

CONTROLLABLE FABRICATION OF BC BASED ON SHAKE REGULATES

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Abstract

To manufacture a high-quality and appropriate standard product its structure and manufacturing procedure must be controlled. Biomaterials have several features including biocompatibility, applicability and effectiveness in different environments; however, there are some limitations associated with development. Bacterial cellulose (BC) achieved from *Gluconacetobacter* / *Acetobacter Xylinum* is a highly crystalline and mechanically stable nanopolymer. BC establishes unique properties containing high-water holding and elevated porosity, high mechanical strength and superior crystallinity. Its properties make it a very useful biomaterial in various processes. However, its physical features (dimension) are challenging and are supposed to be controllable, manageable and invariable in repetitive experiments. Therefore, in this paper, a convenient method of BC fabrication is introduced using Shake regulates. Examining the results of measuring and charting behavior, the effectiveness of this method has been proven. This technique obtains several advantages consisting of invariable BC thickness and interchangeable samples in recapitulation investigations.

Keywords: *Acetobacter Xylinum*, Biomaterial, Bacterial Cellulose, Controllable Fabrication, Shake Regulates, New Generation

Introduction

Science and technology has a tendency to further utilizing renewable raw materials (Peciulyte et al. 2015; Tsouko et al. 2015), which are biocompatible with environment friendly and sustainable resources (Jmel et al. 2016; Carpenter et al. 2015; Reddy, Yang 2015). Undoubtedly, one of them is cellulose and cellulose derivatives which are of growing importance

for the development and application of polymer materials (Usov et al. 2015; Zhong et al. 2015; Carpenter et al. 2015). This development has led cellulose research and application to be known widely all over the world in the last two decades (Usov et al. 2015). Therefore, some researchers concentrated on increasing the current knowledge of polymer chemistries (Bougrini et al. 2015; Figueiredo et al. 2015) and organic especially in the chemistry of low molecular weight carbohydrates (Sulej et al. 2015; Peciulyte et al. 2015). Moreover, polysaccharides are basically investigated for oriented application on the scope of cellulose (Al-Shorgani et al. 2016; Li et al. 2016). Furthermore, various researchers have investigated the interdisciplinary interaction between physics, biology, medicine, pharmacy, material science and chemical engineering (Smith-Moritz et al. 2015; Sulej et al. 2015; Bougrini et al. 2015).

As a consequence of this expanding development, complex structures of cellulose and cellulose derivatives in the solid state and in solution form have been obtained. In the following, it can be seen that the biosynthesis and in-vitro synthesis of cellulose have been developed in the cellulose reactivity, reaction control, selective syntheses, and regarding supermolecule structure formation (Gonçalves et al. 2015; Lee et al. 2015; Smith-Moritz et al. 2015). These achievements include the fast progression of polysaccharide instrumental analysis and a deeper understanding about the relation between product structure, reaction conditions, and application-oriented properties. Accordingly, it has been workable for improving new types of cellulose esters and ethers as “polymers of the future” (Klemm et al. 2005).

Cellulose biosynthesis knowledge has caused the fundamental changes so that cellulose is accepted as a raw material (Jiang et al. 2016; Pan et al. 2016). In fact, efforts are mostly focused on purification and sequence cellulose synthase. Moreover, these efforts are dedicated to the associated proteins for producing a reproducible cell-free system which is capable of generating crystalline cellulose. (Saini et al. 2016)

Cellulose biosynthesis faced a new phenomenon gene that makes a significant difference (Sulej et al. 2015; Smith-Moritz et al. 2015). Undoubtedly, genes modify cellulose biosynthesis in outstanding cellulose-forming organisms such as trees, cotton crops, and bacteria. However, this introducing generates various types of cellulose for pulp, paper, building materials, textiles, and other fields of application. Klemm believed that if cellulose-forming bacteria could be cultivated on a large technical scale, the demand for cellulose could be satisfied entirely by this source in the future (Klemm et al. 2005).

The species of bacteria which produce cellulose is generally called *Acetobacter xylinum*, although bacteria with different names are often used in literature (Zendehbad 2015; Li et al. 2015; Erbas Kiziltas et al. 2015).

