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Review Article

Bacterial Cellulose, Fermentative Production and its Pharmaceutical Application -

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ABSTRACT

Bacterial Cellulose (BC) is a polymer with unique characteristics and structure. Many research studies have focused on using BC to create new materials and nanocomposites for a variety of uses since it can be obtained in various shapes and is simple to modify chemically and physically. Many bacteria, such as *Acetobacter xylinium* and *Glyconoacetobacter hansinii*, can manufacture cellulose with a chemical formula that is similar to plant cellulose but has different physical properties. This difference is due to the reticulated network of their fine fibrils, which have a diameter of around one hundred times that of wood fibers (0.1 m). Enhancing cellulose growth is being studied in a variety of ways right now. This pure form of cellulose has no side effects and zero levels of toxicity. The aim of this review was to provide an overview of BC structure, fermentation parameters for promoting BC processing, unique features of bacterial cellulose, and its industrial and pharmaceutical applications as a very important product.

Keywords: Bacterial cellulose; Plant cellulose; Fermentation parameters; Applications; Bacteria

INTRODUCTION

Biopolymers, which have become valuable for use in a lot of applications, are superior to derived petrochemical in being biodegradable biocompatible, and environmentally.

Polysaccharides are a different group of macromolecules found in nature and widespread. They can be disconnected due to their localization morphology as part of the cytoplasmic membrane or located inside the cell wall as intracellular polysaccharides or located outside the cell wall as extracellular polysaccharides.

Extracellular polysaccharides are found in two structures: non-adherent to the cell which is called loose slime and give it a sticky consistency to the development of bacteria in a solid medium or in a liquid medium give a viscosity form and capsules or microcapsules, which hold to the cell wall. They have a distinct structure and boundary, being only gradually separated in the salt or water solutions. Therefore, it is possible to disconnect microcapsules and capsules by centrifugation from loose slime [1].

Exopolysaccharides are long-chain polysaccharides composed of repeating sugars branched units or derivatives of sugar, fundamentally galactose, rhamnose, and glucose in different ratios. They are divided into two groups: heteropolysaccharides (xanthan and gellan) and homopolysaccharides (curdlan, mutan, cellulose, dextran and pullulan). Homopolysaccharides are composed of only one kind of monosaccharides (D-fructose or D-glucose) connected either by a combination of a set number of linkage types or by a single linkage type. While, Heteropolysaccharides are composed of numerous oligosaccharide copies, including 3 to eight 8 residues, produced by a different microorganism. There are a lot of industrial applications for exopolysaccharides in pharmaceutical, food, and other industries such as cosmetics, gelling agents, medicines for wound dressing, paper, and textiles.

An important example of monosaccharides is cellulose. Cellulose is the most important biopolymer and is produced fundamentally by plants. However, a few bacteria species can also produce cellulose with a chemical formula like that of plant cellulose but with unique physical properties. This variation is because of the reticulated network of their fine fibrils with a diameter of (0.1 μ) is about 100th that of wood fibers.

Also, unlike plant cellulose, BC does not need additional conversion to evacuate undesirable contaminants and impurities like as pectin, hemicellulose, and lignin, in this way have the option to hold a prominent polymerization level. BC also, proved solitary properties, such as in terms of purity, tensile strength, a young's modulus higher than synthetic fibers by around 30-40% [3], and is capable of swelling as much as 700 times its dry weight. The structure of its fiber has a fineness of 0.1-10 micrometers, and high crystallinity is decomposition better than cellulose from plants [4]. Meanwhile, cellulose from plants has lignin, pectin, and hemicellulose as the main compounds. Making it more suitable as crude material for producing high-quality paper, dessert foods, and high-fidelity acoustic speakers [5]. Bacterial cellulose Fibrils are around 100 times thinner than Fibrils of plant cellulose, therefore it is a material of highly porous, which permits the movement of different drugs or antibiotics into the injury while simultaneously working as a physical barrier effective versus any outside contamination. So, it is used broadly in injury healing [6].

Therefore, the goal of this review is to supply an overview of BC structure, fermentation parameters for promoting the production of BC, and as well as its industrial and pharmaceutical applications as a very important product

Plant Cellulose (PC)

In 1838 Cellulose was isolated and discovered from wood for the first time by Anselem Payen a French botanist, after that, the study of cellulose progressively increased. Cellulose is so insoluble, however severe chemical dissolution detects, that cellulose is a glucan where the residues are linked β (1-4). These β-glycoside links play an important key in detecting the strength of the cellulose fibers and thus structural properties of cellulose**.** The extraordinary cellulose properties are due to the relationship of the long chains leading to produce a fiber named microfibrils. The microfibrils support to form a fiber larger in length, which then lay down in a crisscross pattern, and is connected with a polysaccharide that works as bio-cement. Therefore, the cellulose sheets are very strong and tough. The methods of Light scattering demonstrated that, the cellulose chains are about long with 13000 to 14000 glucose residues and that there is no branching [7].

Other than being important to the common world, cellulose is additionally critical to the human being. Since this compound benefits individuals in fundamental ways, such as writing and clothing.

