Review of bacterial cellulose production using agricultural and agroindustrial wastes: Physical and mechanical properties

Alejandro Rincón[1,2*], Gloria M Restrepo[2], Fredy E Hoyos[3]

(1. *Grupo de Investigación en Desarrollos Tecnológicos y Ambientales - GIDTA, Facultad de Ingeniería y Arquitectura, Universidad Católica de Manizales, Carrera 23 No. 60-63, Manizales 170002, Colombia*;
2. *Grupo de Investigación en Microbiología y Biotecnología Agroindustrial—GIMIBAG, Instituto de Investigación en Microbiología y Biotecnología Agroindustrial, Universidad Católica de Manizales, Carrera 23 No. 60-63, Manizales 170002, Colombia*;
3. *Departamento de Energía Eléctrica y Automática, Facultad de Minas, Universidad Nacional de Colombia, Sede Medellín, Carrera 80 No. 65-223, Robledo, Medellín 050041, Colombia*)

Abstract: Bacterial cellulose (BC) is a biopolymer whose properties make it suitable for applications in medicine, pharmaceutics, food, environment, engineering, and chemistry. BC produced from agricultural/industrial material exhibits a wide variety of morphological, physical, and mechanical properties. BC production and yield are dependent on the agricultural material used, the content of nitrogen and phosphorus, the bacterial strain, and culture conditions. In this review, BC production is addressed, focusing on the effect of culture conditions and culture medium on the morphological, physical, and mechanical properties of BC, considering low-cost substrates as culture medium. The main contributions over closely related reviews are: 1) A compilation of BC production data, along with corresponding crystallinity and mechanical or physical properties, is provided to highlight the relationship between crystallinity and these properties within each study; 2) The different culture conditions of each cited study, as well as the blank or control treatment, are included in the data compilation to allow comparison of the effects of the culture conditions within each study; 3) The relationship between microstructure and physical and mechanical properties within each study is discussed; cases of simultaneous achievement of high BC production and either crystallinity or tensile strength are identified. From the data comparison, it follows that the BC production is similar to or better than that for standard Hestrin and Schramm (HS) medium in some studies, and its corresponding crystallinity or mechanical/physical properties are different. Then, a high BC production/yield does not guarantee improvement of crystallinity or physical/mechanical properties. In some cases, the crystallinity index is not significantly modified, but the corresponding physical/mechanical properties are. This implies that considering the physical/mechanical properties corresponding to the BC production/yield data is convenient in experimental studies on BC production using low-cost culture medium.

Keywords: bacterial cellulose (BC), biomaterials, agricultural waste, nanocellulose, *Gluconacetobacter*, *Komagataeibacter*
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1 Introduction

Bacterial cellulose (BC) is a biopolymer produced by bacteria as an exopolysaccharide[1]. BC exhibits high crystallinity (74%-96%)[2-4], high degree of polymerization (2000 to 6000)[3,5,6], high mechanical strength (20-300 MPa tensile strength)[2,7], high water-holding capacity (higher than 95%)[2,8,9], high surface area (higher than 150 m^2/g)[2,10], high permeability to liquid and gas, good biocompatibility, non-toxicity, and biodegradability[10-13]. Also, Young's modulus of 10-65 GPa and tensile strength in the range of 80-800 MPa have been reported[14]. These properties make BC advantageous compared to plant cellulose[15-18].

Applications of BC have been developed in several research areas, mainly in biomedicine, food, pharmaceutics, cosmetics, and bioengineering, including specific applications in wound dressing, food packaging, tissue engineering, paper products, electronics, water treatment[19-21], and coating for fruits or vegetables[20,22]. Also, there are important commercial applications in tissue engineering, biodegradable materials, foods[23,24], cosmetics, and paper industry[25-27]. Due to its porous 3D network, BC has potential for applications related to tissue generation, protein loading and release, and oncology therapy, and also it exhibits capability for holding enzymes, metal, and magnetic nanoparticles, and it can be tuned, impregnated, or functionalized for specific applications[28-30]. Due to its high water-holding capability, it has potential for applications in cosmetics, wound dressing, composite membranes, catalysts, and bioabsorbents[14,31], and as a water-binding food additive[28]. Due to its biocompatibility, non-toxicity, moldability, and water-holding capacity, BC can be applied to tissue engineering and regenerative medicine[14,32,33]. The high mechanical strength of BC implies long service life when used in different applications[34], thus favoring BC application for blood vessels, bone tissue engineering, and cartilage[30]. BC has potential for food packaging, in which mechanical properties (e.g., tensile strength) and moderate barrier properties are important[35]. BC is highly biodegradable in terms of

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Biographies: Gloria M. Restrepo, Professor, research interest: biotechnology, bioeconomy, microbial inoculants, Email: grestrepo@ucm.edu.co; **Fredy E. Hoyos**, PhD, Electrical Engineer research interests: power electronics, nonlinear control, Email: fehoyosve@unal.edu.co.
***Corresponding author: Alejandro Rincón**, Professor, research interest: biotechnology, simulation of biological and environmental systems, data science; Grupo de Investigación en Desarrollos Tecnológicos y Ambientales—GIDTA, Facultad de Ingeniería y Arquitectura, Universidad Católica de Manizales, Carrera 23 No. 60-63, Manizales 170002, Colombia. Tel: +57-4-4309000, Email: arincons@ucm.edu.co.

hydrophilicity and water uptake capability, so that it is feasible for biodegradable packaging materials[14]. Also, it is feasible for chemical/physical surface modification due to the presence of -OH groups and porous network structure[4,13,14]. The degradability of biomaterials is important in medical applications, as it is related to the absence of incompatibility problems. High biodegradability has the following advantages: 1) biomaterial incompatibility (infection or toxicity) issues in the body are avoided; 2) problems in the immune system are avoided, as the immune responses are not driven towards the material as a foreign body; 3) removal from the body is facilitated, which may occur through hydrolytic processes[25].