Simple in nature, the categories of bacteria are discovered, for mention in rotten fruits or vegetables as more than thirty cases have been reported (Jozala et al. 2014). The reason why the microorganisms generate cellulose has been a question of biologists. The aerobic bacteria fabricate pellicle to maintain their position near to the surface of culture solution (Saini et al. 2016). On the other hand, the bacteria generate cellulose to protect themselves from ultraviolet illumination (Bet-moushoul et al. 2015; Iguchi et al. 2000).

Bacterial cellulose (BC) has interested a significant number of researchers due to its academic purpose in addition to captivating commercial sectors owing to its various applications in pharmacy (Huang et al. 2013), biotechnology (Sulej et al. 2015), biomedical science (Gao et al. 2016; Tsalagkas et al. 2016), electro-conductivity (Babaev et al. 2012; Guo et al. 2013; Li et al. 2012) and other scopes in biomaterial science. Particularly, the motivation of such interests can be found in simple production of a fully biobased cellulosic material which has specific properties (Shilin Liu, Jinping Zhou 2013). These mechanical properties consist of high-tensile strength and modulus (Scionti 2010), high purity, water-binding capacity (Roy et al. 2012; Numata et al. 2015), and ultra-fine network structure (Shao et al. 2015; Zendehbad 2015).

Generally, Bacterial cellulose microfibrils properties have been found to be useful as reinforcing agents in composite materials (Saini et al. 2016; Fijałkowski et al. 2015). Moreover, the Raman spectroscopy technique (RST) of the Young's modulus for BC's single filament presents 130–140 GPa (Eichhorn et al. 2010).

Many strains of bacteria control the ability to manufacture cellulose at the surface of a medium embracing nitrogen and carbon as the food source. These include *Sarcina* (Lee et al. 2012), *Agrobacterium* (Huang et al. 2013), *Rhizobium* (Gea et al. 2011), and *Acetobacter* (Zendehbad 2015). Römling et al. believed that the rod-shaped and Gram-negative *Acetobacter xylinum* are the only species capable of fabricating cellulose in widespread commercial quantities. On that ground, methods of producing BC have rapidly been developed aiming at improving yield, structure (Römling, Galperin 2015), and other desired physical properties (Usov et al. 2015; Li et al. 2015).

On the other hand, other related sources to bacterial cellulose have been also investigated such as various production manners (Lee et al. 2012), pH level (Shao et al. 2015), the medium used for culture (Kirdponpattara et al. 2015), and the source of nitrogen / phosphate for *A. xylinum* as food sources (Gea et al. 2011). Doubtless, the purification of cellulose is a crucial factor in the fabrication of cellulose products (Carpenter et al. 2015; Tsouko et al. 2015). Practically, this technique is intended for displacing all the

unsought residual insoluble lignin and additional chemicals bound to the cellulose fibers which are processing and converting into a compound. Consequently, various approaches and different chemicals have been used to fabricate high-quality bacterial cellulose (Atwa et al. 2015).

The laboratory bacterial culture of cellulose-formation is an attractive approach to pure cellulose for both polymer science and organic chemistry (Peciulyte et al. 2015). The cellulose biosynthesis covers plants, fungi, algae, bacteria as well as *Acetobacter*, *Achromobacter* spp. and *Acanthamoeba* (Lakhundi et al. 2015; Atwa et al. 2015).

Approaches to controlling the molar mass /mass distribution, and the supramolecular structure have been found through selecting substrates, cultivation conditions, various additives, and eventually the bacterial strain. Therefore, there is an opportunity to control the cellulose properties, and biosynthesis mean such as kinetics, yield, and other metabolic products (Feng et al. 2012; Ul-Islam et al. 2012).

Practically, among the cellulose-forming bacteria, *Acetobacter*/*Gluconacetobacter* strains are especially applicable to the fabrication and investigation of cellulose (Yang et al. 2014; Zendehbad 2015). These gram-negative (Legnani et al. 2008) and strictly aerobic bacteria from straight, ellipsoidal, or slightly bent rods, which are recognized on naturally grown both fruits and their products. Strains of *Acetobacter xylinus* / *Gluconacetobacter xylinus* species yield extracellular cellulose (Yang et al. 2014; Li et al. 2015). Moreover, this production is easily isolated as fiber material. Generally, a biofilm whose thickness can be changed is constructed under static immersed cultivation conditions (Klemm et al. 2011). It assists the colonized bacteria to support a high volume of oxygen close to the surface. Furthermore, it serves as a protective barrier against drying, radiation and natural enemies (Pan et al. 2016; Saini et al. 2016).

