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Bacterial cellulose production, properties and applications with different culture methods – A review

Jing Wang[a,b], Javad Tavakoli[b], Youhong Tang[b,*]

[a] Key Laboratory of Advanced Textile Composite Materials of Ministry of Education, Institute of Textile Composite, School of Textile Science and Engineering, Tianjin Polytechnic University, Tianjin 300387, China

[b] Institute for NanoScale Science and Technology, Medical Device Research Institute, College of Science and Engineering, Flinders University, South Australia 5042, Australia

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ABSTRACT

Bacterial cellulose (BC) is an organic compound produced by certain types of bacteria. In natural habitats, the majority of bacteria synthesize extracellular polysaccharides, such as cellulose, which form protective envelopes around the cells. Many methods are currently being investigated to enhance cellulose growth. The various celluloses produced by different bacteria possess different morphologies, structures, properties, and applications. However, the literature lacks a comprehensive review of the different methods of BC production, which are critical to BC properties and their final applications. The aims of this review are to provide an overview of the production of BC from different culture methods, to analyze the characteristics of particular BC productions, to indicate existing problems associated with different methods, and to choose suitable culture approaches for BC applications in different fields. The main goals for future studies have also been discussed here.

1. Introduction

The discovery of cellulose by Payen in 1838 resulted in the introduction of a linear organic polysaccharide that is insoluble in water and exhibits hydrophilic behavior (Payen, 1838). When cellulose is treated with concentrated acids at high temperature, its chiral structure can degrade chemically into its glucose units (Béguin & Aubert, 1994). However, due to the lack of appropriate enzymes to break down the beta acetal linkages, cellulose is not degradable in the physiological environment. Cellulose is the main structural component of the primary cell wall of most plants, but it is mainly obtained from the plant sources of cotton fiber, dried hemp, and wood, that contain approximately 90%, 40–50%, and 60% cellulose, respectively. Cellulose has an extraordinary commercial reputation in paper, textile, cellulose nanofibrous mats, and pulp production units (Deng et al., 2010; Klemm, Heublein, Fink, & Andreas, 2005; Piotrowski & Carus, 2011; Zhang et al., 2015). Apart from plants, cellulose is also found in many microorganisms such as fungi, bacteria, and algae. The first report of cellulose produced from bacteria, specifically from Acetobacter *xylinum* (*A. xylinum*), was announced by Brown in 1886. Recent studies have revealed that cellulose can be produced by different bacteria, including Gram-negative bacteria species such as *Acetobacter Azotobacter, Rhizobium*, *Agrobacterium*, *Pseudomonas*, *Salmonella*, *Alcaligenes*, as well as Gram-positive bacterial species such as *Sarcina ventriculi*, as shown in Table 1 (Brown, 1886; Jonas & Farah, 1998). The celluloses produced by different bacteria possess different morphology, structure, properties, and applications. Among the aforementioned bacteria, the most effective sources for the production of bacterial cellulose (BC) are *A. xylinum*, *A. hansenii*, and *A. pasteurianus*. Among these, *A. xylinum*, also called *G. xylinum*, has been used for producing commercially available BC due to its high productivity.

Although both plant-based cellulose (PC) and BC are natural, significant differences have been found between them in terms of purity, macromolecular properties, and characteristics. Compared to PC, BC has high Young's modulus value (Brown, Willison, & Richardson, 1976; Hsieh, Yano, Nogi, & Eichhorn, 2008; Nakagaito, Iwamoto, & Yano, 2005), high water uptake capacity, and fibers of BC have a high aspect ratio (Eliane et al., 2010). Hence, a comparison of the properties of BC and PC is provided in Table 2, with references.

In recent years, many reviews have highlighted the properties and potential applications of BC (Abeer, Mohd, & Martin, 2014; Blanco et al., 2018; Irina, Ute, Thomas, & Antje, 2015; Lin et al., 2013; Mohite & Patil, 2014; Picheth et al., 2017; Ullah, Wahid, Santos, & Khan, 2016). As well, the impact of different factors, such as nutrient (carbon source, nitrogen source, microelement, and so on), pH, speed, density, shear force, viscosity of nutrient, and oxygen delivery, on the

* Corresponding author.
E-mail address: youhong.tang@flinders.edu.au (Y. Tang).

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Table 1
Bacterial sources for production of BC with different structures and biological roles.

Genus	Biological role
Fibril structure	
Aerobacter	Flocculation in wastewater
Agrobacterium	Attachment to plants
Alcaligenes	Flocculation in wastewater
A. hansenii	Maintenance in aerobic bio-reactors for fermentation
Rhozobium	Attachment to plants
Ribbon structure	
Acetobacter	Maintenance of aerobic environment
Achromobacter	Flocculation in wastewater
3D network nanofiber	
Gluconacetobacter	Aerobic environment
Amorphous/ not defined	
Pseudomonas	Flocculation in wastewater
Sarcina	Unknown
Zoogloea	Unknown

production of BC has been discussed (Mohammadkazemi, Azin, & Ashori, 2015; Pacheco et al., 2017). To the best of our knowledge, the literature lacks a comprehensive review of the different methods of BC production, which is critical to the development of BC properties and their final applications. The aim of this review is to provide an overview of the production of BC by different culture methods, analyzing the characteristics of several methods of BC production, indicating the problems of different methods, and selecting suitable culture approaches for BC applications in different fields.

2. Culture methods for BC formation

Current methods for BC preparation include static, agitated/shaking, and bioreactor cultures. The resulting macroscopic morphology, microstructure, and properties of BC are quite different. The static culture method results in the accumulation of a gelatinous membrane of cellulose at the surface of the nutrition solution (Rani & Appaiah, 2011), whereas the agitated/shaking culture results in asterisk-like, sphere-like, pellet-like or irregular masses (Watanabe, Tabuchi, Morinaga, & Yoshinaga, 1998). Selection of the method depends on the final applications of the BC as well as the physical, morphological, and mechanical characteristics required.

The condition of the culture environment, including bacteria strain, nutrition, pH, and oxygen delivery, is also crucial and impacts the properties of BC. The roles of different bacterial strains or of various nutrient sources in BC formation have been discussed (Pourramezan, Roayaei, & Ghezelbash, 2009; Zeng, Small, & Wan, 2011). The bacterium *A. xylinum*, also known as *G. xylinum*, has been shown to have the highest rate of BC production among all the bacteria types. This aerobic bacteria strain is non-photosynthetic and can transform glucose and other organic substrates into cellulose within several days. It has been observed that one bacterium can convert 108 glucose molecules per hour into cellulose. Independent of the type of bacteria, their life cycle can affect the rate of production. It has been well described that, during static culture, the bacterial growth can be modeled with four different phases, as illustrated in Fig. 1(a) (Sushil, 2018). The majority of BC is produced during phases B and C.

In recent years there has been a focus on the feasibility of use of agricultural- and industrial-based wastes as nutrient sources, to reduce production costs. The properties of the produced BC from different waste medium and carbon sources have almost same physicochemical properties as those produced from the commercial H–S media. Meanwhile, these researches provided a broad idea about the potential of waste materials for ecofriendly and inexpensive BC production. (Castro et al., 2011; Revin, Liyaskina, Nazarkina, Bogatyreva, & Shchankin, 2018; Tsouko et al., 2015; Waleed, Taous, Mazhar, Fazli, & Joong, 2015; Wang, Li, Hua, Jia, & Zhang, 2015.). Under different culture medium, the formation process and growth curve of BC are similar. During culture, the morphology of BC transforms from floccus to film. The microfibers intertwine and aggregate with each other, forming irregular meshes or flocculent structures. At the beginning, although the nutrient for the bacteria is adequate, the bacteria concentration is very low and BC films form slowly, as shown in Section B in Fig. 1(a). Subsequently, bacteria strains expand rapidly, and the culture medium still maintains adequate nutrient. Thus, more BC films are secreted, as shown in Section C in Fig. 1(a). However, when nutrients are depleted by constant consumption, the increase in BC film thickness can affect the oxygen supply for strains, suppressing the formation of BC. It has been reported that glucose and acetic acid are essential nutrients for strains. During the early incubation process, the consumption of acetic acid and the production of glucose acid can remain constant in terms of pH and the fermentation environment. However, if the glucose content in the culture medium is greater than that of the acetic acid, the bacteria can actively and continuously convert glucose into gluconic acid during the fermentation process. Finally, media with low pH are suboptimal for bacteria growth, with experimental results indicating that BC biosynthesis terminated when the pH value was outside the suitable pH range for BC formation of $4 < pH < 7$ (Koizumi et al., 2008; Vandamme, Baets, Vanbaelen, Joris, & Wulf, 1998).

