Current Applied Science and Technology Vol. 23 No. 3 (May-June 2023)

Review article

Bacterial Cellulose: A Multipurpose Biomaterial for Manmade World

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Received: 23 July 2022, Revised: 23 August 2022, Accepted: 8 December 2022

DOI: 10.55003/cast.2022.03.23.014

Keywords

bacterial cellulose;
cellulose synthase;
tissue engineering;
biocompatible;
composites

Abstract

Bacterial cellulose (BC) is a flexible biopolymer having valuable properties like high purity (without hemicellulose and lignin), high percentage of crystallinity, water retention, mechanical strength, biodegradability and unique biocompatibility. Unlike plant cellulose, bacterial cellulose is produced by many bacterial species. Recent advances in research have identified several producers of BC but the key producer is *Komagataeibacter xylinus*. BC produced from *K. xylinus* is known to possess captivating structural, physical, and chemical properties, hence making it a significant natural polymer to be used for future innovative research purposes. This review paper discusses the structural and physicochemical properties of BC; its natural production from bacteria as well as its production under optimized culture conditions. Since the demand for useful composites is high, the involvement of BC in the development of BC-based composites has also been discussed in detail in this paper. This review paper also highlights the diverse applications of BC in the biomedical, electronics, food, textile and pharmaceutical industries. The involvement of BC in the food and pharmaceutical industries can lead to further development of several BC-based super foods and next-generation wound dressings. On the basis of the compiled information in this review paper as well as that in the available literature, future studies should be focused on BC-based drug delivery mechanisms and their performance in *in vivo* and *in vitro* experiments; studies that should help to understand this biopolymer in a meticulous manner.

1. Introduction

Cellulose is the most abundant natural biopolymer widely distributed in the ecosystem. Plants and microbes produced cellulose, which can act as a bio-delivered polymer that has plenty of

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applications in various industries. Cellulose-based outcomes have their exclusive commercial value. Bacterial or microbial cellulose is a polymer, and is a biosynthesis byproduct of many types of bacteria like *Komagataeibacter, Salmonella, Aerobacter, Agrobacterium,* and many others but the major producer of cellulose is *Komagataeibacter xylinus* [1]. The ability to synthesise BC is pervasive among bacteria, but the classical and popularly known cellulose producing species is *K. xylinus*, which is a member of the acetic acid producing bacteria (AAB) group. For several years, the species was also known as *Acetobacter xylinum*, but it was later named as *Gluconacetobacter xylinus*, and then reclassified as *Komagataeibacter xylinus* due to further taxonomic changes. Other AAB species have been found to be strong cellulose producers, including *Komagataeibacter hansenii, K. nataicola, K. obediens, K. rhaeticus, K. saccharivorans, K. medellinensis,* and *K. pomaceti* [1]. An important property of BC produced from AAB is that the cellulose is generally recognized as safe for consumption and thus it is used in food products. BC is a naturally occurring polymer with exceptional biocompatible qualities due to its high level of purity, which results from the absence of lignin and hemicellulose [2]. BC is more than a hundred times thinner than plant cellulose and exists in a three-dimensional (3D) network with increased surface area to volume ratio, making BC a superabsorbent with high level of adaptability. The numerous OH- functional groups make it to be functionalized and applicable in the production of polymer modified composites, and the OH- groups also cause extracellular matrix of cellulose to be wet, which may protect it from UV damage. High water holding ability of BC confers advantage of maintaining the moist environment and improved exchange of nutrients and material, making it an excellent choice for industrial and biomedical applications [3]. BC is used in many industrial fields in a variety of products, including foods, packaging, sealing, advanced BC-based composite materials in the medical sector, and tissue design and engineering [4]. BC is extensively used in the biomedical field where it can be used as a biomaterial for vascular grafts, scaffolds for artificial tissue, artificial skin and accessories, manmade blood vessels and wound protection material. The heavy and extensive uses of BC is expected to increase market attraction. However, long cultivation time, low tendency of production yield, and the thickness of cellulosic layers are major limitations in the standard production of BC, which limited its uses in industrial application. Moreover, considerable amount of culture medium required for standard cultivation of BC can cost as much as 40% of the total cultivation capital cost. Further research on choice of bioreactor, type of fermentation and culture conditions to improve production efficiency, BC yield, large scale production and cost-effective production, needs to be undertaken in detail [4-6].

2. Structure and Properties of Bacterial Cellulose

Cellulose $(C_6H_{10}O_5)_n$ is a carbohydrate polymer made up of interconnected straight chains of D-glucose molecules joined by type (β-*1,4*) glycoside bond linkages that connect with intramolecular and intermolecular hydrogen bonds. The formation of the cellulosic structure starts when two glucose monomers join together and the formation of cellobiose takes place, which can be considered as a structural repetition of the glucose monomers [5]. H- bonds that help to produce fibrous cellulose, which are straight, long and stiff molecules, are structurally able to provide rigidity, mechanical opposition and function as the cellulose congregate which is insoluble in most organic solvent and H_2O [6].

