REVIEW ARTICLE

A Review on Bacterial Cellulose: Properties, Applications, Fermentative Production, and Molasses Potential as Alternative Medium

Clara Angela [1] and Putu Virgina Partha Devanthi [1*]

[1]Biotechnology Department, Indonesia International Institute for Life Sciences, Jakarta, Indonesia

**Corresponding author. Email: putu.devanti@i3l.ac.id*

ABSTRACT

Bacterial cellulose (BC) is an extracellular homopolymer produced by certain species of bacteria. It has demonstrated the potential as an alternative to plant cellulose with more appealing features such as unique nanostructure, high porosity, high crystallization index, high tensile strength, high water holding capacity, and a high degree of polymerization. These attributes facilitate BC utilization in various applications, ranging from the food industry, cosmetics, pharmaceutical, medical field, waste treatment, textile, and paper industry. Considering the advantages and wide range of applications, it is necessary to explore and improve the current industrial production to achieve a higher yield at a lower cost. This review article summarizes the BC properties and characteristics as well as its application in different fields. Furthermore, the potential of agricultural waste molasses for low-cost BC production is also discussed.

Keywords: Bacterial cellulose; biopolymer; *Acetobacter xylinum*; molasses; low-cost fermentation

INTRODUCTION

Bacterial cellulose (BC) is an extracellular polysaccharide produced by certain bacterial species (Chawla *et al.*, 2009; Huang *et al.*, 2013). It has a basic microfibrils structure composed of glucan chains interlinked by hydrogen bonds (Chawla *et al.*, 2009). BC was found by Brown in 1886 when he observed the development of a jelly-like translucent layer on the surface of *Mycoderma aceti* culture (mother of vinegar) in the presence of oxygen and glucose (Brown, 1886; Chawla *et al.*, 2009; Lee *et al.*, 2014). Further characterization and identification led to discovering the organism responsible for the layer manifestation, which he named *Acetobacter xylinum* (Brown, 1886). The organism is now reclassified as *Komagataeibacter xylinus* by Yamada (2013), but other synonyms such as *Gluconacetobacter xylinus* might be used before the publication. Extensive studies have shown that the organism can produce cellulose in the form of a gelatinous membrane that could reach a thickness of 25 mm with a strong and tough structure and high liquid resistance (Lee *et al.*, 2014).

BC shares the same molecular formula $(C_6H_{10}O_5)_n$ with plant cellulose. However, BC

provides better physical and chemical properties such as higher tensile strength and water holding capacity, although the microfibril of BC is 100 times thinner than that of plant cellulose (Chawla *et al.*, 2009). BC has also been reported to have a wide range of applications. It can be used as a bio-sorbent in the waste management industry, thickening and stabilizing agent in the food industry, and wound healing agent in the pharmaceutical and medical industry (Chawla *et al.*, 2009; Lee *et al.*, 2014; Jayabalan *et al.*, 2014). The superior quality and the wide range of applications have attracted many researchers to conduct more extensive studies on BC synthesis in bacteria and further optimize yield production.

Traditionally, BC can be produced during Kombucha fermentation using tea fungus or also known as a symbiotic consortium of bacteria and yeast (SCOBY) in a sugar-rich tea (Jayabalan *et al.*, 2014; Chakravorty *et al.*, 2016). Meanwhile, industrial-scale fermentation takes place in a bioreactor using a pure or mixed bacterial culture. One of the limiting factors in industrial BC production is the cost of the fermentation medium. The medium often uses pure sugars such as glucose, sucrose, mannitol, fructose, and arabitol as the sole carbon source, which could consume up to 30% of the total production cost (Çakar *et al.*, 2014). Thus, a new alternative medium that is economically feasible yet still allows high production is urgently needed.

Recent studies of molasses have shown that it could potentially be used as an alternative carbon source in BC production. It is a thick, dark brown syrup obtained as a by-product of sugarcane and sugar beet final processing step (Premjet, Premjet, Ohtani, 2007; Çakar *et al.*, 2014). Molasses contains sugars, nitrogenous compounds, minerals, vitamins, carbohydrates, non-nitrogenous acids, and nucleic acids (Premjet, Premjet, Ohtani, 2007). The composition of molasses might vary, but it could support the growth of many microorganism strains (Çakar *et al.*, 2014). In Indonesia, molasses is a readily available resource. It is usually used as raw material to produce ethanol biofuel, acetic acid, or monosodium glutamate. The availability of molasses reached 499,050 tons/year in East Java, 96,378 tons/year in Central Java, 56,689 tons/year in West Java, 40,789 tons/year in Lampung, 29,686 tons/year in North Sumatra, 29,596 tons/year in South Sumatra, 24,302 ton/year in South Sulawesi, 21,044 tons/year in North Sulawesi (Jaya & Mahendra, 2008). As molasses is available in abundance, it can be utilized as an alternative carbon source in BC production to reduce the total cost and increase molasses' value as agricultural waste.

