Production of Bacterial Cellulose from Acetobacter Species and Its Applications - A Review

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Abstract

Bacterial cellulose (BC) is a natural polymer secreted as a protective cell covering of certain bacterial species. In contrary to plant cellulose, BC possesses some unique features like high moisture-holding capacity, high durability, high liquid absorbing capabilities, biostability, and biodegradability, makes BC an excellent raw material in wide-ranging areas like biomedical, food, agriculture, paper, textile industries and electronics. The main objective of this review is to discuss various aspects of BC production (different sources for bacterial strain isolation, culture media and, its alternatives also major culture techniques). In addition, various applications of BC are also reviewed.

Keywords: Bacterial cellulose, Acetobacter sp, culture medium, Culture techniques, Applications

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INTRODUCTION

The most plentiful organic molecule in the biosphere is cellulose, an unbranched homopolymer of glucose. Fungal and algal cell walls are mainly composed of cellulose. It consists of a linear homopolysaccharide linked by β1, 4 glycosidic bonds. Cellulose is also produced by a number of bacterial species, as a protective covering around their cell. The molecular formula for both plant and bacterial cellulose is the same ($\text{C}_{n}\text{H}_{2n}\text{O}_{n}$) but they differ in their physicochemical properties. BC production is primarily by the utilization of various carbon sources in the culture media, though their rate of production can vary with various additives. BC is synthesized by a multi-biosynthetic pathway catalyzed by a number of enzymes. The major steps involved in BC biogenesis are, phosphorylation of glucose to glucose-6-phosphate, isomerization of glucose-6-phosphate to glucose-1-phosphate, transition of glucose-1-phosphate to uridine diphosphate glucose (UDPG) and integration of linear $\beta$1,4-glucon chains from UDPG. Reactions are catalyzed by glucokinase, phosphoglucomutase, UDPG pyrophosphorylase, cellulose synthase respectively. Finally, polymerized subunits ($\beta$1, 4-glucon chains) crystallized into BC.

Bacterial cellulose is produced mainly by Gram-negative non-pathogenic and free-living bacterial species such as *Acetobacter*, *Salmonella*, *Rhizobium*, *Alcaligenes*, *Agrobacterium*, and *Pseudomonas* also by *Sarcina ventriculi*, a gram-positive bacteria species. Among all these groups of bacteria, the most potent producer belongs to the genus *Komagataeibacter* (initially classified as *Acetobacter sp*). *Komagataeibacter* belongs to acetic acid bacteria (AAB). The different members belong to this genus such as *Komagataeibacter medellinensis*, *Komagataeibacter xylinus*, *Komagataeibacter nataicola*, *Komagataeibacter saccharivorans*, *Komagataeibacter oboediens*, *Komagataeibacter rhaeticus*, *Komagataeibacter hansenii*, and *Komagataeibacter pomacetiare* well-known producer of bacterial cellulose. Among the members, *Komagataeibacter xylinus* forms the model organism for BC biogenesis. The members of the genus *Komagataeibacter* are obligate aerobic, gram-negative, and rod-shaped non-motile organisms, they are positive for catalase and negative for oxidase and can oxidise ethanol to acetic acid.

Investigations on the bacterial extracellular gelatinous fiber led to the discovery of BC by A.J Brown in 1886. He first reported that the white gelatinous pellicle present on the static culture medium is bacterial cellulose. During biosynthesis, BC molecules are first synthesized intracellular and subsequently secreted via squeezing pores on the longitudinal axis of the bacterial cell’s outer membrane. Normally the secreted BC measures about 100nm in width and around 100mm in length. Microbial cellulose forms as an aggregate of nano fibrils of 2-4 nm width. These nanofibers are stabilized by inter and intermolecular Hydrogen bond. Bacterial cellulose is mainly a combination of cellulose I and cellulose II.

The absence of lignin and hemicellulose makes the purity level of BC very high. The purification and isolation process of BC is comparatively effortless than that of plant cellulose, making BC a favorite biopolymer. It exhibits tremendous physicochemical properties such as high mechanical strength, high water holding capacity, biocompatibility, and high purity. Raw material in wide-ranging areas like biomedical, food, agriculture, paper, textile industries, and electronics. Applications of microbial cellulose can be further enhanced by recent techniques such as nanofications and functionalization of BC. Thus, the aim of this review is to discuss various aspects of BC production including different methods of bacterial isolation and also the major application strategies of BC.

