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4

Synthesis, Structure, and Properties of Bacterial Cellulose

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4.1 Introduction

Bacterial cellulose (BC) is produced by several microbial genera belonging to *Acetobacter*, *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia*, and *Sarcina* [1, 2], and a cell-free system [3–6]. It represents the purest form of cellulose compared to plant cellulose, which contains impurities in the form of lignin and hemicellulose. It possesses unique features such as high water holding capacity (WHC), slow water release rate (WRR), greater tensile properties, high crystallinity, better thermal and mechanical properties, ultrafine fiber network, hydrophilicity, polyfunctionality, transparency, nontoxicity, and moldability into three-dimensional structures [7–9]. These features make BC a preferred choice than plant cellulose for various applications such as carrier in drug delivery systems and enzyme immobilization and two-dimensional (2D) and three-dimensional (3D) scaffolds for tissue engineering, wound dressing materials, artificial skin burns, vascular grafts, tissue regeneration, artificial blood vessels, biological films, and biosensors [10–14]. However, its wide range applications are limited by several inadequacies associated with it, such as the lack of antibacterial, antifungal, antioxidant, conducting, and magnetic properties. Nevertheless, BC has the potential to form composites with different materials ranging from organic to inorganic metals, nanoparticles, and biocompatible polymers, which not only improve its existing properties but also impart it additional features [8, 9].

During BC production, the microbial cells polymerize various carbon sources into single liner β-1,4-glucan chains that protrude out through the pores located on the cell membrane, termed as terminal complexes (TCs) [5, 15]. The successively synthesized β-1,4-glucan chains get assembled in the culture medium

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and form protofibrils, which further crystallize into micro- and macro-fibrils, bundles, and ultimately form ribbons-like structures [16]. A single ribbon is composed of about 1000 individual β-1,4-glucan chains [8]. Ultimately, BC is produced in the form of a thick gelatinous membrane at the air–medium interface in a static cultivation [5]. The unique physiological, chemical, mechanical, thermal, and biological properties of BC can be attributed to its unique structural features. BC fibers possess a high aspect ratio with a diameter of 20–100 nm and a high surface area, which confers it a high materials adsorption capability. Electron microscopy of BC demonstrates a well-distributed 3D structure of nanofibers with a high surface area and porosity [17]. These nanofibers are interconnected through inter- and intramolecular hydrogen bonding that stabilizes the reticulate structure of BC and are 100 times smaller than those of plant cellulose [18]. The nanofibers are loosely arranged and contain empty spaces in the form of pores, which increase the overall surface area endowing BC with a highly porous matrix [19]. Any structural variation in BC affects its physico-mechanical and thermal properties: for example, strong, stable, and compact fibers with high density offer better resistance to the applied force while a highly porous structure bestows it with a high WHC, usually 100–200 times more than its dry weight in water [20, 21], and can accommodate different types of materials, thus showing the potential to form composites [22]. These structural variations in BC are attributed to the type of microbial strain, composition of culture medium, variation in culture time and conditions, amount of inoculum, and carbon source [23, 24].

An economically feasible production has always been the main aim of BC research; therefore, several strategies have been developed for improved BC production such as conventional fermentation approaches, genetic engineering strategies, and strain improvement approaches, among others [25]. However, BC production from chemically defined medium is very expensive; thus, researchers are exploring cheap media sources in addition to developing new strategies for cost-effective, efficient, and high yields of BC. This includes the use of various carbon and nitrogen sources, industrial wastes, isolation of high rate production strains, in situ pH control, controlled side product formation, and supplementation with additional substrates [26–28]. However, high yield of BC is overshadowed by several discrepancies associated with microbial production due to the utilization of a large fraction of medium components for growth and proliferation, formation of by-products, formation of negative strain, and risk of contamination, thus making the process ineffective for large-scale production [29–31]. Further, the benefits of whole-cell fermentation are overshadowed by inhibition of microbial cell growth and viability due to membrane fluidity [32]. Together, these factors limit the large-scale production of BC. A few of these limitations can be potentially overcome by using thermo-tolerant microbial strains and immobilized microbial cell systems, which offer improved stability and resistance, low production cost, and high-purity products [33]. Further, the limitations of whole-cell fermentation can be overcome by using a cell-free system for BC production, which offers improved yield [4]. Additionally, this system produces BC with better structural and physico-mechanical features [5] and offers a one-pot in situ composite synthesis method using bactericidal

materials, which are otherwise likely to kill the microbial cells [34]. Therefore, a major objective behind BC research is to improve its production by improving the efficacy of the process, development of advanced fermenters and cultivation strategies, exploring cheap media sources, and supplementation of medium with other components for improved structural features.

This chapter overviews the biogenesis of BC at biochemical and molecular levels and details its various structural, physico-mechanical, and biological features. It further describes various cultivation strategies for BC production with different shapes, sizes, quantity, quality, and physico-mechanical properties. The role of different additives in BC production medium and their effects on various structural features are discussed. Finally, the efforts of different researchers to explore the potential of different raw materials for low-cost BC production are summarized.

4.2 Biogenesis of Bacterial Cellulose

Biosynthesis of BC is a complex process that is regulated by a large number of specific enzymes and regulatory proteins. It is an aerobic process and is directly linked with cellular catabolism; however, it does not interfere with other anabolic processes such as protein synthesis in bacterial cells. In addition to aerobic synthesis of BC by microbial cells, it is also produced anaerobically by a cell-free system using cellulose-producing enzymes and cofactors [4]. The synthesis mechanism of uridine-di-phosphoglucose (UDP-glucose) is well understood; however, this process of BC synthesis involving glucose polymerization is yet to be completely explored.

4.2.1 Biochemistry of BC Synthesis

Sutherland described BC synthesis by microbial cells as a four-step process involving (i) activation of monosaccharides through formation of glucose nucleotides, (ii) polymerization of glucose repeating units through their sequential addition, (iii) simultaneous addition of acyl groups (if present) to individual glucose units, and (iv) excretion of cellulose fibers through the wall/membrane complex into the extracellular environment [35]. BC synthesis involves the production of UDP-glucose, which serves as a precursor for BC synthesis. This process is followed by the polymerization of individual glucose units to form β-1→4 glucan chains. Individual chains are excreted through TCs and form ribbon-like structures composed of hundreds and thousands of individual chains. These ribbon-like structures in turn form fibrils [35]. Each biochemical step in BC synthesis pathway is regulated by the availability of specific enzymes, substrates, and a cofactor (if required), which otherwise results in the abolishment of the metabolic pathway. The synthesis of cellulose fibrils in microbial cells, their transport across TCs into extracellular environment, and the formation of bundles and ribbons are shown in Figure 4.1.

During the first step of BC synthesis, sugar nucleotides provide monosaccharides by means of interconversion through epimerization, dehydrogenation, and

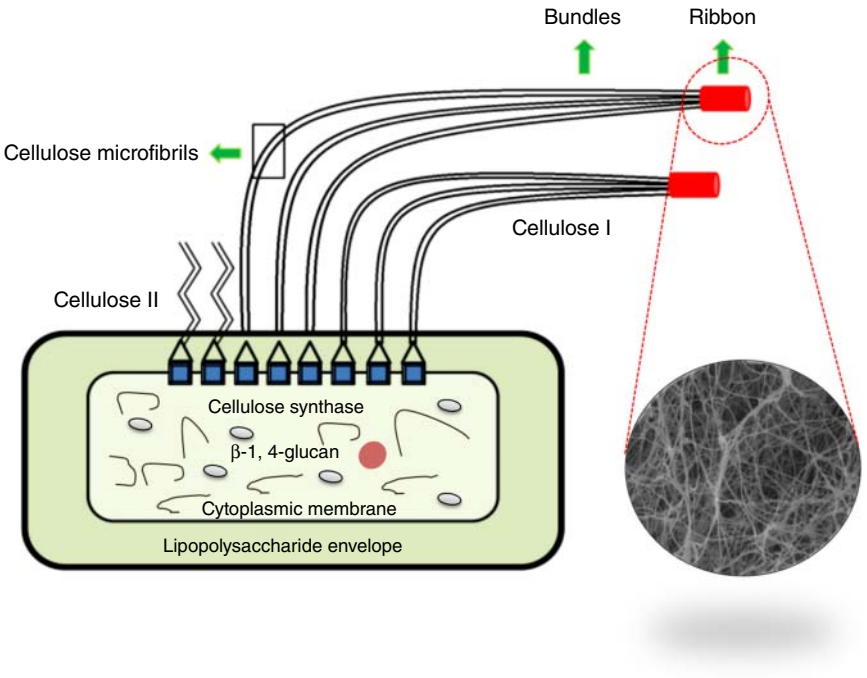

Figure 4.1 Illustration of formation of cellulose chains in microbial cells, their secretion across the cell wall through TCs, and formation of micro- and macro fibrils, bundles, and ribbons. Source: Ul-Islam et al. 2015 [14]. Reproduced with permission of Springer.

decarboxylation reactions, each of which is catalyzed by specific enzymes [35]. The cellulose backbone is formed in the second step through the sequential addition of D-glucose-1-phosphate and UDP-glucose; this process is carried out by UDP-glucose pyrophosphorylase and cellulose synthase, respectively. It is followed by the acylation of cellulose during which the acyl group is transferred to form acetyl Co-A; the process is catalyzed by specific sugar transferases such as 1-acyl-*sn*-glycerol-3-phosphate acyltransferase. The acetyl Co-A formed here serves as a precursor for the tricarboxylic acid (TCA) cycle. It is worth mentioning here that the structure of BC is determined by the sequential transfer of glucose monomers and acyl groups from their respective donors, regulated by highly specific sugar transferases. In the last step, β-1→4 glucan chains produced inside the cytoplasm are crystallized in or near the outer membrane and extruded across the cell membrane through TCs arranged in an orderly manner to the extracellular environment in an energy-dependent process regulated by cellular adenosine triphosphates (ATPs) [35, 36]. This demonstrates that a well-organized system must be present on the microbial cell surface to ensure efficient excretion of cellulose fibrils. Compared to microbial cell systems, a cell-free system bypasses the complicated process of β-1,4-glucan chain extrusion to the extracellular environment since it does not contain any barrier in the form of a cell wall or a membrane [4]. Further, it bypasses the energy utilization and internalization of cellulose within the periplasm, thus avoiding any lethal effects.

4.2.2 Biochemical Pathway of BC Production

Liquid chromatography–mass spectrometry/mass spectrometry linear trap quadrupole (LC–MS/MS LTQ) Orbitrap analysis of the crude cell-free lysate of *G. hansenii* PJK indicated the presence of several key enzymes involved in the cellulose metabolism (Table 4.1). Herein, the glucokinase, phosphoglucomutase, UDP-glucose pyrophosphorylase, and cellulose synthase are directly involved in BC synthesis (Figure 4.2). Likewise, glucose-6-phosphate (G6P) serves as a common intermediate for the principal cellulose synthesis pathway, pentose phosphate pathway (PPP), and TCA cycle. The flux of phosphorylated glucose through the two possible routes determines the BC synthesis level. UDP-glucose also serves as a common substrate for UDP-glucose dehydrogenase and cellulose synthase. Again, the flux of UDP-glucose through the two possible routes determines the BC synthesis level in either a microbial or a cell-free system. A lower concentration of nicotinamide adenine dinucleotide (NAD) in the cell-free lysate may partially inhibit the UDP-glucose dehydrogenase activity, and thus,

Table 4.1 Illustration of the *G. hansenii* PJK enzymes in the cell-free extract involved in bio-cellulose synthesis. The enzymes were analyzed by LC–MS/MS LTQ Orbitrap using the Mascot algorithm.

