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## Overview of Bacterial Cellulose Production and Application

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### Abstract

Bacterial cellulose (BC), produced by aerobic bacteria received ample of attention due to its unique physiochemical properties compared to plant cellulose. Intense researches on BC mainly focus on biosynthetic process to achieve low-cost preparation and high cellulose production. BC has been used as biomaterial for medical field, electrical instrument and food ingredient. However, BC alone has limited capabilities to fulfil current demand on high-performance biomaterials. Hence, BC composite has been introduced to enhance BC properties through addition of reinforcement materials. This review discusses current knowledge in fermentation process and potential application of BC including its application in food industry.

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*Keywords:* Bacterial cellulose; *acetobacter xylinum*; fermentation; composite material

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### 1. Introduction

Cellulose is the most abundant, inexpensive and readily available carbohydrate polymer in the world, traditionally extracted from plants or their wastes. This polymer normally branches with hemicellulose and lignin has to undergo unhealthy chemical process with harsh alkali and acid treatment to obtain the pure product (Sun, 2008). Increasing demand on derivatives of plant cellulose had increased wood consumption as raw material, causing deforestation and global environmental issue (Park et al., 2003).

Although plant is the major contributor of cellulose, various bacteria are able to produce cellulose as an alternative source. Bacterial cellulose (BC) was initially reported by Brown (1988) who identified the growth of unbranched pellicle with chemically equivalent structure as plant cellulose. Due to BC structure that consist only

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glucose monomer, it exhibits numerous great properties such as unique nanostructure (Chen et al., 2010), high water holding capacity (Saibuatong and Phisalaphong, 2010), high degree of polymerization (Dahman et al., 2010), high mechanical strength (Castro et al., 2011) and high crystallinity (Keshk, 2014). The discovery from previous researches had clearly shown that BC and its derivatives have tremendous potential and provide a promising future in various fields such as biomedical, electronic and food industrial (Shah et al., 2013; Zhu et al., 2010).

BC is produced by acetic acid bacteria in both synthetic and non-synthetic medium through oxidative fermentation. *Acetobacter xylinum* is the most studied and the most efficient BC producer (El-Saied et al., 2004) that manage to assimilate various sugars and yields high level of cellulose in liquid medium (Ross et al., 1991; Sani and Dalman 2010; Moosavi-Nasab and Yousefi, 2011). This aerobic gram-negative bacteria actively fermented at pH 3-7 and temperature between 25 and 30°C using saccharides as carbon source (Castro et al., 2011). Rivas et al. (2004) reported that almost 30% of bacterial fermentation is belong to the cost of fermentation medium. High cost and low-yield production has limited the industrial production of BC and its commercial application. Therefore, it is important to look for a new cost-effective carbon source with shorter fermentation process for high yield BC production.

## 2. Bacterial cellulose structure

BC exists as a basic structure of fibril that consists of  $\beta$ -1 $\rightarrow$ 4 glucan chain with molecular formula  $(C_6H_{10}O_5)_n$ . The glucan chains are held together by inter- and intra- hydrogen bonding (Ul-Islam et al., 2012) (Fig. 1). Microfibrils of BC were first described by Muhlethaler in 1949 and about 100 times smaller than plant cellulose (Chawla et al., 2009; Gayathry and Gopaldaswamy, 2014).

