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Sustainable Bioconversion of Industrial Wastes into Bacterial Cellulose for Diverse Applications: A Way Towards Pollution Control and Abatement

Ajay Patel[1] · Payal Patel[1,2] · Arpit Shukla[1] · Jonathan W. C. Wong[3] · Sunita Varjani[4,5] · Haren Gosai[1]

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Abstract
Socio-economic and environmental factors have led scientific community to find alternative approaches for management of agro-industrial wastes. An integrated approach, i.e., clean biotechnology, could be used for the conversion of agro-industrial wastes into industrial important and less toxic end products. Bacterial cellulose (BC) is an incredibly multifaceted biomaterial with desirable attributes including biodegradability, biocompatibility, great tensile strength, cellulose purity, and porosity. An economical BC production is difficult to owing to the cost of expensive synthetic media. By utilizing processed agro-industrial wastes as media substrate, a sustainable large-scale BC production can be achieved along with an effective waste management strategy. Various types of industrial wastes including crop residues, food industry by-products, distillery effluents, and kitchen wastes are used to produce BC. This review is centered on various aspects of cost-effective BC production using industrial wastes and a wide range of probable substrates with alternative methods for enhanced BC production. Novel applications involving BC in the field of environment, wound healing, drug delivery, dental treatment, etc., with an emphasis on new economic opportunities are also discussed. Overall, this study suggests that integrating different methods and techno-economic analysis would be advantageous to researchers in finding way for sustainable production of BC with reduced environmental pollution for diverse applications.

Keywords Bacterial cellulose · Industrial waste · Bioremediation · Environmental sustainability

Introduction

Cellulose is one of the most abundant polysaccharides present on the Earth [1]. Cellulose which is synthesized by plants and algae constitutes the majority of naturally available celluloses, as their cell walls comprising 15–40% of cellulose [2]. While most of the plants produce cellulose, some bacteria also have the capability to synthesize cellulose. The plant cellulose (PC) contains hemicellulose and lignocellulose, whereas the bacterial cellulose (BC), also known as microbial nanocellulose, is produced in pure form which is free from other polysaccharides [3]. BC is a linear polysaccharide comprising more than 10,000 glucose monomers linked together with β (1 → 4) glycosidic bonds and appears as semi-crystalline, insoluble, and gelatinous biomaterial [4]. BC forms microscopic fibers which is hundred times smaller than PC and are attached in 3-D fibrous meshwork which is produced under static condition [5–7]. Its three-dimensional network is 3–5 µm wide and 3–4 nm thick containing ribbon-like microfibrils (20–120 nm wide) [8].

✉ Sunita Varjani
drsvs18@gmail.com

✉ Haren Gosai
dh.haren@gmail.com

1 Department of Biosciences, School of Science, Indrashil University, Rajpur, Mehsana, Gujarat, India 382740

2 Department of Biotechnology, Bhagwan Mahavir College of Basic and Applied Science, Bhagwan Mahavir University, Surat, Gujarat, India 395007

3 Institute of Bioresource and Agriculture and Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong

4 School of Energy and Environment, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong

5 Sustainability Cluster, School of Engineering, University of Petroleum and Energy Studies, Dehradun 248007, Uttarakhand, India

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Bacteria from genus *Komagataeibacter* (previously known as *Gluconacetobacter*) are known to produce BC. *Komagataeibacter xylinus* is the most studied bacteria for BC production, with the first study on the microbe dating back to 1886 by a British chemist A J Brown [9, 10••]. Although a significant number of studies involving BC production employ *K. xylinus*, bacteria from other genera are also being considered for BC production [11]. Generally, the bacteria that are used for cellulose production are Gram-negative, slightly acidic, and aerobic in nature. These are usually found in fruits, juices, vegetables, and vinegar or fermented beverages utilizing raw fruits, vegetables, or lignocellulosic matter as carbon sources to synthesize cellulose [12]. Bacteria synthesize cellulose by converting the glucose molecules into nanocellulose microfibrils after undergoing several enzymatic reactions [1, 13]. The yield of BC is also dependent on the quantity of glucose obtained from the given carbon source [14]. Mostly, synthetic media with high quantity of carbon sources are utilized for synthesis of BC. Since the cost of medium components has a substantial effect on the overall production cost, the refined synthetic media are often a cause of increased expenses [15•, 16]. To overcome this economical barrier and attain cost-effective BC production, several researchers have turned towards industrial wastes as substrate for BC production. Industrial wastes include crop residues from agriculture, wastes or by-products generated through food industries or distilleries, and kitchen wastes. Use of industrial wastes has two important advantages: cost-effective industrial wastes could significantly (i) reduce the production expenditures and (ii) eliminate the problem of waste treatment [17].

Recently, researchers all over the globe has gained interest in BC production owing to their versatile uses in several industries [18•]. BC is biocompatible, biodegradable, non-toxic, flexible polymer with high purity, porosity, excellent crystallinity, and high water-holding capacity [13]. These properties are quite beneficial in the fields such as environmental, biomedical, sensor development, cosmetic, and food packaging. BC is used in the several environmental applications such as bioremediation of organic and inorganic waste like oils, heavy metals, and dyes. Biocompatibility of BC minimal side effects or allergy is observed, which increases the desirability of BC in the biomedical field [19–21]. BC is utilized for therapeutic wound healing, synthesizing artificial skin, organ and vessel grafting, drug delivery, dental treatment, etc. [22]. BC is commercially accessible in many Asian countries in the form of foods like *nata de coco* and beverages like *kombucha tea*. Since the scope of its applicability is diverse, BC is the prime choice for fabricating sustainable cellulose biomaterials; and as a result, it has been studied widely.