Cellulose can also be chemically modified and processed to make rayon, photographic films, and plastics. On the other hand, derivatives of cellulose can be used as explosives, adhesives, moisture-proof coatings, and thickening agents in food. Truly, cellulose made a portion of the first synthetic polymers such as ethyl cellulose, cellulose acetate, rayon, and cellulose nitrate.

Microbial Cellulose

Also, microorganisms that produce cellulose have different applications and properties than those that plant cellulose. Cellulose is produced by different microorganisms like algae, bacteria, and fungi.

In green algae, mannan, xylan, and cellulose serve as polysaccharides in cell wall structures. Although, Cellulose is produced in small amounts in most red algae (*Rhodophyta*), in all of the brown algae (*Phaeophyta*), and the ultimate of the golden algae (*Chrysophyta*) [9]. In Oomycetes, chitin is totally replaced by cellulose representing around 15% of the wall dry mass. Also, Gram-negative species such as *Alcaligenes, Azotobacter, Acetobacter, Sarcina, Rhizobium, Pseudomonas, Salmonella, Agrobacterium, Achromobacter*, and *Aerobacter* produce cellulose [10]. Moreover, Cellulose is synthesized by *Sarcina ventriculi* a Gram-positive bacterium, representing around 15% of the total dry cell mass [11].

Adrian Brown was the first who reported Bacterial Cellulose (BC) when used *Bacterium aceti* in 1886 [12]. He noticed at the surface of the fermentation medium a formation of a solid mass. The solid mass was named the "mother" or "vinegar plant" which is used in the production of homemade vinegar. This component was illustrated as cellulose and the name Bacterium *xylinum* which is accountable for its production. After that, many names were given to this bacterium such as *Bacterium xylinodes* and *Acetobacterium xylinum*. Then, later and according to the International Code of Nomenclature of Bacteria. The official name become *Acetobacter xylinum*. Now, the strictly aerobic, Gram-negative bacterium is named as *Gluconacetobacter xylinus* [13], which is a subspecies of *Acetobacter aceti* [14].

From all microorganisms mention above only *Gluconacetobacter species* (Formerly known as *Acetobacter*) at commercial levels able to produce cellulose. Therefore, *Acetobacter xylinum* has been applied as model microorganism for applied and basic studies on cellulose, due to its capacity to produce high amount of polymer from a different type of nitrogen and carbon sources [15]. It is an obligate aerobic, rod-shaped, Gram-negative bacterium, chemotrophic, ellipsoidal to rod shaped, straight or slightly curved organism belonging to the family of *Acetobacteriaceae*. It can exist in single, pairs or as chains [16]. It produces cellulose as part of primary metabolite in the form of interwoven extracellular ribbons. This bacterium produces cellulose and grows from a different substrate.

On the other hand, table 1 represent some of the different strains producing cellulose and their yields. The Selection of commercially strains was initially determined by its use as a food product for cellulose producing organisms [17]. For example, Nata de Coco, is a public dessert in Philippine, composed of mixing other plant extracts with BC in the fruit juices. The microorganism was then illustrated as *G. xylinus* with optimal culture conditions of temp. 28°C, pH at 5-5.5, glucose or sucrose as carbon source and ammonium salts as nitrogen source [18].

Formation and Description of Bacterial Cellulose Pellicle

As shown in figure 2 in static culture, a film of cellulose is formed on the surface of the liquid after 24 hours and after a further 24 hours, a distinct cellulose pellicle was present [19]. The author found that the pellicle was initially transparent, but as it grew, the denser cellulose matrix became opaque white. When the pellicle became thicker, layers were easily detectable on its underside. When harvested and dried the cellulose pellicle was leathery to touch, with a smooth glossy surface. However, by further drying, the cellulose pellicle developed a plastic texture and had a cracked and wrinkled appearance as the long chains of cellulose appeared to contract.

One of the unique features of this pure cellulose membrane is that it is very strong in the never dried state; however, it can hold hundreds of times its weight in water. This great absorptivity and strength constitute two of the many novel features of microbial cellulose [20].

Bacterial Cellulose Structure

The molecular formula of bacterial cellulose $(C_6H_{10}O_5)$ n is the same as that of plant cellulose, but their physical and chemical features are different. Bacterial cellulose has a basic structure as a polymer strongly connected with a hydrogen chain between a hydroxyl group called microfibrils that has a thickness of 3-8 nanometres and width of 50-80 nanometres, which are composed of glucan chains which held together by intra- and inter- hydrogen bonding so that produced a crystalline domain (Figure 3) [27]. This structure microfibrillar of

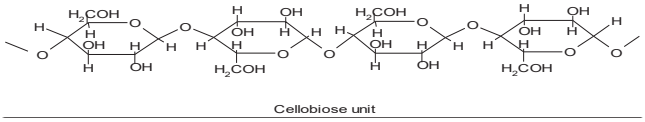

Figure 1: Chemical structure of plant cellulose [8].