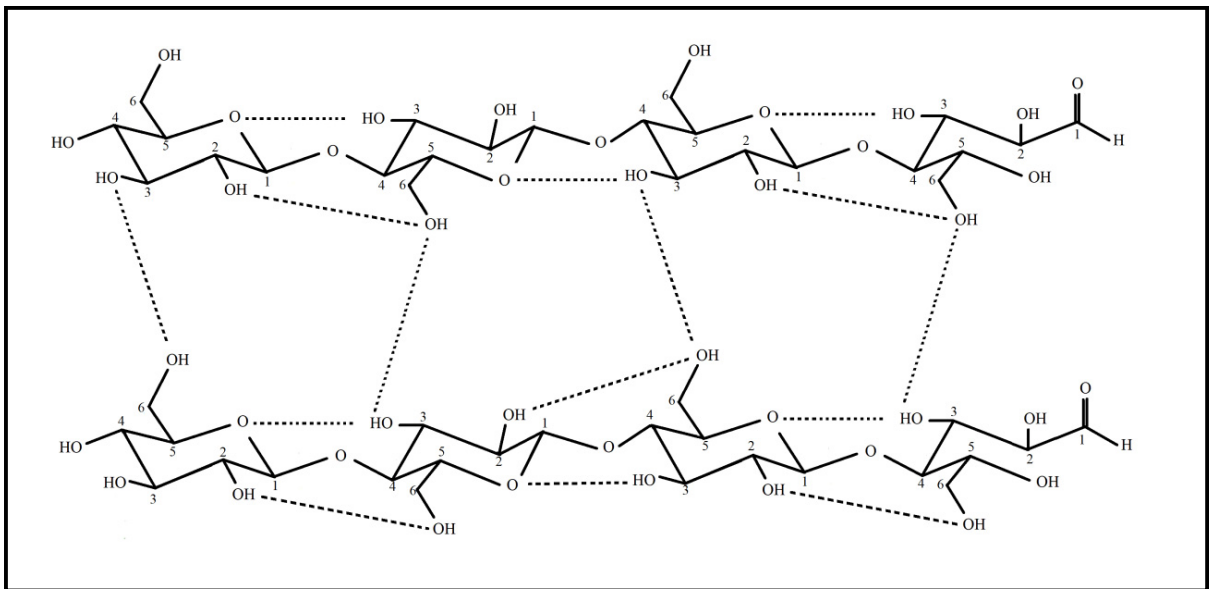


Fig. 1. Inter- and intra-hydrogen bonding of bacterial cellulose (edited from Festucci-Buselli et al. 2007).

The fibrous network of BC is made of three dimensional nanofibres that well-arranged, resulting in formation of hydrogel sheet with high surface area and porosity. *Acetobacter xylinum* produces cellulose I (ribbon-like polymer) and cellulose II (thermodynamically stable polymer) as described in Fig. 2 (Chawla et al., 2009). During the synthesis process, protofibrils of glucose chain are secreted through bacteria cell wall and aggregate together forming nanofibrils cellulose ribbons (Ross et al., 1991). These ribbons construct the web shaped network structure of BC with highly porous matrix (Dahman, 2009; Maria et al., 2010). The cellulose formed has abundant surface of hydroxyl groups that explaining it as hydrophilicity, biodegradability, and chemical-modifying capacity (Klemm et al., 2005). Further mechanism of BC synthesis was clearly explained by Chawla et al. (2009).