Glimpse into research shows that functional nanocomposites or nanohybrids based on biopolymers, and nanometer-scale fillers are of scientific and highly-developed interest owing to their bio functionality and notable performance improvement (Saini et al. 2016; Martínez Ávila et al. 2014). Bacterial cellulose (BC) has a crystalline nano-/microfibril structure, which contains excellent mechanical properties (Feng et al. 2012; Numata et al. 2015). As shown in table 1 BC suits as a reinforcing agent for some productions such as papers and fibers made from glass, carbon, phenol resin, and silicon in small quantities (5%). (Numata et al. 2015; Scionti 2010) Moreover, according to its high modulus of elasticity in combination with a large internal loss factor, it is also a distinguished material for producing headphone and loudspeaker membranes (Wang et al. 2011).

Material	Tensile Strength [MPa]	Young's Modulus [GPa]	Elongation [%]
bacterial cellulose (BC)	200–300	15–35	1.5–2.0
cellophane	20–100	2–3	15–40
polypropylene (PP)	30–40	1–1.5	100–600
polyethylene terephthalate	50–70	3–4	50–300

Table 1 Comparison mechanical properties, Bacterial cellulose and other organic layer materials

One of the major topics in production from renewable feed stock is producing high value-added materials from residual stream's origination (Reddy, Yang 2015). This is an interesting possibility and could be an integral part of existing pulp mills and lignocellulosic bio-refineries. In this category, bacterial cellulose (BC) has been identified as a nanostructured material provided by varying species of acetic acid bacteria (Huang et al. 2015; Huang et al. 2013). However, this natural polymer has attracted significant attention on account of its properties such as biocompatibility, high-tensile strength, excellent water-holding capacity and crystallinity (Klemm et al. 2011; Huang et al. 2013; Zendehbad 2015).

In other views, commercial applications of bacterial cellulose have been rapidly developed over the past few years (Wang et al. 2011; Müller et al. 2011). Accordingly, reference can be identified as extend the use of biological materials such as proteins, cell cultures and microorganisms to product for temporary skin and tissue replacement. The following can be cited to Bio fill, Bioprocess, Gengiflex and additives in the production of lattices and paper (Ul-Islam et al. 2012; Shilin Liu, Jinping Zhou 2013).

The objectives of this research is to examine and evaluate how appropriate is shake regulates for production of BC. It also aims to investigate the possibility to control the production of BC with different layers. In addition, we investigated the metabolic preferences of the bacterium, *Acetobacter /Gluconacetobacter xylinum*, used for the production of bacterial cellulose.

Methods

Materials

In this study, strains of *A. xylinum* for the production of BC were supplied by the Microbiology Laboratory of the Huazhong University of Science and Technology, Wuhan China. The chemicals used for the culture medium were glucose, peptone, disodium phosphate, citric acid and yeast extract. In the purification process, sodium hydroxide, sodium hypochlorite and distilled water were used to assist the growth of the bacteria.

Sample preparation

Preparation of BC pellicle

The culture medium for the production of BC was prepared via the procedure reported by (Zendehbad 2015). *Gluconacetobacter xylinum* (ATCC53582) was used for the biosynthesis of BC. For every litre of distilled water 2% (wt) glucose, 0.5% (wt) yeast extract, 0.5% (wt) peptone, 0.27% (wt) disodium phosphate, and 0.15% (wt) citric acid and the bacterium cultured in a Hestrin and Schramm (HS) medium were added. This culture medium was autoclaved at 121° C for 2 h and cooled to room temperature.

