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INVITED REVIEW PAPER

Production of bacterial cellulose from alternative cheap and waste resources: A step for cost reduction with positive environmental aspects

Mazhar Ul-Islam*, Muhammad Wajid Ullah, Shaukat Khan***, and Joong Kon Park****,†**

*Department of Chemical Engineering, College of Engineering, Dhofar University, Salalah, Sultanate of Oman
**Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan 430074, P. R. China
***Materials Science Institute, PCFM Lab and GDHPRC Lab., School of Chemistry,
Sun Yat-Sen University, Guangzhou 510275, P. R. China
****Department of Chemical Engineering, Kyungpook National University, Daegu 41566, Korea
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Abstract−Bacterial cellulose (BC), an important biopolymer, has gained tremendous interest in several fields in the last few decades. Despite having the same chemical structure as plant cellulose, BC is superior in physical appearance and purity, as well as in mechanical, crystallinic, and biological properties for multiple applications. Despite these features, BC has limitations in production cost as well as physiological features. Notable limitations, including a non-bactericidal nature, low biocompatibility, and lack of conductive and magnetic properties, have been compensated through the development of composites using nanomaterials and polymers. Similarly, the limitation associated with cost has been reduced by developing new BC synthesis strategies, designing novel bioreactors, using genetically modified microbial species, and exploring alternative cheap fermentation media. Successful BC production has been reported from the use of industrial, confectionary, municipal and other wastes, including coconut water and fruit juices. Herein, we overview various efforts made thus far in identifying waste byproducts and inexpensive carbon sources for cost-effective BC production. It also provides information about the BC market and selling price, as well as techno-economic analysis of biotechnological BC production. This review article includes findings reported in the last few decades, and we hope it will be of great interest for readers as well as commercial BC producers.

Keywords: Bacterial Cellulose, Cheap Media, Waste Resources, Low-cost Production

INTRODUCTION

Waste materials are creating serious economic and environmental threats around the globe [1]. Among these, food wastes are of importance, as food manufacturers and processors generate millions of tons of food waste annually, most of which are preventable or recyclable under alternative circumstances. It is estimated that one-third of all food grown worldwide is lost or wasted. Besides direct food product loss, the waste byproducts discarded by various industries are rich in important food ingredients, including sugars. This food wastage not only contributes to economic issues but also creates an ideal environment for the proliferation of microbes that can cause environmental hazards and health risks. Along with formulating an ideal scenario to reduce waste, it is important to seek out opportunities for recycling and conversion into important products.

Cellulose is the most plentiful polymer on Earth and is primarily produced by plants. Certain microbial species (bacteria, fungi, and a few algae) can also produce cellulose from simple sugars and other carbon sources. This cellulose is called microbial cellulose or more commonly, bacterial cellulose (BC) [2,3]. Both plant cellulose and BC possess the same chemical structure, whereas their morphological appearance and physiological behavior are quite different. BC is pure and has better physico-chemical and mechanical properties. The fibril size, arrangement, geometry, crystallinity, and 3D structure of BC is quite different from plant cellulose [4,5]. Its structural features have allowed for application in diverse fields [2].

Currently, BC production at both small and industrial scales utilizes glucose/fructose-based synthetic culture media, which is a costly approach; however, efforts have been devoted towards the exploration of low-cost alternative sources for BC production [3,5]. Waste materials have been impressively recycled and utilized in the development of important bio-products including biofuels (bioethanol, biogas) and biopolymers (biofilms, bioplastic and bio-cellulose) [6]. Wastes generated from agricultural, municipal, and industrial activities, including forestry and food and confectionary production, have been recycled using diverse approaches. Most waste materials are rich in important nutrients that can serve as growth media for microbes and as raw sources in the development of various products. Earlier we reported on waste of beer fermentation broth (WBFB) as a sole nutrient and microbial source in the production of bioethanol [7]. It was determined that the sugar content of WBFB is high enough to allow for BC pellicle development. In addition to wastes, other cheap and readily available carbon sources could be used as potential alternatives to synthetic media.

In the manuscript, we overview multiple cheap resources and

†To whom correspondence should be addressed.
E-mail: parkjk@knu.ac.kr
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waste byproducts that can serve as alternatives to commercial synthetic media. There have been several individual reports about alternative sources used during BC production. All sources have been comparatively analyzed with consideration for the production and productivity of BC. We also provide information about the BC market in terms of market size, growth, selling price, and key manufacturers, as well as techno-economic analysis of biotechnological BC production. We believe that the current work will be eye catching for researchers and BC producers.

BACTERIAL CELLULOSE

Bacterial cellulose (BC) is a natural polymer hydrogel produced by some bacterial species belonging to the genera *Acetobacter*, *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia*, and *Sarcina*. It can also be produced in a cell-free system by utilizing a variety of synthetic and non-synthetic media [8-11]. Its production as an extracellular gelatinous layer was first reported by Brown [12]. Glucose is the main component involved in BC production; however, other sugars including fructose, galactose, and sucrose have been used [3]. The synthetic production of BC involves several steps which are controlled by specific enzymes. The synthetic BC pathway is shown in Fig. 1 [6].

Bacterial species utilize sugar sources to form a glucose chain inside the cell. These chains protrude out from the cell through small pores in the cell wall and combine to form microfibrils through hydrogen bonding. The fibrils develop, through further inter- and intramolecular interactions, a reticulated porous web-shaped structure on the media surface. This thin layer of BC becomes thicker with time as newly produced glucose chains unite from the bottom layer. This ultimately results in a hydrogel layer on the media surface [13]. Another approach used to produce BC is agitation culture, where BC is produced as small granules in a submerged culture [2,3]. BC production through various static and agitation strategies is shown in Fig. 2.

BC has the same chemical structure as plant cellulose. Both BC and plant cellulose are polymers of glucose linked through β-1,6-glycosidic bonds. However, the morphology and physico-mechanical properties of BC are much different from those of plant cellulose [4,15-17]. BC is superior to plant cellulose as a result of its purity, porous fibrous structure, high crystallinity, and post synthetic moldability. Numerous studies have reported on the improved mechanical, thermal, and biological properties of BC, as compared to those of plant cellulose. Additionally, the synthetic methodologies, structural features, and purity are ideal for the development of BC composites using a variety of other polymeric and nanomaterials that have additional applications in multiple fields [18-20].