BC commercialization is limited by the cost of culture medium, BC production, and yield, where the culture medium accounts for more than 30% of BC production cost[31,36–38], whereas the end application of BC depends on its physical and mechanical properties[14,36,37,39]. In turn, mechanical and physical properties of BC are strongly influenced by morphological properties, whereas morphological properties and BC production are dependent on culture conditions (operation conditions, bacterial strain, culture medium)[34,36,40,41]. In particular, the carbon source (type and composition) has a significant effect on microstructure and physical and mechanical properties[11,40,41]. To reduce the cost of culture medium, the use of agroindustrial materials is commonly studied, which results in a wide variation of the BC production and properties, compared to conventional Hestrin and Schramm (HS) medium[11,14,42,43]. However, BC production from agrowastes involves quality issues, including: 1) BC may absorb unwanted compounds and be colored, so that strict purification is needed; 2) low mechanical strength is obtained for some agrowastes; 3) the obtained BC stiffness and high fragility are unsuitable for certain applications; 4) its toughness is not sufficient for applications requiring impact resistance. To this end, *in situ* and *ex situ* methods lead to significant improvement of BC properties, such as an increase of tensile strength, toughness, and bacterial activity, for specific commercial applications in fields such as medicine, pharmaceutics, and the food industry[20,44,45].

Many review reports on BC production compare production and yield of obtained BC as a function of culture medium and culture conditions, considering agroindustrial materials (for instance[32,36,39,46]. This review addresses the effect of cultivation conditions and culture medium on morphological and mechanical properties of produced BC, including the effect of low cost substrates. The aim of this review on BC production is to give insight into the crystallinity and the corresponding mechanical properties and the corresponding data of BC production and blank or control treatment, as a function of culture media variation, with emphasis on low-cost materials as culture medium. Common literature reviews on BC production exhibit the following limitations: 1) the data on BC production are provided, but the corresponding microstructure and mechanical properties are not; 2) the different experimental cases (e.g., culture conditions) of each reference and also the blank or control case are not given in the data compilation. Therefore, the effect of culture media on both the BC production and properties is not clear. Thus, the above limitations are overcome in this review, as depicted by the following contributions with respect to closely related studies (e.g.[36,37,39,46]):

1) A compilation of BC production data, along with corresponding data on crystallinity and either mechanical or physical properties, is provided individually for each reference to highlight the relationship between crystallinity and these properties within each reference. In contrast, in review studies on BC production, the data on BC production are provided, but the corresponding microstructure and mechanical properties are not. To the authors' knowledge, Urbina et al. (2021) is the only exception among the data that were surveyed[14].

2) The different experimental cases (e.g., culture conditions) of each reference, as well as the blank or control case, are included in the data compilation, and the effects of culture conditions on BC production, crystallinity, and tensile strength are assessed individually for each reference.

3) The relationship between microstructure and physical and mechanical properties within each study is discussed; cases of simultaneous achievement of high BC production and either crystallinity or tensile strength are identified.

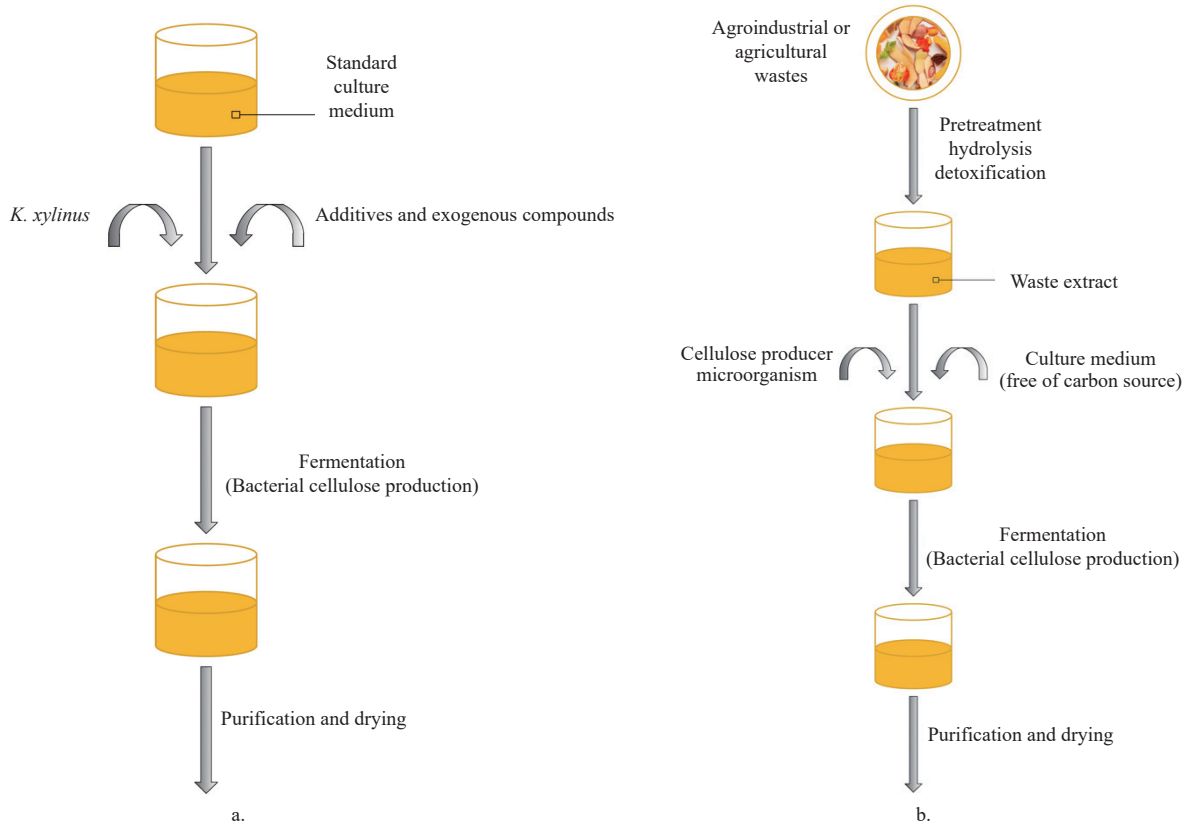
2 Bacterial cellulose production using traditional substrates: Effects of process parameters

