Production of Bacterial Cellulose

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Contributor: Dr. Siddhartha Pati

Cellulose is the most liberal and viable sustainable polymer, and it is the one that is predominantly utilized. Though cellulose is considered a plant-based product, several fermentation techniques showed positive results in producing alternative sources of cellulose from bacterial genera.

natural polymer biocomposite nanocomposite antimicrobial

1. Introduction

Cellulose is a prime biopolymer due to its extensive productive importance. Chemically, it is linear homopolysaccharide, composed of β-D-glucopyranose units that remain linked by β-1,4 glycosidic bonds. Apart from being the most important structural component of the primary cell wall of green plants, cellulose is found to also be present in bacteria [1][2][3]. Cellulose is a group of carbohydrates that contains considerable amounts of hydroxyl groups that remain existent in the form of polymer chain [4] and has wide applications in pulp and paper and pharmaceutical industries and as a renewable fuel source. However, the plant cellulose is often associated with lignin, pectin, hemicellulose, and other biogenic products, which has made it difficult to obtain pure cellulose as substrate [5][6].

2. Cultivation of Bacteria for the Production of Bacterial Cellulose

Fermentation for the production of BC is conducted in static and agitated or stirred mode, and with the change of mode, different forms of cellulose are produced. Under the static condition, three-dimensional interconnected reticular pellicles are formed, whereas sharp, irregular sphere-like cellulose particles (SCP) are produced in agitated or stirred conditions. Cellulose formation under static conditions is regulated by the supply of carbon and air into the medium. BC formation is increased with the increase in growth time and the C-H bonding. When the pellicle growth slows down and all the bacteria are entrapped, the synthesis of BC reaches its threshold. Compared to continuous processes, semi continuous processes are put forward in all the industrial scale in order to achieve maximum BC productivity. For commercial production of BC with high yield, agitated fermentation has been used over static fermentation.

The production of BC can be achieved by both agitated and static fermentation. The process involved in the production of BC depends on the morphologies and the properties of BC to be produced [7]. The formation of gelatinous pellicles takes place in static culture at the air–liquid interface of the culture media, whereas in an

agitated fermentation system, the irregular pellets are developed and remain totally suspended in the culture media. Since higher genetic stability is found among the bacterial species, cultured by static fermentation technique [4], the agitated fermentation can be more easily scaled up for the purpose of industrial production [8], although there may be a chance of the appearance of non-cellulosic bacterial mutant that can drastically decrease the productivity [9]. Varied microscopic morphology with 3D reticulate network has been observed with the BC obtained by static or agitation-based fermentation mechanisms [10]. The BC obtained from the agitation fermentation possesses a very low degree of polymerization and also exhibits a lower level of crystallinity in comparison to those obtained from the static fermentation techniques [11]. CP/MAS 13C NMR analysis reveals that the proportion of Iα is lower but Iβ is quite higher in agitation fermentation obtained BC than that of BC yielded from static fermentation [12]. The mechanical properties vary in BC obtained from static to agitation fermentation, since Young's modulus of the BC obtained from static fermentation technique exhibits a higher value in comparison to those obtained from the agitation fermentation technique [13]. It has been observed that BC produced by the technique of static fermentation requires raw materials possessing fixed geometrics, high water holding capacity, and good wet tensile strength. The optimized culture media required for production of BC include 0.5 wt% peptone, 0.5 wt% yeast extract, 0.27 wt% Na_2HPO_4, 2.0 wt% glucose, and 0.115 wt% citric acid [14]. The cost of production of BC is too high for it to be sustained for various industrial processes; thus, alternative strategies are being studied for the development of cost-effective mechanisms [15]. Various mechanisms involve promotion in the production of BC, including the isolation of bacterial strains that are responsible for the production of BC and the detection of high-yielding strains with the use of genetic engineering and traditional mutagenic methods [16] and optimization of the various culture conditions [13]. Various types of carbon sources such as sucrose, fructose, molasses, arabitol, and mannitol, and nitrogen sources such as peptone, yeast extract, and corn steep liquor, are used for the purpose of producing BC [17]. Various types of agricultural residues can be also used for the production of BC [18].

