric methods. Since these techniques are well established to vinegar and fermented beverages production, they could be applied in BC production to improve the cell growth control and product formation. However, alternative techniques are useful for monitoring cell growth and analyzing microorganism behavior. These methodologies should be applicable to fermentation routine to monitoring cell growth and ensure the bioprocess control.

Plating methods to cultivate and enumerate AAB in synthetic culture media could be affected by the presence of cells in the VBNC which leads to an underestimation of cell count and limits cultivation, isolation and cell maintenance (*116,129,130*). The VBNC state has been associated with discrepancies between the target inoculation rate and plate counting results in fermentation systems. This state can disrupt the direct correlation between biomass formation, substrate consumption, and product yield, as VBNC cells cannot be enumerated (*41,130–132*).

Another limiting factor in cell determination is the attachment of cells to the BC during inoculum propagation and fermentation. During the inoculum preparation on a liquid medium the simultaneous cell growth and BC formation result in the cell holding inside of the biopolymer structure. An alternative to overcome this limitation is the use of cellulase to release cells attached to BC fibers, increasing the number of free cells in the liquid medium and improving cell enumeration (*113,133*).

In addition to growth monitoring, it is crucial to use rapid methods to quantify and identify the cell state (*i.e.* live, dead, or VBNC). Several techniques have been used to quantify both live and dead cells in acetic fermentation. Fluorescence technology has proven useful for this purpose,

showing good results for AAB enumeration compared to the plating method (*113*). Similarly, flow cytometry has been used to assist in cell enumeration during acetic fermentation (*134*) and to assess cell viability after exposure to stress factors (*125*). Additionally, real-time polymerase chain reaction (RT-PCR) offers an alternative to traditional AAB enumeration methods (*12,135*).

DESIGNING CULTURE MEDIA TO BACTERIAL CELLULOSE PRODUCTION

Culture media significantly impact the total cost in bacterial cellulose production and require strategies to overcome this limitation and increase the bioprocess economic feasibility (*126,127*). The conventional medium used for BC production was developed by Hestrin and Schramm (*121*). Hestrin-Schramm (HS) composition consists in (%, *m*/*V*): glucose (2 %), peptone (0.5 %), yeast extract (0.5 %), anhydrous sodium phosphate (0.27 %), and citric acid (0.115 %). The composition of the culture medium must provide sufficient macro- and micronutrients for cell growth and biopolymer synthesis. Considering the HS composition, each component plays an essential role on microorganism metabolism and BC formation. Carbon is supplied by glucose, which is the ideal precursor for the formation of BC chains (*4,12,136*). Peptone and yeast extract provide amino acids for protein synthesis and essential compounds, such as vitamins and minerals, for microorganism growth (*117,137,138*). Finally, anhydrous sodium phosphate (Na_2HPO_4) and citric acid exert a buffering effect during cell cultivation (*139*).

Increasing BC yield and reducing media costs are essential for bioprocess viability. Nowadays, different approaches have been used to design economically feasible nutrient sources to BC production. These strategies involve modifying individual components of the standard media, supplementing culture media, formulating synthetic media, and using low-cost materials. All approaches must consider an ideal carbon and nitrogen ratio for BC production. In microbial biopolymer production, excess nitrogen increases biomass formation while limiting biopolymer production, whereas an excess of carbon over nitrogen decreases protein synthesis and reduces microorganism growth. Thus, the energy from excess carbon is used to produce the polysaccharide (*28,140,141*).

Many BC studies have reported higher production when HS carbon and nitrogen sources were modified by changing the concentration or the type of the sources. Basu, Vadanan, and Lim (*142*) used response surface methodology to determine the optimal HS composition for the *G. hansenii* strain. In this case, glucose and sucrose were evaluated at different concentrations and the author found higher BC yields with sucrose, a cheaper carbon source compared to glucose. Similarly, Jacek *et al*. (*94*) reported the increase in BC production when replaced glucose by eucalyptus

biomass hydrolysate in HS supplemented with ethanol. Considering N influence on cell growth and biopolymer formation, Santoso *et al*. (*100*) used different nitrogen sources, namely, yeast extract, peptone, malt extract, and ammonium sulfate as substitutes for the nitrogen source in HS composition. The results suggested peptone was the more suitable source for *K*. *intermedius* (BCRC 910677) while no BC was produced using ammonium sulfate as N substitute.

Synthetic media formulation is another alternative to improve BC yield from different strains. Gomes, Ida and Spinosa (*17*) evaluated the effect of amino acid supplementation on *K. intermedius* V-05 metabolism for BC production. The authors reported aspartic acid (1.5 g/L), phenylalanine (1.5 g/L), and serine (3.0 g/L) as essential elements on the media formulated (50 g/L sucrose, 10 g/L $(NH_4)_2SO_4$), 2 g/L Na_2HPO_4, 1 g/L $MgSO_4 \cdot 7H_2O$, and 10 mL/L ethanol) achieving 3.02 g/L from the optimized media.

In recent years, the use of low-cost materials, particularly agro-industrial waste, has gained significant attention for biosynthesis of bacterial cellulose (BC). These materials not only reduce production costs but also contribute to environmental sustainability by utilizing waste that would otherwise be discarded. Several agro-industrial by-products have been successfully used as substrates for BC production, such as cashew apple juice, soybean molasses (*119*), potato peel waste (*143*), sugar beet molasses, cheese whey, tobacco waste (*20*), oat hulls (*144*), and brewing by-products (*e.g.* beer and distillery waste) (*145*).

These waste materials provide a rich source of carbon and nutrients necessary for bacterial growth and BC synthesis. However, their complex and variable compositions present challenges to the fermentation process. The undefined nature of these substrates can lead to inconsistencies in the bioprocess, making it difficult to achieve reproducible results and potentially impacting the quality and yield of the BC produced. For example, the presence of inhibitors or non-fermentable components in these waste materials can hinder bacterial growth or BC production efficiency.

To overcome these challenges, some agro-industrial waste materials require pre-treatment processes such as acid or enzymatic hydrolysis to break down complex polysaccharides and increase the concentration of fermentable sugars. While these treatments can enhance BC production, they also introduce additional costs and can complicate the overall process. Furthermore, extensive purification may be necessary to remove residual contaminants, which further adds to the operational expenses (*114,144*).

Designing an optimal culture medium for BC production requires careful consideration of cost-effectiveness, environmental sustainability, and desired application outcomes. Agro-industrial residues, such as cantaloupe peels (CP) (*146*), starchy kitchen wastes (SKW) (*147*) and paper sludge

(*148*), have shown great potential as alternative substrates for BC biosynthesis. Enzymatic hydrolysis of these substrates enhances fermentable sugar availability, which significantly boosts BC production. For instance, hydrolyzed CP supported a BC yield of 3.49 g/L, while SKW hydrolysates yielded 2.11 g/L. Similarly, paper sludge enzymatically processed in a fed-batch system increased BC production to 3.10 g/L, outperforming batch fermentation. These results highlight the feasibility of using waste-derived substrates to reduce production costs and minimize environmental impact, while offering pathways to valorize cellulosic and starchy wastes (*146–148*).

Optimizing media formulations through techniques such as the Box-Behnken design (BBD) can further enhance BC production and application-specific performance. For instance, BBD optimization of SKW hydrolysis maximized reducing sugar availability, researchers can tailor culture media to achieve sustainable BC production with enhanced functionality for diverse applications, including wastewater treatment, biomedical materials, and environmental remediation (*146–148*).

On the other hand, the use of defined (synthetic) media offers advantages in terms of process control, reproducibility, and scalability. These media have a known and consistent composition, which allows for better monitoring and optimization of fermentation parameters. Additionally, the use of defined media can simplify the recovery and purification steps, leading to higher-quality BC production. However, synthetic media are typically more expensive than agro-industrial wastes, which can offset some of the cost-reduction benefits (*114,144*).

Despite the limitations, the exploration of agro-industrial waste as an alternative culture medium for BC production remains a promising area of research. With further optimization and pre-treatment strategies, agro-industrial by-products can serve as a sustainable and cost-effective source for BC production, contributing positively to both economic and environmental goals. Additionally, BC produced from waste materials could hold higher added value in diverse applications, such as in biocomposites, packaging, and medical products, making it a potential key player in the circular economy (*20,147,149*).

CULTIVATION METHOD

Bacterial cellulose fermentation can be carried out under static or agitated cultivation, and the method used influences both the yield and material properties. However, the success of each process depends on strain adaptation and the interaction of these variables with the culture medium. Under static conditions, AAB are inoculated into fermentation flasks or bioreactors containing sterile culture medium and incubated at predefined temperature and time conditions. In this method, BC formation occurs at the air-liquid interface as a gelatinous pellicle, shaped according to the flask used

for cultivation (*150*).

Although static cultivation is the most used technique for BC production, agitated (stirred or shaking) culture has been proposed as an alternative due to its potential to overcome some limitations inherent to the static method. In agitated cultivation, the culture medium inoculated with AAB is incubated under various agitation speeds, and the biopolymer is synthesized as ellipsoidal, stellate, or fibrous components dispersed throughout the culture medium (*1*). Compared to static cultivation, crystallinity is the most significant characteristic affected by agitation, particularly at high rotation speeds. This parameter reflects the structure organization that would be affected by shear force resulting in a less organized network. Under the agitation system, spherical BC formation results from cell aggregation around air bubbles, following a ribbon-like arrangement. However, the mechanism is also influenced by inoculum, carbon sources, and the temperature of the medium volume (*59,151*).