2.1 Effect of culture conditions and culture medium on BC production and bacterial growth

The steps for bacterial cellulose production are shown in Figure 1. The main factors influencing BC production are: the bacteria species and strain[40]; culture medium, carbon source (concentration and type), nitrogen source, and supplement addition[11,34,40]; static/dynamic mode[40]; pH[24,31], temperature[47,48], dissolved oxygen, and fermentation time[5,31,47,49]. Genera of cellulose-producing bacteria include *Komagataeibacter*, *Gluconacetobacter*, *Azotobacter*, *Enterobacter*, *Alcaligenes*, *Achromobacter*, *Pseudomonas*, and *Salmonella*[21,36,37,46]. Genus *Komagataeibacter* is the most widely used for BC production, due to its capability of using a wide range of carbon and nitrogen sources[36,38,50]. *Komogataeibacter xylinus* is the most common bacteria used in BC production studies, due to its BC production capability. *K. xylinus* was formerly known as *Acetobacter xylinum*, then classified as *Gluconacetobacter xylinus*, and later reclassified as *Komagataeibacter xylinus*[14,30,46,51]. In addition, *Gluconacetobacter hansenii*, *Gluconacetobacter pasteurianus*, and *Komagataeibacter rhaeticus* also exhibit a high BC production capability[4,50]. Culture conditions depend on the bacterial strain[14,37]. Each bacterial genus has a different capability for metabolizing the compounds of the culture medium, resulting in a high effect on BC yield[11]. The conventional culture medium is the Hestrin and Schramm (HS), which is composed of glucose, peptone, yeast extract, sodium phosphate, and citric acid[14].

Several of the culture conditions and culture media concentrations can be optimized, resulting in a significant BC productivity increase[37,52]. The highest BC production is achieved for a pH range of 4-7[3,14,31] and a temperature range of 25°C-30°C[3,14]. Excessive carbohydrate concentration leads to growth inhibition and reduced BC production for many bacterial genera. This is related to enzyme saturation and low pH caused by the production of acids[53]. The nitrogen source (type and concentration) is of paramount importance for achieving maximum BC production[37]. For commercial production of BC, agitated fermentation is preferred over static fermentation, due to its higher BC yield[5,47,54]. However, some commercial applications use static fermentation[23]. In addition, BC production is favored with the addition of inorganic salts, antioxidants (for instance, vitamin C), organic acids (e.g. acetic acid, lactic acid, citric acid), vitamins and fatty acids[31,42,55,56], minerals and water-soluble polymers[37], alcohol (e.g. ethanol), and ethylene[21,56,57].

High values of Young's modulus and tensile strength are important for food packaging, bone tissue engineering, and artificial



Note: a. Using standard culture media; b. Using agricultural materials.

Figure 1 General steps for bacterial cellulose production

heart valve manufacturing, while elasticity and stretching are important for membrane compatibility in wound dressing applications[38]. The mechanical and physical properties of BC depend on culture conditions and bacteria species[11,14,26]. The polymerization degree, crystallinity index, tensile strength, and water-holding capacity may be influenced by the carbon source[58]. Also, agitated culture results in lower BC crystallinity,[14,47,57] lower tensile strength[3], higher water-holding capacity, and lower Young's modulus[59-60] compared to static culture. BC can be modified via physical or chemical methods in order to improve BC productivity or its mechanical and physical properties, such as tensile strength, surface area, Young's modulus, water absorption capacity, antimicrobial effect, and porosity[10,31,61,62], thus favoring its applications[20,36]. However, knowledge of the characteristics of cellulose before using it for composites is useful for improving the mechanical and thermal properties of composites[62]. Modification methods are commonly grouped into *in situ* and *ex situ* modifications. For *in situ* modifications, additive materials are incorporated into BC culture medium before BC film is formed (that is, at the beginning of the BC production)[6,36,61] and these materials are not essential components of the culture medium[60]. Additive materials include water-soluble polymers, water-insoluble polymers, vitamins (including ascorbic acid), and minerals. Examples of water-soluble polymers include polysaccharides (e.g. xanthan gum, starch, alginate, pectin), cellulose derivatives (e.g. methylcellulose, carboxymethyl cellulose (CMC)), proteins (e.g. gelatin), polyethylene oxide (PEO), polyvinyl alcohol (PVOH), and polyvinyl alcohol (PVA). Examples of water-insoluble polymers include paraffin and poly-beta-hydroxybutyrate (PHB)[37,60,61]. *In situ* modification can improve BC production, whereas crystallinity and mechanical properties may be altered.

Physical modifications allow the enhancement of mechanical properties of BC[27,30,54]. The method used for treating (purifying and drying) the BC sample can affect the morphological, physical, and mechanical properties. It has been found that alkali purification treatment partially removes amorphous cellulose[29], and improves mechanical properties and thermal stability[16,59].

2.2 Effect of culture conditions on microstructure and physical and mechanical properties

The mechanical and physical properties of obtained BC, including surface properties of BC nanofiber, are determined by its morphological and microstructure characteristics (e.g. nanofiber width, fibril density, size, and arrangement)[10,63] and microstructure (crystallinity)[64,65]. The morphology of BC depends on culture conditions (e.g. media composition, pH, temperature, agitation, O_2 content), bacterial strain, drying treatment, and fermentation duration[34,36,40,65]. The carbon source (type and composition) has a significant effect on morphology, microstructure, and physical and mechanical properties[11,29,40].

Crystallinity is crucial in the study of mechanical and physical properties of macromolecules[25,66]. The crystallinity index (CI) is important for describing BC structure and for its application[64]. In fact, BC exhibits high thermal stability for high crystallinity, and BC flexibility decreases with crystallinity[25,64]. However, crystallinity index (CI) of cellulose strongly depends on the measurement method used[62,67].