BC has been impressively applied in medical, pharmaceutical, food, textile, electronic, and agricultural industries and is used in several commercially available products [21-24]. The most import-

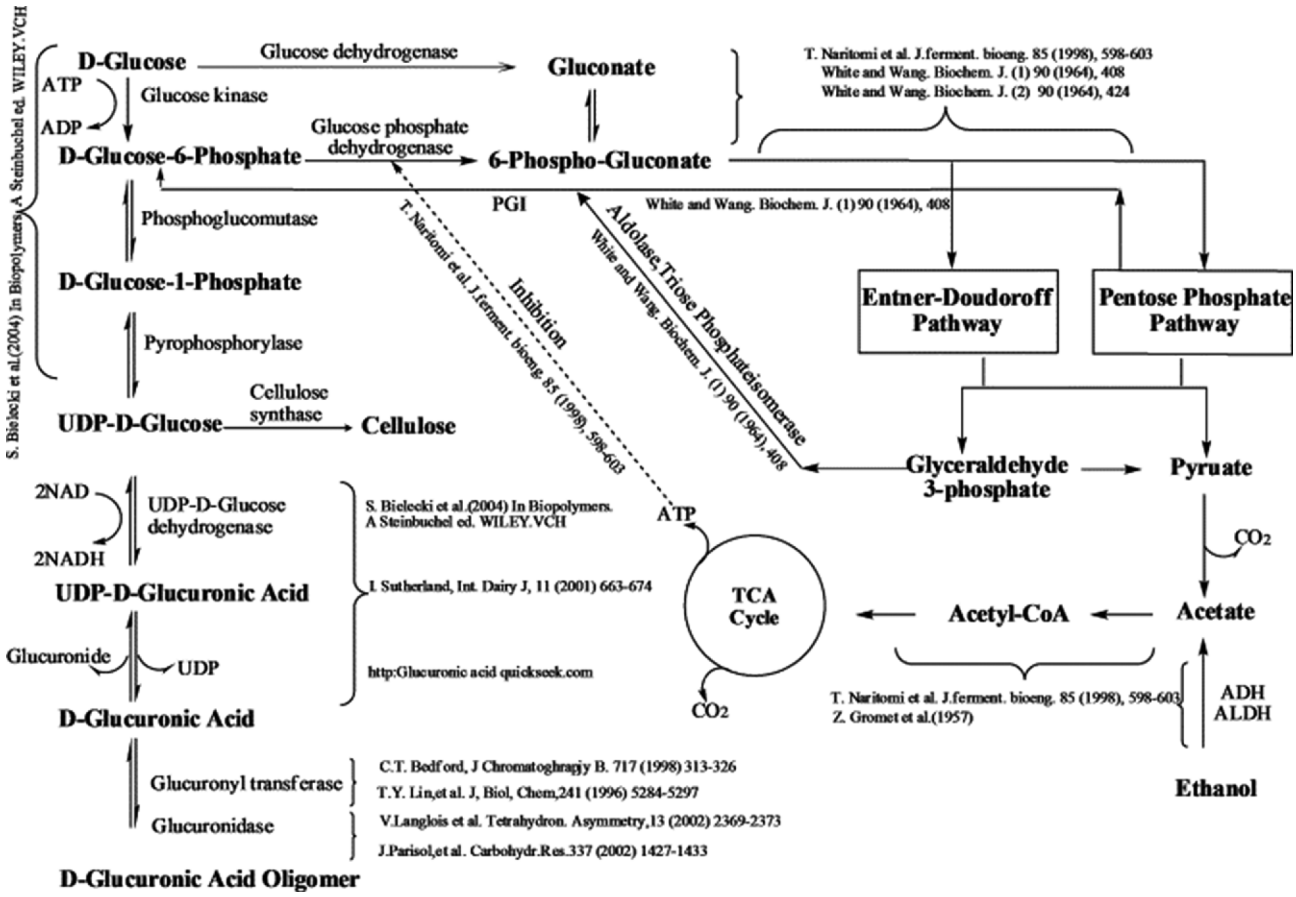

Fig. 1. Proposed biochemical pathways for BC production. Figure reproduced from [6].

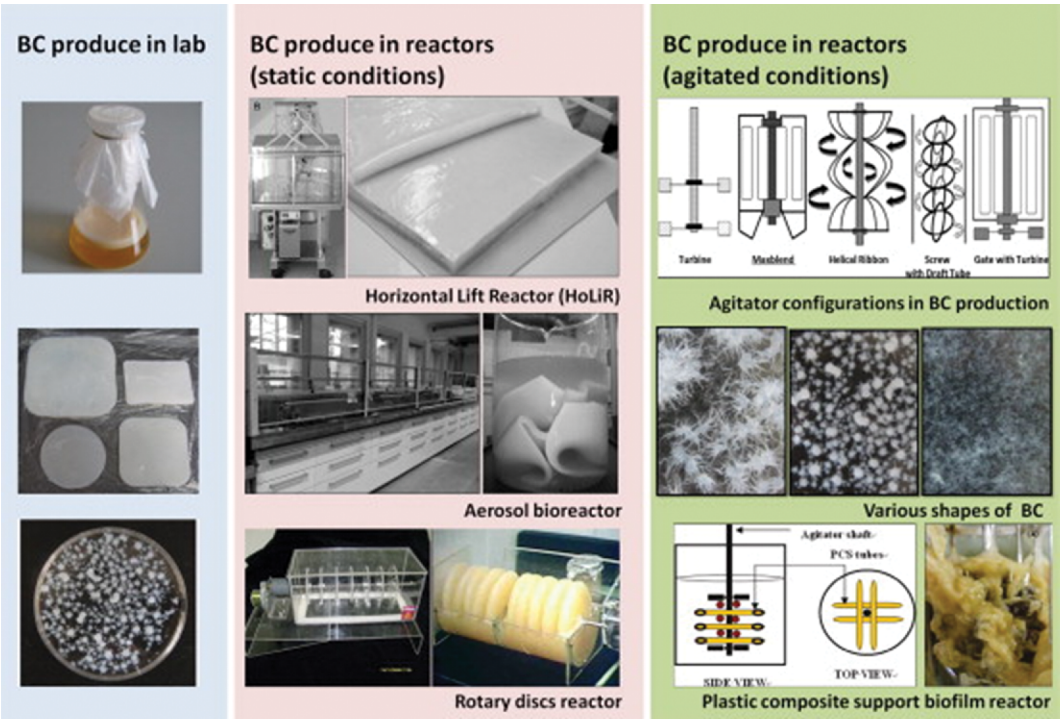

Fig. 2. BC production through static and shaking cultivation strategies using variety of designed reactors. Figure reproduced from [14].

ant applications of BC have been in the medical field, in products like facial masks used to cure acne and pimples, arterial stent coatings, topical coverings for severe wounds, artificial skin, 3D printed objects, drug delivery, and durometer prostheses [25-32]. BC products used in burn and wound healing have provided insight into the improvement of skin regeneration and pain relief. BC has been also been used in the development of display devices, conducting papers, food items, water purifications membranes, textile products (clothes, jackets, shoes), and filter papers [3]. The applications of BC and BC-based products continue to increase in multiple fields.

One of the hurdles faced in BC production and commercialization is production cost. Utilization of commercial synthetic sugar sources for BC production increases cost and hence limits applicability. There is much interest in developing BC from cheap, readily available resources, including waste products, in order to match market demand and reduce production cost, in addition to the interest in discovering novel features and innovative applications of BC. Researchers have reported success in exploring alternative media for BC production. Some of the most successfully used alternative media include coconut water, fruit juices, waste products from beer and confectionary industries, and waste generated during municipal and agricultural activities [2,3,15,33]. The use of alternative culture media for BC production has decreased synthesis and processing costs, therefore improving the scaling up and industrialization processes. The details of such alternative media will be discussed in the following sections.