2.1. Static culture method

The static culture method is a traditional approach for production of BC and has been widely used. In this method, containers are filled with fresh nutrient solution and incubated for 1–14 days at suitable temperature and pH, namely 28–30 °C and $4 < pH < 7$. BC produced by the static culture method is a hydrogel sheet with excellent structure and properties. The membrane of BC harvested via the static culture is shown in Fig. 1(b–f). The fresh BC harvested was primrose yellow, as shown in Fig. 1(b) (Auta, Adamus, Kwiecien, Radecka, & Hooley, 2017). After purification using hot water and sodium hydroxide, samples were rinsed with plenty of water to reach a neutral pH, when the BC became white, as shown in Fig. 1(c). In the static culture, membranous BC is formed on the gas-liquid interface where entrapped carbon dioxide is generated from the bacterial metabolism. The thickness of the BC membrane increases with the increase in culture time, as shown in Fig. 1(d). Because BC film is formed on the surface of the nutrient solution, the BC production is directly related to the surface area of the air-liquid interface. The sub-fibrils of cellulose are continuously extruded from linearly ordered pores at the surface of the *A. xylinum*, crystallized into microfibrils. Therefore, the BC pellicle, supporting the population of *A. xylinum*, included overlapping and intertwined cellulose ribbons, forming parallel but disorganized planes (Krystynowicz et al., 2002). Then the cellulose assembles into a thick membrance on the surface of the medium. The produced BC as shown in Fig. 1(e) and (f) has 3D networks with high porosity. The static culture method is a relatively simple technique with low shear force environment; therefore, it is the most frequently used technique for the formation of BC at lab scale.

2.2. Agitated/shaking culture

High cost and low rate of production are the two main problems in static culture systems. To solve these problems, use of an agitated/shaking culture has been suggested. The delivery of oxygen is directly associated with the production of BC and is known as a major drawback of the static culture method. However, excessive oxygen supply has been shown to result in a decrease in BC formation. The basic idea behind the design of an agitated/shaking culture was to increase or optimize the delivery of oxygen to the bacteria during culture. It was found that, despite increasing the rate of oxygen delivery to the culture

Table 2
Comparison of properties for bacterial and plant-based cellulose.

Properties	BC	PC	References
Tensile strength (MPa)	20 – 300	25 – 200	Feng et al., 2015; Gibson, 2012
Young's modulus (MPa)	Sheet: 20,000 Single fibre:130,000	2.5 – 0.170	Lynd, Weimer, van Zyl, & Pretorius, 2002; Nishi et al., 1990
Water holding capacity (%)	> 95	25 – 35	Rebelo et al., 2018; Islam, Taous, & Joong, 2012; Boulos, Greenfield, & Wills, 2000; Goto & Yokoe, 1996
Size of fibers (nm)	20-100	micrometer scale	Monika, Justyna, & Artur, 2011; Genet et al., 2005
Crystallinity (%)	74 – 96	40 – 85	Park, Baker, Himmel, Parilla, & Johnson, 2010
Relative hydrophilicity (%)	40 – 50	20 – 30	Bishop, 2007
Purity (%)	> 99	< 80	Klemm et al., 2005
Degree of polymerization	14000 – 16000	300 – 10000	Tahara et al., 1997
Porosity (%)	> 85	< 75	Elham & Amir, 2013
Total surface area (m^2/g)	> 150	< 10	Islam et al., 2012; Alexander, Ibon, & Jürgen, 2002

media, both agitation/shaking and static resulted in production of the same quantity of BC over an equal duration. Moreover, some studies reported that a lower quantity of BC was produced by the agitated/shaking culture method than that by the static culture method (Czaja, Romanovicz, & Brown, 2004; Inagaki & Phillips, 1989; Toyosaki et al., 1995). Contributing factors were the appearance of a non-cellulose mutant and the genetic instability of bacteria under agitated conditions, which may reduce the BC productivity (Chawla, Bajaj, Survase, & Singhal, 2009; Yang et al., 2014). A time course of BC synthesis in both static and agitated cultures is shown in Fig. 2(a), indicating no significant difference in the final concentration of the BC produced by the two culture methods (Czaja et al., 2004).

Although studies revealed that the agitated/shaking culture method was not suitable for all bacterial strains to increase the BC yield, it did allow the bacteria to produce BC of different particle sizes ranging from 10 μm to 10 mm in diameter and in various shapes including spherical

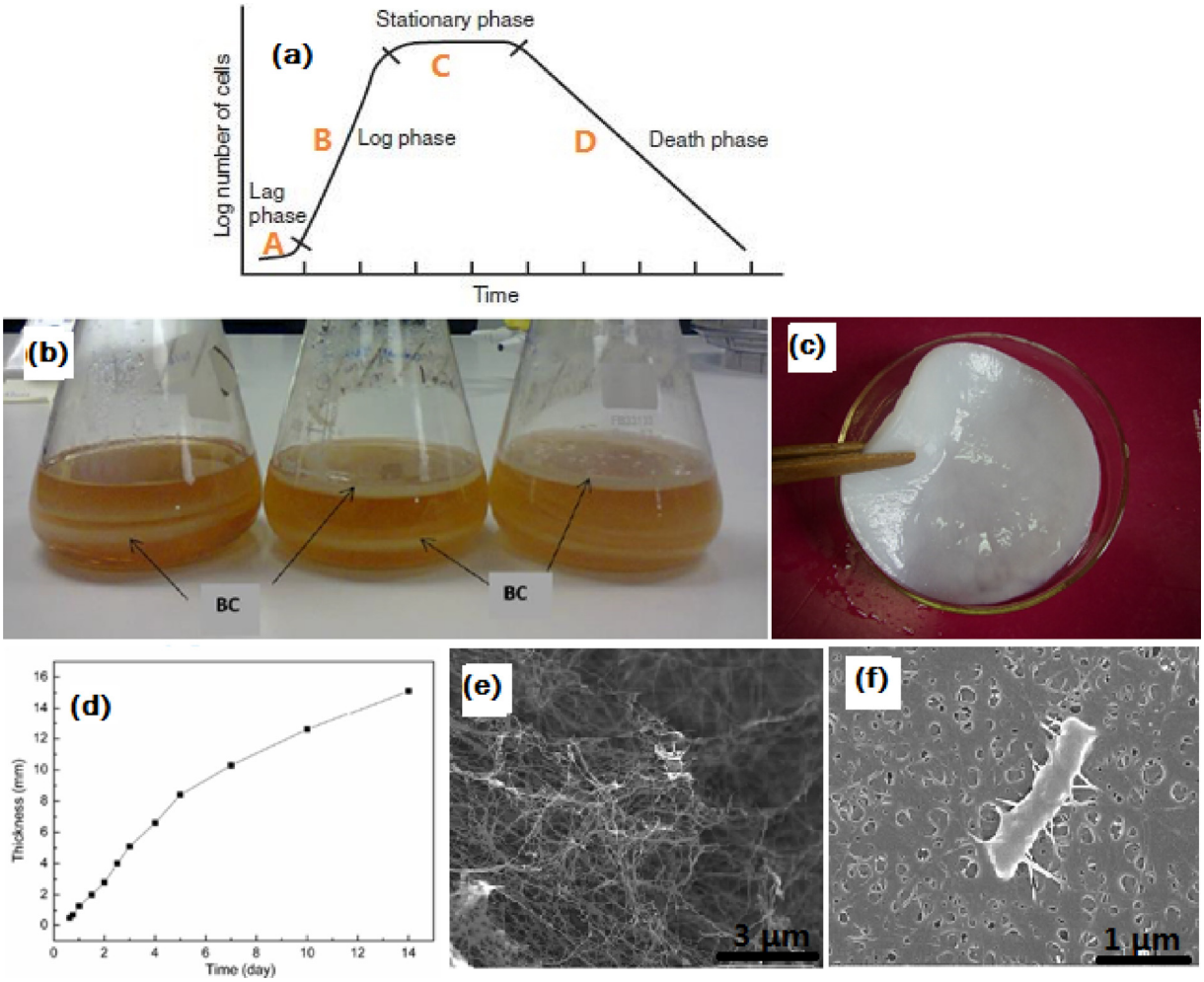

Fig. 1. (a) BC growth curve. Section A. Lag phase: Duration depends on conditions and species of bacteria. Section B. Exponential or log phase: Several hours (depending on species and density of bacteria, and conditions). Section C. Stationary phase: Cell growth = cell death, a constant bacterial population. Section D. Death phase or decline phase: An exponential process, number of cells decreases (Source: Reprinted with permission from Sushil, 2018), (b) Preparation of BC membrane using static culture method (Source: Reprinted with permission from Auta et al., 2017), (c) BC membrane after purification with water and sodium hydroxide, (d) BC membrane thickness at different culture times (Source: Reprinted with permission from Luo, Zhang, Xiong, & Wan, 2014), (e) SEM images of BC obtained in a static culture, and (f) SEM images of BC including bacteria.