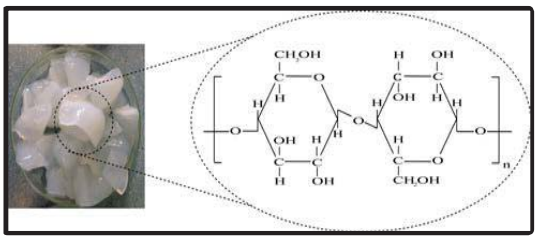

Figure 2: Chemical structure of bacterial cellulose and the shape of bacterial cellulose.

Table 1: Some of different strains producing microbial cellulose.

Microorganism	Carbon Source	Supplement	Culture Time	Yield/(g/L)	Reference
A. xylinum ATCC 10245	Black strip molasses	no	144 h	4.695	[21]
G. hansenii PJK (KCTC 10505 BP)	Glucose	oxygen	48 h	1.72	[22]
Acetobacter xylinum ssp. *sucrofermentans* BPR2001	Fructose	oxygen	52 h	10.40	[23]
Acetobacter xylinum ssp. *sucrofermentans* BPR2001	Fructose	agar oxygen	44 h	8.70	[23]
G. xylinus strain (K3)	Mannitol	green tea	7 days	3.34	[24]
Gluconacetobacter xylinus IFO 13773	Glucose	lignosulphonate	7 days	10.10	[25]
Acetobacter xylinum NUST4.1	Glucose	sodium alginate	5 days	6.00	[26]
Gluconacetobacter xylinus IFO 13773	Sugar cane molasses	no	7 days	5.76	[25]

bacterial cellulose was first demonstrated by Mühlethaler who proved that each bacterial strain produced different structures of cellulose, as shown in table 2 [28].

Observations of Electron microscopic showed that, the cellulose produced by *Gluconacetobacter xylinus* (Formerly known as *Acetobacter xylinum*) produced in fibres form. The BC fibrous network is synthetic of three-dimensional nanofibers that well-arranged, so that of hydrogel sheet with porosity and high surface area was formed. *Acetobacter xylinum* synthesis two forms of cellulose: (i) cellulose I, the ribbon-like polymer, and (ii) cellulose II, the thermodynamically more stable amorphous polymer as showed in figure 4 [30].

During the process of synthesis, protofibrils of glucose chain are secreted through bacteria cell wall and aggregate together forming nanofibrils cellulose ribbons. These ribbons build the network structure shaped of BC which have highly porous matrix [31]. The cellulose produced has abundant surface of hydroxyl groups that explaining it as chemical-modifying capacity, biodegradability and hydrophilicity [32].

Synthesis of Bacterial Cellulose

Bacterial cellulose synthesis is an exact and specifically regulated many-step process; as shown in figure 5; containing a large number of both complexes of catalytic and regulatory proteins and individual enzymes, whose supramolecular structure has not yet been well known. Mechanisms and Pathways of Uridine Phosphoglucose (UDPGlc) synthesis are comparatively well known, while mechanisms of glucose polymerization into long and unbranched chains still need searching [33].

Colvin and Leppard propose in cellulose biosynthesis a cycle of lipid-phosphate-carbohydrate intermediates, where glucose reacts to produce glucose-6-phosphate, then glucose-1-phosphate, Uridine Diphosphoglucose (UDP-glc) and finally cellulose [34]. This bacterial cellulose biosynthesis produces a multi-regulation network include many key enzymes to control the synthesis of cellulose (like cellulose synthase, isomerase, glucokinase, UDPG-pyro phosphorylase and phosphoglucomutase) where tricarboxylic acid cycle and the branched Hexose Monophosphate Pathway (HMP) are included [35].

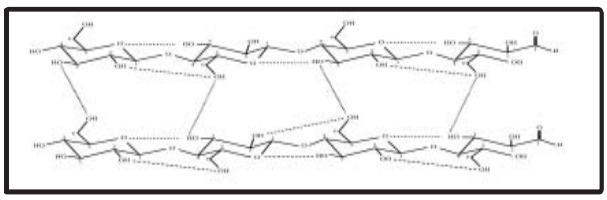

Figure 3: Intra and Inter hydrogen bonding of bacterial cellulose [29].

Table 2: Bacterial cellulose structure of various microorganism.

Microorganism	Structure of Cellulose
Acetobacter	Fibers like ribbon
Acetobacter	Fibers
Alcaligenes	Fibers
Agrobacterium	Short fibres
Achromobacter	Fibers
Rhizobium	Short fibre
Pseudomonas	Unclear fibres
Sarcina	Amorphous
Zoogloea	Unidentified

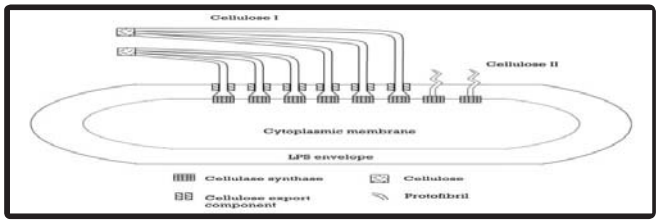

Figure 4: Microfibrils of cellulose by *Acetobacter xylinum*.