LIMITATIONS IN COMMERCIALIZATION OF BC

BC structural and physico-mechanical features have led to implementation of the material in extensive applications in multiple fields. However, BC has several limitations that reduce applicability, production scaling potential, and industrial use. These limitations can be classified into two categories: physiological features and production costs.

Pure BC lacks certain important features including antimicrobial properties, biocompatibility, magnetic properties, conductivity, and transparency. These limitations consequently reduce its applicability in different fields. For instance, BC is used for wound care in medical fields; however, the lack of antimicrobial properties reduces its applications in contaminated environments [25]. Similarly, pure BC cannot be used in the manufacturing of magnetic devices, owing to lack of magnetic properties. Furthermore, the non-conducting and semitransparent nature limit application in the development of optoelectronic devices. These limitations have been addressed by developing BC composites in conjunction with bactericidal, biocompatible, conductive, and magnetic materials. Herein, we focus on the second limitation associated with BC.

The second major limitation associated with BC is production costs. Synthetic media components are quite expensive, which increases the production costs of BC. Furthermore, slow production processes and low productivity hinder process scaling up. The practical solution to this problem is to explore alternative cheap, waste, and renewable carbon sources for BC production. This will reduce the media cost and augment up-scaling possibilities. Herein, we review various alternative sources that can be employed for low cost BC production. We focus on the potential of BC production from waste resources and cheap natural resources. Along with providing cost effective BC production, the use of waste resources reduces the risks associated with environmental pollution. Poten-

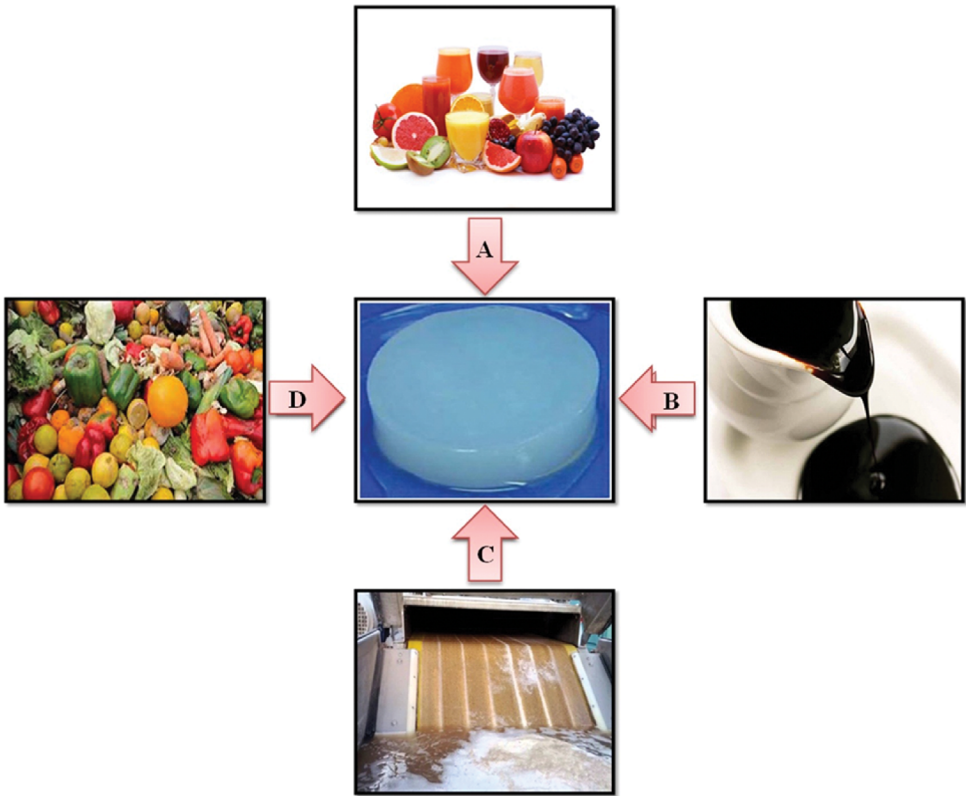

Fig. 3. Production of bacterial cellulose from various cheap sources including (A) fruit juices, (B) sugar cane molasses, (C) agricultural wastes, and (D) brewery wastes. Figure reproduced from [3].

tial of various waste sources for BC production are shown in Fig. 3.

BC PRODUCTION FROM WASTES

The high cost of fermentation media is the limiting factor for economic production of BC, which accounts for about 30% of its total production cost. To minimize this cost, extensive efforts in the last few decades have been devoted to exploring the utilization of various wastes as low-cost media, which has the alternative benefit of helping to address the environmental challenges caused by disposal of waste products [34]. Therefore, an ideal scenario would involve recycling and the conversion of these wastes to value-added products like BC. This would require different pre-treatment approaches including simple separation of solid materials and sterilization to remove contaminants, acid hydrolysis, or sub/supercritical water hydrolysis. Sub/supercritical water hydrolysis is an advanced technique performed between 100 °C and 374 °C while pressure is applied to keep water in liquid form; this results in the breakdown of cellulose and lignocellulose into small components [35]. Several types of waste, including those of agro-industrial, brewery, bakery, municipal, and textile origins, have been extensively utilized as media for BC production. Such wastes are rich sources of monosaccharides (e.g., glucose, fructose), polysaccharides (e.g., starch and cellulose), proteins, vitamins, and minerals, thus providing nutrients for the growth of microbial cells as well as serving as carbon sources for BC production. The following sections overview the potential of different industrial wastes as media for BC production.

1. Agro-industrial Wastes

Agricultural wastes are an important resource with economic significance worldwide. Being environmentally friendly, they are a potential source of renewable energy. Of the tons of agro-industrial waste produced on a daily basis, less than 10% is utilized as alternative raw material in other industries [36]. Several agro-industrial wastes with potential for use in BC production are summarized in Table 1.

1-1. Corn Stalk

Corn stalk hydrolysate consists of different sugars, including glucose (3.87 g/L), xylose (29.61 g/L), and mannose (1.84 g/L), furfural (2.95 g/L), lignin (4.01 g/L), and acetic acid (18.73 g/L). A study reported on the green synthesis of 2.86 g/L BC using acetic acid prehydrolysis liquor of agricultural corn stalk as a carbon source under optimized detoxification and pretreatment conditions. The resulting BC fibrils were 20 to 70 nm in diameter and 300 nm to several micrometers in length [37]. Additionally, rice bark obtained from agricultural residues can be used as a medium for BC production. Utilization of enzymatically hydrolyzed rice bark produced 2.42 g/L and 1.57 g/L BC under static and shaking conditions, respectively [38]. These examples highlight the potential of crop waste as a cost-effective feedstock for BC production.