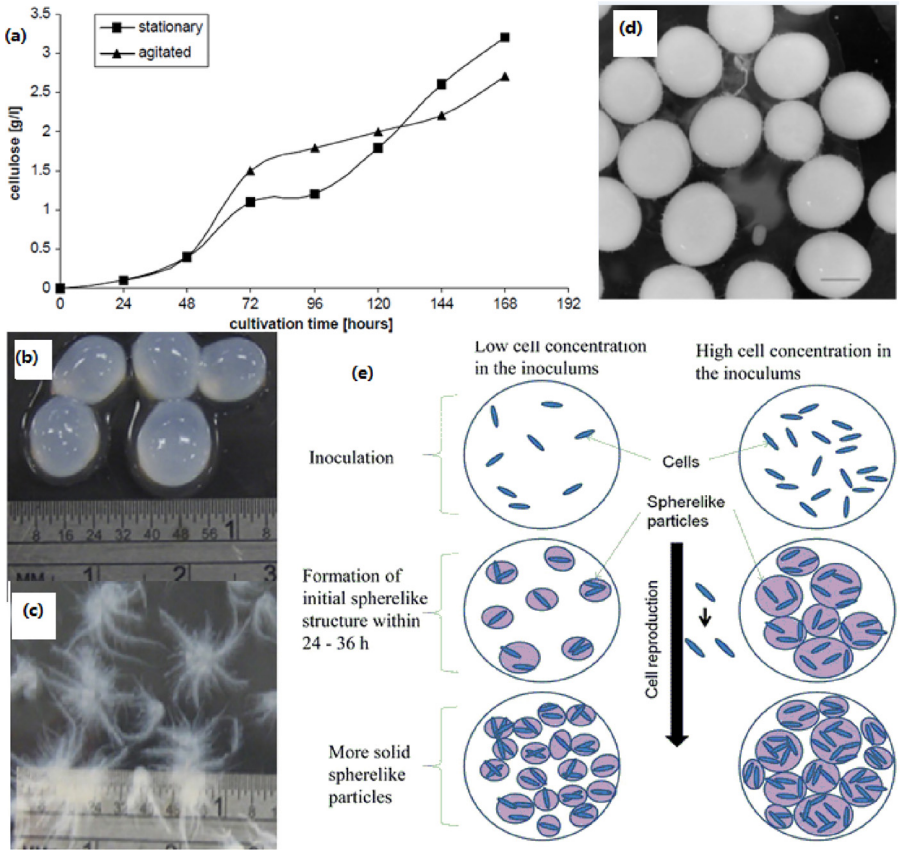

Fig. 2. (a) Time course of bacterial cellulose synthesis in static and agitated cultures. Optical images of BC assembly formed in the presence of (b) arabinogalactan and (c) xyloglucan and pectin (1:3). (d) Sphere-like BC formed in agitated culture and (e) schematic illustration of the formation of sphere-like BC using different bacteria concentrations in the inoculums (Source: Reprinted with permission from Czaja et al., 2004; Gu & Catchmark, 2012; Hu et al., 2013).

with the size of 1–9 mm, ellipsoidal with the size of 3–5 mm, stellate, fibrous suspensions, pellets, or irregular masses. The size and shape of the BC is related to the rotating speed, culture time, and additive types in the culture medium (Watanabe et al., 1998). Fig. 3(b) and (c) shows different shapes of BC obtained during agitated culture in the presence of different additives (Gu & Catchmark, 2012). Fig. 2(d) displays sphere-like BC formed in an agitated culture without additives (Czaja et al., 2004). Furthermore, *A. xylinum* strain NQ5 (ATCC 53,582) and *A. xylinum* JCM 9730 strain in agitated culture were found to produce sphere-like BC in the form of isolated spheres (Czaja et al., 2004; Hu & Catchmark, 2010a). Under rotationally agitated cultivation, the continuous shear force during agitated culture is the first influence that forms a spherical structure. Duration of culture is another factor influencing the size and quantity of spherical BCs. The produced BCs become larger with an increase in duration. However, after 60 h no further change in size was observed by various researchers (Czaja et al., 2004; Hu and Catchmark, 2010a; Hu, Catchmark, & Vogler, 2013). It was also found that the concentration of bacteria could affect the size and quantity of sphere-like BC. A higher concentration of bacteria can lead to the production of more BC spheres. Fig. 2(e) is a schematic illustration of the formation of sphere-like BC under different bacterial strain concentrations (Hu et al., 2013).

Rotation speed in an agitated culture also plays an important role in the formation of sphere-like BC. With a rotational speed less than 100 rpm, it is difficult to find any sphere-like BC particles; rather, irregular shapes of the synthesized BC have been observed. In experiment, the spherical BC shape became evident at 125 rpm and the sphere-like BC was about ˜8 mm, which was larger than at other speeds. Increasing the rotational speed to 150 rpm resulted in a change in shape, with a tail-like feature and the BC diameter reduced to about ˜2.5 mm. With speeds above 200 rpm, the formation of sphere-like BC was inhibited and a few interconnected BC particles with diameter of ˜1 mm were found. When the rotational speed was much greater than 200 rpm, scarcely any sphere-like BC could be found in the culture medium. Meanwhile, increasing the rotational speed did not increase the quantity of BC production (Hu and Catchmark, 2010a).

In general, the instability of the bacterial strain, the non-Newtonian behavior during mixing of the BC, and the high shear force are some drawbacks of the agitated/shaking method. However, BC produced by agitated culture displays some changes of microstructure and properties, such as a low degree of polymerization, a low crystallinity index, and inferior mechanical properties (Kouda, Naritomi, Yano, & Yoshinaga, 1997; Kouda, Yano, & Yoshinaga, 1997; Kouda, Naritomi, Yano, & Yoshinaga, 1998; Kouda, Yano, Yoshinaga, Kaminoyama, & Kamiwano, 2016). The microstructure of the sphere-like BC is quite different from that produced by other culture methods. That is because the process of BC formation may be affected by the agitation. The geometries of sphere-like BC are quite different between the static and agitated/shaking cultures. A BC membrane can be formed on the air-liquid interface of the nutrient in the static culture, whereas in the agitated/shaking culture, BC is produced from the center of the particle and then develops outwards. Hence, a layered structure can be observed in the microstructure of sphere-like BC. The interior region of sphere-like BC is hollow. Significantly denser fibers of BC have been found within the layered structure, also containing bacterial strains (Hu and Catchmark, 2010b). Despite these problems, some research has suggested that the agitated culture might be the most suitable technique for economical scale production (Hu et al., 2013).

2.3. Bioreactor cultures

As already explained, the agitated/shaking culture was proposed to increase the mass transfer rate by inducing a low shear stress and a high oxygen transfer rate. However, the appearance of a non-cellulose mutant usually results in a reduction of BC productivity in the agitated/shaking culture. Such culture methods could have limited utility for up-

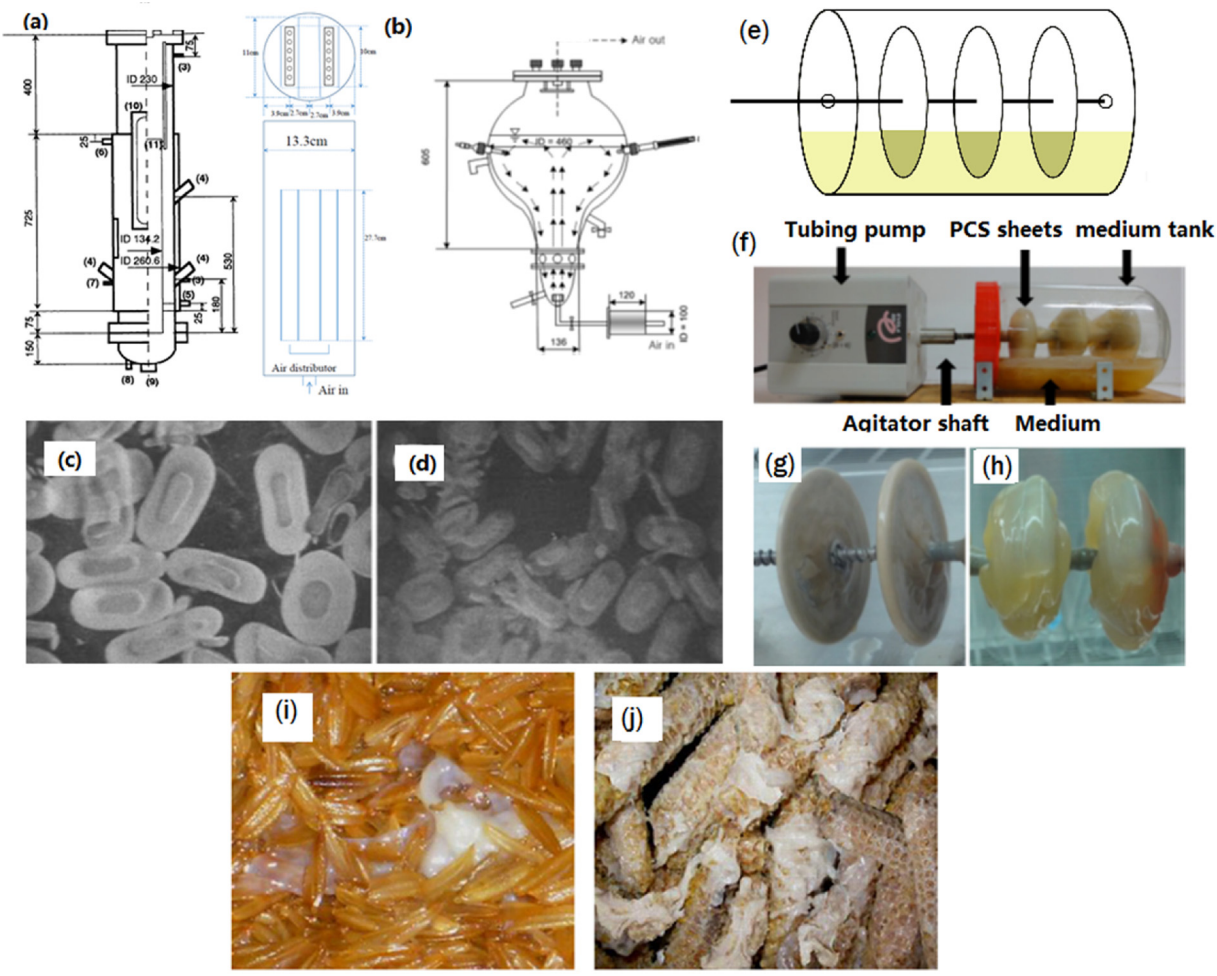

Fig. 3. (a) Schematic diagram of a 50 L internal-loop airlift reactor (left) and a modified airlift bioreactor (right). (Units: mm) (1) nozzle for inoculation; (2) gas outlet; (3) nozzles for addition of acid and base; (4) sensor nozzles; (5) and (6) inlet and outlet for temperature-controlled water; (7) sampling nozzle; (8) temperature sensor; (9) drain; (10) sight glass; (11) draft tube (134.2 mm inner diameter × 800 mm height). (b) Schematic diagram of a modified 50 L bubble column bioreactor (ID: inner diameter, unit: mm). Morphology of BC pellets formed in an airlift reactor after 24 h cultivation time from the start of experiments at (c) 40 g/l fructose, (d) 70 g/l fructose (Source: Reprinted with permission from Chao et al., 2000 and 2001; Choi, Song, Kim, Chang, & Kim, 2009; Wu & Li, 2015), (e) Schematic of RDB, (f) Illustration of PCS-RDB composed of tubing pump, agitator shaft, PCs sheets and medium tank, (g) BC produced from stainless steel rotating disk bioreactors, (h) PCS-RDB by G. xylinum ATCC 700178 after 5 days of cultivation (Source: Reprinted with permission from Lin et al., 2014), and (i–j) morphological appearance of BC obtained from trickling cultivation (Source: Reprinted with permission from Lu & Jiang, 2014).