The existing BC production technologies need to be critically reviewed to determine sustainable approach. The main purpose of this review is to emphasize the latest findings and developments in the production of BC from agro-industrial wastes. Since the major obstruction to large-scale BC production is the huge investment costs, the review features catalogue of studies with inexpensive substrate for BC production. Here, sustainable, low-cost BC production using a wide range of waste products including agricultural wastes, food-industry wastes, distillery effluents, and kitchen wastes has been discussed in details. Additionally, their impact on BC yield and structure has been also discussed in details. Applications of BC in a wide variety of fields including bioremediation, wound healing, drug delivery, dental care, and foods have been also highlighted. A strong emphasis on underutilized environmental applications, which includes utilization of BC for absorption of heavy metals, dyes, oil, pesticides, and other toxic compounds, is another unexplored area that has been discussed in length. While other review articles are focused on single aspect of BC, in the present comprehensive review, the authors have tried to compile several important aspects of BC, starting from selection of medium or substrate and production techniques followed by its application in diverse fields. The authors believe that this review will benefit academic researchers and formulation scientists by providing fresh perspectives on BC in regard to developing some unique BC-based functional nanocomposites that are helpful in various sectors such as environment, healthcare, and food. In addition, the readers will be able to understand the multifaceted nature of BC with the help of this all-inclusive review.

Production of Bacterial Cellulose

Sources and Synthesis of Bacterial Cellulose

BC is synthesized by bacteria at liquid–air interface of the medium [23]. Production of BC is influenced by multiple factors including the type of bacterial strain, temperature, pH, medium components, and incubation time [10••]. Generally, the bacteria used for cellulose production are Gram-negative and aerobic in nature [24]. *K. xylinus* is the most commonly reported bacteria for BC production, owing to its high purity and ease of production. However, several genera including *Agrobacterium*, *Rhizobium*, S*almonella*, *Aerobacter*, *Pseudomonas*, *Sarcina*, *Achromobacter*, *Azotobacter*, and *Alcaligenes* have also successfully exhibited BC production [25]. Table 1 describes isolation of BC-producing bacteria from rotten fruits and vegetables, vinegar, fermented beverages, and other sources. In 2020, Bagewadi et al. have demonstrated BC production from a novel thermophilic *Bacillus licheniformis* ZBT2 strain isolated from forest soil. Many researchers have reported the use of bacterial and fungal consortia known as SCOBY

Table 1 Bacterial cellulose-producing strains isolated from various sources

Strain	Source	BC yield (g/L)	Production media	References
G. sucrofermentas H 110, *K. hansenii* C 110	Kombucha, Tibisco	2.19 and 1.76	Hestrin-Schramm media	[28]
G. medellensis	Colombian home-made vinegar	4.50	Hestrin-Schramm media	[29]
L. hilgardii IITRKH159	Sapodilla fruit	7.23	Yamanaka media	[30]
G. xylinus TJU-S8	Chinese Persimmon vinegar	4.62	GYC media	[31] IG
K. rhaeticus TJPU03	Rotten orange peel	8.28	Hestrin-Schramm media	[32, 33]
K. saccharivorans MD1	Fermented beverage	3.90	Hestrin-Schramm media (with sugarcane molasses as C source)	[34]
K. europaeus SGP37	Rotten grapes	9.98	Hestrin-Schramm media	[35]
A. pasteurianus RSV-4	Kinnow-rich fruit residues	5.60	Whey medium	[36]
B. licheniformis ZBT2	Forest soil	9.20	Hestrin-Schramm media (with (yeast extract 1.5% *w/v* and peptone 1.5% *w/v*)	[37]
G. hansenii ATCC23769	Glycerol	3.40	Hestrin-Schramm media	[38]
Pseudomonas sp.	Sugarcane rhizosphere	9.30	Hestrin-Schramm media	[39]
K. rhaeticus SU12	Kombucha tea	25.31	Mango extract	[40]
G. hansenii UAC 09	Contaminated grapes wine	-	Hestrin-Schramm media	[41]
G. xylinus C18	Corn steep liquor/molasses	4.34	Sugar cane molasses-modified Hestrin-Schramm media	[42]
G. intermedius CIs26	Mandarin fruit rotten	7.20	Citrus water solution	[43, 44]
K. rhaeticus	Kombucha SCOBY (symbiotic colony of bacterial and yeast)	2.90	Minimal media and crude glycerol	[45]
T. mepensis strain	rotten ramie leaf	5.02	Hestrin-Schramm media	[46, 47]
E. amnigenus GH-1	Rotten apple	4.10	Modified Hestrin-Schramm media	[48]
Komagataeibacter sp.	Rotten banana	1.60	Hestrin-Schramm media, corncob (CC), and sugarcane bagasse (SCB)	[49]
B. bruxellensis MH393498, *K. saccharivorans* LN886705, and *B. anomalus* KY103303	Kombucha consortium	18.68	Glucose and black tea	[50]
G. xylinus TJU-S8	Chinese persimmon vinegar	3.02	Glucose yeast extract	[31]
K. maltaceti	grape and apple vinegars	6.45	Hestrin-Schramm media	[51••]

(Symbiotic Culture of Bacteria and Yeast) for production of BC [26, 27]. These bacteria break down glucose or other biological substrate and convert them into cellulose to produce BC. It is estimated that a single bacterium can convert around 108 molecules of glucose into one unit of cellulose per hour (h) [10••].

BC synthesis is a systematic, stepwise process driven by different enzymes at each step, beginning with adsorption of glucose from the environment, followed by four major enzymatic reactions: (i) breakdown of glucose into glucose-6-phosphate by glucokinase, (ii) conversion of glucose-6-phosphate into glucose-1-phosphate by phosphoglucomutase, (iii) breakdown of glucose-1-phosphate into uridine diphosphate glucose(UDP-glucose) by UDP-glucose pyrophosphorylase, and (iv) synthesis of cellulose from UDP-glucose by cellulose synthase [13]. BC is synthesized in form of linear 1,4 glucan chains secreted out through pores of cell wall by bacteria. These linear chains form inter- and intra-chain hydrogen bonds which result in the formation of cellulose nanofibers. The nanofibers or ribbons further aggregate to form a gel pellicle at the air–liquid interface known as BC. For a wide range of applications, BC is put through a purification process to remove bacteria culture from pellicle; after forming a BC pellicle, we thoroughly rinsed it with distilled water to remove the medium that had become trapped inside the BC structure, boiled it in a 0.1 M NaOH/KOH solution at 80 ºC for 60 min to kill any remaining bacteria, and washed it with distilled water until pH 7.0, and a soft, gelatine-like gel with around 99% water content is derived [12, 18•].