In their work, Saleh *et al.* (*152*) reported that BC production is highly influenced by fermentation conditions, with static fermentation consistently outperforming agitated fermentation in terms of yield. Incorporating hydroxyapatite (HA) nanoparticles into the culture medium further enhanced BC production, with static conditions yielding 4.10 g/L, approximately 1.25 times higher than in agitated fermentation. Static fermentation supports BC formation at the air-liquid interface, optimizing oxygen availability, while agitation can cause excessive oxygen diffusion, genetic instability, and reduced yields. Additionally, structural analysis of BC/HA composites confirmed improved functional properties, particularly under static conditions, highlighting their potential in biomedical applications like bone tissue engineering due to enhanced cell viability and attachment (*152*).

The evaluation of the strain performance under both systems should consider the effect of the culture medium on cell viability and cell ability to adapt the metabolic mechanics under each cultivation method used. Therefore, it is useful to consider the media composition and the strain when comparing the static and agitated methods. The main differences observed in studies comparing both methods, considering the same strain and nutrient sources in both cultivations, include yield, crystallinity index, water retention, porosity, and BC form (Table 4 (*24,34,71,103,153*)).

These aspects are essential to define the final biopolymer application (*16,71*). BC production is influenced by surface area, volume of culture medium, and nutrient availability. Under static cultivation, the surface area-to-volume ratio significantly influences oxygen availability; a larger surface area favors oxygen consumption. Since AAB are aerobic microorganisms, the oxygen supply is essential for cell growth. In this case, cells are suggested to use the BC synthesized, as well as the remaining from inoculum, as a support to access the air–liquid interface improving their oxygen

access. In contrast, aeration in the agitated method ensures a greater oxygen supply and improves nutrient availability, thereby enhancing cell growth (*34,103,154,155*).

Despite higher oxygen diffusion under agitation, BC production may be negatively affected by the emergence of mutant cells (non-producing cells), by-product formation, and simultaneous production of water-soluble polysaccharides (WSPS), leading to lower production compared to the static method (*38,103,153*). The by-product formation is a consequence of the nutrient consumption, carbon source metabolization and aeration. For example, using glucose as a carbon source Chen *et al*. (*16*) reported higher glucose consumption and gluconic acid production in the shaking system. Krusong *et al*. (*156*) observed gluconic acid formation increased with the aeration rate while BC production and cell content were reduced. Besides these limiting-factors, recent research confirms the ability of some AAB strains to grow and produce BC under agitation, and the yield could be similar or higher than static method (*24,71,103*).

DOWNSTREAM METHODS OF ISOLATION AND PURIFICATION OF BACTERIAL CELLULOSE

The downstream processes for isolating and purifying bacterial cellulose (BC) can be categorized into three main steps: harvesting, purification, and drying. At the end of the fermentation stage, the produced BC is harvested from the liquid medium and separated using centrifugation or filtration methods (*32,71,153*).

The harvested BC must be purified to remove residues from the culture medium and cells, as these materials can influence the biopolymer's properties, such as crystallinity and color, and may also induce BC contamination (*157,158*). The conventional approach used is alkaline treatment. In this method, BC is purified in an aqueous solution of sodium hydroxide (NaOH) and then neutralized by washing with distilled water. To achieve optimal purification efficiency, literature suggests various combinations of NaOH concentration, temperature, and time. Typically, BC has been purified with 0.1 to 1 M NaOH solution at 80 to 90 ºC, for 30 to 60 minutes (*10,17,100*). Alkaline treatment is suggested to be able to remove remaining nutrients from the culture medium and to lyse bacterial cells attached into BC (*102*).

BIOPROCESS CONTROL, OPTIMIZATION AND SCALE-UP

As mentioned, bacterial cellulose has unique properties to industrial applications. However, implementing a highly productive bioprocess is essential to scale up biopolymer production. Each fermentation process can be optimized by using statistical tools to assess the effect of critical parameters on BC production or properties. In addition to the one-factor-at-a-time approach, statistical

optimization is often used to define the ideal conditions for BC production by analyzing a wide range of parameters, including the interaction of process variables.

Studies highlight the use of innovative substrates like enzymatically hydrolyzed prickly pear peels (PPP), yielding 6.01 g/L of BC under optimized conditions (68 % PPP substrate, pH 4, 20°C, 11 days). Functionalized BC membranes loaded with fruit byproducts, such as pomegranate peel extract, demonstrated antimicrobial properties and extended the shelf life of strawberries, highlighting their potential in sustainable packaging (*159*).

Statistical models provide time-efficient and cost-effective alternatives for exploring fermentation conditions aimed at large-scale production. Considering their influence on BC yield, the main parameters used in bioprocess optimization include the type and concentration of carbon and nitrogen sources, ethanol, pH, temperature, cultivation method, rotation speed, inoculum concentration, and culture medium volume (*12,32,141,160*).

Statistical optimization techniques, such as Plackett-Burman and Box-Behnken designs, have refined parameters like yeast extract concentration, temperature, and incubation time, significantly enhancing BC yields. For instance, *Gluconacetobacter hansenii* ATCC 23769 achieved 2.91 g/L BC with optimized conditions. Comprehensive characterization of BC membranes revealed high purity, crystallinity, and thermal stability, reinforcing their suitability for diverse applications, from packaging to biomedical and environmental uses (*161*).

Currently, the limitations faced in large-scale implementation are mainly related to raw material costs, energy and water consumption, by-product formation, carbon source metabolism, and the ability to reproduce yields obtained in the initial stages (*105,144*). Scale-up studies and alternatives operation mode have been successfully carried out using different nutrient sources and acetic acid bacteria, confirming that this bioprocess can be more explored to improve BC application (*102,162*).

CONCLUSIONS

Bacterial cellulose is a high-value product obtained through acetic acid fermentation and has the potential for widespread use in industrial applications due to its unique characteristics. To achieve this, the key challenge is optimizing the yield to establish a production system capable of meeting industrial demand. Designing a profitable bioprocess requires consideration of the interaction between the three key elements of fermentation: the strain, the culture medium, and the cultivation method. This review aims to present the general aspects of BC production and summarize the main challenges and strategies for increasing production and reducing bioprocess costs. The findings

presented in this review provide insights into alternatives for improving bacterial cellulose production. Designing bioprocess for bacterial cellulose production requires: 1) a high-productivity strain, either wild-type or genetically engineered; 2) a low-cost nutrient source, achievable using agro-industrial waste or substitution of carbon sources in a synthetic medium; and 3) an optimized cultivation method. An effective combination of these strategies must be explored to ensure bacterial cellulose production and its application at an industrial scale.

ACKNOWLEDGEMENTS

The researchers thank the State University of Londrina, and Brazilian agencies CNPq and CAPES for their financial support.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTION

R.P.F. Catarino contributed to the conception of the work, data collection, drafting of the article, and critical revision. V.A.B. Mascareli and A.C.L. Pavanello participated in preparation and revising the manuscript. V.L.P. Costa contributed to the preparation of the paper. W.A. Spinosa was responsible for preparation and revising the manuscript, critical revision and provided final approval of the version to be published.

ORCID ID

R.P.F. Catarino https://orcid.org/0000-0003-1539-2150
V.A.B Mascareli https://orcid.org/0000-0002-8327-8790
V.L.P Costa https://orcid.org/0000-0002-7274-7475
A.C.L. Pavanello https://orcid.org/0000-0002-7492-9133
W.A. Spinosa https://orcid.org/0000-0001-9532-0135

REFERENCES

1. Azeredo HMC, Barud H, Farinas CS, Vasconcellos VM, Claro AM. Bacterial cellulose as a raw material for food and food packaging applications. Front Sustain Food Syst. 2019;(3):7. https://doi.org/10.3389/fsufs.2019.00007.
2. Mandenius CF, Brundin A. Bioprocess optimization using design-of-experiments methodology. Biotechnol Progr. 2008;24(6):1191–203. https://doi.org/10.1002/btpr.67.
3. Shi Z, Zhang Y, Phillips GO, Yang G. Utilization of bacterial cellulose in food. Food Hydrocoll. 2014;35:539–45. https://doi.org/10.1016/j.foodhyd.2013.07.012
4. Cacicedo ML, Castro MC, Servetas I, Bosnea L, Boura K, Tsafrakidou P, *et al.* Progress in bacterial cellulose matrices for biotechnological applications. Bioresour Technol. 2016;213:172–80. https://doi.org/10.1016/j.biortech.2016.02.071
5. Marestoni LD, da Silva Barud H, Gomes RJ, Catarino RPF, Hata NNY, Ressutte JB, *et al.* Commercial and potential applications of bacterial cellulose in Brazil: Ten years review. Polimeros. 2021;30(4). https://doi.org/10.1590/0104-1428.09420
6. Li JM, Nie SP. The functional and nutritional aspects of hydrocolloids in foods. Food Hydrocoll. 2016;53(2016):46–61. http://doi.org/10.1016/j.foodhyd.2015.01.035
7. Xavier JR, Ramana KV. Development of slow melting dietary fiber-enriched ice cream formulation using bacterial cellulose and inulin. J Food Process Preserv. 2022;46(5). https://doi.org/10.1111/jfpp.15394
8. Cazón P, Velázquez G, Vázquez M. Characterization of bacterial cellulose films combined with chitosan and polyvinyl alcohol: Evaluation of mechanical and barrier properties. Carbohydr Polym. 2019;216:72–85. https://doi.org/10.3382/ps.0750417
9. Cai Q, Hu C, Yang N, Wang Q, Wang J, Pan H, *et al.* Enhanced activity and stability of industrial lipases immobilized onto spherelike bacterial cellulose. Int J Biol Macromol. 2018;109:1174–81. http://doi.org/10.1016/j.ijbiomac.2017.11.100
10. Jayani T, Sanjeev B, Marimuthu S, Uthandi S. Bacterial cellulose nano fiber (BCNF) as carrier support for the immobilization of probiotic, *Lactobacillus acidophilus* 016. Carbohydr Polym. 2020;250:116965. https://doi.org/10.1016/j.carbpol.2020.116965
11. Moradi M, Tajik H, Almasi H, Forough M, Ezati P. A novel pH-sensing indicator based on bacterial cellulose nanofibers and black carrot anthocyanins for monitoring fish freshness. Carbohydr Polym. 2019;222:115030. https://doi.org/10.1016/j.carbpol.2019.115030
12. Bilgi E, Gokce EH, Bayir E, Sendemir A, Ozer KO, Tuna EEH. Bacterial cellulose based facial mask with antioxidant property and high moisturizing capacity. Cellulose. 202;28(16):10399–414. https://doi.org/10.1007/s10570-021-04106-z
13. Pasaribu KM, Ilyas S, Tamrin T, Radecka I, Swingler S, Gupta A, *et al.* Bioactive bacterial cellulose wound dressings for burns with collagen *in-situ* and chitosan *ex-situ* impregnation. Int J Biol Macromol. 2023;230:123118. https://doi.org/10.1016/j.ijbiomac.2022.123118