This review article discusses several aspects of BC, including its characteristics, biosynthetic pathway, and application. The fermentative production of BC and the use of molasses as the alternative medium are also reviewed.

PHYSICAL AND CHEMICAL PROPERTIES OF BACTERIAL CELLULOSE

BC is a homopolymer that consists of glucose with a repeating ß-1→4 conformation glycosidic bond (Chawla *et al.*, 2009). First described by Mühlethaler in 1949, BC has a basic microfibrils structure, which are glucan chains linked together by inter and intra hydrogen bonds (Chawla *et al.*, 2009; Esa *et al.*, 2014). Being said as an equal substitute of plant cellulose, BC and plant cellulose's molecular structure is identical. They only differ in the chemical and physical properties, in which the properties of BC are more outstanding compared to its plant counterpart. One of the remarkable features that differentiate them is the chemical purity.

Impurities such as lignin and hemicellulose are present in plant cellulose, making the downstream process complex and costly. On the other hand, BC comes naturally as pure cellulose, adding an appealing feature to BC. Other remarkable properties of BC include unique nanostructure, high porosity, high crystallization index, high tensile strength, high water holding capacity (WHC), and a high degree of polymerization (DP) (Chawla *et al.*, 2009; Huang *et al.*, 2014; Esa *et al.*, 2014).

DP of BC ranges between 300 and 10,000 depending on the cultivation conditions, fermentation additives, and bacterial strains (Huang *et al.*, 2014). Wanichapichart *et al.* (2002) reported that DP and molecular mass of BC are 793 and 142.73 kDa, respectively. Under a scanning electron microscope (SEM), BC develops a tunnel-like structure with a diameter of about 7 µm where the external surfaces are made up of irregular clusters of fibrils. In contrast, the inside is composed of neatly organized fibrils (Chawla *et al.*, 2009). With such arrangement and 100 times smaller fiber size, BC exhibits better mechanical properties, higher crystallinity, and higher thermal properties. These are reasons why BC applications are commonly found in the pulp and paper industry since pure BC can enhance paper's performance (Gao *et al.*, 2011). Furthermore, porous structure and thinner fiber also account for higher surface area, facilitating higher water holding capacity and lower water release rate than plant cellulose, which is about 100 times higher by mass. This attribute is most favorable in biomedical utilization as wound dressing and nanocomposites (Tsouko *et al.*, 2015).

BIOSYNTHETIC PATHWAY OF BACTERIAL CELLULOSE IN BACTERIA

The mechanism of BC synthesis by *A. xylinum* has been well described. The mechanism is initiated with the formation of uridine diphosphoglucose (UDPGlc), which is a cellulose precursor. UDPGlc itself is formed through a series of enzymatic reactions that starts with glucose phosphorylation to glucose-6-phosphate (Glc-6-P) by glucokinase, followed by the intermediate isomerization to glucose-1-phosphate (Glc-1-P) by phosphoglucomutase (Chawla *et al.*, 2009; Lee *et al.*, 2014). Glucose-1-phosphate is then synthesized into UDPGlc by UDPGlc pyrophosphorylase (UDPase) (Chawla *et al.*, 2009; Lee *et al.*, 2014). UDPase is thought to be the main enzyme in cellulose synthesis; its activity is 100 times higher in cellulose-producing bacteria than in non-cellulose-producing bacteria (Valla *et al.*, 1989).

Cellulose synthesis happens in two intermediary steps: polymerization of glucose into ß-1→4 glucan chain by cellulose synthase and assembly and crystallization of cellulose chains (Chawla *et al.*, 2009; Lee *et al.*, 2014). The mechanism of glucose polymerization is still not well understood, and there are currently two hypotheses that attempt to explain this process. The first hypothesis suggests that the glucan chain contains a lipid intermediate, in which glucose from UDPGlc is transferred into lipid molecule in the plasma membrane by glycosyltransferase (De Jannino, Couso, Dankert, 1988). The second hypothesis suggested by Brown and Saxena (2005) describes that no lipid intermediate is involved in the process. Instead, glucose residues are attached to the non-reducing end of polysaccharides in the extracytoplasmic space. Despite these debates, it is known that *A. xylinum* can polymerize approximately 200,000 glucose molecules per second (Hestrin and Schramm, 1954).