BC and plant-synthesized cellulose have the same chemical composition that is an additive shaped chain structure homopolymer of glucan (glc) monomers or residues with (β-*1,4*) linkages and has the chemical formula $(C_6H_{10}O_5)_n$ (Figure 1). However, BC does not possess the same macromolecular union of chemical composition and properties as plant cellulose [6]. Comparing the

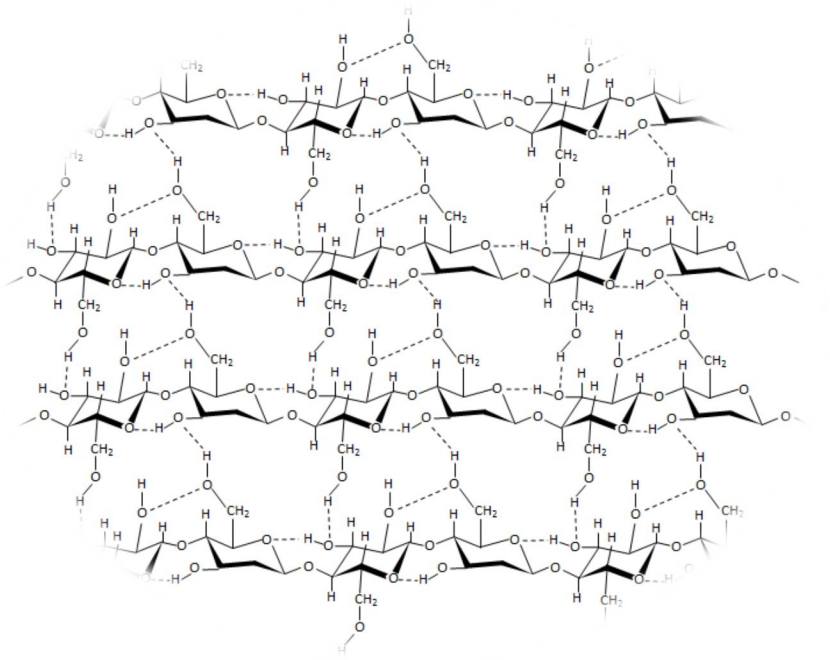

Figure 1. Structure of cellulose polymer [4]

power of polymerization of plant and BC, both plant cellulose (PC) and BC have different ranges of polymerization. PC ranges from 1300-1400 whereas BC ranges 300-1000. BC has the purest form of cellulose, in which glucose residues are attached with glycosidic bonds with stacking chain units rotated at 180^{o}, and the cellulose sheets are strongly connected with both intra and intermolecular H- bonding, which results in a semi-transparent cellulosic structure (Figure 1). The structural density of the semi-transparent cellulose ribbon like structure differs in species with growth medium pathology. For example, BC produced by *K. xylinum* ranges 3-5 × 10^{-6} mm (3-5 nm) in thickness and 70-80 nm in width [7].

BC is the final-product of saturation by oxygen consuming (aerobic) microbes and its end products present as nanofibrous structures (10-50 nm), which are also called biocellulose, and whose filaments are uniaxially arranged with microbands of width of 1-9 nm, making it extra remarkable nano-structure. BC has high degree of polymerization in comparison to plant cellulose, with average degree of polymerization in the range of 14,000-16000. It offers mechanical properties that can be used to improv natural materials and support bio-based composites in a superior way to other reinforcing materials [7].

WHC (water holding capacity), WRR (water release rate), thermal and purity properties, and mechanical properties are the prime physiological features of BC and many characteristics features of BC lead to its broad-spectrum applications (Figure 2) [7]. These impressive properties of BC are connected with common features such as fibrils architecture and the compliance of its permeable framework, which are properties that depend upon the strains used, ingredients of growth medium, modification in cultivation time and conditions, inoculum size, and carbon sources [8]. High level of biocompatibility, hydrophilic nature, high holding capacity of water and slow release rate, makes BC applicable in medical industry as an effective carrier of chemicals that can be used in wound dressing that offers superior healing ability without damaging wound healing process and

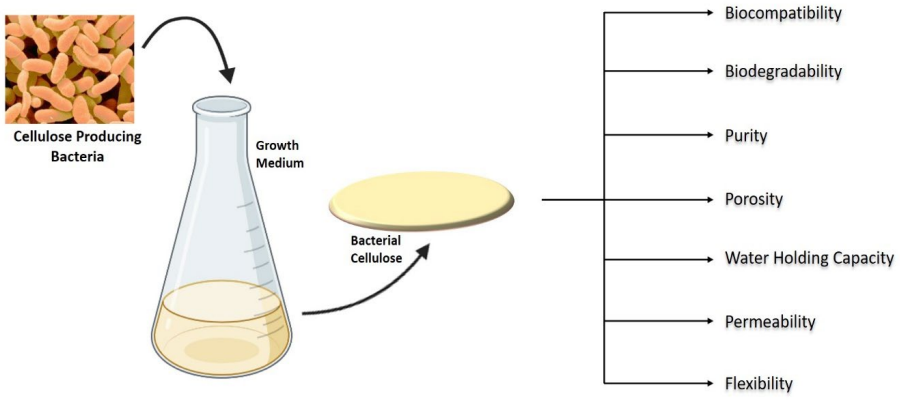

Figure 2. Properties of BC [9]

skin tissues. As per a study, BC can bind approximately 100 to 200 times its dry mass in water. The exceptionally permeability features of BC favor its holding capacity as a permeable grid of cellulose fibers with the help of H-bonds. Any modification in BC, results in variations in physico-mechanical and thermal properties, which is an important feature for its commercial applications [9].

3. Biosynthesis of BC

Many bacterial species are able to biosynthesize cellulose; however, *G. xylinus* or *K. xylinum* is considered to be a novel organism for the study of the biopolymer. BC biosynthesis is a multistep process involving a number of proteins with cellulase synthase being the key protein. The process beings with the polymerization of glucose molecules via interconnection of β-1,4-glucan units, which results in the formation of a chain structure. The cellulose chains then aggregate into a more organized structure called cellulose microfibrils. BC microfibrils are stabilized by intra-and intermolecular forces such as hydrogen bonds, electrostatic interactions and van der waals forces [3, 9]. Cellulose biosynthesized by *K. xylinus* has the extraordinary attributes of multidirectional polarity power and fluctuating thickness [10]. Two types of cellulose form in the crystallization mechanism of the microfibrils in *K. xylinus*: parallel arranged microfibril-based (cellulose I) and antiparallel arranged microfibril-based (cellulose II).

