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REVIEW PAPER

Recent advances in bacterial cellulose: a low-cost effective production media, optimization strategies and applications

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Abstract Bacterial cellulose (BC), a promising polysaccharide of microbial origin, is usually produced through synthetic (chemically defined) or natural media comprising of various environmental wastes (with exact composition unknown), through low-cost and readily available means. Various agricultural, industrial, and food processing wastes have been explored for sustainable BC production. Both conventional (using one variable at a time) and statistical approaches have been used for BC optimization, either during the static fermentation to obtain BC membranes (pellicle) or agitated fermentation that yields suspended fibers (pellets). Multiple studies have addressed BC production, however, the strategies applied in utilizing various wastes for BC production have not been fully covered. The present study reviews the nutritional requirements for maximal BC production including different optimization strategies for the cultivation conditions. Furthermore, commonly-used applications of BC, in various fields, including recent developments, and our current understanding have also been summarized.

Keywords Bacterial cellulose · Low-cost media · Optimization strategies · Applications

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Introduction

The growing environmental challenges and the human race has been facing since the last few decades have made implementation of efficient and sustainable eco-friendly products, absolutely imperative (Kardung et al. 2021; Witek and Kuźniar 2021). Bacterial cellulose (BC), among other cellulose sources, meets the required eligibility for green applications (Urbina et al. 2021a). Unlike plant cellulose, BC is produced as an extracellular polymer, which facilitates its extraction in high purity, completely free from lignin and hemicellulose (Betlej et al. 2021). BC polymer is also characterized by its high degree of polymerization and crystallinity with a unique fiber network in the micro or nano-size, which increases its surface

to volume ratio, a unique feature over other cellulose sources (Zhong 2020). These salient properties qualify BC for several applications in various medical (Seddiqi et al. 2021; Swingler et al. 2021), environmental (de Medeiros et al. 2021; Saleh et al. 2021), and industrial sectors (Lin et al. 2020).

BC production depends mainly upon the availability of the nutritional components of the production medium, especially carbon (Fernandes et al. 2020). Different carbon sources and cultivation media have been screened and reported, however, Hestrin–Schramm (HS) medium remains the most commonly used for BC production (Lu et al. 2020). The high production cost attributed to the medium (30% of the total cost) represents a major challenge for fulfilling the commercial manufacturing requirements (Fernandes et al. 2020; Rahman et al. 2021). In recent decades, the implementation of the agro/industrial wastes for sustainable BC production represent a promising cost-effective alternative for the current applied media (Raiszadeh et al. 2020; Saleh et al. 2021). On the other hand, BC producing organism, is another important element regarding commercial production. Till date, *Gluconacetobacter xylinus* is the model producer for the BC at the commercial levels (Aswini et al. 2020; Wang et al. 2019). The high production capacity of this organism is attributed to the multiple copies of BC synthesis genes in its genome (Lu et al. 2020). Research for novel BC producers in different niches, in addition to deeper studies at the molecular level for BC production pathway continues toward improving the production titer.

Controlling and optimizing the production conditions and media also improves the yield and reduces production costs (Rahman et al. 2021). The one-variable at a time (OVAT) approach is the most applied strategy for BC production optimization (Alemam et al. 2021; Bera et al. 2021). Due to the intensive labor and high cost involved in this strategy, more attention, in recent times, has been directed toward the implementation of statistical numerical designs as a more reliable and cost-effective alternative for BC production optimization (Bagewadi et al. 2020; Singh et al. 2017). The scope of this review is to discuss the current advances in BC production, the application of different agro/industrial wastes as alternative media for sustainable BC production, process optimization, and its applications in various medical and industrial fields also discusses.

Nutritional requirements for bacterial cellulose production

BC yield depends mainly on the availability and quality of the carbon sources on the production medium (Fernandes et al. 2020; Gullo et al. 2019). Various simple and complex carbon sources through different organisms, including glucose, fructose, sucrose, mannitol, starch, and glycerol have been used for BC production (Lu et al. 2020; Mikkelsen et al. 2009Rangaswamy et al. 2015). Many studies have reported that, ethanol indirectly influences the enzymes involved in the BC synthesis pathway (glucokinase and fructokinase) thereby improving ATP yield (Andriani et al. 2020; Fernandes et al. 2020; Jacek et al. 2021). Ethanol also diminishes the spontaneous mutation rate of BC producing strain in agitating culture (Buldum et al. 2018; Son et al. 2001). Li et al., signified the role of ethanol supplementation in the reduction of glycerol formation (main by-product) during BC production (Li et al. 2012). Though BC production is a growth-dependent process (Singhania et al. 2021), some nutritional sources could support bacterial growth without any BC production (Aswini et al. 2020). The impact of the carbon source types on the final quality and crystallization of the BC is not fully determined; some studies have reported the direct impact of various nutritional sources on the final BC production and quality (Mohammadkazemi et al. 2015; Ruka et al. 2012), while others have reported the opposite (Aswini et al. 2020; Mikkelsen et al. 2009; Saleh et al. 2021).

The independent synthesis of BC, regardless of the carbon substrate, may be attributed to the ability of the producing organism to synthesize glucose from various carbohydrate sources, followed by polymerization to cellulose (Aswini et al. 2020; Rangaswamy et al. 2015). A suitable nitrogen source is also crucial for maximum BC production (Lahiri et al. 2021). The production of BC is commonly supported with complex organic nitrogen sources such as yeast extract (Aswini et al. 2020), peptone (Rangaswamy et al. 2015), casein hydrolysate with peptone (Lahiri et al. 2021), and soybean molasses (Souza et al. 2020), contrary to inorganic nitrogen that usually retards the bacterial growth and hence inhibits the BC production (Aswini et al. 2020). Determination of optimum nitrogen source for maximum BC production is influenced by the organism type as well as the carbon

source. Lahiri et al. reported maximum BC production by *Acetobacter xylinum* using casein hydrolysate with sucrose, while peptone was the most effective nitrogen source in presence of mannitol (Lahiri et al. 2021). Recently, more attention has been directed toward the implementation of agricultural and industrial-waste products as alternative cost-effective carbon/nitrogen sources (Ogrizek et al. 2021; Saleh et al. 2021; Singh et al. 2017). Several by-products successfully applied for cost-effective BC production included: a 50:50 mixture of date syrup and cheese whey (Raiszadeh et al. 2020), sugar beet molasses and cheese whey (Salari et al. 2019), and starchy kitchen wastes (Saleh et al. 2021).

Numerous additives have been screened and evaluated regarding their effect on BC production including, vitamins, minerals, and water-soluble polymers. Ascorbic acid (vitamin C) supplementation to the BC production media was investigated through many reports (Cielecka et al. 2021; Raiszadeh et al. 2020). Enhancement in BC production (one fold increase) from four isolates of *Gluconacetobacter xylinus* through ascorbic acid (0.5% w/w) supplementation in the production medium was previously reported, due to reduction in by-product concentration (gluconic acid) in the production medium (Keshk 2014). Water-soluble polymers including agar, alginate, and carboxymethyl cellulose (CMC) also play an important role in BC production by avoiding clumping and coagulation of BC (Cheng et al. 2009). In the same context, Aswini and his colleagues reported an increase in the BC production through the addition of polyethylene glycol-6000 to the production medium (Aswini et al. 2020).

Bacterial cellulose from a low-cost media

Culture or growth medium is a liquid or solid substrate, containing nutritive components for growth of microorganisms, cells, or small plants. Culture media are classified into two major subtypes based on their composition and application: defined (synthetic) media and undefined (natural) media (Fan et al. 2014; Meenakshi 2013). Defined media has known chemical compositions and concentrations, like Hestrin and Schramm (HS) media which used for the cultivation of the BC-producing bacteria is expensive and requires additional resources like glucose, peptone, yeast extract, ethanol, etc. (Ayed et al. 2017; Hestrin and Schramm 1954). The undefined media, on the other hand, depend on the use of natural sources without a specific chemical composition and is characterized as the unknown composition of the media, like pineapple peel, sugar beet molasses, etc. used for BC biosynthesis (Revin et al. 2021; Santoso et al. 2021).

BC production process is costly, owing to the low productivity of known strains and the use of extremely expensive fermentation medium (defined media). For BC production, where the defined medium represents around 30% of the total cost, this high expense becomes an obstacle for expanding into large scale production and further applications (Jozala et al. 2016). As a result, the major challenge of the fermentation process is the identifying of new effective and low-cost culture media that can promote a high yield within a short cultivation time. This can be obtained from various sources such as agriculture, industrial and food processing wastes (Table 1), and include low-price organic waste products that are easily available in high quantities (undefined media). Using these wastes as media effectively remove the wastes from the environment and reduces pollution associated with industrial waste disposal (Castro et al. 2011; Fan et al. 2016).