3 Bacterial cellulose production using agricultural and agroindustrial materials: Effects of process parameters and culture medium

3.1 BC production using agricultural and agroindustrial materials

The industrial scale production of BC is limited by the high cost of the components of the conventional HS medium (mainly

glucose, yeast extract, and peptone)[11,14,46]. The cost depends on culture medium and BC yield. The culture medium accounts for approximately 30% of the total BNC production cost[28,34,37,58]. Hestrin-Schramm (HS) medium is the most common medium for BC production, and it is expensive[37]. To reduce the culture medium cost, BC studies use different low-cost carbon sources (industrial, agricultural, and food-processing by-products), which leads to significant changes in the BC productivity, yield, morphology, and surface properties[11,14,37]. Low-cost substrates include foods (by-products), foods (non-by-products), algae (non-by-product), lignocellulosic biomass (non-by-product), and by-products of the following industries: biodiesel, pulp and paper, textile, microbial oil, fermentation, soap, and tobacco. In turn, agricultural residues include wheat straw, rice straw, rice bark, rice husk, corn straw (stalk and cob), durian shell, pecan nutshell, bagasse, oat hulls, coffee cherry husk, corn stover, and sugarcane bagasse[68–71], and also walnut shells and pistachio shells[72].

The results of several studies confirm the feasibility of BC production from agroindustrial materials at industrial scale[14]. Indeed, some agricultural materials lead to higher BC production than HS medium[40,46,57]. For instance, the combination of sugarcane molasses and coffee grounds with ethanol supplementation can increase BC production[73]. In some studies, the agroindustrial waste is used as carbon or nitrogen source, whereas the other components are the same as those of HS medium[39]. For instance, a fruit juice is used as carbon source in[74].

Agroindustrial materials provide monosaccharides (e.g. glucose, fructose), oligosaccharides (e.g. sucrose), polysaccharides (e.g. cellulose, starch), proteins, vitamins, and minerals, thus favoring bacterial cell growth and BC production[39,74-76]. They may contain nitrogen source[14], as is the case with molasses[77], sweet potato residues (SPR),[34] and green tea[78]. SPR contain small amounts of aminoacids and proteins[34]. Also, some agricultural materials contain polyphenols, which can promote BC production[53], and trace elements (e.g. Mg^{+2}, Mn^{+2}, Na^{+}, K^{+}) which have a significant promoting effect on cell growth and BC production by *G. xylinus*[53]. In particular, lignocellulosic materials contain lignin, cellulose, hemicellulose, and a small quantity of proteins, pectins, and ash[36,62,79].

BC production using alternative culture media includes pretreatment, hydrolysis, detoxification, fermentation and purification[34,36,37,80], although detoxification is not always performed (Figure 1). Nitrogen supplementation and removal of bacterial growth inhibitors (e.g. furfuraldehyde) are necessary for high BC production in many cases[14,39,81]. The general characteristics of these steps are as follows. Pretreatment includes the modification of size, structure, and chemical composition, in order to favor hydrolysis. Pretreatment may be chemical or physical [37]. The main advantages and disadvantages of common pretreatment methods are shown in Table 1. Hydrolysis is the main step for BC production using alternative culture media. It is used for transforming polysaccharides (cellulose and hemicellulose) to fermentable sugars (e.g. glucose and fructose)[14,36,37]. In [82], it was found that hydrolysis conditions have a strong influence on crystallinity. Hydrolysis may be acid or enzymatic[37,83]. Detoxification aims at removing inhibitors in lignocellulosic hydrolysates, for instance, aldehydes, aliphatic acids, and phenolic compounds. Detoxification includes chemical, physical, and biological methods[37,83,84]. Bacterial growth inhibitors may appear in the hydrolysis or in the fermentation step, as is the case with corn stalk and wheat straw[14,39]. Inhibitors are commonly removed from the fermentation medium through different physical, physicochemical, and chemical methods[14,36,39,80]. In addition, in some studies, the BC produced from wastes exhibits a biocompatible and nontoxic nature, which is important for biomedical materials[32]. However, due to undesirable substances and coloring, a correct purification is needed, but BC application is limited in areas with highly restrictive regulations, for instance, biomedicine, pharmaceutics, cosmetics, and food industry[14,85]. Modification of culture medium (*in situ* modification) consists of the combination of the culture medium with either additives (e.g. water water-soluble polymers, water-insoluble polymers, vitamins, proteins, minerals, or natural extracts), components of culture medium (e.g. nitrogen or phosphorus source), or other culture medium[60,61,86]. Fermentation consists of bacteria growth in the culture medium leading to BC formation, under adequate conditions, e.g. temperature, pH, and static/agitated fermentation mode[37]. Purification aims at removing bacterial cells, medium components, and byproducts from produced BC[87,88]. Drying reduces transport costs, storage space, and avoids microbial decomposition[89–90].

Table 1 Major advantages and disadvantages of common pretreatment methods[91-93]