14. Legnani C, Barud HS, Caiut JMA, Calil VL, Maciel IO, Quirino WG, *et al.* Transparent bacterial cellulose nanocomposites used as substrate for organic light-emitting diodes. J Mater Sci: Mater Electron. 2019;30(18):16718–23. https://doi.org/10.1007/s10854-019-00979-w
15. He Y, Xie Z, Zhang H, Liebl W, Toyama H, Chen F. Oxidative fermentation of acetic acid bacteria and its products. Front Microbiol. 2022;13. https://doi.org/10.3389/fmicb.2022.879246
16. Chen G, Wu G, Chen L, Wang W, Hong FF, Jönsson LJ. Performance of nanocellulose-producing bacterial strains in static and agitated cultures with different starting pH. Carbohydr Polym. 2019;215:280–8. https://doi.org/10.1016/j.carbpol.2019.03.080
17. Gomes RJ, Ida EI, Spinosa WA. Nutritional supplementation with amino acids on bacterial cellulose production by *Komagataeibacter intermedius*: effect analysis and application of response surface methodology. Appl Biochem Biotechnol. 2022;194(11):5017–36. https://doi.org/10.1007/s12010-022-04013-4
18. Lotfy VF, Basta AH, Abdel-Monem MO, Abdel-Hamed GZ. Utilization of bacteria in rotten Guava for production of bacterial cellulose from isolated and protein waste. Carbohydr Polym Technol Applicat. 2021;2:100076. https://doi.org/10.1016/j.carpta.2021.100076
19. Lin D, Liu Z, Shen R, Chen S, Yang X. Bacterial cellulose in food industry: current research and future prospects. Int J Biol Macromol. 2020;158:1007–19. https://doi.org/10.1016/j.ijbiomac.2020.04.230
20. Salari M, Khiabani MS, Mokarram RR, Ghanbarzadeh B, Kafil HS. Preparation and characterization of cellulose nanocrystals from bacterial cellulose produced in sugar beet molasses and cheese whey media. Int J Biol Macromol. 2019;122:280–8. https://doi.org/10.1016/j.ijbiomac.2018.10.136
21. Foresti ML, Vázquez A, Boury B. Applications of bacterial cellulose as precursor of carbon and composites with metal oxide, metal sulfide and metal nanoparticles: A review of recent advances. Carbohydr Polym. 2017;157:447–67. https://doi.org/10.1016/j.carbpol.2016.09.008
22. Lin SP, Calvar IL, Catchmark JM, Liu JR, Demirci A, Cheng KC. Biosynthesis, production and applications of bacterial cellulose. Cellulose. 2013;20(5):2191–219. https://doi.org/10.1007/s10570-013-9994-3
23. Armitano J, Méjean V, Jourlin-Castelli C. Gram-negative bacteria can also form pellicles. Environ Microbiol Rep. 2014;6(6):534–44. https://doi.org/10.1111/1758-2229.12171
24. AydIn YA, Aksoy ND. Isolation and characterization of an efficient bacterial cellulose producer strain in agitated culture: *Gluconacetobacter hansenii* P2A. Appl Microbiol Biotechnol. 2014;98(3):1065–75. https://doi.org/10.1007/s00253-013-5296-9
25. Valera MJ, Torija MJ, Mas A, Mateo E. Cellulose production and cellulose synthase gene detection in acetic acid bacteria. Appl Microbiol Biotechnol. 2015;99(3):1349–61. https://doi.org/10.1007/s00253-014-6198-1
26. Wu JM, Liu RH. Cost-effective production of bacterial cellulose in static cultures using distillery wastewater. J Biosci Bioeng. 2013;115(3):284–90. https://doi.org/10.1016/j.jbiosc.2012.09.014
27. Gomes RJ, de Fatima Borges M, de Freitas Rosa M, Castro-Gómez RJH, Spinosa WA. Acetic acid bacteria in the food industry: Systematics, characteristics and applications. Food Technol Biotechnol. 2018;56(2):139–51.

https://doi.org/10.17113/ftb.56.02.18.5593

28. Lima HLS, Nascimento ES, Andrade FK, Borges MF, Cassales AR, Muniz CR, *et al.* Bacterial cellulose production by *Komagataeibacter hansenii* ATCC 23769 using sisal juice - an agroindustry waste. Braz J Chem Eng. 2017;34(03):671–80. https://doi.org/10.1590/0104-6632.20170343s20150514
29. Lin SP, Huang YH, Hsu K Di, Lai YJ, Chen YK, Cheng KC. Isolation and identification of cellulose-producing strain *Komagataeibacter intermedius* from fermented fruit juice. Carbohydr Polym. 2016;151:827–33. https://doi.org/10.1016/j.carbpol.2016.06.032
30. Machado RTA, Meneguin AB, Sábio RM, Franco DF, Antonio SG, Gutierrez J, *et al. Komagataeibacter rhaeticus* grown in sugarcane molasses-supplemented culture medium as a strategy for enhancing bacterial cellulose production. Ind Crops Prod. 2018;122:637–46. https://doi.org/10.1016/j.indcrop.2018.06.048
31. Molina RC, Enciso C, Torres TM, Zuluaga R, Gañán P, Rojas OJ, *et al.* Effects of alternative energy sources on bacterial cellulose characteristics produced by *Komagataeibacter medellinensis*. Int J Biol Macromol. 2018;117:735–41. https://doi.org/10.1016/j.ijbiomac.2018.05.195
32. Du R, Zhao F, Peng Q, Zhou Z, Han Y. Production and characterization of bacterial cellulose produced by *Gluconacetobacter xylinus* isolated from chinese persimmon vinegar. Carbohydr Polym. 2018;194:200–7. https://doi.org/10.1016/j.carbpol.2018.04.041
33. Fernández J, Morena AG, Valenzuela S V, Pastor FIJ, Díaz P, Martínez J. Microbial cellulose from a *Komagataeibacter intermedius* strain isolated from commercial wine vinegar. J Polym Environ. 2019;27(5):956–67. http://doi.org/10.1007/s10924-019-01403-4
34. Li J, Chen G, Zhang R, Wu H, Zeng W, Liang Z. Production of high crystallinity type-I cellulose from *Komagataeibacter hansenii* JR-02 isolated from kombucha tea. Biotechnol Appl Biochem. 2019;66(1):108–18. https://doi.org/10.1002/bab.1703
35. Rangaswamy BE, Vanitha KP, Hungund BS. Microbial cellulose production from bacteria isolated from rotten fruit. Int J Polym Sci. 2015;2015. https://doi.org/10.1155/2015/280784
36. Semjonovs P, Ruklisha M, Paegle L, Saka M, Treimane R, Skute M, *et al.* Cellulose synthesis by *Komagataeibacter rhaeticus* strain P 1463 isolated from kombucha. Appl Microbiol Biotechnol. 2017;101(3):1003–12. https://doi.org/10.1007/s00253-016-7761-8
37. Bagewadi ZK, Bhavikatti JS, Muddapur UM, Yaraguppi DA, Mulla SI. Statistical optimization and characterization of bacterial cellulose produced by isolated thermophilic *Bacillus licheniformis* strain ZBT2. Carbohydr Res. 2020;491:107979. https://doi.org/10.1016/j.carres.2020.107979
38. Liu D, Cao Y, Qu R, Gao G, Chen S, Zhang Y, et al. Production of bacterial cellulose hydrogels with tailored crystallinity from *Enterobacter* sp. FY-07 by the controlled expression of colanic acid synthetic genes. Carbohydr Polym. 2019;207:563–70. https://doi.org/10.1016/j.carbpol.2018.12.014
39. Khan H, Kadam A, Dutt D. Studies on bacterial cellulose produced by a novel strain of *Lactobacillu*s genus. Carbohydr Polym. 2020;229:115513. https://doi.org/10.1016/j.carbpol.2019.115513
40. Saleh AK, El-Gendi H, Soliman NA, El-Zawawy WK, Abdel-Fattah YR. Bioprocess development for bacterial cellulose biosynthesis by novel *Lactiplantibacillus plantarum* isolate

along with characterization and antimicrobial assessment of fabricated membrane. Sci Rep. 2022;12(1):2181. https://doi.org/10.1038/s41598-022-06117-7

