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Bacterial Cellulose as High Performance Nano Biopolymer.

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ABSTARCT

Cellulose is one of the most widely used natural materials for paper and food productions, textile and biomedical applications, etc. Due to the limitations of the original source of cellulose, which is wood and conservation of natural resources, researchers have made efforts in producing new materials cellulose. Four routes have been identified for the formation of biopolymer cellulose. The most important industrially biosynthesis of cellulose is by different microorganisms, such as bacteria. Bacterial cellulose (BC) molecular structure is similar to plants cellulose, but they are different physical and chemical properties. Its fibers are very stiff and it has high tensile strength, high porosity, high purity and nanofibrillar structure as compared to the native cellulose, it can be used in high performance materials and goods. This article presents a critical review of the available information on bacterial cellulose as a biomaterial with biocompatible and sustainable properties, production methods and applications.

Keywords: Bacterial Cellulose, Biopolymer, High Performance, Nano Fiber

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INTRODUCTION

Cellulose is the most abundant biological polymer on earth, which combines to form the main body of the plant and a representative of microbial extracellular polymers as well [1-4]. Cellulose is a linear polymer made of β -1-4 glucopyranose molecules, $- [C_6H_{10}O_5]_n -$ which are covalently linked through acetal functions between the OH groups of C_1 and C_4 carbon atoms (Figure1). The glucose rings are rotated in the plane by 180° in order to accommodate the bond angles of acetal oxygen bridges. As a result of this rotation the linear polymer has screw rotational axis symmetry and hence two adjacent structural units constitute the basic repeat unit called disaccharide cellobiose. The cellulose synthesized by both plant species as well as bacteria has identical molecular structure and repeat units [1, 5].

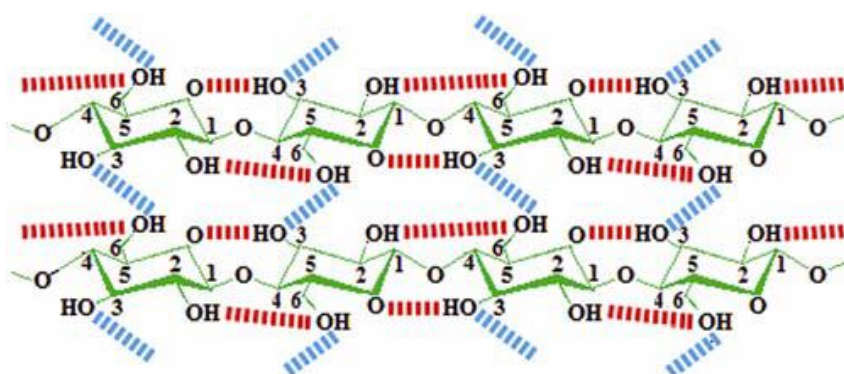


Figure 1: Molecular structure of cellulose and the dimolecular cellobiose unit with intra- and inter-chain weak hydrogen bonds [5]

Micro-organisms are a source for industrial fabrication of many polymers which have characteristics of fiber forming polymers [6-9]. These are compounds from the class of polysaccharides, proteins, and polyesters. Several microbial derived polysaccharides with novel and interesting physical and biological properties already have been applied in biotechnology products. Among them microbial cellulose is one of the most promising classes of microbial polysaccharides [10, 11].

Four routes have been identified for the formation of biopolymers cellulose. Cellulose biosynthesis by plants is the most common and most important industrially biosynthesis of cellulose by different microorganisms, including bacteria (*Gluconacetobacter xylinus*), algae, and fungi among others [1,6,7,12]. The other two less common sources include the enzymatic in vitro synthesis starting from cellobiosyl fluoride, and the chemosynthesis from glucose by ring-opening polymerization of benzylated and pivaloylated derivatives [5,13,14]. Bacterial cellulose (BC) is produced by strains of the bacterium *G. xylinus*, which is a gram-negative, rod shaped and strictly aerobic bacterium [2,15-17].