Production of Bacterial cellulose

For the systematic production of bacterial cellulose, the prime need is an efficient and stable bacterial strain, which should have cheap growth requirements and also the ability to maintain effortlessly. Fabrication of different BC shapes like sheets, pellicle, granules, gels, and films can be achieved by manipulating the culture conditions. Previous studies showed *Acetobacter* sp can be isolated from natural sources. The various sources of *Acetobacter* sp include fruits, vinegar, agricultural waste, and industrial by-products.

Culture media for *Acetobacter* Sp

Components of the growth medium,
environmental conditions, accumulation of metabolic by-products may affect bacterial cellulose production; therefore ideal designing of the culture medium is essential for optimum bacterial growth and cellulose production\textsuperscript{10}.

Past studies showed that Hestrin Schramm (HS) medium is the best known synthetic complex medium for the isolation of \textit{Acetobacter} sp\textsuperscript{6,16-19}. A study by Dubey \textit{et al}\textsuperscript{16} reported that modification of HS media with hot water-sweet lime extract can improve BC production. Modified forms of HS medium and Yamanaka medium were used in another study by Krystynowicz \textit{et al}\textsuperscript{17}.

Molina \textit{et al}\textsuperscript{18} discussed the usage of alternative energy sources (AES) along with conventional growth mediums like ethanol, acetic acid, lactic acid, sodium citrate, amino acid, and vitamin C to improve bacterial yield and cellulose production.

Another study conducted by Mohammadkazemi \textit{et al}\textsuperscript{19} explained the comparative production of BC by \textit{Acetobacter xylinus} in three different media. The media used were Hestrin Schramm (HS) and Yamanaka, Zhou, and he found out that more bacterial cellulose production was on the first two media and a decreased production in the third medium. The study also concluded that the combination of nitrogen and carbon sources was very effective for BC synthesis.

Ruka \textit{et al}\textsuperscript{20} published a study in which they investigated the effect of different carbon sources on the BC yield by modifying the media. The four media used were Hestrin Schramm (HS), Yamanaka, Zhou, and CSL (corn steep liquor).

Media added with glucose, mannitol and sucrose showed improved bacterial cellulose yield. A study done by Kouda \textit{et al}\textsuperscript{21} mentioned the modified use of CSL medium with fructose, results showed improved BC production. Various parameters which affect the bioprocess are mainly, pH, temperature, agitation, and the level of dissolved oxygen, optimization of all these parameters are the key factor for bacterial cellulose production\textsuperscript{22-25}. Gorgieva \textit{et al}\textsuperscript{4} reported that the highest yield of BC can be achieved by modifying HS media by substituting the glucose with mannitol, sucrose, or galactose. Wang \textit{et al}\textsuperscript{26} observed that fructose can improve BC production when compared to other carbon sources. The composition of the different culture media is presented in Table 1.

\textbf{Table 1. Different types of culture media and their composition}

| Composition | Media |
|-------------|-------|
|             | Hestrin Schramm (HS) | Yamanaka (Y) | Zhou (Z) |
| Glucose    | ✔       | ✔      | ✔       |
| Corn-steep liquor | —    | —      | ✔       |
| Yeast-extract | ✔  | ✔      | —       |
| Peptone    | ✔       | —      | —       |
| Na\textsubscript{2}HPO\textsubscript{4} | ✔     | —      | —       |
| Citric acid. Water (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} | ✔    | —      | —       |
| KH\textsubscript{2}PO\textsubscript{4} | ✔    | —      | ✔       |
| MgSO\textsubscript{4}.7H\textsubscript{2}O | —   | ✔      | ✔       |

✔ = item present in the media, — = item absent in the media

\textbf{Table 2. Alternate medium used for the enumeration of \textit{Acetobacter} sp}

| Media type                              | Features                                      | Reference |
|----------------------------------------|-----------------------------------------------|-----------|
| Plant Extract (Common tea, unroasted Coffee, cacao) | Stimulating effect of Xanthine | 28        |
| Beverage industrial waste (Citrus peel and Pomace Enzymolysis) | Enzyme treatment increases the reducing sugar content | 27        |
| Wheat straw acid hydrolysate Tomato juice | Wheat straw acid hydrolysate higher carbon level | 29        |
| Water melon and mandarin juice Litchi extract | increased level of carbon and Nitrogen higher crystallinity | 30        |
|                                         |                                              | 31        |

