The Future of Bacterial Cellulose and Other Microbial Polysaccharides

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Received October 15, 2012; Accepted October 23, 2012

ABSTRACT: Biobased polymers have been gaining the attention of society and industry because of concerns about the depletion of fossil fuels and growing environmental problems. Cellulose fibers are one of the most promising biopolymers to be explored as a component of composite materials with emergent properties for new applications. Bacterial Cellulose (BC), a special kind of cellulose produced by microorganisms, is endowed with unique properties. In this context, this perspective offers an overview about the properties of BC that would enable it to become a commodity. This includes an appraisal of the current BC market, as compared with other available biopolymers. The steps of the biosynthesis and purification of BC are also outlined, together with the difficulties that may be responsible for its future development, including the needs for making its production process(es) more attractive to industry. Other microbial polysaccharides are also discussed.

KEYWORDS: Bacterial Cellulose, market, biopolymers, other microbial polysaccharides

1 INTRODUCTION

Bacterial Cellulose (BC) is a fascinating exopolysaccharide produced by bacteria, extruded to the external environment and deposited over the bacterial colonies, as a protective membrane to ensure their survival in their natural habitat [1,2].

Cellulose is a homopolysaccharide of glucose monomers linked by β-1,4 bonds, a chemical structure found in all celluloses, from vegetal to microbial or animal source, (Fig. 1). Thus, at a molecular scale, all celluloses have the same composition, but variable properties and morphologies, according to their sources. BC displays some intrinsic characteristics that differentiate it from other forms of cellulose, such as its membranous character, composed of highly pure cellulose, unique in nature, and its tridimensional arrangement formed by entangled nano- and micro-fibrils [2]. The BC membrane is weaved when the BC nano and microfibrils are synthesized and extruded to the external environment by the pores at the surface of the bacterial cell wall. When they reach the external environment, while still bonded to the bacteria, the extruded microfibrils fold onto each other due to the movements of the microorganism, resulting in a nano- and micro-fibrilar entangled tridimensional structure [3]. BC, with its pronounced hydrophilic character and the presence of water in its natural environment, is synthesized in a highly hydrated state, with 98–99% (wt) of water, adsorbed between the microfibrils. When cultivated without agitation in this aqueous environment, the BC membrane is synthesized at the surface of the media [2]. Under a shaking culture, where the oxygen is dispersed into the water, the membranes are not formed because the turbulence does not allow their assembly, giving rise, instead, to pellets, which may differ in form (long, circular, stellate) and size [4]. The aspect of dried BC membranes is similar to that of a sheet of paper, with a discrete surface gloss. Fig. 2 shows the BC at the surface of culture media before purification (A), after purification (B) and after drying (C).

2 THE PRODUCTION OF BC

The microorganisms able to produce and secrete cellulose in high amounts belong to the *Acetobacteriaceae* family, distributed worldwide, more frequently in tropical regions. In Southeast Asia, the cellulose producing microorganisms are easily found as the natural flora of coconut water.

Coconut water is an industrial residue produced in high volume by the coconut industry, one of the major

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DOI: 10.7569/JRM.2012.634104

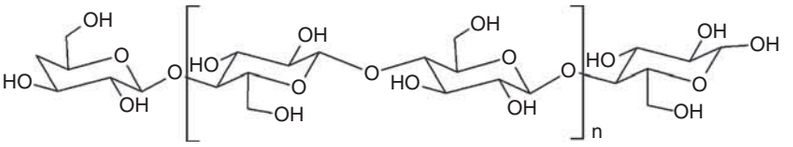

Figure 1 Chemical structure of cellulose.

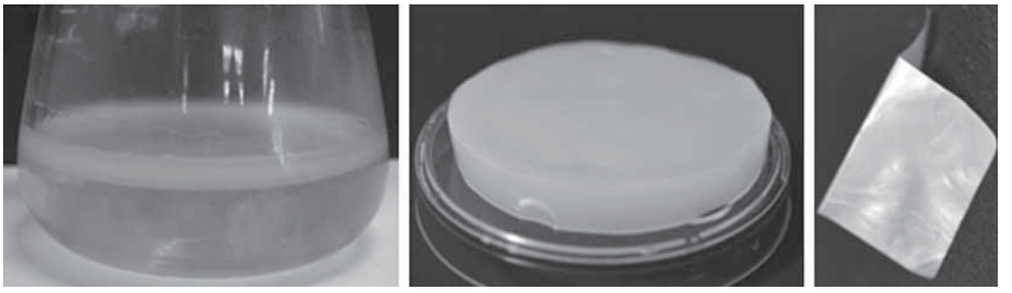

Figure 2 Bacterial cellulose at the surface of the culture medium (A), as a purified wet membrane (B), and as a dried membrane (C).

pillars in employment generation and foreign exchange earnings in the Phillippines and Indonesia [5]. It is rich in sugar and other nutrients, sufficient to support the growth and reproduction of microorganisms, which secrete cellulose as their main by-product, known as "Nata de Coco" (NC). The NC obtained by natural fermentation, under a non-controlled process, is produced by bacteria, which are part of a consortium of microorganisms that also includes bacteria able to produce weak acid (acetic acid, lactic acid and gluconic acid), and yeasts. The natural contamination of an industrial residue results in a beneficial fermentation process generating this valuable product for the food industries (diet food and dessert), which led to the development of the process for BC production in Southeast Asia, two decades ago.