1-2. Wheat Straw

Wheat straw is an abundant biomass resource available across the world, particularly in China. Generally, this important biomass is burned after the kernel is harvested, causing heavy air pollution. It can be pretreated via acid or enzymatic hydrolysis, followed by

Table 1. Agro-industrial wastes utilized as a feedstock for BC production

Agro industrial wastes	Additional nutrients	Microbial strain	Maximum BC production	Ref.
Waste as complex medium without additional nutrients				
Citrus peels (lemon, mandarin, orange and grapefruit)	---	*Komagataeibacter hansenii* GA2016	3.92 BC/100 g peel	[44]
Sugar cane juice and pineapple residues	---	*Gluconacetobacter medellinensis*	3.24 g/L	[45]
Discarded waste durian shell	---	*G. xylinus* CH001	2.67 g/L	[46]
Waste as carbon source with additional nutrients				
Enzymatic hydrolysate of wheat straw	Other components are same as of HS medium	*G. xylinus* ATCC 23770	8.3 g/L	[47]
Coffee cherry husk	Urea and corn steep liquor	*Gluconacetobacter hansenii* UAC09	8.2 g/L	[48]
Juice samples watermelon, pineapple and paw	Other components are same as that of HS medium	*Gluconacetobacter pasteurianus* PW1	7.7 g/L	[49]
Cashew tree exudates	Other components are same as of HS medium	*Komagataeibacter rhaeticus*	6.0 g/L	[50]
Extracted date syrup	Other components are same as of HS medium	*G. xylinus* 0416 MARDI	5.8 g/L	[51]
Pineapple waste medium and Pawpaw waste medium	Other components are same as of HS medium	*G. pasteurianus* PW1	3.9 g/L	[49]
Orange peel fluid and orange peel hydrolysate	Acetate buffer, peptone and yeast extract	*G. xylinus* BCRC 12334	3.40 g/L	[41]
Cheap agricultural product konjac powder	Yeast extract and tryptone	*G. aceti* ATCC 23770	2.12 g/L	[52]
Sago by-product	Other components are same as of HS medium	*Beijerinkia fluminensis* WAUPM53 and *G. xylinus* 0416 (Reference strain)	0.47 g/L and 1.55 g/L for reference strain	[53]
Grape skins aqueous extract, cheese whey, crude glycerol and sulfite pulping liquor	Organic or inorganic nitrogen	*Gluconacetobacter sacchari*	0.1 g/L	[54]
Waste as nitrogen source				
Pineapple peel and sugar cane juice	Glucose, fructose and sucrose	*Gluconacetobacter swingsii*	2.8 g/L	[55]
Others				
Poor quality apple residues in combination with glycerol	Apple glucose equivalents, glycerol, ammonium sulfate and citric acid	*G. xylinus* DSMZ-2004	8.6 g/L	[56]
Pineapple and watermelon peels	Sucrose, ammonium sulfate and cycloheximide	*G. hansenii* MCM B-967	125 g/L (on a wet weight basis)	[57]

bacterial fermentation to produce BC. Hong et al. successfully utilized dilute acid hydrolyzed wheat straw as a starting feedstock for BC production using *Gluconacetobacter aceti subsp. xylinus*. The bacterial growth inhibitors were removed by detoxifying the wheat straw hydrolysates with various alkali, like sodium hydroxide, calcium hydroxide, and ammonia, in combination with laccase or activated charcoal. The results showed about a 50% improved production of BC in calcium hydroxide and charcoal-treated hydrolysate, as compared to chemically defined medium, under identical conditions [39]. Another study reported on BC production from chemically and thermally pretreated wheat straw containing 52.12 g/L sugar. After initial chemical and thermal pretreatment, the wheat straw was subjected to enzymatic hydrolysis using cellulase, β-glucosidase, and xylanase. The hydrolyzed wheat straw medium produced up to 10.6 g/L BC [40].

1-3. Peels

Peels of some fruits and vegetables are inedible, thus discarded. The inedible peels account for 5-40% of the total weight of fruits and vegetables and are rich sources of reducing sugars, vitamins, proteins, and acids and thus can be used as substrates for value-added products such as BC. Several studies have reported on the biological conversion of peels from different fruits and vegetables

into BC. For example, orange peel waste from the juice processing industry was utilized as a medium for BC production. The peel with 10% moisture content was composed of 30-40% sugars, 15-25% pectin, 8-10% cellulose, and 5-7% hemicellulose. The pretreatment of peel with cellulase and pectinase increased the concentration of fermentable sugars to 60-80 g/L that then resulted in the production of 4.2-6.32 times more BC, compared to HS medium, and the BC also contained a dense pack of nanofibrils [41].

1-4. Oat Hulls

Oat hulls are a cheap and renewable resource that account for 28% of grain weight and contain up to 45% cellulose [42]. On an industrial scale, hulls are accumulated at grain-processing facilities that produce oat cereals, slices, and cookies. Oat hulls are an industrially sustainable and global waste product that is standardized against chemical composition (i.e., chemical composition is not affected by growth conditions) and mechanical features (i.e., similar size, strength, and elasticity). These features indicate that BC production using oat hulls as a medium can be effectively standardized. Oat hulls are hydrolyzed to a sugar solution by various pre-treatment approaches prior to their use as a medium. A recent study reported pilot production of BC from oat hulls that involved four different production steps: (1) exclusive chemical pretreatment with HNO_3 under atmospheric pressure at concentrations of 2-6 wt%, (2) enzymatic saccharification with commercial enzymes that resulted in 79.5% sugar yield (carried out in a 100-L fermenter), (3) mixed-culture fermentation of the hydrolysate by *Medusomyces gisevii* in a Binder KB-400 incubator, and (4) purification. The resulting BC yield was 10% of the reducing sugar concentration. The produced BC was highly pure with a crystallinity index of 93% [43].

2. Industrial Brewery Wastes

2-1. Fermentation Wastewater

The fermentation wastewaters from acetone, butanol, and ethanol (ABE) factories are a rich source of sugars (glucose and xylose), organic acids (acetic acid and butyric acid), and alcohols (ethyl alcohol and butyl alcohol); thus, they can be used as a medium for BC production. Huang et al. reported on the production of 1.34 g/L BC by *G. xylinus* CH001 after seven days by utilizing ABE medium. Further, structural analysis via FTIR and XRD revealed that the ABE medium did not affect the structure of BC, as compared to BC produced using an HS medium. Thus ABE waste can be effectively used for routine and low-cost BC production [58].

2-2. Sludges

Different kinds of sludges such as thin stillage (TS), vinasse, Makgeolli sludge (MS), waste beer yeast (WBY) from waste beer fermentation broth (WBFB), and lipid fermentation wastewater are rich sources of useful ingredients like carbohydrates, vitamins, minerals, and proteins, and thus can be potentially used as media for BC production.