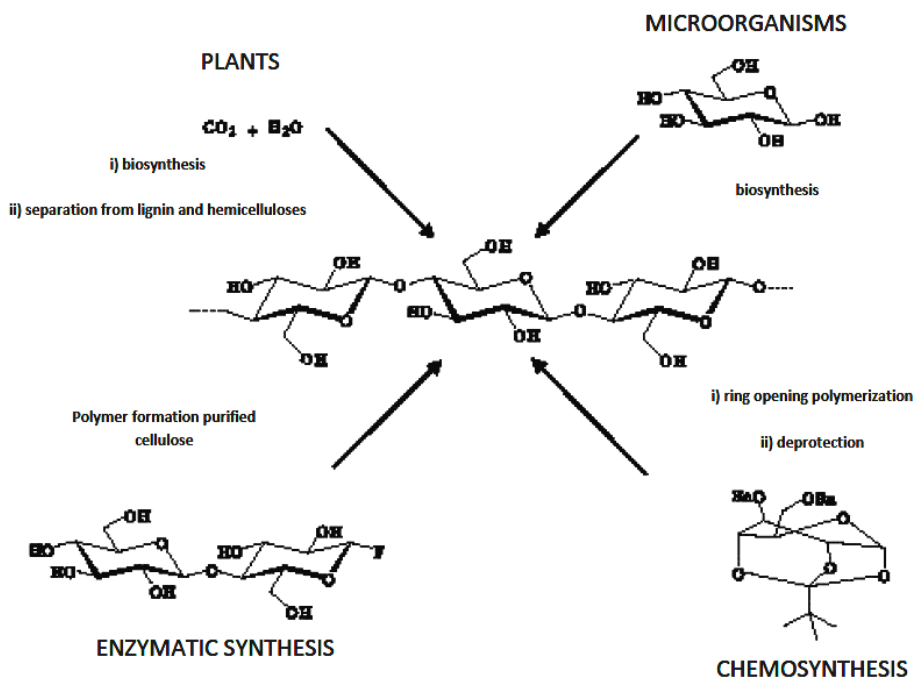


Figure 2: Cellulose synthesis routes

BC is a type of cellulose synthesized by some bacteria. BC differs from plant cellulose in its higher purity, crystallinity, degree of polymerization and tensile strength [7,18-21]. BC has I_{α} and I_{β} crystalline forms, unlike the cellulose of plants that present mainly the I_{β} structure [8,22]. Biocompatibility of BC-based products have made them suitable for several biomedical applications, including membranes for wound dressings [9,23,24], scaffolds for tissue engineering [10,11], substrates for cell seeding [12,13,25], structures for biomineralization of hydroxyapatite [1,14], etc. Its Nano fibers (Figure 3) are very stiff and it has high tensile strength, high porosity, high purity as compared to the native cellulose, it can be used in high performance materials and goods.

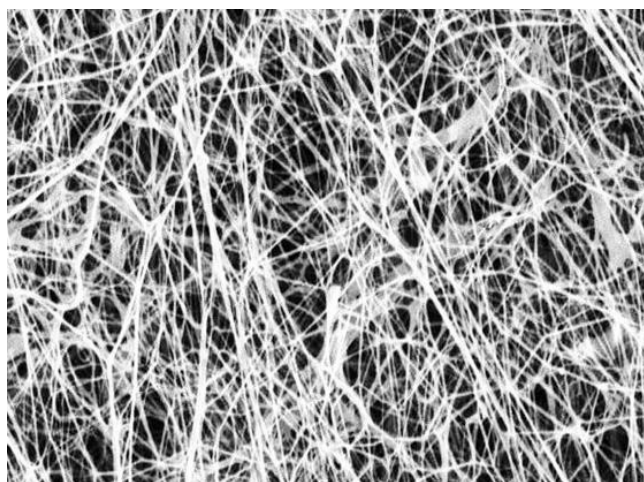


Figure 3: Bacterial cellulose Nano fibers[1]

BIOSYNTHESIS OF BACTERIAL CELLULOSE

In general, cellulose consists of five basic steps that are dependent enzymes includes:

- Conversion of D - glucose to glucose by glucose parmehas
- Phosphorylation of glucose to glucose 6 - phosphate by glucokinase
- Conversion of glucose 6 - phosphate to glucose 1 - phosphates by the phosphoinositideglucocorticosteroidsMvtaz
- Conversion of glucose 1 - phosphate to UDP-glucose by UDP-glucose Pyrophosphorylase
- Cellulose synthesis from UDP-glucose by cellulose synthase

Figure 4 shows schematic steps of biosynthesis BC[28,27].