In 1989 the "Nata de Coco Technology" program was implemented in several cities of the Phillippines. It was produced in a homemade manner as a source of livelihood, based on its easy technology, low initial capital investment and fast source of income. In this case, the fermentation was performed in trays stacked on top of each other, without any control of such parameters as temperature and pH, giving nevertheless rise to large amounts of BC [6]. Although NC is a non-traditional coconut product, representing 8,7% of the coconut derivatives, its volume of production was accelerated in 1991, when countries in Southeast Asia lived through the "NC boom", with a production reaching 22 tons/month [6]. The exportation to the USA, and Japan, among other countries, substantially increased then both in volume and value, where it was used as a healthy food and for industrial applications. The volume of exports of NC from the Philippines increased from 200 MT in 1990 to 6,000 MT in 1997, representing an average annual growth rate of 320%, which reached a export value close to 6 FOB million US$ in 1997 [5].

Ten years later, the volume exported by the Philippines reached 4,500 MT in 2007 [7], with values of 5,100, 6,000 and 5,300 MT in 2008, 2009 and 2010, respectively, corresponding to export values of 5.45, 6.03 and 5.89 FOB million US$, respectively [8].

The volume of BC production is in fact low, possibly because it is a biotechnological product generated by fermentation, that would need an optimized process to reach good yields, as opposed to coconut oil (the main product of the coconut industry), coco cream powder and liquid coconut milk, extracted directly from the coconut and sold as such [8].

After over a century since its discovery, description and characterization, in 1886, by Brown [9], NC has recently attracted renewed attention, because of the growing search for new renewable resources aimed at replacing fossil-based counterparts in the production of chemicals and materials. Hence the considerable increase in publications since 1993, related to BC production, characterization and uses, which tripled in the last five years, as shown in Table 1.

These studies made it possible to transform the simple homemade process into controlled, easy and quick laboratory approaches, which take into account the peculiarities of the microorganisms. These cellulose-producing microorganisms normally belong to the well-known *Acetobacteriaceae* family, whose members are non-fastidious microorganisms [10], able to

Table 1 Nanocelluloses citations in scientific papers in the last twenty years.

Year	BC	MFC	NC
1993–1997	121	11	0
1997–2002	230	12	0
2002–2007	327	19	12
2007–2012	1013	260	129

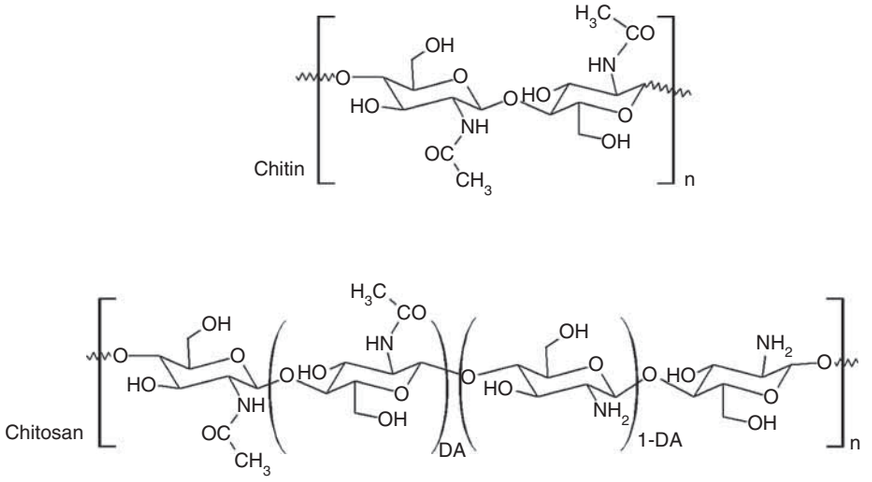

Figure 3 Chitin and chitosan chemical structure. DA is the degree of acetylation, i.e. the percentage of residual acetylated units from chitin.

grow from different carbon sources, glucose, fructose, sucrose and glycerol, among others [11–13]. The microorganism easily grows with low amounts of nitrogen source, such as in Kombucha tea [14], with around 0.7g/L of nitrogen, and coconut water, a poor nutrient source that leads nevertheless to high BC yields. Sugars such as sucrose, glucose and fructose are found in most of the industrial residues and can be used as an economic nutrient source for cellulose producing microorganisms. For instance, coconut water, molasses from beet or sugar cane and glycerol from biodiesel synthesis [15], lead to similar yields as synthetic media, thus helping to decrease BC costs.

On both laboratory and industrial scales, besides the composition of the culture media and the conditions, such as optimum pH and temperature, the isolation of a pure culture is an important step in the production of BC. A pure strain of microorganism can lead to high yields, since there is no competition with other forms of life, like moulds and other bacteria.

However, despite the know-how and technology for the development of a competitive industry, a higher volume of BC, exported as NC, is still obtained today by the traditional homemade method in the Philippines. In conclusion, the edible Nata de Coco is the only established industry of BC that provides consolidated data on its production and market and offer products at accessible costs.

The availability of any raw material on the market is fundamental to ensure the feasibility for the development of industrial processes and sustainable applications, and this should precisely be the goal for the production of BC. Given that BC is a material with outstanding properties and hence numerous potential applications, it is surprising that limitations associated with the low volumes available on the market, are preventing its proper exploitation. What is urgently required are both technological improvements and process scale up.