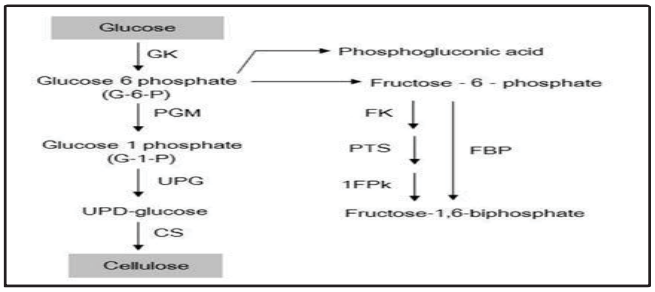

Figure 5: The bacterial cellulose synthesis pathway of *Acetobacter* sp. 1FPk: Fructose Phosphatekinase; PTS: System of Phosphotransferase; FBP: Fructose-1,6-bi-phosphate phosphatase; FK: Fructokinase.

On the other hand, Chawla, et al. proved that, the bacterial cellulose Production depends on carbon sources, like dicarboxylic acids, dihydroxyacetone, hexoses, glycerol, D-gluconolactone, pyruvate, glucose, arabinose, sucrose, starch, fructose and xylose [30]. These substances to enter the main metabolism must be able to change to intermediates. When enter through the pathway of the main metabolism, Uridine Diphosphoglucose (UDP-Glucose) will be present, which used as a precursor for cellulose producing by glucose phosphorylation to glucose-6-phosphate (Glc-6-P), catalysed by glucokinase, then isomerization of this intermediate to Glc-α-1-P, catalyzed by phosphoglucomutase and by UDPGlc Pyrophosphorylase (UGP) enzyme the transformation of the latter metabolite to UDPGlc happened. Afterward, by the function of the Cellulose Synthase (CS) enzyme, uridine UDP-Glucose will be linked to the lines of cellulose or β-1, 4-glucan chain [30].

The UDPGlc Pyrophosphorylase (UGP) enzyme appears to be the critical one implicated in cellulose synthesis, due to several phenotypic Cellulose-negative mutants (Cel^-) are specifically weakly in this enzyme, although the activity of display Cellulose Synthase (CS), which was confirmed in vitro by means of cellulose synthesis observation, catalyzed by cell-free extracts of Cel^- strains [36]. Furthermore, the activity of pyro phosphorylase is different between different strains of *A. xylinum,* and the most effective cellulose producers detect the highest activity, like *A. xylinum ssp. sucrofermentans BPR2001* [15].

On the other hand, *A. xylinum* Cellulose synthase is a typical membrane-anchored protein having a molecular mass of 400-500 kDa. It is tightly bound to the cytoplasmic membrane and appears to be very unstable. During the synthesis of bacterial cellulose, the bacterial cellulose synthase (Bcs) enzyme B and A play key

roles to boost cellulose fiber elongation. Morgan, et al. proved the crystal structure of a complex of BcsB and BcsA and consist of a translocating polysaccharide, which proved the BcsA-BcsB complex forms a cellulose-conducting channel to extend polysaccharide by one glucose [10].

Also, in a cell-free system BC can also be synthesized. Glaser, 1958 first reported that cellulose synthesis in vitro can be gained with only broken cells UDPG and ATP [37]. However, the efficiency of the production was low, which could be due to the membrane potential requirement during cellulose synthesis [38].

Crystallization of Cellulose Chains

To observe cellulose formation Dark field microscopy was employed. The bacteria are secreted cellulose in the form of a ribbon, composed of approximately 46 microfibrils, at a rate of 2 μm/min. the cell envelope Electron micrographs indicated that, the presence of 50-80 pore-like sites coordinated in a uniform row along the cell long axis and in clear group with the extracellular cellulosic ribbon [39]. Brown demonstrated that, these discrete structures are the extrusion sites for cellulosic microfibrils in combination of 10-15 chains. Rather than individual β-1, 4-glucan chains, microfibrils are assumed to be the initial form of the produced cellulose [40].

Most cellulose in nature is produced as crystalline cellulose, which is defined as cellulose I. In an attempt to detect the relationship between crystallization and polymerization of β-1, 4-glucans into microfibrils of *A. xylinus*, Benziman, et al. added a commercial brightener for cellulose called Calcofluor White ST, to the production medium [41]. This disrupted the association of crystalline cellulose I fibrils while accelerating the process of the polymerization. Once the chemical agent was removed, polymerization rates and ribbon production and return to normal. They concluded that, the crystallization and polymerization are coupled in a consecutive process, and the polymerization rate was restricted by the crystallization rate [41]. Deslandes and Marchessault continued this work and developed a mechanism for the self-assembly of cellulose microfibrils [42]. The first step is the polymerization of several β-1, 4-glucan chains at an extrusion site on the cell surface. A single *A. xylinus* cell may polymerize up to 200,000 glucose molecules per second into β-1, 4-glucan chains on the lined-up extrusion sites, which later secreted into the surrounding medium [43]. Parallel glucan chains then aggregate and crystallize into microfibrils, and finally these microfibrils aggregate into discontinuous bundles. Native cellulose microfibrils occur in a spectrum of dimensions, ranging from 1 to 25 nm in width (corresponding to 10-250 chains) and from 1 to 9 μm in length (2,000-18,000 glucose residues). On the other hand, the presence of certain substances that cannot penetrate inside the cells or stronger aeration can form competitive hydrogen bonds with the β-1, 4 glucan chains (fluorescent brightener, carboxymethyl cellulose, calcofluor white) and make important changes in the supramolecular organization of cellulose chains.