The mechanism of fermentation is followed by the removal of impure raw pellets of BC that comprise metabolic substances and nutrient residues along with BC. The mechanism of purification can be achieved by the treatment of BC with alkaline solution at a temperature of 1000 °C for 15–20 min to remove the bacterial cells. This is followed by the washing of the pellets with distilled water to recover the BC pellets and recover the value of pH [19].

Interestingly, a cell-free enzyme system is also developed to produce BC, which might transform into a cell-free factory for BC production in the future. The cell-free enzyme system is developed from BC-producing strains and contains whole enzymes and cofactors required for BC synthesis. Quantitative analysis reveals that the system produces BC with a higher yield than the corresponding bacteria [20]. Further study demonstrates that the cell-free enzyme system produces BC via an anaerobic biosynthesis process, and the premature BC pellicles formed in the culture media move to the air–liquid interface and assemble into a sheet [21]. B-(1-4)-glucan chains become polymerized into the cell wall before being delivered into the culture medium. The mess structure of the BC gives it pores through which cellulose-synthesizing complex perceives place between plasma membrane and outer membrane of the cells. In this mechanism, the initial material, uridine diphosphate glucose (UDP glucose), is expanded into the cellulose chain, resulting in the development of basic fibril, which is assembled with the elementary fibrils in order to develop microfibrils and strips [22][23][24].

The fermentation medium is incubated for 1–14 days in pH 4–7, 28 to 30 °C with the inoculum, until the vessel gets filled by cellulose. The proper aeration and formation of CO_2 control the metabolic activity in the production of BC. Compared to static cultivation, agitated cultivation is expensive due to the continuous agitation, which increases production yield [25]. In the stirred cultivation process, cellulose is produced in the form of solid balls. The increase in the shear rate may increase the bio productivity, although elevated share rate results in formation of turbulence force in the medium, leading to the change of cellulose-producing strains to the cellulose negative strains. Both the stirred tank bioreactor and air-lift bioreactor showed positive results with high productivity of the BC in highly viscous and dense fibrous suspensions. In the context of oxygen mixing, an air-lift bioreactor showed more efficiency over a stirred tank reactor, as from the bottom, the vessel oxygen is transferred continuously to the culture medium in order to provide an aerobic atmosphere. An airlift bioreactor showed efficiency in controlling energy and shear stress to control the production of cellulose-negative mutants.

3. Parameters Controlling BC Production

Several parameters such as dissolved oxygen, pH, and temperature, independent of static or agitated cultivation, need to be optimized to improve BC yield[26]. Crystalline polymorph, crystallinity index (CI), cellulose Iα, and size are the factors that determine microstructure, and these are dependent on culture conditions [27].

3.1. Temperature

One of the most important parameters is the temperature, which can regulate the adaptation pattern of an organism for its survival by influencing the normal homeostatic physiology. A temperature range of 25 to 30 °C was found to be best for the production of BC, as a Komagataeibacter sp. Was cultivated at 30 °C for 7 days under static conditions [28], whereas 28 °C is the optimum temperature for the BC production by *Acetobacter xylinum* [29] [30]. A slightly higher temperature of 33.5 °C was required by *Acetobacter senegalensis* MA1 [31]. For BC production by *Gluconacetobacter,* sp. RV28, *Pseudomonas* sp. RV14, and *Enterobacter* sp. RV11 preferred a range of temperature that was found to be 28–30 °C [32], as high temperatures cause denaturation of the culture environment, whereas low temperatures slow down cellular metabolism by supplying low energy for cell development.

3.2. pH

pH is another important factor in controlling oxidative fermentation of BC production. Acidic or near-neutral pH is suitable for BC production. During the fermentation process of BC, production of secondary metabolites such as acetic acid, gluconic acid, and lactic acid independently shifts the pH of fermentation culture media [26]. Thus, pH 4–6 is considered the ideal pH for the fermentation culture medium of BC. Experimental observations indicate that pH of 5.50 for *Acetobacter xylinum* [33], 4.5 to 7.5 for another strain of *Acetobacter xylinum* [29], and 6.0 for *Komagataeibacter* spp. [28] are required.