The second step of cellulose synthesis, assembly, and crystallization of cellulose chains occurs between the outer and cytoplasmic membranes of the cell (De Ley, Gillis, Swings, 1984). Individual cellulose molecule is spun through cellulose export components, forming protofibrils with a diameter of about 2-4 nm (Iguchi, Yamanaka, Budhiono, 2000). These protofibrils will self-assemble into a ribbon-shaped microfibril of approximately 80 nm (Bielecki *et al.*, 2005).

FERMENTATIVE PRODUCTION OF BACTERIAL CELLULOSE USING KOMBUCHA CULTURE

BC is naturally produced by bacteria in the genera *Gluconacetobacter*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Alcaligenes*, *Azotobacter*, *Rhizobium*, *Sarcina*, *Salmonella*, and *Escherichia*. In Kombucha tea fermentation, BC is produced by SCOBY, which is a microbial consortium composed of lactic acid bacteria, acetic acid bacteria, and yeast. The composition of bacteria and yeast in Kombucha tea fermentation may be diverse, but several studies have reported the most dominant bacteria and yeast species. *A. xylinum* is the most common bacteria found during the Kombucha fermentation (Danielova, 1954; Konovalov and Semenova, 1955; Sievers *et al.*, 1995; Roussin, 1996). Other cellulose-producing bacteria such as those mentioned above have also been identified (Jayabalan *et al.*, 2014). Furthermore, many yeast species involved during Kombucha fermentation have also been identified, including *Saccharomyces, Saccharomycodes, Schizosaccharomyces, Zygosaccharomyces, Brettanomyces/Dekkera, Candida, Torulospora, Koleckera, Pichia, Mycotorula*, and *Mycoderma* (Teoh, Heard, Cox, 2003; Goh *et al.*, 2012; Jayabalan *et al.*, 2014). Yeast species found in Kombucha culture tend to be osmotolerant, acid-tolerant and can produce alcohol and other flavor compounds (Teoh, Heard, Cox, 2003).

Bacteria and yeast in Kombucha fermentation exist as a symbiotic consortium where the yeast turns sucrose into glucose and fructose to produce ethanol through hydrolysis and glycolysis, respectively (Teoh, Heard, Cox, 2003; Jayabalan *et al.*, 2014). The glucose and ethanol derived from yeast metabolism are utilized as a food source by acetic acid bacteria to oxidize them into gluconic acid and acetic acid (Teoh, Heard, Cox, 2003; Jayabalan *et al.*, 2014). The metabolism process can be seen in Fig 1. A similar symbiotic relation between *Saccharomyces cerevisiae* and *Acetobacter* isolated from Haipao fermentation was reported by Liu *et al.* (1996). The production of ethanol by *S. cerevisiae* drives the *Acetobacter* growth and promotes higher acetic acid production. Liu *et al.* (1996) also observed positive feedback, in which the produced acetic acid can stimulate more ethanol production. Furthermore, ethanol supplementation at 10 g/L was proven effective in enhancing BC production in *A. xylinum* (Naritomi, 1998). Krystynowicz *et al.* (2002) also found that 1% ethanol significantly increased the cellulose production up to 6-fold, from 0.52 g/L to 3.31 g/L. The symbiosis that happened in mixed culture creates an environment where the chance of contamination during fermentation is reduced in the presence of ethanol and acetic acid (Liu *et al.*, 1996). Villareal-Soto *et al.* (2018) also mentioned that the dead yeast cells could further stimulate bacterial growth since they provide additional vitamins and nutrients. Besides gluconic acid, glucuronic acid, and ethanol, other secondary metabolites, including glucuronic, lactic, citric, tartaric, malic, oxalic, succinic, and malonic pyruvic, usnic acids are also produced (reviewed in Jayabalan *et al.*,