Pretreatment method	Advantages	Disadvantages
Mechanical milling	Inhibitory compounds are not produced; particle size is reduced, thus facilitating handling of material and increasing hydrolysis yield.	Energy consumption is high.
Extrusion	Energy consumption is low; inhibitory compounds are not produced; waste effluents are not produced.	Its effect is limited when no chemical agents are used; requires the use of additives; the yield of hydrolysis is low.
Ammonia fiber explosion (AFEX)	Inhibitory compounds are not produced; a high cellulose content is retained; no sugar loss takes place; degradation of cellulose and hemicellulose is negligible.	The cost depends on price and quantity of ammonia used; requires highly controlled environment; the equipment must be constructed with special materials.
Liquid hot water hydrolysis	The cost of the process is relatively low; the formation of degradation products is negligible; corrosion issues and need for recycling are limited; the recovery of hemicellulose sugar is high; it is environmentally friendly, as no chemical agents are used.	High water and energy consumption; the obtained concentration of product is low; down-stream processing is energy-intensive because of the large amount of water used.
Acid pretreatment	It significantly improves the further hemicellulose and cellulose hydrolysis.	Inhibitory by-products form as result of sugar degradation; acid causes corrosion and implies high cost of construction material.
Alkaline pre-treatment	Lime can be used, which is relatively safe and cheap; it exhibits high removal of lignin and xylan side chains, leading to increased efficiency of the further enzymatic hydrolysis.	Inhibitory by-products are formed; the residence time is long; the cost of downstream processing is high.
Organosolv process	The degradation of carbohydrates into undesired compounds is significantly limited; it is very effective for high lignin lignicellulosic materials; separate fractions of lignin, hemicellulose, and cellulose are produced.	The cost of organic solvents is high; the need of solvent recycling implies additional cost.
Ionic liquid pretreatment	It is performed under moderate reaction conditions.	The process is expensive due to the high cost of ionic liquid; inhibitory by-products are formed.

When using agricultural culture medium, BC productivity is strongly influenced by composition factors (e.g. pH, excess sugars, nitrogen source), which depends on bacterial strain[53]. Indeed, concentration and type of nitrogen source, carbon source and other culture medium components, pH, temperature, and fermentation time can be optimized, resulting in a significant improvement of BC production[66,74,90]. In particular, low pH and absence of nitrogen source have a strong detrimental effect[53]. In some cases, pH control is highly important for obtaining high BC production[14]. Also, addition of nitrogen (for instance yeast extract and peptone) and phosphate sources significantly improves BC production and yield[14,40,53].

3.2 Comparison of data on crystallinity and physical and mechanical properties of BC

Some studies on BC production are discussed below, considering agro-industrial materials in the culture medium and focusing on the main characteristics. The culture conditions are given in Table 2, whereas the corresponding BC production, crystallinity, and physical and mechanical properties are listed in Table 3. For Akintunde et al. 2022, the mechanical properties are shown for HS case and one case among I agitation/C agitation/ static, which is chosen as the one with higher tensile strength[40].

Table 2 Bacterial species, culture medium and fermentation conditions used for BC production, considering agroindustrial materials

Microorganism	Culture medium	Culture conditions	Ref
Komagataeibacter sp., strains CCUG73629 (K29) and CCUG73630 (K30)	Three culture media: CC: corncob enzymatic hydrolysate supplemented with nitrogen and phosphate sources. SCB: sugarcane bagasse enzymatic hydrolysate supplemented with nitrogen and phosphate sources. HS: HS medium.	Static, continuous agitation (C agitation at 100 r·min^{-1}) and intermittent agitation (I agitation at 100 r·min^{-1} for 6 hours daily), at 30°C for 10 days in blue-capped bottles. Only static mode is considered for HS medium.	[40]
Gluconacetobacter sucrofermentans	Culture media: Molasses and ethanol: molasses 75 g·L^{-1}+1.5% ethanol. HS: HS medium.	Static and dynamic, with or without immobilized cells. In the case of immobilized cells, only HS medium is used. Dynamic regime is performed in BIOSTAT A Plus bioreactors at 200 r·min^{-1}.	[42]
Gluconacetobacter xylinus	Two media: SM (synthetic medium): glucose (50 g·L^{-1}), peptone (5 g·L^{-1}), yeast extract (5 g·L^{-1}), citric acid (1 g·L^{-1}), KH_2PO_4 (1 g·L^{-1}), Na_2HPO_4 (2 g·L^{-1}). SPR: glucose (30 g·L^{-1}), yeast extract (4 g·L^{-1}), citric acid (1 g·L^{-1}), KH_2PO_4 (1 g·L^{-1}), Na_2HPO_4 (2 g·L^{-1}), ethanol (0.8% v·v^{-1}). In the preparation of the SPR medium, the glucose is replaced by sweet potato residues hydrolysate, which contains glucose at 193 g/L.	Static, at 30°C for 6 days; 50 mL of fermentation medium in 250 mL Erlenmeyer flasks.	[34]
K. saccharivorans	Culture media: HS: HS medium. D-HS: HS medium being glucose replaced by extract of date fruit wastes. F-HS: HS medium being glucose replaced by extract of fig fruit wastes. M-HS: HS medium being glucose replaced by treated sugarcane molasses.	Static at 28°C for 7 days; 50 mL of fermentation medium in 250 mL beakers. Initial pH 6.0.	[58]
Gluconacetobacter xylinus	HS culture medium, being peptone and yeast extract replaced by okara extracted protein at concentrations 0, 0.5%, 1%, 2%, 3% w·v^{-1}. The obtained BC samples are denoted by BCx, where x is the okara protein concentration.	Static at 30°C for 7 days; 100 mL fermentation medium. Initial pH 5.0.	[94]
Kombucha strains including *Gluconacetobacter xylinus*	Culture media: BC_O: Sucrose, tea, and orange peel extract. BC_c (control): Sucrose and tea.	Static at 28°C for 21 days; 1 L of fermentation medium. Initial pH 3.0.	[95]
Acetobacter xylinum	Culture media: CSL_Fru: Corn steep liquor with fructose, KH_2PO_4, $MgSO_4$, $(NH)_2SO_4$, $FeSO_4$, $CaCl_2$, Na_2MoO_2, $ZnSO_2$, $MnSO_4$, $CuSO_4$, inositol, nicotinic acid, pyridoxine, thiamine, HCl, pantothenic acid, riboflavin, benzoic acid, folic acid, biotin, and an additive, including carboxymethyl cellulose (CMC). Control: the control has the same components as CSL_Fru but with no additive.	Dynamic mode (200 r·min^{-1}) at 30°C for 5 days; 100 mL of fermentation medium in 250 mL flasks. Initial pH 5.0.	[80]

Experimental studies on BC production providing BC production data but also the corresponding data of crystallinity and mechanical properties (e.g. tensile strength) and also the blank or control data corresponding to standard culture medium are quite few. In Table 3, a few studies with BC production data and the corresponding data of either microstructure or mechanical/physical properties and the data for the standard culture medium are those of Xu et al. 2022, Akintunde et al. 2022, Atykyan et al. 2020, Senthilnathan et al. 2022, and Abol-Fotouh et al. 2020. These facts hamper comparing data and assessing the simultaneous achievement of high BC production and either high crystallinity or high tensile strength. Also, in review studies on BC production, the compilation of BC production data with corresponding data of crystallinity and mechanical properties (e.g. tensile strength) and the blank/control data corresponding to standard HS culture medium are usually not provided.