Treatment of BC pellicle

This medium was divided and poured into the pipes which are selective of several kinds of tubes and flasks in different group times. In this work, the number of glass tubes was used for experiments was 100 tubes of 70× 20mm sizes; whereas, the value and volume of them are measured. Then, we added the suitable culture with specific value and wait for incubating statically for specific number of days at 26 °C. All samples were investigated daily and were recorded by the time subsequently (Zendehbad 2015). After harvesting, the BC membranes were dipped into distilled water for two days, and later keep in a 1 wt% NaOH solution for 30 min to eliminate bacteria and proteins. Afterwards, the BC membranes were purified through washing in distilled water various times, and were then stored in it at 4° C.

Processing of shake regular

The traditional method of preparing bacterial cellulose was mentioned above (Klemm et al. 2005) in which this process served as a control method during our investigation. Our previous experiment (Zendehbad 2015) was supposed to analyze time effect in BC process. It was presented in the same conditions. Therefore, here, the focus was on shake effect on the process. After 7days, it was found out that there was a thin membrane (layer) above liquid and sticking to the inside of the glass tube (figure 1).

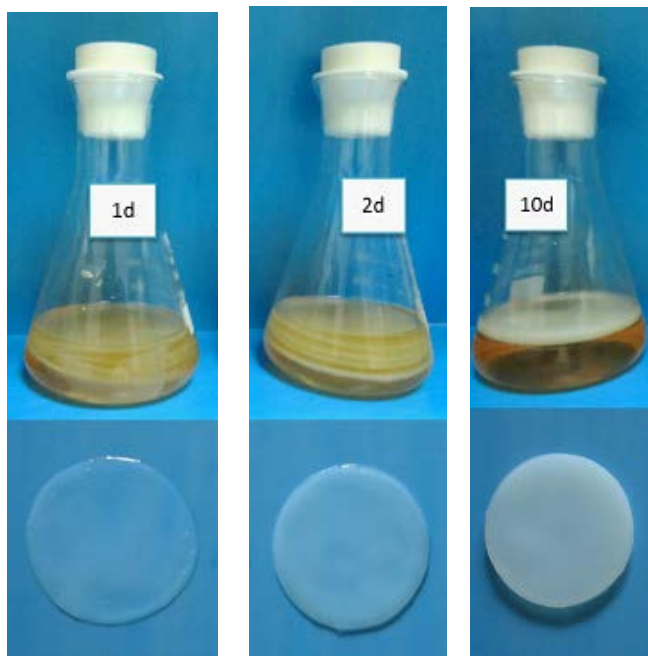


Figure 1: The bacterial cellulose preparation: according to the days, the BC membrane thickness is increased. (1d, 2d, 10d) (Zendehbad 2015)

The process was going when test tube was shaken slowly until the membrane was gotten free from the tube well and was immersed in the medium (figure 2). Then, all the target tubes were put back in the ordinary place and were kept for the next seven days. This method was expected to continue for selective test tubes while other tubes were in constant condition and normal situation.

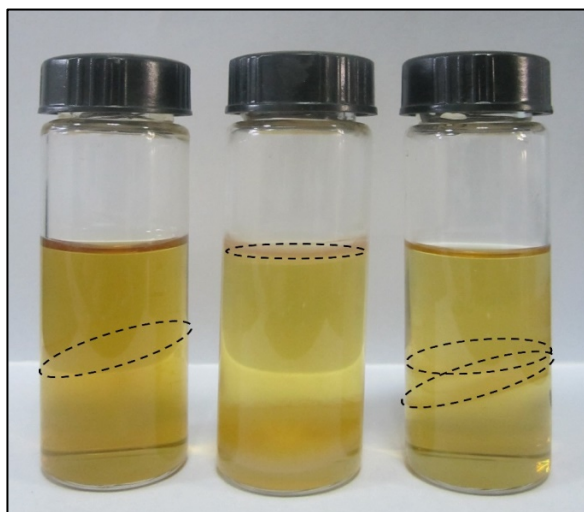


Figure 2: Bacterial cellulose under treatment, control thickness of membrane, From left to right: after one shake, without shake, after two shakes

Characterization

The morphology of the bacterial cellulose pellicle was investigated using an optical microscope. Advanced microscopy techniques (AMT) were exploited for the characterization of cellulose structure and cellulose-cellulose interactions and some instrumentations. After removing BC from solution we measure the weight and thickness of each membrane. Actually, using an optical microscope we were able to investigate the surface of BC; however, a scanning electron microscope (SEM) and X-ray diffraction (XRD) were mostly used for the deep survey of them.