41. Lynch KM, Zannini E, Wilkinson S, Daenen L, Arendt EK. Physiology of acetic acid bacteria and their role in vinegar and fermented beverages. Compr Rev Food Sci Food Saf. 2019;18(3):587–625. https://doi.org/10.1111/1541-4337.12440
42. Saichana N, Matsushita K, Adachi O, Frébort I, Frebortova J. Acetic acid bacteria: a group of bacteria with versatile biotechnological applications. Biotechnol Adv. 2015 Nov;33(6):1260–71. https://doi.org/10.1016/j.biotechadv.2014.12.001
43. China S La, Zanichelli G, Vero L De, Gullo M. Oxidative fermentations and exopolysaccharides production by acetic acid bacteria: a mini review. Biotechnol Lett. 2018;40(9–10):1289–302. https://doi.org/10.1007/s10529-018-2591-7
44. Jacek P, Dourado F, Gama M, Bielecki S. Molecular aspects of bacterial nanocellulose biosynthesis. Microb Biotechnol. 2019;12(4):633–49. https://doi.org/10.1111/1751-7915.13386
45. Ross P, Mayer R, Benziman M. Cellulose biosynthesis and function in bacteria. Microbiol Rev. 1991:55(1):35-58. https://doi.org/10.1128/mr.55.1.35-58.1991
46. Ullah H, Santos HA, Khan T. Applications of bacterial cellulose in food, cosmetics and drug delivery. Cellulose. 2016;23(4):2291–314. https://doi.org/10.1007/s10570-016-0986-y
47. Costa AFS, Almeida FCG, Vinhas GM, Sarubbo LA. Production of bacterial cellulose by *Gluconacetobacter hansenii* using corn steep liquor as nutrient sources. Front Microbiol. 2017;8(OCT):1–12. https://doi.org/10.3389/fmicb.2017.02027
48. Shah N, Ul-Islam M, Khattak WA, Park JK. Overview of bacterial cellulose composites: A multipurpose advanced material. Carbohydr Polym. 2013;98(2):1585–98. https://doi.org/10.1016/j.carbpol.2013.08.018
49. Habibi Y, Lucia LA, Rojas OJ. Cellulose nanocrystals: Chemistry, self-assembly, and applications. Chem Rev. 2010;110(6):3479–500. https://doi.org//10.1021/cr900339w
50. Gibson LJ. The hierarchical structure and mechanics of plant materials. J R Soc Interface. 2012;9(76):2749–66. https://doi.org/10.1098/rsif.2012.0341
51. Nascimento DM, Nunes YL, Figueirêdo MCB, de Azeredo HMC, Aouada FA, Feitosa JPA, *et al.* Nanocellulose nanocomposite hydrogels: technological and environmental issues. Green Chemistry. 2018;20(11):2428–48. https://doi.or/10.1039/c8gc00205c
52. Parte FGB, Santoso SP, Chou CC, Verma V, Wang HT, Ismadji S, *et al.* Current progress on the production, modification, and applications of bacterial cellulose. Crit Rev Biotechnol. 2020;40(3):397–414. https://doi.org/10.1080/07388551.2020.1713721
53. Ul-Islam M, Khan S, Ullah MW, Park JK. Comparative study of plant and bacterial cellulose pellicles regenerated from dissolved states. Int J Biol Macromol. 2019;137:247–52. https://doi.org/10.1016/j.ijbiomac.2019.06.232
54. Kwak MH, Kim JE, Go J, Koh EK, Song SH, Son HJ, *et al.* Bacterial cellulose membrane produced by *Acetobacter sp*. A10 for burn wound dressing applications. Carbohydr Polym.

2015;122:387–98.
https://doi.org/10.1016/j.carbpol.2014.10.049

55. Rani MU, Appaiah KAA. Production of bacterial cellulose by *Gluconacetobacter hansenii* UAC09 using coffee cherry husk. J Food Sci Technol. 2013;50(4):755–62. https://doi.org/10.1007/s13197-011-0401-5
56. Machado RTA, Gutierrez J, Tercjak A, Trovatti E, Uahib FGM, de Padua Moreno G, *et al. Komagataeibacter rhaeticus* as an alternative bacteria for cellulose production. Carbohydr Polym. 2016;152:841–9. https://doi.org/10.1016/j.carbpol.2016.06.049
57. Vasconcelos NF, Feitosa JPA, da Gama FMP, Morais JPS, Andrade FK, de Sá Moreira de Souza Filho M, *et al.* Bacterial cellulose nanocrystals produced under different hydrolysis conditions: Properties and morphological features. Carbohydr Polym. 2017;155:425–31. http://doi.org/10.1016/j.carbpol.2016.08.090
58. Bi JC, Liu SX, Li CF, Li J, Liu LX, Deng J, *et al.* Morphology and structure characterization of bacterial celluloses produced by different strains in agitated culture. J Appl Microbiol. 2014;117(5):1305–11. https://doi.org/10.1111/jam.12619
59. Brandes R, Carminatti C, Mikowski A, Al-Qureshi H, Recouvreux D. A mini-review on the progress of spherical bacterial cellulose production. Journal of Nano Research. 2017;45:142–54. https://doi.org/10.4028/www.scientific.net/JNanoR.45.142
60. de Oliveira Barud HG, da Silva RR, da Silva Barud H, Tercjak A, Gutierrez J, Lustri WR, *et al.* A multipurpose natural and renewable polymer in medical applications: bacterial cellulose. Carbohydr Polym. 2016;153:406–20. https://doi.org/10.1016/j.carbpol.2016.07.059
61. Feng X, Ullah N, Wang X, Sun X, Li C, Bai Y, *et al*. Characterization of bacterial cellulose by *Gluconacetobacter hansenii* CGMCC 3917. J Food Sci. 2015;80(10):E2217–27. https://doi.org/10.1111/1750-3841.13010
62. Fijałkowski K, Peitler D, Rakoczy R, Zywicka A. Survival of probiotic lactic acid bacteria immobilized in different forms of bacterial cellulose in simulated gastric juices and bile salt solution. LWT - Food Sci Technol. 2016;68:322–8. https://doi.org/10.1016/j.lwt.2015.12.038
63. Guo Y, Zhang X, Hao W, Xie Y, Chen L, Li Z, *et al.* Nano-bacterial cellulose/soy protein isolate complex gel as fat substitutes in ice cream model. Carbohydr Polym. 2018;198:620–30. https://doi.org/10.1016/j.carbpol.2018.06.078
64. Marchetti L, Muzzio B, Cerrutti P, Andrés SC, Califano AN. Bacterial nanocellulose as novel additive in low-lipid low-sodium meat sausages. Effect on quality and stability. Food Struct. 2017;14(July):52–9. http://doi.org/10.1016/j.foostr.2017.06.004
65. Olivera AAN, Guimarães J de T, Furtado AAL, Mesquita E de FM de. Effect of the addition of bacterial cellulose on the texture and color properties of sausages obtained from mechanically separated meat from Nile tilapia (*Oreochromis niloticus L.*). Food Sci Technol. 2023;43: e81522. https://doi.org/10.1590/fst.81522
66. Farooq U, Ullah MW, Yang Q, Aziz A, Xu J, Zhou L, *et al.* High-density phage particles immobilization in surface-modified bacterial cellulose for ultra-sensitive and selective electrochemical detection of *Staphylococcus aureus*. Biosens Bioelectron. 2020;157:112163. https://doi.org/10.1016/j.bios.2020.112163
67. Shafipour Yordshahi A, Moradi M, Tajik H, Molaei R. Design and preparation of antimicrobial

meat wrapping nanopaper with bacterial cellulose and postbiotics of lactic acid bacteria. Int J Food Microbiol. 2020;321:108561. https://doi.org/10.1016/j.ijfoodmicro.2020.108561

68. Liu H, Shi C, Sun X, Zhang J, Ji Z. Intelligent colorimetric indicator film based on bacterial cellulose and pelargonidin dye to indicate the freshness of tilapia fillets. Food Packag Shelf Life. 2021;29:100712. https://doi.org/10.1016/j.fpsl.2021.10071
69. Marchetti L, Andrés SC, Cerruti P, Califano AN. Effect of bacterial nanocellulose addition on the rheological properties of gluten-free muffin batters. Food Hydrocoll. 2020;98. https://doi.org/10.1016/j.foodhyd.2019.105315
70. Yan H, Chen X, Song H, Li J, Feng Y, Shi Z, *et al.* Synthesis of bacterial cellulose and bacterial cellulose nanocrystals for their applications in the stabilization of olive oil pickering emulsion. Food Hydrocoll. 2017;72:127–35. https://doi.org/10.1016/j.foodhyd.2017.05.044
71. Gao H, Sun Q, Han Z, Li J, Liao B, Hu L, *et al.* Comparison of bacterial nanocellulose produced by different strains under static and agitated culture conditions. Carbohydr Polym. 2020;227. https://doi.org/10.1016/j.carbpol.2019.115323
72. Martins D, de Carvalho Ferreira D, Gama M, Dourado F. Dry bacterial cellulose and carboxymethyl cellulose formulations with interfacial-active performance: processing conditions and redispersion. Cellulose. 2020;27(11):6505–20. https://doi.org/10.1007/s10570-020-03211-9
73. Paximada P, Tsouko E, Kopsahelis N, Koutinas AA, Mandala I. Bacterial cellulose as stabilizer of o/w emulsions. Food Hydrocoll. 2016;53:225-32. https://doi.org/10.1016/j.foodhyd.2014.12.003
74. Zhai X, Lin D, Liu D, Yang X. Emulsions stabilized by nanofibers from bacterial cellulose: new potential food-grade pickering emulsions. Food Res Int. 2018;103:12–20. https://doi.org/10.1016/j.foodres.2017.10.030
75. Zhang X, Zhou J, Chen J, Li B, Li Y, Liu S. Edible foam based on pickering effect of bacterial cellulose nanofibrils and soy protein isolates featuring interfacial network stabilization. Food Hydrocoll. 2020;100:105440. https://doi.org/10.1016/j.foodhyd.2019.105440
76. Gorgieva S, Trček J. Bacterial cellulose: production, modification and perspectives in biomedical applications. Nanomaterials. 2019;9(10):1352. https://doi.org/10.3390/nano9101352
77. Dutta SD, Patel DK, Lim KT. Functional cellulose-based hydrogels as extracellular matrices for tissue engineering. J Biol Eng. 2019;13(1):55. https://doi.org/10.1186/s13036-019-0177-0
78. Swingler S, Gupta A, Gibson H, Kowalczuk M, Heaselgrave W, Radecka I. Recent advances and applications of bacterial cellulose in biomedicine. Polymers (Basel). 2021;13(3):412. https://doi.org/10.3390/polym13030412
79. Chantereau G, Sharma M, Abednejad A, Vilela C, Costa EM, Veiga M, *et al.* Bacterial nanocellulose membranes loaded with vitamin B-based ionic liquids for dermal care applications. J Mol Liq. 2020;302:112547. https://doi.org/10.1016/j.molliq.2020.112547
80. Ul-Islam M, Alhajaim W, Fatima A, Yasir S, Kamal T, Abbas Y, *et al.* Development of low-cost bacterial cellulose-pomegranate peel extract-based antibacterial composite for potential biomedical applications. Int J Biol Macromol. 2023;231:123269. https://doi.org/10.1016/j.ijbiomac.2023.123269
81. Beekmann U, Schmölz L, Lorkowski S, Werz O, Thamm J, Fischer D, *et al.* Process control