In *K. xylinus*, cellulose biosynthesis universally depends on the oxidation of functional carbohydrates as well as organic acids and metabolic cycle of the pentoses and krebs cycle [11]. Notably, *K. xylinus* is an aerobic microbe, due to the absence of phosphofructokinase-1, which catalyzes the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate. Thus, glycolysis is inhibited and there is a failure to utilize glucose anaerobically [12]. The combination of the metabolic regulation of hexose monophosphate or the pentose phosphate pathway and gluconeogenesis results in synthesis of cellulose [13]. The conversion of cellulose to hexose phosphate is direct and independent of the intermediary divisions of the carbon skeleton. The pathway of cellulose synthesis from glucose is shown in Figure 1, and it begins with the glycolytic intermediate glucose-6-phosphate [14, 15]. The first step is the phosphorylation of glucose (C6) by the enzyme glucokinase, yielding glucose-6-phosphate which is then isomerized in glucose-1-phosphate (G-1-P) by the enzyme phosphoglucomutase (EC 5.4.2.). Glucose-1-phosphate then reacts with UTP, resulting in the formation of uridine-5′-diphosphate-α-D-glucose (UDP-glucose), which is a rate limiting step catalyzed by UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9).

Cellulose synthesis in *K. xylinum* involves UDP-glucose as an intermediate sugar nucleotide precursor [16]. Finally, glucosyl residues are transferred from UDP-glucose to a nascent β-1,4-glucan chain by cellulose synthase. Subsequently, it is polymerized into BC through the BC synthase complex. The cellulose synthase complex, located in the cytoplasmic membrane, is the most important enzyme involved in the cellulose biosynthesis, as shown in Figure 3 [17].

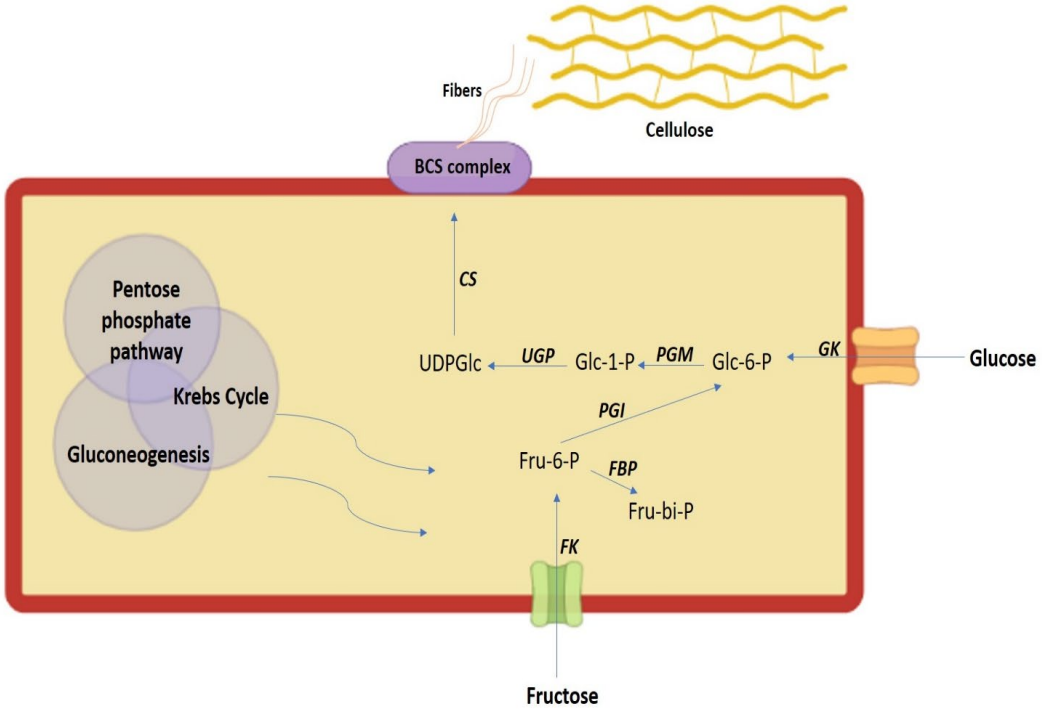

Figure 3. Biosynthetic pathway for BC synthesis (FBP-fructose-1,6-biphosphate, FK-fructose-1-phosphate kinase, GK- glucokinase, PGI-phosphoglucoisomerase, PGM-phosphoglucomutase, CS-cellulose synthase), (Fru-bi-P- fructose-1,6-bi-phosphate, Fru-6-P- fructose-6-phosphate, Glc-6-P-glucose-6-phosphate, Glc-1-P-glucose-1-phosphate, UDPGlc-uridine diphosphate glucose) [17]