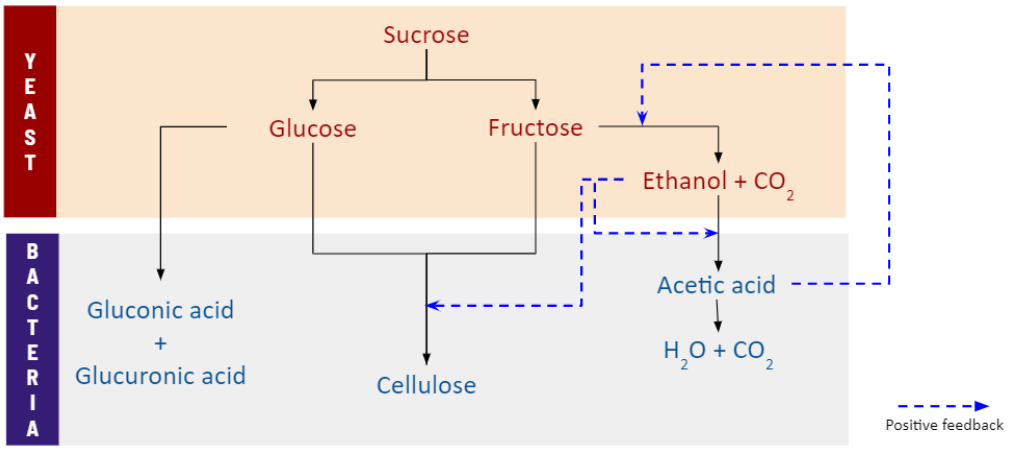

Figure 1. Microbial Interaction in Kombucha Fermentation (Villarreal-Soto *et al.*, 2018, after modification)

2014). Those organic acids contribute to the sour taste and beneficial health effects of the Kombucha tea (Malbaša *et al.*, 2007).

MOLASSES AS THE ALTERNATIVE CARBON SOURCE FOR BACTERIAL CELLULOSE PRODUCTION

Despite better physical and chemical properties, industrial BC production is still in the research and development stage to produce high yield at low production cost. The current bottleneck of the production cost comes from the utilization of pure sugars as a carbon source, which consumes about 30% of the total cost. In this section, the potential of agricultural waste molasses as the alternative cheaper carbon source is reviewed.

Molasses is a thick, dark brown syrup that is by-produced by the sugarcane and sugar beet processing industry (Malbaša *et al.*, 2007; Premjet, Premjet, Ohtani, 2007; Çakar *et al.*, 2014). The cost of molasses is only 1/250 of fructose (Bae & Shoda, 2004), but it is very rich in minerals, organic compounds, and vitamins. Due to this reason, the application of molasses can be easily found in many industrial productions such as lactic acid, polyhydroxybutyrate, ethanol, pullulan, and xanthan gum production (Çakar *et al.*, 2014). A study by Malbaša *et al.* (2007) showed that molasses consisted of 84.2% dry matter, 50.4% sucrose, 0.83% invert sugar, 1.8% total nitrogen, 0.29% amino nitrogen, 5.5 ug/100 g biotin, and 6.7 pH. Meanwhile, a characterization conducted by Keshk & Sameshima (2006) demonstrated that molasses consisted of 35% sucrose, 16% reducing sugar, 88% total dissolved solids, 12% ash content, 0.35% total nitrogen, and 0.04% phosphorus. The composition of molasses may vary, depending on the source and the production process, but it contains essential compounds to support many microorganism species.

Besides the essential nutrients, molasses also contains organic and inorganic toxic compounds that might harm microorganism by interfering with the metabolism, thus lowering the overall BC yield (Rahman *et al.*, 2016; Tyagi & Suresh, 2016). The toxic heavy metal in molasses could be removed before fermentation through acid, heat, or acid-heat pretreatment. Tyagi & Suresh (2016) mentioned that the H_2SO_4-heat pretreatment aided in

eliminating the coloring material and heavy metals of molasses, and it gave maximum BC yield amongst other pretreatments. Improvement in BC yield after molasses pretreatment has been demonstrated by Bae & Shoda (2004), Çakar *et al.* (2014), and Tyagi & Suresh (2016).

Bae & Shoda (2004) achieved a two-fold increase in the specific growth rate of *A. xylinum* BPR2001 and 76% improvement on BC production when the molasses was subjected to heat pretreatment at 120^{O}C and acid pretreatment using H_2SO_4. They also reported that a fed-batch fermentation in molasses medium at low total sugar concentration (12 g/L) produced the highest BC concentration at 7.82 g/L. Subsequently, Çakar *et al.* (2014) and Tyagi & Suresh (2016) used *G. xylinus* FC01 and *G. intermedius* (synonym of *Komagataeibacter intermedius*) SNT-1, and they observed a BC yield of 1.64 g/L and 12.6 g/L, respectively.