In comparison with BC, a polysaccharide that rapidly reached industrial production and an increasing market is chitosan, a polysaccharide constituted of a random copolymer with variable compositions of anhydro-N-acetyl-D-glucosamine and anhydro-D-glucosamine residues, derived from the deacetylation of chitin, the second most abundant polysaccharide in nature (Fig. 3) [16–18]. The main sources of chitin are presently the shells of shrimps, prawns and crabs,

with high expectation from Antarctic krill and cultured fungi becoming major alternative sources [19]. The increasing costs associated with the disposal of the chitin wastes is rapidly becaming the focus of research about their recovery, alternatively to expensive disposals. Thus, converting the industrial wastes in high-added value products, the fish plants enhanced thei economic return.

Chitosan and its derivatives have a vast array of applications in the pharmaceutical and cosmetics industries, flocculating and chelating agents for water treatment, medical suture and fibers, agriculture, chromatographic separations, solid state batteries, besides its high potential to be used in other biomedical fields [16]. The reinforcement of chitosan films with natural fibers, such as BC and microfibrillated cellulose (MFC) was recently developed to increase the polymer properties for application in food and biomedical products [20]. The discovery of all these application niches led to the sound establishment of the product on the market, based in its consolidated production, with steadily decreasing costs.

The success story of chitosan within the realm of materials from renewable resources should have represented a stimulus for developing a similar venture applied to BC, because a successful material is based on both economic production and applications in innovative materials. Curiously, however, this has not happened as yet, despite frequent unverifiable claims of the arrival of large-scale implementations.

The main properties of BC that enables it to be used as a very promising nanomaterial are its high purity, high degree of crystallinity, ranging from 70–80%, high mechanical properties (Young modulus around 4GPa, tensile strength around 150 MPa and an elongation at break of around 2% in the dried state), high water holding capacity, mainly in the never dried state and, finally, the tridimentional nano and microfibrillar network morphology and membranous form. The other nanocelluloses, MFC and Cellulose Nanocrystals (CN) display similar properties, but with different aspects, as discussed below.

3 NANOCELLULOSES

BC, MFC and NC are cellulose nanofibers obtained from different sources and different processes that have been gaining growing interest in the last few decades, due to their high potential as materials for industrial applications. Examples include composites, rheology modifiers in the food industry, paints, cosmetics and pharmaceutical products, as well as novel paper-based materials [21–23]. Those and other applications of nanocelluloses are based on their properties, which enable preparing low density materials, many of them optically transparent, besides the reinforcing effect of the nanofibres. Table 1 illustrates this trend through the increasing number of citations related to each type.

MFC are long nanosized, individualized cellulose fibrils produced by the fibrillation of cellulose fibers through intensive mechanical treatment, often with a preliminary chemical treatment (acid, basic, enzymatic, among others) [22]. The typical form of MFC consists in aggregates of microfibrils with diameters ranging from 10 to 40 nm.

CNs are the fundamental constitutive polymeric crystalline motifs of cellulose fibers, whose whiskers (another term for CN) are 100–200 nm long with a diameter ranging between 3 and 5 nm. Their preparation is based on the acid hydrolysis of cellulose fibers, where the non-organized regions are preferentially hydrolyzed, while the acid resistant crystalline regions, remain intact. The most typical hydrolytic agent employed for the production of CNs is sulfuric acid, which also reacts with the surface of hydroxyl groups of the final whiskers yielding charged surface sulfate esters that promote their dispersion in water. The purification of NC suspension is performed through dialysis against distilled water [22,24].

BC, as mentioned before, is a nano and microfibrilar tridimensional net of continuous nanocellulose fibers 20–100 nm wide. Apart from its unique morphology, BC is also the only non-vegetable nanocellulose, arising from a microorganism fermentation process.

Scanning electron micrographs (SEM) of BC, MFC and CN are shown in (Fig. 4), together with the corresponding visualization of the respective suspensions.

Nanocomposites based on nanocelluloses, in which the fibers are used as fillers for the reinforcement of natural and/or synthetic matrices, reflect a recent research domain [22], and represent a remarkable approach to the development of new materials with improved properties. The inclusion of fibers into a polymeric matrix results in an improvement in their mechanical behavior, leading to the increase in Young's Mmodulus and stress at break, thanks to the stress transfer from the matrix to the reinforcing phase [25], at the molecular level, and obviously depends on the load of nanocellulose fibers [26]. Compared with composites incorporating normal cellulose fibers, these positive effects attained with much lower loads, associated with the vast increase in specific surface, which allows percolation to be reached, typically with as little as 1% nanofiber addition. The use of a highly crystalline reinforcement also enhances the mechanical strength of the composite. Optically transparent nanocomposites based on nanocellulose can also be obtained, especially when CNs are used, because the

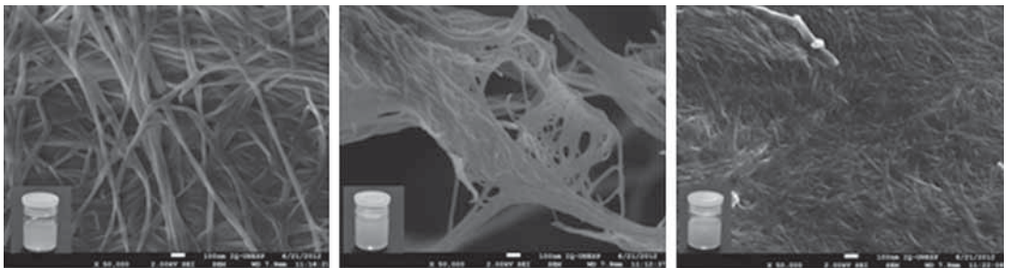

Figure 4 SEM micrograph of BC (A), MFC (B) and CN (C), 50.000X, and their respective suspension (insert).

characteristic diameters are smaller than the range of visible wavelengths [27].