scaling due to their low productivity and high production cost. The agitated culture cannot markedly increase the production of BC compared with the static culture. To resolve this problem, achieve industrial scale production, and expand the applications of BC, establishment of an economical production process with high productivity, low production cost, and short cultivation time is needed. Hence, many studies have been conducted involving switching on the oxygen supply, adding nutriment to the culture medium, and developing the culture medium. The production of BC can be increased by continuous cultivation with high-oxygen or nutrient solution transfer rate. Some bioreactor cultures have been reported to produce high levels of BC. In recent years, some research about the fermentation process and preparation technology of BC introduced some bioreactor cultures of BC (Campano, Ana Balea, Blanco, & Negro, 2016; Yang et al., 2014). They can be characterized by the way they function, such as producing BC under oxygen-enriched air, use of a rotating disc, or through biofilm support.

2.3.1. Enriched oxygen bioreactors

To increase oxygen delivery, a stirred tank bioreactor has been used. However, this method entails high energy consumption. Another common type of fermentation reactor is the airlift bioreactor. An airlift bioreactor using a low power supply was first reported for BC production by Chao et al. in 1997 (Chao, Sugano, Kouda, Yoshinaga, & Shoda, 1997). This reactor was more energy efficient and produced less shear stress than stirred-tank reactors because it could supply a sufficient amount of oxygen during BC production. With the air lift bioreactor setup, when the bacteria were cultured for 67 h, 3.8 g of BC was harvested. In another study, Chao and colleagues (Chao, Ishida, Sugano, & Shoda, 2000) improved the airlift reactor's efficiency via an internal loop, whereby BC was harvested with the highest concentration of 10.4 g/l. The BC thus obtained was in pellet form similar to that from an agitated culture and possessed low mechanical strength. Later, an internal-loop airlift reactor was developed (Chao, Sugano, & Shoda, 2001) to study the effect of different fructose concentrations on BC production rate. With the use of oxygen-enriched air, the BC production rate increased to 0.093 g/l/h and the BC yield was enhanced from 11% in air to 18%. The highest BC yield of 35% with the highest production rate of 0.22 g/l/h was observed at 60–70 g/l fructose. A greater amount of BC was produced using the internal-loop airlift reactor than with the airlift bioreactor. Fig. 3(a) shows schematic diagrams of an internal-loop airlift reactor and a modified airlift bioreactor (Chao et al., 2000; Wu & Li, 2015). Nevertheless, all these airlift reactor types produced BC

with elliptical pellet morphology, as displayed in Fig. 3(c) and (d) (Chao et al., 2000). BC membrane with greater water-holding capacity was harvested via the modified airlift bioreactor (Wu & Li, 2015). The bioreactor employed rectangular net plates instead of the simple draft tube used in the general airlift bioreactor. The properties of BC could be manipulated by varying the number of net plates. For example, BC showed greater water holding capacity and the highest Young's modulus using 6 net plates. With the airlift bioreactor, the process of BC formation should occur at the medium/pellicle interface, and the bacteria strains should be near this interface, with no BC formation observed elsewhere in the medium. In this modified airlift bioreactor, BC could be formed in the medium and BC membrane was obtained in the bioreactor.

Another modified airlift-type bubble column bioreactor as shown in Fig. 3(b), reported by Choi et al. (2009) and Song, Li, Seo, Kim, & Kim, 2009, produced BC with the high productivity of 2.27 g/l/day. This bioreactor could provide low shear stress and a high oxygen rate. When pure oxygen was supplied into the scaled-up culture conditions, 5.6–6.8 g/l of BC was produced, demonstrating an efficient method for BC mass production. However, the prepared BC had low mechanical properties, low crystallinity, low molecular weight, and a low degree of polymerization.

2.3.2. Rotating disc bioreactors

The first report of BC prepared via a rotating disk bioreactor was in 2002 (Serafica, ·Mormino, & ·Bungay, 2002). Fig. 3(e)) displays the simplified schematic diagram. In that design, several circular disks are fitted on a rotating central shaft, with an inlet for inoculation. In a rotating disc bioreactor, many different kinds of solids and fibers can be added directly to the medium and become incorporated into the cellulose to improve the properties of BC and BC-based composites (Kuure-Kinsey, Weber, Bungay, Plawsky, & Bequette, 2005; Mormino & Bungay, 2003). The purpose of the rotating disc bioreactor is to obtain BC with a homogeneous structure. In the rotating disc bioreactor, the circular discs can continue rotating while their surfaces alternatively interact with air and liquid media. BC with additional mechanical strength can attach to the disk surfaces. Although the BC prepared via rotating disk bioreactor is homogeneous, the yield is not significantly greater than that obtained from the static culture. However, many reports have testified that plastic composites (PCs) made from agriculture wastes can enhance the total productivity of ethanol, acetic acid, and pullulan. Therefore, a new rotating disc bioreactor was made using PCs to increase the yield of BC production, hence reducing the cost. The productivity of BC in that bioreactor was reported to reach approximately 0.24 g/l/day. The produced BC exhibited lower crystallinity and lower mechanical properties but similar water content and thermostability compared to BC obtained via the static culture (Mormino & Bungay, 2003; Zahan, Pa'e, & Muhamad, 2016). Usually, bioreactors were designed so that half of the area of the discs was submerged in the medium and the other half was exposed to the atmosphere. Therefore, they suffered re-inoculation after each BC harvest. However, plastic composites supporting a rotating disk bioreactor (PCS-RDB) can be fully immersed into the culture media and provide a rough surface for bacteria attachment, resulting in a high BC yield. In fact, the PCS-RDB can produce BC without re-inoculation, consequently retaining its productivity for at least five cycles. This bioreactor hence can produce BC in a semi-continuous manner and is easy to be scaled up to meet commercial production (Lin, Hsieh, Chen, Demirci, & Cheng, 2014). Figs. 3(f)–5 (h) illustrate a PCS-RDB (Lin et al., 2014). Research has been undertaken to evaluate the efficiency of PCS-RDB systems with different additives. For example, carboxymethylcellulose (CMC), avicel, agar, and sodium alginate were added to the PCS-RDB to increase the BC productivity and properties. It was found that the presence of CMC and avicel in the medium significantly increased the production rate of BC. The highest BC production (0.64 g/slice) was reached with the addition of 0.8% avicel into the bioreactor (Lin et al., 2016). The final BC in pellet form had similar water uptake capacity but lower mechanical properties than BC obtained under a bioreactor without additions.

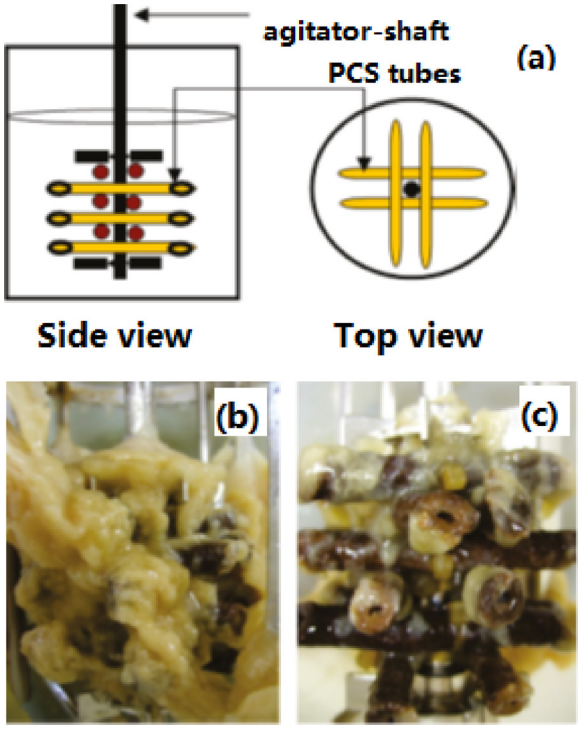

Fig. 4. Plastic composite support tubes bound to agitator shaft and bioreactor design (a) and results of BC production in reactor (b) without and (c) with the addition of CMC (1.5%) (Source: Reprinted with permission from Cheng et al., 2011).