4. Cellulose Synthase Machinery of BC Biosynthesis

The BC bio assembly is very complex machinery, and consists in a series of reactions that result in BC synthesis. The cellulose synthase enzyme complex includes subunits that jointly work in a way that synthesizes and exports the beta-glc chains in the outer space. The cellulose synthase enzyme complex uses uridine-diphosphate (UDP-glucose) as a parent molecule and cyclic di-guanosine monophosphate(c-di-GMP) as a regulator molecule and as an important secondary messenger of the biofilm biosynthesis regulation cycle [18]. BC synthase A (*BCSA*) -galactosyltransferase, an enzyme from GT-2 glycosyltransferase family, is the functional subunit of cellulose synthase (CS). It consists of 8 transmembrane regions and two cytoplasmic domains along with the PilZ (C-terminal *BCSA*) domain which facilitates c-di-GMP binding [15-17]. The catalytic subunits of *BCSA*, TM-4 and the TM-5 consist of GT domain. The reducing and nonreducing ends of the cellulose are formed

by the unmodified C1- and C4-hydroxyl groups of cellulose. A nucleophilic substitution process that transfers a glucose unit from UDP-glucose to the polymer's C4-hydroxyl group lengthens cellulose at its nonreducing end and process is facilitated by Mn^{2+} and Mg^{2+} [17]. *BCSA* is closely connected with the subunit *BCSB* periplasmic protein, which is bound to the cytoplasmic membrane by a C-terminal located α-helix, a periplasmic space helix. The convex shaped protein gives rise to two copies of a function domain, a carbohydrate-binding domain and a flavodoxin-like domain. Based on the *BCSA-BCSB* complex architecture, the universal function of the *BCS*B subunit is to help in the translocation of the synthesized glucose chain which is synthesized by the *BCS*A subunit. For most bacteria in AAB group, the cellulose synthase enzyme complex units constitute an additional two *BCS* functional subunits (*BCSC* and *BCSD*) (Figure 4). The *BCS*C is a complex subunit with a C- terminal of ~300 residues (18-stranded β-barrel) placed in the outer most membrane and periplasmic region with an N-terminal that performs the transportation of the BC fibers in the extracellular location (Table 1) [17].

The periplasmic space (N-terminal) incorporates tetra-trico peptide, comprised of 34-units repeats with helix-turn-helix tertiary constructions, which is associated with protein-protein interconnection work, and the *BCS*D protein itself forms a chamber with the presence of a utilitarian complex unit as an octamer in the periplasmic space with a direction corresponding to the long pivot of the cell [17]. The linear terminal complex architecture within the longitudinal axis of the cell is functionalized by *BCS*D. The cellulose synthase (CS) complex of AAB is a combination of *CcpAx, CMCax*, and *BlgAx*, which are interacting accessory proteins that are encoded by the regulatory operon of *BCS* proteins, during BC synthesis [18], meaning that the *CcpAx* accessory functional protein plays an important role in the structure management and management of synthesis of BC. It is a singular domain GH or glycosyl hydrolase family protein, and the N-terminal presents 11 α-helixes and 7 β-strands organized in a 6-barrel structure in the periplasmic space [19].

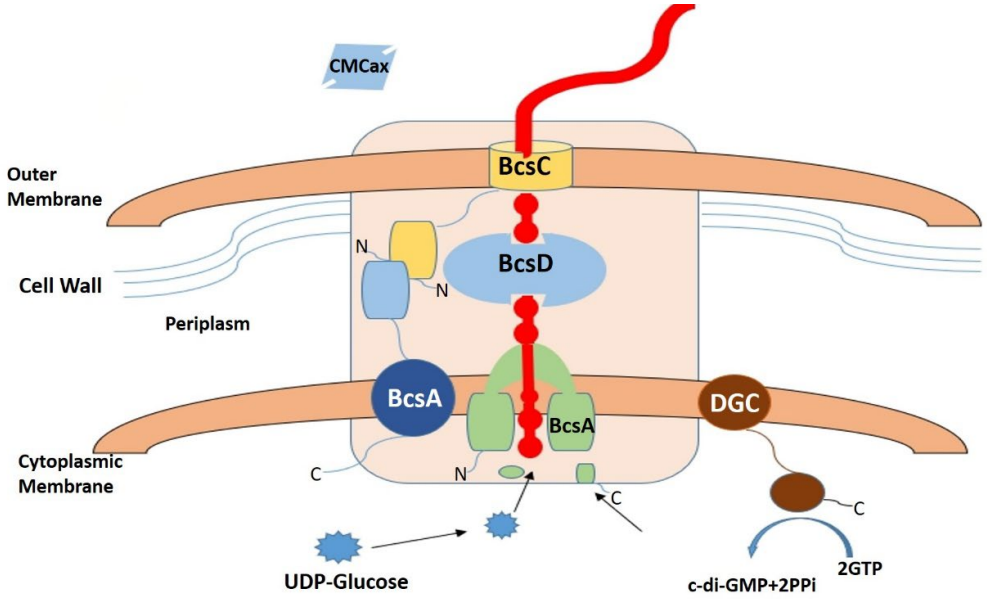

Figure 4. Structure of cellulose synthase complex [18]

Table 1. Products of the core genes of various cellulose synthase operons

Protein type	Length, Amino acid	Function	References
BcsA	750-870	Catalytic activity in *BCS* subunit A (polymerization and translocation)	[15]
BcsB	770-8S00	Catalytic activity in *BCS* subunit B^c (periplasmic activity)	[16, 17]
BcsC	1150	Catalytic activity in *BCS* subunit C (TPR or tetratricopeptide repeat domains)	[17]
BcsD	155	*BCS* subunits arrangement at the cell axis(longitudinally)	[17]
BcsE	500-750	bind c-di-GMP and synthesis regulation	[18]
BcsF	60	Membrane-anchored subunit	[18]
BcsG	550	4 Transmembrane segments and 1 periplasmic AlkP domain	[19]
BcsQ	250	*BCS* subunits cellular localization	[19]
BcsR	65	Likely regulatory subunit	[19]
BcsZ	370	Functional periplasmic subunit, cellulase like activity	[16, 19]

5. Production of BC

The production of BC can be operated under static, agitated or stirred conditions. Different forms of BC, such as irregular shape or spherical cellulose particles of BC, can be produced by different production techniques.