2-2-1. Vinasse and TS

Vinasse and TS are industrial byproducts produced during the distillation of ethanol from the fermentation of molasses, which is usually produced from sugarcane or sugar beet and corn starch. They are generally brown with a low pH (4-5), are highly turbid, rich in various carbohydrates and organic acids, and contain appreciable amounts of inorganic salts composed of the sulfate and phosphate forms of Ca, K, Na, and Mg. TS is a liquid waste obtained from the fermentation of grain-based feedstock. TS obtained from rice wine distilleries is rich in carbon and organic acids. In an early study, Wu and Liu utilized TS to supplement HS medium for the growth of *G. xylinus* used in BC production. Additionally, the replacement of TS with water in the preparation of HS medium further increased BC production by 2.5-fold to a concentration of 10.38 g/L [59]. Revin et al. reported on the production of 6.19 g/L BC using TS from wheat and 5.45 g/L BCusing whey, as compared to 2.14 g/L BC obtained from HS medium after three days under dynamic cultivation. Additionally, the BCs produced from these different sources showed variation in their micromorphology and crystallinity, despite possessing identical chemical structures [60].

2-2-2. Makgeolli Sludge

Makgeolli sludge produced in traditional rice wine distilleries, typically discarded as waste, is a rich carbon source for the growth of *G. xylinus*, as it contains glucose (10.24 g/L), nitrogen (0.81 g/L), organic acids (1.15 g/L), alcohol (0.93% v/v), and metal ions. BC produced from MS has a compact fibrous network and characteristic cellulose in polymorphic form [61].

2-2-3. Beer Waste

Different types of wastes produced by the beer industry are rich sources of nutrients for the growth of microorganisms and thus can be used for BC production. For example, waste beer yeast (WBY) is a byproduct of the fermentation of various cereals. It is mainly composed of proteins (48-55%), carbohydrates (23-28%), RNA (6-8%), vitamins (2%), and glutathione (1%), in addition to small amounts of phosphorus, potassium, calcium, iron, and magnesium [62]. This rich chemical composition makes it an attractive candidate for BC production; however, it cannot be directly utilized by microorganisms due the presence of large polymers of carbohydrates and proteins. Such large polymers are disrupted via pretreatment with ultrasonication and acid or using alkaline hydrolysis. Lin et al. used WBY as a sole nutrient source for growth of *G. hansenii* CGMCC 3917 that resulted in up to 7.02 g/L and 1.21 g/L BC from ultrasonicated and untreated WBY, respectively. Ha et al. utilized the waste from beer fermentation broth (WBFB) as a medium for BC production. WBFB contains a considerable amount of semi-solid waste at the end of beer production process and includes reasonable quantities of non-fermented sugars. Depending on the initial raw materials, these sugars can be simple ones, including glucose, or polysaccharides like starch. Prior to its use for BC production, WBFB should be sterilized to kill the yeast used in the production of ethanol. A comparatively high level of BC production under shaking conditions was reported, as compared to static incubation [7].

2-2-4. Lipid Fermentation Waste

Lipid fermentation wastes such as glycerol and residual waters are rich sources of lipids, residual sugars, such as glucose, xylose, and arabinose, and exopolysaccharides, produced as byproducts during biodiesel production; thus, this waste can serve as a rich medium for BC production. Supplementation of a medium with glycerol as a carbon source from biodiesel production and grape bagasse produced up to 10 g/L BC. The microfibrils of BC were several micrometers long with rectangular cross-sections with a width and thickness in the range of 35-70 and 13-24 μm, respectively, with a high crys-

tallinity of up to 79% [63]. Huang et al. used wastewater from lipid fermentation as a substrate for BC production using *G. xylinus* with a yield of up to 0.659 g/L after five days [64]. This study supports the possibility of using lipid fermentation wastewaters containing low-value carbohydrates to produce high-value BC polymer products.

In addition to the above-described sludges, a study also reported BC production from wastes of fiber sludges obtained from pulp factories. This was carried out in three steps: enzymatic hydrolysis of fiber sludge, microbial production of BC, and production of cellulase enzyme. The hydrolysis of fiber sludge was done using Cellic CTec2 hydrolyzing enzyme under shaking at 150 rpm at 50 °C for two days. The medium obtained from hydrolysis of the sludge was then used as a carbon source for BC production by *G. xylinus*, and finally the leftover sludge was converted to BC by using the cellulase [65].

2-3. Corn Steep Liquor

Corn steep liquor (CSL) is a byproduct of wet corn milling. It is a rich source of nitrogen, carbon, and vitamins; thus, it effectively supports microbial growth and fermentation [66]. Additionally, its use as a growth and production medium for microbial cells aids in minimizing the environmental pollution resulting from disposal, as well as reducing deforestation caused by use of vegetal cellulose. A study reported up to 3.12±0.03 g/L BC production by *Acetobacter* sp. V6 after eight days from CSL under shaking cultivation with supplemented molasses as a carbon source. This yield was twofold higher than that obtained when using a conventional medium. Additionally, the BC possessed high crystallinity (83.02%), as compared to that produced using a conventional medium (67.27%) [67]. In another study, Costa et al. investigated the BC-producing potential of a mixture of various carbon and nitrogen sources and CSL, as compared with HS medium. The results showed medium composed of 2.5% CSL and 1.5% glucose produced the highest yield of BC (dry and hydrated mass) using *G. hansenii* UCP1619, which corresponded to 73% of that produced with the HS medium. Additionally, the BC produced from CSL exhibited greater thermal stability, high crystallinity, and higher tensile strength [68].

3. Bakery Wastes

Bakery wastes, including breads, dough, and flour, which are generally discarded by catering agencies due to expiration or spoilage, can be utilized for BC production. They can be hydrolyzed to glucose by boiling in HCl or H_2SO_4 in high pressure vessels. Similarly, high quantity and quality BC can be produced from fermentation media obtained from the waste byproducts of confectionery industries. Tsouko reported on the production of 13 g/L high-quality BC from batch fermentation of flour-rich hydrolyzed bakery wastes using *Komagataeibacter sucrofermentans* DSM15973. The produced BC demonstrated a high water-holding capacity of up to 102-138 g water/g of dry BC and increased mechanical properties such as stress at break (72.3-139.5 MPa) and Young's modulus (0.97-1.64 GPa). These properties are comparable to those of BC obtained from an expensive chemically defined medium [69].

4. Municipal Wastes

The wastewaters from many industries contain important nutrients that are commonly discarded, leading to a great economic and environmental loss. Utilization of such streams as media to produce value-added products, such as BC, thus has economic and environmental importance. Li et al. reported on BC production from wastewater from the Candied Jujube processing industry. In their study, both untreated and acid-treated wastewaters were utilized as nutrient sources for BC production using *G. xylinus* CGMCC 295. The pretreatment with acid increased the glucose content of wastewater, as compared to the untreated wastewater. The acid-treated wastewater produced up to 2.25 g/L BC, which was 1.5-times more than that produced using untreated wastewater. The fiber diameter of BC produced from acid-treated wastewater was 3-14 nm, with an average value of 5.9 nm; however, the crystallinity was lower than that of BC produced using untreated wastewater [70].