2.3.3. Modified static bioreactors

In dynamic cultures it has been found that shear stress applied to the bacteria during culture can significantly facilitate cellulose-negative mutants that are more enriched than the cellulose producing cells and that reduce productivity. Therefore, static culture that is simple and produces little shear stress has been widely used to obtain BC despite its low productivity, long culture time, and considerable resources. During fermentation, if the bioreactor can be coupled with an enriched oxygen transfer capability, it could successfully lead to BC formation. Hence, a trickling bed reactor, a kind of vinegar manufacturing equipment, has also been used for BC production (Lu & Jiang, 2014). Compared to the static culture and agitated/shaking methods, the trickling bed reactor can provide high oxygen concertation and low shear force. This bioreactor, which provides high biomass density systems, can supply a greater surface to volume ratio than that from a conventional static culture. BC obtained from the trickling bed reactor has excellent properties, such as high degree of −OH association, as well as high polymerization, purity, water holding capacity, porosity, and thermal stability. Fig. 3(i) and (j) show the morphological appearance of BC obtained from trickling cultivation. The BC is in the form of irregular sheet films. Each thin wet film varied from 1 to approximately 5 mm in thickness and grew among gaps in rice husk or affixed to the surface of corncobs.

2.3.4. Other kinds of bioreactor

It has been proven that high biomass density is useful for BC production. Biofilm is a natural form of cell immobilization that can increase biomass density. Hence, PCs have been used as biofilm for BC production. Fig. 4 shows a schematic diagram of PC tubes bound to an agitator shaft and bioreactor design and BC production in the reactor.

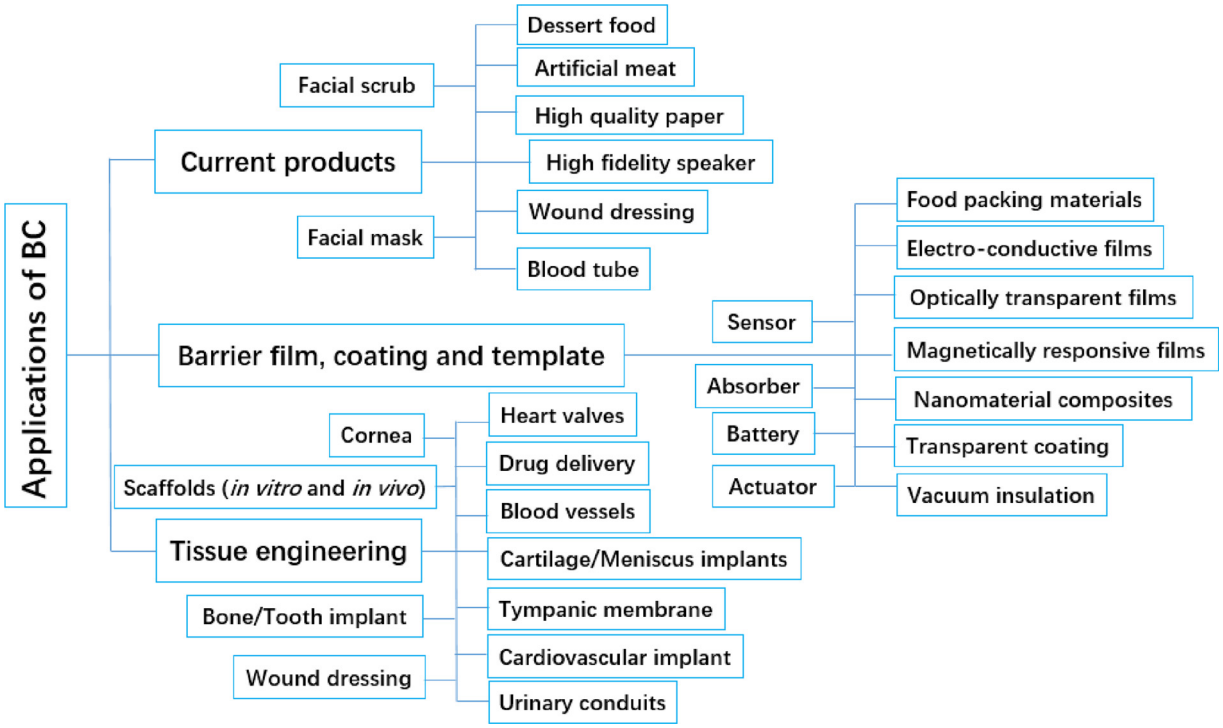

Fig. 5. Applications of BC in different industrial and medical areas.

The production of BC has been successfully enhanced by using a biofilm reactor. It has been reported that BC production achieved in a biofilm reactor was 7.05 g/l, which was 2.5-fold more than that obtained from static culture with a yield of 2.82 g/l. The BC had higher crystallinity (93%), a similar crystal size (5.2 nm), and superior thermal performance than that obtained from the static culture. Moreover, mechanical strength analysis indicated that the BC produced, like the pellicle form, restored its tensile strength, a feature that could broaden its potential applications. However, the water retention ability was lower than that of a BC produced within a suspended-cell reactor (Cheng, Catchmark, & Demirci, 2009). In another study, BC production was increased (˜13 g/l) in the presence of 1.5% CMC, which was 1.7-fold that of BC produced by a bioreactor without additions. However, the crystallinity (80%) and crystal size (4.2 nm) of the BC produced with CMC were lower than those of the BC obtained without additions that developed crystallinity of 86% and crystal size of 5.3 nm (Cheng, Catchmark, & Demirci, 2011).

Various additives or modified methods have been used to improve the production of BC. The BC harvested from other bioreactors has been characterized in terms of structure and properties such as macroscopic morphology, microstructure, degree of crystallinity, chemical structure, polymerization degree, purity, water holding capacity, porosity, and thermogravimetric ability. Table 3 shows the production specifications, modifications, and advantages of different reactors.

3. Applications of BC

Due to its outstanding properties, BC can be applied as a natural renewable polymer in many areas. Food packing, transparent coating or film, battery separator, adsorbent, pharmaceutical industries, water treatment, cosmetics, biomaterials, ethanol production, electric conductors or magnetic materials, artificial blood vessels, and scaffolds for tissue engineering are some applications of BC in different industrial and medical fields, as shown in Fig. 5 (Cacicedo et al., 2016; Oliveira Barud et al., 2016).

3.1. Applications of BC made by static culture

BC has been in situ and ex situ modified via different methods and used as scaffolds (Stumpf, Yang, Zhang, & Cao, 2018). With in situ modification, the culture conditions are changed by the use of additives or reinforcement materials, whereas ex situ modification is carried out after BC is harvested. The additive materials can be incorporated into growing BC nanofiber networks to prepare BC composites with desired properties. This type of modification in the static method can also be employed to control the structure, shape, and properties of BC. For example, paraffin wax microspheres have been added into culture medium to obtain BC scaffolds with microporous structure for bone tissue engineering (Zaborowska et al., 2010). Meanwhile, commercially available BC membrane has been widely used for wound dressing. For example, Dermafill (i.e., *Xylinum* cellulose) cellulose membrane dressing is a translucent, semi-opaque biosynthetic cellulose membrane dressing. BC closely resembles the extracellular matrix of tissues and yields a high vapor transfer rate while providing a normal matrix covering the entire wound bed. Glucose and dextrin have been added into media to obtain BC/glucose and BC/dextrin composites for wound dressing (Stumpf, Pértile, Rambo, & Porto, 2013). These composites display high porosity and biocompatibility. The moldability of BC during culture is an important feature that can extend the range of its applications. For example, BC-tubes have been studied for blood vessels. The addition of CMC to the culture medium to produce BC/CMC tubes has been reported (Orelma et al., 2014). Chitosan and heparin with appropriate potential applications in wound healing can also be added into the medium to improve the properties of BC. Table 4 shows BC and BC-based composites harvested in static culture with different applications.

3.2. Application of BC made by agitated/shaking culture method

Compared to BC prepared by static culture, sphere-like BC has loose, layered, porous character, a large surface area and highly hydrophilic network structures that result in additional advantages. More importantly, its spherical surface area increases with decrease in the size

Table 3
Advantages and production specification of different reactors for BC production.