Some other notable features of nanocellulose fibers are their potential availability, renewable character, light weight, biodegradability, hydrophilicity (which can also play a negative role in some applications, e.g. with hydrophobic matrices), and unique morphology [24,25].

All these characteristics of nanocelluloses have accelerated the search for their industrial application. Among them, the MFC is showing the fastest development and recent results suggest a potential scale up for industrial production. At present MFC is being produced by Borregaard Chemcell (Sarpsborg, Norway) in small amounts, CelluForce (Canada), one ton/day, and Stora Enso (Imatra, Finland), on a pre-commercial plant. CN is produced by UPM Fibril Cellulose (Espoo, Finland), in a pre-commercial scale [28]. BC is produced in undisclosed, but certainly modest, amounts by a few companies, like Dermafill in the USA and Bionext in Brazil, which commercialize BC dressing in the form of sterilized sheets in several dimensions, at around U$S1,000/$m^2$ [29]. BC is also produced for biomedical application by Jenpolymers, Germany [28]. As mentioned above, this situation reflects a nebulous market, from which only two negative certainties can be drawn, viz. (i) low production, and hence availability, and (ii) abnormally high prices. Conversely, BC from NC dessert is readily available on the market at low costs, with well documented production ad price information.

4 BC-BASED PRODUCTS ON THE MARKET

The highest volume of BC in the world is designed for the food industry, its oldest and largest application, however, the homemade BC, obtained from industrial residues of coconut water or wastes of fruit juice, can also contain cellulose fibers from fruits (coconut or pineapple, for instance). The presence of these fibers limits its application in the areas that require fibers with homogeneous size and thickness to ensure the reproducibility of the results.

In the biomedical field, BC membranes produced under controlled condition and synthetic culture media are used in its dried form for treatment of skin burns and chronic wound healing. Some trademark products like Dermafill [30] and Bionext [29] are available on the market. In order to assess the healing effect of BC dressing, its action was studied and compared that of with standard wound care counterparts in human volunteers with skin tears. The time to close a wound, reduce pain, and its ease of use were studied. The results showed that the healing time were equivalent to the commercial controls, with an advantage for pain relief and ease of use, leading to a greater satisfaction for the patient and nurse [31].

In the cosmetic field, BC is used for facial beauty masks and skin care treatment. BC membranes impregnated with cosmetic emulsion compositions (whitening or anti-ageing formulations) [32] or anti-cellulite wraps [33] are used directly on the skin. In both wound dressing and cosmetic masks, the performance of the membranous form of BC is fundamentally important.

The first high technological application of BC was the use of dried BC fibers in a composite material for acoustic diaphragms in speaker systems of headphones commercially available on the market, developed by Sony Corporation. The acoustic diaphragms have superior physical properties such as Young's modulus and tensile strength as compared to those of conventional materials, and the composite material containing reinforced BC may also be very profitably produced with [34].

The physical properties of BC in its wet unmodified state are associated with an attractive material for surgical implants. Its mechanical properties, hydroexpansivity, biocompatibility, structural stability within a wide range of temperatures and pH levels, and the versatility of the material to be molded in various sizes and shapes are important characteristics for

implantable biomedical materials [35]. Based on these properties, a BC based composite was designed with a suitable shape for implantation as a replacement material for damaged tissues such as the meniscus and the cartilage. The composite has sufficient porosity for oxygen to pass through it and some pores are large enough to let at least a portion of animal cells enter. The composite is produced by a single fermentation process, under specific conditions of oxygen level, which control the proportion of the exopolysaccharides secreted by the microorganisms. Under high oxygen tensions (above 21%), cellulose is almost exclusively produced, whereas, under relatively low oxygen tensions (20% or lower), a hydrogel material composed of copolymers of beta glucan is almost exclusively produced [36]. The invention is the main product of the recently created BC Genesis company of BC-based products for biomedical applications [37].

In all the examples above, BC-based products are high-value added, which is not relevant for these application, because the production costs are low if compared to the product profit and to the low amounts of BC required. Nevertheless, BC is still a high- cost raw material, produced by a biotechnological process, which needs a long period of time to be synthesized, besides its purification and sterilization, i.e. additional processes that increase costs and time to deliver the end product. As for any other raw material, low amounts of BC can be used in high-value added products or in high amounts of low-cost products. Research on BC production and applications is on its way to make this biopolymer become an important raw material for new developments.

The chemical modification of cellulose is one of the most important aspects that allows it to be used in a wide range of industrial applications, generating derivatives with properties totally different at from the pristine cellulose, but conserving its intrinsic features. Just like vegetable cellulose, BC has been chemically modified, but these modifications are performed in modest volumes and a small number of modifications have been reported, as described below.