Economical and Sustainable Bacterial Cellulose Production Using Industrial Wastes

The lab-scale BC production is accomplished through synthetic media, for instance, Hestrin-Schramm (HS), Hassid-Barker (HB), glucose-yeast extract-calcite (GYC), yeast extract-peptone-dextrose (YPD), and Yamanaka [52]. Use

of these media can drive up the excessive cost of production at industrial level. An approach for cost-cutting is to replace synthetic media with cheap industrial wastes leading to cost-effective BC production and sustainable usage of waste products [53]. A typical HS media most likely cost around US $4/L, i.e., for every liter of HS media, $4 will be needed [54]. While this valuation may seem quite economical on its own, when viewed from industrial perspective where bioprocesses are carried out using thousands of liters of media, this seemingly cheap price could be amplified to a great amount. If we look at Indian scenario, then cost of HS media will be relatively cheaper at approximately ₹231, i.e., around $2.8. This suggest that BC production could be accomplished with less expenses in India. However, in both scenarios whether BC production is carried out in India or outside India, agro-industrial wastes can be easily acquired at low expense. By using such sustainable waste as media substrate, the cost for media could be reduced by 40–50% with less than $2/L media. Earlier reports on BC production have revealed use of different types of wastes and by-products, i.e., (i) agro-wastes or residues, (ii) distillery wastes or effluents, (iii) by-products or wastes of food industries, and (iv) kitchen wastes. Figure 1 represents different types of industrial wastes used for BC production. Industrial wastes are either used directly as a production medium or as a substitute for carbon and/or nitrogen sources [55–58]. Table 2 summarizes the reports of BC production using different industrial wastes, recently.

Agro-Industrial Wastes

The agri-based industries generate tons of biomass every day, but their utility as raw materials is incomplete as only 10% of waste is recycled [83, 84]. Agro-based industries or residues include any material leftover after processing or usage of vegetables, fruits, or crops including vegetables and fruit peels, pomace, rotten fruits/vegetables, or lignocellulosic crop residues such as husks, bagasse, and shells [12]. These agro-wastes contain a wealth of nutrients, including polysaccharides, proteins, and secondary metabolites like phenolic acids, flavonoids, anthocyanidins, anthocyanins, carotenoids, and saponins [85]. These waste materials can be obtained at economical rates from local farms, vendors, or shops. However, they can be used for formulating a production media,agro-wastes must be processed to remove any undesirable impurities and any contamination. Thus, agro-wastes undergo thermal or enzymatic extraction for media formulation followed by sterilization. In a recent study by Fatima et al., rotten tomatoes were used to develop the production media for BC production. Rotten tomatoes initially autoclaved at 121℃ for 15 min, blended, filtered, and sterilized by placing them in oven at 70 °C for 6 h. The processed tomato juice was used as it is without any additional supplements for BC synthesis [86•]. Moreover, higher BC yield is observed using agro-wastes compared to synthetic media on several instances. Abol-Fotouh et al. have carried out BC production by replacing pricier D-glucose with equivalent concentration of sugarcane molasses residues. A significant