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2. Journal of Pure and Applied Microbiology
Alternate media for *Acetobacter* Sp isolation

Past studies proved that the role of alternate media with complex organic constituents is more productive and profitable than the traditional synthetic defined medium. The use of agricultural and industrial scrap as a growth medium is not only economical but also an eco-friendly waste management system. The alternate medium used for the enumeration of *Acetobacter* sp is shown in Table 2.

Use of additives for BC production

Incorporation of various chemicals in BC culture medium can improve the production of bacterial cellulose in both static and submerged cultures. Past studies showed that Carboxymethyl cellulose (CMC) is a better additive to improve bacterial cellulose production. CMC is water-soluble cellulose with a carboxymethyl group. It helps in the prevention of large BC aggregates formation and enhances its solubility. Incorporation of CMC in culture medium keeps the BC fibers thin and turns it more suitable for its special applications.

Ishida et al. mentioned that the effect of water-soluble polysaccharides such as agar can improve bacterial cellulose production by enhancing the viscosity of the culture medium. The addition of agar in the medium may promote the dispersion of pellets and thus prevents their aggregation and maximize BC production. Bae et al. also viewed the yield of bacterial cellulose is improving with the effect of agar.

BC nanofibers are an excellent candidate for drug delivery systems; experiments conducted by Beekman et al. have proved that the addition of Poly Ethylene Glycol (PEG) to the culture medium modifies the BC fibers with increased transparency, loading capacity, and also the effective drug delivery properties.

In a study conducted by Zhou et al. observed that sodium alginate, a water-soluble polysaccharide can promote bacterial cellulose production by hindering the formation of large BC production.

Cultivation techniques

Since bacterial cellulose is a biopolymer with extensive applications its efficient and cost-effective production is of utmost importance. Soon after the discovery of this material intense investigations have been progressing for the same. Several techniques were proposed out of which some are possible methods for economic, and commercial tools for bacterial cellulose production. Static cultivation, agitated/shaking cultivation, and airlift reactors are currently using fermentation techniques for better BC yield. However, researchers are trying to develop new reactors for improved BC production. Large scale production for the commercial application requires BC with optimum shape, composition and maximum BC properties. BC secretes as a pellicle under shaking condition or as a sheet at air-liquid interface in static culture. Advanced techniques for the fabrication of biomolecules using microbial cellulose are in progress and the cell-free system is one such technique.

Static cultivation technique for BC production

Frequently applied method for the production of bacterial cellulose, is a relatively simple method for lab-scale production of BC. Broth medium is filled in shallow containers or trays used for cultivation, incubated up to 5-14 days at optimum temperature (28-30°C) and pH. The thick pellicle formed on the vacuum/liquid interface is directly related to the surface area. Here, the time of incubation is the main factor that controls the thickness of BC. Decreased productivity and increased cultivation time limit industrial application of this culture technique. Pellicle formation is influenced by the surface of the culture medium, and also aeration at the vacuum/liquid interface will yield high-quality cellulose. A newly invented bioreactor system, Horizontal Lift Reactor (HoLiR) was developed for semi-continuous production of BC. This system offers the benefits of both static and continuous cultivation techniques in a cost-effective manner.

Submerged/Agitated cultivation technique for BC production

The two chief drawbacks of static culture are high cost and low rate of production. To minimize these issues researchers suggested the submerged/agitated technique. The basic concept behind this culture technique was to optimize the oxygen supply to the growing bacteria, rotating speed, culture time, and additive types used in the culture medium, are the factors which contribute to the size and shape of the BC. In this method, maximum yield in comparatively
less time can be possible. The higher yield of BC is possibly due to the homogeneous distribution of nutrients, cells, air, and substrates\textsuperscript{44,51,52}. In agitating cultivation method cells have improved contact with circulating air leads to an improved growth rate\textsuperscript{44}. Previous studies showed that the BC yield from the agitated technique is lower when compared to the static method, probably due to the emergence of cellulose mutant strains under agitated technique\textsuperscript{6,50,53} which may lower the BC yield. Even though there are drawbacks like instability of bacterial strain, high shear force, asymmetrical shape of the produced BC, and the accumulation of BC mutants, some research has suggested that the submerged (agitated) culture method is ideal for large scale production\textsuperscript{44,50}.