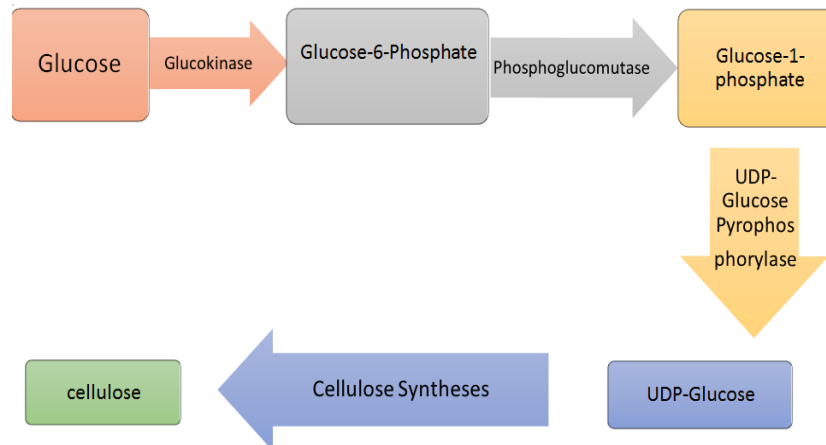


Figure 4: Bacterial Cellulose biosynthesis steps

Different methods for the production of bacterial cellulose

NanoFibers of BC can form static and agitated cultures. In the stationary culture conditions, a thick, gelatinous membrane of BC is accumulated on the surface of a culture medium; whereas under agitated culture conditions, cellulose can be produced in the form of a fibrous suspension, irregular masses, and pellets or spheres. While stationary culture has been quite widely investigated and applied for production of some successful commercial cellulose products (Nata de Coco, transducer diaphragms, wound care dressing materials, etc.), agitated culture is still considered as a cultivation technique which is more suitable for the commercial production of bacterial cellulose mainly due to the higher production rates which potentially can be achieved. However, it is also well known that cellulose production in fermentors with continuous agitation and aeration encounters many problems, including spontaneous appearance of Cel-mutants (cellulose non-producers), which contributes to a decline in the polymer synthesis [1,28-30].

Various factors on bacterial cellulose production

Effect of Temperature and PH: Temperature is a crucial parameter that affects both growth and cellulose production. In the majority of experiments, the maximal cellulose production was observed between 28 and 30°C [31,32]. The optimum pH of the culture medium for bacterial cellulose production is in the range of 4.0 to 6.0 [33]

Effect of nitrogen sources: Nitrogen is a main component of proteins necessary in cell metabolism, and comprises 8–14 % of the dry cell mass of bacteria. The effect of various nitrogen sources on the production of BC has been reported; casein hydrolyzate gave yield of 5 g/L, and peptone gave yield of 4.8 g/L of cellulose in *A. xylinum* [34]. The addition of extra nitrogen favours the biomass production, but diminishes cellulose production [1,34].

Effect of vitamins: Vitamins like pyridoxine, nicotinic acid, p-aminobenzoic acid and biotin were also found to be important for the cell growth and cellulose production, but vitamins like pantothenate and riboflavin were found to have contradictory effects [35].

Effect of oxygen concentration: Oxygen concentration is important in this regard so that the increase in oxygen concentration from 10 to 25% have no impact on bacterial growth, increased cellulose production [33,34].

Effect of Gzantnyn: Xanthine, including caffeine and theophylline as a potent stimulus for production of bacterial cellulose has been [32,35].