5.1 Static cultivation for cellulose production

The static cultivation method is the simple and most commonly used technique for BC production at the laboratory scale. The culture medium is sterilized and inoculated with the desired microorganism, resulting in BC production, which forms as a floating gelatinous sheet at the liquid-air interface. The cultivation time required for the BC to be produced at optimum yield ranges from 5-15 days. BC production largely depends upon the carbon source composition [20-22]. BC sheets acquire the shape of the containers used for cultivation, and therefore containers of different shapes and sizes are used to obtain BC films [23]. Bacteria become functionally inactive due to low levels of oxygen supply and depleted levels of nutrients during long incubation periods, which are factors limiting BC production [24]. As a result, static cultivation is a very slow and time-consuming process and results in a low production rate of BC. To overcome this issue, semi-continuous or fed-

batch cultivation is recommended to improve yields [20, 25]. The addition of nutrients during cultivation results in two to three times higher yield of BC in fed-batch cultivation than batch cultivation [25].

5.2 Production in shaking culture

Continuous delivery of oxygen to the growth medium enhances the production of BC. BC development can be very slow and continuous. Shaking types of production system make it somewhat faster but the structure or geometry of produced BC differs from the static produced BC. Generally, BC produced by shaking is in the form of irregular pellets. The shape and size of the produced pellets depend upon the rotational speed. Several studies showed that morphology of the BC depends on the agitation speed and can either produce a mass with irregular shape or spherical forms of different diameters. Irregular shape and non-BC producing mutant formation are the two major drawbacks of shaking production [25].

5.3 Agitated culture for producing BC

In agitated condition, production of BC can be in spherical, irregular, fibrous, or pellet shapes; however, the product is inferior to statically cultured product in terms of properties like crystallinity, mechanical strength, and degree of polymerization. Agitation does though facilitate high production of BC because the process fulfils the requirements such as air supply and growth medium in better and more efficient way [26]. BC shaped as stringy suspension, pellets, circles or unpredictable masses forms in an unrestricted at the air-fluid interface since air (O_2) and supplements are used in larger amount for microbial development [26]. The size, shape and amount of BC in agitation fermentation are identified with variables like agitator setup, air (O_2) supply, rotation speed, duration of culture, persistent shear power and added substance types in the growth medium [27, 28]. To improve the efficiency of BC creation, various kinds of bioreactors were utilized Some of these bioreactors produced exceptional returns of BC. To improve the yield of BC creation, different substance compounds were added to the BC synthesis cycle in order to examine the impact on BC yield. These mixtures incorporated ethanol, nutrients, agar, avicel, sodium alginate, gelatin, polyacrylamide, lignosulfonate and microcrystalline cellulose [29]. The examples of BC production and application are presented in Table 2 and Table 3.

Table 2. Bacterial cellulose production by *K. xylinus* from different additives [29].

Types and concentration of additives	Weight of BC(g/l)
0.2 Avicel	2
0.5 Avicel	4.5
0.2 CMC	4.8
0.5 CMC	7.2
0.2 Alginate	0.8
0.5 Alginate	1
0.2 Agar	4

Table 3. Major BC cultivation techniques and their application [30].

Technique	Application	Reference
Static	Blood vessel; vascular grafts	[31]
Agitation	Adsorbent, immobilization gel	[32]
Modified air lift bioreactor	Biomedical, tissue engineering, packaging industry	[32, 33]
Rotating disk bioreactor	loud speaker, fuel cells, packaging industry	[32]
Biofilm support	Artificial muscles and blood vessels, sensors, flexible electrodes and displays	[34]

BC production has improved significantly over the past few years as a result of the integration of contemporary molecular biology methods and various fermentation strategies; however, some areas still need further exploration and investigation. In order to meet the demands of large-scale commercial production, further research focusing on the use of novel and inexpensive wastes as substitute carbon/nitrogen substrates that reduce fermentation costs and times while increasing BC yields need to be done, as does more research into the use of Gram-positive bacteria and extremophiles for BC production in addition to BC made from the widely used Gram-negative strains [35]. In this regard, the use of contemporary gene-editing tools like CRISPR and metagenomics analysis of different strains may broaden the profiles of BC-producers, Furthermore, extensive research should be done to improve the cultivation conditions for BC production, e.g., research on the impact of oxygen tensions, particularly at hyper and hypoxic conditions. Moreover, co-culturing is a recent strategy for increasing yield that needs more research, and the utilization of BC as a green and sustainable biopolymer to address the urgent environmental concerns should be further developed [35].

6. Development of BC- Based Composite Material

In this era of industrialization, requirement of both natural and synthetic composite material with useful features are in huge demand. Composites are the modified material composed of scaffolds and reinforcement material. BC is an outstanding natural polymer used in the development of composites [36].