Various studies have recently focused on using alternative, natural and effective nutritional sources like agricultural, industrial and food processing wastes as a carbon source in order to reduce the cost of BC biosynthesis. Spruce hydrolysate (Guo et al. 2013), wood hot water extracts (Kiziltas et al. 2015), pineapple agro industrial residues (Algar et al. 2015), citrus juices (Andritsou et al. 2018), rotten fruit (Hungund et al. 2013; Jozala et al. 2015), cotton-based waste textiles (Hong et al. 2012), beet and sugar cane molasses (Cakar et al. 2014), food processing waste (Saleh et al. 2021; Suwanposri et al. 2014), wine fermentation waste broth (Ogrizek et al. 2021; Wu and Liu 2012), waste water of candied jujube-processing industry (Li et al. 2015a, b),waste and by-product streams from biodiesel and confectionery industries (Tsouko et al. 2015), acetone-butanol-ethanol fermentation wastewater (Huang et al. 2015), citrus peel (Fan et al. 2016; Güzel and Akpınar 2018), hemicellulose (Penttilä et al. 2017), konjac (Liu et al. 2020), rice husk (Goelzer et al. 2009), wheat straw (Chen et al. 2013), maple syrup (Zeng et al. 2011a, b), coffee cherry husk (Rani 2013), dried olive mill

Table 1 General process for bacterial cellulose production from a low cost effective media

Waste source	Source	Pretreatment	Hydrolysis	Fermentation	Purification	BC (g/l)		Microbial strain	Reference
						Defined media	Undefined media		
Agricultural wastes	Corn stalk	Chemical, using acetic acid and detoxification by activated carbon and ion exchange resin	ND	Static at 30 °C for 7 days	0.5 M NaOH at 80 °C for 90 min	2.93	2.86	*Acetobacter xylinum* ATCC 23,767	Cheng et al. (2017)
	Wheat straw	Chemical, by ionic liquid 1-allyl-3 methyl-imidazolium chloride	Enzymatic, by cellulase	Static at 30 °C for 7 days	ND	3.7	8.3	*Gluconacetobacter xylinus* ATCC 23,770	Chen et al. (2013)
	Sugarcane straw	Physical, using hot water	Enzymatic, by (240 FPU) Cellic ®CTec2	Static at 30 °C for 15 days	0.1 M NaOH at 80 °C for 90 min	1.06	4.51	*Komagataeibacter xylinus* ATCC 11,142	Dhar et al. 2019)
	Sweet sorghum	Chemical, by phosphoric acid and hydrogen peroxide	Enzymatic, by 20 FPU) Cellic® CTec2	Static at 30 °C for 6 days	0.3 M NaOH at 80 °C for 1 h	0.98	Stalk 1.82 Leaf 2.54 Root 2.28	*Acetobacter xylinum* ATCC 23,767	Wang et al. 2021)
		Physical, by juice extraction using blander	ND				Juice 0.87		
	Tobacco extract	Physical, by boiling in water and steam distillation for nicotine removal	ND	Static at 30 °C for 7 days	0.2 M NaOH at 80 °C for 2 h	0.51	5.2	*Acetobacter xylinum* ATCC 23,767	(Ye et al. 2019)
	Cashew tree residues	ND	ND	Static at 28 °C for 7 days	0.1 M NaOH at 80 °C for 45 min	5.8	6	*Komagataeibacter rhaeticus*	(Pacheco et al. 2017)

Table 1 (continued)

Waste source	Source	Pretreatment	Hydrolysis	Fermentation	Purification	BC (g/l)		Microbial strain	Reference
						Defined media	Undefined media		
	Red maple	Physical by hot water extraction under high pressure	ND	Static at 30 °C for 28 days	1% NaOH at 100 °C for 30 min	0.04	0.15	*Acetobacter xylinus* 23,769	(Kiziltas et al. 2015)
	Pecan nutshell	ND	ND	Static at 28 °C days	0.5 M NaOH, at 90 °C for30 min	ND	2.81	*Gluconacetobacter entanii*	(Dórame-Miranda et al. 2019)
	Caragana Korshinskii Kom	Chemical by Ethylene diamine	Enzymatic, by (15 FPU) Cellic CTec2 and (9 U) Cellic HTec2	Static at 30 °C for 7 days	Soaking in water for several days	3.0	4.6	*Gluconacetobacter xylinus* CGMCC 2955	(Li et al. 2021)
		Chemical by sodium hydroxide					ND		
		Physical by hydrothermal					ND		
	Oat hulls Silver grass	Physical, by hydrothermobaric measures and Chemical, by nitric acid, or, sodium hydroxide, or combining both	Enzymatic, by (40 FPU) CelloLux-A and (15 FPU) BrewZyme BGX	Static at 27 °C for 7 days	Purified by water, NaOH and HCl	ND	ND	*Medusomyces gisevii* Sa-12	(Kashcheyeva et al. 2019)
	Oat hulls	Chemical by nitric acid	Enzymatic, by (25 FPU) CelloLux-A and (15 FPU) BrewZyme BGX	Static at 27 °C for 24 days	Purified by water, NaOH and HCl	2.2	0.7 – 2.2	*Medusomyces gisevii* Sa-12	(Skiba et al. 2020)

Table 1 (continued)

Waste source	Source	Pretreatment	Hydrolysis	Fermentation	Purification	BC (g/l)		Microbial strain	Reference
						Defined media	Undefined media		
	Elephant grass	ND	Acid by sulphoric acid	Static at 28 °C for 14 days	1.5% NaOH at 80 °C for 2 h	ND	6.4	*Gluconacetobacter xylinus* CH001	(Yang et al. 2013)
Industrial wastes	Waste water of candied jujube	ND	Acid by dilute sulphuric acid	Static at 30 °C for 6 days	0.1 M NaOH at 100 °C for 1 h	ND	2.25	*Gluconacetobacter xylinum* CGMCC 2955	(Z. Li et al. 2015a, b)
	Rice noodle Processing	Physical, by filtration	ND	Static at 30 °C for 10 days	0.5 M NaOH at 80 °C for 30 min	2.67	11.76	*Komagataeibacter* sp. PAP1	(Sutthiphatkul and Ochaikul 2020)
	Industrial hardwood	Chemical, by acidic sulfite pulping	ND	Static at 30 °C for 4 days	0.5 M NaOH at 90 °C for 30 min	2.7	0.27	*Gluconacetobacter saccharii*	(Carreira et al. 2011)
	Citrus pulp water	ND	Enzymatic, by 50 U cellulase and 150 U pectinase	Static at 30 °C for 4 days	0.2 M NaOH at 85 °C for 30 min	1.6	8.77	*Gluconacetobacter xylinum*	(Cao et al. 2018)
	Coconut water						9.91		
	Sugar beet molasses	ND	ND	Static at 28 °C for 5 days	1% NaOH at 80 °C for 1 h	1.6	2.9	*Komagataeibacter sucrofermentans* H-110	(Revin et al. 2021)
	Sugar beet molasses	Chemical, by sulphuric acid	ND	Static at 28 °C for 14 days	0.1 M NaOH at 90 °C for 1 h	3.26	4.65	*Gluconacetobacter xylinus* PTCC 1734	(Salari et al. 2019)
	Cheese whey	ND	Enzymatic, by lactase				3.55		
	Acerola byproduct	Physical, by hydrothermal	ND	Static at 30 °C for 15 days	0.1 M NaOH at 50 °C for 24 h	ND	4.0	*Komagataeibacter rhaeticus*	(Leonarski et al. 2021)
	Bovine whey powder	ND	ND	Static at 30 °C for 4 days	0.5 M NaOH at 90 °C for 30 min	2.7	0.31	*Gluconacetobacter saccharii*	(Carreira et al. 2011)

Table 1 (continued)

Waste source	Source	Pretreatment	Hydrolysis	Fermentation	Purification	BC (g/l)		Microbial strain	Reference
						Defined media	Undefined media		
	Sago liquid waste	ND	ND	Static at 28–30 °C for 14 days	Soaking in water for 24 h	ND	4.12	Acetobacter xylinum LKN6	(Yanti et al. 2017)
	Sweet lime pulp	Physical, by hot water extract	Acid by dilute sulphuric acid	Static at 30 °C for 16 days	0.5 N NaOH at 100 °C for 1 h	9.98	26.2	*Komagataeibacter europaeus* SGP37	(Dubey et al. 2018)
	Rice Washing Drainage	ND	ND	Static at 25 °C for 8 days	0.1 N NaOH at 90 °C for 10 min	ND	0.20	*Komagataeibacter nataicola* Li1	(Sudying et al. 2019)
	Rice wine distillery	ND	ND	Static at 30 °C for 7 days	1 N NaOH at 95 °C for 3 h	4.21	10.38	*Gluconacetobacter Xylinus* BCRC 12,334	(Wu and Liu 2012)
	Waste fiber sludges	Chemical by sulfate and sulfite-based process	Enzymatic by Cellic CTec2 (Novozymes)	Static at 30 °C for 7 days	0.1 M NaOH at 80 °C for 1 h	ND	11	*Gluconacetobacter xylinus* ATCC 23,770	(Cavka et al. 2013)
	Vinasse	Physical, by centrifugation and dilution	ND	Static at 30 °C for 10 days	0.1 M NaOH at 100 °C for 20 min	2.18	7.02	*Komagataeibacter xylinus* PTCC 1734	(Barshan et al. 2019)
	olive pomace mill	Physical by water extraction	ND	Static at 30 °C for 4 days	0.5 M NaOH at 90 °C for 30 min	2.5	0.81–0.85	*Gluconacetobacter saccharii*	(Gomes et al. 2013)
		Chemical by sulphuric acid extraction					ND		
	Grains and yellow water from Baijiu production	Physical, by stirring under heat and pressure	Enzymatic, by Cellulase and Glucoamylase	Static at 30 °C for 7 days	1% NaOH at 80 °C for 90 min	0.86	7.42	*Gluconacetobacter xylinus* G29	(He et al. 2020)