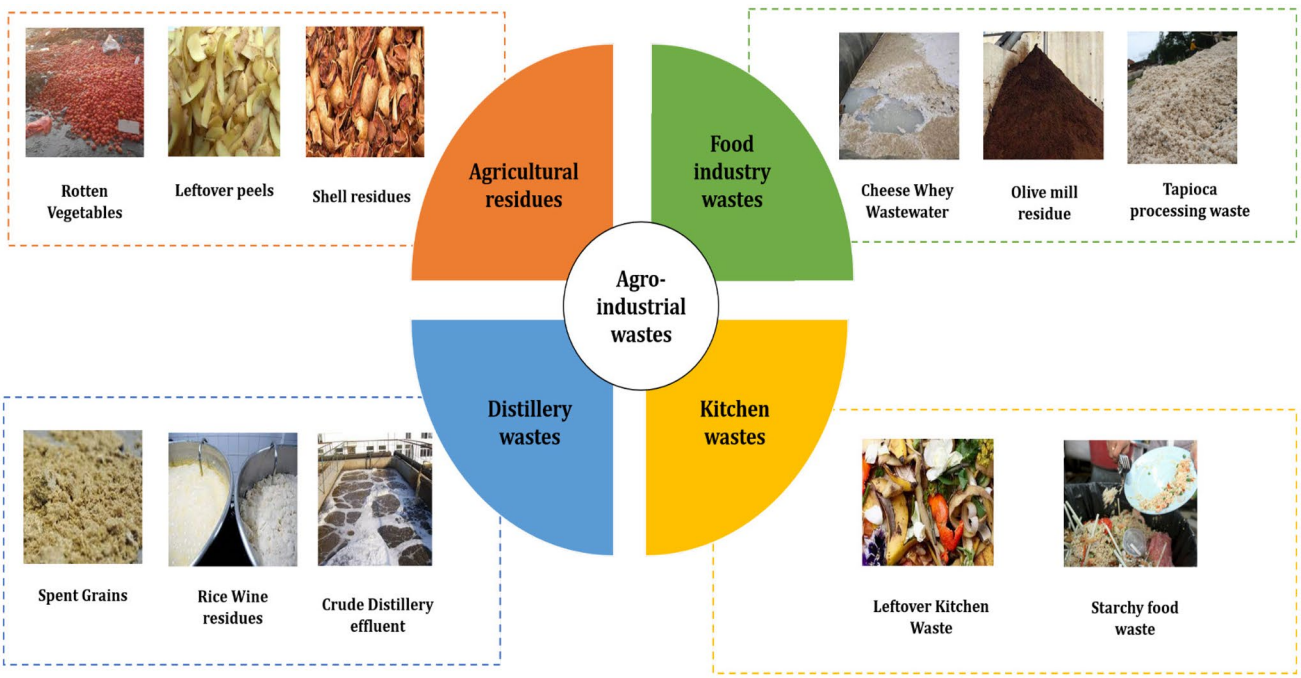

Fig. 1 Types of agro-industrial wastes used for bacterial cellulose production

Table 2 Bacterial cellulose production employing agro-industrial wastes

Carbon source	Bacterial strain	Incubation time (days)	Production (g/L)	References
Litchi extract	*G. xylinus*	14	2.53	[59]
Tobacco waste extract	*A. xylinum* ATCC 23767	16	5.2	[60]
Grapefruit juice and orange juices	*K. sucrofermentans* DSM 15973	13	6.7 6.1	[61]
Soapberry shell extract	*K. xylinus* B2-1	7	1.31	[62]
Waste beer yeast	*G. hansenii* CGMCC 3917	10	7.02	[63]
Sago by-product	*B. fluminensis* WAUPM53	2	0.47	[64]
	G. xylinus 0416	2	1.55	
Glycerol residual from biodiesel production	*G. xylinus*	14	10.0	[65]
Sugar cane juice, pineapple residual	*G. medellinensis*	7	0.82	[66]
Beet molasses	*G. xylinus* ATCC 10245	6	7	[67]
Pineapple peels waste	*K. xylinus*	9	3.8	[68]
Pineapple juice and corn starch hydrolyzed medium	*G. xylinus* strain	14	9.1 and 3.82	[69]
Fig fruit extract and date fruit extract	*K. saccharivorans* MD1	7	1.1 and3.2	[34]
potato tuber juice	*K. xylinus*	7	4.26	[70]
Mango pulp	*K. xylinus*	16	6.32	[71]
Wine and pulp industries	*G. sacchar*	3	0.6 and 0.3	[72]
Aloe vera gel pulp	*G. hansenii* ATCC 23769	10	-	[73]
Citrus peels and pomace	*K. xylinus*	8	5.7	[74]
Grape bagasses	*G. xylinus*	14	8	[65]
Pawpaw juice and watermelon juice	*A. pasteurianus* PW1	15	7.7 and 0.4	[75]
Musk melon and orange juice	*G. persimmonis*	14	6.18 and 5.98	[48]
Elephant grass acid hydrolysate	*G. xylinus* strain	14	6.4	[43, 44]
Cashew tree residue	*K. rhaeticus*	7	2.8	[76]
Date syrup	*G. xylinus* strain	7	1.2	[77]
Banana peels	*A. xylinum* strain	15	19.46	[78]
Carrot juice medium	*G. hansenii*	7	1.35	[79]
Wheat straw	*G. xylinus* strain	7	10.6	[80]
Vinasse	*K. xylinus* PTCC 1734	10	7.02	[81]
Sugar jaggery or gur from of brewery industry	*G. xylinum* ATCC 23768	10	2.51	[82]

increase in BC production (3.9 g/L) was observed using sugarcane molasses compared to HS medium [34]. Tobacco waste extract (TWE) juice, wasted grapefruits, pecan nutshells, and potato peel waste (PPW) have been also utilized for BC production of 4.7 g/L, 5.2 g/L, 2.8 g/L, and 5.2 g/L, respectively [17, 60, 61, 87]. Guzel et al. have also explored few fruits and vegetables peels as media alternative for the production of BC. Among melon, kiwi fruit, apple, cucumber, pomegranate, and quince, highest BC production was obtained using kiwi fruit peels [88].

Distillery Wastes or Effluents

Distilleries generate substantial wastes during alcohol production that include effluent wastewater, spent yeast, vinasse, and other by-products. Hence, distilleries are considered as one of the most pollution-causing industries, and the total waste generated is around 15 times of the total alcohol production [89]. The liquid effluents from the distilleries are disposed into environment after wastewater treatment, while the some of the solid wastes are generally used as compost [90]. An eco-friendly option is to utilize the distilleries and alcohol production-related wastes as medium for BC production. Lin et al. have reported BC production by *Gluconacetobacter hansenii* CGMCC 3917 using waste beer yeast with the highest productivity of 7.02 g/L. Crude distillery effluents was used by Gayathri and Srinikethan to synthesize 1.24 g/L of BC after 8 days of static fermentation through *Komagataeibacter saccharivorans* BC1 strain [63]. In a study by He et al., by-products of *baijiu* (a traditional Chinese alcohol) production underwent enzymatic treatment to develop the best distiller's grains—yellow water medium and BDY medium. After 7 days of static fermentation by *Gluconacetobacter xylinus*, a 3.72 times elevation in BC

yield was recorded compared to the regular HS medium [32, 33]. Hyun et al. have utilized makgeolli sludge (Korean rice wine waste) for production of BC and obtained 4–5-folds higher BC production compared to synthetic media [91].

By-Products and Effluents from Food Industries

Wastes from food industries are fascinating for many researchers due to various properties of the effluent and nutrients present in the wastes [92]. Several researchers have successfully incorporated such waste materials as substrate for eco-friendly BC production. Voon et al. and Ghozali et al. have used *sago* and *tapioca* wastes to synthesize BC, respectively [15•, 64]. Khattak et al. have used wastes of sugarcane as a media for the production of BC. They have cultivated BC-producing bacteria in both static and shaking conditions for 10 days without the supplementation of any additional nutrients. Considerable BC production was obtained after 10 days of incubation, i.e., 2.51 g/L in shaking condition and 2.13 g/L in static condition [82]. Carreira et al. have used cheese whey for production of BC, where additional nutrients including yeast extract, nitrogen, and phosphate were used, and BC production with a yield of 0.1 g/L is obtained after 96 h [72]. Coffee cherry husk (CCH) extract can also be utilized as a carbon source. Rani et al. have used CCH as carbon source in varying concentrations, while corn steep liquor and urea were used as nitrogen source for BC production. Additionally, some additives like ethyl alcohol and acetic acid have been also used in the minute amounts. A threefold increase in the yield of BC (5.6–8.2 g/L) was achieved compared to HS media [41]. Dry olive mill residue combined with nutrition sources was also found to be useful for synthesis of BC and yielded 1.28 g/L of BC following incubating period of 96 h [93]. Another interesting study by Li et al. has showed utilization of hydrolyzed wastewater from *candied jujube* (a type of fruit) to achieve low-cost BC production with a yield of 2.25 g/L after 6 days of incubation [94].