Purification of bacterial cellulose

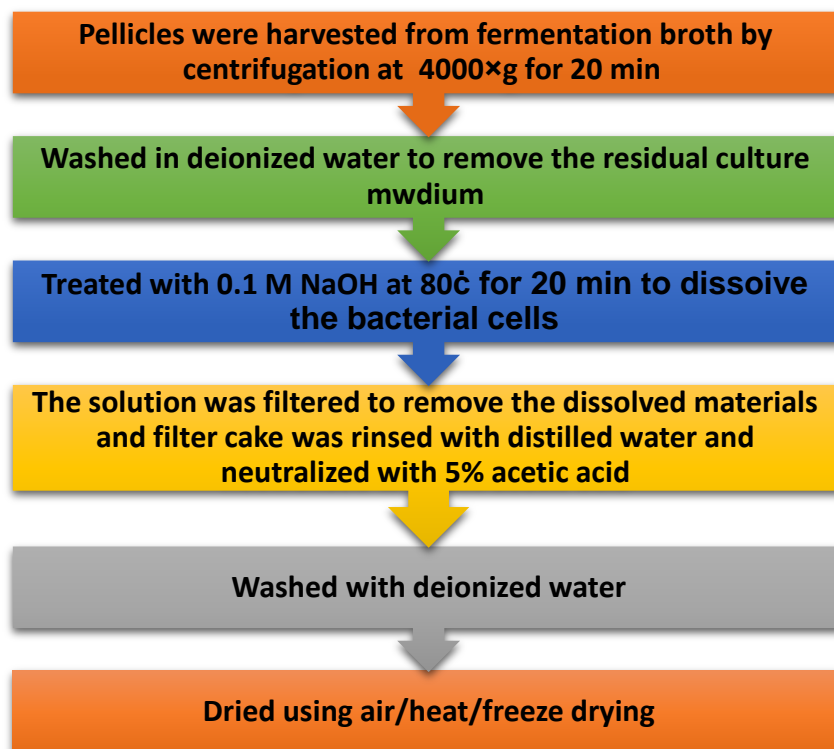


Figure 5: Bacterial cellulose pellicles Purification [41]

The most widely used process of purification of BC in the culture medium is the treatment with alkali (sodium hydroxide or potassium hydroxide), organic acids like acetic acid or repeated washing of the mixtures with the reverse osmosis water or hot tap water for a period of time [1,36,37]. BC containing entrapped cells was treated with solutions like NaOH/KOH/Na₂CO₃ at 100°C for 15–20 min to lyse the microbial cells; thereafter the solution was filtered using an aspirator to remove the dissolved materials. The filter cake

was repeatedly rinsed with distilled water until the pH of the filtrate became neutral. The dry mass of BC without any microbial cells was measured after drying for 4–6hr. As such, the dry cell mass was considered to be the difference between the mass of the dried BC containing the cells and the dried BC after the treatment with NaOH [38-41]. The culture medium was treated with acetic acid after the addition of NaOH solution for neutralization and then with distilled water [1,42-44]. The cells can be treated with aqueous solution of sodium dodecyl sulfate (SDS) and washed with aqueous NaOH, followed by neutralization with acetic acid or by repeated washing with distilled water and then drying in the air or at 60–80°C to a constant mass [38,45,46]. The common step for purification of BC is shown in figure 5 and the unpurified and purified pellicles of BC are shown in figures 6 & 7.

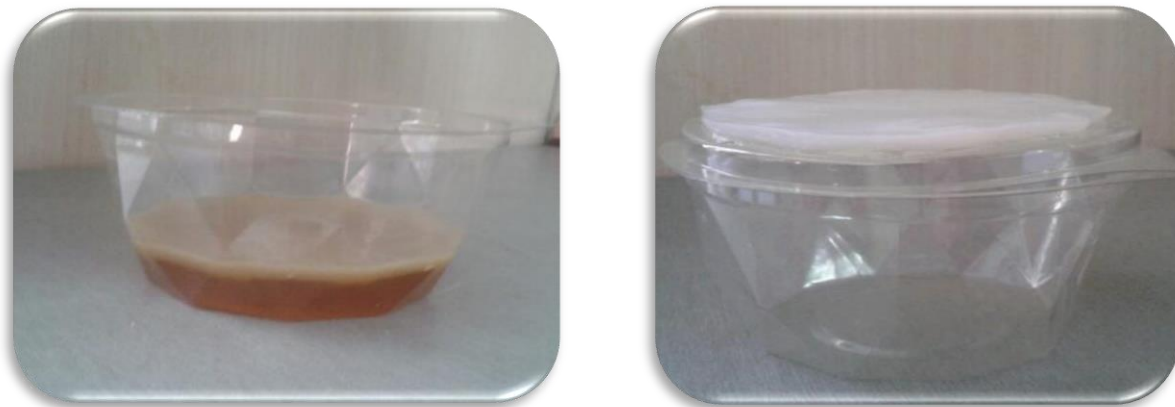


Figure 6: Unpurified bacterial cellulose pellicle Figure 7: Purified bacterial cellulose pellicle