5. Textile Mills Waste

The tons of waste produced in the form of textiles and fibers by textile industries and that thrown away by consumers is a significant environmental challenge. This highlights the demand for sustainable utilization, recycling, and management of these textiles and fibers. One important solution could be their use in the production of value-added products because of the high cellulose content. This utilization requires detoxification and pretreatment through processes like hydrolysis. For example, pretreatment of cotton-based textiles with ionic liquid and enzymatic hydrolysis produces a hydrolysate containing as high as 17 g/L of sugar. Hong et al. utilized this hydrolysate as a medium for BC production, wherein *G. xylinus* produced as high as 10.8 g/L BC, corresponding to an 83% yield and BC with high tensile properties, as compared to that produced from HS medium [71]. In another study, pretreatment of cellulose-based textiles with 85% concentrated phosphoric acid, N-methylmorpholine oxide monohydrate, ionic liquid 1-butyl-3-methylimidazolium chloride, and a NaOH/urea solution, followed by enzymatic hydrolysis, produced up to 1.88 g/L and 1.59 g/L BC from discolored hydrolysate and colored hydrolysate, respectively [72].

BC PRODUCTION FROM CHEAP SOURCES

The primary barrier to industrial-scale production and broad-spectrum applications of BC is the high production cost due to expensive culture media which limits the use of BC in high value-added applications. In addition to utilizing different wastes from various sources as described above, another solution is to utilize low-cost materials as substrates. To this end, extensive efforts have been devoted towards the exploration of cheap natural carbon and nitrogen sources [3]. Several cheap sources with potential of BC production are shown in Fig. 4. The following sections discuss low-cost BC production from various cheap natural carbon and nitrogen sources.

1. Molasses

Molasses is a viscous liquid obtained as a byproduct during the manufacturing or refining of sugar [73]. It is widely used as a microbial fermentation substrate and has different levels of readily biodegradable sugars such as sucrose, fructose, and glucose, in addition to N, Fe, Ca, K, Mg, and vitamins [74]. Because of the low cost and high content of total reducing sugars, as well as other useful components, molasses has attracted attention for use as a substrate in BC production [75,76]. A study reported that 12.6 g/L BC was produced by *Gluconacetobacter intermedius* SNT-1 under static con-

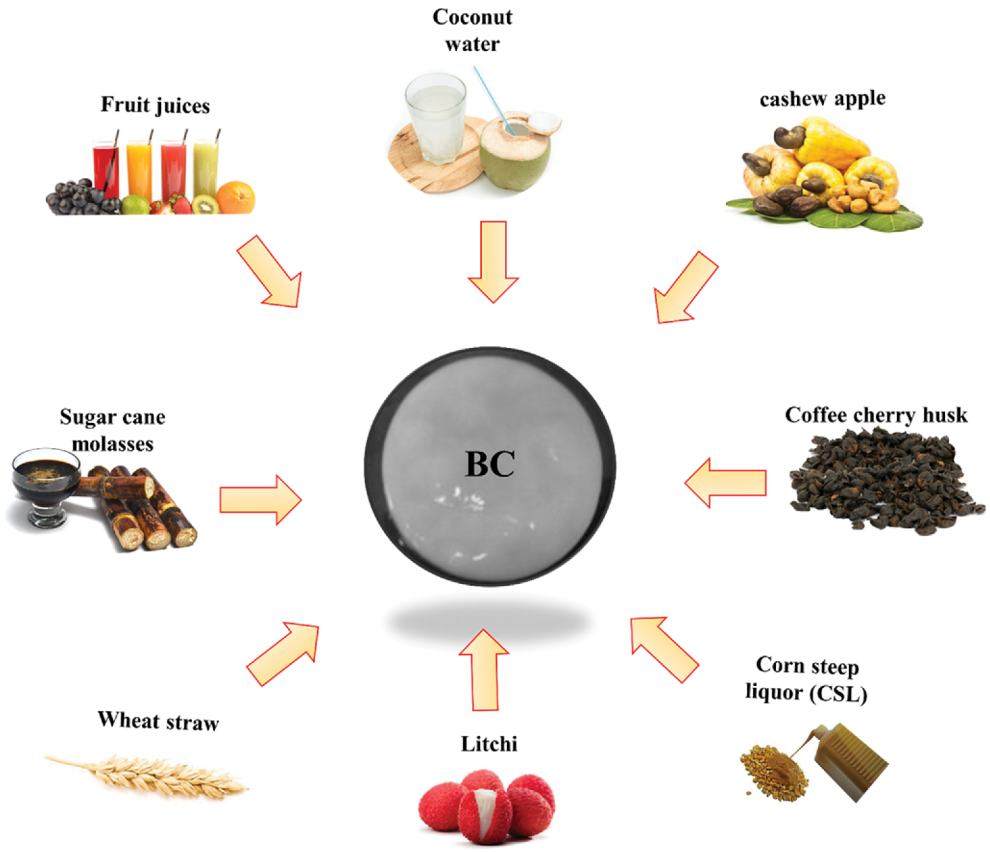

Fig. 4. Bacterial cellulose production from various cheap resources.

ditions using 45.8 g/L of diluted (1 : 4 v/v) molasses after heat pre-treatment with H_2SO_4. The yield of BC pellicles was comparable with yields produced using heat pre-treated molasses and corn steep liquor or yeast extract as the nitrogen sources. Additionally, the BC from this alternative source had high mechanical properties [75]. Another interesting study formulated 36 alternative culture media by utilizing rawhide sugar cane molasses, molasses inverted by high temperature, and molasses inverted by acid and high temperature, combined with nutrients like glucose, peptone, yeast extract, Na_2HPO_4, and citric acid. The results showed that, compared to the standard HS medium, the alternative medium formulated with 15 g/L of rawhide molasses, 5 g/L glucose, 1.5 g/L acid citric, and 2.7 g/L Na_2HPO_4, without any nitrogen source addition, had the lowest production cost and yields of 52, 59, and 65% in dry mass, hydrated mass, and yield, respectively [76]. These studies elucidate the suitability of using sugar cane molasses to minimize the BC production cost for industrial use. Additionally, the utilization of molasses as a medium for BC production lowers the environmental impact in terms of energy consumption and pollution load.

2. Fruit Juices and Extracts

Wasted food items and effluents produced by food industries are rich sources of important nutrients. For example, palm oil mill waste, pineapple juice industry waste, pineapple peel juice wastes, sugar cane molasses, sweet potato pulp, rotten apples, and apple juice industry wastes are all rich sources of glucose, sucrose, proteins, vitamins, and other useful ingredients; thus, they can serve as media for low-cost BC production. A study reported an effective BC producing medium comprising different fruits like grapes, Japanese pear, apple, orange, and pineapple. *G. xylinus* NBRC 13693 produced a high amount of BC from this medium, which was further increased when additionally supplemented with nitrogen sources [77]. The following sections overview the potential of different fruits to be used as media for BC production.

2-1. Oranges

Peel and pulp from oranges can be used as media for BC production. A study reported the production of about 0.65 g dry weight of BC by utilizing 17.2 g of solid waste residues from oranges, including peel and squeezed residues [77]. Kim et al. utilized citrus fruit juice as a medium supplemented with 10% sucrose, 1% acetic acid, and 1% ethanol for the growth of *Gluconacetobacter* sp. gel_SEA623-2 at 30 °C and pH 3.5 that produced BC with a soft physical structure, high tensile strength, and high water retention capability [78].