Table 1 (continued)

Waste source	Source	Pretreatment	Hydrolysis	Fermentation	Purification	BC (g/l)		Microbial strain	Reference
						Defined media	Undefined media		
	Rice bark	ND	Acid by sulphuric acid and then Enzymatic, by Avizyme® (α-xylanase, α-glucanase and polygalacturonase)	Aerated and Static at 28 °C for 10 days	Immersed in 0.1 M NaOH for one day	Aerated ~ 0.9 Static ~ 1.3	Aerated 1.57 Static 2.42	*Acetobacter xylinum* ATCC 23,769	(Goelzer et al. 2009)
	Waste beer yeast	Chemical, by sodium hydroxide		Static at 30 °C for 14 days	0.1 M NaOH at 80 °C for 2 h	1.21	2.33	*Gluconacetobacter hansenii* CGMCC 3917	(D. Lin et al. 2014a, b)
		Physical, by homogenizer					~ 1.20		
		Physical, by microwave					~ 1.20		
		Physical, by ultrasonic	Acid under mild condition (pH 2)				7.02		
	Coffee cherry husk	Physical, by dried, grounded and then hot water extract	No	Static at 27 °C for two weeks	Immersed in 1 N NaOH for one day	1.5	5.6- 8.2	*Gluconacetobacter hansenii* UAC09	(M. U. Rani and K. A. Appaiah 2013)
	Cotton based waste textiles	Chemical, by ionic liquid (1-allyl-3-methyl-imidazolium chloride) Physical by hydrothermal	Enzymatic by Cellulase 11 U	Static at 30 °C for 7–14 days	1% NaOH at 80 °C for 120 min	5.9	10.8	*Gluconacetobacter xylinus* ATCC 23,770	(Hong et al. 2012)

Table 1 (continued)

Waste source	Source	Pretreatment	Hydrolysis	Fermentation	Purification	BC (g/l)		Microbial strain	Reference
						Defined media	Undefined media		
	Waste dyed cotton fabrics hydrolysate	Chemical, by ionic liquid (1-allyl-3-methyl-imidazolium chloride)	Enzymatic by Cellulase 8.1 U	Static at 30 °C for 10 days	Washed thoroughly with deionized water	8.7	12.8	*Gluconacetobacter xylinus*	(Guo et al. 2016)
	Cellulose based textile	Chemical by NaOH, Urea, Phosphoric acid, N-methyl-morpholine-N-oxide and 1-butyl-3-methylimidazolium chloride	Enzymatic by Cellulase 10 U	Static at 30 °C for 7 days	1 N NaOH at 95 °C for 20 min	1.14	1.88	***Gluconobacter xylinus***	(Kuo et al. 2010)
	Crude glycerol residue	ND	ND	Static at 30 °C for 4 days	0.5 M NaOH at 90 °C for 30 min	2.7	2.07	*Gluconacetobacter saccharl*	(Carreira et al. 2011)
	Glycerol remaining	Physical, by dilution, pH adjustment, precipitation and then separation	ND	Static at 28 °C for 14 days	Homogenized in blender and then treated by 5% KOH for 14 h	ND	10	*Gluconacetobacter xylinus*	(Vazquez et al. 2013)
	Grape bagasse	Physical, by homogenized in a blender and then filtration					8		
	Corn Steep Liquor	ND	ND	Static at 30 °C for 10 days	0.1 M NaOH at 80 °C for 2 h	8	5.80	*Gluconacetobacter hansenii* UCP1619	(Costa et al. 2017)

Table 1 (continued)

Waste source	Source	Pretreatment	Hydrolysis	Fermentation	Purification	BC (g/l)		Microbial strain	Reference
						Defined media	Undefined media		
Food wastes	Starch kitchen wastes	ND	Enzymatic, by amylase 313 U	Static at 30 °C for 7 days	0.5% NaOH at 90 °C for 30 min	ND	2.11	*Komagataeibacter hansenii AS.5*	(Saleh et al. 2021)
	Pear peel and pomace	Physical, by mixed with water and blended	ND	Static at 30 °C for 7 days	0.1 M NaOH at 80 °C for 1 h	5.1	10.9	*Komagataeibacter rhaeticus*	(Ma et al. 2021)
	Grape skins	Physical, aqueous extraction at refluxing temperature	ND	Static at 30 °C for 4 days	0.5 M NaOH at 90 °C for 30 min	2.7	2.60	*Gluconacetobacter sacchari*	(Carreira et al. 2011)
	Citrus peel and pomace	ND	Enzymatic, by complex enzymes	Static at 30 °C for 8 days	0.1 M NaOH at 100 °C for 1 h	3.9	5.7	*Komagataeibacter xylinus* CICC 10,529	(Fan et al. 2016)
	Potato peel	ND	Acid by nitric, sulfuric, hydrochloric and phosphoric acid	Static at 30 °C for 4 days	1 N NaOH at 60 °C for 90 min	1.21	4.7	*Gluconacetobacter xylinus*	(Abdelraof et al. 2019)
	Orange Peel	ND	Enzymatic, by cellulase 30,000 U and pectinase 1200 U	Static at 30 °C for 8 days	1 N NaOH at 95 °C for 20 min	0.97	6.1	*Gluconacetobacter xylinus*	(Kuo et al. 2019)
	Cashew apple juice	ND	ND	Static at 30 °C for 7 days	1 M NaOH at 80 °C for 1 h	4.03	4.5	*Gluconacetobacter xylinus*	(Souza et al. 2020)
	Orange juice	ND	ND	Agitation for 1-2 days at 150 RPM and then static at 30 °C for 13 days	1 M NaOH at 100 °C for 30 min	ND	6.1	*Komagataeibacter sucrofermentans* DSM 15,973	(Andritsou et al. 2018)
	Grapefruit juice	ND					6.7		
	Lemon peel	Physical, by aqueous extraction					5.2		

Table 1 (continued)

Waste source	Source	Pretreatment	Hydrolysis	Fermentation	Purification	BC (g/l)		Microbial strain	Reference
						Defined media	Undefined media		
	Carrot juice	Physical, by juice dilution	ND	Static at 30 °C for 14 days	Boiled in water for 1 h and then 0.5 M NaOH for 12 h	0.60	1.35	*Gluconacetobacter hansenii* ATCC 23,769	(Gündüz and Aşık 2018)
	Maple syrup	ND	ND	Rotary shaking at 25 °C for 10 days	1% NaOH at 90 °C for 30 min	1.60	1.51	*Acetobacter xylinum* BPR 2001	(X. Zeng et al. 2011a, b)
	Pineapple peels	Physical, by aqueous extraction	ND	Static at 28 °C for 16 days	0.5 M NaOH at 60 °C	2.57	11.44	*Komagataeibacter xylinus* IITR DKH20	(Khan et al. 2021)
		Physical, by crushing, drying and grounding to powder,	Acid by dilute sulphuric acid and detoxification of hydrolysate by atmospheric cold plasma	Static at 28 °C for 9 days	0.1 M NaOH at 90 °C for 30 min	1.73	3.82	*Komagateibacter xylinus*	(Santoso et al. 2021)
		Physical, by adjusting the pH at 3.6 by citric acid	ND	Static at 28 °C for 13 days	5% KOH at 25 °C for 14 h	2.1	2.8	*Gluconacetobacter swingsii*	(Castro et al. 2011)
	Carob and haricot bean	Physical, by grounded and autoclaved	ND	Static at 30 °C for 9 days	0.1 M NaOH at 100 °C for 20 min	0.52	3.2	*Gluconacetobacter xylinus* ATCC 700,178	(Bilgi et al. 2016)
	Mango peels	Physical, by using of a 5 HP fruit-pulping machine	ND	Static at 30 °C for 16 days	0.1 M NaOH at 90 °C for 30 min	1.49	6.32	*Komagataeibacter xylinus*	(García-Sánchez et al. 2020)
	Mangifera indica extracts	Physical, by blended and aqueous extraction	ND	Static at 30 °C for 21 days	Washed by 70% ethanol and then 0.1 M NaOH at 50 °C for 24 h	1.61	25.43	*Komagataeibacter rhaeticus* SU12	(Calderón et al. 2021)

Table 1 (continued)

Waste source	Source	Pretreatment	Hydrolysis	Fermentation	Purification	BC (g/l)		Microbial strain	Reference
						Defined media	Undefined media		
	Rotten banana	Physical, by blended and diluted	ND	Static at room temperature for 10 days	Rinsed many times in 5% KOH	2.75	4.81	*Komagataeibacter medellinensis* NBRC 3288	(Molina-Ramírez et al. 2018)
	Rotten tomatoes	Physical, by autoclaving and then blending for juice extraction	ND	Static at 30 °C for 7 days	0.3 M NaOH at 103 kPa and 121 °C for 15 min	3.83	3.71	*Gluconacetobacter hansenii* PJK KCTC 10505BP	(Fatima et al. 2021)
	Cacao mucilage exudate	Physical, by autoclaving	ND	Static at 30 °C for 15 days	Immersed in boiling water for 30 min then 5% NaClO for 72 h	4.20	13.13	*Gluconacetobacter xylinus* ATCC 23,768	(Saavedra-Sanabria et al. 2021)
	Vegetable oil	ND	ND	Static at 28 °C for 7 days	0.1 M NaOH at 90 °C for 30 min	~1	~7.5	*Komagataeibacter xylinus* DSM 46,602	(Żywicka et al. 2018)
	Colored rice	Physical, by grounding to powder using an electric grinder	Enzymatic, by starch degrading enzyme 300 U and glucoamylase 2 U	Static at room temperature for 9 days	Washed by water several times	ND	8.15	*Komagataeibacter xylinus* AGR 60	Noree et al. (2021)
	Tapioca waste	ND	ND	Static at room temperature for 10 days	Immersed in ethanol	ND	ND	*Acetobacter xylinum* InaCC B422	Ghozali et al. (2021)

ND: Not Detected

residue (Gomes et al. 2013), waste beer yeast (Lin et al. 2014a, b), date syrup (Mohammadkazemi et al. 2015) have been reported. BC production process from low-cost effective media can be divided into four general steps (Fig. 1). Each step includes several options, but the overall effect remains the same (Algar et al. 2015; Hong et al. 2012; Vasconcelos et al. 2017).