and scale-up of modified bacterial cellulose production for tailor-made anti-inflammatory drug delivery systems. Carbohydr Polym. 2020;236:116062. https://doi.org/10.1016/j.carbpol.2020.116062

82. Potočnik V, Gorgieva S, Trček J. From nature to lab: sustainable bacterial cellulose production and modification with synthetic biology. Polymers (Basel). 202318;15(16):3466. https://doi.org/10.3390/polym15163466
83. Lima HLS, Gonçalves C, Cerqueira MÂ, do Nascimento ES, Gama MF, Rosa MF, *et al.* Bacterial cellulose nanofiber-based films incorporating gelatin hydrolysate from tilapia skin: production, characterization and cytotoxicity assessment. Cellulose. 2018;25(10):6011–29. https://doi.org/10.1007/s10570-018-1983-0
84. Jang EJ, Padhan B, Patel M, Pandey JK, Xu B, Patel R. Antibacterial and biodegradable food packaging film from bacterial cellulose. Food Control. 2023;153:109902. https://doi.org/10.1016/j.foodcont.2023.109902
85. Ju S, Zhang F, Duan J, Jiang J. Characterization of bacterial cellulose composite films incorporated with bulk chitosan and chitosan nanoparticles: a comparative study. Carbohydr Polym. 2020:37:116167. https://doi.org/10.1016/j.carbpol.2020.116167
86. Salari M, Sowti Khiabani M, Rezaei Mokarram R, Ghanbarzadeh B, Samadi Kafil H. Development and evaluation of chitosan based active nanocomposite films containing bacterial cellulose nanocrystals and silver nanoparticles. Food Hydrocoll. 2018 Nov;84:414–23. https://doi.org/10.1016/j.foodhyd.2018.05.037
87. Oliveira AAN, Mesquita E de FM de, Furtado AAL. Use of bacterial cellulose as a fat replacer in emulsified meat products: review. Food Sci Technol. 2022;42. https://doi.org/10.1590/fst.42621
88. Gullo M, Caggia C, Vero L De, Giudici P. Characterization of acetic acid bacteria in “traditional balsamic vinegar.” Int J Food Microbiol. 2006;106(2):209–12. https://doi.org/10.1016/j.ijfoodmicro.2005.06.024
89. Kim DH, Chon JW, Kim H, Seo KH. Development of a novel selective medium for the isolation and enumeration of acetic acid bacteria from various foods. Food Control. 2019;106:106717. https://doi.org/10.1016/J.FOODCONT.2019.106717
90. Vegas C, Mateo E, González Á, Jara C, Guillamón JM, Poblet M, *et al.* Population dynamics of acetic acid bacteria during traditional wine vinegar production. Int J Food Microbiol. 2010;138(1–2):130–6. https://doi.org/10.1016/j.ijfoodmicro.2010.01.006
91. Ryngajłło M, Jędrzejczak-Krzepkowska M, Kubiak K, Ludwicka K, Bielecki S. Towards control of cellulose biosynthesis by *Komagataeibacter* using systems-level and strain engineering strategies: current progress and perspectives. Appl Microbiol Biotechnol. 2020;104(15):6565–85. https://doi.org/10.1007/s00253-020-10671-3
92. Nascimento FX, Torres CAV, Freitas F, Reis MAM, Crespo MTB. Functional and genomic characterization of *Komagataeibacter uvaceti* FXV3, a multiple stress resistant bacterium producing increased levels of cellulose. Biotech Reports. 2021;30:e00606. https://doi.org/10.1016/j.btre.2021.e00606
93. Gomes RJ, de Sousa Faria-Tischer PC, Tischer CA, Constantino LV, de Freitas Rosa M, Chideroli RT, *et al. Komagataeibacter intermedius* V-05: an acetic acid bacterium isolated from vinegar industry, with high capacity for bacterial cellulose production in soybean molasses medium. Food Technol Biotechnol. 2021;59(4):432–42. https://doi.org/10.17113/ftb.59.04.21.7148

94. Jacek P, Silva FAGS da, Dourado F, Bielecki S, Gama M. Optimization and characterization of bacterial nanocellulose produced by *Komagataeibacter rhaeticus* K3. Carbohydr Polym Technol Appl. 2021;2:100022. https://doi.org/10.1016/j.carpta.2020.100022

95. Zhang H, Xu X, Chen X, Yuan F, Sun B, Xu Y, *et al*. Complete genome sequence of the cellulose-producing strain *Komagataeibacter nataicola* RZS01. Sci Rep. 2017;7(1):4431. https://doi.org/10.1038/s41598-017-04589-6

96. Thongwai N, Futui W, Ladpala N, Sirichai B, Weechan A, Kanklai J, *et al.* Characterization of bacterial cellulose produced by *Komagataeibacter maltaceti* P285 isolated from contaminated honey wine. Microorganisms. 2022;10(3):528. https://doi.org/10.3390/microorganisms10030528

97. Gullo M, Giudici P. Acetic acid bacteria in traditional balsamic vinegar: Phenotypic traits relevant for starter cultures selection. Int J Food Microbiol. 2008;125(1):46-53. https://doi.org/10.1016/j.ijfoodmicro.2007.11.076

98. Barja F, Andrés-Barrao C, Pérez RO, Cabello EM, Chappuis ML. Physiology of *Komagataeibacter spp*. during acetic acid fermentation. In: Acetic Acid Bacteria. Springer Japan; 2016: 201–21. https://doi.org/10.1007/978-4-431-55933-7_9

99. Pacheco G, Nogueira CR, Meneguin AB, Trovatti E, Silva MCC, Machado RTA, *et al.* Development and characterization of bacterial cellulose produced by cashew tree residues as alternative carbon source. Ind Crops Prod. 2017;107:13–9. http://doi.org/10.1016/j.indcrop.2017.05.026

100. Santoso SP, Chou CC, Lin SP, Soetaredjo FE, Ismadji S, Hsieh CW, *et al.* Enhanced production of bacterial cellulose by *Komactobacter intermedius* using statistical modeling. Cellulose. 2020;27(5):2497–509. https://doi.org/10.1007/s10570-019-02961-5

101. Dubey S, Sharma RK, Agarwal P, Singh J, Sinha N, Singh RP. From rotten grapes to industrial exploitation: *Komagataeibacter europaeus* SGP37, a micro-factory for macroscale production of bacterial nanocellulose. Int J Biol Macromol. 2017;96:52–60. https://doi.org/10.1016/j.ijbiomac.2016.12.016

102. Dubey S, Singh J, Singh RP. Biotransformation of sweet lime pulp waste into high-quality nanocellulose with an excellent productivity using *Komagataeibacter europaeus* SGP37 under static intermittent fed-batch cultivation. Bioresour Technol. 2018;247:73–80. https://doi.org/10.1016/j.biortech.2017.09.089

103. Singhsa P, Narain R, Manuspiya H. Physical structure variations of bacterial cellulose produced by different *Komagataeibacter xylinus* strains and carbon sources in static and agitated conditions. Cellulose. 2018;25(3):1571–81. https://doi.org/10.1007/s10570-018-1699-1

104. Chen SQ, Mikkelsen D, Lopez-Sanchez P, Wang D, Martinez-Sanz M, Gilbert EP, *et al.* Characterisation of bacterial cellulose from diverse *Komagataeibacter* strains and their application to construct plant cell wall analogues. Cellulose. 2017 Dec;24(3):1211–26. https://doi.org/10.1007/s10570-017-1203-3

105. de Araújo e Silva R, Brígida AIS, de Freitas Rosa M, da Silva Neto RM, Spinosa WA, de Sá Filho EB, *et al.* An approach for implementing ecodesign at early research stage: a case study of bacterial cellulose production. J Clean Prod. 2020;269. https://doi.org/10.1016/j.jclepro.2020.122245