Accession no.	GI no.	Description	Taxonomy	Mass (Da)	PI
EFG85049.1	gi|295978312	Glucokinase	*G. hansenii*	34 282	6.51
EFG84192.1	gi|295977434	Phosphoglucomutase	*G. hansenii*	59 762	6.03
EFG85649.1	gi|295978924	UDP-glucose pyrophosphorylase	*G. hansenii*	22 755	4.36
EFG83224.1	gi|295976446	Cellulose synthase catalytic subunit (UDP-forming)	*G. hansenii*	175 801	6.94
EFG84542.1	gi|295977790	UDP-D-glucose dehydrogenase	*G. hansenii*	47 825	5.61
EFG83101.1	gi|295976316	1-Acyl-*sn*-glycerol-3-phosphate acyltransferase	*G. hansenii*	41 281	11.15
EFG83324.1	gi|295976549	Diguanylate cyclase/ phosphodiesterase	*G. hansenii*	84 871	6.11
EFG83841.1	gi|295977078	Glucose dehydrogenase	*G. hansenii*	84 604	5.73
WP003620002.1	gi|489715879	Aldolase	*G. hansenii*	29 824	6.42
EFG84043.1	gi|295977283	Triosephosphate isomerase	*G. hansenii*	25 791	4.96
EFG82935.1	gi|295976147	Pyruvate dehydrogenase (acetyl-transferring)	*G. hansenii*	34 832	5.35
EFG85628.1	gi|295978903	Glucose-6-phosphate dehydrogenase	*G. hansenii*	57 043	6.04

Source: Ullah et al. 2015 [4]. Reproduced with permission of Elsevier.

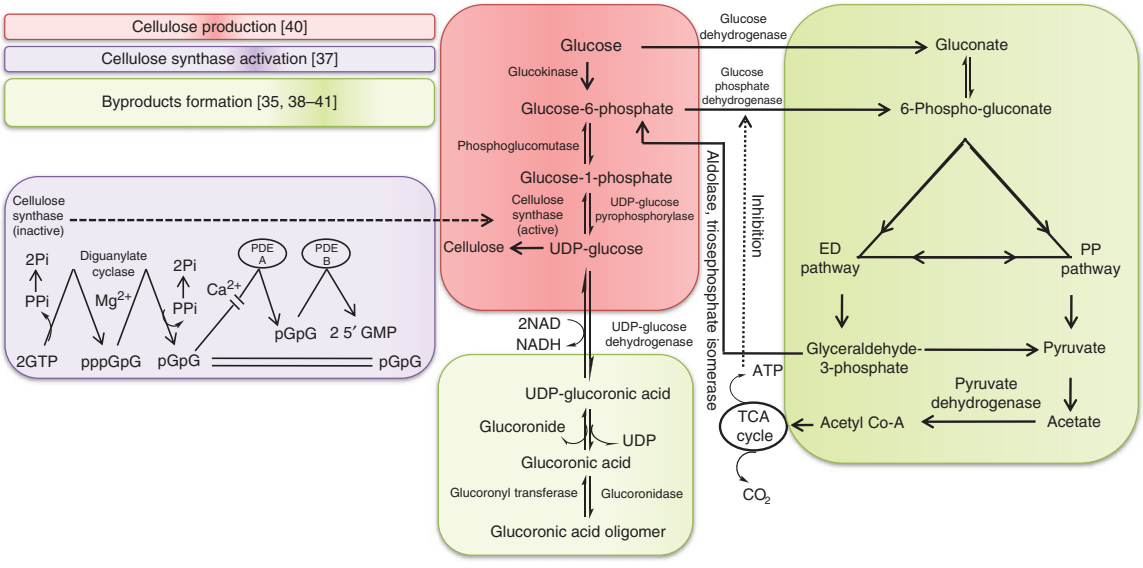

Figure 4.2 Schematic representation of bio-cellulose production by the cell-free system through the principal glucose pathway, and other pathways interconnected through the activation of cellulose synthase. The scheme was developed based on literature review and the results of LC-MS/MS LTQ Orbitrap analysis. Source: Ullah et al. 2015 [4]. Reproduced with permission of Elsevier.

may favor high level of cellulose synthesis (Figure 4.2). Importantly, the presence of diguanylic cyclase and two phosphordiesterases (PDE-A and PDE-B) serves to regulate cellulose synthase activity through a specific activator bi-(3′→5′)-cyclic diguanylic acid. Notably, PDE-A and PDE-B are involved in the synthesis and degradation of the activator, respectively [37]. It may aid in accelerating the polymerization of UDP-glucose into cellulose. Similarly, glucose can also enter other pathways in the microbial metabolism [38]. A small portion of triose sugar produced by the Entner Doudorouff (ED) and PP pathways can be converted into pyruvate [42], which may subsequently be converted into acetyl-CoA by pyruvate dehydrogenase (Table 4.1, Figure 4.2). Acetyl-CoA serves to produce bulk ATPs to enhance the efficacy of BC synthesis [39]. Generally, a high concentration of ATP (Table 4.1) inhibits G6P dehydrogenase, and hence, reduces its activity by several folds. Thus, it may retard the PP pathway and favor BC synthesis through the principal pathway in either a microbial or a cell-free system (Figure 4.2).

4.2.3 Molecular Regulation of BC Synthesis

BC production and its extrusion to the extracellular environment is a complex process that is readily effected by several mutations in the genes regulating the enzymes involved in its synthesis. Any mutations in key genes lead to the accumulation of cellulose in the periplasm with inevitable lethal effects [35].

BC production by microbial cells is regulated by at least four different genes, *axcess A*, *axcess B*, *axcess C*, and *axcess D,* which are organized in the form of an operon (Figure 4.3) and encode specific proteins, AxCESA, AxCESB, AxCESC, and AxCESD, respectively, which perform specific functions [15]. Among these, AxCESA and AxCESB catalyze and regulate the polymerization of individual β-1,4-glucan chains. Specifically, AxCESA contains amino acid motifs suggestive of processive β-glycosyltransferase and binds the UDP-glucose [43]. Similarly, the AxCES subunit reversibly binds large quantities of cyclic di-guanosine monophosphate (c-di-GMP), which in turn activates AxCESB [15, 44]. During this process, c-di-GMP is released and auto-inhibits the state of enzyme by breaking the salt bridge, which otherwise tethers a conserved gating loop that controls the access to and substrate coordination at the active site. The disruption of salt bridge by mutagenesis generates a constitutively active cellulose synthase. It has also been demonstrated that the c-di-GMP-activated BcsA-B complex contains a nascent cellulose polymer whose terminal glucose unit resides at a new location above BcsA's active site where it is positioned for catalysis. The other two subunits AxCESC and AxCESD have been proposed to mediate extrusion of β-1,4-glucan chains and crystallization of cellulose sub-elementary fibrils (SEF), respectively, and are considered to be the rate-limiting steps during cellulose assembly [45, 46]. AxCESD is reported to be a cylinder-shaped protein formed by an octamer as a functional unit [47]. All N-termini of the octamer are positioned inside the AxCeSD cylinder and create four passageways from which four β-1,4-glucan chains are extruded individually along the dimer interface in a twisted manner to the extracellular environment. This suggests that AxCESD protein resides at the extracellular side of the TC. These four subunits and perhaps other components from the cellulose-producing complexes can be viewed

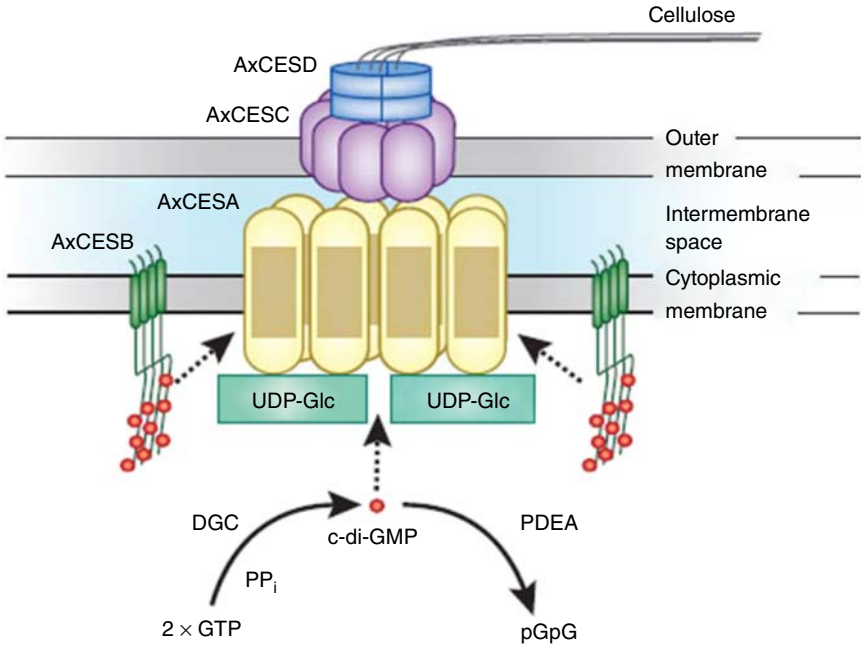

Figure 4.3 Cellulose synthesis in *A. xylinum*. AxCESA, with 8 to 10 putative transmembrane regions (TMDs), is located in the cytoplasmic membrane and binds to UDP-Glc. Free c-di-GMP allosterically activates the AxCESA. The majority of the cellular c-di-GMP is bound to AxCESB, which is in close proximity to AxCESA. The diguanylate cyclase (DGC) catalyzes the synthesis of c-di-GMP, whereas phosphodiesterase A (PDEA) degrades the molecule. AxCESC is located in the outer membrane, whereas AxCESD might be located in the intermembrane space or extracellularly. Source: Endler et al. 2010 [15]. Reproduced with permission of Nature Publishing Group.

as pores (i.e. TCs) at the cell surface. These complexes assist the extrusion of the nascent β-1,4-glucan chains that aggregate to form the twisted SEF, which later crystallize into ribbons. This unique molecular configuration gives microbial cellulose different features compared to plant cellulose, such as high crystallinity and lower degree of polymerization despite its chemical structure being similar to that of plant cellulose.

4.3 Structure and Exciting Features of Bacterial Cellulose

BC has received immense consideration due to its purity, unique structural, physicochemical, mechanical, and biological properties. These unique features and its potential to form composites with a wide range of materials, including biocompatible polymers, bactericidal elements, and conducting materials, provide BC with a high potential to find broad spectrum applications in different areas. The following sections describe the exciting features of BC.