Pretreatment of wastes

Pretreatment of different wastes, especially agricultural wastes, is a vital step in BC production. It includes altering the size, structure and chemical properties of the biomass thus optimizing the conditions for efficient hydrolysis (Saini et al. 2015). The yield of reducing sugars from hydrolysis of natural biomass is low; therefore, one of the major challenges is to demonstrate effective pretreatment methods that affect the structure, chemical composition, and hydrolysis rate of pretreated biomass (Podgorbunskikh et al. 2019; Sun et al. 2016). Consequently, pretreatment must meet the following: (1) enhance sugar yield or the ability to subsequently form sugars by enzymatic hydrolysis; (2) eliminate carbohydrate degradation; and (3) be cost-effective. Chemical and physical processes have been used for a common pretreatment of agricultural wastes (Abol-Fotouh et al. 2020; Li et al. 2021). A variety of chemical (acid, alkali, ozone) and, physical (comminution and hydrothermolysis) techniques have been established for the pretreatment of biomass for BC production (Palamae et al. 2017).

Chemical pretreatment

Chemical pretreatments are considered promising, being quite effective in degrading more complex-structured substrates (Song et al. 2014). Various acid, alkali, combined acid–alkali and ionic liquid have been adopted to pretreat lignocellulosic biomass. By comparing the quantity of reducing sugars obtained from hydrolysis of pretreated wastes, the effectiveness of various chemical pretreatments was investigated.

Acid pretreatment

Organic (acetic) or inorganic (nitric) acids can decompose the biomass wastes and enhance cellulose availability, further facilitating BC production. Acetic acid at 2.0% (w/v) is an organic acid used for pretreatment of 200 g corn stalk at the desired temperatures of 140 °C, 160 °C, 180 °C, and the reaction time was 30 min, 60 min, 90 min respectively, and the optimized parameters include 160 °C, 60 min of pretreatment, with 28.84% of total sugars (Cheng et al. 2017). Nitric acid at 2, 4 and 6 wt% as mineral acid used for

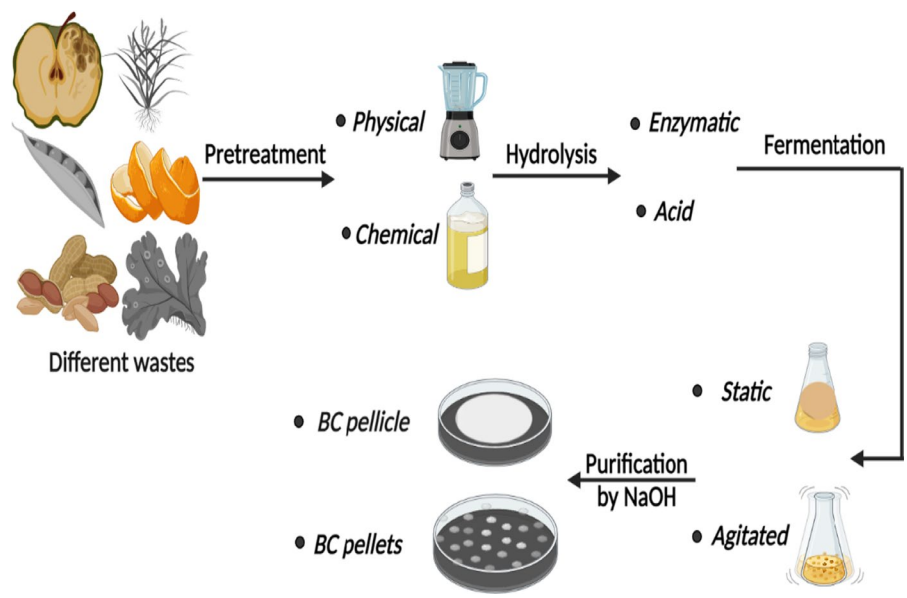

Fig. 1 The general steps for bacterial cellulose production from a low-cost media

pretreatment of 150 g oat hulls at 90–95 °C for 1 h in 4 l flask has been reported. The optimum nitric acid concentration for oat hulls pretreatment was found to be 4 wt%, with 79.5% reducing sugars (Skiba et al. 2020).

Alkali pretreatment

Alkali pretreatment is the most widely used method to enhance the digestibility of the lignocellulose, by swelling of biomass and thus causing an increased surface area, decreased crystallinity and lignin structure disruption (Singh et al. 2016). This process presents a simple way to modify the surface of the wastes to remove cationic species (Ponce et al. 2021). Caragana Korshinskii Kom (20 g) was pretreated by mixing with 1.8–7.2 g of NaOH. The mixture was then autoclaved at 150 °C for 30 min, to yield 40–45% total sugars by using 7.2 g NaOH (Li et al. 2021). Silver grass at 10 kg was pretreated by a 3–6 wt% NaOH solution at 90–95 °C for 6–8 h, to yield 85% reducing sugars (Kashcheyeva et al. 2019). In NaOH pretreatment, the OH^- targets the carbon of the ester linkage between lignin and hemicellulose, resulting in the irreversible hydrolysis of the ester bond and weakening the structural integrity of the lignocellulose (Modenbach and Nokes 2014).

Ionic liquid pretreatment

Imidazolium-based ionic liquids (ILs), having anions of chloride, acetate or alkyl phosphonate as soluble cellulose liquids, are being used for pretreatment of wastes like switchgrass (Singh et al. 2009) and wheat straw (Chen et al. 2013). 1-allyl-3- methylimidazolium chloride is a type of novel ionic liquid that is recognized as one of the most effective reagents for improving enzymatic saccharification of wheat straw and cotton cloth wastes for BC production. Wheat straw of 150, 250 and 500 mg were pretreated by dissolving in 1-allyl-3-methylimidazolium chloride of 5 g at 100 °C for 2 h to obtain dosages of 3, 5 and 10 w/w%, respectively. The optimum conditions for pretreatment was found to be 3 w/w% dosage of ionic liquid, to yield 71.2% of total sugars, compared to 19% of untreated wheat straw after enzymatic hydrolysis (Chen et al. 2013). Cotton cloth of 0.05 g was pretreated with 10 g of 1-allyl-3-methylimidazolium chloride in a 50 ml solution, agitated at 500 rpm and incubated in an oil-bath heater at 90 °C, 110 °C, and 130 °C. The optimum condition was found to be 110 °C for 90 min for pretreatment of cotton cloth (Hong et al. 2012).

Physical pretreatment

Physical pretreatments, commonly used to degrade wastes, include steaming, grinding and milling, irradiation, temperature and pressure. These methods increase the accessible surface area and size of pores. Several researchers reported that, different wastes were beat in a blender for shredding and grinding, as a physical pretreatment, and then used for BC production. Aqueous extracts of fruit peels (ex. pineapple, orange, sweet lime and banana) wastes have been used as a nutrient and carbon source for the production of BC through physical pretreatment. The dried peels were ground and immersed in distilled water at 90 °C for 60 min. The supernatant was used as a substrate for BC production by using *Komagataeibacter xylinus* IITR DKH20, to achieve BC of 11.4 g/l (Khan et al. 2021). Extracts from citrus processing waste peels of grapefruit and lemon have also been reported (Andritsou et al. 2018). Table 1 summarizes the physical pretreatments used for processing of simple sources like fruit peels and other food processing wastes.

Hydrolysis of wastes

Hydrolysis is the main step for BC production from alternative media. It is responsible for the conversion of treated or untreated wastes to sugars, formed during fermentation by producer strains to BC. The rate of hydrolysis of wastes depends on two factors: pretreatment technology and type of hydrolysis (acid or enzymatic).

Acid hydrolysis

Acid hydrolysis has several detrimental effects including considerable energy consumption equipment corrosion, and the formation of inhibitory chemicals (Chen et al. 2013). This process also reduces the complexity of the carbon source to simple sugars which can otherwise be easily assimilated by the microorganism for BC production (Jaramillo et al.