Kitchen Wastes

Wastes from home kitchens, canteens, or restaurants are rich in starch and carbohydrates which can be utilized by bacteria to produce BC. Wu et al. have used leftover starchy food from canteen to produce BC where the collected food was valorized before being used as the key substrate. After removing NaCl from the valorized food, the BC yield showed an increase from 0.11 to 2.07 g/L. Several other researchers have also successfully used kitchen wastes for BC synthesis [95, 96]. Wang et al. have designed a closed loop biorefinery system to separate oil from waste cooking oil which was subsequently used as the substrate to produce BC [97]. It is during the transportation of perishable food items such as fruits and vegetables that they are damaged or rotten and not suitable for consumption. Molina-Ramírez et al. have utilized rotten mango, rotten banana, and cheese whey for production of BC. Maximum production of BC having highest tensile strength was obtained from *Komagataeibacter medellinesis* using banana extract [98].

Production Methods for Bacterial Cellulose

The methods employed for the production of BC play a major role in determining a particular structure, consistency, and shape. Generally, BC production is carried out by either of the two methods: (i) static cultivation or (ii) agitated/shaking cultivation. Both are distinct techniques and impart different properties of cellulose such as macroscopic and microstructure of cellulose, respectively.

Bacterial Cellulose Production Through Static Cultivation

Currently, the most widespread method used for BC production is the static cultivation method. In this method, bacteria grown at static state create biofilms of cellulose on the interface of the media. In the static condition, the cellulose shape is determined by the type of vessel that is being used to cultivate the bacteria [12]. Mostly, such BC production is carried out in a plastic tray and beaker with large area surface in order to enhance the aeration. Both large surface area and incubation period are important factors in BC production during static cultivation. Cellulose microfibrils produced by the bacteria bind with each other at initial stage and move towards the surface. A cellulose aggregation is formed at the interface of media and air appears as a thin layer. Slowly, more such layers are formed, and they combine to form thick cellulose pellicles. Figure 2 illustrates the process of BC synthesis during static cultivation. Usually, the incubation time for BC production is 7–14 days. Maximum BC production has been observed after 168 to 336 h of incubation dependent on culture conditions [99, 100]. In static condition, pellicle formation on the surface of media is observed due to low aeration responsible for slow synthesis of cellulose. Meanwhile in agitated culture, the high aeration rate also affects BC production and properties of synthesized BC. Static culture has certain disadvantages such as large vessels are required for scale up production. However, BC produced using static condition has more advantages in biomedical fields because BC produced using static condition could be used in wound healing due to higher crystallinity and linear structure [23, 101].

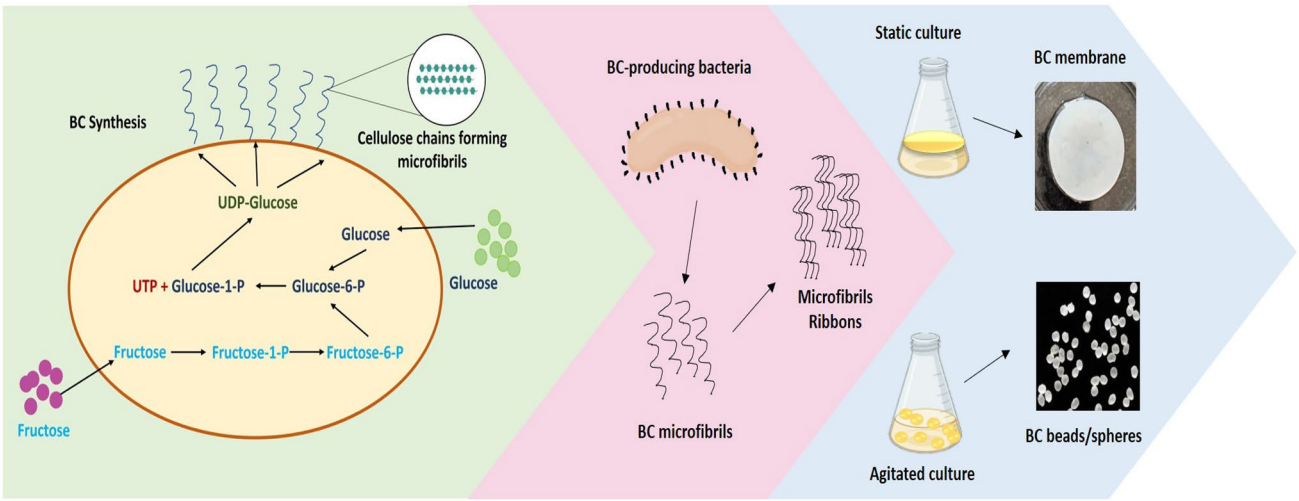

Fig. 2 Bacterial cellulose synthesis during static cultivation

Bacterial Cellulose Production Through Agitated Cultivation

Agitated cultivation is the preferred method for industrial scale production of BC. Less space is required in agitated cultivation compared to the static condition, and rate of aeration is higher during BC production. Generally, shaking culture is used for improving or optimizing oxygen supply in media during the bacterial culture, but excessive aeration has also been reported to decrease the production of cellulose [10••]. Cellulose produced in agitated condition has different properties including microfibril size, cellulose crystallinity of cellulose, and shape. During agitated culture, different shapes of BC are produced such as spherical ellipsoidal or irregular shapes and pellets, depending on the agitation rate and the bacterial strain used. BC synthesized using agitation culture has superior water-holding ability in comparison to BC synthesized in the static condition [102]. The agitated condition can also form non-cellulose mutant strains due to the genetic instability of bacteria which could lower BC productivity [10••]. Bacterial strains after mutation change into (cel^{-}) mutants, and they lack genes that are responsible for the synthesis of cellulose [103].

Bacterial Cellulose Production Through Intermittent Fed-Batch Culture

BC production at industrial levels usually employed batch culture or continuous culture for production. The batch culture involves adjustment of nutrients, pH, aeration, and agitation in the bioreactor so as to achieve the desired end product, and the final product is harvested in batches. When a continuous culture is in progress, fresh media are constantly replaced by new ones, and at the same time, the product is continuously being harvested. An alternative method of production known as intermittent fed-batch culture or semi-continuous culture could be used for enhanced BC production. It is a modified form of batch culture where fresh nutrients or additives could be added during cultivation because of semi-open nature of production to increase the overall yield. In the last few years, some reports have mentioned using industrial wastes as substrate for BC production through fed-batch culture. In one such study, beer fermentation wastes were utilized as media for BC synthesis using fed-batch culture method; the BC production increased up to 2–3 times compared to the regular static culture fermentation [104]. Dubey et al. have carried out intermittent fed-batch culture using sweet lime pulp waste as media, and the highest BC production of 26.2 ± 1.50 g/L was reported, an increase of about 4–5-folds compared to static culture fermentation [105]. In another study, black tea was fermented for the production of BC using fed-batch method, and the productivity was 2–3 times higher (29.2 g/L) with respect to the regular batch culture, i.e., 13 g/L BC [106]. Therefore, intermittent fed-batch culture could be considered as a superior culture technique for industrial scale BC production.