106. Shafiei R, Zarmehrkhorshid R, Mounir M, Thonart P, Delvigne F. Influence of carbon sources on the viability and resuscitation of *Acetobacter senegalensis* during high-temperature gluconic acid fermentation. Bioprocess Biosyst Eng. 2017;15;40(5):769–80.

https://doi.org/10.1007/s00449-017-1742-x

107. Dong K, Pan H, Yang D, Rao L, Zhao L, Wang Y, *et al.* Induction, detection, formation, and resuscitation of viable but non-culturable state microorganisms. Compr Rev Food Sci Food Saf. 2020;19(1):149–83. https://doi.org/10.1111/1541-4337.12513

108. Singh A, Walker KT, Ledesma-Amaro R, Ellis T. Engineering bacterial cellulose by synthetic biology. Int J Mol Sci. 2020;21(23):9185. https://doi.org/10.3390/ijms21239185

109. Li G, Wang L, Deng Y, Wei Q. Research progress of the biosynthetic strains and pathways of bacterial cellulose. J Ind Microbiol Biotechnol. 2022;49(1). https://doi.org/10.1093/jimb/kuab071

110. Montenegro-Silva P, Ellis T, Dourado F, Gama M, Domingues L. Enhanced bacterial cellulose production in *Komagataeibacter sucrofermentans:* impact of different PQQ-dependent dehydrogenase knockouts and ethanol supplementation. Biotechnol Biofuel Bioprod. 2024;17(1):35. https://doi.org/10.1186/s13068-024-02482-9

111. Yang F, Cao Z, Li C, Chen L, Wu G, Zhou X, *et al.* A recombinant strain of *Komagataeibacter xylinus* ATCC 23770 for production of bacterial cellulose from mannose-rich resources. N Biotechnol. 2023;76:72–81. https://doi.org/10.1016/j.nbt.2023.05.002

112. Jacek P, Ryngajłło M, Bielecki S. Structural changes of bacterial nanocellulose pellicles induced by genetic modification of *Komagataeibacter hansenii* ATCC 23769. Appl Microbiol Biotechnol. 2019;103(13):5339–53. https://doi.org/10.1007/s00253-019-09846-4

113. Zou X, Zhang S, Chen L, Hu J, Hong FF. Determination of live and dead *Komagataeibacter xylinus* cells and first attempt at precise control of inoculation in nanocellulose production. Microb Biotechnol. 2020;3(2):458–69. https://doi.org/10.1111/1751-7915.13494

114. Sperotto G, Stasiak LG, Godoi JPMG, Gabiatti NC, De Souza SS. A review of culture media for bacterial cellulose production: complex, chemically defined and minimal media modulations. Cellulose. 2021;28(5):2649–73. https://doi.org/10.1007/s10570-021-03754-5

115. Santos JV dos, Nizoli É, Galvan D, Gomes RJ, Biz G, Ressutte JB, *et al.* Micronutrient requirements and effects on cellular growth of acetic acid bacteria involved in vinegar production. Food Sci Technol. 2022;42. https://doi.org/10.1590/fst.05121

116. Mamlouk D, Gullo M. Acetic acid bacteria: physiology and carbon sources oxidation. Indian J Microbiol. 2013;53(4):377–84. https://doi.org/10.1007/s12088-013-0414-z

117. Gopu G, Govindan S. Production of bacterial cellulose from *Komagataeibacter saccharivorans* strain BC1 isolated from rotten green grapes BC1 isolated from rotten green grapes. Prep Biochem Biotechnol. 2018;0(0):1–11. https://doi.org/10.1080/10826068.2018.1513032

118. Leonarski E, Cesca K, Zanella E, Stambuk BU, de Oliveira D, Poletto P. Production of kombucha-like beverage and bacterial cellulose by acerola byproduct as raw material. LWT-Food Sci Technol. 2021;135:110075. https://doi.org/10.1016/j.lwt.2020.110075

119. Souza EF, Furtado MR, Carvalho CWP, Freitas-Silva O, Gottschalk LMF. Production and characterization of *Gluconacetobacter xylinus* bacterial cellulose using cashew apple juice

and soybean molasses. Int J Biol Macromol. 2020;146:285–9. https://doi.org/10.1016/j.ijbiomac.2019.12.180

120. Entani E, Ohmori S, Masai H, Suzuki KI. *Acetobacter polyoxogenes* sp. nov., a new species of an acetic acid bacterium useful for producing vinegar with high acidity. J Gen Appl Microbiol. 1985;31(5):475–90. https://doi.org/10.2323/jgam.31.475

121. Hestrin S, Schramm M. Synthesis of cellulose by *Acetobacter xylinum*. II. preparation of freeze-dried cells capable of polymerizing glucose to cellulose. Biochem J. 1954;58(2):345–52. https://doi.org/10.1042/bj0580345

122. Vashisht A, Thakur K, Kauldhar BS, Kumar V, Yadav SK. Waste valorization: Identification of an ethanol tolerant bacterium *Acetobacter pasteurianus* SKYAA25 for acetic acid production from apple pomace. Sci Total Environm. 2019;690:956–64. https://doi.org/10.1016/J.SCITOTENV.2019.07.070

123. Gupte Y, Kulkarni A, Raut B, Sarkar P, Choudhury R, Chawande A, *et al.* Characterization of nanocellulose production by strains of *Komagataeibacter sp.* isolated from organic waste and Kombucha. Carbohydr Polym. 2021;266(May):118176. https://doi.org/10.1016/j.carbpol.2021.118176

124. Sokollek SJ, Hertel C, Hammes WP. Cultivation and preservation of vinegar bacteria. J Biotechnol. 1998;60(3):195–206. https://doi.org/10.1016/S0168-1656(98)00014-5

125. Shafiei R, Delvigne F, Babanezhad M, Thonart P. Evaluation of viability and growth of *Acetobacter senegalensis* under different stress conditions. Int J Food Microbiol. 2013;163(2–3):204–13. https://doi.org/10.1016/j.ijfoodmicro.2013.03.011

126. Revin V, Liyaskina E, Nazarkina M, Bogatyreva A, Shchankin M. Cost-effective production of bacterial cellulose using acidic food industry by-products. Braz J Microbiol. 2018;49:151–9. https://doi.org/10.1016/J.BJM.2017.12.012

127. Reiniati I, Hrymak AN, Margaritis A. Recent developments in the production and applications of bacterial cellulose fibers and nanocrystals. Crit Rev Biotechnol. 2017;37(4):510–24. https://doi.org/10.1016/j.bej.2017.07.007

128. Vazquez A, Foresti ML, Cerrutti P, Galvagno M. Bacterial cellulose from simple and low-cost production media by *Gluconacetobacter xylinus*. J Polym Environ. 2013;21(2):545–54. https://doi.org/10.1007/s10924-012-0541-3

129. Bartowsky EJ, Henschke PA. Acetic acid bacteria spoilage of bottled red wine - A review. Int J Food Microbiol. 2008;125(1):60–70. https://doi.org/10.1016/j.ijfoodmicro.2007.10.016

130. Roos J De, Vuyst L De. Acetic acid bacteria in fermented foods and beverages. Curr Opin Biotechnol. 2018;49:115–9. https://doi.org/10.1016/j.copbio.2017.08.007

131. Hutchinson UF, Ntwampe SKO, Ngongang MM, Chidi BS, Hoff JW, Jolly NP. Product and microbial population kinetics during balsamic-styled vinegar production. J Food Sci. 2019;84(3):572–9. https://doi.org/10.1111/1750-3841.14429

132. Tran T, Grandvalet C, Verdier F, Martin A, Alexandre H, Tourdot-Maréchal R. Microbial dynamics between yeasts and acetic acid bacteria in kombucha: impacts on the chemical composition of the beverage. Foods. 2020;9(7):963. https://doi.org/10.3390/foods9070963

133. Wang ZG, Xiang D, Wang XB, Li CF. Preparation of an inoculum of *Gluconacetobacter*

xylinus without mutants in shaken culture. J Appl Microbiol. 2016;121(3):713–20. https://doi.org/10.1111/jam.13193

134. Baena RS, Jiménez OC, Santos DIM, Cantero MD, Barja F, García GI. Rapid method for total, viable and non-viable acetic acid bacteria determination during acetification process. Process Biochem. 2006;41(5):1160–4. https://doi.org/10.1016/j.procbio.2005.12.016

135. González A, Hierro N, Poblet M, Mas A, Guillamón JM. Enumeration and detection of acetic acid bacteria by real-time PCR and nested PCR. FEMS Microbiol Lett. 2006;254(1):123–8. https://doi.org/10.1111/j.1574-6968.2005.000011.x

136. Jozala AF, Pértile RAN, dos Santos CA, de Carvalho Santos-Ebinuma V, Seckler MM, Gama FM, *et al.* Bacterial cellulose production by *Gluconacetobacter xylinus* by employing alternative culture media. Appl Microbiol Biotechnol. 2015;99(3):1181–90. https://doi.org/10.1007/s00253-014-6232-3

137. Campano C, Balea A, Blanco A, Negro C. Enhancement of the fermentation process and properties of bacterial cellulose: a review. Cellulose. 2016;23(1):57–91. https://doi.org/10.1007/s10570-015-0802-0

138. Lee KY, Buldum G, Mantalaris A, Bismarck A. More than meets the eye in bacterial cellulose: Biosynthesis, bioprocessing, and applications in advanced fiber composites. Macromol Biosci. 2014;14(1):10–32. https://doi.org/10.1002/mabi.201300298

139. Molina RC, Castro M, Osorio M, Torres TM, Gómez B, Zuluaga R, *et al*. Effect of different carbon sources on bacterial nanocellulose production and structure using the low pH resistant strain *Komagataeibacter medellinensis*. Materials. 2017;10(6). https://doi.org/10.3390/ma10060639