Also, it follows that BC production is higher than that of standard HS medium in several studies, but it does not guarantee that crystallinity or tensile strength is similar or higher than that of standard HS medium, according to the cases from Table 3 discussed in what follows. BC production and crystallinity values obtained with agricultural materials are similar or higher than those of standard HS medium in the studies of Atykyan et al. 2020 (case molasses+1.5 % ethanol); Xu et al. 2022 (case SPR); Senthilnathan et al. 2022 (case HS-PPJ); and Abol-Fotouh et al. 2020 (case D-HS)[42]. BC production is higher than that of HS medium, whereas crystallinity is high (higher than 78%) but lower than that of HS medium, for the studies of Abol-Fotouh et al. 2020 (case M-HS)[77]. BC production and tensile strength values are higher than those of standard medium in the studies of Xu et al. 2022 (case SPR), and Abol-Fotouh et al. 2020 (case D-HS)[34,58]. BC production is higher than that of standard medium, whereas tensile strength is lower, in the study of Akintunde et al. 2022 (all cases)[40].

Table 3 Production, crystallinity, and physical and mechanical characteristics of BC, considering agro-industrial materials in the culture medium

Microorganism	BC production and productivity	Crystallinity	Mechanical and physical properties	Ref. and culture medium
Komagataeibacter sp., strain CCUG73629 (K29)	**BC dry weight:** 1.6 g·L^{-1} approx. (c agitation-CC) 1.4 g·L^{-1} approx. (I agitation-CC) 0.8 g/L approx. (HS) **BC yield (%):** 8.6 (static-CC) 14.1 (C agitation-CC) 11.9 (I agitation-CC) 7.9% (HS)	-	Tensile strength: 57.8 MPa (I agitation-CC). 76.2 MPa (HS). Young's modulus: 7.0 GPa (I agitation-CC) 7.1 GPa (HS)	[40], CC and HS
Komagataeibacter sp., strain CCUG73629 (K29)	**BC dry weight:** 1.18 g·L^{-1} (c agitation-SCB) 0.28 g·L^{-1} (HS) **BC yield (%):** 5.9 (static-SCB) 9.4 (C agitation-SCB) 7.9 (I agitation-SCB) 3.2% (HS)	-	Tensile strength: 40 MPa approx. (c agitation-SCB) 76.2 MPa (HS) Young's modulus: 3.2 GPa approx. (c agitation-SCB) 7.1 GPa (HS)	[40], SCB and HS
Komagataeibacter sp., strain CCUG73630 (K30)	**Dry weight of BC:** 0.4 g·L^{-1} approx. (I agitation-CC) 0.45 g·L^{-1} approx. (HS) **BC yield (%):** 3.0 (static-CC) 3.0 (C agitation-CC) 3.4 (I agitation-CC) 4.5% (HS)	-	Tensile strength: 70.9 MPa (I agitation-CC) 48 MPa approx. (HS) Young's modulus: 10.8 GPa (I agitation-CC) 5.0 GPa approx. (HS)	[40], CC and HS
Komagataeibacter sp., strain CCUG73630 (K30)	**Dry weight of BC:** 0.4 g·L^{-1} (I agitation-SCB) 0.3 g·L^{-1} approx. (HS) **BC yield (%):** 6.2 (static-SCB) 12.7 (c agitation-SCB) 4.7 (I agitation-SCB) 4.4% (HS)	-	Tensile strength: 58.4 MPa (I agitation-SCB) 48 MPa approx. (HS) Young's modulus: 9.9 GPa (I agitation-SCB) 5.0 GPa approx. (HS)	[40], SCB and HS
G. sucrofermentans (no immobilization)	BC content: 2.86 g·L^{-1} (static cultivation) 1.98 g·L^{-1} (dynamic cultivation)	Crystallinity of BC: 73% approx. (static cultivation) 75% approx. (dynamic cultivation)	-	[42], Molasses +1.5% ethanol
G. sucrofermentans (no immobilization)	BC content: 1.25 g·L^{-1} approx., approximately the same for static and dynamic regimes	Crystallinity: 43% approx. (static cultivation) 47% approx. (dynamic cultivation).	-	[42], HS
G. sucrofermentans (Immobilized cells)	BC content: In the range 0.8-2.12 g·L^{-1} (static cultivation) In the range 1.22-2.59 g·L^{-1} (dynamic cultivation)	Crystallinity: 52.3% (static cultivation) 66.0% (dynamic cultivation)	-	[42], HS
G. xylinus	BC production: 11.35 g·L^{-1} (SPR) 10.29 g·L^{-1} (SM)	Crystallinity (%): 87.39% (SPR) 83.31% (SM)	Tensile strength: 6.87 MPa (SPR) 6.33 MPa (SM) Young's modulus: 13.33 MPa (SPR) 10.51 MPa (SM)	[34]
K. saccharivorans	BC produced: 2.6 g·L^{-1} (HS) 1.1 (F-HS) 3.9 g·L^{-1} (M-HS) 3.2 (D-HS)	Crystallinity index (CI): 87% (HS) 81% (F-HS) 84% (M-HS) 94% (D-HS)	**Tensile strength:** 42 MPa (HS) 33 MPa (F-HS) 38 MPa (M-HS) 57 MPa (D-HS) **Water-holding capacity (WHC):** 99.0 g·g^{-1} approx. (HS) 97.3 g·g^{-1} approx. (F-HS) 103.8 g·g^{-1} approx. (M-HS) 99.9 g·g^{-1} approx. (D-HS)	[58]
G. xylinus	BC yield: 6.5% (BC0) 7.6% (BC0.5) 9.4% (BC1) 10.5% (BC2) 11.6% (BC3)	Crystallinity: 70% approx. (BC0) 90.4% (BC1) 90% (BC2) 91% approx. (BC3)	**Shear modulus at 0.01% strain:** 5 kPa approx. (BC0) 15.1 kPa approx. (BC1) 24 kPa approx. (BC2) 27 kPa approx. (BC3) **Shear modulus at 1% strain:** 2 kPa approx. (BC0) 7 kPa approx. (BC1) 8 kPa approx. (BC2) 9 kPa approx. (BC3)	[94]
Kombucha strains including *G. xylinus*	BC pellicle thickness at 21 days cultivation: 10 mm approx (BC_O) 6 mm approx (BC_c)	Crystallinity: 85.31% (BC_O) 72.52% (BC_c)	WHC: 101 (BC_O) 45 (BC_c)	[95]
Acetobacter xylinum	BC production: 8.5 g·L^{-1} approx. (CSL_Fru with CMC as additive, 1.0% CMC) 1.4 g·L^{-1} approx. (control)	Crystallinity: 80.0% (CSL_Fru with CMC as additive, 1.0% CMC) 85.0% (control)	Young's modulus: 325 MPa approx. (CSL_Fru with CMC as additive, 1.0% CMC) 300 MPa approx. (control)	[80]