2014). However, hydrolysis of lignocellulosic materials causes disruption of lignocellulosic hydrogen bonds with byproducts like carboxylic acids (formic acid) and furan aldehydes (furfural and 5-hydroxymethylfurfural) which can be toxic to bacterial growth and inhibit fermentation. To reduce the formation of these toxic compounds, additional detoxification step is necessary to promote proper bacterial growth and fermentation (Santoso et al. 2021; Steinbach et al. 2017). The most commonly used acid for saccharification of wastes for BC production is diluted sulphuric acid and the hydrolysate is detoxified by atmospheric cold plasma (Santoso et al. 2021). In another study, elephant grass was hydrolysed by 2.5% (w/v) sulphuric acid at 135 °C for 1 h to achieve (g/l) 12 glucose, 20.3 xylose and 2.3 arabinose (Yang et al. 2013).

Enzymatic hydrolysis

Enzymatic hydrolysis is a promising waste-saccharification process, performed at moderate pH and temperature conditions. It is eco-friendly and does not generate toxic compounds or degraded sugars (Kuo et al. 2019). Different enzymes used for saccharification of wastes for BC production include cellulases for cotton cloth (Hong et al. 2012), Cellic®CTec2 for sugarcane straw (Dhar et al. 2019), Avizyme® for rice bark (Goelzer et al. 2009), amylase for starch kitchen wastes (Saleh et al. 2021) and lactase for cheese whey (Salari et al. 2019). Pretreated sugarcane straw (10% (w/v)) was hydrolyzed by enzymatic treatment of 15 FPU/g cellulase, the hydrolysis was performed using citrate buffer (pH 5) at 250 rpm and 50 °C to achieve (g/l) 49.2 glucose; 6.1 xylose; 1.8 cellobiose and 2.9 acetic acid after two days of enzymatic hydrolysis (Dhar et al. 2019).

Fermentation of bacterial cellulose

Bacterial cellulose producing strains

One of the crucial factors controlling the BC yield is the producing strain. The strain also influences the quality and polymerization of the produced BC membranes (Lu et al. 2020; Wang et al. 2019). Many bacterial genera have been reported for BC production including *Gluconacetobacter*, *Azotobacter*, *Pseudomonas*, and *Salmonella* (Arrebola et al. 2015; Lu et al. 2020; Zhong 2020). Gram-negative bacteria, particularly *Gluconacetobacter xylinus* (*Komagataeibacter xylinus*) fulfill the industrial production level with a high yield of extracellular BC especially under static conditions (Keshk 2014; Lin et al. 2013; Wang et al. 2019). *Gluconacetobacter* species are regularly isolated from rotten fruits (Khan et al. 2020; Rangaswamy et al. 2015), vinegar (Du et al. 2018; Top et al. 2021), sugarcane juice (Aswini et al. 2020), and kombucha (Zhang et al. 2018). On the other hand, BC production using Gram-positive bacteria is quite uncommon and few organisms only including *Lactiplantibacillus plantarum* (Saleh et al. 2022), *Lactobacillus hilgardii* (Khan et al. 2020), *Bacillus licheniformis* (Bagewadi et al. 2020), and *Rhodococcus* sp (Tanskul et al. 2013) have been studied.

At the molecular level, the full genome for several wild type, potent BC producing strains, have been sequenced and investigated including; *Gluconacetobacter xylinus* CGMCC 2955 (Liu et al. 2018b), *Komagataeibacter xylinus* K2G30 (UMCC 2756) (Gullo et al. 2019), and *Komagataeibacter uvaceti* FXV3 (Nascimento et al. 2021). Together these genomic data provide a deeper insight into the polymer synthesis pathway that mainly engaged the BC synthase operons (*bcs* operon). This operon encoded the four essential cellulose synthesis genes, namely *bcsA*, *bcsB*, *bcsC*, and *bcsD* (Buldum and Mantalaris 2021; Liu et al. 2018b). In addition, several regulating genes were also identified and located up and downstream from the cellulose synthase operons (Lu et al. 2020; Singhania et al. 2021). The exceptional cellulose production capacity of *Gluconacetobacter xylinus* is attributed to multiple built-in copies of cellulose synthase operons (up to 4 copies) on the strain genome (Lu et al. 2020). Based upon the genomic data, numerous attempts have been reported for intensifying the BC yield to the commercial level through genetic manipulation (Lu et al. 2020; Singhania et al. 2021). To reduce the production time, the cellulose synthase operon was heterogeneously expressed in *E.coli,* as a faster-growing host (Buldum et al. 2018; Imai et al. 2014). Though the BC production was detected in the early phase of the fermentation course (after 3 h), the resulted BC yield did not fulfill the expected commercial level (Buldum and Mantalaris 2021). More recent research has been directed toward the endogenous and heterologous expression of the

cellulose regulating genes in the potent BC producing organisms (Jacek and Kubiak and et al. 2019; Jacek and Ryngajłło et al. 2019), with regards to cheaper and more available carbon sources, enhancing the oxygen assimilation under hypoxic conditions (Liu et al. 2018a), or knocking-out the genes responsible for by-product accumulation (gluconic acid) during the fermentation course (Chun et al. 2014), all causing an increase in BC productivity. Till date, exploring new BC producers is completely dependent on the culturing techniques, though only 1% of the microbial population can be cultured (Bodor et al. 2020). Application of modern molecular strategies, such as the metagenomics library, may extend the scope of BC production through exploring these microbial communities (ex. in wild niches and extreme environments), that cannot yet be cultured.

Co-culturing is a recent effort for improving the quality and productivity of BC. Hu et al. studied the co-culturing of *Aureobasidium pullulans* in the BC-production medium of *Komagataeibacter hansenii* and reported enhanced BC productivity with better mechanical properties, attributed to the introduction of pullulan polysaccharide in the produced BC microstructure (Hu et al. 2021). Liu and Catchmark evaluated the co-culturing of *Escherichia coli* (*E. coli*) in the fermentation medium of *Gluconacetobacter hansenii* and reported enhancement in the productivity and quality of the produced BC through the incorporation of mannose-rich exopolysaccharide, generated by *E.coli*, into the growing cellulose network (Liu and Catchmark 2019).

Cultivation conditions affecting the bacterial cellulose production

BC production is directly affected by the various cultivation conditions such as temperature, medium pH, incubation time, and nature of cultivation (Fernandes et al. 2020). The physiological state of the applied microorganism governs its optimum cultivation conditions, thereby determining the medium nutrients and maximizing the yield. Medium pH is a vital cultivation parameter that directly affects carbon assimilation and hence BC production (Rangaswamy et al. 2015; Yassine et al. 2016). The optimum pH for BC yield is strain-dependent, a slightly-acidic pH is most recommended for maximum BC production (Dirisu et al. 2017; Fernandes et al. 2020), although an optimum alkaline pH has been reported as well (Abdelraof et al. 2019; Farrag et al. 2019; Lin et al. 2016). In their research for potent BC producers, Lin et al. isolated *Komagataeibacter intermedius* from fermented fruit juice that can produce BC at a wide pH range (4–9) where the maximum production (1.2 g/l/4 days) was at pH 8 (Lin et al. 2016). Assimilation of carbon sources usually resulted in pH dropping in the fermentation medium below the initial optimum level, and this is attributed to the production of numerous organic acids (gluconic and acetic acids) as by-products (Blanco et al. 2020; Esa et al. 2014). Hence, medium pH control during BC production is very important as any pH below 4 does not support BC production at all (Klemm et al. 2001). Additionally, the applied carbon source has a direct influence on the optimum pH for BC production. It was reported that when bacteria were cultured on glucose, the optimal initial pH was 5.5. However, when mannitol was applied, the optimal pH was raised to 6.5 (Hutchens et al. 2007).

Surface to volume ratio (S/V ratio) represents another important parameter influencing BC yield especially under the static production conditions (Kumar et al. 2021). S/V ratio has a direct influence on the aeration level in the production medium. At static cultivation conditions, BC production usually takes place at a higher oxygen level in the liquid-surface interface, and hence increases the oxygenation level resulting in higher BC yield (Rodrigues et al. 2019). It was reported that the optimum level of S/V ratio for BC production is a strain-dependent trait and is crucial, since the higher/lower ratios drastically declined the BC membrane thickness and yield (Kumar et al. 2021). Various studies reported different S/V ratios for optimum BC production including 1.22 cm^{-1} (Aytekin A et al. 2016), 0.4 cm^{-1} (Rodrigues et al. 2019), and 0.22 cm^{-1} (Kumar et al. 2021), however, most of them consider the S/V ratio among the most significant variables for enhanced BC production at static condition.

Types of bacterial cellulose fermentation

There are two main models for BC fermentation using microorganisms: static and agitated fermentation. The application of BC depends on the type of

fermentation, as well as, the physical, and mechanical features of the formed BC (Cacicedo et al. 2016).

Static fermentation

Static fermentation forms a thick and gel-like white BC pellicle at the air–liquid interface, thus limiting the oxygen (at the side of the pellicle exposed to media) and nutrient supply (at the upper aerobic zone of the pellicle) (Sharma et al. 2021). It requires a longer culture period, larger cultivation area and intensive manpower, thus resulting in low productivity (Kuo et al. 2016). Static fermentation is more suitable for the medical production of BC as wound dressing materials (Portela et al. 2019) or in dye removal (Saleh et al. 2021).