4.3.1 Chemical Structure and Properties

BC possesses the same chemical structure as plant cellulose and is a linear homopolymer of glucose monomers linked by β-(1→4) glycosidic linkage with the chemical formula $(C_6H_{10}O_5)_n$. However, it possesses different macromolecular structure and properties than plant cellulose. The two successive monomers in BC are linked in such a way that the former glucose unit is rotated at 180° with reference to the preceding. BC represents the purest form of cellulose; however, its degree of polymerization only ranges between 2000 and 6000 compared to plant cellulose, which lies in the range of 13 000–14 000 [48]. The repeated glucose monomers in BC form a continuous long unbranched polymer chain. Several cellulose chains in BC are held together through strong intra- and intermolecular hydrogen bonds that form a sheet. BC is produced at the air–medium interface in static cultivation where its shape is maintained by the hydrophilic interactions [5, 49]. The crystalline structure of BC results from the hydrogen bonding between the cellulose sheets. The thickness of cellulose fibers varies from species to species beside the culture conditions. For example, BC fibers produced by *Gluconacetobacter xylinum* consist of microfibril ribbons that are 3–4 nm thick and 70–80 nm wide.

Chemical structure analysis by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) demonstrated slight variations in BC produced by different methods (e.g. static and shaking cultivation) and obtained from different sources (e.g. microbial and the cell-free system) [5, 50]. Structural analysis by NMR revealed that cellulose produced by microbial and cell-free systems demonstrated cellulose I and cellulose II polymorphic structures, respectively. X-ray diffraction (XRD) analysis of cellulose produced by microbial and the cell-free systems demonstrated cellulose I and cellulose II polymorphic structures, respectively [5]. Surface analysis of BC by scanning electron microscopy (SEM) reveals random distribution of fibers while the cross section shows layers of clustered fibers [19]. CP/MAS ^{13}C NMR spectroscopy, wide-angle X-ray diffractometry, and transmission electron microscopy (TEM) have been used to study the solid-phase nitration and acetylation of BC. The relative reactivity of OH groups in BC was found to be in the order of 6′OH > 2′OH > 3′OH [51].

4.3.2 Physiological Features

The broad spectrum applications of BC are highly dependent on its physiological features such as WHC, WRR, thermal properties, and mechanical features. These properties of BC are associated with its structural features including the arrangement of fibrils and the conformation of porous matrix, which are in turn associated with the microbial strain, synthesis method, chemical composition of culture medium, variation in culture time and conditions, amount of inoculum, and carbon source [23, 24].

Appropriate moisture content is required for a wound dressing material for the effective adsorption of bioactive substances into the BC matrix, which in turn offers several advantages, including easy and painless wound dressing, accelerated wound healing, and prevention of any damage to the newly formed skin

tissues [6, 24]. Therefore, owing to the high WHC and slow WRR in addition to its hydrophilic nature, BC has received immense consideration as a wound dressing material. According to an estimate, BC can accommodate 100–200 times its dry weight in water [20, 21]. Highly porous BC favors a high WHC where the water molecules remain within the porous matrix by binding to the cellulose fibrils via hydrogen bonding [52]. Any structural variations in BC account for its altered physico-mechanical and thermal properties. For example, the fiber density is improved by prolonged BC production through supplementation of an additional carbon source or by extending its availability, which allows the newly formed β-1,4-glucan chains to be continuously added to the preexisting fibrils [6]. These strong and stable fibrils account for improved resistance to the applied force [53]. Further, a compact structure also imparts thermal stability to BC, which is an important feature for its commercial applications.

4.3.3 Self-assembly and Crystallization

The unique structural and physico-mechanical features of BC are attributed to the extrusion and self-assembly of β-1,4-glucan chains in the extracellular environment. The synthesized fibrils after extrusion through TCs subsequently form 2–4 nm protofibrils, bundles, and finally 80 × 4 nm ribbon-shaped microfibrils [54].

Electron micrographs of cell envelope revealed the presence of about 50–80 pore-like TCs along the axis of a microbial cell and in combination with the extracellular ribbon [15]. These TCs are assumed to be the sites for the extrusion of β-1,4-glucan chains, which form the initial assembly of cellulose in the form of aggregates. The formation of such aggregates suggests that simultaneous synthesis of several β-1,4-glucan chains is a common feature of the assembly of cellulose microfibrils in both higher and lower organisms [55]. This mutual orientation and association of β-1,4-glucan chains, their aggregation, and formation of microfibrils, bundles, and ribbons are governed by the original pattern of TCs while the process of self-assembly and crystallization of cellulose is cell directed [56]. Specifically, *G. xylinum* synthesizes two distinct physical forms of cellulose, the ribbon-like cellulose I and the thermodynamically more stable amorphous cellulose II [57]. The β-1,4-glucan chains of cellulose I are aligned in parallel and arranged uniaxially whereas those of cellulose II are arranged in a random manner. The microfibrillar arrangements of fibers and their extreme purity compared to plant cellulose mainly contribute to the exciting and unique features of BC such as greater tensile strength, better crystallinity, high WHC, slow WRR rate, and improved thermal stability.

4.3.4 Ultrafine Thin Fibrous Structure

Compared to plant cellulose, pure BC possesses a 100 times thin ultrafine fibrous structure that bestows it with unique features and provides the base for its broad spectrum applications [18, 49]. Its fibers are well distributed and form a reticulate web-shaped structure, giving a firm appearance to BC. This arrangement of fibers accounts for higher crystallinity, better mechanical features, and high

thermal properties of BC. Further, this arrangement of fibers plays a vital role in the development of BC-based composites with different types of materials for various biomedical and optoelectronic applications [58, 59].

4.3.5 Macrostructure Control and Orientation

The structural features of BC are exciting both in dry (powder or sheet) and wet (hydrogel) forms and their applications vary accordingly [60]. The physical structure of BC is flexible enough to be controlled at the macro, micro, and nano scales. Further, BC can be produced in different shapes (sheets, pellets, or granules) by varying the culture conditions (static, shaking, or agitation) and microbial strains [58]. Further, several of its characteristic features such as surface chemistry, porosity, and fiber arrangement and orientation can also be controlled at the micro, macro, and nano scales. This microstructure control offers advantages for its broad-spectrum applications [8, 61].

In nature, BC demonstrates anisotropic patterns in the culturing plane. The movement of microbial strains in the culture medium accounts for the randomness of BC fibers. The arrangement of fibers can be controlled by controlling the movement of microbial cells. For example, Uraki et al. obtained a honeycomb pattern of BC by using honeycomb-patterned microgrooves in an agarose film [62]. In another study, Wang et al. controlled microbial cell movement by using microfluidic channels and successfully obtained aligned BC fibers [63]. Shi et al. used a magnetic field to control the movement of *G. xylinum* for the production of patterned BC fibers [64]. Despite the natural random behavior of BC fibers, its overall structure is quite compact and mechanically strong. BC can achieve comparable isotropic alignment and prove useful for a wide range of applications.

4.3.6 Porosity and Materials Absorption Potential of BC for Composite Synthesis

BC is porous in nature and its level of porosity varies to a great extent depending upon its synthesis method and pre- and post-synthesis treatment processes. Its porous geometry provides an ideal environment for the adsorption of different types of materials such as solid particles and liquids, including water, cells, solutions, medium components, wound exudates, nanoparticles, and polymer solutions. The small natural porosity of BC is advantageous in that it prevents the invasion of microbial cells; however, it is a limitation from the medical perspective as it prevents the penetration of larger particles and mammalian cells deep into its matrix [65].

Currently, efforts have been devoted toward the development of artificially porous BC by using different materials such as salts, paraffin particles, ice crystals, gelatin, and various sugars, collectively termed as "porogens." A porogen is any material that is first incorporated as a space holder, followed by its removal in a careful manner to avoid any damage or interference to the geometry of the pores formed [61, 66]. Porogens are adsorbed only physically and not chemically into the BC matrix for different time intervals depending upon the objectives of the study. The sizes and shapes of the porogens are also varied according to the

requirements of the culturing cells or other materials to be incorporated into the BC matrix. Bäckdahl et al. reported the use of potato starch and paraffin wax particles as porogens for the development of BC tubes containing pores of various sizes, which were interconnected with each other. Further, they also effectively controlled the pore size and created partial particle fusion through heat treatment of initial paraffin wax particles at specific temperatures. The structures formed effectively supported the growth of smooth muscle cells inside the pores [61].

4.3.7 Biocompatibility

Biocompatibility of BC refers to its ability to remain in contact with the living tissue without causing any toxic or allergic side effects [14, 67]. Biocompatibility of BC is directly linked with its higher water and other liquid absorbing capabilities to support the sustained growth and proliferation of living cells and tissues. To date, BC has been reported to show moderate to high levels of biocompatible behavior toward different types of mammalian cells and tissues [18, 68, 69]. For example, in vivo study in rate by implanting BC subcutaneously has shown biocompatibility up to 12 weeks. Microscopic observation demonstrated the complete absence of any fibrotic capsule or giant cells, indicating no foreign body reaction. Further, implantation of BC avoided any redness, swelling, and formation or accumulation of exudates around the BC implant [70]. In a recent study, Khan et al. reported the fabrication of highly biocompatible microporous BC scaffold for skin tissue regeneration application, which demonstrated in vitro adhesion and proliferation of human keratinocytes (HaCaT) and complete regeneration of skin tissues within two weeks in experimental mice [71]. However, the biocompatibility of BC in certain cases is not up to the desired levels and thus requires further improvement. One possible solution is to form composites with other biocompatible materials to improve its biocompatibility. For example, development of composites with various materials has been reported to enhance the biocompatibility, cell adhesion, and proliferation for wound dressing application and development of scaffold in tissue engineering [72, 73].

4.3.8 Biodegradability

The potentials of biodegradable polymers have long been recognized [74]. A biomaterial must be biodegradable: it must be capable of eliciting an appropriate host response in a specific application. This response can be described in terms of chemical, physical, and biological properties of the materials to the shape and structure of the implant. Biodegradation is an exciting and rather the most difficult feature of BC to meet the requirements for various biomedical applications. Difficulty in biodegradation of BC is attributed to its high degree of crystallinity and compact structure. Lack of cellulase enzyme in mammalian cells or tissues necessitates the development of alternative strategies for degradation of BC implants. To this end, Li et al. reported an enhanced degradation of BC in vivo through periodate oxidation, which showed improved degradation in

water, phosphate buffered saline (PBS), and simulated body fluid (SBC) without disturbing its original fibrous network [75].

4.4 Production of Bacterial Cellulose: Synthesis Approaches

The chemically defined medium for BC production contains several components including yeast extract, glucose, peptone, disodium phosphate, and citric acid. BC production is carried out in acidic conditions (pH 5–6); however, variation in other growth conditions such as pH, temperature, and carbon source and its concentration optimizing BC production eventually affect both its quality and quantity. In addition, different cultivation methods lead to the production of BC with different structures and properties [76]. Therefore, efforts have been made to develop a low-cost and efficient method for BC production with high yield and improved properties. The following sections overview the commonly used fermentation strategies including static, shaking, and agitation fermentation for BC production with different structural and physiological properties and yields [77].