Modification	Advantages of airlift reactor for BC production Production specification and advantages	References
High oxygen concentration	BC concentration: 5.63 g/l in 28 h Productivity: 0.20 g/l Production deteriorated after 30 h **Advantages:** High oxygen transfer rate, low power requirement, higher productivity.	Chao et al., 1997
Internal loop airlift with enriched oxygen	Production: 3.8 g in 67 h culture or 0.116 g/l/h **Advantages:** Formed a unique ellipse, high volumetric oxygen transfer, high hydrodynamic characteristic	Chao et al., 2000
Internal loop airlift with controlled pH/ fresh and glucose medium	The highest production rate: 0.22 g/l/h The highest concentration: 10.4 g/l at 60-70 g/l fructose **Advantages:** Formed a unique ellipse, high volumetric oxygen transfer, high hydrodynamic characteristic, low mechanical strength	Chao et al., 2001
Shaking flask with controlled pH/ Hestrin & Schramm medium	Produced a membrane-type BC **Advantages:** High water holding capacity, Young's modulus could be manipulated by varying the number of net plates	Wu & Li, 2015
Bubble column with controlled pH Aeration rate:1.0 vvm (30 L/min)	**Advantages:** High production: 5.6-6.8 g/l/3 days Low mechanical properties: 17.15 to 11.66 MPa Low crystallinity: 86 to 79.6% Low molecular weight and degree of polymerization Low shear stress, high oxygen transfer rate Low concentrated solution state culture	Choi et al., 2009
Production specification of rotating disc bioreactors for BC production		
A rotating disk bioreactor	**A consistent product** Higher tensile strength; pore size of BC: 10-15 μm; cellulosic matrix remains intact and strong	Mormino & Bungay, 2003; Zahan, Pa'e, & Muhamad, 2016
Plastic composites supporting rotating disk bioreactor	**A semi-continuous process** Higher productivity: 0.24 g/l/day; lower crystallinity: 66.9%; lower mechanical property (Young's modulus of 372.5 MPa); water content and thermostability similar to that of BC obtained by static culture	Lin et al., 2014
Plastic composites supporting rotating disk bioreactor with different additions	**A semi-continuous process** No re-inoculation; concentration of fructose reduced from 50 to 10 g/l; oxygen concentration and disc rotation speed enhance the fermentation process; BC productivity: 0.64 g/slice; high water retention ability (98.6-99 %); similar strain but lower stress; highest BC production with 0.8% carboxymethylcellulose and avicel, respectively	Lin et al., 2016
Rotating magnetic field	Did not increase the number of mutants unable to produce cellulose Increased biochemical properties; positive impact on growth of bacteria; increased water molecules; obtained BC with altered micro-structure and degree of porosity.	Fijałkowski et al., 2015, 2016; Fijałkowski, Zywicka et al., 2017, 2017b
Production specifications of several other bioreactors for BC production		
Bioreactor equipped with a spin filter	High cell density Cel + cells can convert into Cel- mutants in abundance Higher BC productivity: from 0.55 to 1.61 g/l/day Cell mass: from 5.65 to 11.52 g/L (140 h)	Jung, Khan, Park, & Chang, 2007
Fed batch principle	High quality cellulose; Culture box: low cost BC slices or layer (3-4 cm); Best time interval: 6 h High tensile strength: 114 N, DP: 5200; Gradient of graph in load-displacement diagram: 34.7 N/10 mm	Hornung, Ludwig, & Schmauder, 2007
Biofilm reactor	High biomass density; High production: 7.05 g/L Higher crystallinity: 93%; Crystal size: 5.2 nm Better thermal performance; Water retention ability: 95 %	Cheng et al., 2009
Biofilm reactor with additives	High biomass density; Continuous BC production High production: 13 g/L; Lower crystallinity: 80% Crystal size: 4.2 nm; BC paper sheets: higher tensile strength and Young's modulus	Cheng et al., 2011

of sphere-like BC, an effect that is related to both rotation speed and additives. In the past, in order to prepare sphere-like BC, a hydrogel fiber cultivation method and electrospinning have been used to form spherical BC (Higashi & Mik, 2018). Compared with such reported methods for the formation of spherical BC, the agitated culture method is simple, selectable, controllable, environmentally friendly, and visible. Hence agitated culture is useful for expanding the potential applications of sphere-like BC in other fields. Sphere-like BC has been used in some research as a carrier to adsorb, carry, and crosslink various substances including lipases, Fe_3O_4, graphene, carbon nanotube, protein, enzyme, human osteoblast, nucleic acid, and compounds. It also has been applied in bioseparation, drug delivery, immobilized enzyme, cell suspension culture, and as an adsorbent for heavy metal ions, oil, organic solvents, and sewage treatment. Hu et al. (2013) prepared sphere-like BC particles under agitated culture using *G. xylinum* for human osteoblast growth. Sphere-like BC particles of various sizes were produced under 125 rpm and 150 rpm rotating speeds. Human osteoblast cells can attach and grow into BC particles. In comparisons of the BC particles obtained under two different rotating speeds, cells exhibited better attachment and viability on the larger sphere-like BC particles that were produced under the 125 rpm rotating speed. That was because the surface area, porosity, stiffness, and microstructure were different between BC particles developed under the two rotating speeds (Hu et al., 2013). Cai et al. (2018) found that spherical BC particles could enhance the activity and stability of industrial lipases. They reported that enzymatic stability and hydrolytic activity of lipases immobilized on a series of spherical BC particles with several sizes were quite different. Spherical BC particles with the diameter of 6.10 ± 0.05 mm could lead to two optimal hydrolytic activities of lipases under both acidic and alkaline conditions. However, these authors did not study the effect of rotation speed and agitated culture conditions. They showed that spherical BC particles possessed

Table 4
Several applications and properties of BC and BC-based composites.

BC/BC composites	Structure and properties	Applications	References
BC	Dense, thin layers; good mechanical properties	Blood vessel; Vascular grafts	Putra, Kakugo, Furukawa, Gong, & Osada, 2008
	High mechanical strength and moldability, smooth inner surface	Synthetic blood vessels in microsurgery	Klemm, Schumann, Udhardt, & Marsch, 2001
	Suitable mechanical properties and patient-specific shapes	Implant material for ear cartilage replacement	Nimeskern et al., 2013
	High mechanical properties and compression strain	Potential meniscus implant	Bodin, Concaro et al., 2007
	High conformability, moisture donation, and fast healing	BC mask	Brown & Saxena, 2007
	High water holding capacity and mechanical strength	Substitution of diseased arteries and blood vessels	Charpentier, Maguire, & Wan, 2006
BC/chitosan & sliver	High transparency. Antibacterial activity	Membranes and scaffolds for skin tissue regeneration	Legeza et al., 2004
BC/ciprofloxacin	No cytotoxicity, genotoxicity or mutagenicity effects.	Contact lens used for regeneration or protection against bacteria.	Messaddeq et al., 2008
BC/polycaprolactone	High transparency and mechanical properties	Tissue substitutes in rabbit cornea	Sepúlveda et al., 2016
BC/chitosan	Improved fibroblast adhesion and proliferation	Wound dressing	Kim et al., 2011
BC/polyvinyl alcohol	Creation of optimal moist condition; Skin cell support	BC gloves	Osorio et al., 2017

potential as green carrier for the immobilization of lipases. Enzymatic immobilization onto spherical BC particles could enhance the recycling hydrolytic capability of oil and fats in various industrial divisions compared to BC of other sizes and shapes (Cai et al., 2018). Hence, it has been demonstrated that spherical BC particles have broad applications in material science or the biomedical fields.

Furthermore, due to the excellent microstructure of sphere-like BC, it also can be used as an adsorbent for heavy metal ions, oil, and organic solvents. Zhu, Jia, Wan et al. (2011) prepared spherical BC/Fe_3O_4 as shown in Fig. 6(a) and (b). Introducing Fe_3O_4 particles could improve the properties of BC. Spherical BC/Fe_3O_4 with 41 emu/g of saturated magnetization and 27 *Oe* of related coercively has been used for adsorbing heavy metal ions such as Pb^{2+}, Mn^{2+}, and Cr^{3+}. The results indicated that sphere-like BC/Fe_3O_4 particles could absorb heavy metal ions efficiently and were recyclable after the elution. Sphere-like BC can also be used as an adsorbent with other superparamagnetic particle materials such as Fe_2O_3, Ni-Fe, and Fe-Co. Apart from superparamagnetic particle materials, carbon nanotubes, graphene, and graphene oxide can be introduced into sphere-like BC to prepare nanocomposites. Hu (2014) obtained sphere-like BC/graphene composites on a rotary shaker with a rotating speed of 160 rpm. Fig. 6(c)–6(j) show macrographs and SEM images of spherical BC and spherical BC/graphene before and after carbonization, respectively. The samples had a honeycomb-like surface morphology and a 3D interconnected porous structure that was expected to possess high absorption capacity. It was found that after carbonization the BC/graphene spheres showed excellent capability for absorbing oils and organic solvents due to their highly porous, honeycomb-like surface, 3D interconnected structure, high mechanical stability, good hydrophobicity, and elasticity.

Sphere-like BC possesses excellent properties that have been studied for larger scale production. Table 5 shows applications and specifications of spherical BC and spherical BC-based composites prepared via agitated culture systems. It is known that several extrinsic factors exist for the formation of such spherical BC particles, including bacterial strains, inoculums, initial glucose concentration, pH of culture medium, culture time, rotating speed, culture temperature, cellulose fiber residue in the inoculums, cell density, and culture space. However, the mechanism behind the formation of spherical BC particles has yet to be conclusively determined. Further studies are required to develop more detailed understanding of the formation of spherical BC particles in agitated cultures to expand its application.