Agitated fermentation

Agitated fermentation, wherein the BC is synthesized in the fermentation medium as randomly distributed pellets or suspended fibers. The process has higher yield in lesser time but the shearing stress generated due to agitation causes the bacterial strain to revert to non-BC producing mutants (*cele*) thereby inhibiting the BC production. These mutants have a higher growth rate compared to the wild type, thus decreasing BC production even more (Sharma et al. 2021; Singhania et al. 2021). The agitated fermentation process is more suitable for the industrial production of BC and can be used for commercial applications in various fields. Moreover, mutations in the applied strains are more probable thus influencing BC production (Chawla et al. 2009; Tyagi and Suresh 2016).

Conventional and statistical optimization strategies for bacterial cellulose production

The relatively high production cost of the BC represents one of the major challenges facing its commercial implementation (Aswini et al. 2020; Fernandes et al. 2020). About 30% of BC production cost is attributed to the growth medium and cultivation conditions (Fernandes et al. 2020), therefore, besides mining for new superior BC producing organisms, reducing the production cost and time represent a step ahead for wide commercial applications (Fernandes et al. 2020; Jozala et al. 2015; Rahman et al. 2021). Two approaches were widely reported for improving the cultivation conditions and medium composition for BC production, including conventional and statistical optimization approaches. The conventional optimization approach depends upon the OVAT, wherever optimum conditions were elaborated through changing one variable while all other parameters are fixed. Once the optimum level of this variable is attained, another variable is evaluated in the same manner (Shojaei et al. 2021). The OVAT strategy is straight forward, and many studies rely on this strategy for optimizing different nutritional and physical parameters for BC production, as indicated in Table 2. Two fundamental drawbacks of the OVAT strategy include the long experimentation time and the strenuous labor (Abdel-Fattah et al. 2009). Furthermore, the OVAT strategy is incapable of elucidating the factor interactions and their consequences upon the final yield (Abdel-Fattah et al. 2009; Fernandes et al. 2020; Yousef et al. 2021).

The second optimization approach relies upon statistical mathematical designs. Unlike the OVAT method, in this approach multiple factors are evaluated simultaneously in one experiment (Singh et al. 2017). Besides, the data could be fitted in mathematical models, where the experimental results may be manipulated, analyzed and optimum expected (Bagewadi et al. 2020; Fernandes et al. 2020). The statistical optimization approach is a sequential process involving three main steps: (1) setting the range for studied variables and developing the design matrix and model, (2) conducting the experiment in the laboratory, recording the process yield, fitting the results to the applied model, and expecting the optimum levels toward maximum productivity, and (3) validating computationally expected results to ensure the adequacy of the applied model in terms of the expected response (Das and Dewanjee 2018). Analysis of variance (ANOVA) and regression analysis are the most applied methods for evaluating the accuracy and significance of the results of the statistical designs (Rahul and Pretesh 2018; Shojaei et al. 2021).

The statistical designs could be categorized into screening and optimization designs. The screening design is usually applied to elucidate the impact of each studied variable upon the final process yield, assuming that variable interactions are neglected (Das and Dewanjee 2018). In this process, all studied variables are evaluated for their positive/negative

Table 2 Optimization strategies for bacterial cellulose production medium and cultivation conditions

Optimization approach	Factors implemented	Optimum conditions	Strain applied	Maximum BC yield	Reference
OVAT	Different carbon sources; mannitol, glucose, glycerol, fructose, sucrose or galactose	Sucrose 20 g/l and glycerol 20 g/l as separate additives for glucose free-Hestrin and Schramm medium	*Gluconacetobacter xylinus* strain ATCC 53,524	3.81 g/l/4 days	Mikkelsen et al. 2009)
OVAT	Different carbon and nitrogen sources, different inoculum density, temperature, pH, and agitation	Sucrose 2%, peptone 0.5%, inoculum size of 5% at temperature 30 °C and pH 6.0	*Gluconacetobacter* sp. RV28. (local isolate)	4.7 g/l/3 days	Rangaswamy et al. (2015)
OVAT	Different temperatures (20–40 °C), different pH (3.0–9.0), different carbon sources and nitrogen sources (0.5%), Na_2HPO_4 (0–1%), ethanol and citric acid	Glucose 4%, yeast extract 0.1%, polypeptone 0.7% and Na_2HPO_4 0.8% in addition to ethanol 1.4%	The *Acetobacter* sp. A9 (local isolate)	15.2 g/l/8 days	Son et al. (2001)
OVAT	Different carbon sources including glucose, fructose, raffinose, sucrose, lactose, maltose, mannitol, and starch, different nitrogen sources including $(NH_4)_2SO4$, NH_4NO_3, NH_4Cl, tryptone, peptone, beef extract, yeast extract and urea, different incubation times, inoculum sizes, inoculum ages, pH values, and temperatures	Mannitol 2% and yeast extract 0.5% at 30 °C with 8% inoculum size and 8-day incubation period at pH 7	*Komagataeibacter xylinus* SB3.1 (local isolate)	6.54 g/l/8 days	Alemam et al. (2021)
OVAT	Whey hydrolysis through B-galactosidase, different temperature and surface to volume ration	Whey hydrolyzed with B-galactosidase (1.5 IU/ml), at pH 4, and temperature 30 °C with surface to volume ration 0.22 cm^{-1}	*Acetobacter pasteurianus* RSV-4 (MTCC 25,117)	5.6 g/l/8 days	Kumar et al. (2021)

Table 2 (continued)

Optimization approach	Factors implemented	Optimum conditions	Strain applied	Maximum BC yield	Reference
OVAT	Different additives to HS medium including (3 salts, 5 trace elements, 19 amino acids and 9 vitamins), different glucose concentrations, different co-substrates, different ethanol concentrations	Glucose 1.5%, $(NH_4)_2SO_4$ 0.2%, KH_2PO_4 0.3%, Na_2HPO_4 0.3%, $MgSO_4$ 0.08%, $FeSO_4$ 0.0005%, H_3BO_3 0.0003%, nicotinamide 0.00005%, and 0.6% ethanol at 200 rpm	Acetobacter sp. V6 (Local isolate)	4.16 g/l/8 days	Son et al. (2003)
Statistical approach: PBD and BBD	**PBD:** mannitol, yeast extract, ethanol, pH, inoculum size, temperature, incubation time **BBD (3 variables at 3 levels):** yeast extract, temperature and incubation time	Mannitol 25 g/l, yeast extract 13 g/l, ethanol 7 ml/l, pH 7, inoclulum size 7%, temperature 26.3 °C and incubation time 12 days	*Gluconacetobacter hansenii* ATCC 23,769	2.91 g/l/12 day	Farrag et al. (2019)
Statistical approach: BBD	**BBD (3 variables at 3 levels):** glucose concentration, citric acid concentration, and inoculation amount	Glucose 3.62%, citric acid 0.45%, inoculum size 9.39%, added to the pear residue medium under static cultivation conditions at 30℃	*Komagataeibacter intermedius* 6–5 (local isolate)	11.54 ± 0.42 g/l//7 days	Zhu et al. (2021)
Statistical approach: CCD	Eucalyptus biomass hydrolysate as carbon source, ethanol, and nitrogen source (yeast extract/peptone)	Eucalyptus biomass hydrolysate 31.4 g/l, ethanol 2.89% and yeast extract/peptone 10.8 g/l	*Komagataeibacter rhaeticus* K3 (local isolate)	5.46 g/l/15 day	Jacek et al. (2021)
Statistical approach: PBD and CCD	**PBD:** sugar content of carob (carbon source), protein content of haricot bean (nitrogen source) initial pH, surface/volume ratio, inoculum ratio, incubation time, citric acid content and temperature, **CCD (3 variables at 5 levels):** protein amount, incubation time and inoculum ratio	Carbon source 2.5 g/l, protein source 2.75 g/l, inoculum ratio 9.3%, citric acid 1.15 g/l, Na_2HPO_4 2.7 g/l, at 30℃ with an initial pH 5.5 for 9 days of incubation	*Gluconacetobacter xylinus* (ATCC 700,178)	1.8 g/l/9 days	Bilgi et al. (2016)

Table 2 (continued)