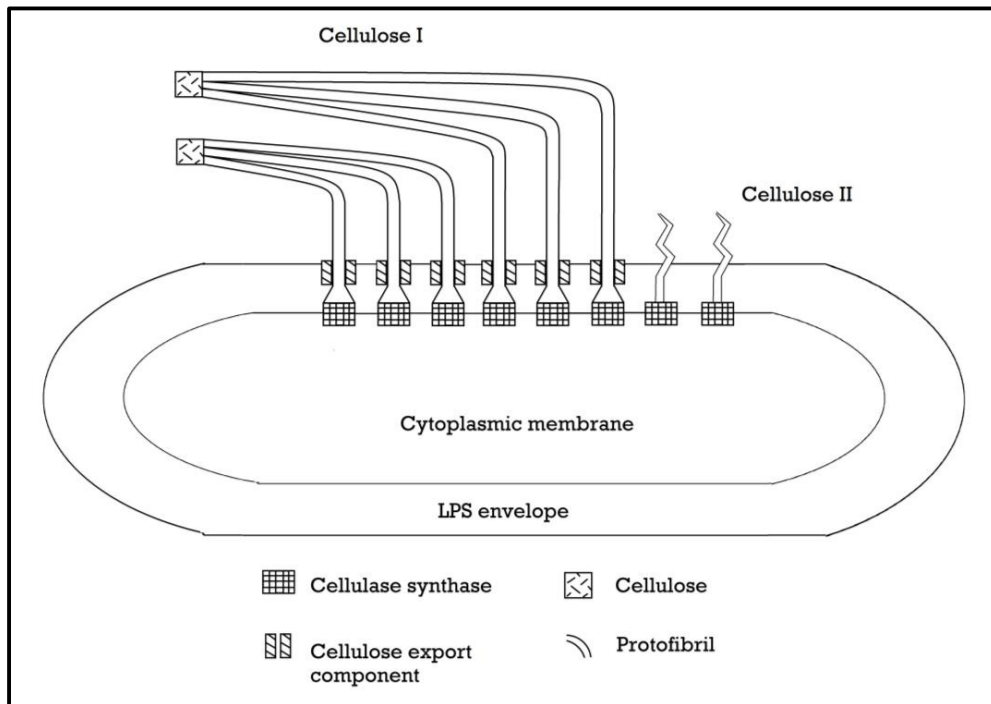


Fig. 2. Production of cellulose microfibrils by *Acetobacter xylinum* (Chawla et al. 2009).

### 3. Fermentation process

#### 3.1 Culture condition

Fermentation of BC production is conducted in either static, agitated or stirred conditions. Different forms of cellulose are produced under these conditions. Three dimensional interconnected reticular pellicle was reported under static condition while both agitated and stirred condition produced irregular shape sphere-like cellulose particle (SCP) (Tanskul et al., 2013). The process of cellulose formation under static condition is regulated by air supply from medium surface and the yield depends on the carbon source concentration moderately (Budhiono et al., 1999). The increasing of growth time will increase the formation of BC along with hydrogen and C-H bonding (Sheykhnazari et al., 2011). Synthesis of BC reach its limit when the pellicle growth downward and entraps all bacteria. Bacteria become inactive due to insufficient oxygen supply (Borzani and Desouza, 1995). Semi-continuous process in static condition is recommended at industrial scale as it manage to increase BC productivity compared to continuous process (Çakar et al., 2014).

In contrast, due to low yield of the static production, most of cellulose used in commercial purpose is generated through agitated fermentation. Agitated condition causes formation of SCP, an irregular forms of cellulose either in fibrous suspension, spheres, pellets or irregular masses (Yan et al., 2008, Wu and Lia, 2008). The SCP has lower crystallinity, mechanical strength and degree of polymerization compared to pellicle from static culture (Shi et al., 2013). The altered microfibrils organization was proposed related to the disruption effects of aeration on the hydrogen bonds formations between cellulose (Bootten et al., 2008). Study by Hu et al. (2013) found that the number of SCP decreased with the increasing volume of inoculums while different initial glucose concentration only gave an impact to the mean diameters of SCP. However, the mechanism of SCP formation is still remain unknown.

### 3.2 Culture medium

In biorefinery concept, a progressive transition to economically renewable materials as feedstock for chemical, materials and fuels is predicted to occur due to depletion of fossil resources (Octave and Thomas, 2009). In order to find a new economical culture medium for industrial scale production of BC, many studies have focused on agriculture waste and industrial by-product as potential medium (Kurosumi et al., 2009; Gomes et al., 2013; Çakar et al., 2014). Some of them have been proven as beneficial carbon source for BC production such as waste beer yeast (Lin et al., 2014), dry oil mill residue (Gomes et al., 2013), thin stillage (Wu and Liu, 2012), grape skin (Carreira et al., 2011) and maple syrup (Zeng et al., 2011). Besides that, the use of such products gives a positive impact on corresponding industry by decreasing the environmental problems associate with disposal of waste. In addition, medium supplementation with nitrogen and phosphorus sources were confirmed would increase BC production (Gomes et al., 2013).

The usage of glucose as carbon source during BC production is associated with the formation of gluconic acid as by-product in the culture medium. This by-product will decrease the pH of the culture and negatively affect the quantity of BC production. However the presence of antioxidant and polyphenolic compounds manages to inhibit the gluconic acid formation (Keshk and Sameshima, 2006).

### 4. Bacterial cellulose composite

BC has been applied in multiple field such as wound dressing (Muangman et al., 2011), blood vessel regeneration (Wippermann et al., 2009), and paper restoration (Santos et al., 2014). Although BC has unique properties, there is limitation that restricts its applications such as lack of antibacterial properties, optical transparency, and stress bearing capability. To overcome these limitations, BC composite has been introduced which consist of a matrix and reinforcement materials. BC owns a porous nature arrangement of fibres. It acts as matrix for housing a variety of particles from different reinforcement materials. The anchored reinforcement materials provide an additional properties to BC that impart its nature biological and physiochemical properties (Shah et al., 2013).