SPECIFIC FEATURES OF BACTERIAL CELLULOSE

Water absorption

Hydration properties of cellulose sheet pore and tunnel structures within Wet sheet, the internal surface area of small pores in the matrix depends showing the power of water conservation in the BC brings us to the value of about 1000% [1].

Very fine grid structure:

Comparison between the BC and thrombocytes as a blood component with a size smaller than 3 mm can be realized as the space between the cellulose networks. BC is preferred over the plant cellulose as it can be obtained in higher purity. Nanofibrillar structure of BC is responsible for most of its properties such as high tensile strength, higher degree of polymerization, water holding capacity, and crystallinity index [47-50]. Fibrils of BC are about 100 times thinner than that of plant cellulose, making it a highly porous material, which allow transfer of antibiotics or other medicines into the wound while at the same time serving as an efficient physical barrier against any external infection [9,25,51].

The ability of light to pass

Figure 8 shows a Pellicle of pure cellulose is dried in air has been found on a paper form. If the transparency of the structure is well visible [1,41].

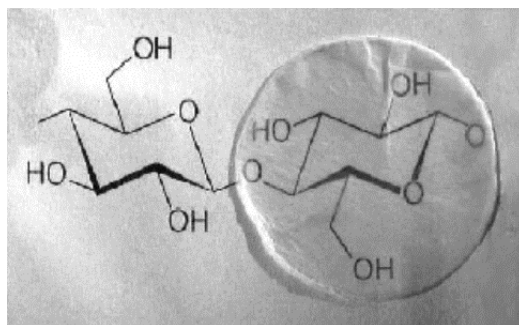


Figure 8: Light ability on a dried cellulose pellicle in air

APPLICATION OF BACTERIAL CELLULOSE

The bioNanofabric of BC has properties like high purity, high degree of crystallinity, high density, good shape retention, high water binding capacity, and higher surface area as compared to the native cellulose, it can be used in various areas including textile industry, nonwoven cloth, paper, food, pharmaceutical, waste treatment, broadcasting, and mining and refinery[24,25-52-55]. BC gels have been used for wound dressings [9].The biocompatibility ofBC-based wound dressings is related to its distinctive nanofibrillar structure, which serves as anoptimal wound healing environment. This nanofibrillar structure eliminates pain symptoms (byisolating the nerve endings) and enhances the absorption of wound exudates [56]. BC accelerates theprocess of healing of the skin in comparison with conventional wound dressings, such as wet gauzeand ointments. Studies have shown that BC-based coverings reduce wound pain, acceleratere-epithelization and reduce wound infection rates and scarring [9,57,58].

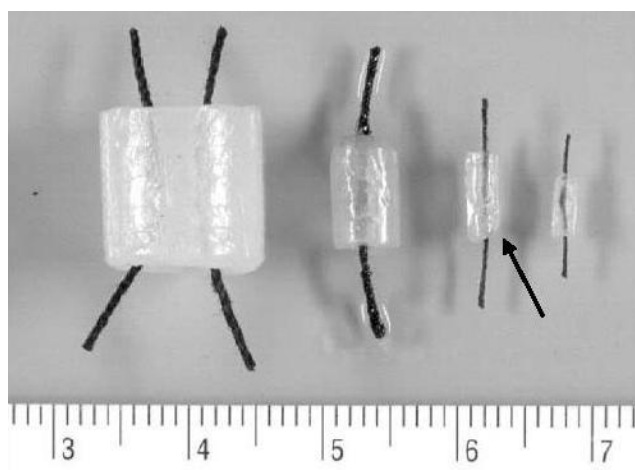


Figure 9: Tubes of BC which can be used for arterial grafting applications [4]