BC acquires a fibrous ultra-finely arranged structure, unique level of porosity, high degree of crystallinity, power of transparency, biocompatibility and biodegradability, and all these properties together make BC a highly market-oriented material for applications in the medical and other industrial fields [36]. BC-based composites are modified materials with increased degrees of mechanical strength, biocompatibility, biodegradability, antibacterial activity, conductivity, and other structural and effective level magnetic properties compared to normal or naturally occurring BC [37]. The most commonly used method for biopolymer composite production is the mixed cultivation of reinforcement materials to the culture medium or in situ composite production protocols with major reinforcement materials such as chitosan, which is a flavorless food ingredient like gelatin, biodegradable plastics like poly-3-hydroxybutyrate, carbon nanotubes or CNTs, and other solid particles, including clays and silica. The reinforcement materials become well saturated inside the growing BC architecture. The post-synthetic modification of BC with reinforcement materials is a major drawback of this strategy. To overcome these limitations, the *ex situ* composite synthetic strategy was introduced for composite development (Table 4) including BC-based composites with reinforcement materials like chitosan, gelatin, polyethylene glycol, silver and gold nanoparticles, and CNTs [38].

Table 4. Various BC-based composite materials, their properties and applications

Substitute approach	Complex type	Supplement	Upgraded properties	Applications	Reference
Ex-situ solution perforation	Polymer-polymer	Chitosan	Potential in moist injury dressing	Biomedical and Industrial research	[39]
		Gelatin Polyaniline	Improved tensile strength and moisture barrier properties	Biomedical field and conducting elements.	[40]
		Polyethylene glycol	Better tensile strength and crystallinity	Biomedical research	[40, 41]
		Graphene oxide	Drug loading capacity and better cell viability	Biomedical research	[42]
In-situ particle perforation		Collagen	Better physical stability	Biomedical research	[40, 42]
		Aloe Vera gel	Wound dressing	Biomedical and Industrial research	[43]
Ex-situ particle perforation	Polymer-particle	Montmorillonite	Antimicrobial	Biomedical and Industrial research	[43]
Ex-situ particle perforation		Carbon Nano-tubes	Conductivity	Electronic devices, electrode material	[42]
In-situ incorporation		Silica	Structural integrity	Industrial research and development	[43, 44]

The most advanced technique for BC-based composites production is synthesis of BC composites from dissolved BC solution. In this technique, BC is mixed with some unique solvents to produce BC composites, BC with ZnO and TiO_2 were synthesized using this advanced new technique [38]. Some BC-based composites are BC tubes, BC films, BC-membranes, BC sheets impregnated with drugs, BC-chitosan with silver nanoparticles, BC-MMT, BC-hydroxyapatite, and BC-gelatin [39].

7. Applications of Bacterial Cellulose in Different Industrial Fields

7.1 Food industry

Toxicological evaluation of food sources and food substances has emerged as an important and necessary part of the food industry. Consequently, several toxicological examinations for BC ought to be performed before utilization by human. As per standards followed by many national testing organizations, BC is a suitable material for use in the food industry. BC is a naturally low-calorie building element which can be used in fiber enriched utilitarian food varieties in various forms like powder, jelly, or shreds. Some researchers suggested that BC utilized in dietary fiber supplements could efficiently reduce human weight [39].

The thickening and emulsifying properties of BC makes it better material for food industry and finds application in enhancing the quality attribute of variety of food products. BC has also been used as rheology modifying agent in several beverages. In consonance with the ongoing research, BC can keep frozen yoghurt at its consistency for at least an hour after being taken out of the refrigerator. BC can be added as a supplementary ingredient to frozen yoghurt because it lowers the overall number of calories and improves the softening obstruction and textural qualities [39].

In one such study, the nanofibrils extracted from BC were explored as a potential biodegradable material for food coating. It is known that the BC nanofibrils (BCN) show better transparency and homogeneity of particle size when compared to BC. During the study, 2% weight of BCN was coated on fresh cut apples. It was observed that the coated samples showed delayed browning time and weight loss as well as improved firmness [40]. Another study involved the use of BC as well as guar gum-based hydrogel film in food packaging. Guar gum was used mainly to enhance the mechanical strength of the biofilm. The prepared films were used to make biodegradable packets which demonstrated enhanced shelf life of the fruit samples. Moreover, the prepared biofilms showed enhanced load bearing capacities and improved elasticity. In addition to this, the prepared biofilms were 80% biodegradable in vermicomposting, and hence could be used as better and safer alternatives for food product packaging [43]. Besides, the essential oil from cloves was used as an antimicrobial agent to prepare antimicrobial films with an aim to increase shelf life of food products. In this study, a blend of BC and polydroxybutyrate was used to prepare the biofilm. To this film, the essential oil from cloves was added as an antimicrobial agent. The results showed that addition of clove oil was observed to reduce microbial growth by 65%. In addition to this, the mechanical properties of the biofilms were also improved [41].

7.2 Textile industry

The textile industry has a direct influence on the environment due to the use of hazardous chemicals, excessive energy and water use, and improper disposal. Therefore, it is vital to develop ways to reduce the negative environmental, social, and economic repercussions of this business in order to establish a more sustainable situation [42, 45]. Investing in fields like biotechnology and biofabrication that look at alternatives, such the use of microorganisms, for the production of textiles, both for apparel and the footwear sector, is one way to find a solution. Since the biofabrication of BC is a significant green economy concept, it is important to closely examine the sustainability of this process and its impact on downstream processing and finishing. In order to investigate the unique characteristics of BC including its great purity, absence of lignin and hemicellulose, high crystallinity, high polymerization, good flexibility, tensile strength, and nanofibril network structure, several studies were conducted on BC. Resources made from bacteria have primarily been created as high-end leather substitutes [42]. Textile fiber production amounts to 10^6 million tons per year and caters for the worldwide market. The current BC or BNC (bacterial

nanocellulose) worldwide creation is far less than this and limited in scope to creation units. Currently cotton fiber is at the price of 1.40 $/kg, viscose at 1.56 $/ kg and polyester for 1.02 $/ kg. BNC's cultivation costs are for the most part thought to be high, keeping the volume of production from expanding. The significant expense of the growth medium is said to be a fundamental limitation on high-end production of BNC [43].