2-2. Sisal Juice

Sisal juice was utilized as a medium for the growth of *G. hansenii* ATCC 23769 under static conditions that produced up to 3.38 g/L BC after ten days of cultivation at pH 5 when supplemented with 15 g/L sugar and 7.5 g/L yeast extract. This yield was three-times higher than that produced using the HS medium, indicating the suitability of sisal juice as a substrate for BC production [79].

2-3. Watermelon

Kosseva et al. utilized watermelon and mandarin juices as a medium for the growth of *G. xylinus* CICC10529. The medium was

further supplemented with 1% ethanol (v/v), 1.5% (w/v) $MgSO_4 \cdot 7H_2O$, and 0.1% (w/v) K_2HPO_4. BC was effectively produced under static and shaking cultivations at 30 °C in seven to ten days. Highest BC yield up to 12 g/L and 11 g/L was obtained from 70% (v/v) watermelon juice followed by a mixture of 80% (v/v) watermelon and 80% (v/v) mandarin juices, respectively. Specifically, the width of BC ribbons produced after two days was larger (40-50 μm) than those produced under static conditions (25-37 μm). Additionally, the BC produced under shaking conditions was thermally more stable [80].

2-4. Coconut

Coconut is mainly produced in Indonesia and India and is available year around. Coconut juice is a rich source of carbon and nitrogen and thus can be effectively utilized as a medium for BC production. An early study by Hungund reported that coconut water contains about 1.6% sugar and was utilized as a medium for BC production by *Gluconacetobacter persimmonis*. When supplemented with 2% peptone, 0.5% yeast extract, and 0.115% citric acid, *G. persimmonis* produced 6.18 g/L BC at pH 6 after 14 days, which was comparable to the amount of BC produced from orange juice under identical conditions [81]. In another study, BC production was reported by *G. xylinus* cultured in a medium containing coconut water under static and shaking cultivations. A high rate of BC production and significant conversion of coconut water to BC by *G. xylinus* was shown, as compared to pineapple juice [82].

In another study, the optimum conditions, such as carbon source percentage, cultivation time, and pH, for producing BC from coconut water were determined. *G. xylinus* was cultured in a medium containing coconut water supplemented with 3%, 5%, or 7% sugar, with the cultivation time fixed at three, five, or seven days. The pH conditions were maintained at pH 3, pH 5, or pH 7. The BC produced under different conditions was dried at 100 °C until the moisture content reached 4-5%. Overall, results showed that optimal BC production was carried out with a 5% carbon source at pH 5 after seven days. The produced BC had pores covered by fibrils [83]. In another study, *Gluconacetobacter* sp. sju-1 was used to produced BC from mature coconut water and cashew apple juice under optimal conditions of incubation time of 20 days, pH of 6.5, incubation temperature of 31 °C, fructose as the carbon source, yeast extract as the nitrogen source, and indole acetic acid as a growth hormone. Under static cultivation, 14.92±0.30 g/L dry weight BC was produced with a biosynthetic yield of 74.7±0.32%. In contrast, 9.63±0.20 g/L BC was produced with a biosynthetic yield of 48.15±0.98% under shaking conditions. The produced BC was utilized in products such as reinforced paper, nata, and vinegar under optimized conditions [84].

3. Vegetables

3-1. Coffee Cherry Husk

Coffee cherry husk is produced during dry processing of coffee cherry. Dry processing of one ton of coffee cherries produces about 0.18 ton of coffee cherry husk [48]. Coffee cherry husk is a rich source of polyphenols, proteins, carbohydrates, and minerals, but its use in agriculture has been restricted due to the presence of undesirable substances like tannins, caffeine, and other polyphenols, and disposal poses a significant pollution problem. Rani and Appaiah utilized coffee cherry husk as medium for BC production using *G. hansenii* UAC09, along with other nutritional constituents including water (at a coffee cherry husk ratio of 1 : 1), 10% CSL, 0.5% alcohol, and 1.13% acetic acid. At pH 6.64, 6.24 g/L BC was produced after 14 days [85]. In another study, Rani and Appaiah utilized coffee cherry husk with other nutrients such as nitrogen (CSL, urea) and additives (acetic acid, ethyl alcohol). About 5.6-8.2 g/L BC was produced using these concentrations of nutrients: coffee cherry husk extract 1 : 1 (w/v), 8% (v/v) CSL, 0.2% (w/v) urea, 1.5% ethyl alcohol, and 1.0% (v/v) acetic acid. Under these conditions, the BC yield was three-fold higher, as compared to the control. Further, the BC produced from coffee cherry husk extract had a high mechanical strength [48]. These studies indicate the potential of coffee cherry husk as a cheaper alternative feedstock for BC production, which additionally helps to minimize a major environmental issue.

3-2. Litchis

Litchi has a high nutrient content and edible value; however, its marketability is limited by an extremely short shelf-life [86]. A study reported on the utilization of litchi extract as a feedstock for BC production by *G. xylinus*. Under static cultivation, *G. xylinus* produced 2.53 g/L BC membrane (dry weight) after 14 days that had a regularly reticulated nanostructure and high crystallinity (94%), which was an improvement over BC produced from an HS medium. Interestingly, the produced BC was doped with sodium and magnesium elements, indicating *in situ* composite synthesis [87]. The BC produced from litchi extract can be utilized as a food source or a food additive.

3-3. Tea

Yim et al. evaluated the potential of four types of tea and different sugars as carbon sources on the production (i.e., yield) and features (i.e., diameter, appearance, and structure) of BC. BC production was highest when green tea and sucrose were used as nitrogen and carbon sources, respectively. The BC fabric was 0.213 mm thick with high crystallinity (74.26%) and mechanical strength [88]. Black tea broth (known as kombucha) can also be used as a nitrogen source for BC production. Goh et al. carried out fermentation of kombucha and investigated the effect of sucrose concentration and fermentation time on BC yield. A yield of 66.9% BC was obtained when 90 g/L sucrose was included as the carbon source during fermentation. The thickness and yield of BC was further increased with fermentation time [89].