Optimization of Bacterial Cellulose Production

The factors affecting cellulose production mainly include environmental conditions including pH, Temperature and dissolved oxygen (static or agitated) and growth medium including (carbon sources and nitrogen sources).

The current methods of BC production are static culture, agitating culture, and the airlift reactor [39]. Continuous fermentation, semi-continuous and large scale will be controlling to meet commercial demand. In all cases, the main objective is to achieve maximum BC production with suitable properties and optimum form for the application uses.

It was already believed that, the *A. xylinus* wild strains were not able to produce cellulose in an aerated and submerged system due to the accumulation of cellulose-negative (Cel^-) mutants [21]. Unlike wild-type cells (Cel^+), Cel^- cells produce water-soluble polysaccharides and they are identified as acetan [16]. However, through strain selection or genetic modification, many strains can now produce cellulose in an aerated and agitated bioreactor [23].

A Three-dimensional interconnected reticular pellicle was proved under static condition while both stirred and agitated condition produced irregular Shape-like Cellulose Particle (SCP) [44]. BC produced by these new strains under airlift and agitated cultures is reticulated cellulose slurry formed with restricted applications.

The cellulose formation process under static condition is adjust by air supply from medium surface and the amount produced depends on the carbon source concentration moderately [45]. When the growth time increase, the formation of BC increases along with hydrogen and C-H bonding [34]. The BC synthesis reach its end when the growth pellicle downward and entraps all bacteria and due to insufficient oxygen supply the bacteria become inactive [6]. Therefore, the process of semi-continuous in static condition at industrial scale is recommended, because it manage to increase the productivity of BC compared to continuous process [10].

Agitated condition causes formation of Sphere-like Cellulose Particle (SCP), an irregular form of cellulose either in fibrous suspension, spheres, pellets or irregular masses [46]. The SCP has lower crystallinity, mechanical strength and degree of polymerization compared to pellicle from static culture [35]. The altered microfibrils organization was proposed related to the disruption effects of aeration on the hydrogen bonds formations between cellulose [5]. Hu Y, et al. [22] found that the number of SCP decreased with the increasing volume of inoculums while different initial glucose concentration only gave an impact to the mean diameters of SCP. However, the mechanism of SCP formation is still remained unknown.

On the other hand, many researchers such as Romano R, et al. [31] studied the effect of different temperatures on BC production and found that, *Acetobacter xylinum* grow normally and produce cellulose only if a temperature of 30-35°C was insured during the cultivation period. These results were with accordance to those of Son HJ, et al. [37] who showed that, the optimum temperature for cellulose production was observed at 30°C with no significant difference in its production at 25°C however, it obviously decreased above 35°C. This latter result was previously reported by Hestrin S, et al. [20] who found that, at a temperature of 37°C *Acetobacter xylinum* failed completely to multiply even in an optimal medium.

Furthermore, Schmauder HP, et al. [33] proved that, the bacterial cellulose synthesized at 30°C, had a lower degree of polymerization and higher water binding capacity in comparison to that produced at 25°C and 35°C. Also, McCormick SP, et al. [47] proved that, *Acetobacter xylinum* ATCC 10245 can grow at a temperatures range of 12°C to 35°C.

Moreover, the effect of the initial pH of the fermentation medium is an important factor for the growth of microorganism and the production of BC. This factor was studied by several scientists and most of them indicated that the optimal pH range for cellulose

production by *Acetobacter xylinum* is 4-6 and the yield of cellulose decreasing below pH = 4 [14]. However, Fiedler M, et al. [48] stated that, *Acetobacter xylinum* grow over a pH range of 3 to 8.

The pH decreases during fermentative production because of the accumulation of gluconic, acetic or lactic acids in the culture broth therefore, it is important to control the pH within the optimal range [49].

On the other hand, Cheng HP, et al. [11] stated that, the pH is one of the most critical factors for efficient BC production. He used CSL both as a nitrogen source and in order to maintain the pH of the fermentation medium within an optimal range. The buffering capacity of CSL resulted in high bacterial cellulose production was achieved in shaken flask, stirred tank or an airlift reactor, almost equal to that produced in cultivations where the pH was controlled manually.

Furthermore, the effect of the fermentation period on BC production was also studied by different scientists. For example, Cheng HP, et al. [11] demonstrated that, maximum production of BC was obtained after 72 hours of cultivation period. However, most of the scientists proved that, the highest level of cellulose was obtained only after 7-9 days of incubation period [40].

Also, Ramana KV, et al. [30] found that, the degree of polymerization of BC increases as the duration of biosynthesis is prolonged up to 6 days. A further prolongation of the process to 28 days lowers the Degree of Polymerization (DP) value and increases polydispersity.