3.3. Culture Media

Carbon, in the form of fructose, glycerol, maltose, starch, and xylose, and nitrogen, in the form of casein hydrolysate and peptone, are the main components of the growth medium required for BC fermentation. Alteration of growth media has direct or indirect effects on microbial growth patterns. A 5 g/L yield of BC and a 4.8 g/L yield of BC from *A. xylinum* were reported in presence of casein hydrolysate and peptone, respectively [34]. Vitamins are important in regulating cellular metabolism and growth. Apart from pantothenate and riboflavin, vitamins such as pyridoxine, nicotinic acid, biotin, and p-aminobenzoic acid are required for cellular synthesis [35]. It was observed that in the presence of lignosulphonate, a low formation rate of gluconic acid increases the productivity of BC [36]. A medium with 0.38% agar, 2.85% corn steep liquor, 4.99% fructose, and 28.33% dissolved O2 is suitable for the formation of 14.0 g/L of BC [37]. Optimized cultivation of *Gluconacetobacter* sp. RKY5 in agitated and static culture produces 5.63 g/L and 4.59 g/L of BC, respectively [38]. Using dual carbon sources such as fructose and sucrose, around 8.79 g/L yield of BC was reported. Cheaper carbon sources such as molasses, muskmelon, orange juice, and watermelon can be used for BC production, and among them, muskmelon is recognized for the highest yield of BC 0.08 g/L. Even so, BC-producing cells have the unique feature of using the nutritional medium for the inexpensive production process.

3.4. Agitation Rate

Production of BC is affected by another important parameter, i.e., agitation rate. At a lower agitation rate of 100 rpm, a uniform solid ball of 0.5 to 1 cm diameter is formed. With an increase of agitation rate around 150 to 250 rpm, BC size decreases, and at 300 rpm, irregularly shaped clumps are produced. Net 3, 10, 11.46, 7.73, and 3.91 g/L yields of BC were obtained at 100, 150, 250, and 300 rpm, respectively, in the case of synthesis of cellulose from *G. xylinus* DSM46602. According to this observation, the moderate rpm is suitable for the optimal production of BC. The generation of shear stress during agitation provides stability to the mutant of cellulose-producing strains in order to enhance the net BC productivity. An increase in the impeller speed from 80 to 500 rpm may decrease the cellulose-negative strains to almost zero. Agitation rate has an impact on the size of the BC. It may decrease the size of BC from 8 mm to <1 mm [39]. The agitation rate needs to be optimized for the production of BC on a large scale as it varies for different microbes along with the culture medium.

3.5. Oxygen Level

Oxygen plays an important role in controlling aeration within the media. An adequate supply of oxygen is needed as all the microbes in the culture medium are aerobic in nature. Within the media, a low level of dissolved oxygen obstructs bacterial growth, leading to the retardation of BC production. O_2 is essential for cellular metabolism and BC production. A restricted O_2 supply not only collapses the BC production but also reduces BC quality. O_2 transfer rate has an inverse relationship with the viscosity of the broth. An increase in viscosity decreases the O_2 transfer rate as well as the BC productivity. Ten percent saturation of dissolved oxygen provides the highest yield of BC in the fed-batch cultivation [40]. Therefore, to obtain the highest yield of BC, a two-stage cultivation system has been developed by Liu et al., wherein in the first-stage dissolved O_2 within the culture media is increased before reaching the log phase of the growth curve followed by maintaining the hypoxic condition at the secondary phase of the growth curve in the second phase for BC production [41].

3.6. Growth Curve

Analogously to the other bacterial strains, BC-producing strains have identical growth curve patterns with lag, log, stationary, and death phases. *Komagataeibacter mendellinensis* has a lag phase of 25 h when it is cultivated in growth media with carbon sources glucose, fructose, and sucrose in 2% *w/v* [42]. A five percent fructose in the culture medium is longer than the lag phase by around 10–15 h in the case of *G. liquefaciens*, leading to the long-time consumption in BC synthesis.

3.7. Yield of BC

Different bacterial strains favor different nutritional media independently of the cultivation technique. Cheap media such as citrus waste solution, pineapple peel, or molasses also assist in the production of BC. The 2 to 14 days range is optimal for regulating cultivation of BC in order to obtain the highest yield. Overall increase in incubation days may increase the yield of BC. Among all other sources, glucose is the main nutrient source of carbon, which is remarkably consumed by bacterial cells to attain the metabolic demands and high energy yield.

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