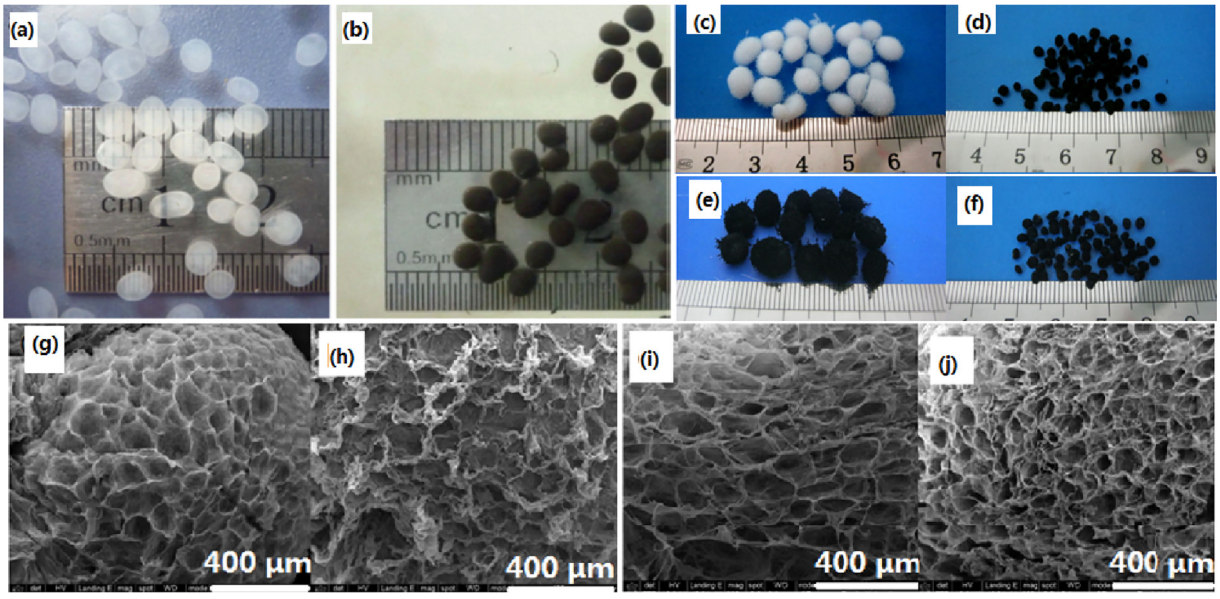

Fig. 6. Photo images of (a) and (c) sphere-like BC, (b) BC/Fe_3O_4, (d) carbonized spherical BC, (e) spherical BC/graphene, and (f) carbonized spherical BC/graphene. SEM surface images of spherical (g) and (h) BC and (i) and (j) BC/graphene (g) and (i) before and (h) and (j) after carbonization (Source: Reprinted with permission from Zhu, Jia, Wan et al., 2011; Hu, 2014; Wan et al., 2015.).

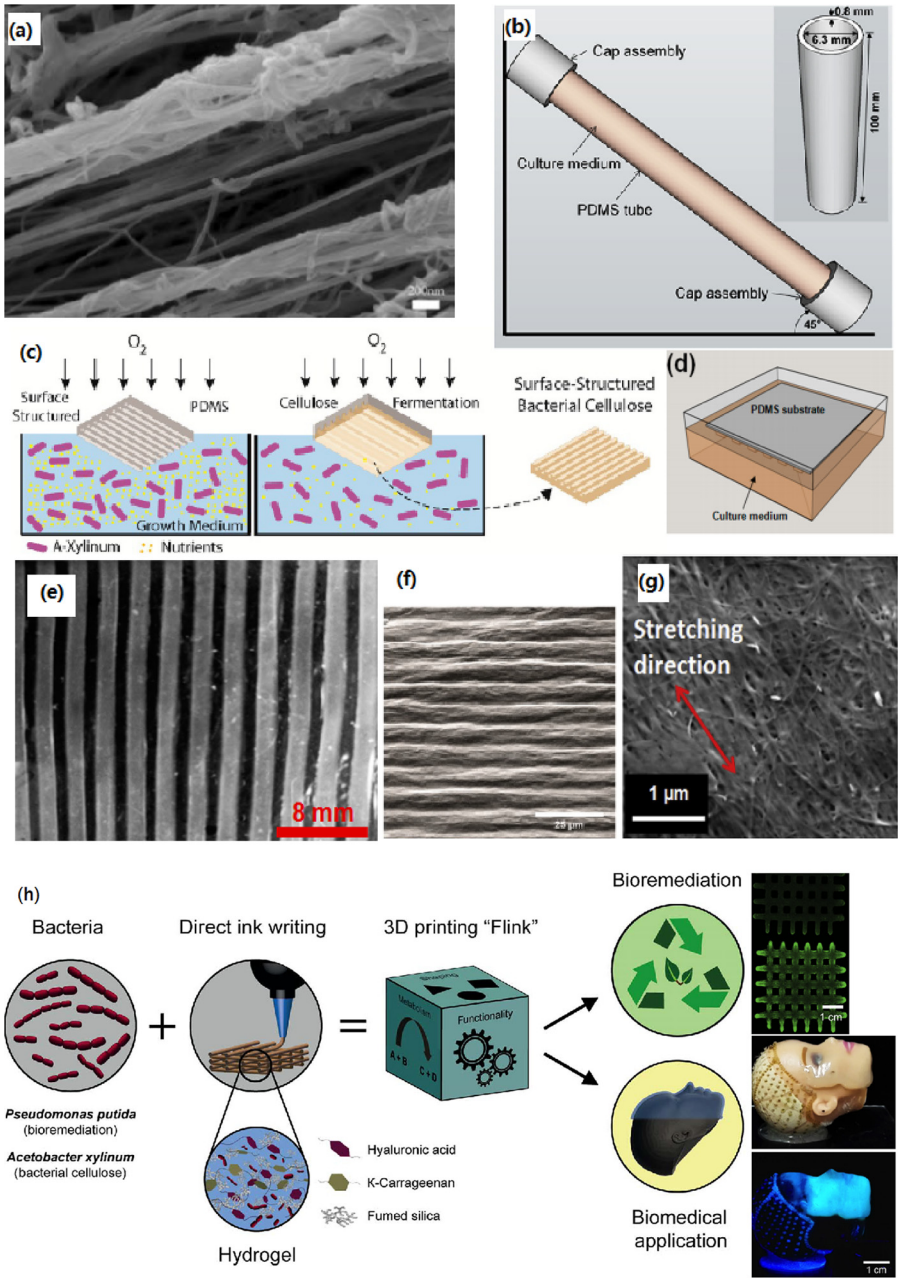

Fig. 7. (a) SEM images of BC produced under a 0.45 V/cm electric field, (b) Tube-shaped BC cultured in PDMS tubes, (c) BC cultured with surface-structured PDMS mode, (d) BC cultured in 3D printed reticulated fashion, (e) BC obtained in 3D printed reticulated fashion, (f) BC obtained in surface-structured PDMS mode, (g) BC obtained in PDMS tubes (Source: Reprinted with permission from Sano et al., 2010; Bottan et al., 2015; Rahman & Netravali, 2016 and 2017), and (h)Schematics of 3D bacteria-printing platform for the creation of functional living materials (Source: Reprinted with permission from Schaffner et al., 2017).

3.3. Applications of BC fabricated by bioreactor culture

As we know, the purpose of bioreactor culture was to increase production and achieve the industrial scale production, so the applications of BC made by bioreactor culture were rarely discussed before. BC made by bioreactor culture can be used for many industrial application fields, such as, high quality paper, high-fidelity speakers, wound dressings, dessert foods, structural/non-structural composites of vehicle, high performance reinforced composites in wind energy, civil infrastructure, hydrokinetic energy and marine infrastructure fields, other industries of food packaging, sorbents, water treatment) and so on. If there is no special requirement for size and shape, its application made by bioreactor culture overlaps with those made by other cultivation methods.

4. Future opportunities and challenges

Recently, BC has been successfully prepared via several culture methods. Owing to the unique properties developed, BC as a unique functional material has been shown to have great potential for many different fields and will certainly continue to do so. The results for BC and BC-based composites in various applications have attracted many research groups. Furthermore, many new preparation techniques have also been developed for BC production.

It is known that *A. xylinum* or *G. xylinum* can produce dense cellulose networks and they are difficult to control in traditional fermentation technology. Agitated/shaking and bioreactor methods can regulate the structure and properties of BC. However, to improve properties, many other approaches have been used for BC production. A set of

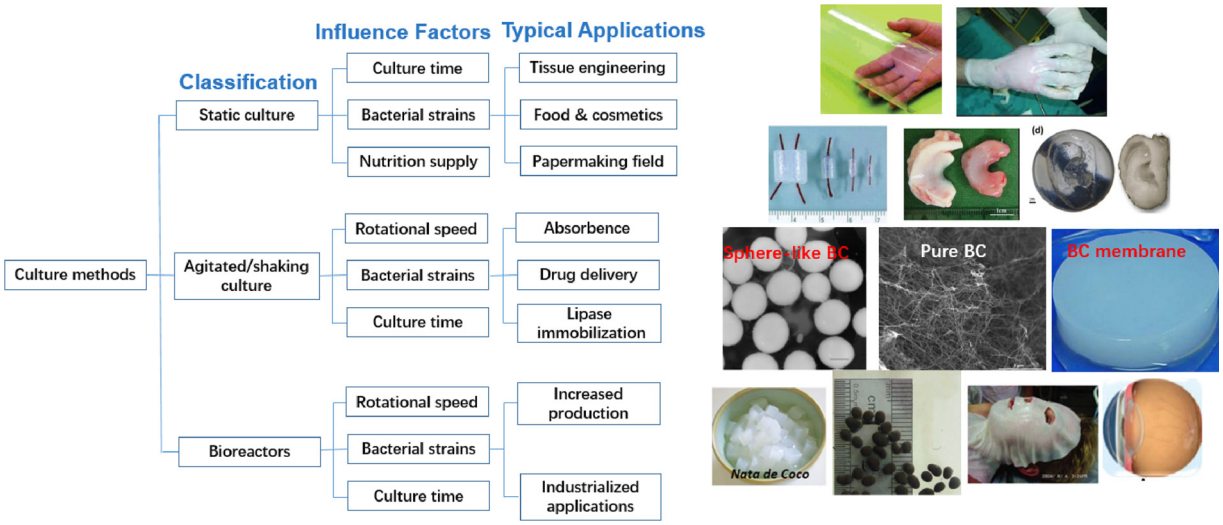

Fig. 8. Classification, influencing factors, and typical applications of culture methods (Source: Reprinted with permission from Bodin, Concaro et al., 2007, 2007b; Fu et al., 2013; Gatenholm & Klemm, 2010; 2007; Nimeskern et al., 2013; De Oliveira Barud et al., 2016; Fu et al., 2013; Ifuku et al., 2007).

genetic toolkits has been created for BC production (Florea et al., 2016). By means of rational reprogramming, this toolkit can control the biosynthesis, functionalization, and production of BC. Using this tool, BC and BC-based composites can be controlled through genetic engineering. New BC-based materials with modification can be prepared for a variety of ideal applications. The pore size, micro-structure, physical and chemical properties of BC can be tunable by altering gene expression. Due to the outstanding advantages of this method, it is likely to be used widely in future research.