5 CHEMICAL MODIFICATION OF BC

In traditional processes, the chemical modification of cellulose involves predominantly the free hydroxil groups of the saccharide units, which undergo typical condensation reaction of primary and secondary alcohols, giving cellulose derivatives, mostly ethers and esthers [38,39]. Additionally, others derivatives can be obtained by alternative synthetic paths such as nucleophilic displacement reactions, 'click reactions' and controlled oxidation [40]. The chemical modification of cellulose can be performed under homogeneous or heterogeneous condition, leading to derivatives with properties which can be completely different. The bulk modifications, conventionally carried out in a homogeneous phase, after the dissolution of cellulose in an appropriate solvent, whereby the reaction modifies the entire fiber, destroying its morphology and its semi-crystalline phase [41], resulting in materials with novel characteristics. On the contrary, the supramolecular structure of cellulose and its properties, mainly its mechanical properties, are unchanged if the modification reaction is carried out at the fiber surface, normally in heterogeneous conditions. The physical and chemical properties of the modified fiber are also related to the degree of substitution [39,41].

Cellulose derivatives like acetates, nitrates, ethylcellulose and hydroxypropilcellulose, among others, have a large industrial interest as such, or as a component of composite materials or blends, increasing the compatibility between the phases of the system, in agreement with the nature of the polymeric matrix. The modification of the cellulose surface can be modulated by the extent and distribution of the appended functional groups [42], and enables furthermore the ensuing derivatives to be used in grafting and crosslink reactions for the formation of new materials [39].

Following the path of vegetable cellulose, BC has been chemically modified under homogeneous or heterogeneous conditions in order to develop new functional derivatives with original properties and to compare them with those of the pristine material. Despite the enormous variety of potential chemical or physical modifications, BC is just starting to be used as a substrate for these treatments.

Acetylation is the most studied BC modification, reached through several different approaches. For instance, the use of dimethylacetamide/lithium chloride was effective in dissolving and acetylating BC with a yield of around 43%, which is regenerated in water giving a totally transparent wet membrane [43]. The use of ionic liquids such as 1-N-butyl-3-methylimidazolium chloride leads to the dissolution of BC and allows it to be efficiently acetylated with acetic anhydride in a homogeneous system [42]. The *in situ* acetylation of BC membranes is also possible, since the water held within the fibers is replaced by acetone, followed by a sequential treatment with acetic acid, toluene, and perchloric acid, followed by hot-pressing to form dried sheets [44]. The acetylation with acetic anhydride in the presence of the iodine as a catalyst preserves the nanostructural morphology of the nanofibers when performed under moderate conditions [45]. Acetylated BC fibers display a hydrophobic surface and good mechanical properties, useful

to enhance the compatibility with hydrophobic non-polar polymeric matrix.

Carboxymethylated BC can be prepared by the conventional heterogeneous reaction with sodium monochloroacetate and NaOH in isopropanol [46]. The nanostructure of BC is important for the reactivity and the properties of the synthesized CMBC, like water solubility.

The alkyl ketene dimmer (AKD) dissolved in a supercritical CO_2 medium can be used to modify the BC fibers with increased hydrophobicity. This process leads to the covalent grafting of AKD at the BC surface, preserving its porous tridimensional network structure and morphological aspect [47].

The esterification of BC nanofibers has been performed with carboxylic acids (acetic, hexanoic and dodecanoic acid). The hydrophobicity of the ensuing membranes increased with increasing carbon chain length, even when decreasing the degree of substitution. The degree of crystallinity was not significantly changed and the thermal stability decreased with increasing carbon chain length.These modifications produce BC fibers to be used as fillers for hydrophobic matrices [48].

Other interesting studies include the grafting of the polylactide-glycidyl methacrylate copolymer [49] and other modification approaches such as carbanilation in an ionic liquid [42].

The application of bulk-modified BC produces properties close to those of similarly treated vegetable cellulose, because the BC pristine morphology is destroyed and the modifications are carried out at the molecular level resulting in similar products. For instance, the bulk carboxymethylation of BC, gives rise to a gel, whose characteristics are the same as those of standard CMC. The same applies to regenerated cellulose.

6 THE POTENTIAL APPLICATION OF BC-BASED COMPOSITES

Nanocomposites based on BC are innovative materials with improved performance and new properties such as electrically-conductive flexible membranes, photoluminescent films, flexible organic light emitting devices, magnetically responsive polymers, and optically transparent devices, among others.

Some original developments have been emerging in recent years, involving the preparation of nanocomposite materials based on the physical mixture of BC with polymeric matrices, where the modified or unmodified BC fibers are used to reinforce these matrices or where the BC membranes represent the matrix to be loaded with polymeric or mineral fillers.

For instance, the use of a conductive and flexible support represents an advance in the field of conductive materials because the conventional counterparts are normally metals, which are not readily foldable. BC has shown appropriate characteristics for the development of such materials, due to its elastic stretching and bending properties, that metals cannot offer [50]. BC based conductive composites could thus be useful to supply the demands of materials that requires these specific properties, such as flexible electronic devices, actuators, sensors and loudspeakers. Based on a recent strategy for the development of stretchable conductors by backfilling a connected network of conducting fillers (such as graphene foams [51]) with an elastic polymer, freeze-dried BC membranes were pyrolyzed and embedded in a polydimethylsiloxane polymeric matrix [50]. The resulting composite is a new stretchable material with a relatively high electrical conductivity (0.20–0.41 S cm^{-1}), exhibiting electromechanical stability even under high tensile and bending strain. The explanation for these properties is attributed to the 3D networks morphology of BC, which is preserved even after pyrolysis, providing an interconnected pathway through which electrons can quickly move. The advantages of the method and the applicability of the product can lead to a scale up of the fabrication process to produce flexible, stretchable and foldable electronic devices [50].