The optimal design of the medium is very important for the growth of a microorganism and thus stimulating the formation of products. The fermentation medium contains carbon, nitrogen and other macro- and micronutrients required for the growth of organism. The changes in the medium components affect the growth and the product formation directly or indirectly. Secretion of exopolysaccharides is usually most noticeable when the bacteria are supplied with an abundant carbon source and minimal nitrogen source [50]. Sometimes a complex medium supplying amino acids and vitamins is also used to enhance the cell growth and production [49].

This make many investigations concerning optimal composition of medium for bacterial growth and cellulose production. The standard medium used for BC cultivation, the Hestrin-Schramm medium is expensive and requires additional supplements for effective cultivation [51]. Saccharified liquid medium, created by the enzymatic treatment of food wastes, appears as an inexpensive alternative to complex commercial BC production medium. Products from beets (molasses, sugar syrup, and saccharose), corn (starch, hydrolysed starch, glucose syrup, and glucose), and potatoes (starch and starch hydrolyzates) can be used for BC production [51].

Yamanaka S, et al. [52] modified media produced high levels of cellulose, and more specifically the Yamanaka-mannitol combination reached the highest cellulose yield. The results also showed that glucose, mannitol and sucrose were the carbon sources that produced consistently high yields of cellulose, regardless of the composition of the media. The maximum BC production was set at 10 g produced in 14 days using a large surface area and high cellulose-producing media [53].

One of the most very important composition of the media is carbon sources, glucose and sucrose are used as carbon sources for cellulose production, although other carbohydrates such as fructose, maltose, xylose, starch and glycerol have also been tried [19]. Mikkelsen D, et al. [54] evaluated mannitol, glucose, glycerol, fructose, sucrose and galactose as carbon sources in HS medium. They found that after 96-h of fermentation, the highest yields of BC 3.83 and 3.75 g/l were obtained with sucrose and glycerol, respectively. They also observed that different carbon sources did not markedly alter the micro-architecture of the resulting cellulose pellicles. Furthermore, Fructose and glycerol gave almost the same cellulose yield as glucose [55].

Park JK, et al. [56] (KCTC 10505 BP) produced 1.72 g/L of cellulose when glucose was provided as carbon source. *Acetobacter* sp. *V6* strain isolated from the traditionally fermented vinegar produced 4.16 g/L cellulose in a complex medium containing glucose as a carbon source [51]. Moreover, the effect of initial glucose concentration on cellulose production is important, since the formation of gluconic acid as a by-product in the medium decreases the pH of the culture and ultimately decreases the production of cellulose. For example, Cellulose yields at initial glucose concentrations of 6, 12, 24 and 48 g/L were studied, and the consumption of glucose was found to be 100, 100, 68 and 28 % of the initial concentration, respectively [19].

Therefore, to overcome the problem of gluconic acid formation investigated the formation of gluconic acid and bacterial cellulose production in the presence of lignosulphonate [57]. Gluconic acid production was decreased and bacterial cellulose production was increased when the medium was supplemented with lignosulphonate. This was attributed to the inhibition of gluconic acid formation in the presence of antioxidant and polyphenolic compounds in lignosulphonate.

On the other hand, Oikawa T, et al. [55] reported that D-mannitol was an effective substrate for cellulose production of *G. xylinus* [58]. The optimal medium containing polypeptone and yeast extract, in addition to mannitol, produced three times more cellulose than a glucose medium under identical culture conditions. The author presumed that mannitol is first converted to fructose before entering the cellulose synthesis pathway [48]. Also, *Gluconacetobacter xylinus* strain isolated from kombucha gave maximum cellulose production with mannitol as a carbon source [49]. Therefore, recent studies have investigated the replacement of carbon and nitrogen sources in Hestrin-Schramm media.

Furthermore, Ishihara M, et al. [58] used xylose as a carbon source for the production of cellulose by *A. xylinum* IFO 15606 and obtained a yield of 3.0 g/L. Sucrose, mannitol and glucose were found to be the optimal carbon sources for cellulose production by *A. xylinum* NCIM 2526.

Also, Ethanol is used as additional carbon source and to degenerate the cellulose non-producing cells of *G. hansenii* (Cel–), which can appear under submerged culture conditions. Addition of ethanol increased cellulose production from 1.30 to 2.31 g/L in *G. hansenii* [56]. Moreover, Son HJ, et al. [37] studied the effect of the addition of ethanol on cellulose production by using newly isolated *Acetobacter* sp. A9 strain. It was observed that with the addition of 1.4% (by volume) ethanol to the medium, cellulose production was 15.2 g/L, which was about four times higher than that without ethanol addition.