4.4.1 Static Fermentative Cultivation: Production of BC Membrane, Film, or Sheet

Static fermentative cultivation is the most commonly used method for BC production. During static cultivation, the cellulose-producing microbial cells are inoculated into the culture medium at optimum pH and incubated at appropriate temperatures. Undisturbed incubation is carried out for 5–10 days during which the microbial cells grow and proliferate by partly utilizing the available carbon source and other medium components and produce BC. In static cultivation, BC is produced across the surface at the air–medium interface as an assembly of reticulated crystalline ribbons that forms a gel and ultimately a membrane, film, or sheet with increasing cultivation time [5, 78]. The BC pellicle grows downward until all microbial cells are entrapped inside the pellicle and become inactive or die due to depletion of nutrients or oxygen deficit [58].

Most of the BC used for biomedical applications is obtained by static cultivation because it forms a sheet with 3D interconnected reticulate structure, which makes it suitable for preparing different types of scaffolds [77, 79]. Unfortunately, the low yield and longer incubation time of the static cultivation approach limits its large-scale production and hence restricts its commercial applications. Several efforts have been made to overcome the limitations associated with static fermentative cultivation. For example, Jung et al. developed a novel technique for static cultivation that utilized an intermittent feeding strategy for BC production. Since the BC pellicles become separated from the culture medium due to depletion of nutrients and limited oxygen availability in static cultivation, the thickness of BC produced after 7 and 10 days cultivation is generally around 2 mm. However, using intermittent feeding, the thickness of BC produced after 30 days of cultivation was significantly increased to 30 mm using the same culture conditions [31].

4.4.2 Shaking Fermentative Cultivation: Production of BC Pellets

Shaking fermentative cultivation refers to BC production in an incubator containing a rotor or shaker that can be easily differentiated from agitation, which is the production of BC in a reactor that offers controlled speed [78]. During shaking cultivation, the cellulose-producing microbial cells are inoculated into the culture medium at optimum pH and incubated at appropriate temperature under shaking conditions. Shaking is generally measured in terms of revolutions per minute (rpm). This type of cultivation is generally carried out for 24–36 hours during which microbial cells grow and proliferate by partly utilizing the available carbon source and other medium components and produce BC. In shaking cultivation, BC is produced within the culture medium in the form of small pellets. The shape of the pellet varies according to the type of microbial strain, incubation period, and shaking speed. A study by Hu and Catchmark reported that the shape of the BC pellet was sphere-like when the shaking speed was kept at 125 rpm. With further increase in the shaking speed, the pellets exhibited a tail-like feature that appeared to grow in size as the particle size decreased or the shaking speed increased. At further high rotational speeds, the tail-like BC pellets were found interconnected with each other [80].

4.4.3 Agitation Fermentative Cultivation: Production of BC Granules

In agitation cultivation, BC is produced in the form of granules. A comprehensive model for BC production by agitation cultivation has been developed by optimizing various factors such as bioreaction (substrate consumption and product formation), transport of oxygen and carbon sources, and removal of cellulose fibrils. BC production by agitation cultivation takes place at a much faster rate compared to static and shaking fermentative cultivation. The high cell density and better contact with available oxygen result in very high volumetric productivity in agitated cultures. Further, the improved productivity can also be correlated to the high density of microbial cells. The fermenters offer controlled pH, temperature, supply of nutrients, availability of oxygen, and prevent the formation of foam.

Despite the high productivity of BC by agitation cultivation, there are several limitations associated with such cultivation strategies. For example, high supply of power is required, which usually produces cellulose pellets instead of pellicles under submerged and aerated cultivation, which limits its application spectrum [79–81]. Moreover, BC produced through agitated cultivation possesses a lower degree of polymerization, mechanical strength, and crystallinity than those produced in static or shaking cultivations. Sometimes, irregular or sphere-like cellulose particles (SCPs) are also produced due to agitation or stirring [77], which are less crystalline, possess low mechanical strength, and demonstrate low degree of polymerization [82]. Further, the BC granules cannot be utilized directly for biomedical and several other applications. Furthermore, continuous agitation converts the cellulose-producing microbial strains into more enriched cellulose-negative (Cel^-) mutants, which grow much faster compared to the wild type and result in lower BC production [83].

Several modified reactors such as rotating disk bioreactor, cylindrical silicone membrane vessel, and a direct oxygen and glucose feeding reactor have been developed to improve microbial cellulose production (Figure 4.4). Some of these are briefly described in the following sections.

4.4.3.1 Rotating Disk Reactor

This is a designed bioreactor that enhances BC production by ensuring that one half of its disk remains inside the culture medium while the other half is present at the air surface. During BC production, the cells present on the surface of the disk are able to obtain the nutrients when they come in direct contact with the medium and are exposed to oxygen in the atmosphere [84]. Previous reports indicate that using horizontal fermenters with rotating discs effectively improved the culture conditions and BC production. *G. xylinum* ATCC 700178 were able to attach on the plastic composite surface (PCS) and increased BC production (0.24 g/l/day) in a 5-day cultivation while retaining its important structural features [85, 86].

4.4.3.2 Trickling Bed Reactor

Generally, this kind of reactor is used for vinegar production and is composed of inlets that help in air circulation. In such a reactor, the fermentation liquid from the collection reservoir is circulated to the top of the tank by a pump until the desired product is obtained. Such a reactor has also been successfully employed for BC production. For this purpose, cellulose-producing microbial cells such as *G. xylinum* are adsorbed on to packings such as husks of rice or corncobs and exposed to the fermentation medium and air space. The oxygen supply to the fermenter further enhances the capability of the system. This cultivation method increases the provision of oxygen and hence reduces the sheer force, which in turn increases BC production [87].

In such reactors, BC films grow among gaps of husk or are fixed to the surface of the corncobs. The presence of plenty of packings in the reactor provides a larger surface to volume ratio, which enhances the oxygen supply to an adequate level; thus bacteria can easily attach and grow in the semisolid and liquid–solid microenvironments [87]. BC produced by trickling bed reactors appeared to be irregular sheet films with different thicknesses ranging between 1 and 5 mm. BC produced by such reactors possesses better degree of polymerization, purity, WHC, porosity, and thermal stability compared to that produced in static and shaking cultivations. Thus, BC produced by trickling reactors paves the way for various industrial applications.

4.5 Additives to Enhance BC Production

The main idea behind the supplementation of culture medium with additives in BC production is to improve its morphology in one way or another. For this purpose, the effect of different additives has been evaluated for improved BC production with enhanced features. The following sections describe a few tested additives.

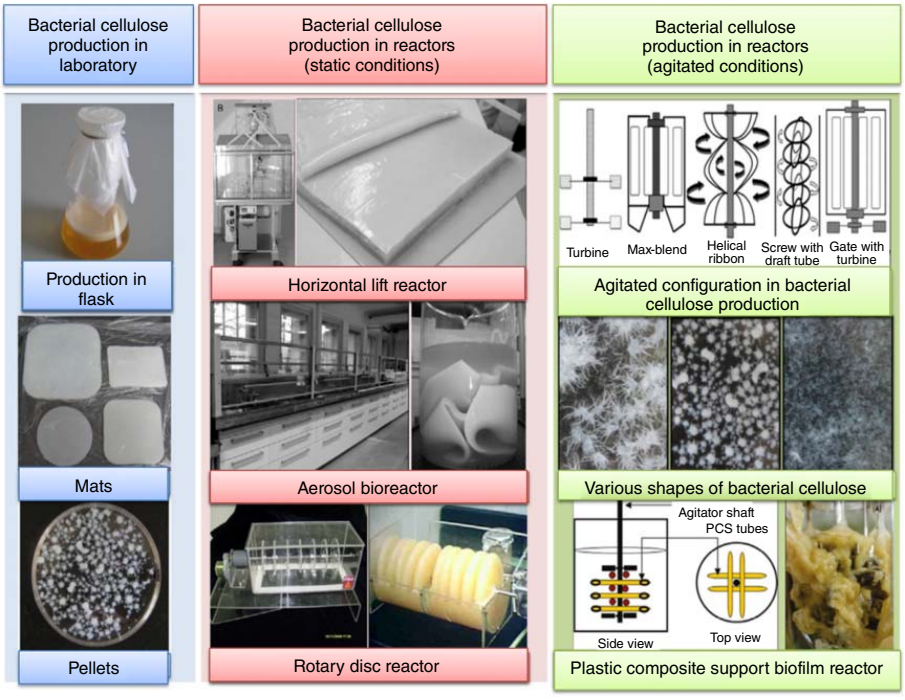

Figure 4.4 Various bioreactors designed for enhanced production and productivity of BC. Source: Ul-Islam et al. 2015 [14]. Reproduced with permission of Springer.

4.5.1 Carboxymethylcellulose

Addition of carboxymethylcellulose (CMC) to the culture medium significantly improves BC production. Chang et al. reported that the addition of 1% CMC increased BC production from 1.3 to 8.2 g/l. Besides the yield, its addition to the culture medium also increased the crystallinity and crystal size of the produced BC by attaching onto the cellulose microfibrils during the fermentation process. Moreover, the addition of CMC-supplemented BC retained its fibrous structure and accounted for enhanced WHC. However, the mechanical strength of CMC-supplemented BC was reduced to a significant level [88]. Another study showed that the supplementation of 1.5% CMC into the *G. xylinum* culture enhanced BC production by 1.7 folds; however, the crystallinity of CMC-modified BC was much lower, with a much smaller pore size compared to pristine BC [85]. The addition of CMC increases the viscosity of the culture medium, which lowers the shear stress to the microbial cells. It was also reported that BC pellicles are transformed into pellet when modified by CMC in a biofilm reactor [85]. Carboxymethylated BC (CM-BC) is used for biomedical applications as a hemostatic material and has been established to enhance its functionality among minor cuts and less in larger wounds due to its solubility in water with a high degree of substitution.

4.5.2 Organic Acids

The effect of different organic acids as additive on BC production has been investigated. It was noted that oxalic acid and tartaric acid inhibit BC synthesis. For example, BC production was 0.16 and 0.17 g/l, respectively upon the addition of oxalic and tartaric acids, which were lower than those achieved without the addition of any acid to the culture medium (1.48 g/l). In contrast, BC production levels with malic acid, pyruvic acid, and citric acid were found to be 2.83, 2.34, and 2.27 g/l, respectively, which were higher than for the ethanol-supplemented sample (1.93 g/l). Similarly, the addition of lactic acid, acetic acid, and succinic acid to the culture media significantly improved BC production to 1.67, 1.85, and 1.49 g/l, respectively. All this could be due to a synergistic effect on BC production; therefore, the accumulation of oxalic acid and tartaric acid resulted in low BC production while citric and acetic acid accumulation improved its production (Figure 4.5) [89].

4.5.3 Vitamin C

The addition of vitamin C to Hestrin and Schramm (HS) medium for BC production resulted in lowering the crystallinity of BC due to decreased inter-hydrogen bonding between cellulose sheets because it interferes with the cellulose planes. The resulting interference could be attributed to high water solubility, low molecular weight, and less steric hindrance of vitamin C. However, BC production was increased in the presence of vitamin C and reached 0.47/30 ml compared to 0.25/30 ml in its absence. Being an antioxidant, vitamin C decreased the production of gluconic acid produced by *G. xylinus* during BC production. Thus, the addition of vitamin C resulted in overall increased production of BC [94].