BC is also used to generate conductive organic/inorganic materials, since it is used as substrate for TiO_2 nanoparticles generating TiO_2/BC hybrids, thanks to the hydrophilic character of the cellulose fibers. The composite shows a conductive behavior arising from the electrostatic dipole–dipole interactions generated by hydrogen bonds between the hydroxyl groups of BC and TiO_2 [52].

The idea to use BC as the substrate for electronic papers was recently developed and deserves special attention. The electronic paper is built by impregnating the dried BC membrane with an electronic dye. The device is based on the "ink on paper" principle, in which the ink is electronically addressable and is dynamically switchable in its light absorption, by applying an appropriate voltage [53, 54]. The electronic paper finds potential utilizations in e-book tablets, e-newspapers, dynamic wall papers, rewritable maps and learning tools.

Among the new BC-based materials, nanocomposite of BC films with cadmium and selenium (CdSe/BC) exhibit quantun dots optical property, showing green photoluminescence, characterized by a strong emission peak at 529 nm, when excited by UV light (330–385 nm). One advantage of the composite is its long-time stability. These flexible composites endowed with the intrinsic high mechanical properties of BC and the luminescent

properties of the nanoparticles, are promising materials for security papers and sensors [55].

Transparent bionanocomposites from renewable resources are potential materials with applications in opto-electronic devices. Several approaches have been developed to produce BC-based composites in the form of transparent films endowed with improved mechanical properties. Thus, (i) the nanocomposite of BC with epoxidized soy-bean oil generates transparent flexible membranes with increased mechanical behavior [56]; (ii) flexible transparent plastic substrates with an ultra-low coefficient of thermal expansion (4 ppm K^{-1}) are prepared by reinforcing low Young's Modulus transparent acrylic resin with BC [57]; (iii) the deposition of boehmite–epoxi-siloxane on the surface of BC leads to biphasic composites with high tensile strength (116 MPa) and Young's Modulus (13.7 GPa) [58]; and (iv) composites of BC or BC nanocrystals and PLA also exhibit high transparency, [59–61]. The PLA composites with BC and acetylated BC showed improved mechanical properties [60], while the use of BC nanocrystals decreased considerably the water uptake capacity and the oxygen permeability, without substantial changes in the thermal and mechanical properties [61].

Additional advantages associated with BC-based nanocomposite, are (i) the preparation of potential substrates for transparent flexible organic light emitting diode (OLED) displays, (ii) flexibility and high coefficient of thermal expansion that overcome the limitations of glass [62–64] and conventional polymers, respectively [65] (iii) dimensional stability (coefficient of thermal expansion below 18 ppm/K), coupled with flexibility and high optical transparency in BC-polyurethane nanocomposites [65].

The incorporation and deposition of palladium within the BC membranes generate composites capable of catalyzing the generation of hydrogen when incubated with sodium dithionite. The invention is suitable for biosensors and biofuel cells construction due to its high thermal stability and low gas crossover [66].

Magnetically responsive BC sheet have been developed by simple and cost-effective methods, to be used in biosensors and microwave absorption devices [67].

In the packaging field, films of BC with PVC and the antimicrobial agent sorbic acid can be prepared dispersing a BC powder in a PVA solution under vigorous stirring, followed by drying at room temperature in plastic Petri dishes. These biobased polymeric films have a potential application in food packaging with antimicrobial activity [68].

BC is a promising material for the reinforcement of vulnerable historic fabrics as an alternative to conventional polymers, which, after the loss of their protective properties, are difficult to remove without damaging the relic textiles. This totally innovative method for restoring cultural textiles was developed using Chinese silk fabrics and showed a positive result. The method consists in immersing the historic fabric in a liquid nutrient culture media containing the cellulose-producing microorganisms. After approximately 15 hours, several fibers of BC are synthesized over the fabric. The sparse fibers are enough to coat the fabric, maintaining the silk fibers connected and thus preventing the deterioration of the textile. Moreover, the low amount of fibers over the fabric does not change the intrinsic characteristics of silk, such as shine and flexibility [69].

Finally, the admicellar polymerization method conventionally used to increase the compatibility of plant cellulose with hydrophobic matrices has been applied for the first time to nanocelluloses, including BC, in our research group. The method involves the adsolubilization of a cationic surfactant, below its critical micellar concentration, on the surface of fibers. The surfactant interacts with the hydroxyl groups of cellulose forming a double layer around the fiber. A hydrophobic monomer is then incorporated intop the aqueous suspension and migrates to the surfactant sleeve, where it is conventionally polymerized using a water soluble initiator, under adequate conditions of temperature and agitation. The remarkable advantage of this process is the use of an aqueous medium, i.e. the natural green medium for nanocelluloses and BC in particular, avoiding the laborious operations used in other modification approaches, where organic solvents are required.

A high number of biobased materials composed of BC and natural polymers have been developed in the last few decades in order to improve mechanical performances, increase the barrier to oxygen and the hydrophobicity, and enhance matrix-fiber interface compatibility, among other features. Table 2 displays the basic compositions and features of a selection of such materials.

7 PROJECTION AND EXPECTATIONS FOR THE DEVELOPMENT OF BC PRODUCTION

The growing number of patents and scientific publications related to BC clearly points to an increasing demand, which requires a serious scale-up in its production, as well as a decrease in its cost, which however should, in principle, be a consequence of the scale-up, at least in part.

The conventional technology for BC production is quite straightforward and economically viable,

Table 2 BC-based composites.