BC MARKET AND SELLING PRICE

Nata de Coco is the main commercial form of BC and is in huge demand in the international market, especially in Japan, which largely introduced it to the rest of the world. It first entered into the market in 1980 when Del Monte Corporation exported snack/dessert-sized servings of a tropical fruit mix that contained Nata de Coco cubes. From 2006-2013, Japan was the major importing country with an aggregate 30,501 tons, followed by the United States (3,327.5 tons), the United Arab Emirates (926 tons), Canada (758 tons), and Malaysia (683 tons). During this period, Japan imported 77% of all internationally sold product, followed by the United States (8.37%). During this time, the Philippines exported Nata de Coco to 40 other countries, primarily in Europe and the Middle East; however, its exportation decreased from 2011-2013 and the country

began importing Nata de Coco from mainly Asian countries. According to data from 2006-2013 obtained from Department of Trade and Industry (DTI), various countries began importing Nata de Coco from China, Taiwan, Malaysia, and the United States, totaling 78.6 tons valued at US$ 35,145. Since then, the export of Nata de Coco by Malaysia has exponentially increased and accounted for 85.7% of the total amount from just January to February 2015. Export from Taiwan and China accounted for 5.4% and 8.8%, respectively. Over the past few decades, BC production has exponentially increased. According to a report by ResearchMoz, the BC market was valued at US$ 207.36 million in 2016, and is expected to reach US$ 497.76 million by the end of 2022, with a capital annual growth rate (CAGR) of 15.71% [90]. According to this report, the BC market is expected to surpass a valuation of US$ 700 million in 2026. Currently, there are several key players in the BC market, including Celluforce, American Process, Innventia AB, University of Maine, US Forest Service, Borregaard, and Nippon, based in countries including the USA, Canada, Europe, Japan, and China.

The Nata de Coco form of BC is mostly sold as slabs or diced pieces on the buyers' requirements. It is also sold in the form of 'reject', which refers to Nata de Coco obtained during the trimming of cubes or slabs, or sliced Nata de Coco with dark spots or blemishes. Depending on the traded amount and finishing of the final product, the price of Nata de coco for external markets ranges between US$ 200-1,000 per ton, corresponding to US$ 1-10 per kg on a small scale [91]. However, the pricing of Nata de Coco differs between manufacturers and is dependent on the finished product. For example, the price of slabs ranges between US$ 0.31-0.36 per kg, while that of slices ranges between US$ 0.27-0.36 per kg. Similarly, the 'reject' product is sold at a retail price between US$ 0.33-0.45 per kg. These prices may also vary according to the type of buyer (e.g., walk-in or regular customers). However, as the buying potential of walk-in buyers is low, manufacturers prefer to mostly sell Nata de Coco to regular buyers [92].

TECHNO-ECONOMIC ANALYSIS OF BC PRODUCTION

Dourado et al. carried out a comprehensive techno-economic analysis of an industrial-scale fermentation process for BC production [93]. The analysis was carried out using Super-Pro Designer software (version 9) for a Windows operating system.

1. Input Data

For analysis, the authors first collected data on the type of strains, culture media, and fermentation conditions used in production. The authors selected a publication by Keshk et al. [94] for retrieval of relevant data including strain type (e.g., *Komagataeibacter xylinus* ATCC 10245), waste resource (e.g., 570 g/L beet molasses), chemically defined culture medium (e.g., yeast extract, peptone, disodium phosphate, and citric acid), yield (e.g., 7 g/L), cultivation time (e.g., 7 days), and cultivation strategy (e.g., static). The plant was arbitrarily projected to process 60,000 L/month of culture medium. Assuming hydrated BC contains about 99% water, this production volume was expected to yield 42 tons/ month, corresponding to 504 tons of BC per year. The inoculum propagation was assumed to be at a 1 : 10 ratio of biomass to culture medium. Furthermore, the software estimated the equipment size as well as cost (~US$ 4.83 million) by using built-in cost correlation from data obtained from a number of vendors and literature resources, based on the input data and with consideration for unit operations, reaction kinetics, and raw materials.

2. Capital Investment

According to the results, the total capital investment for an industrial facility capable of producing 504 tons of BC per year was about US$ 13 million, of which 71% corresponds to direct costs for equipment and installation, piping, instrumentation, insulation, electrical facilities, building cost, yard improvements, auxiliary facilities, and land, while the remaining 29% corresponded to the indirect cost associated with engineering and construction. In addition to the above estimated cost, an additional estimated US$ 0.966 million was included as contingency charges to compensate for unpredicted expenses, minor process charges, price fluctuations, and estimation errors.

3. Manufacturing Cost

The annual manufacturing cost was estimated to be US$ 7.4 million which included the factors directly contributing to production, such as direct operational costs, fixed charges, and plant overhead, as well as general expenses. The operating labor cost was estimated to be US$ 2.18 million, corresponding to 58% of the direct cost. Fixed charges, comprised of depreciation, local tax, insurance, and rent costs, accounted for US$ 2.1 million. The overhead cost was estimated to be about US$ 1 million, which included charges for various services such as medical facilities and a cafeteria, as well as janitorial, administrative, and accounting services.

4. Profitability Analysis

The profitability analysis, usually carried out in terms of gross margin analysis, representing the percent of total sales revenue retained after incurring direct costs, considered the market price of BNC to be US$ 25/kg for a packed BC cube final product. According to the software analysis results, the net profit was estimated to be US$ 3.3 million/year. Importantly, the estimated payback period, representing the time required to recover the capital investment, was four years.

CONCLUSIONS AND PERSPECTIVE

Techno-economic analysis through process simulation revealed that the biotechnological production of BC is highly capital-intensive. Despite the use of low-cost substrates, low yield, high capital investment requirements, and associated high operating costs present major economic constraints to the commercialization of BC production. In addition, the sugars used as carbon sources, particularly glucose and fructose, have resulted in high selling prices of BC. Researchers are aiming to explore alternative ways to minimize production cost, including isolation and engineering of high BC-producing microbial strains, development of advanced reactors, and utilization of low-cost substrates. Together, these efforts have contributed greatly to minimizing BC production costs. Various low-cost substrates, including wastes from agro-industry, breweries, food production, and municipalities, described in this manuscript, have shown great potential for high quality BC production at a much lower cost than use of chemically defined synthetic media. In addition, the use of such wastes as media for BC production has

greatly contributed to waste disposal and management, thus illustrating positive economic and environmental impacts.

In contrast to the use of wastes from various sources, the utilization of edible feed stocks, like fruits and vegetables, is questionable. Although the use of feedstocks has shown promising potential in high quality BC production, considering nutritional value and pricing, these cannot be the preferred choice for production of products like Nata de Coco; nevertheless, BC production from food sources for the manufacturing of high value products, like medical implants and energy storage devices, still receives economic acceptance. Alternatively, the utilization of sugars from wasted food is a wise approach for general BC production, as globally, approximately one-third of food is wasted and is a rich source of sugars. By utilizing low-cost substrates and wastes from agro-industry, breweries, food producers, and municipalities, it is possible to devise an economically feasible biotechnological process for BC production, though its high selling cost would restrict BC to high-value markets.

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Joong Kon Park is a Professor at the department of Chemical Engineering, Kyungpook National University in Korea. He received his B.S. degree (Seoul National University, Korea), M.S. and Ph.D. degrees (KAIST, Korea), all in Chemical Engineering and was a postdoctoral fellow at the University of Michigan at Ann Arbor. His research interests include transport phenomena, and biochemical engineering, especially, bacterial cellulose nanocomposite. His paper "Overview of bacterial cellulose composites: A multipurpose advanced material" has been cited more than 350 times. He had worked as Editor-in-Chief of Korean J Chem Eng for 8 years from 2009 to 2016 and also worked as Director of Engineering at Korean NRF for 2 years from 2017 to 2019.