4 Discussion and conclusions

4.1 Prospects of commercial bacterial cellulose production

In recent years, there has been a significant increase in the number of patents on BC products. Also, many BC products are expected to be developed and commercialized due to the wide range of possible innovations. More research is needed to study lab scale BC production using agricultural and industrial waste materials and improvement of BC productivity and mechanical and physical properties.

The growth of the BC market is limited by the high product cost, which is mainly due to the high cost of the standard carbon source and the limited BC yield. A schematic diagram of the production cost of BC is shown in Figure 2, which includes operational and capital costs, considers tea and sucrose as substrates instead of glucose, and is based on the work of[96–97]. To tackle the high BC production cost, significant research has been devoted to the use of industrial and agricultural wastes or byproducts, which are promising for large-scale BC production as they lead to high BC productivity with low cost of culture media. These materials act as carbon and nitrogen sources, are available in large amounts, and contribute to reducing waste disposal and the associated environmental hazards. Quite often the obtained physical and mechanical properties, crystallinity, and water-holding capability of obtained BC are comparable to those for standard culture medium. In addition to lowering the cost associated with culture media, a high BC productivity can be achieved through the following strategies: 1) improvement or optimization of the BC culture conditions (e.g. temperature and pH); 2) improvement or optimization of the BC culture medium (components and its composition, including micronutrients and additives); 3) using strains or bacteria species with BC production capability higher than that of *K. xylinus*; 4) using genetic engineering technologies to obtain high BC productivity; 5) improvement of bioreactor design. More research focused on the application of these strategies in the case of agricultural and agroindustrial materials is needed. Optimization of the culture medium and culture conditions leads to a significant increase in BC yield.

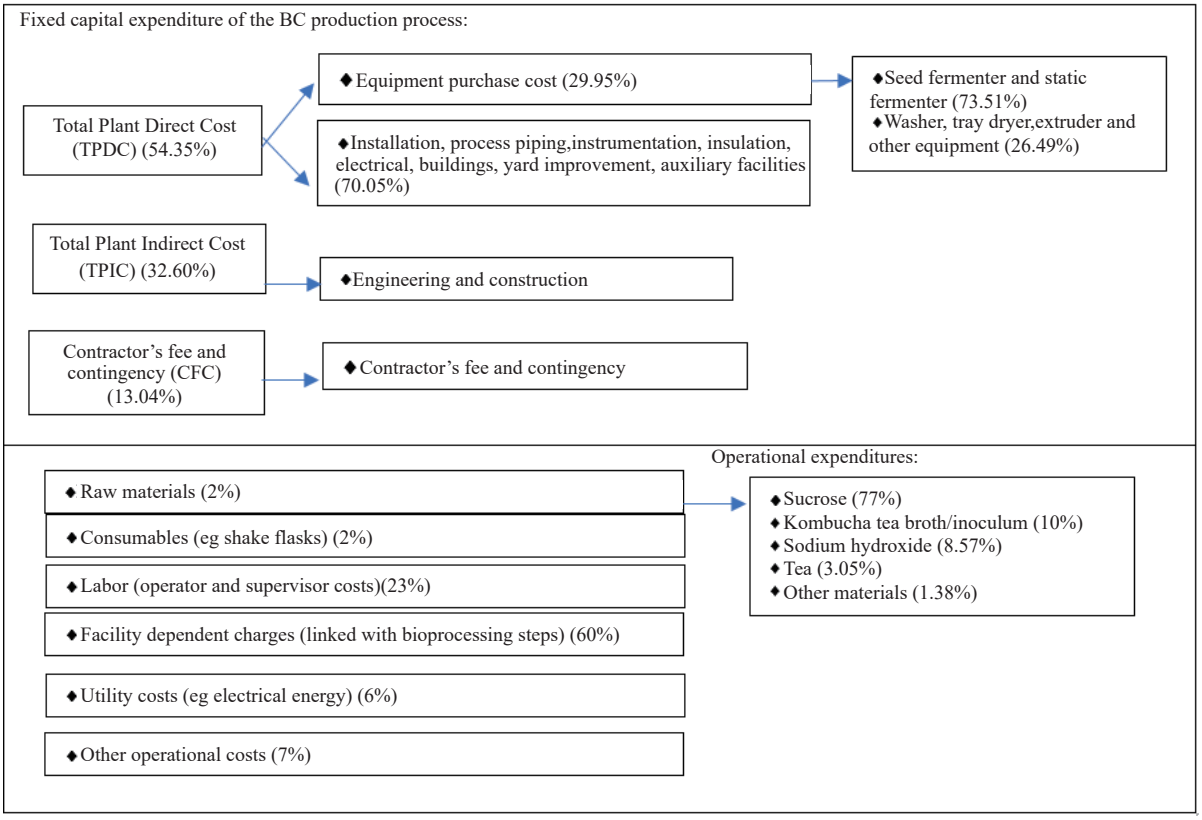


Figure 2 Schematic diagram of the production cost of BC, based on Behera et al. 2022