Improvement in BC production up to 31% from 3.35 g/L to 4.38 g/L was observed in *G. xylinus* ATCC 10245 when glucose was replaced with beet molasses as the sole carbon source (Keshk, Razek, Sameshima, 2006). This drastic improvement was driven by the sulfur and the nitrogen contents inside the beet molasses. The study also revealed that glucose replacement with molasses did not alter BC's crystallinity index as confirmed through FT-IR spectra, indicating that molasses can substitute glucose medium. More remarkable improvement up to 174% (5.97 g/L) and 180% (5.99 g/L) was achieved when the fermentation was carried out using *A. xylinum* IFO 13772 on molasses in the absence and the presence of lignosulfonate, respectively (Keshk & Sameshima, 2006). The physical properties of the BC produced from molasses medium, either with or without lignosulfonate, were also found to be similar to that of BC produced from the Hestrin-Schramm (HS) medium. The crystallinity and the content of the cellulose *Iα* slightly decreased from 88 and 42% (HS medium) to 87 and 41% (molasses medium). All studies reporting molasses' feasibility to be a cheap carbon source for cost-effective BC production using various *Gluconacetobacter* strains are summarized in Table 1.

Table 1. BC Production through Fermentation on Molasses Medium

Bacteria	Culture time (h)	Yield (g/L)	Ref
A. xylinum BPR2001	72	7.82 g/L	Bae & Shoda, 2004
A. xylinum ATCC 10245	168	1.86* and 1.48**	Keshk & Sameshima, 2006
A. xylinum IFO 13772	168	5.97* and 5.99**	
A. xylinum IFO 13773	168	2.82* and 3.21**	
A. xylinum IFO 14815	168	1.38* and 1.24**	
A. xylinum IFO 15237	168	2.34* and 2.25**	
G. xylinus ATCC 10245	168	4.38 g/L	Sherif *et al.*, 2006
G. xylinus FC01	168	1.64 g/L	Çakar *et al.*, 2014
G. intermedius SNT-1	168	12.6 g/L	Tyagi & Suresh, 2016

* Carbon source: molasses medium without lignosulfonate
** Carbon source: molasses medium with lignosulfonate

APPLICATION OF BACTERIAL CELLULOSE

As a natural polymer that is high in purity, crystallinity, density, water binding capacity, and surface area, BC has been extensively explored for its potential to be used in a wide range of applications such as in the food industry, cosmetics, pharmaceutical, and medical field, waste treatment, textile, and paper industry (Chawla *et al.*, 2009; Lee *et al.*, 2014; Jayabalan *et al.*, 2014). The applications of the BC in the food, cosmetics, and medical field are summarized in the following sections.

Food Applications

Food and Drug Administration (FDA) has given BC a Generally Recognized as Safe (GRAS) status since 1992. Cellulose is generally hard to digest; however, BC is edible due to its high water holding capacity and has a chewy texture similar to squids and grapes (Ullah, Santos, Khan, 2016). In the food industry, BC is used both as food packaging materials and as food additives such as gelling, thickening, and stabilizing agents (Chawla *et al.*, 2009; Ullah, Santos, Khan, 2016). Nata de coco, the first BC used as food ingredients, has been acknowledged for its soft texture and high fiber content (Chawla *et al.*, 2009; Ullah, Santos, Khan, 2016). It has also been widely consumed worldwide as dessert or candy. A study by Chau, Yang, Yu, Yen (2008) showed that nata de coco, or BC in general, could be a promising source of insoluble fiber. High water holding capacity and cation exchange capacity, which is higher than plant cellulose, also make BC more attractive in the food industry. Due to the properties above, consuming BC could significantly lower serum lipids and cholesterol; it has been demonstrated previously in the *in-vitro* study through absorption or binding (Stephens *et al.*, 1990) and *in-vivo* study in mice (Chau, Yang, Yu, Yen, 2008). Other than that, BC is also often used for immobilizing enzymes or cells. Chen *et al.* (2015) demonstrated that immobilizing laccase derived from *Trametes versicolor* fungus on BC, compared to free or adsorbed form, offered better catalytic activity and higher stability.

Cosmetics

Huge interest has been put on cosmetics, which is a substance that could improve the appearance and quality of body parts without any side effects. Nowadays, consumers prefer to use a skincare product made of herbal and/or natural ingredients. BC, being a natural ingredient, may attract many consumers for reasons that it is biodegradable and skin-hydrating. BC can be processed as a skincare product to produce facial masks and scrub (Ullah, Santos, Khan, 2016). Facial mask from BC has been proven to have beautifying, nourishing, moisturizing, exfoliating, and brightening properties (Legendre, 2008). Also, it is mentioned that BC-based facial masks might potentially treat severe skin conditions such as xerosis, atopic dermatitis, and psoriasis due to its moisturizing effect (Ullah, Santos, Khan, 2016).