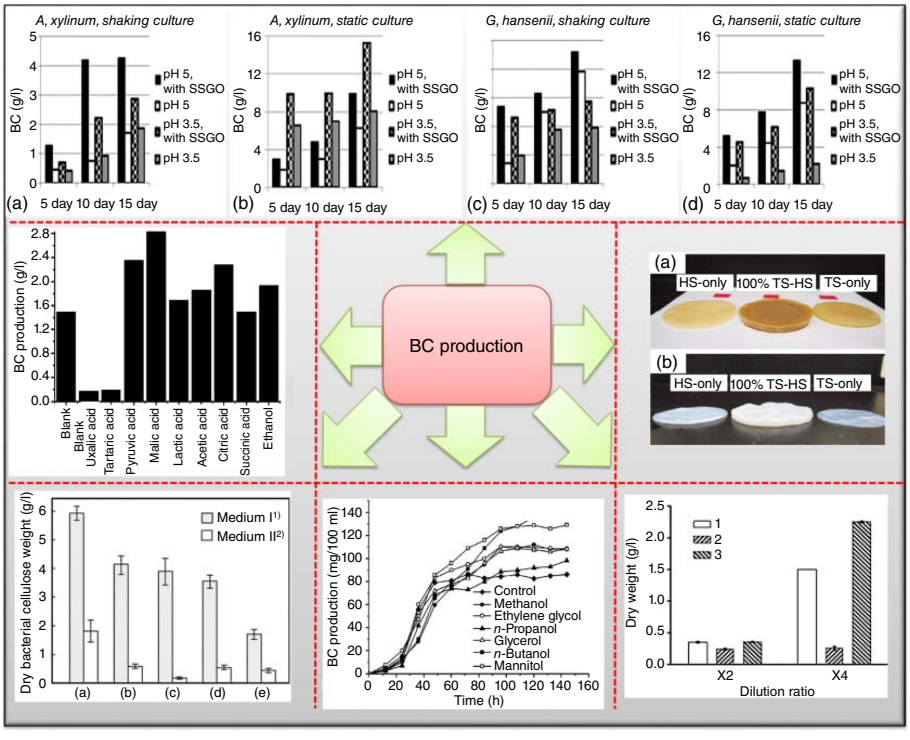

Figure 4.5 Effect of different additives on BC production. Figure modified from Refs. [27, 89–93].

4.5.4 Sodium Alginate

Sodium alginate is an anionic polysaccharide made up of a linear unbranched copolymer of 1,4-linked β-d-mannuronate (M) and α-d-guluronate (G) and is mainly extracted from a brown marine algae [95]. It offers favorable characteristic features such as nontoxicity, gelled structure, transparency, biocompatibility, and biodegradability [96]. The addition of sodium alginate to the BC culture medium significantly improves BC production. Zhou et al. reported that the addition of 0.04% (w/v) sodium alginate enhanced BC production from 3.7 to 6.0 g/l by *G. xylinum* NUST4.1 in a stirred-tank reactor. Moreover, it changed the morphology of BC from irregular clumps and fibrous masses entangled in the internals to discrete masses dispersed in the broth. This demonstrates that sodium alginate functions to prevent the formation of large BC clumps and improves its production. Moreover, sodium alginate forms hydrogen bonds with BC, as confirmed by structural morphology (SEM) and chemical structure analysis (FTIR). However, the incorporation of sodium alginate to the BC matrix lowers its crystallinity and crystal size [97].

4.5.5 Alcohols

Ethanol, on addition to the BC production medium, functions as an energy source for ATP generation at the early stage of fermentation [98, 99]. Further, it reduces glycerol production in the hexose monophosphate pathway, thereby improving overall BC production [100]. Investigations on the addition of different types of alcohols to the culture medium showed improved BC production to different levels. For example, the addition of 1.0% (v/v) methanol to the HS medium accounted for 21.8% improved BC production compared to the control group. Similarly, the addition of 0.5% ethylene glycol to the culture medium resulted in 24.1% improved BC production compared to the control group. A 13.4% improvement in BC production was observed when 0.5% of *n*-propanol was added to the culture medium. Further, the addition of 3.0% glycerol accounted for 27.4% improved BC production compared to the control group. Similarly, the addition of 0.5% *n*-butanol and 4.0% of mannitol to the culture media accounted for 56.0% and 47.3% improved BC production, respectively, compared to the control group. These observations suggest that the addition of alcohol seems to have a stimulatory effect at the later stage of fermentation, which enhanced BC production [90] (Figure 4.5).

4.5.6 SSGO

The production of BC follows a biosynthetic pathway that does not completely convert glucose into BC but also forms oligomers. A study by Ha et al. reported that the addition of single sugar-linked glucuronic acid-based oligosaccharide (SSGO) into the culture medium inhibits the glucuronic acid oligomers synthesis during BC production where these also serve as alternative source of glucose [38]. BC produced by the addition of SSGO showed improved mechanical properties; for instance, the fibrils became thicker and denser, which

further accounted for improved crystallinity [49]. In another study, Ha and Park reported that the addition of 1.0% of SSGO to the chemically defined medium resulted in increased BC production up to 89.3% and 52.3% by *G. xylinum* and *G. hansenii* after 15 days of cultivation under static condition. Therefore, it is definite that the addition of SSGO to the culture medium successfully improves BC production [91] (Figure 4.5).

4.5.7 Lignosulfate

Lignosulfonate is an antioxidant containing polyphenolic compounds. Keshk and Sameshima reported that the addition of lignosulfate to the HS medium resulted in lowering the production of gluconic acid, which is commonly produced in the hexose monophosphate pathway of BC production and led to overall increased BC production. Further, the addition of lignosulfonate to the culture medium enhanced the crystallinity of BC. These findings can be justified by the presence of hydrogen bonding in cellulose, which is not affected by the lignosulfonate, and therefore does not interfere with cellulose planes due to its high water solubility and steric hindrance [101].

4.5.8 Agar and Xanthan

Studies have shown that the addition of both agar and xanthan as additives to the culture media resulted in increased BC production. Microbial strains producing BC are always sensitive to shear force; therefore, it should be minimized to the lowest possible level for efficient BC production. Agar has been identified as one of the components that can be added to the culture medium to lower the sheer force for yield of BC. Agar as a polymeric compound tends to increase the viscosity of the culture medium, which in turn improves the yield of BC. The effect of addition of agar into the culture medium on BC production was further enhanced when agar concentration was increased to 0.4%. Thus, it can be concluded that soluble viscosity-inducing materials such as agar have rather more effect on BC productivity due to its mass production; therefore, such soluble polymers are recommended for enhanced BC production [102]. Chao et al. showed that the addition of 0.1% (w/v) agar and 0.06% (w/v) xanthan to the culture medium increased BC production from 6.3 to 8.7 g/l and 7.2 g/l, respectively, using *G. xylinum* in batch cultivation with an airlift reactor. BC was produced in the form of pellets, with the size decreasing as productivity of BC increased, indicating that an increase in relative viscosity with the addition of polysaccharides hindered the formation of large clumps [103].

4.5.9 Thin Stillage

Thin stillage (TS) is a wastewater from rice wine distillery, which has been used as a supplement to the traditional HS medium for enhanced BC production because it is rich in organic acid. The addition of TS (100% TS–HS medium) enhanced BC production by 3.4-folds to 10.38 g/l, which is about 2.5-folds higher than that obtained in HS-only medium. This could be attributed to the reduction

of sugar consumption rate by *G. xylinus*, leading to higher BC production. Use of TS as a supplement is also a way of reducing the pollution caused by wine industry and employing cheap materials for enhanced BC production [92] (Table 4.2, Figure 4.5).

4.6 Strategies Toward Low-Cost BC Production

The different chemically defined media used for BC production are generally very expensive. A typical BC production medium containing glucose as the carbon source and other nutrient sources increases the overall BC production cost, thus limiting its use in value-added applications. Therefore, finding a low-cost medium for BC production has always been the target of research right from its discovery. The use of raw and cheap carbon sources is one such strategy that can lower the overall BC production cost [26, 27]. The following sections summarize a few low-cost BC production media (Figure 4.6).

4.6.1 Fruit Juices

Fruits are used as a routine food. However, these usually have short shelf-life and often become rotten if not consumed in time. When bad quality fruits are not shipped or processed in time, these must be essentially discarded, which results in wastes. However, fruits are known to contain a large amount of simple sugars, especially fructose, glucose, and sucrose, which can be used for the production of useful products, in addition to being used as a carbon source in BC production [27]. It has been reported that various types of sugars are present in different levels in various fruit juices; for example, sucrose is found highest in orange and pineapple juices while grape, apple, and Japanese pear juices contain high amounts of fructose. Various juices were reportedly utilized as media for production of BC by *G. xylinum* NBRC 13693. Orange and Japanese pear juices yielded the highest BC production, thus proving to be the most suitable medium for BC production. The BC yield could be further enhanced by adding nitrogen to the fruit juices [27].

4.6.2 Sugarcane Molasses

Molasses is a low-cost sugar industrial product that is often used in microbial fermentation. It is mainly composed of glucose, fructose, and sucrose sugars, which are readily biodegradable. Besides, it contains some nitrogen and vitamins, which can stimulate BC production by microbial cells. Molasses is pretreated by two different methods: chemical and physical. Chemically treated molasses usually gives a high BC yield compared to the physically treated molasses. However, the production cost of physically treated molasses is much lower than that of chemically treated molasses because of the low concentration. A dense fibrils-web was clearly seen during the molasses medium production compared with HS and M1A05P5 media [115]. Pretreatment of molasses with acid helps in the production of high quantity of sugars such as fructose, glucose, and sucrose, which could aid in BC production. It further helps in the removal of coloring materials and

Table 4.2 Production of BC in static and agitation cultures with a variety of BC-producing strains, carbon sources, and supplementary materials.

Microorganism	Carbon source	Supplementary materials	Culture time (days)	Yield (g/l)	Cultivation mode	References
G. xylinus, Trichoderma reesei	Glucose	Fiber sludge	14	6.23	Static	[104]
G. xylinus	Glucose	Cellulosic fabrics	14	10.80	Static	[105]
G. medellensis	Glucose	None	14	4.50	Static	[106]
G. hansenii PJK (KCTC 10505 BP)	Glucose	SSGO	10	7.4	Static	[38]
G. xylinus (PTCC, 1734)	Glucose	Date syrup	14	40.35	Static	[107]
G. persimmonis (GH-2)	Glucose	Fructose, beef extract	14	5.14	Static	[108]
G. xylinus (ATCC 53524)	Sucrose	None	4	3.83	Static	[109]
G. hansenii PJK (KCTC 10505 BP)	Waste from beer culture	None	14	8.6	Static	[110]
G. xylinus (K3)	Mannitol	Green tea	7	3.34	Static	[111]
G. xylinus (IFO 13773)	Sugar cane molasses	None	7	5.76	Static	[101]
G. xylinum (ATCC 700178)	CSL-Fru	Carboxymethyl cellulose	5	13.00	Agitated	[88]
G. xylinus	CSL-Fru	Sodium alginate, agar, CMC	5	7.05	Agitated	[85]
Gluconacetobacter sp. (RKY5)	Glycerol	None	6	5.63	Agitated	[112]
G. xylinus (BPR2001)	Molasses	None	3	7.80	Agitated	[113]
G. xylinus (BPR2001)	Fructose	Agar/oxygen	3	14.10	Agitated	[114]
G. hansenii PJK (KCTC 10505 BP)	Glucose	Ethanol	3	2.50	Agitated	[28]

Source: Wang et al. 1991 [42]. Reproduced with permission of Elsevier.