With growing interest in applications of BC, the biopolymer has been used widely as an active agent in the textile industry. In one such study, the potential of BC membranes was investigated for the filtration properties of waste water from the textile industry. The effect of cultivation time on filtration properties showed positive results. In addition to this, BC was also found to be successful in removal of dye color and COD [43]. In another study, TiO_2 and clay were successfully placed on the surface of BC membranes. New modified BC composites showed improved mechanical strength as well as morphology. TiO_2 was observed to impart plastic like strength properties whereas clay was observed to impart porous membrane properties to modified BC, indicating its potential to become an integral component in the textile industry [43]. Besides this, BC was modified with the addition of antimicrobial agent as well as emulsified acrylated epoxidized soybean oil (AESO). The modified BC composites showed better mechanical strength, wettability as well as thermal and antimicrobial properties. These biocomposites have been widely used in the textile industry because of their modified properties [44].

7.3 Pharmaceutical applications and participation in tissue engineering

The ability of a substance or material system to be therapeutically effective when it is applied to a receiver without leading to a systemic or local adverse reaction is referred to as biocompatibility. Due to its ability to meet the requirements of biocompatible system testing, BC has been designated as a suitable material to make wound dressings. This is explained by its high purity and nanofibrous structure, which permit the host cells to unite and multiply, assisting in the wound. A study was done to ascertain the inherent biocompatibility of BC with various morphologies, such as membranes, pellicles, or pellets, which were exposed to various purification and sterilizing processes [44]. Modification of BC enhanced its use as a wound-healing material in the medical field and in the production of advanced materials with desirable properties, such as good permeability to gases and substances, simple dressing and removal, and infection prevention at a low cost. Table 5 represents the modified versions of bacterial cellulose produced by different companies for varied applications [46-53].

Table 5. Some commercially available BC products of medical use

Product	Company	Reference
DermaFill™	Cellulose Solutions (USA)	[46]
Suprasorb® X	Lohmann & Raucher (Germany)	[47]
SyntheCel®	Synthes (USA)	[48]
BioFill®	Fibrocel Produtos Biotechnologicos (Brazil)	[49]
BioProcess®	Fibrocel Produtos Biotechnologicos (Brazil)	[49]
NexFill®	Fibrocel Produtos Biotechnologicos (Brazil)	[49]
Gengiflex®	Fibrocel Produtos Biotechnologicos (Brazil)	[49]
BASYC®	POLYMET Jena (Germany)	[50]
Securian®	Xylos (USA)	[51]
BioCelltrix	BC genesis (USA)	[52]
CELMAT®	Bowil Biotech (Poland)	[53]

According to recently published literature, there has been more research into the use of BC as a wound dressing with a variety of approaches, although it is uncommon to find studies that test the theory on actual people. Carvalho *et al*. [54] were able to demonstrate that a BC wound dressing, which was applied to venous leg ulcers for 120 days, did not cause any negative effects and decreased the overall depth of the ulcer, indicating that it facilitated the remodelling of the dermal layers without being toxic in comparison to conventional wound dressings. BC's nano-porous matrix was successfully converted to a microporous replica by Ye *et al*. [55]. Gelatin microspheres were used as surface-altering agents and porogens to produce this. Since gelatin is a raw material for the production of the hydrolysis of collagen, it has also been demonstrated to have very minimal cytotoxicity. Due to the fact that gelatin mimics the structure of collagen, which promotes cellular attachment to BC, it has been utilized as a surface modification. The HaCaT keratinocyte model cell line was used to test the cytotoxicity of bacteria-cellulose composite *in vitro*. It was determined that the cells could penetrate and multiply within the modified cellulose matrix's microporous structure, which is vital for tissue regeneration treatments. Similarly, studies using *in vivo* study of C57BL/6 mice with dorsal dermal wounds found that applying BC-gelatin wound dressings improved re-epithelialization with enhanced healing processes and minimal inflammatory cell responses [56].

In one such study, the BC has been modified with the live cells of *Bacillus subtilis*. It was found that immobilization of the live cells onto the BC sheets enhanced the attenuating potential against the pathogens for wound healing like *E.coli, S. aureus, S. epidermidis* and *P. aeruginosa*. The results also proved that the application of modified BC composites resulted in enhanced healing time as well as reparative processes in *in- vivo* models [57]. Similarly, BC was also modified with polylactic acid (PLA) and showed enhanced antimicrobial capability. In this study, the commercial BC sheets were coated with PLA at different concentrations. The coating of PLA proved to exhibit strong antimicrobial activities against *E. coli* and *S. aureus* as well as wound healing properties, hence reducing the dependency on plant based cellulose materials [58]. Another application of BC has also proved its use in wound healing (Table 6). The modified BC was composed of BC fibers that conjugated with tragacanth gum which further enhanced the porous structure as well as the fiber diameter. This further increased the cell adhesion and proliferation during the wound healing process [59].