When used as a facial scrub, the BC must be turned into powder first and then mixed with natural ingredients such as olive oil, ascorbic acid, and *Aloe vera* extract. Parameters including transdermal permeation of active ingredients, skin moisturizing effect, sebum absorption, and skin exfoliation are reported to be enhanced after adding the fragments of BC film up to 1.0% concentration into the facial scrub formulation (Lin *et al.*, 2015; Ullah, Santos, Khan, 2016). It is because BC has a high water-holding capacity and good gas permeability, allowing it to be a great carrier of active cosmetic ingredients.

Pharmaceutical and Medical Field

High tensile strength, high porosity, microfibrillar structure, high water holding capacity, and antibacterial properties of BC enable it to be utilized in the pharmaceutical and medical field (Chawla *et al.*, 2009; Ullah, Santos, Khan, 2016). BC, due to those properties, has excellent potential as a drug carrier. Kaplan *et al.* (2014) showed that BC could be used to deliver two antibiotics: ampicillin and gentamicin, in a prolonged drug release manner. The antimicrobial properties of cellulose to fight *Pseudomonas aeruginosa, Staphylococcus epidemidis, Staphylococcus aureus, Enterococcus feacalis*, and *Escherichia coli* could last up to 3 days (Kaplan *et al.*, 2014).

The wound dressing system could also utilize BC; it has been tested previously *in-vivo* in guinea pigs and rabbits. A wound dressing system composed of never-dried BC and polyhexamethylene biguanide recovered 70% of the wound, providing the promising potential for treating venous leg ulcers and deep pressure wounds (Serafica *et al.*, 2010; Ullah, Santos, Khan, 2016). Applying a BC-based wound dressing system is also associated with pain management and bacterial load reduction (Ullah, Santos, Khan, 2016).

CONCLUSION

BC is a pure cellulose homopolymer produced extracellularly by various microbial strains that has gained tremendous interest in the past decades. The unique structure and physical-chemical properties of BC make it suitable for multiple applications, including in the food industry as food packaging and food additives, in cosmetics as facial masks, and most commonly in the biomedical field as drug carriers and wound dressing. Despite its attractiveness, the current industrial production is still limited by relatively low yield and high production cost due to the utilization of pure bacterial strain and pure sugar as a carbon source. Many studies have addressed the high-cost issue by exploring few cheaper alternative carbon sources such as agricultural waste molasses. It has been proven that molasses contains the essential compounds to support BC producers' growth, and with few pretreatment steps, further improvement on BC yield has been observed up to two-fold.

While research has explored the potential of many alternative fermentation media, a very limited number of studies focus on exploring the microbial strains used in fermentation. Most of the study performed to identify individual strain potential, while a symbiotic consortium of bacteria and yeast can enhance the BC yield. More research on this aspect should be addressed in future research to identify the composition of SCOBY that best fits each fermentative production. Further research that combines both the utilization of SCOBY and cheap alternative medium can also add extra value to BC industrial production.

REFERENCES

Bae, S., & Shoda, M. (2004). Bacterial cellulose production by fed-batch fermentation in molasses medium. *Biotechnology Progress*, *20*(5), 1366–1371. doi:10.1021/bp0498490

Bielecki, S., Krystynowicz, A., Turkiewicz, M., & Kalinowska, H. (2005). Bacterial cellulose. *Polysaccharides and Polyamides in the Food Industry: Properties, Production, and Patents*, 31-84.

Brown, Adrian J. (1886). An acetic ferment which forms cellulose. *Chemical Society 49*, 432–439.

Çakar, F, Özer, I., Aytekin, A.Ö., Şahin, F. (2014). Improvement production of bacterial cellulose by semi-continuous process in molasses medium. *Carbohydrate Polymers 106*, 7–13.

Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., & Gachhui, R. (2016). Kombucha tea fermentation: microbial and biochemical dynamics. *International Journal of Food Microbiology*, *220*, 63–72. doi:10.1016/j.ijfoodmicro.2015.12.015

Chau, C., Yang, P., Yu, C., & Yen, G. (2008). Investigation on the lipid- and cholesterol-lowering abilities of biocellulose. *Journal of Agricultural and Food Chemistry*, *56*(6), 2291-2295. doi:10.1021/jf7035802

Chawla, P. R., Bajaj, I. B., Survase, S. A., & Singhal, R. S. (2009). Microbial cellulose: fermentative production and applications. *Food Technology and Biotechnology*, *47*(2), 107–124.

Chen, L., Zou, M., Hong, F. F. (2015). Erratum: Evaluation of fungal laccase immobilized on natural nanostructured bacterial cellulose. (2015). *Frontiers in Microbiology*, *6*. doi:10.3389/fmicb.2015.01327

Danielova, L., T. (1954). K morfologii "čajnogo griba". *Trudy Erevanskogo Zooveterinarnogo Institute, 17*, 201–16.