Figure 4.6 Cost-effective production of BC from various waste sources including (a) fruit juices, (b) brewery waste, (c) sugarcane molasses, (d) agricultural and food wastes, and (e) waste from beer fermentation broth.

heavy metals in molasses and enhances BC production. Sulfuric acid pretreated molasses yielded high BC production with relatively better mechanical properties compared to the one obtained from glucose [116]. Khattak et al. reported BC production from sugarcane jaggery (gurr), obtained from brown sugar industry and identified to contain a high quantity of glucose. The BC produced from sugarcane jaggery demonstrated better structural properties and possessed high mechanical and thermal properties. Moreover, the BC produced showed better biocompatibility properties and supported the adhesion and growth of skin cells, which demonstrates its usefulness in medical application [117].

4.6.3 Agricultural and Industrial Wastes

Most of the organic wastes from agro industries are available but are never used due to their poor quality although these are a rich source of carbon such

as glucose, fructose, and sucrose. These waste materials can be effectively utilized for BC production. Such waste materials cannot only serve as a cheap carbon source but can also prove to be an environment cleaning step [118]. For example, wastewater from candied Jujube (WWCJ) processing industry as a low-cost and an economical product was studied as agro-industrial waste for BC production in fruit-producing areas in China. The wastewater contained low level of glucose, glucan, and other carbohydrates. Therefore, hydrolysis by acid treatment was performed to increase the glucose concentration, which increased to 58% compared to unhydrolyzed WWCJ. The pretreated medium produced BC with improved yield (0.375 g/l/d) after 6 days of cultivation, although with a much lower crystallinity [93]. In another study, a high yield of BC was produced by *K. sucrofermentans* DSM 15973 when fermented in the presence of by-product streams from oil seed-based biodiesel industries and waste streams from confectionery industries as the sole source of nutrients [119]. A carbon source for production of BC by *G. sacchari* was employed by using a residue of olive oil production industry, which also proved to be a good source that showed improved BC production [120]. This concludes that most of the agro-industrial wastes provide all the nutrients required for bacterial growth and BC production, mostly with improved properties.

4.6.4 Food Wastes

Most of the flour-rich waste (FRW) streams produced in the manufacturing industries (bread and confectionery industries) contain high levels of starch, proteins, and micronutrients, which are useful in the fermentation processes for production of other industrial products, thus reducing environmental pollution [119]. Most of the available food wastes used for BC production are fruit peels such as rinds or skins of various foods. The use of such food wastes as substrates for BC production helps in reducing the production cost and hence proves very economical. BC production by using pineapple (PA) and watermelon (WM) peels as culture media was analyzed and compared with the HS medium. The wet weight of BC obtained from pineapple medium was highest (i.e. 12.5 g/100 ml) as compared to watermelon medium (i.e. 10 g/100 ml) and HS medium (3 g/100 ml). The fibers in BC produced by pineapple peel medium (PA-BC) were highly disordered, showing an irregular arrangement. Similarly, the ribbons were shorter in length for WM-BC and PA-BC and longer and uniform in HS-BC. In contrast, the BC fibers produced by PA were thicker compared to those produced by WM and HS after seven days. The texture for PA-BC was hard, adhesive, and cohesive followed by WM-BC, and lowest for HS-BC [121]. In another study, food wastes of rotten fruits and whey milk were evaluated for BC production, which showed high BC production among all the other culture media used due to the high percentage of carbon in the rotten fruits. BC production by whey milk was limited by the presence of lactose, which is not favorable for BC production and thus results in lowering the overall yield. In contrast, the culture medium containing rotten fruits blended with whey milk accounted for improved bacterial cell growth and BC production

due to the presence of rich nutrients. This demonstrates that a combination of different culture media of different food wastes can be useful for improved BC production. These findings show that food waste medium can be a potential substrate for low-cost BC production for different applications [122]. Besides, the recycling of food wastes helps in reducing environmental pollution and increasing ecological awareness.

4.7 Conclusions and Future Prospects

BC is an important natural biopolymer produced by various microbial strains and a cell-free system. It possesses unique structural and physicochemical, mechanical, and biological features, which make it an attractive material for various applications. Different methods of synthesis of BC greatly affect its yield and structural and physico-mechanical properties. The broad spectrum applications of BC have been limited by its low yield and high production cost, mainly due to the expensive medium components. Therefore, extensive efforts have been made to minimize BC production cost including (i) the development of novel production strategies (static, shaking, and agitation cultivation), (ii) designing of new bioreactors, (iii) replacement of common carbon sources with new and cheaper sources, (iv) exploration of novel and cheap waste sources, (v) discovery of new BC-producing microbial species and genetic modification of known bacterial strains, and (vi) the production of BC through cell-free systems, which ideally convert all available substrate to product. BC yield and its properties have been significantly improved by adding different supplements to the culture medium. The utilization of various wastes as nutrient sources has significantly reduced the overall production cost.

Efforts are required to further increase the BC yield and explore and utilize cheap media sources. A mixture of different waste materials from different sources could lead to improved productivity. This can be an important aspect of future research for improved BC production with better characteristic features for various applications. Development of advanced low-cost BC production strategies will open the gateways for industrial-scale applications. Nevertheless, the most important future aspects of BC research are to identify cheap, easily available, and renewable media resources and microbial strains for cost-effective BC production. With increased BC production, we can expect the development of new useful BC-based materials in the near future.

Acknowledgment

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References

1 Seo, C., Lee, H.W., Suresh, A. et al. (2014). Improvement of fermentative production of exopolysaccharides from *Aureobasidium pullulans* under various conditions. *Korean Journal of Chemical Engineering* 31 (8): 1433–1437.

2 Shoda, M. and Sugano, Y. (2005). Recent advances in bacterial cellulose production. *Biotechnology and Bioprocess Engineering* 10 (1): 1–8.

3 Ullah, M.W., Ul Islam, M., Khan, S. et al. (2017). Recent advancements in bioreactions of cellular and cell-free systems: a study of bacterial cellulose as a model. *Korean Journal of Chemical Engineering* 34 (6): 1591–1599.

4 Ullah, M.W., Ul-Islam, M., Khan, S. et al. (2015). Innovative production of bio-cellulose using a cell-free system derived from a single cell line. *Carbohydrate Polymers* 132: 286–294.

5 Ullah, M.W., Ul-Islam, M., Khan, S. et al. (2016). Structural and physico-mechanical characterization of bio-cellulose produced by a cell-free system. *Carbohydrate Polymers* 136: 908–916.

6 Ullah, M.W., Khattak, W.A., Ul-Islam, M. et al. (2016). Metabolic engineering of synthetic cell-free systems: strategies and applications. *Biochemical Engineering Journal* 105: 391–405.

7 Di, Z., Shi, Z., Ullah, M.W. et al. (2017). A transparent wound dressing based on bacterial cellulose whisker and poly(2-hydroxyethyl methacrylate). *International Journal of Biological Macromolecules* 105: 638–644.

8 Czaja, W., Krystynowicz, A., Bielecki, S., and Brown, R.M. (2006). Microbial cellulose – the natural power to heal wounds. *Biomaterials* 27 (2): 145–151.

9 Li, H.X., Kim, S.J., Lee, Y.W. et al. (2011). Determination of the stoichiometry and critical oxygen tension in the production culture of bacterial cellulose using saccharified food wastes. *Korean Journal of Chemical Engineering* 28 (12): 2306–2311.

10 Khan, S., Ul-Islam, M., Ullah, M.W. et al. (2017). Nano-gold assisted highly conducting and biocompatible bacterial cellulose-PEDOT:PSS films for biology-device interface applications. *International Journal of Biological Macromolecules* 107: 865–873.

11 Aljohani, W., Ullah, M.W., Zhang, X., and Yang, G. (2018). Bioprinting and its applications in tissue engineering and regenerative medicine. *International Journal of Biological Macromolecules* 107: 261–275.

12 Abdalla, A.M.E., Xiao, L., Ullah, M.W. et al. (2018). Current challenges of cancer anti-angiogenic therapy and the promise of nanotherapeutics. *Theranostics* 8 (2): 533–548.

13 Jasim, A., Ullah, M.W., Shi, Z. et al. (2017). Fabrication of bacterial cellulose/polyaniline/single-walled carbon nanotubes membrane for potential application as biosensor. *Carbohydrate Polymers* 163: 62–69.

14 Ul-Islam, M., Khan, S., Ullah, M.W., and Park, J.K. (2015). Bacterial cellulose composites: synthetic strategies and multiple applications in bio-medical and electro-conductive fields. *Biotechnology Journal* 10 (12): 1847–1861.

15 Endler, A., Sánchez-Rodríguez, C., and Persson, S. (2010). Cellulose squeezes through. *Nature Chemical Biology* 6 (12): 883–884.

16 Ul-Islam, M., Khattak, W.A., Ullah, M.W. et al. (2014). Synthesis of regenerated bacterial cellulose-zinc oxide nanocomposite films for biomedical applications. *Cellulose* 21 (1): 433–447.
17 Ul-Islam, M., Khan, T., and Park, J.K. (2012). Nanoreinforced bacterial cellulose-montmorillonite composites for biomedical applications. *Carbohydrate Polymers* 89 (4): 1189–1197.
18 Klemm, D., Schumann, D., Udhardt, U., and Marsch, S. (2001). Bacterial synthesized cellulose – artificial blood vessels for microsurgery. *Progress in Polymer Science* 26 (9): 1561–1603.
19 Ul-Islam, M., Khan, T., and Park, J.K. (2012). Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. *Carbohydrate Polymers* 88 (2): 596–603.
20 Lin, S.B., Hsu, C.P., Chen, L.C., and Chen, H.H. (2009). Adding enzymatically modified gelatin to enhance the rehydration abilities and mechanical properties of bacterial cellulose. *Food Hydrocolloids* 23 (8): 2195–2203.
21 Schrecker, S.T. and Gostomski, P.A. (2005). Determining the water holding capacity of microbial cellulose. *Biotechnology Letters* 27 (19): 1435–1438.
22 Clasen, C., Sultanova, B., Wilhelms, T. et al. (2006). Effects of different drying processes on the material properties of bacterial cellulose membranes. *Macromolecular Symposium* 244: 48–58.
23 Kaewnopparat, S., Sansernluk, K., and Faroongsarng, D. (2008). Behavior of freezable bound water in the bacterial cellulose produced by *Acetobacter xylinum*: an approach using thermoporosimetry. *AAPS PharmSciTech* 9 (2): 701–707.
24 Tang, W., Jia, S., Jia, Y., and Yang, H. (2010). The influence of fermentation conditions and post-treatment methods on porosity of bacterial cellulose membrane. *World Journal of Microbiology and Biotechnology* 26 (1): 125–131.
25 Kojima, Y., Seto, A., Tonouchi, N. et al. (1997). High rate production in static culture of bacterial cellulose from sucrose by a newly isolated acetohacter strain. *Bioscience, Biotechnology, and Biochemistry* 61 (9): 1585–1586.
26 Islam, M.U., Ullah, M.W., Khan, S. et al. (2017). Strategies for cost-effective and enhanced production of bacterial cellulose. *International Journal of Biological Macromolecules* 102.
27 Kurosumi, A., Sasaki, C., Yamashita, Y., and Nakamura, Y. (2009). Utilization of various fruit juices as carbon source for production of bacterial cellulose by *Acetobacter xylinum* NBRC 13693. *Carbohydrate Polymers* 76 (2): 333–335.
28 Park, J.K., Jung, J.Y., and Park, Y.H. (2003). Cellulose production by *Gluconacetobacter hansenii* in a medium containing ethanol. *Biotechnology Letters* 25 (24): 2055–2059.
29 Park, J., Park, Y., and Jung, J. (2003). Production of bacterial cellulose by *Gluconacetobacter hansenii* PJK isolated from rotten apple. *Biotechnology and Bioprocess Engineering* 8: 83–88.
30 Khan, T. and Park, J.K. (2008). The structure and physical properties of glucuronic acid oligomers produced by a *Gluconacetobacter hansenii* strain

using the waste from beer fermentation broth. *Carbohydrate Polymers* 73 (3): 438–445.