Optimization approach	Factors implemented	Optimum conditions	Strain applied	Maximum BC yield	Reference
Statistical approach: CCRD	**CCD (5 variables at 5 levels):** temperature, pH, incubation time, molasses concentration, and corn steep liquor concentration	Sugarcane molasses 10.77%, supplemented with 12.47% corn steep liquor at 31℃, pH 6.5, and incubation time of 172 h	*Gluconacetobacter xylinus* C18 (local isolate)	4.34 g/l/7.17 days	Singh et al. (2017)
Statistical approach: PBD and BBD	**(BBD):** different concentration of glucose, yeast extract, KH_2PO_4, $MgSO_4$ and ethanol, varied pH, inoclume size, incubation time and temperature **(BBD) (4 variables at 3 levels):** $MgSO_4$, ethanol, pH and yeast extract	Glucose 25 g/l, yeast extract 13 g/l, $MgSO_4$ 0.15 g/l, KH_2PO_4 2 g/l, ethanol 7.18 ml/l, pH 5.5, inoculum size 7% at 20 °C, and incubation time 9 days	*Komagataeibacter hansenii* AS.5	6.30 g/l/9 days	Saleh et al. (2020)
Statistical approach: PBD and BBD	**PBD:** Maple syrup concentration, incubation period, yeast extract, citric acid and trisodium citrate, ethanol, acetic acid, $MgSO_4$, agar, inoculum size, age of inoculum, temperature, shaker speed **BBD (4 variables at 3 levels):** Maple syrup concentration, incubation period, inoculum size, shaking speed	Maple syrup 30 g carbohydrate/l, $(NH_4)_2SO_4$ 3.3 g/l, KH_2PO_4 1 g/l, yeast extract 20 g/l, citric acid 1.6 g/l, tri-sodium citrate 2.4 g/l, ethanol 0.5%, acetic acid 0.5 g/l, $MgSO_4$ 0.8 g/l, inoculum age 3 days, inoculum volume 6%, shaking speed 135 rpm, and incubation temperature 25 °C	*Acetobacter xylinum* BPR 2001 (ATCC #700,178)	1.51 g/l/10 days	Zeng et al. (2011a, b)
Statistical approach: PBD and CCD	**PBD:** different concentration of Sucrose, KNO_3, $CaCl_2$, Na_2HP4, $MgSO_4$, with varied pH, temperature and agitation speed **CCD (3 variables at 5 levels):** Sucrose, $CaCl_2$, and pH	Sucrose 2.81%, $CaCl_2$ 1.26%, KNO_3 2%, agitation speed 170 rpm, Na_2HPO_4 100 mg%), $MgSO_4$ 0.1%, at temperature 25 °C and pH 3.88	*Gluconacetobacter hansenii* NCIM 2529	7.0 g/l/5 days	Mohite et al. (2012)
Statistical approach: Taguchi method	**TM:** initial pH, medium volume, inoculum size, temperature, and incubation time	Temperature 35 °C, pH 9, medium volume 55 ml and 8% inoculum size with incubation time of 6 days	*Gluconacetobacter xylinum ATCC 10,245*	4.7 g/l/6 days	Abdelraof et al. (2019)

Table 2 (continued)

Optimization approach	Factors implemented	Optimum conditions	Strain applied	Maximum BC yield	Reference
Statistical approach: CCRD	**CCD (5 variables at 5 levels):** pH, corn steep liquor, alcohol, acetic acid, and water to coffee cherry husk ratio	pH 6.64, corn steep liquor 10%, alcohol 0.5%, acetic acid 1.13%, and water to coffee cherry husk ratio 1:1	*Gluconacetobacter hansenii* UAC09 (Local isolate)	6.24 g/l/14 days	Rani et al. (2011)
Statistical approach: CCD	**CCD (4 variables at 3 levels):** Incubation time, volume changing ratio, glucose concentration and surface/volume ratio)	Incubation time 7 days, volume changing ratios 66%, glucose concentrations 50 g/l and surface/volume ratios1.22 cm^{-1}	*Gluconacetobacter xylinus (FC01)* (Local isolate)	0.284 g/l/day	Aytekin A et al. (2016)
Mixed strategy: OVAT and BBD	**OVAT:** Different carbon sources; sucrose, fructose, mannitol, or glycerol, and nitrogen sources; yeast extract/peptone, yeast extract, peptone, malt extract, or ammonium sulfate, pH and incubation period **BBD (3 variables at 3 levels):** fructose, peptone, and pH	Fructose 41 g/l, peptone 38 g/l, pH of 5.2 and incubation time of 6 days	*Komactobacter intermedius* (BCRC 910,677)	3.906 g/l/6 days	Santoso et al. (2020)
Mixed strategy: OVAT and CCD	**OVAT:** different carbon, nitrogen, precursor, polymer additive, pH, temperature, inoculum concentration, and incubation time **CCD (5 variables at 3 levels):** glycerol, yeast extract, pH, temperature, PEG 6000,	Glycerol 50 ml/l, yeast extract 7.50 g/l along with 7.76 g/l of PEG 6000, supplemented to Hestrin and Schramm (HS) medium at pH 6.0 and temperature of 33.5 °C	*Acetobacter senegalensis* MA1	469.83 g/l/30 days (wet weight)	Aswini et al. (2020)

Table 2 (continued)

Optimization approach	Factors implemented	Optimum conditions	Strain applied	Maximum BC yield	Reference
Mixed strategy: OVAT, PBD and CCD	**OVAT:** different carbon sources; fructose, galactose, lactose, sucrose and maltose (2% w/v), apple juice, orange juice, grape juice and sugarcane juice (5%, v/v). Effect of different NaCl concentration with varied temperature and pH **PBD:** lactose, yeast extract, peptone, citrate, Na_2HPO_4, $MgSO_4$, $CaCl_2$ and NaCl **CCD (2 variables at 5 levels):** yeast extract and peptone	Lactose 2%, citrate 0.3%, NH_2PO_4 0.3%, $MgSO_4$ 0.2%, $CaCl_2$ 0.4%, NaCl 1%, yeast extract 1.5% and peptone 1.5% at pH 7; temperature initial 50 °C for 3 days then shifted into 37 °C for another 5 days	*Bacillus licheniformis* strain ZBT2 (local isolate)	9.2 g/l/8 days	Bagewadi et al. (2020)
Mixed strategy: OVAT and BBD	**OVAT:** fructose, corn steep liquor, dissolved oxygen, and agar concentration **BBD (4 variables at 3 levels):** fructose, corn steep liquor, dissolved oxygen, and agar concentration	Fructose 4.99%, corn steep liquor 2.85%, dissolved oxygen 28.33%, and 0.38% agar concentration	*Acetobacter xylinum* BPR2001	14.3 g/l/3 days	Bae and Shoda (2005)

effects on the process yield. The insignificant factors are excluded, to obtain a smaller controllable set of factors, which are likely to elicit optimal or nearly optimal responses. Plackett–Burman design (PBD) represents one of the widely applied screening designs that rely upon the first-order reaction model as follows: $Y = A + \sum_{k=1}^{n} BX$, where Y is the process response (dependent variable) and X is the studied factors (independent variable). Plackett–Burman design is a two-levels screening where the effect of each factor is evaluated between low settings, high one coded + 1, and low value coded -1 (Plackett and Burman 1946). In this design, up to N−1 factors could be screened with N trials, however, screening reasonable numbers of variables (between 12–20 variables) is more applicable, as with screening a large number of factors, a partial confounding between factors and factor interactions was reported which may lead into unreliable results (Kulahci and Bisgaard 2007).

Taguchi method (TM) is another statistical screening design that elucidates the effect of each factor at a lower cost and a better quality outcome (Shojaei et al. 2021). This design is based upon formulating the lowest amount of experiments (fraction of all factorial combinations) called orthogonal array, without affecting the product quality (Das et al. 2014). The orthogonal array guarantees that all factors are weighted equally, and the evaluation of the one-factor effect, within the experiment, does not influence the other factors implemented in the experiment (Das and Dewanjee 2018). Taguchi method involves two types of factors; controlled factors (under our control) called inner array and noise factors (can't be controlled) called outer array (Malhotra and Chapadgaonkar 2020). In this design, the signal-to-noise (S/N) ratio was proposed to measure design results quality, where S is the targeted signal and N is the interfering noise. The S/N ratio is usually determined in two categories: larger-the-best, when maximizing the response is the target or smaller-the-best, in the opposite situation (El-Moslamy et al. 2017; Rahul and Pretesh 2018). Though the Taguchi method is a straightforward and cost-effective method for process optimization, it also revealed some limitations as the results are approximate, in addition, the design's inability to specify the parameters influenced the highest effect on the process performance (Rahul and Pretesh 2018).

The second category includes optimization designs where the exact values of the most significant studied variable are determined to achieve the maximum desired responses. These statistical designs are three-or-more-level designs, where each variable is studied in three or more levels to ensure attending the real optimum conditions. Box–Behnken (BBD) and central composite design (CCD) are examples of the most applied 'three-five level' optimization designs (Table 2). Both belong to the response surface methodology (RSM) proposed by (Box and Behnken 1960), where the optimization results could be analyzed through surface plots (2 or 3 dimensional) representing the change in the response (Y), according to the applied parameters (X). The optimization designs rely upon polynomial quadratic models, where the nature of variable interactions and the implication of these on the process yield can also be elucidated (Rahman et al. 2021; Yousef et al. 2021). For any two studied variables, the quadratic polynomial model represents a second-order reaction as: $Y = A + B_1X_1 + B_2X_2 + B_{12}X_1X_2 + B_{11}X_1^2 + B_{22}X_2^2$ where Y = process response, A = model intercept (represents the fitted response at the design's center point), X_1, X_2 = studied variables, B_1, $B_{2=}$ linear coefficients; B_{12} = cross-interaction coefficients; and $B_{11,}$ B_{22} = non-linear quadratic coefficients. Integration between OVAT method and statistical designs have recently become widely adopted by many authors as an effective strategy for medium optimization. In this, the variable under study is generally screened through the OVAT approach. The levels of the potent and most significant variables, defined in the previous step, are further optimized through statistical optimization designs (Table 2). Integration between the OVAT and statistical approaches could be useful in preliminary studies to determine the appropriate ranges for studied variables (Aswini et al. 2020; Bagewadi et al. 2020). There have been debates in the past regarding the accuracy of the screening designs (Kulahci and Bisgaard 2007) and their misleading results, especially when faced with a huge number of factors. This has influenced several authors to rely upon the OVAT strategy in variables screening despite the laborious effort and time involved (Aswini et al. 2020; Santoso et al. 2020).