De Iannino, N. I., Couso, R. O., & Dankert, M. A. (1988). Lipid-linked intermediates and the synthesis of acetan in Acetobacter xylinum. *Microbiology*, *134*(6), 1731-1736.

De Ley, J., Gillis, M. and Swings, J. (1984) Family VI. Acetobacteriaceae, Bergey's Manual of Systematic Bacteriology. In: Krieg and Holt, J.G., Eds., The Williams and Wilkin Co., Baltimore, Vol. 1, 267-278.

Esa, F., Tasirin, S. M., & Rahman, N. A. (2014). Overview of bacterial cellulose production and application. *Agriculture and Agricultural Science Procedia*, *2*, 113–119. doi:10.1016/j.aaspro.2014.11.017

Gao, W., H., Chen, K., F., Yang, R., D., Yang, F., Han, W., J. (2011). Properties of bacterial cellulose and ist influence on the physical properties of paper. *BioResources*, *6*(1), 144-153.

Goh, W. N., Rosma, A., Kaur, B., Fazilah, A., Karim, A. A., & Bhat, R. (2012). Fermentation of black tea broth (Kombucha): I. effects of sucrose concentration and fermentation time on the yield of microbial cellulose. *International Food Research Journal*, *19*(1), 109–117.

Hestrin, S., & Schramm, M. J. B. J. (1954). Synthesis of cellulose by Acetobacter xylinum. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochemical Journal*, *58*(2), 345.

Huang, Y., Zhu, C., Yang, J., Nie, Y., Chen, C., & Sun, D. (2014). Recent advances in bacterial cellulose. *Cellulose*, *21*(1), 1–30. doi:10.1007/s10570-013-0088-z

Iguchi, M., Yamanaka, S., & Budhiono, A. (2000). *Journal of Materials Science, 35*(2), 261–270. doi:10.1023/a:1004775229149

Jaya, A.K, & Mahendra, S. (2008). Pra desain pabrik etanol dari molasses. https://www.slideshare.net/mah3ndr4/indonesia-mandiri (accessed 12 May 2021)

Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on Kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 538–550. doi:10.1111/1541-4337.12073

Kaplan, E., Ince, T., Yorulmaz, E., Yener, F., Harputlu, E., & Laçin, N. T. (2014). Controlled delivery of ampicillin and gentamycin from cellulose hydrogels and their antibacterial efficiency. *Journal of Biomaterials and Tissue*

Engineering, 4(7), 543-549. doi:10.1166/jbt.2014.1198

Keshk, S. M. a S., Razek, T. M. a, & Sameshima, K. (2006). Bacterial cellulose production from beet molasses. *Acta Biomater*., *5*(17), 1519–1523.

Keshk, S., & Sameshima, K. (2006). The utilization of sugar cane molasses with/without the presence of lignosulfonate for the production of bacterial cellulose. *Applied Microbiology and Biotechnology*, *72*(2), 291–296. doi:10.1007/s00253-005-0265-6

Konovalov, I., N., Semenova, M., N. (1955). K Fiziologii "Cajnogo Griba". *Bot Žurnal (Moscow) 40*, 567–70.

Krystynowicz, A., Czaja, W., Wiktorowska-Jezierska, A., Gonçalves-Miśkiewicz, M., Turkiewicz, M., & Bielecki, S. (2002). Factors affecting the yield and properties of bacterial cellulose. *Journal of Industrial Microbiology & Biotechnology, 29*(4), 189-195. doi:10.1038/sj/jim/7000303

Lee, K. Y., Buldum, G., Mantalaris, A., & Bismarck, A. (2014). More than meets the eye in bacterial cellulose: Biosynthesis, bioprocessing, and applications in advanced fiber composites. *Macromolecular Bioscience*, *14*(1), 10–32. doi:10.1002/mabi.201300298

Legendre, J. Y. (2008). *U.S. Patent No. US20090041815A1*. Washington, DC: U.S. Patent and Trademark Office.

Liu, C., Hsu, W., Lee, F., & Liao, C. (1996). The isolation and identification of microbes from a fermented tea beverage, Haipao, and their interactions during Haipao fermentation. *Food Microbiology, 13*(6), 407-415. doi:10.1006/fmic.1996.0047

Lin, Y. C., Wey, Y. C., Lee, M. L., & Lin, P. C. (2015). *U.S. Patent No. US20150216784A1*. Washington, DC: U.S. Patent and Trademark Office.