Table 6. Different BC-based composites with their properties

Composites	Properties	Reference
BC-vaccarin, BC-dextran, BC-collagen, BC-acrylic acid, BC- lidocaine, BC-acrylamide, BC- thymol	Wound healing	[59]
BC/hyaluronic acid/poly (vinyl alcohol) (PVA), BC–heparin–chitosan, BC/gelatin- (VEGF)	Cardiovascular implants	[60, 61]
BC-silicate, BC-doxycycline	Dentistry	[61]
BC-gelatin	Urethral implants	[61]
ϵ-caprolactone/BC, Graphene oxide-BC	Neural implants	[61]
BC-polycaprolactone (PCL)	Artificial cornea and retina	[62]
BC- HA ($Ca_{10}(PO_4)_6(OH)_2$)	Bone regeneration	[62]
BC-poly(3-hydroxybutyrate) (P(3HB)	Cartilage regeneration	[59, 62]
Graphene oxide-BC	Drug delivery	[62]

Numerous BC-based composites such as silk sericin-BC control blood clotting while speeding up the healing of wounds. BC-based composites with polyhexamethylene served as an antimicrobial agent and sped up the application of BC to wounds. BC-HA ($Ca_{10}(PO_4)_6(OH)_2$) and BC-poly(3-hydroxybutyrate) (P(3HB)) composites were used for bone and cartilage regeneration, respectively [59, 62].

The versatility of natural BC helps in the modification of BC into many human friendly products with the properties of biocompatibility that can be used in medical science, such as drug delivery system. Its porosity means it can be loaded with drugs that have antibacterial and anti-cancer properties. Its high-water insolubility percentage, high water-holding power, ultrafine network of cellulose fiber and porosity, permeability, cell adhesion and ease of production into desired shapes makes BC a novel material for drug delivery [60]. Vascular grafts, artificial diaphragm, artificial blood vessels, bones, artificial cornea, dental grafting, neural implants, diagnostic sensors and many other very helpful inventions makes BC more applicable in medical and tissue engineering fields as shown in Table 6. Its interacting characteristics, together with properly mixed composites, make BC a novel material in tissue and biomedical engineering [60]. Impregnation of medicine into BC membranes involves immersion of membranes in the drug solution, typically after lyophilization to ensure maximum drug absorption. Anti-inflammatory medications, such ibuprofen and diclofenac, and antibacterial pharmaceuticals are the most often used drugs to be included into BC. Utilizing tensile strength and water uptake to load the cellulose with antimicrobial substances, such as antibiotics, can increase the effectiveness of BC as a drug delivery material and offers new features and functionalities [60-64].

BC has been demonstrated to be an appropriate starting material for tissue regeneration, a field of biotechnological study currently at the forefront of medical science. As a result of its intracellular matrix, BC makes an excellent scaffold for a variety of cell types. By utilizing the porosity of BC, Roman *et al*. [37] were able to promote cartilage development. To do this, sterile BC was seeded with bovine chondrocytes in Dulbecco's Modified Eagle Medium and allowed to develop for an entire night at 37°C. After SEM analysis, it became clear that the chondrocytes had started to move into the BC matrix, where they began to differentiate and exhibit chondrogenic phenotypes in cartilaginous matrix secretions. Laser perforation was used to create channels that aided cell movement, promoting the seeding of the chondrocytes. Additionally, *in vivo* experiments demonstrated that adding alginate to BC before seeding it with human nasoseptal chondrocytes led to highly stable chondrocyte cell development [65, 66].

Using BC patches rather than the traditional fat or Temporalis fascia graft, Mandour *et al*. [67] developed a novel treatment for tympanic membrane (TM) perforation repair (myringoplasty) and the restoration of the eardrum. BC has previously demonstrated promising re-epithelialization in tissue implantation studies. It was discovered that patients with TM perforation responded well to the BC patches [66-68].

However, considerable scepticism has been expressed because not all outcomes were consistently in line with researcher projections. The handling, manufacture, and storage of BC derivatives have given rise to a number of problems, particularly in the context of biomedical applications. The process of "bioprinting" BC into practical bioactivated membranes produced a phenomenon in which the final printed object had significantly lower tensile strength than anticipated. This was made worse by the challenges associated with autoclaving BC membranes in order to sterilize them for use as tissue grafts and wound dressings. The bioactive components of the BC are typically temperature sensitive and become inactive, making the material inefficient for its intended function even though the BC itself is relatively stable under high heat. Alternative sterilization techniques, like radiation, can address this, but they come with their own set of issues, like the denaturation of proteins and enzymes, which is a concern with sterilization in general [67, 68].

It is evident that using BC as a biomaterial has both benefits and drawbacks, particularly for biomedical applications; the latter must be immediately resolved before BC-based biomaterials may be used often. However, it is evident from global research that there is a greater level of interest in producing biomaterials from bacterial sources than from vegetable sources due to the more affordable synthesis of pure cellulose fibers that can be further modified.

8. Conclusions

Acetobacter xylinum or *K. xylinus* can naturally form thick networks of cellulose that can be sometimes complicated to control in simple lab scale fermentation technology. In addition to advanced cultivation techniques, agitated or shaking and bioreactor bioprocess methods were developed for the large-scale production of BC. These methods can control the structure and properties of BC. Using the integrated approach based on genetic engineering, and metabolic and bioprocess engineering, BC can be produced with unique physiochemical properties, and at high production rates. The biosynthesis of BC by potential cellulose producers is a multistep process regulated by the complex of the cellulose synthase enzyme. Because of its special attributes and versatility, BC is useful in a variety of industrial sectors, including the food and textile industries, as well as biosensors, pharmaceuticals, tissue engineering, and other important sectors. Many BC oriented products are commercially available with high revenue. Future research in this area must concentrate on enhancing the yield of BC and expanding the scope of BC as a green sustainable natural polymer for various industrial applications. It is a substance that should have many applications in the overcoming of environmental challenges.

9. Acknowledgements

The authors are grateful to the Department of Biotechnology, Jaypee Institute of Information Technology, Noida, Uttar Pradesh, India for providing necessary facilities to execute this work.

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