Production of Bacterial Cellulose with Different Types of Bioreactors

Due to significant disadvantages, static and submerged cultures have been unsuitable for BC production for industrial scale. During the agitated culture, the amount of BC is significantly reduced due to the large expansion of the cellulose-negative mutants, the build-up of undesirable acids, and the adhesion of the BC broth to the reactor wall [107]. Throughout the past few years, many numerous efforts are taken to overcome these limits. Many attempts have been

made to develop a reactor design that can boost productivity, lower production cost, and minimize cultivation time to achieve BC production at industrial scale [108]. Different types of bioreactors are utilized for this purpose including rotating disk reactors [109], rotary biofilm contactor reactor [110], and reactor with silicone membranes [111]. In recent years, there are so many reports available of the different modified bioreactors to enhance the production of BC. Zywicka et al. have utilized magnetically assisted external-loop airlift bioreactor for the production of BC, and they have used *Komagataeibacter xylinus* strain in HS media for production, and they have obtained 7.26 g/L dry weight of BC [112]. Hu et al. have used a benchtop bioreactor and used *Komagataeibacter hansenii* for production of BC [113]. Tsouko et al. have employed BC production in tray bioreactor under air sparging. They utilized orange peel waste as a carbon source for cultivation. They have used *Komagataeibacter sucrofermentans* strain for the production of BC and reported 1.55 g/L of BC production per day 1.55 g/L [114]. Lin et al. have employed a plastic composite support rotating disk bioreactor (PCS-RDB). They modified CSL-Fru medium and used a variety of additives, including microcrystalline cellulose, carboxymethylcellulose (CMC), agar, and sodium alginate and produced 0.64 g/slice of BC [115].

Applications of Bacterial Cellulose in Various Fields

Environmental Applications

The standard of living has continuously increased in the last few decades, and it is expected to continue in the following years. The enormous growth in the human population and dependence on industrial products has resulted in major environmental pollution [116]. Numerous industries use chemicals that contaminate the environment significantly, by releasing their effluents in the water for, e.g., heavy metals, organic wastes, antibiotics, oils, dyes, and pesticides [117, 118]. BC has a beneficial feature—high adsorption capacity which can be employed for bioremediation. Many reports have demonstrated the use of BC for bioremediation of pollutants either directly or by modifying into a composite. Hu et al. have carried out a study for treatment of wastewater containing dyes and antibiotics using BC. They have synthesized BC and Ca-montmorillonite (Ca-MMT) composite for this purpose. The BC/Ca-MMT composites have provided abundant adsorption sites due to its microporous structure. Results have revealed that BC/Ca-MMT exhibits superior removal efficiency towards methylene blue (MB) and tetracycline (TC) [119]. Alves et al. have utilized BC as a membrane filter for polluted environmental water and industrial wastewater. Water samples with high load of dye and pathogenic microorganisms from textile industries and dairy industries were taken for the study. BC membranes were effective at removing pathogenic microorganisms and dye effluent, and a single BC membrane could be repeatedly used for filtration up to 10 cycles. The filtrate obtained by treatment of river water showed no microbial growth when transferred to growth medium [120]. Li et al. have synthesized adsorbent by combining BC/chitosan composite (BC/CH) aerogel with zeolitic imidazolate framework-67 (zif-67, a metal–organic framework) for the removal of organic pollutants and heavy metals. The aerogel successfully absorbed organic dye (reactive brilliant blue X-3B) and heavy metal ions like cupric ion (Cu^{2+}) and chromium ion (Cr^{6+}. Additionally, the aerogel has demonstrated good reusability; even after five reuses, the removal efficiencies for Cr^{6+} and Cu^{2+} remained at 72% and 81% of initial values, respectively [121]. Various studies have reported use of BC for removal of heavy metals from wastewater such as manganese (Mn^{2+}), lead (Pb^{2+}), copper (Cu^{2+}), and chromium (Cr^{6+}) [122–124]. Claudio et al. have studied the effectiveness of BC for treatment of oily water using wet purified BC membranes as filter. Water with different concentrations of oil such as 10 ppm, 150 ppm, and 230 ppm could be purified using wet BC membranes. The filter flow rate and filter diameter (25 mm, 50 mm, and 110 mm) were investigated, and separation of oil and water was observed in all instances [125]. He et al. have also reported the synthesis of BC membranes incorporated with silica to produce BC/SiO_2 network aerogels, which have even achieved oil recoveries of up to 88% [126].Yang et al. have synthesized three-dimensional nanocomposite using BC, polydopamine, and titanium dioxide. These nanocomposites can adsorb dyes and degrade them by photocatalysis under ultraviolet/visible radiation. The BC/PDA/TiO_2 composites displayed greater adsorption capacities for methyl orange, rhodamine B, and methylene blue compared to commercial photocatalyst P25. Additionally, the photocatalytic properties were considerably enhanced within 30 min of irradiation. A 5.5% loss in photocatalytic capacity after five cyclic tests demonstrated the high stability of BC/PDA/TiO_2 [127]. Figure 3 depicts application of BC for dye removal and oil recovery from polluted water.