**Purification and Characterisation of Bacterial Cellulose**

Many studies have shown that the method employed for the purification of harvested bacterial cellulose was treated with sodium hydroxide. Characterization of bacterial cellulose was done by Scanning electron microscope (SEM). A study conducted by Rangaswami \textit{et al}\textsuperscript{54} concluded that the bacterial cellulose fibers are made of a random assembly of nano fibrils with the aid of SEM, these findings are parallel with many other studies\textsuperscript{15,54,55}. The crystalline structure of harvested bacterial cellulose can be examined by X-ray diffraction study\textsuperscript{14,34}. The thermal degradation behavior of the harvested BC can be assessed by thermogravimetric analysis (TGA)\textsuperscript{15}.

**Bacterial Cellulose-Applications**

Microbial cellulose, a biopolymer with distinctive attributes such as high durability, biodegradability, and high-water holding capacity has made it a demanding product in the field of biotechnology with a number of applications\textsuperscript{56,57}. Moldability of BC at the time of culture has crucial features that impart an extended application to BC. The major applications are summarised in the following discussions and Fig. 1.

**Bacterial Cellulose Composites**

Fascinating structural and physiological features of BC turn it into an absolute tool for several applications. Particularly the nanofibrillar
structure of BC turns it into an ideal for composites fabrication. In spite of all the features, BC has some limitations like lack of antibacterial activity and antioxidant properties which limit its acceptability in the medical field. Moreover, BC does not have properties such as electrical conductivity, optical transparency, and hydrophobicity which limit its use in electronic industries. Pure BC is not suitable for direct use in electrical devices, batteries, sensors, and electromagnetic shielding. To overcome these constraints and also to extend its application, scientists have developed various BC composites. BC is ideal to use as a reinforcing and matrix material for composite design.

BC composite fabrication requires a series of synthetic procedures; a vast number of nanomaterials (Au, Ag, ZnO, etc.) and polymers (chitosan, PEG, etc.) are involved in composite making. All this turns pure BC with additional properties such as antibacterial activity conductivity and transparency; Current advances in this field are the development of fuel cells made...
of BC composites, fabricating display devices, and synthetic organs. Further comprehensive studies in this field are a necessity into develop novel BC composites to meet new applications in assorted areas. Various classes of BC composites and their applications are shown in Fig. 2.

**Biomedical Applications of BC**

BC composed of randomly distributed microfibrillar networks with unique structural properties, bio absorbency, high water holding capacity, gaseous exchange property, high-level of crystallinity, biodegradability is highlighted features of bacterial cellulose for use in the biomedical field. It forms a compatible material with a nontoxic, non-pyrogenic nature. The basic limitations of BC as a biomedical tool are its inability to trigger initial cell adhesion, very slow degradation capacity, etc. It can be eliminated by chemical and physical modifications of prepared BC. In-situ (done by alteration of culture media, carbon source, and use of additives) and ex-situ (performed by chemical and physical treatments of BC yield) methods are used in BC modification. Cellulose digesting enzymes such as cellulase and beta glucanases are absent in human beings moreover BC is poorly soluble in various physiological media and turns it into a potential constituent of various biomaterials with attractive features. Lack of both antibacterial and antioxidant properties limits its use in the medical field. Fabrication of various BC composites helps to overcome this. Modifications during the fermentation process helps to mold BC into any form, size or thickness and thus it can be specifically fit for varied applications. Fig. 3 shows examples of biomedical applications of bacterial cellulose.

**Food Applications of BC**

The consumption of bacterial cellulose originated long back, the fermented food product Nata de coco (prepared from coconut water and bacteria) has been popularized in the Philippines from 1973. A number of studies reported that consumption of BC does not cause any type of toxicity to humans and also studies proved the safety side of BC as a food material. Therefore, BC has been classified as generally recognised as safe (GRAS) by the US Food and Drug Administration since 1992. The incorporation of bacterial cellulose to various food items can improve their quality and
character. Attractive suspending, water retention, thickening, emulsifying and stability properties turn BC into a recommended candidate in the food industry. Yang et al\textsuperscript{53} reported that addition of BC in pasty food can upgrade firmness and better texture. The addition of BC in meat food items can improve its juiciness and chewiness due to its ability to maintain humidity\textsuperscript{60}. BC has great potential in the food packaging industries because it is compatible with the number of food items\textsuperscript{60,4}. Various food applications of BC are listed in Table 3.