Results and discussion

Figure 1 shows several kinds of BC membranes in terms of time. Mainly, plant cellulose is naturally bound with some materials such as lignin, hemicellulose and other chemicals while BC fortunately is of relative high-purity and is not bound with other chemicals (Gea et al. 2011). According to these properties, it is expected that the purification process used for this study would be different from purification process of plant cellulose. In fact, the purification process aimed to remove the remaining organic material part, whereas, this is a food supply for the microbes in the medium. BC was fabricated, besides, we investigated shake effect on its growth behavior (figure 2). Principally we made a standpoint to shake regularly and record effective outcomes. As it is shown in figure 2, the number of layers depends on shake times in a suitable growth time. Further, the shake effect is an important factor when there is a thin and visible layer sticking to the inside of the glass tube.

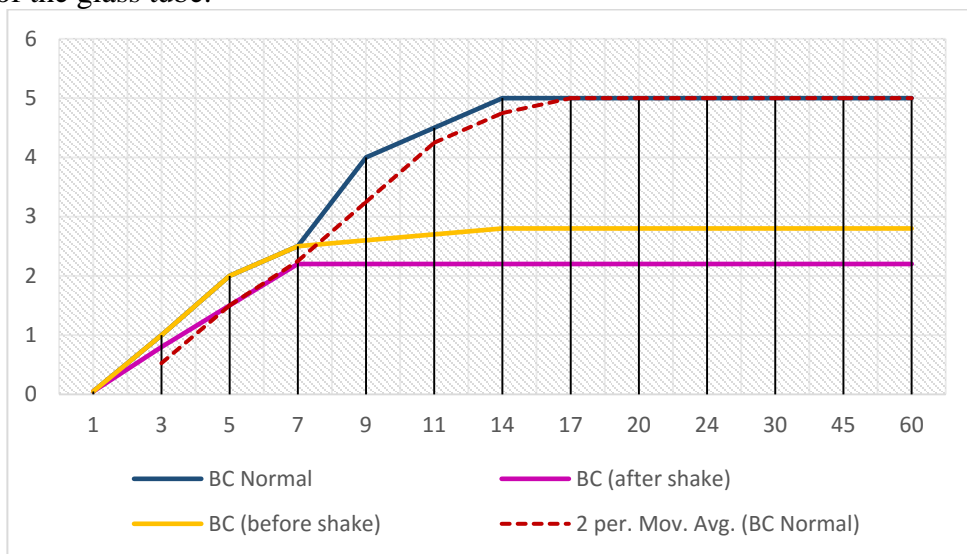


Figure 3 Behavior of BC under air and shake control

However, we measured the thickness of each membrane from selective test tubes and found that the sum of thickness size was equal. According to the database and daily recorders, one graph was achieved to identify behavior of bacterial cellulose membrane during this process. Figure 3 shows the graph which depicts slow BC growth movement before, and after shake effect while the control samples were naturally growth. The main point for all specimens is the constant conditions and materials. Certainly, in this method, we reproduced from initial membrane to another using regular shakes. Moreover, we can say two membranes are from one family.

Conclusion

The laboratory bacterial culture of cellulose-formation is an attractive approach to obtaining pure cellulose for both polymer science and organic chemistry. Although it is practical to control the supramolecular structure or the molar mass distribution through selecting substrates, cultivation conditions, various additives and bacterial strain, its dimension (size and shape) is disputed and must be controllable and invariable in repetitive experiments. As it was, even though we fabricated bacterial cellulose using the common approach, we reproduced new BC's membrane from another one. This laboratory method helps to provide the same quality and quantity of bacterial cellulose samples which is an essential factor in an experiment. Our study approach has several advantages such as interchangeable samples, invariable BC thickness and cost variables in recapitulation investigations.

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Abbreviations

BC: Bacterial cellulose; *G. xylinus*: *Gluconacetobacter xylinus*; *A. xylinus*: *Acetobacter xylinus*; SEM: Scanning electron microscope; AMT: advanced microscopy techniques; XRD: X-ray diffraction; RST: Raman spectroscopy technique.

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