BC modification via *in situ* and *ex situ* methods leads to significant improvement of BC properties, such as an increase in tensile strength, toughness, and bacterial activity, for specific commercial applications in fields such as medicine, pharmaceutics, and the food industry. Also, these modifications allow tackling quality issues of BC production from agricultural wastes, including: 1) BC may absorb unwanted compounds and be colored, so that strict purification is needed; 2) Low mechanical strength is obtained for some agrowastes; 3) The obtained BC stiffness and high fragility is unsuitable for certain applications; 4) Its toughness is not sufficient for applications requiring impact resistance. Absorption of unwanted compounds implies limited commercial use in fields such as medicine, pharmaceutics, and cosmetics, where high purity is crucial and there are stringent standards.

4.2 Discussion

In this review, BC production is addressed, focusing on the effect of culture conditions and culture medium on the microstructure and physical and mechanical properties of BC, including low-cost substrates as culture medium. The main contributions over closely related reviews include: 1) A compilation of BC production and the corresponding data of crystallinity and either mechanical or physical properties is provided; 2) The different culture conditions of each reference and also the blank or control case are included in the data compilation; 3) The relationship between microstructure (crystallinity), physical and mechanical properties is discussed, and cases for simultaneous

achievement of high values of BC production and either crystallinity or tensile strength are identified. Although many experimental studies provide BC production data, studies providing the crystallinity and mechanical properties (e.g. tensile strength) corresponding to the BC production data, and the corresponding blank or control data corresponding to standard medium are few. Indeed, the blank or control data corresponding to standard medium are not given in several experimental studies. These facts hamper comparing data and assessing the simultaneous achievement of high BC production and either high crystallinity or high tensile strength.

Bacterial cellulose (BC) is suitable for applications in medicine, pharmaceutics, food, environment, engineering, and chemistry. The effect of nutrient addition and *in situ* and *ex situ* modifications on the mechanical and physical properties implies wider application possibilities. BC commercialization is limited by the cost of culture medium, BC production, and yield, whereas the end application of BC depends on its physical and mechanical properties. BC production/yield and physical/mechanical properties can be modified through *in situ* methods, *ex situ* methods, and also by modification of culture conditions (e.g. static/agitated culture mode, bioreactor type), culture medium, and microbial species (including genetic modification). Also, mechanical and physical properties of BC are strongly influenced by morphological and microstructure (crystallinity) properties, whereas morphological and microstructure properties and BC production are dependent on culture conditions (operation conditions, bacterial strain, culture medium). In particular, the carbon source (type and composition) has a significant effect on morphology, microstructure, and physical and mechanical properties. Also, the morphological properties and physical and mechanical properties can be affected by the method used for treating the BC sample.

Future applications of this study could focus on bacterial cellulose (BC) production that utilizes agricultural or agro-industrial materials while achieving the desired mechanical or physical properties. Thus, successful cases of this approach that are depicted in this study can be further addressed through the following research directions: 1) Enhancing mechanical or physical properties by varying the culture and processing conditions (e.g., pH, temperature, O_2 content, concentration of culture components, fermentation time, as well as the species and strain of bacteria used, and drying treatments); 2) Improving mechanical or physical properties through *in situ* modifications by using different additive materials in varying concentrations; 3) Improving productivity and material properties through repeated harvest; see[98]; 4) Evaluating the performance of BC in various fields, such as food packaging, paper products, biodegradable materials, and food additives.

4.3 Conclusions

Low-cost materials can substitute standard HS culture medium in bacterial cellulose production, leading to lower cost and high quality BC, as confirmed by several BC production studies. BC produced from agricultural/industrial material exhibits a wide variety of BC production values, as well as morphological, physical, and mechanical properties, which are dependent on the agricultural material used, the content of nitrogen and phosphorus, the bacterial strain, and culture conditions. In many cases in which low-cost carbon sources are used, the resulting BC production is higher than that of standard HS medium. From the data comparison, it follows that the BC production and the required morphological, mechanical, and physical properties are either similar or better than those for conventional HS medium in only some studies. In cases in which BC production is higher than that of standard medium, the improvement of crystallinity or mechanical/physical properties is not guaranteed. In some cases, the crystallinity is not significantly modified, but the corresponding physical/mechanical properties are. This implies that a more complete characterization is convenient in experimental studies on BC production using low-cost culture medium, including the physical and mechanical properties corresponding to the BC production/yield data. Also, further research is required considering *in situ* methods, *ex situ* methods, and modifications of culture conditions, in addition to the use of low-cost substrate, but a complete characterization is necessary in this case.

Using agroindustrial materials results in a significantly different culture medium for BC production. The carbon source differs from glucose, for example, fructose or sucrose, but other components may also be present, such as polysaccharides, proteins, vitamins, minerals, amino acids, polyphenols, and trace elements. In particular, lignocellulosic materials contain lignin, cellulose, hemicellulose, and small amounts of proteins, pectins, and ash. These components and their concentrations affect the biosynthesis process of cellulose as well as the BC microstructure, including the polymeric structure of BC, the interfibrillar binding in the fiber network, the hydrogen bonds between OH groups of cellulose, the molecular weight, the crystalline ordering of the BC fibrils, the density of fiber arrangement, and the pellicle thickness. As a result, the physical and mechanical properties are also affected.

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