31 Jung, J.Y., Khan, T., Park, J.K., and Chang, H.N. (2007). Production of bacterial cellulose by *Gluconacetobacter hansenii* using a novel bioreactor equipped with a spin filter. *Korean Journal of Chemical Engineering* 24 (2): 265–271.

32 Khattak, W.A., Khan, T., Ha, J.H. et al. (2013). Enhanced production of bioethanol from waste of beer fermentation broth at high temperature through consecutive batch strategy by simultaneous saccharification and fermentation. *Enzyme and Microbial Technology* 53 (5): 322–330.

33 Kourkoutas, Y., Bekatorou, A., Banat, I.M. et al. (2004). Immobilization technologies and support materials suitable in alcohol beverages production: a review. *Food Microbiology* 21 (4): 377–397.

34 Ullah, M.W., Ul-Islam, M., Khan, S. et al. (2016). In situ synthesis of a bio-cellulose/titanium dioxide nanocomposite by using a cell-free system. *RSC Advances* 6 (27): 22424–22435.

35 Sutherland, I.W. (2001). Microbial polysaccharides from gram-negative bacteria. *International Dairy Journal* 11 (9): 663–674.

36 Shi, Z., Zhang, Y., Phillips, G.O., and Yang, G. (2014). Utilization of bacterial cellulose in food. *Food Hydrocolloids* 35: 539–545.

37 Ross, P., Weinhouse, H., Aloni, Y. et al. (1987). Regulation of cellulose synthesis in *Acetobacter xylinum* by cyclic diguanylic acid. *Nature* 325 (6101): 279–281.

38 Ha, J.H., Shah, N., Ul-Islam, M. et al. (2011). Bacterial cellulose production from a single sugar alpha-linked glucuronic acid-based oligosaccharide. *Process Biochemistry* 46 (9): 1717–1723.

39 Naritomi, T., Kouda, T., Yano, H., and Yoshinaga, F. (1998). Effect of ethanol on bacterial cellulose production from fructose in continuous culture. *Journal of Fermentation and Bioengineering* 85 (6): 598–603.

40 Bielecki, S., Krystynowicz, A., Turkiewicz, M., and Kalinowska, H. (2002). Bacterial cellulose. In: *Biopolymers: 5. Polysaccharides* (ed. A. Steinbuchel), 37–90.

41 White, G.A. and Wang, C.H. (1964). The dissimilation of glucose and gluconate by *Acetobacter xylinum*. 2. Pathway evaluation. *Biochemical Journal* 90 (2): 424–433.

42 Wang, H.M., Loganathan, D., and Linhardt, R.J. (1991). Determination of the pKa of glucuronic-acid and the carboxy groups of heparin by 13C-nuclear-magnetic-resonance spectroscopy. *Biochemical Journal* 278: 689–695.

43 Lin, F.C., Brown, R.M., Drake, R.R., and Haley, B.E. (1990). Identification of the uridine 5′-diphosphoglucose (UDP-Glc) binding subunit of cellulose synthase in *Acetobacter xylinum* using the photoaffinity probe 5-Azido-UDP-Glc. *Journal of Biological Chemistry* 265 (9): 4782–4784.

44 Morgan, J.L.W., McNamara, J.T., and Zimmer, J. (2014). Mechanism of activation of bacterial cellulose synthase by cyclic di-GMP. *Nature Structural and Molecular Biology* 21 (5): 489–496.

45 Saxena, I.M., Kudlicka, K., Okuda, K., and Brown, R.M. (1994). Characterization of genes in the cellulose-synthesizing operon (acs operon) of *Acetobacter xylinum*: implications for cellulose crystallization. *Journal of Bacteriology* 176 (18): 5735–5752.

46 Benziman, M., Haigler, C.H., Brown, R.M. et al. (1980). Cellulose biogenesis: polymerization and crystallization are coupled processes in *Acetobacter xylinum*. *Proceedings of the National Academy of Sciences of the United States of America* 77 (11): 6678–6682.

47 Hu, S.-Q., Gao, Y.-G., Tajima, K. et al. (2010). Structure of bacterial cellulose synthase subunit D octamer with four inner passageways. *Proceedings of the National Academy of Sciences* 107 (42): 17957–17961.

48 Jonas, R. and Farah, L.F. (1998). Production and application of microbial cellulose. *Polymer Degradation and Stability* 59 (1–3): 101–106.

49 Ul-Islam, M., Khattak, W.A., Kang, M. et al. (2013). Effect of post-synthetic processing conditions on structural variations and applications of bacterial cellulose. *Cellulose* 20 (1): 253–263.

50 Watanabe, K., Tabuchi, M., Morinaga, Y., and Yoshinaga, F. (1998). Structural features and properties of bacterial cellulose produced in agitated culture. *Cellulose* 5 (3): 187–200.

51 Yamamoto, H., Horii, F., and Hirai, A. (2006). Structural studies of bacterial cellulose through the solid-phase nitration and acetylation by CP/MAS ^{13}C NMR spectroscopy. *Cellulose* 13 (3): 327–342.

52 Gelin, K., Bodin, A., Gatenholm, P. et al. (2007). Characterization of water in bacterial cellulose using dielectric spectroscopy and electron microscopy. *Polymer (Guildf)* 48 (26): 7623–7631.

53 Ul-Islam, M., Ha, J.H., Khan, T., and Park, J.K. (2013). Effects of glucuronic acid oligomers on the production, structure and properties of bacterial cellulose. *Carbohydrate Polymers* 92 (1): 360–366.

54 Iguchi, M., Yamanaka, S., and Budhiono, A. (2000). Bacterial cellulose – a masterpiece of nature's arts. *Journal of Materials Science* 35 (2): 261–270.

55 Delmer, D.P. and Amor, Y. (1995). Cellulose biosynthesis. *American Society of Plant Physiologists* 7 (July): 987–1000.

56 Ross, P., Mayer, R., and Benziman, M. (1991). Cellulose biosynthesis and function in bacteria. *Microbiological Reviews* 55 (1): 35–58.

57 Yu, X. and Atalla, R.H. (1996). Production of cellulose II by *Acetobacter xylinum* in the presence of 2,6-dichlorobenzonitrile. *International Journal of Biological Macromolecules* 19 (2): 145–146.

58 Shah, N., Ul-Islam, M., Khattak, W.A., and Park, J.K. (2013). Overview of bacterial cellulose composites: a multipurpose advanced material. *Carbohydrate Polymers* 98 (2): 1585–1598.

59 Khan, S., Ul-Islam, M., Khattak, W.A. et al. (2015). Bacterial cellulose-poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) composites for optoelectronic applications. *Carbohydrate Polymers* 127.

60 Shi, Z., Gao, X., Ullah, M.W. et al. (2016). Electroconductive natural polymer-based hydrogels. *Biomaterials* 111: 40–54.

61 Bäckdahl, H., Esguerra, M., Delbro, D. et al. (2008). Engineering microporosity in bacterial cellulose scaffolds. *Journal of Tissue Engineering and Regenerative Medicine* 2 (6): 320–330.

62 Uraki, Y., Nemoto, J., Otsuka, H. et al. (2007). Honeycomb-like architecture produced by living bacteria, *Gluconacetobacter xylinus*. *Carbohydrate Polymers* 69 (1): 1–6.

63 Wang, G., Chen, X.F., Shi, X.D. et al. (2008). Bio-fabrication of patterned cellulose nano-fibers. *Advanced Materials Research* 47: 1359–1362.

64 Shi, X., Shi, Z., Wang, D. et al. (2016). Microbial cells with a Fe_3O_4 doped hydrogel extracellular matrix: manipulation of living cells by magnetic stimulus. *Macromolecular Bioscience* 16 (10): 1506–1514.

65 Hutchens, S.A., Benson, R.S., Evans, B.R. et al. (2006). Biomimetic synthesis of calcium-deficient hydroxyapatite in a natural hydrogel. *Biomaterials* 27 (26): 4661–4670.

66 Khan, S., Ul-Islam, M., Ikram, M. et al. (2016). Three-dimensionally microporous and highly biocompatible bacterial cellulose-gelatin composite scaffolds for tissue engineering applications. *RSC Advances* 6 (112).

67 Ul-Islam, M., Shehzad, A., Khan, S. et al. (2014). Antimicrobial and biocompatible properties of nanomaterials. *Journal of Nanoscience and Nanotechnology* .

68 Schumann, D.A., Wippermann, J., Klemm, D.O. et al. (2009). Artificial vascular implants from bacterial cellulose: preliminary results of small arterial substitutes. *Cellulose* 16 (5): 877–885.

69 Khan, S., Ul-Islam, M., Ullah, M.W. et al. (2015). Engineered regenerated bacterial cellulose scaffolds for application in in vitro tissue regeneration. *RSC Advances* 5 (103).

70 Helenius, G., Bäckdahl, H., Bodin, A. et al. (2006). In vivo biocompatibility of bacterial cellulose. *Journal of Biomedical Materials Research Part A* 76 (2): 431–438.

71 Khan, S., Ul-Islam, M., Ikram, M. et al. (2018). Preparation and structural characterization of surface modified microporous bacterial cellulose scaffolds: a potential material for skin regeneration applications in vitro and in vivo. *International Journal of Biological Macromolecules* 117 (1): 1200–1210.

72 Ciechańska, D. (2004). Multifunctional bacterial cellulose/chitosan composite materials for medical applications. *Fibres and Textiles in Eastern Europe* 12 (4): 69–72.

73 Kim, J., Cai, Z., Lee, H.S. et al. (2010). Preparation and characterization of a bacterial cellulose/chitosan composite for potential biomedical application. *Journal of Polymer Research* 18 (4): 739–744.

74 Ul-Islam, M., Khan, S., Ullah, M.W., and Park, J.K. (2015). Structure, chemistry and pharmaceutical applications of biodegradable polymers. *Handbook of Polymers for Pharmaceutical Technologies* 3: 517–540.