On the other hand, Ishihara M, et al. [58] observed that, lactate had a stimulating effect on cellulose production when it was added

with 4 % (by mass per volume) fructose containing corn steep liquor, yeast extract or peptone as a nitrogen source. Bae S, et al. [1] also studied the effect of lactate on bacterial cellulose production from fructose in continuous culture by *Acetobacter xylinum* ssp. They reported that supplementing 12.5 g/L of lactate to the feed medium increased the cell concentration and fructose consumption at a steady state, resulting in a production rate of 0.90 g/L and a cellulose yield of 36% at a dilution rate of 0.1 h^{-1}. The Adenosine Triphosphate (ATP) content of viable cells was maintained at a higher level by feeding with a lactate-supplemented medium rather than the unsupplemented corn steep liquor-fructose medium [1].

Furthermore, they indicated that lactate functioned as an energy source, not as a substrate for cellulose biosynthesis. Increased intracellular ATP resulting from lactate oxidation may have improved the fructose consumption and cellulose production in the continuous culture [1].

Moreover, in order to compensate its low sugar conversion yield and to reduce the feed-stock cost of BC production, BC has been produced by fermenting the hydrolysates of agricultural wastes such as sugarcane molasses [60], hemicelluloses [16], konjac powder [15], and waste cotton fabrics [61]. Several successful efforts have been made to use certain industrial food wastes as growth medium for the BC producer organisms, which is not only a cheap way but also works as a basin for environmental cleaning [62].

Keshk T, et al. [57] investigated the production of bacterial cellulose using sugarcane molasses in a Hestrin-Schramm medium, and indicated it to be a better carbon source than glucose for cellulose production. However, Bae S, et al. [1] studied the production of bacterial cellulose by *Acetobacter xylinum* BPR2001 using molasses medium in a jar fermenter. They also reported that, subjecting molasses to H_2SO_4-heat treatment gave a maximum cellulose concentration that was 76% more than that achieved using untreated molasses, and also that the specific growth rate increased twofold. They also varied the initial sugar concentrations in the H_2SO_4-heat treated molasses from 23 to 72 g/L, and concluded that maintaining a lower concentration in the molasses is essential for efficient cellulose production in jar fermenters, the effect being attributed mainly to the complex nature of molasses [63].

Also, Premjet S, et al. [62] added components of sugarcane molasses such as sucrose, fructose, glucose, nitrogenous compounds, non-nitrogenous acids, nucleic acids, vitamins, other carbohydrates, minerals and black colour substances individually or in combined forms into Hestrin-Schramm medium, and investigated their effect on bacterial cellulose production by *Acetobacter xylinum* ATCC 10245. They concluded that the addition of vitamins, amino acids, other carbohydrates, minerals and black colour substances to the molasses in the Hestrin-Schramm medium with a mixture of sucrose and fructose as the carbon source increased the bacterial cellulose yield [64]. The black colour substance was the most effective in increasing the production of bacterial cellulose.

On the other hand, Thin Stillage (TS) is a wastewater from rice wine distillery rich in carbon sources and organic acids. Wu JM, et al. [63] discovered that TS, when employ to replace distilled water for preparing Hestrin and Schramm medium can enhance the BC production 2.5-fold to a concentration of 10.38 g/l, with a sugar-BC conversion yield of 57% (0.57 g BC/g reducing sugar) after 7 days of static cultivation [65]. Also, in 2012, Ha and Park further improved the BC production, 15.28 g/l of BC was obtained after 15 days of cultivation [66].

The Nitrogen source is the main component of proteins necessary in cell metabolism, and comprises 8-14 % of the dry cell mass of bacteria. The effect of various nitrogen sources on the production of bacterial cellulose has been reported; casein hydrolyzate gave yield of 5 g/L, and peptone gave yield of 4.8 g/L of cellulose in *A. xylinum* [55]. The addition of extra nitrogen favours the biomass production, but diminishes cellulose production [58]. They observed that, corn steep liquor had a stimulating effect on cellulose production when it was added at 0.15 % (by volume) to the medium with 4% (by mass per volume) fructose [58]. This was attributed to the presence of lactate in corn steep liquor, which is absent from other nitrogen sources as mention before.

Also, the effect of yeast extract concentrations on cell growth and cellulose production in different carbon media has been studied. Yeast extract was added to the medium in the range from 5 to 60 g/L, while carbon sources comprised 20 g/L; the medium containing 40 g/L of yeast extract yielded maximum concentration (6.7 g/L) of cellulose [67].

Furthermore, Yeast extract and peptone are the most commonly used nitrogen sources in BC production as they provide nitrogen and growth factors *for Gluconacetobacter* strains. However, many researchers are trying to find efficient substitutes due to their high cost. Therefore, Matsuoka M, et al. [50] determined that, Corn Steep Liquor (CSL) was the most effective undefined nutrient and they found that lactate and methionine had the greatest effect within CSL. The defined medium based on this analysis was able to synthesize cellulose at 90% of the rate of the undefined medium [58].