Table 3. Food applications of bacterial cellulose

| Applications                                                                 | Ref.   |
|------------------------------------------------------------------------------|--------|
| Use as thickening agent, Low calorie desert, fabricated food, stabilizer,   | 49,56,61 |
| Texture modifier, salads                                                     |        |
| Cell immobilization agent in wine production                               | 56,62  |
| In vitro fermentation of plant dietary fibre                               | 56,63  |
| Food packaging                                                              | 56,60,64 |

**Other Applications**

BC can be successfully used in the field of electronics\textsuperscript{55,56} like the manufacturing of speaker diaphragm\textsuperscript{65}. The Shape retention ability of bacterial cellulose is the main property used for this purpose. Sony corps developed loudspeakers and diaphragm with bacterial cellulose\textsuperscript{56,66}. Recent studies also disclosed the perspective of nano cellulose fibres for the manufacture of organic light emitting diode (OLED)\textsuperscript{56,67,68}.

Previous studies observed that high quality paper can be prepared from bacterial cellulose pellets from agitated culture. Mixing of paper pulp with crushed bacterial cellulose can also be used as a method of preparing the paper with high tensile strength\textsuperscript{69-71}. Specialized paper with high kaolin retention and fire resistance can be achieved by the addition of modified BC\textsuperscript{70}. A variety of BC composites are also used for the paper manufacturing industry\textsuperscript{71}.

Studies proved that bacterial cellulose is a promising biopolymer in the textile industry mainly because of its high unit tensile strength. It is the spectacular feature of bacterial cellulose to be used in the synthesis of geotextiles\textsuperscript{55,72}. Chan et al\textsuperscript{73} also discussed novel applications of bacterial cellulose in the textile industry.

Hassan et al\textsuperscript{74} tested that high-efficiency membrane filters can be prepared from BC. These membranes can be effectively used in oil refineries for separating oil from stabilized or non-stabilized oil in water emulsions. Galdino et al\textsuperscript{75} developed bacterial cellulose membrane filters for the treatment of oil filters, and achieved a 100% oil removal technique from all emulsions. Other studies also discussed the use of bacterial cellulose composites as an efficient filtration membranes\textsuperscript{76}.

Recently, bacterial cellulose composites have been successfully used in the treatment of waste water from the food industry, pharmaceutical and oil fields. Graphene oxide and bacterial cellulose composites exhibit good permeability to different organic or inorganic ions particularly in nano-scale from angstrom-scale\textsuperscript{77}. Novel BC membrane composite is another attraction of BC for water treatment plants\textsuperscript{78,79}.

Bacterial cellulose nanofibers have unique properties to transform into nanosized materials with optical, electrical, and magnetic properties\textsuperscript{80-82}. BC electronic applications include fabrication of piezoelectric material for different engineering applications, Photoluminescent paper optical sensor etc\textsuperscript{83,84}. Various features of bacterial cellulose make it an excellent tool in tissue engineering. Modification of bacterial cellulose for tissue engineering use can be done by in-situ or ex situ methods\textsuperscript{84}.

**CONCLUSION**

As a biopolymer with fascinating features bacterial cellulose is real material with wide applications. It can replace plant cellulose with many advantages. The unique physicochemical properties of bacterial cellulose mainly include biocompatibility, high moldability and tensile strength, high water retention capacity. Basically, microbial cellulose can be obtained from a number of renewable sources, the Acetobacter sp is the role model for bacterial cellulose biogenesis. This review is primarily focusing on the synthesis of cellulose by Acetobacter sp. Different sources for Acetobacter sp isolation and various culture media or their alternatives and cultural techniques have been updated in this review. To meet the elevated...
demand for bacterial cellulose, a strong, feasible method should be employed. Extensive studies on biochemical and genetic features of Acetobacter sp are required to be performed in order to understand and improve the cellulose biogenesis within the same.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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