Modification	Properties/Applications	Reference
PLA	Reinforcement of PLA matrix with acetylated BC	[60]
PHA	Biocompatible and biodegradable reinforced PHAs	[70,71]
Chitosan	Reinforcement of the polymeric matrix for packaging and biomedical applications	[20,72]
Pullulan	Reinforcement of the polymeric matrix for fod industry	[73]
Alginate	Composite with improved mechanical properties and good miscibility between the components	[74]
CMC	The incorporation of CMC into the culture medium during the BC synthesis interfered with the formation of BC fibers, leading to composites with low mechanical strength and low crystallinity	[75]
PVA	Biodegradable composite materials for application in the packaging industry	[76]
Starch	Composite prepared by incorporating starch in the culture medium during the BC synthesis showed no significant improvement of mechanical properties	[77]
Acrylic resin	Improved mechanical properties	[26]

since it calls upon static conditions in trays containing the liquid culture medium under controlled temperature, namely 28–30°C. In general, the microorganisms require 15–30 g/L of carbohydrate and around 5 g/L of a nitrogen source, as well as traces of salts and vitamins, to grow and secrete the BC. Systematic studies have shown that the BC production in the presence of different carbohydrate sources, including industrial residues, contribute to the implementation of the biorefinery concept for the production of such a high added-value product [15, 78–80]. Several reactors have been conceived for the production of relatively large amounts of BC [81], but the results were not very encouraging. Bioreactors of smaller proportions designed for the direct preparation of various artifacts, such as tubes and microtubes for blood vessel replacement [82] and menisci [83], have been more successful. Studies about microbial engineering [84,85], using molecular biology to attain overproducing bacteria, have also been reported, but the results were not promising.

The purification of BC is time-saving and economically advantageous when compared to the extraction and purification of vegetable cellulose, because BC is free of lignin and hemicelluloses [2]. After the withdrawal of BC from the culture medium, the purification is performed through abundant washing with water to remove the soluble wastes, followed by a heat treatment with diluted NaOH [86] to disrupt and remove the bacteria cells from the fibers. Thereafter, the cellulose is washed with distilled water to neutral pH. This relatively easy and quick process is only possible thanks to the high purity of BC in its native form. The main steps of BC production are resumed in (Fig. 5).

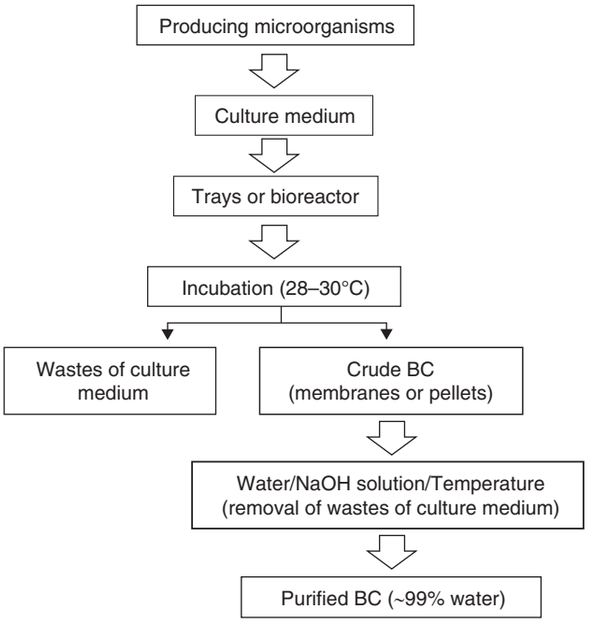

Figure 5 Schematic steps of BC production and purification.

8 OTHER MICROBIAL POLYSACCHARIDES

Compared with BC, other polysaccharides produced by microbes are the subject of only a few novel investigations in terms of scientific and industrial interest, but seem to hold a bright future.

8.1 Kefiran

Kefiran is a capsular microbial exopolysaccharide produced by kefir grains during milk fermentation

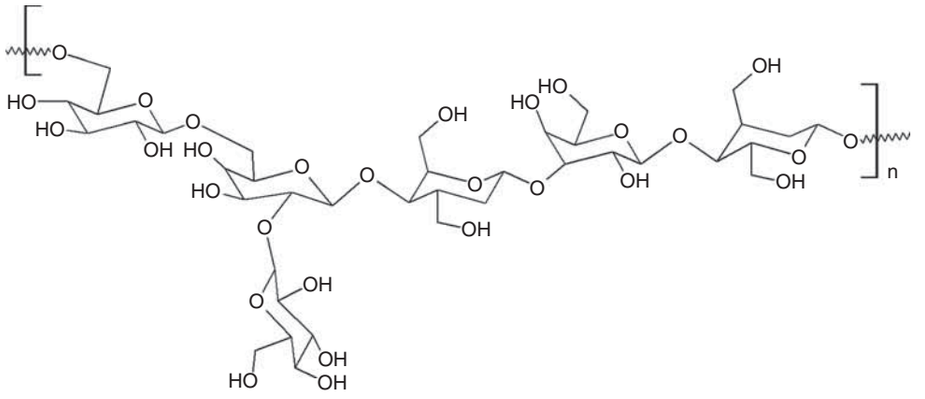

Figure 6 The structure of kefiran monomer unit.