75 Li, J., Wan, Y., Li, L. et al. (2009). Preparation and characterization of 2,3-dialdehyde bacterial cellulose for potential biodegradable tissue engineering scaffolds. *Materials Science and Engineering: C* 29 (5): 1635–1642.

76 Castro, C., Cordeiro, N., Faria, M. et al. (2015). In-situ glyoxalization during biosynthesis of bacterial cellulose. *Carbohydrate Polymers* 126: 32–39.

77 Tanskul, S., Amornthatree, K., and Jaturonlak, N. (2013). A new cellulose-producing bacterium, *Rhodococcus* sp. MI 2: screening and optimization of culture conditions. *Carbohydrate Polymers* 92 (1): 421–428.

78 Ruka, D.R., Simon, G.P., and Dean, K.M. (2014). Bacterial cellulose and its use in renewable composites. In: *Nanocellulose Polymer Nanocomposites: Fundamentals and Applications* (ed. V.K. Thakur), 89–130.

79 Shezad, O., Khan, S., Khan, T., and Park, J.K. (2010). Physicochemical and mechanical characterization of bacterial cellulose produced with an excellent productivity in static conditions using a simple fed-batch cultivation strategy. *Carbohydrate Polymers* 82 (1): 173–180.

80 Hu, Y. and Catchmark, J.M. (2010). Formation and characterization of spherelike bacterial cellulose particles produced by *Acetobacter xylinum* JCM 9730 strain. *Biomacromolecules* 11 (7): 1727–1734.

81 Kralisch, D., Hessler, N., Klemm, D. et al. (2010). White biotechnology for cellulose manufacturing – the HoLiR concept. *Biotechnology and Bioengineering* 105 (4): 740–747.

82 Shi, Q.-S., Feng, J., Li, W.-R. et al. (2013). Effect of different conditions on the average degree of polymerization of bacterial cellulose produced by *Gluconacetobacter intermedius* bc-41. *Cellulose Chemistry and Technology* 47: 503–508.

83 Kim, Y.J., Kim, J.N., Wee, Y.J. et al. (2007). Bacterial cellulose production by *Gluconacetobacter* sp. PKY5 in a rotary biofilm contactor. *Applied Biochemistry and Biotechnology* 137–140 (1–12): 529–537.

84 Chawla, P.R., Bajaj, I.B., Survase, S.A., and Singhal, R.S. (2009). Microbial cellulose: fermentative production and applications (review). *Food Technology and Biotechnology* 47 (2): 107–124.

85 Cheng, K.C., Catchmark, J.M., and Demirci, A. (2011). Effects of CMC addition on bacterial cellulose production in a biofilm reactor and its paper sheets analysis. *Biomacromolecules* 12 (3): 730–736.

86 Lin, S.P., Hsieh, S.C., Chen, K.I. et al. (2014). Semi-continuous bacterial cellulose production in a rotating disk bioreactor and its materials properties analysis. *Cellulose* 21 (1): 835–844.

87 Lu, H. and Jiang, X. (2014). Structure and properties of bacterial cellulose produced using a trickling bed reactor. *Applied Biochemistry and Biotechnology* 172 (8): 3844–3861.

88 Cheng, K.C., Catchmark, J.M., and Demirci, A. (2009). Effect of different additives on bacterial cellulose production by *Acetobacter xylinum* and analysis of material property. *Cellulose* 16 (6): 1033–1045.

89 Lu, H., Jia, Q., Chen, L., and Zhang, L. (2015). Effect of organic acids on bacterial cellulose produced by *Acetobacter xylinum*. *Journal of Microbiology and Biotechnology* 5 (2): 30–37.

90 Lu, Z., Zhang, Y., Chi, Y. et al. (2011). Effects of alcohols on bacterial cellulose production by *Acetobacter xylinum* 186. *World Journal of Microbiology and Biotechnology* 27 (10): 2281–2285.

91 Ha, J.H. and Park, J.K. (2012). Improvement of bacterial cellulose production in *Acetobacter xylinum* using byproduct produced by *Gluconacetobacter hansenii*. *Korean Journal of Chemical Engineering* 29 (5): 563–566.

92 Wu, J.M. and Liu, R.H. (2012). Thin stillage supplementation greatly enhances bacterial cellulose production by *Gluconacetobacter xylinus*. *Carbohydrate Polymers* 90 (1): 116–121.
93 Li, Z., Wang, L., Hua, J. et al. (2015). Production of nano bacterial cellulose from waste water of candied jujube-processing industry using *Acetobacter xylinum*. *Carbohydrate Polymers* 120: 115–119.
94 Keshk, S.M.A.S. (2014). Vitamin C enhances bacterial cellulose production in *Gluconacetobacter xylinus*. *Carbohydrate Polymers* 99: 98–100.
95 Aljohani, W., Li, W., Ullah, M.W. et al. (2017). Application of sodium alginate hydrogel. *IOSR Journal of Biotechnology and Biochemistry* 03 (3): 19–31.
96 Lee, K.Y. and Mooney, D.J. (2013). Alginate: properties and biomedical applications. *Progress in Colloid and Polymer Science* 37 (1): 106–126.
97 Zhou, L.L., Sun, D.P., Hu, L.Y. et al. (2007). Effect of addition of sodium alginate on bacterial cellulose production by *Acetobacter xylinum*. *Journal of Industrial Microbiology and Biotechnology* 34 (7): 483–489.
98 Ullah, M.W., Khattak, W.A., Ul-Islam, M. et al. (2014). Bio-ethanol production through simultaneous saccharification and fermentation using an encapsulated reconstituted cell-free enzyme system. *Biochemical Engineering Journal* 91: 110–119.
99 Ullah, M.W., Khattak, W.A., Ul-Islam, M. et al. (2015). Encapsulated yeast cell-free system: a strategy for cost-effective and sustainable production of bio-ethanol in consecutive batches. *Biotechnology and Bioprocess Engineering* 20 (3).
100 Li, Y., Tian, C., Tian, H. et al. (2012). Improvement of bacterial cellulose production by manipulating the metabolic pathways in which ethanol and sodium citrate involved. *Applied Microbiology and Biotechnology* 96 (6): 1479–1487.
101 Keshk, S. and Sameshima, K. (2006). Influence of lignosulfonate on crystal structure and productivity of bacterial cellulose in a static culture. *Enzyme and Microbial Technology* 40 (1): 4–8.
102 Kim, S.J., Li, H., Oh, I.K. et al. (2012). Effect of viscosity-inducing factors on oxygen transfer in production culture of bacterial cellulose. *Korean Journal of Chemical Engineering* 29 (6): 792–797.
103 Chao, Y., Mitarai, M., Sugano, Y., and Shoda, M. (2001). Effect of addition of water-soluble polysaccharides on bacterial cellulose production in a 50-L airlift reactor. *Biotechnology Progress* 17 (4): 781–785.
104 Cavka, A., Guo, X., Tang, S.J. et al. (2013). Production of bacterial cellulose and enzyme from waste fiber sludge. *Biotechnology for Biofuels* 6 (1).
105 Feng, Y., Zhang, X., Shen, Y. et al. (2012). A mechanically strong, flexible and conductive film based on bacterial cellulose/graphene nanocomposite. *Carbohydrate Polymers* 87 (1): 644–649.
106 Castro, C., Zuluaga, R., Álvarez, C. et al. (2012). Bacterial cellulose produced by a new acid-resistant strain of Gluconacetobacter genus. *Carbohydrate Polymers* 89 (4): 1033–1037.
107 Moosavi-Nasab, M. and Yousefi, A. (2011). Biotechnological production of cellulose by *Gluconacetobacter xylinus* from agricultural waste. *Iranian Journal of Biotechnology* 9 (2): 94–101.

108 Hungund, B. (2013). Production of bacterial cellulose from *Gluconacetobacter persimmonis* GH-2 using dual and cheaper carbon sources. *Journal of Microbial and Biochemical Technology* 05 (02).

109 Mikkelsen, D., Flanagan, B.M., Dykes, G.A., and Gidley, M.J. (2009). Influence of different carbon sources on bacterial cellulose production by *Gluconacetobacter xylinus* strain ATCC 53524. *Journal of Applied Microbiology* 107 (2): 576–583.

110 Ha, J.H., Shehzad, O., Khan, S. et al. (2008). Production of bacterial cellulose by a static cultivation using the waste from beer culture broth. *Korean Journal of Chemical Engineering* 25 (4): 812–815.

111 Nguyen, V.T., Flanagan, B., Gidley, M.J., and Dykes, G.A. (2008). Characterization of cellulose production by a *Gluconacetobacter xylinus* strain from Kombucha. *Current Microbiology* 57 (5): 449–453.

112 Kim, S.Y., Kim, J.N., Wee, Y.J. et al. (2006). Production of bacterial cellulose by *Gluconacetobacter* sp. RKY5 isolated from persimmon vinegar. *Applied Biochemistry and Biotechnology* 131 (1–3): 705–715.

113 Bae, S. and Shoda, M. (2004). Bacterial cellulose production by fed-batch fermentation in molasses medium. *Biotechnology Progress* 20 (5): 1366–1371.

114 Bae, S., Sugano, Y., and Shoda, M. (2004). Improvement of bacterial cellulose production by addition of agar in a jar fermentor. *Journal of Bioscience and Bioengineering* 97 (1): 33–38.

115 Çakar, F., Kati, A., Özer, I. et al. (2014). Newly developed medium and strategy for bacterial cellulose production. *Biochemical Engineering Journal* 92: 35–40.

116 Tyagi, N. and Suresh, S. (2016). Production of cellulose from sugarcane molasses using *Gluconacetobacter intermedius* SNT-1: optimization and characterization. *Journal of Cleaner Production* 112: 71–80.

117 Khattak, W.A., Khan, T., Ul-Islam, M. et al. (2015). Production, characterization and biological features of bacterial cellulose from scum obtained during preparation of sugarcane jaggery (gur). *Journal of Food Science and Technology* 52 (12).

118 Castro, C., Zuluaga, R., Putaux, J.L. et al. (2011). Structural characterization of bacterial cellulose produced by *Gluconacetobacter swingsii* sp. from Colombian agroindustrial wastes. *Carbohydrate Polymers* 84 (1): 96–102.

119 Tsouko, E., Kourmentza, C., Ladakis, D. et al. (2015). Bacterial cellulose production from industrial waste and by-product streams. *International Journal of Molecular Sciences* 16 (7): 14832–14849.

120 Gomes, F.P., Silva, N.H.C.S., Trovatti, E. et al. (2013). Production of bacterial cellulose by *Gluconacetobacter sacchari* using dry olive mill residue. *Biomass and Bioenergy* 55: 205–211.

121 Kumbhar, J.V., Rajwade, J.M., and Paknikar, K.M. (2015). Fruit peels support higher yield and superior quality bacterial cellulose production. *Applied Microbiology and Biotechnology* 99 (16): 6677–6691.

122 Jozala, A.F., Pértile, R.A.N., dos Santos, C.A. et al. (2014). Bacterial cellulose production by *Gluconacetobacter xylinus* by employing alternative culture media. *Applied Microbiology and Biotechnology* 99 (3): 1181–1190.