Due to its flexibility coupled with strength and conformability, BC could be used in different contour locations, including moving parts of the human body. Another stringent biological requirement comes from an application such as cardiovascular bypass implant. The material to be used for this must conform to blood and tissue compatibility, endothelization, cell ingrowth, surgical handling and common methods of sterilization. Klemm and co-workers[59-61] used tubes made of BC as arterialgrafts in rats and pigs for

extended periods of time (Figure 9). It was found that BC tubes facilitated the growth of neointima and active fibroblasts. The grafts in carotid. Artery of pigs showed that 87.5% did not develop any blocks after 3 months in the native environment, indicating the potential of BC for tubular implants [62].

Some studies have shown that BC composites could be used as cartilage replacement material [64]. Concluded that BC-poly (dimethyl acrylamide) double network gel has mechanical properties similar to the mechanical properties of cartilage and that may meet the requirements of artificial cartilage. However, in vivo tests that could confirm the biocompatibility of BC-based cartilage replacements have not been reported yet. BC-poly (vinyl alcohol) composites evaluated using unconfined compressions testing also have elastic modulus values similar to those reported for native articular cartilage [65]. To broaden the biomedical applications of BC, various attempts have been made to produce BC composites with high functionality[20,66,67]. Among them, BC/polyethylene glycol (PEG) composite is one of candidates that have great potential applications for tissue engineering and drug delivery. BC also has applications in mineral and oil recovery. Based on the tensile strength, low oxygen transmission (barrier property) rate and its hydrophilic nature, the processed cellulose membrane appears to be of great relevance for its application as packaging material in food packaging, where continuous moisture removal and minimal oxygen transmission properties play a vital role [15]. The unique dimensional stability of BC gives rise to a sound transducing membrane which maintains high sonic velocity over a wide frequency ranges, thus being the best material to meet the rigid requirements for optimal sound transduction. Sony Corporatio (Japan), in conjunction with Ajinimoto (Japan), developed the first audio speaker diaphragms using BC. However, the production of the speaker membrane by using BC is not justifiable for the market because of the high costs[68].

The unique physical and mechanical properties of BC such as high reflectivity, light mass and ease of portability, wide viewing angles, and its uniformity determine the applications in the electronic paper display [54]. Some application of BC shows in figure 10.

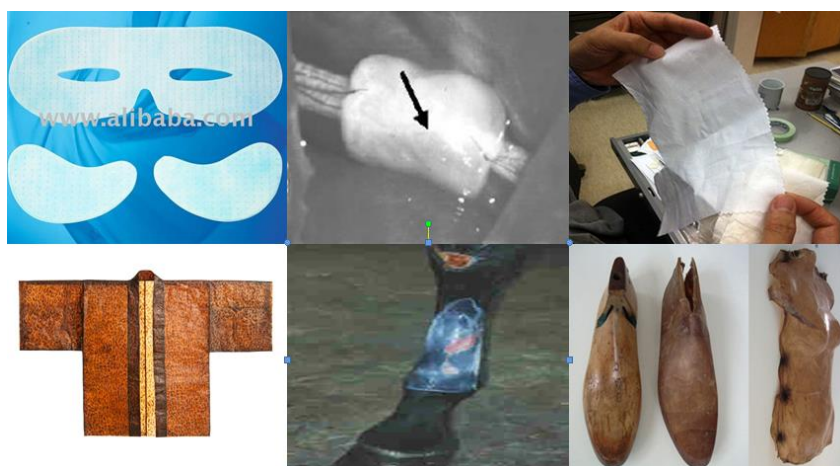


Figure 10: Some application of bacterial cellulose

CONCLUSION

Cellulose is the most abundant biopolymer on earth. Different methods for the production of biopolymers cellulose has been identified, one of which produce BC as a source of microbial cellulose. Several different techniques for BC production have been reported so far, that can be agitated culture, cultivation in the horizontal fermenters, stationary culture or cultivation in the internal-loop airlift reactors. Its Nanofibers are very stiff and it has high tensile strength, high purity, high crystalline, high water holding capacity, large surface area. Unique features of microbial cellulose possibility enjoying in various cases it has provided.

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