Malbaša, R., Lončar, E., Djurić, M., & Došenović, I. (2007). Effect of sucrose concentration on the products of Kombucha fermentation on molasses. *Food Chemistry*, *108*(3), 926-932. doi:10.1016/j.foodchem.2007.11.069

Mühlethaler, K. (1949). The structure of bacterial cellulose. *Biochimica et Biophysica Acta*, *3*, 527-535

Naritomi, T., Kouda, T., Yano, H., & Yoshinaga, F. (1998). Effect of ethanol on bacterial cellulose production from fructose in continuous culture. Journal of Fermentation and Bioengineering, *85*(6), 598-603. doi:10.1016/s0922-338x(98)80012-3

Premjet, S., Premjet, D., & Ohtani, Y. (2007). The Effect of ingredients of sugar cane molasses on bacterial cellulose production by *Acetobacter xylinum* ATCC 10245. *Fiber*, *63*(8), 193-199. doi:10.2115/fiber.63.193

Rahman, Gomaa, N. A., Nassar, Nadia, R. A., Heikal, Yehia, A., Donia, Mohmoud, A. M. A., Naguib, Mohamed, M., & Fadel, M. (2016). Effect of different treatments on heavy metal concentration in sugar cane molasses. *International Journal of Agricultural and Biosystems Engineering, 10*(1), 43–48.

Roussin, M., R. (1996). Analyses of Kombucha ferments: Report on growers. Information Resources, LC, Salt Lake City, Utah, USA. Retrieved on 31 Jan, 2021, from http://www.Kombucha-research.com

Saxena, I. M., & Brown Jr, R. M. (2005). Cellulose biosynthesis: current views and evolving concepts. *Annals of Botany*, *96*(1), 9-21.

Serafica, G., Mormino, R., Oster, G. A., Lentz, K. E., & Koehler, K. P. (2012). *U.S. Patent No. US7704523B2*. Washington, DC: U.S. Patent and Trademark Office.

Sievers, M., Lanini, C., Weber, A., Schuler-Schmid, U., Teuber, M. (1995). Microbiology and fermentation balance in a Kombucha beverage obtained from a tea fungus fermentation. *Syst Appl Microbiol 18*, 590–4.

Stephens, R. S., Westland, J. A., & Neorgi, A. N. (1990). *U.S. Patent No. US4960763A*. Washington, DC: U.S. Patent and Trademark Office.

Teoh, A. L., Heard, G., & Cox, J. (2004). Yeast ecology of Kombucha fermentation. International *Journal of Food Microbiology*, *95*(2), 119–126. doi:10.1016/j.ijfoodmicro.2003.12.020

Tsouko, E., Kourmentza, C., Ladakis, D., Kopsahelis, N., Mandala, I., Papanikolaou, S., Paloukis, F., Alves, V., & Koutinas, A. (2015). Bacterial Cellulose Production from Industrial Waste and by-Product Streams. *International Journal of Molecular Sciences*, *16*(12), 14832–14849. doi:10.3390/ijms160714832

Tyagi, N., & Suresh, S. (2016). Production of cellulose from sugarcane molasses using *Gluconacetobacter intermedius* SNT-1: Optimization & characterization. *Journal of Cleaner Production,112*, 71-80. doi:10.1016/j.jclepro.2015.07.054

Ullah, H., Santos, H. A., & Khan, T. (2016). Applications of bacterial cellulose in food, cosmetics and drug delivery. *Cellulose*, *23*(4), 2291-2314. doi:10.1007/s10570-016-0986-y

Valla, S., Coucheron, D. H., Fjærvik, E., Kjosbakken, J., Weinhouse, H., Ross, P., ... & Benziman, M. (1989). Cloning of a gene involved in cellulose biosynthesis in *Acetobacter xylinum*: complementation of cellulose-negative mutants by the UDPG pyrophosphorylase structural gene. *Molecular and General Genetics MGG*, *217*(1), 26-30.

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J., & Taillandier, P. (2018). Understanding Kombucha tea fermentation: a review. *Journal of Food Science, 83*(3), 580-588. doi:10.1111/1750-3841.14068

Wanichapichart, P., Kaewnopparat, S., Buaking, K., Puthai, W. (2002). Characterization of cellulose membranes produced by *Acetobacter xylinum*. *J. Sci. Technol., 24*, 855–862

Yamada, Y., Yukphan, P., Vu, H. T. L., Muramatsu, Y., Ochaikul, D., Tanasupawat, S., & Nakagawa, Y. (2012). Description of Komagataeibacter gen. nov., with proposals of new combinations (Acetobacteraceae). *The Journal of General and Applied Microbiology*, *58*(5), 397-404.