140. Miqueleto AP, Dolosic CC, Pozzi E, Foresti E, Zaiat M. Influence of carbon sources and C/N ratio on EPS production in anaerobic sequencing batch biofilm reactors for wastewater treatment. Bioresour Technol. 2010;101(4):1324–30. https://doi.org/10.1016/j.biortech.2009.09.026

141. Rastogi A, Banerjee R. Statistical optimization of bacterial cellulose production by Leifsonia soli and its physico-chemical characterization. Process Biochem. 2020;91:297–302. https://doi.org/10.1016/j.procbio.2019.12.021

142. Basu A, Vadanan SV, Lim S. Rational design of a scalable bioprocess platform for bacterial cellulose production. Carbohydr Polym. 2019;207:684–93. https://doi.org/10.1016/j.carbpol.2018.10.085

143. Abdelraof M, Hasanin MS, El-Saied H. Ecofriendly green conversion of potato peel wastes to high productivity bacterial cellulose. Carbohydr Polym. 2019;211:75–83. https://doi.org/10.1016/j.carbpol.2019.01.095

144. Skiba EA, Budaeva V V, Ovchinnikova E V, Gladysheva EK, Kashcheyeva EI, Pavlov IN, *et al.* A technology for pilot production of bacterial cellulose from oat hulls. Chem Eng J. 2020;383:123128. https://doi.org/10.1016/j.cej.2019.123128

145. Tsouko E, Pilafidis S, Dimopoulou M, Kourmentza K, Sarris D. Bioconversion of underutilized brewing by-products into bacterial cellulose by a newly isolated *Komagataeibacter rhaeticus* strain: a preliminary evaluation of the bioprocess environmental impact. Bioresour Technol. 2023; 387:129667. https://doi.org/10.1016/j.biortech.2023.129667

146. Saleh AK, El-Gendi H, El-Fakharany EM, Owda ME, Awad MA, Kamoun EA. Exploitation of cantaloupe peels for bacterial cellulose production and functionalization with green synthesized copper oxide nanoparticles for diverse biological applications. Sci Rep.

2022;12(1):19241.

147. Saleh AK, El-Gendi H, Ray JB, Taha TH. A low-cost effective media from starch kitchen waste for bacterial cellulose production and its application as simultaneous absorbance for methylene blue dye removal. Biomass Convers Biorefin. 2023;13(14):12437–49. https://doi.org/10.1007/s13399-021-01973-1

148. Saleh AK, Salama A, Badawy AS, Diab MA, El-Gendi H. Paper sludge saccharification for batch and fed-batch production of bacterial cellulose decorated with magnetite for dye decolorization by experimental design. Cellulose. 2023;30(17):10841–66. https://doi.org/10.1007/s10570-023-05545-6

149. Qi GX, Luo MT, Huang C, Guo HJ, Chen XF, Xiong L, *et al*. Comparison of bacterial cellulose production by *Gluconacetobacter xylinus* on bagasse acid and enzymatic hydrolysates. J Appl Polym Sci. 2017;134(28):1–7. https://doi.org/10.1002/app.45066

150. Khattak WA, Khan T, Ul-Islam M, Ullah MW, Khan S, Wahid F, *et al.* Production, characterization and biological features of bacterial cellulose from scum obtained during preparation of sugarcane jaggery (gur). J Food Sci Technol. 2015;52(12):8343–9. https://doi.org/10.1007/s13197-015-1936-7

151. Mohite B V, Patil S V. Physical, structural, mechanical and thermal characterization of bacterial cellulose by *G. hansenii* NCIM 2529. Carbohydr Polym. 2014;106(1):132–41. https://doi.org/10.1016/j.carbpol.2014.02.012

152. Saleh AK, Tolba E, Salama A. *In situ* development of bacterial cellulose/hydroxyapatite nanocomposite membrane based on two different fermentation strategies: characterization and cytotoxicity evaluation. Biomass Convers Biorefin. 2024;14(16):18857–67. https://doi.org/10.1007/s13399-023-03940-4

153. Lu T, Gao H, Liao B, Wu J, Zhang W, Huang J, *et al.* Characterization and optimization of production of bacterial cellulose from strain CGMCC 17276 based on whole-genome analysis. Carbohydr Polym. 2020;232:115788. https://doi.org/10.1016/j.carbpol.2019.115788

154. Gullo M, Sola A, Zanichelli G, Montorsi M, Messori M, Giudici P. Increased production of bacterial cellulose as starting point for scaled-up applications. Appl Microbiol Biotechnol. 2017;101(22):8115–27. https://doi.org/10.1007/s00253-017-8539-3

155. Rodrigues AC, Fontão AI, Coelho A, Leal M, da Silva FAGS, Wan Y, *et al.* Response surface statistical optimization of bacterial nanocellulose fermentation in static culture using a low-cost medium. New Biotechnol. 2019;49:19–27. https://doi.org/10.1016/j.nbt.2018.12.002

156. Krusong W, Pothimon R, China S La, Thompson AK. Consecutive bacterial cellulose production by luffa sponge enmeshed with cellulose microfibrils of *Acetobacter xylinum* under continuous aeration. 3 Biotech. 2021;11(1):1–9. https://doi.org/10.1007/s13205-020-02569-8

157. Dima SO, Panaitescu DM, Orban C, Ghiurea M, Doncea SM, Fierascu RC, *et al.* Bacterial nanocellulose from side-streams of kombucha beverages production: Preparation and physical-chemical properties. Polymers (Basel). 2017;9(8):5–10.

158. Sulaeva I, Henniges U, Rosenau T, Potthast A. Bacterial cellulose as a material for wound treatment: Properties and modifications. A review. Biotechnol Adv. 2015 Dec;33(8):1547–71. https://doi.org/10.3390/polym9080374

159. El-Gendi H, Salama A, El-Fakharany EM, Saleh AK. Optimization of bacterial cellulose production from prickly pear peels and its *ex situ* impregnation with fruit byproducts for antimicrobial and strawberry packaging applications. Carbohydr Polym. 2023; 302:120383.

https://doi.org/10.1016/j.carbpol.2022.120383

160. Hu H, Catchmark JM, Demirci A. Co-culture fermentation on the production of bacterial cellulose nanocomposite produced by *Komagataeibacter hansenii*. Carbohydr Polym Technol Applic. 2021;2:100028. https://doi.org/10.1016/j.carpta.2020.100028

161. Ahmed A, Saleh A, Soliman N, Ibrahim M, El-Shinnawy N, Abdel-Fattah Y. Biocellulose production by *Gluconacetobacter hansenii* ATCC 23769: Application of statistical experimental designs and cellulose membrane characterization. Egypt J Chem. 2019;62(11):2077-92. https://doi.org/10.21608/ejchem.2019.9975.1662

162. Jahan F, Kumar V, Saxena RK. Distillery effluent as a potential medium for bacterial cellulose production: A biopolymer of great commercial importance. Bioresour Technol. 2018;250:922–6. https://doi.org/10.1016/j.biortech.2017.09.094