BC possess a potential as both matrix and reinforcement materials. Various BC composites have been synthesized through either in situ or ex situ methods. For the in situ method, reinforcement materials is added to the polymer during its synthesis (Saibuatong and Philsalaphong, 2010) while in the ex situ process, BC is impregnated with reinforcement materials (Ul-Islam et al., 2012). As shown in Table 1, various BC composites are synthesized with different function. BC composite can be either organic or inorganic material such as polymers (Kim et al., 2011), metal or metal oxides (Maneerung et al., 2007), solid materials (Meng et al., 2009) and nanomaterials (Yan et al., 2008).

Table 1. Bacterial cellulose composites and application.

No.	Application field	Reinforcement material	Function	References
1.	Electronic	Graphite nanoplatelet	Electrical conductivity	Zhou et al., 2013
2.	Electronic	Poly-4-styrene sulfonic acid	Redox flow battery	Gadim et al., 2014
3.	Biomedical / Industrial	Chitosan	Nanofilm	Fernandes et al., 2009
4.	Biomedical	Hydroxyapatite	Bone tissue engineering	Tazi et al., 2012
5.	Biomedical	Silver nanoparticles	Antimicrobial wound dressing	Wu et al., 2014, Zhang et al., 2013
6.	Biomedical	Paraffin	Bone scaffolding	Zaborowska et al., 2010
7.	Electronic	Polyurethane	Film substrate of light emitting diod	Ummartyotin et al., 2012.

### 5. Application of bacterial cellulose in food industry

BC is traditionally used to make nata de coco, an indigenous dietary fiber of South-East asia that served as gelatinous cube. Nata de coco has textural properties like chewy, soft and smooth surface. It has no cholesterol, low in fat and low calories. During the production process, BC was synthesized in static culture of coconut water (Jagannath et al., 2008). Coconut water served as carbon source for *Acetobacter xylinum* and later converted to extracellular cellulose (Cannon and anderson, 1991). The thick sheet of cellulose was washed, boiled and cooked in sugar syrup for food applications such as desserts, fruit cocktails and jellies. High fiber supplement mixture of nata

de coco and cereal was reported able to reduced lipid level of consumer (Mesomya et al., 2006). On the other hand, nata de coco is also a very promising medium for continuous bioethanol production in term of structural strength and cost effectiveness (Montealegre et al., 2012).

Packaging plays an important role to protect and preserve the food. Bio-based materials in packaging industry is more preferable nowadays due to concern arise on non-biodegradable packaging impact to the environment. BC has been identified as one of the suitable material (Tang et al., 2012) as it consist of fine network, biodegradable (Sonia and Dasan, 2013), and high water resistance performance (Arrieta et al., 2014). Even BC is an excellent choice for food packaging, unfortunately it has no antibacterial and antioxidant properties to prevent food contamination. Therefore, BC composites are used to gain these properties (Gao et al., 2014). Further BC application are summarized in Table 2.

Table 2. Application of modified bacterial cellulose (BC) and its composites in food industry.

No.	Materials	Function	Types of food	References
1.	BC/nisin	Antimicrobial food packaging	meat	Nguyen et al., 2008
2.	BC/polylysine	Biodegradable food packaging	sausage	Zhu et al., 2010
3.	BC	Emulsifier	surimi	Lin et al., 2011
4.	Carboxymethylcellulose	Regulate gough rheology	Flour dough	Correa et al., 2010
5.	Hydroxypropyl methyl cellulose	Texture enhancer	Whipped cream	Zhao et al., 2009
6.	Methyl cellulose	Enhancing shelf life	Egg	Suppakul et al., 2010
7.	Methyl cellulose	Enhance bioavailability	Vitamin C	Perez et al., 2013

## 6. Conclusions

Acetic acid bacteria are capable to replace plant as an alternative source of cellulose through oxidative fermentation under static, agitated and stirred conditions. However, high cost and low-yield production have limits its commercial applications and industry potentials. Therefore, researches have focus on agriculture waste and industrial by-product as new cost-effective carbon sources. Besides that, the modification and incorporation of particles in BC matrix enhance its nature physiochemical properties and bring up the new opportunity for applications.

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