Wound Healing

Skin is the largest organ in our body that covers the entire body, and any accidental cut or burn in the skin can lead to a wound. Since bacterial exposure can infect the wound, it is necessary to take adequate precautions while dressing the wounds. Factors like proper oxygen exchange, retention of moisture, adsorption of wound secretion, reduction in pain, and protection from contamination are essential for an ideal wound dressing [22]. BC has high adsorption rate, moisture retention, and biocompatibility, and therefore, the

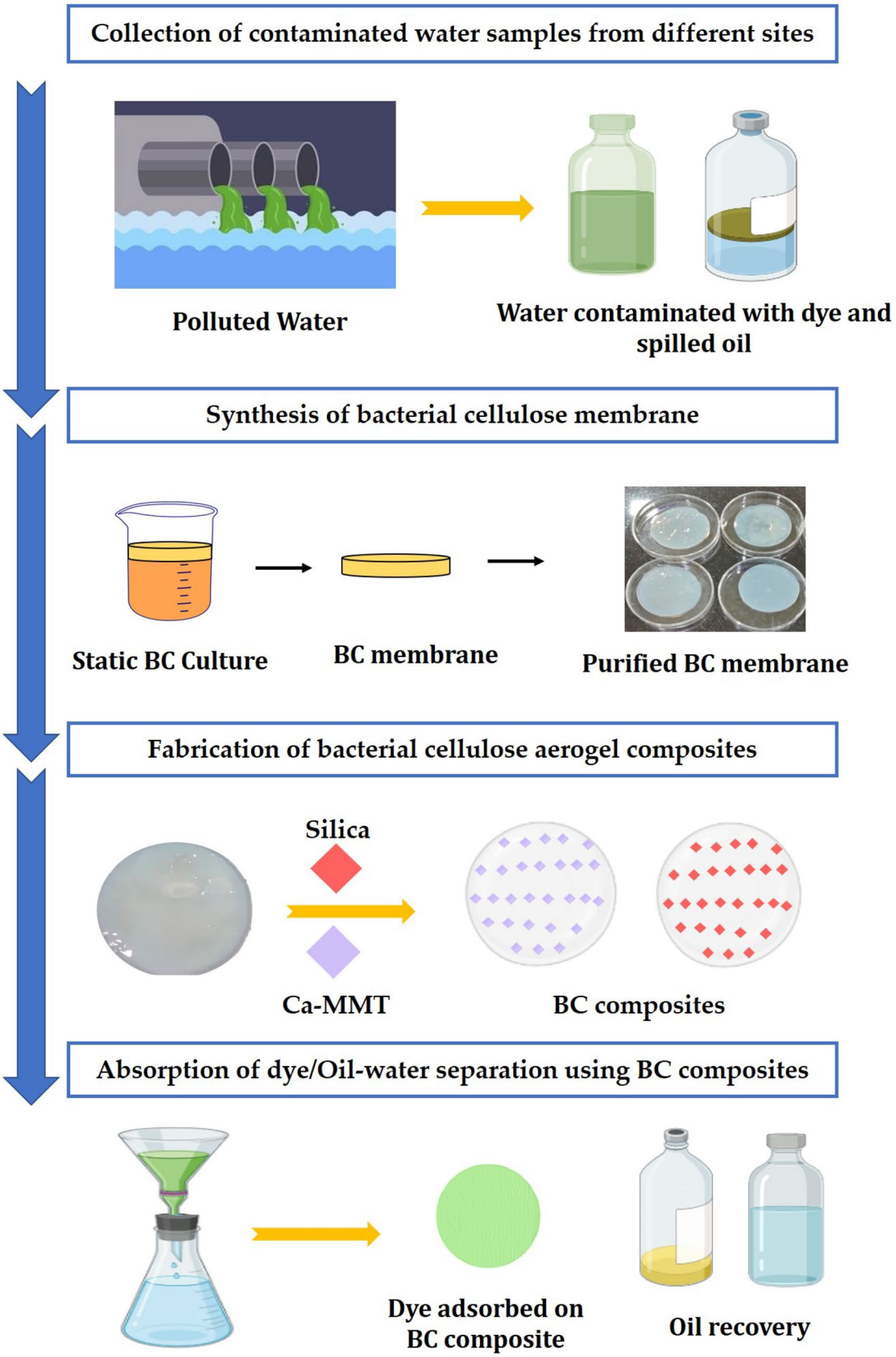

Fig. 3 Bacterial cellulose for dye removal and oil separation

biopolymer has found application in wound healing. BC lacks antimicrobial activity, despite being non-allergic, so in order to overcome this drawback, BC is combined with an additive to form a hydrogel, hydrocolloid, or nanoconjugate/particles. The modified form of BC should be able to prevent any bacterial infection and provide suitable microenvironment for the wound healing [128]. Several researchers have reported studies that suggest BC as a good wound healing material [129].

Qiu et al. have studied BC-vaccarin membrane for its wound healing properties, and the study proved that BC has no cytotoxic activity, also helps with cell growth, and increases the rate of wound healing in rat [130]. Many researchers have explored BC for antimicrobial activity by adding some compounds and elements [46, 47]. Pal et al. have worked on BC silver nanoparticle. Zmejkoski et al. have studied BC hydrogel having dehydrogenative polymer of coniferyl alcohol (DHP). Kim et al. have studied BC composite of chitosan, poly(ethanlene) glycol, and gelatine [131–134]. BC is used in distinctive of wound healing treatments of diabetic wounds, bedsore, cuts, burns, venous stasis wound, and grafting sites of skin donor [135]. Aside from wound healing, BC has also become the most desirable skin replacement material. The first BC product to be used as skin replacement was trademarked as BioFill® and presently known as Dermafill® also used as an artificial skin, Membracel® used as wound care dressing, and XCell® and Bionext® used as wound care [136].

Drug Delivery

Biocompatibility and low toxicity of BC fulfil the criteria necessary for a drug delivery carrier. BC can potentially be utilized as enhanced tropical drug delivery carrier that resembles the oil based formulation [137]. Many studies have reported the fabrication of BC composite comprising of additional substrates like nanoparticles or antibiotics. These studies have shown that BC hydrogel with properties like high porosity, gel fraction, and high swelling capacity can be used to customize and create carriers that can easily carry and release desired drugs to targets [138]. One such study by Tamahkar in 2021 utilized BC/polyvinyl alcohol (BC/PVA) hydrogel filled with ampicillin. The hydrogel had a swelling ratio of 188–240% and 50% of ampicillin was released from hydrogel after 24 h with good antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* [139]. Weyell et al. have demonstrated the efficacy of BC-doxycycline hydrogel used as prophylaxis against infection involving different concentration of periodate on BC, oxodized-nonoxidized form of BC to treat infection. The study also considered in vitro toxicity through MTT assay to confirm the biocompatibility along with agar diffusion test to verify antibiotic effectiveness against pathogenic bacteria [140]. Badshah et al. have examined difference in drug release of two different type of drugs—hydrophobic and hydrophilic. Drugs were loaded into individual BC hydrogel, water-insoluble famotidine in one and water-soluble tizanidine in another. It was observed that famotidine was released within 0.5–3 h, while tizanidine was released within 0.25–0.5 h. Therefore, the study showed that physiochemical properties also play an important role in drug releasing. BC surface modification was also a key factor in controlling drug release [141].