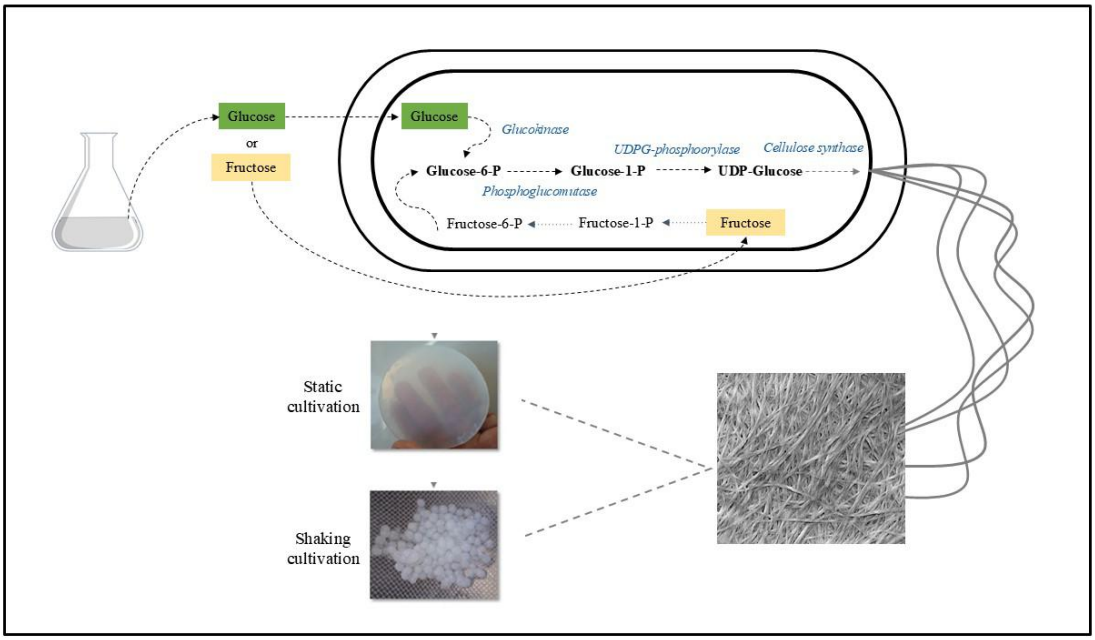

Fig. 1. Biosynthesis of bacterial cellulose from glucose and fructose

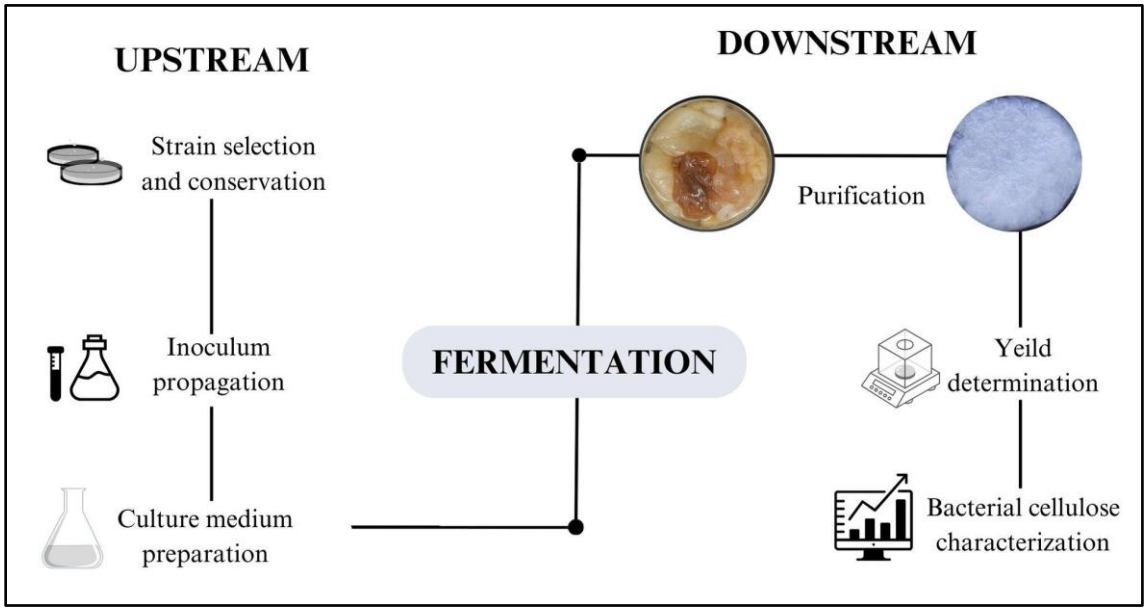

Fig. 2. Scheme of bacterial cellulose production

Table 1. Bacterial cellulose properties and potential application fields

Application	Function	Structure-propertie	Reference
Meat products; ice cream.	Fat replacer	Water holding capacity; emulsion stabilization; amphiphilic nature.	(*63–65*)
Dietary fiber source; low calorie products; low cholesterol diet.	Functional food ingredients	Insoluble dietary fiber; high water holding; ion exchange capacities.	(*7,19,46*)
Food packaging, edible films and coatings, active and intelligent packaging film; immobilization of cell, enzyme, antimicrobial agents	Food packaging and support for bioactive compounds	High surface area; porosity; high pore volume; gelling behavior; high crystallinity; hydrophilicity; rehydration property; chemical, thermal, and mechanical stability; barrier properties.	(*19,66–68*)
Pickering emulsion; edible foam; beverages; bakery products; dairy products.	Thickener and stabilizing agent in emulsion, suspensions, and foam stabilizer	Amphiphilic nature; high surface area; crystallinity; three-dimensional structure.	(*69–75*)
Bioengineering; tissue engineering;	Controlled release	High purity and crystallinity; porosity;	(*76–78*)

	systems; biosensors; scaffold for regeneration; vessel substitute;	biocompatibility; nanofibrillar matrix; mechanical strength; durability; flexibility; elasticity	
Cosmetics	Bioactive compounds delivery	High water-holding capacity; biocompatibility; nanofibrillar porous structure;	(*79*)
Wound dressings; drug delivery systems	Controlled release systems; water retainer	High water absorption capacity; biocompatibility; porosity; crystallinity; thermal stability.	(*80,81*)
Electronic field	Flexible substrates for electronic devices, conductive materials, and biosensors	High purity and crystallinity; porosity; biocompatibility; nanofibrillar matrix; mechanical strength; durability; flexibility; large surface area.	(*78,82*)

Table 2. Acetic acid bacteria performance on bacterial cellulose production under different conditions

Strain	Source	Nutrient source	Method	Parameter	BC/ (g/L)	BC/ (g/(L·h))	Reference
K. europaeus SGP37	Rotten grapes	Hestrin Schramm (glucose)	Static	30 ºC/384 h	5.61	0.01 46	(*101*)
		Hestrin Schramm (fructose and ethanol)	Static	30 ºC/384 h	9.98	0.0260	
		Sweet lime pulp waste	Static	30 ºC/384 h	6.30	0.0164	(*102*)
		Sweet lime pulp waste supplemented with HS	Static batch	30 ºC/384 h	26.20	0.0682	
		Sweet lime pulp waste supplemented with HS	Static intermittent fed-batch	30 ºC/384 h	38.00	0.0990	
K. intermedius BCRC 910677	Fermented fruit juice	Hestrin Schramm	Static	28 ºC/120 h	1.20	0.0100	(*29*)
		Synthetic optimized medium	Static	28 ºC/144 h	3.91	0.0271	(*100*)
K. intermedius V-05	Vinegar	Soy molasses with ethanol	Static	30 °C/336 h	10	0.0297	(*93*)
		Hestrin Schramm	Static	30 °C/336 h	3.7	0.0110	

		Synthetic with amino acids (optimized)	Static	30 °C/240 h	3.02	0.0125	(*17*)

(continue)

Table 2. – Acetic acid bacteria performance on bacterial cellulose production under different conditions (continued)

Strain	Source	Nutrient source	Method	Parameter	BC/ (g/L)	BC/ (g/(L·h))	Reference
K. rhaeticus AF-1	Kombucha tea	Hestrin-Schramm with ethanol	Static	28 °C/96 h	6.70	0.0698	(*56*)
		Cashew tree exudate	Static	28 °C/168 h	2.80	0.0167	(*99*)
		Cashew gum	Static	28 °C/168 h	2.30	0.0137	
		Hestrin-Schramm	Static	28 °C/168 h	~ 6.0	0.0357	
		HSCTE	Static	28 °C/168 h	~ 6.0	0.0357	
		HSCG	Static	28 °C/168 h	~ 6.0	0.0357	
		Sugarcane molasses-supplemented	Static	30 °C/120 h	3.46 - 4.01	0.0288 - 0.0334	(*30*)
		Sugarcane molasses	Static	30 °C /120 h	1.90	0.0158	
		Hestrin Schramm	Static	30 °C/120 h	3.00	0.0250	

Table 3. Main culture media for acetic acid bacteria isolation, cultivation, and preservation

Culture media	Composition	Function	Reference
AE	1.5 % glucose; 0.2 % yeast extract; 0.3 % peptone; 2.0 % ethanol; 6.5 % acetic acid.	Isolation and enrichment	(*120*)
Glucose-yeast extract-carbonate (GYC)	10 % glucose; 1.0 % yeast extract; 2.0 % $CaCO_3$.	Isolation and enrichment	(*97*)
Hestrin-Schramm (HS)	2.0 % glucose; 0.5 % yeast extract; 0.5 % peptone; 0.27 % Na_2HPO_4; 0.115 % citric acid.	Isolation, cultivation, and bacterial cellulose production	(*18,33,116,121)*
Mannitol, yeast extract, peptone (MYP)	2.5 % mannitol; 0.5 % yeast extract; 0.3 % peptone.	Isolation and enrichment Preservation	(*116*)
Carr	2.5 % yeast extract; 2.0 % ethanol; 0.02 % peptone.	Preservation	(*122*)
Glycerol	15 % glycerol	Preservation	(*123*)
Malt extract	20 % malt extract	Preservation	(*124*)

Table 4. Bacterial cellulose production by acetic acid strains under static and agitated cultivation

Strain	Static/ (g/L)	Agitated/ (g/L)	BC properties Static	BC properties Agitated	Reference
A. xylinum BCA263	3.97	1.70	Higher crystallinity; stronger tensile strength; denser network structure; higher temperature resistance.	Larger porous; lower crystallinity; higher water retention.	(*71*)
K. xylinus BCC529	2.48	1.66			
G. xylinus P1	1.40	1.72			
K. xylinus (KX)	1.14–1.84	0.60 - 1.20 (~)	Higher crystallinity and smaller crystallite sizes;	Disorderly reticulated structures of microfibrils; higher cellulose Iα content in the flocky asterisk-like BC than in the solid sphere-like.	(*103*)
K. xylinus (TISTR 086)	0.14–0.39	0.00 - 0.10 (~)			
K. xylinus (428)	0.09–0.22	0.20 - 0.40 (~)			
K. xylinus (975)	1.11–1.55	(~) 2.40 - 3.54			
K. xylinus (1011)	0.57–1.46	(~) 3.20 - 4.69			
Komagataeibacter sp. nov. CGMCC 17276	8.85	3.22	Higher crystallinity; high water-holding capacity; denser network.	Network structure looser and more porous; higher porosity.	(*153*)
K. hansenii JR-02	4.62	3.14	Thicker fibers; higher thermal degradation temperature and lower	Higher weight loss; higher moisture content and amorphous proportion.	(*34*)

			moisture content; higher crystallinity.		

Table 4 – Bacterial cellulose production by acetic acid strains under static and agitated cultivation (continued)

Strain	Static g/L	Agitated g/L	BC properties Static	BC properties Agitated	Reference
G. hansenii P2A	1.89	3.25	Ordered and dense network of fibrils with (8–10 nm diameter); the network was composed of interconnected layers.	Slight decrease in the crystallinity index; looser clump of disordered short and thin fibrils; lower molecular weights; increased thermal due the gradual increase in Iβ phase content.	(*24*)