Application of Bacterial Cellulose (BC)

Bacterial cellulose offers a wide range of applications, whereas plant cellulose can hardly be used. This is mainly due to its high purity, high crystallinity, high water absorption capacity, and its mechanical strength in the wet state. Therefore, many authors studied the applications of BC**.**

In the field of Paper manufacturing Yamamoto H, et al. [68] as well as, Johnson DC, et al. [69] demonstrated that, because of its unique physical and structural properties, BC is seen as a potential material to be used in the production of high-quality paper. Also, Johnson DC, et al. [69] reported that highly branched, reticulated BC pellets produced from agitation culture are suitable for the production of high-quality paper. Many other investigators, referred to the importance of BC as high-quality additives to paper such as [70]. Also, Lin S, et al. [71] proved that BC is a valuable component of synthetic paper, since although nonpolar polypropylene and polyethylene fibers prove insulation, heat resistance, and fire-retarding properties; they cannot form hydrogen bonds like the papers containing BC and this type of paper is usually of very good quality.

In the field of food Okiyama A, et al. [72] suggested several applications for BC in the food industry, such as thickening agents, low-calorie desserts, salads, and fabricated food. Therefore, BC has been determined to be "Generally Recognized as Safe" (GRAS) and accepted by the Food and Drug Administration in 1992.

Also, it has important applications in a variety of food formulations, especially when low use levels, lack of flavor interactions, foam stabilization, and stability over wide pH range, temperature, and freeze-thaw conditions are required. BC in combination with other agents such like sucrose and CMC improve the dispersion of the product. Potential applications also include low-calorie additive,

thickener, stabilizer, texture modifier, pasty condiments, and ice cream additive [73].

On the other hand, Budhiono A, et al. [45] investigated the introduction of microbial cellulose into a food product known as the Nata de Coco in which *Acetobacter xylinum* was incubated with the coconut milk. They showed that Nata do Coco has a plasma cholesterol-lowering effect, protects against bowel cancer, arteriosclerosis and coronary thrombosis, and also prevents the sudden rise of glucose in the urine therefore, "Nata de Coco" is becoming increasingly popular.

Another popular BC-containing food product which also protect against cancer is the Chinese Kombuchar or Manchurian Tea. This tea is obtained by growing the microorganism in a medium containing tea extract and sugar.

In the fields of medicine and pharmacy, bacterial cellulose has unique properties such as high elasticity, high wet surface tension, great porosity, and high-water holding capacity, together with having a fibre structure [74]. This result allows bacterial cellulose to be a new alternative in medical applications, like artificial arteries, or being used for treating chronic wounds that are hardly curable, like leg wounds, bedsores, and chronic wounds from diabetes [75].

Wound healing is a dynamic process that involves the complex interaction of various cell types, Extracellular Matrix (ECM) molecules, and soluble compounds [76]. Moniri M, et al. [77] discovered that, healing, and specifically re-epithelialization, was accelerated if the wound was kept moist. Because of its unique properties, Bacterial cellulose is important for producing wound dressings for burned skin, especially for large skin areas, accelerating tissue regeneration and healing to reduce inflammation.

On the other hand, bacterial cellulose can be used as a novel potential scaffold for the tissue engineering of cartilage, due to its unusual material properties and degradability [78].

Furthermore, Bacterial cellulose presenting in a 3D network structure has low cytotoxicity and excellent mechanical properties and biocompatibility therefore, artificial skin, dental implants, medical pads, and blood vessels are features that make it a biomaterial of choice [79].

Also, due to its previously mentioned properties, Takai M, et al. [80] demonstrated the high potential of BActerial SYnthesized Cellulose (BASYC) as artificial blood vessels and ureters in microsurgery.

Moreover, for tablet modification, a new preparation method of microcrystalline cellulose from *G. xylinus* (BC) and kenaf (KF) is reported [81]. The developed cellulose materials showed different crystalline structures with high crystallinity of (85%). The physical properties of this microcrystalline cellulose were compared with those of the commercially available microcrystalline cellulose Avicel® PH 101. Microcrystalline cellulose exhibited a lower value of loose density than Avicel PH 101 [52]. Both microcrystalline and Avicel PH 101 demonstrated similar behavior during the flow and binding processes.

Other applications of BC include its use as an ultra-filtration membrane [82], as a culture substrate for mammalian cells [83], and as a row material for acoustic speakers and conductive membranes [82].

Also, Ammon HP, et al. [83] studied the improvement of the long- term stability of an Amperometric Glucose Sensor system by introducing a cellulose membrane of bacterial origin.

On the other hand, purified bacterial cellulose can be a raw material for preparation of cellulose derivatives such as carboxy methyl cellulose, hydroxy methyl cellulose, cellulose acetate and methyl cellulose [84]. Also, they proved that, BC appeared to be a good binder in nonwoven fabric-like products [84].

CONCLUSION

In addition to being biodegradable and biocompatible, it also has many advantages over plant cellulose, including a high capacity for absorbing and holding water, purity, high porosity and permeability to gas and liquid, and high crystallinity. These qualities have made this natural polymer an excellent medium to be used for dental grafts, wound dressings, gels, and composites. The main limitation is BC's low productivity, but the antibiofilm efficacy of BC bio-composite has opened up a new avenue for treating infections and chronic wounds caused by biofilms. Because of its unique mechanical properties as well as its antibiofilm efficiency bacterial cellulose will eventually be produced in large quantities, allowing for the safe manufacturing of essential biomaterials for biomedical devices.

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