Dental Treatment

BC has also great potential in dental applications because of its physiochemical properties like bio-absorbability which is quite essential in dental medicine for periodontal disease caused by various types of microorganisms. Inoue et al. [142] have studied the effect of BC composites loaded with chlorhexidine (CHX) and $NaIO_4$ on periodontal disease. BC composites showed good zone of inhibition ($p < 0.05$) against *Escherichia coli* and *Candida albicans* which have demonstrated its potential application as bioactive agent with high bio-absorbability. In a study reported by Voicu et al., a combination of powdered BC and silicate cement was used to create an improved cementing agent that can be used as a dental filling or capping material. The study also showed that BC worked as an excellent cementing agent due to its high biocompatibility with hydro-compounds and helps with teeth setting time and other biological activity as they had no cytotoxic effects. They have demonstrated the capacity to maintain cell viability and encourage cell division. BC has the potential to be of use in other dental applications such as root channel obturation and dentine mineralization or filling holes in teeth [143]. In a study by Yoshino et al., tooth canal treatment using plant-based and bacterial-based cellulose was compared. To treat infected dental root canal, it is dried and sterilized using a cotton pellet and paper point (PP) composed of plant cellulose in traditional treatment. In this study, BC-based material for dental canal therapy has favorable biological and material traits compared to traditional PP. The rate of absorption for BC was ten times higher, delivering a substantially larger expansion of BC compared to PP. Under the same conditions, BC had greater tensile strength than PP in wet conditions [144]. However, BC with high absorbency, great tensile strength, and biocompatibility should be able to easily replace plant cellulose fillings.

Food Industries

BC can benefit the food industries because it may create hydrogels with favorable rheological characteristics. It can be easy to modify BC such that the cellulose produced by the

bacteria has flavor, color, texture, and shape, making it suitable for use in the food industries [145]. BC is consumed as a dessert known as *nata de coco* in South-East Asian nations like the Philippines, Thailand, Vietnam, and Malaysia. BC is also classified as GRAS (generally regarded as safe) by the Food and Drug Administration in 1992 [146]. There are a variety of beverages manufactured with bacterial cellulose that are sold in the market such as kombucha tea. The composition of kombucha tea is black or green tea with sugar. Microbial consortium that forms a BC and cellulose barrier on the surface is removed after 1 or 2 weeks, and the liquid phase is then fit for consumption [147]. However, due to its complex and inadequately microbial community, kombucha tea as a medicinal infusion is still debatable [148]. BC contains fibers that are beneficial for digestion and lowers risk of chronic illnesses like diabetes, cardiovascular disease, and obesity [145]. Products from BC have enjoyed tremendous global success, particularly in the food sector. The BC market is estimated to be worth roughly US $207.36 million in 2016, US $ 497.76 million in 2022, and US $ 700 million or more by 2026, as per report, by ResearchMoz [149]. BC is consumed all over the globe in the form of dessert, vegetarian meat, low calorie, and low cholesterol products and as food additives [6, 7]. Another fascinating use of native or modified BC as a nano-reinforcement component or casting films is food packaging. Starch and PLA composite films with the addition of BC fibers have increased transparency and thermo-resistance and decreased in gas diffusibility and water sensitivity [148, 150].

Knowledge Gaps and Future Prospects

BC is a valuable biological macromolecule with diverse properties and applications. By looking at recent developments and advancements, it can be argued that BC will continue to be a sought-after biomaterial for a wide range of industries. As more research is conducted for BC production using agro-industrial wastes, it is possible that cost-effective, large-scale BC production will be feasible in the future. An unexplored application of BC that could prove to be beneficial in upcoming years is in the field of environmental bioremediation. As of now, scientists have successfully carried out bioremediation of contaminated soil and water at lab-scale studies by fabricating BC composites, though actual implementation of these BC composites at in situ levels is still lagging behind. As concrete results are obtained through multiple studies, it may be possible that in the future, BC composites would be used for absorption of heavy metals, pesticides, dyes, and other toxic compounds. Once the process is optimized and appropriate machinery is constructed, BC membranes could also serve as filter for water purification and oil recovery.

Apart from bioremediation, BC-based wound dressing is readily available for commercial usage. Their cost is relatively higher compared to regular dressings, but as large-scale BC production becomes more efficient, it may become more economical. BC has shown great promise in pharmaceutical sector as a drug carrier for targeted drug delivery. However, most of these studies are at either lab-scale or clinical trials, and commercial BC-based drug delivery system is currently not in the market. Since numerous studies have had positive outcomes, it is possible that a BC-based wound dressing that releases drug can be fabricated and made available in the markets in the future. The treatment of dental conditions is another untapped BC application that might receive greater attention in the upcoming years. Being a multifaceted biomaterial, the applications of BC are numerous, and in the next 15–20 years, emergence of novel, untapped BC applications is highly probable.

Conclusions

Enormous amount of waste is produced by different agro-industrial sectors on daily basis. A significant chunk of these agro-industrial wastes would have either been released to the environment or subject to waste treatment, and hence, their use as substrate for BC production eliminates the cost of waste management while making BC production relatively cheaper. From an environmental lookout, appropriate management of these industrial wastes will reduce environment and health hazards associated with these wastes. Owing to the various applications of BC in the aspects of bioremediation, wound healing, organ regeneration, drug delivery, and dental treatment are currently being studied. This surge in areas of application of BC also bestows an objective to be addressed by the researchers, specifically pertaining to optimization of production parameters for large-scale sustainable BC production. The varied applications of BC also necessitate the engagement of the scientific community with the policy makers for ethical, judicious, and apt use of bacterial cellulose.

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- • Of importance
- •• Of major importance

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valuable biomaterials from wastes for value added applications in biomedical and pharmaceutical fields.

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