Moreover, it has proved that bacterial motion is related to the production of BC. Traditional culture methods cannot precisely predict the motion of bacteria strains. Researchers have used several methods

Table 5
Production of BC and BC composites under agitation culture systems.

Num.	Structure and properties	Applications	References
BC	Diameter 0.5-6 mm, unique or adhering to each other, loose and porous structure	Adsorption of bovine serum albumin and Pb^{2+}, bio-separation, immobilized reaction, sewage treatment, reusable	Zhu, Jia, Yang et al., 2011
	Diameter 5-10 mm, flocky asterisk-like, crystallinity: 81.43-84.35 %, IR: 6.52-3.85	Choice of different species or strains and fermentation methods	Bi et al., 2014
	Diameter 5-10 mm, unique and large spheres, crystallinity/IR: 84% / 4.48, slightly thinner micro fibrils	Good production yield	Czaja et al., 2004
	Diameter 2-8 mm, more solid structure, temperature affects the time of sphere formation.	Cells exhibit good attachment and viability on the particles	Hu et al., 2013
	Diameter 3-9 mm, lipase immobilized BC sphere, high operational and hydrolytic activity, low active temperature.	Green carrier. Excellent for enzymatic immobilization. High-efficiency lipase-immobilization system for large-scale industrial hydrolysis of oils and fats	Cai et al., 2018
	At 150 rpm, diameter < 1-8 mm, hollow with layered outer shell, solid but the central region is not layered. Layer spacing 20 μm (125 rpm) and 10 μm (150 rpm).	Food, healthcare, and materials applications	Hu and Catchmark, 2010a
	Xyloglucan: aster-like, 4-5 mm Xylan: spheres 7-8 mm Arabinogalactan: spheres, 4-5 mm Pectin: aster-like, 5-6 mm Xyloglucan: No obvious central core, layered structure, densely packed cellulose bundles Xylan: A few tails formed on the surface of the spheres, layered structure, pore structure of cellulose bundles Arabinogalactan: layered structure, cellulose linkage between layers Pectin: layered structure, densely packed cellulose bundles	Xyloglucan has dominant impact on the assembly of cellulose. Xyloglucan and pectin may interact with cellulose at different points in the assembly process, or in different regions. BC and biomass yields indicate that xyloglucan and pectin can also stimulate the growth of cellulose	Gu and Catchmark, 2012
	BC spheres with different ranges of 0.5-1.5, 2–3 and 4–5 mm. Relatively higher activity of smaller spheres. Smaller spheres provide a greater surface area and larger functional groups to connect with enzymes.	BC beads are a promising support for the preparation of immobilized glucoamylase for industrial applications.	Wu & Lia, 2008
BC/CNT	BC: snow like, crystallinity 67.2 %, IR crystallinity index 2.23 BC/CNT: rice like, 2-5 mm, crystallinity 76.2 %, IR crystallinity index 2.56	–	Yan, Chen, Wang, Wang, & Jiang, 2008
BC/Fe_3O_4	Diameter 3-5 mm Fe_3O_4 particles (15 nm) distributed uniformly in spheres	Adsorption: $Pb^{2+} > Mn^{2+} > Cr^{3+}$ Elution: $Mn^{2+} > Pb^{2+} > Cr^{3+}$ Superparamagnetic	Zhu, Jia, Wan et al., 2011
BC/GO	Diameter 3-7 mm Honeycomb-like surface pattern, interconnected structure, superior absorption capacity	A promising superabsorbent for water environmental protection; reusable	Hu, 2014

in attempts to manipulate bacterial motion during culture. An electric field or electromagnetical field can control the tendency of the culture medium to induce bacteria move in a certain direction (Liu et al., 2017; Sano, Rojas, Gatenholm, & Davalos, 2010). Fig. 7(a) shows a BC network produced under a 0.45 V/cm electric field. It was found that the 3D structure of the BC could be regulated via controlling the movement of bacteria. BC can be prepared with an oriented structure, a weave structure, a multiple fiber layer, or a gradient structure, and with desirable properties for particular applications such as blood scaffolds, tendon tissue, and transparent materials. This culture method has many advantages such as simplicity, high production level, and easy realization. Apart from applying electrical or electromagnetical fields, many other culture methods, especially in static culture, have been used to control BC networks. For example, *A. xylinums* were cultured in a surface-structured polydimethylsioxane (PDMS) mold (Bottan et al., 2015), in a 3D printed reticulated fashion (Rahman & Netravali, 2016), and in PDMS tubes (Rahman & Netravali, 2017) to obtain aligned BC fiber. Fig. 7(b)–(g) show the culture molds used in these culture methods and BC images obtained by thereby.

As well known, 3D printing technology is widely used for manufacturing. Metals, plastics, and carbon fiber have been printed via 3D printing technology. Printing living materials is a greater challenge than printing any other materials. However, bacteria-derived functional materials have been created by 3D printing technology. Through the addition of bacteria in multifunctional print ink, bacteria-derived living material has been obtained by 3D printing technology (Kyle, 2018). Schaffner, Rühs, Coulter, Kilcher, and Studart, (2017) embedded two model bacterial strains (*P. putida* and *A. xylinum*) in biocompatible hydrogel ink and printed living material capable of producing BC scaffolds that were in situ formed on nonplanar surfaces as relevant for personalized biomedical applications. Fig. 7(f) shows a 3D bacteria-printing platform for the creation of functional living materials. The results showed that 3D print technology could prepare BC scaffolds having functionalized structures with complex shapes for bioremediation and biomedical applications. Apart from bioremediation and biomedical applications, these functionalized 3D printed BC-based materials could be used for manufacturing new generation biological intelligent materials. Thus, 3D print technology can expand applications of BC-based materials in future work. There is enormous potential for introducing 3D print technology into BC-based materials (Schaffner et al., 2017).

Although many technologies have been improved for BC research applications, it is difficult to produce BC on an industrial scale while controlling structure and properties through standard manufacturing and digital techniques. Recent developments within BC-related technology have improved production of this material. Many bio-fabrication methodologies have also been developed to modify BC production, such as self-assembling manufacturing, a biosynthetic approach, and bio-inspired fabrication methodologies (Derme, Mitterberger, & Tanna, 2016). Through optimizing culture approaches, evaluating fermentation systems, drawing comparisons with genetic engineering, and assessing modification methods, BC can be produced to offer inestimable development matrixes in different fields. It has been shown that modification of properties, increasing yields, reducing production costs, and choosing appropriate industrial fabrication lines are the main future goals for all researchers.

5. Conclusions

As a natural renewable polymer, BC has unique specific structure and properties that include high purity, 3D nanofiber network, high crystallinity, remarkable mechanical properties, high moldability during formation, and biocompatibility. The production of BC by different culture methods has been updated in this study. To reduce production costs, scale up optimization and improve productivity, many approaches have been progressively developed. In the static culture condition, large-scale and thickness-controlled BC membrane can be prepared steadily, although this method still requires a long culture time and has a low yield rate. More importantly, BC obtained using the static method has excellent properties compared to that produced by other culture methods. Many studies have been tried to enhance the synthesis process for BC, significantly reducing production cost. With the agitated/shaking culture that was the first chosen to increase the BC yield, the BC was prepared with different sizes and shapes. Although that culture approach had no significant effect on BC production, sphere-like BC particles that were related to rotation speed and culture time also had unique properties and structures. Based on their unique advantages, sphere-like BC particles can be used for bio-separation, as adsorbents for heavy metal iron, oil, origin solvent, and protein, immobilized reactions. They can also be used as carriers to crosslink with other substances such as multiwall carbon nanotubes, graphene, and Fe_3O_4 for absorbency. Compared the use of static and agitated/shaking cultures, fermentation in a bioreactor can significantly increase the yield and reduce production time of BC. These methods can produce BC on an industrial scale. Many kinds of bioreactors have been used for BC production, examples including airlift bioreactor, biofilm bioreactor, rotating disk bioreactor, and trickling bed reactor. Hence, BCs obtained from different culture methods possess particular structures and properties that can be used in various fields. To summarize the influencing factors and typical applications for choosing ideal culture methods, the main information regarding culture methods is presented in Fig. 8, showing the classification, influencing factors, typical applications, and application examples of several culture methods (Bodin, Concaro, Brittberg, & Gatenholm, 2007, 2007b; Fu, Zhang, & Yang, 2013; Gatenholm & Klemm, 2010; 2007; Nimeskern et al., 2013).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2019.05.008.

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