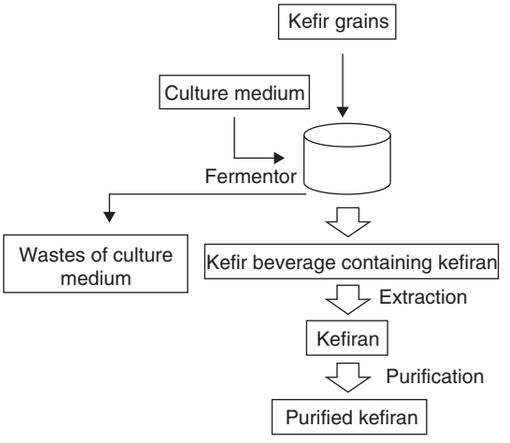

Figure 7 Schematic production and extraction of Kefiran.

to produce a traditional homemade beverage, the kefir. The kefir grains are composed by several species of microorganisms, including the Lactobacillus, responsible for milk fermentation and kefiran secretion. Kefiran is a water-soluble polysaccharide constituted of glucose and galactose (approximately 1:1), as shown in (Fig. 6) [87]. The separation of kefiran from the fermentation broth is conducted through precipitation with an organic solvent like ethanol or acetone, followed by centrifugation (Fig. 7) [88]. Since kefiran is a component of a traditional diet food, it is completely biocompatible and non toxic at the normal levels found in the beverage.

Kefiran has an excellent potential as a film-forming agent, giving transparent sheets with good appearance, but little information is available about their properties [89]. Their mechanical properties are satisfactory for certain applications, with a tensile strength of around 11 MPa and elongation at break at ~40%. These properties are modified by the addition of glycerol or oleic acid, which act as plasticizers and, in the case of oleic acid, as hydrophobizier [89,90]. Some

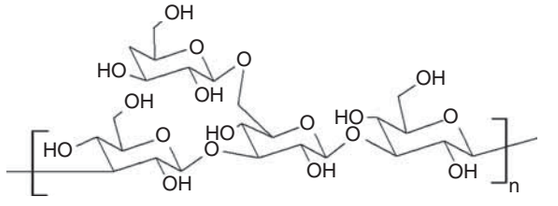

Figure 8 Structure of schyzophyllan monomer unit.

important features of kefiran are its antibacterial, antifungal, and antitumour action [91]. This polymer has found applications in the food industry as a texturing and gelling agent, as well as its possible use as an alternative to synthetic packaging for food [89]. Moreover, its antimicrobial and antiflamatory activities suggest its use as a potential natural polymer to the pharmaceutical industry, but this requires a systematic evaluation.

8.2 Schyzophyllan

Schyzophyllan is a non-ionic water soluble exopolysaccharide, discovered in 1971 [92] and produced by fermentation from the filamentous fungus *Schizophyllum commune*. Its basic homoglucan structure is composed of a β-(1,3)-D-linked backbone of glucose residues and a single β-(1–6)-glucoside side chain at approximately every third residue (Fig. 8) [93]. After production, the microbial cells are mechanically withdrawn from the fermentation broth and schyzophyllan is isolated by gel chromatography [94]. Among its properties and applications, rheology modification and natural bioactivity should be highlighted.

The viscosity of schyzophyllan aqueous solutions decreases with the increasing shear rate, showing a thixotropic, pseudoplastics and viscoelastic behavior. Such properties enable the polymer to be used in oil recovery, food industry and cosmetics [95]. Concerning its bioactivity, schizophyllan has been

shown to display immunomodulatory, antineoplastic and antiviral activities [96], higher than other glucans and similar to those of conventional synthetic medicines, with the obvious advantage of the safety associated to the use a polysaccharide. Recently, its ability to inhibit mammary carcinomas and suppress liver lesions was shown to be similar to that of the conventional synthetic drug (tamoxifen) used in the treatment of these diseases [97].

8.3 Other polysaccharides

Hemicelluloses like dextran, xantan, carregenan and alginates can also be produced by microorganisms [98], but are usually obtained from their vegetable or marine sources. All of them are well established on the market thanks to their remarkable properties and applications [99].

8 CONCLUSIONS

It seems appropriate at this point to draw some comparisons related to the progress of three polymers derived from renewable resources, which have encountered different histories. Polyh(ydroxyalkanoate)s (PHAs), an important family of bacterial polyesters, were first publicized by ICI some 40 years ago as potential substitutes for a number of fossil-derived commodity materials, like poly(propylene). Regrettably, they never reached substantial industrial production, although recent claims to that effect are again being put forward in several countries. The major obstacle here is economic (quite apart from some aspects related to lower specific performances), since the expected progressive reduction in their production costs never reached competitive levels compared with the petrochemical counterparts. Although this does not imply that this problem will never be solved, four decades represent a very long period of time to find a viable process. At the other end of the spectrum, chitosan, as emphasized above, is witnessing a rapid ascent as a very useful and diverse biomaterial, with an accompanying growth in industrial production and a corresponding decrease in price. Bacterial cellulose sits in an intermediate position, because its potential applications are multiplying, with some already in full use, but the implementation of its large-scale production is lagging behind its societal needs. This situation is moreover characterized by a lack of clear information about the state of art regarding industrial availability or even near-future projects. One of the purposes of this perspective was precisely to underline this nebulous context, which conspires against a more rapid development in the exploitation of this beautiful material.

ACKNOWLEDGEMENTS

I am grateful to CNPq (National Counsel of Technological and Scientific Development) for Grant 161535/2011-9 and FAPESP and CNPq for the financial support. I thank Mina G. Talatala from Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA) for providing the data about BC, and Joan Pennifold for her help with improving the English of my manuscript.

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