Purification of bacterial cellulose

The BC obtained after fermentation is usually not pure, containing impurities like cells and medium components. Several purification methods have been applied by soaking BC for several days in water without any additives or heating (Li et al. 2021). Otherwise, BC could be washed several times by water and then treated with NaOH and HCl (Skiba et al. 2020), or heating (50–100 °C) with 0.1–0.5 M NaOH for 30–120 min, which facilitates the removal of certain metabolites. This treatment increases viscosity, promoting surface purification and the elimination of low molecular mass BC, and finally conferring better characteristics to the biomaterial (Costa et al. 2017). The purification of BC obtained through different fermentation strategies is illustrated in Fig. 2.

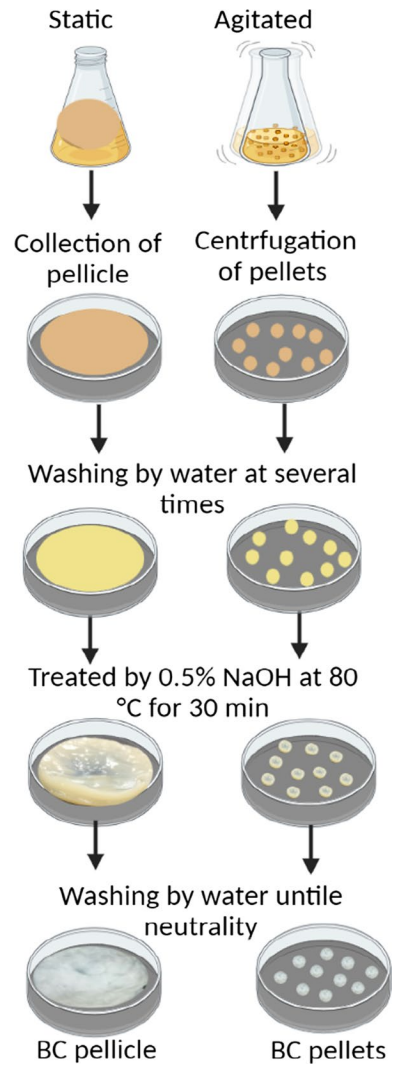

Fig. 2 Steps for bacterial cellulose purification obtained from static and agitated fermentation

Applications of bacterial cellulose

Biomedical applications

The unique physico-mechanical properties of BC such as, high water absorption capacity, good permeability, high tensile strength, crystalline structure and biocompatibility, have made it useful in different biomedical applications especially in wound dressing and tissue engineering. As an ideal wound dressing material, BC is able to accelerate the healing process, can prevent microbial infections, and can restore the structure and function of the skin (Abrigo et al. 2014). The BC fibers have the advantage of being randomly aligned with nanometer-size distribution which makes it able to mimic the shape and structure of natural extracellular matrix that encourage the epithelial cells' proliferation and enhance the formation of new tissues (Frone et al. 2020). Moreover, BC is an excellent wound healing membrane that has the ability to increase the sorption of wound liquids and hence cleans the wound exudates, allows the proper respiration of the cells, and facilitates the painless dressing change to keep the intact of the newly formed epithelial lining of the skin (Shalumon et al. 2011; Ul-Islam et al. 2013; Zou et al. 2012).

Biocompatibility of BC has been tested in rats with dural defect, for 120 days, showing good mechanical stability, absence of inflammatory reactions, and similar properties to the local tissues (de Lima et al. 2017). Researchers have succeeded in modifying the BC in the shape of nanofibers that are highly similar to the collagen fibers in the body, thereby making it attractive for tissue engineering applications (Torgbo and Sukyai 2018). BC has also been used as scaffold materials (Kumbhar et al. 2017), artificial skin (Keskin et al. 2017), dental implants (Voicu et al. 2017), and artificial blood vessels (Lee and Park 2017). BC is still under investigation in multiple other biomedical applications such as cartilage replacement tissue substitutes (Pang et al. 2020), tissue-engineered corneal stroma

(Zhang et al. 2020), dura mater (Binnetoglu et al. 2020), and nasal septa (Mandour et al. 2019).

Food industry applications

BC membrane has a gelatinous consistency and a smooth texture that make it a good candidate in various food industry applications. It can become as edible when processed with compounds such as sugar alcohol or alginate polymer and calcium chloride, gives it the consistency of fruits or molluscs (Okiyama et al. 1992). Subsequent to, USA Food and Drug Administration classifying it as a safe type of dietary fiber, BC can now be accepted as a food ingredient or food additive (Okiyama et al. 1992). BC has been used as a water binding, thickening, and gelling agent (Fig. 3) that improves the rheological profiles of BC-stabilized emulsions with promising food industry applications (Paximada et al. 2016a; Paximada et al. 2016b). From a dietary point of view, BC can substitute fats and lower the cholesterol concentration (Dourado et al. 2017), it can also be used in ice creams to reduce the fat content, show observable improvement in stability and rheological properties with higher resistance to melting (Guo et al. 2018), or as a fat replacer in mayonnaise with acceptable sensory characteristics and other physical properties (Akoğlu et al. 2018).

BC has a strong network and barrier properties that makes it suitable for food packaging applications. In the shape of cellulosic nanofiber, use of BC in nanopaper production gives the latter high strength, optical transparency, and thermal stability. BC also has good oxygen barrier properties, effectively reducing the penetration of oxygen molecules, thereby making it a good replacement for traditional micro-sized pulp papers, currently used for bags and other packages (Samyn et al. 2018).

Recently, BC has been applied in active packaging where the packing materials may have components that extend the shelf-life conditions through the absorbing or releasing of certain substances from or into the packaged food or the surrounding environment (Urbina et al. 2021b). In this context, BC, impregnated with lyophilized bacteria of *Lactobacillus plantarum* has been used as an antimicrobial nanopaper wrapping of the ground meat in order to stop the growth and activity of *Listeria monocytogenes* (Yordshahi et al. 2020). In another study, BC and potato peel films loaded with the phenolic compound curcumin, used for food packaging showed an improvement in the tensile strength and noticeable

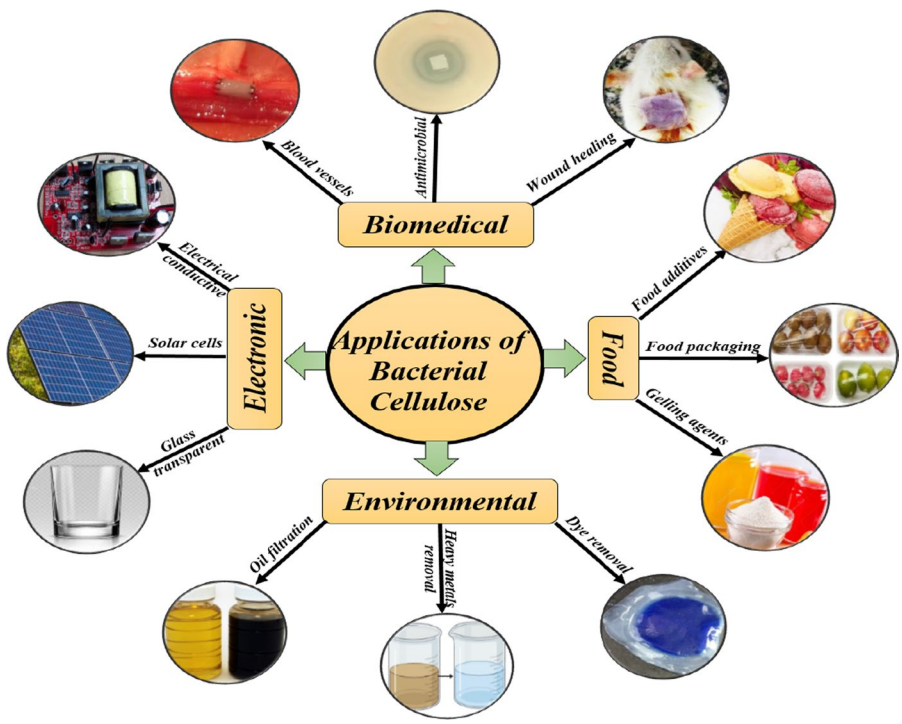

Fig. 3 General applications of bacterial cellulose in diverse fields

reduction in the water vapor and oxygen permeability (Xie et al. 2020).

Environmental applications

BC, a green bio-based material, being biodegradable and sustainable, conforms to the new environmental regulations. Accordingly, it is considered a promising alternative for current wastewater treatment technologies. Recent studies in our laboratory have shown that BC, loaded with charcoal or graphite compounds, has effectively removed cationic dyes such as methylene blue from contaminated water. When tested alone, BC membrane was able to clear 53% of methylene blue, while, this percentage improved to 98.7% and 100% when loaded with graphite and charcoal, respectively (Saleh et al. 2021). BC membrane has also been combined with polydopamine and TiO_2 nanoparticles to improve the surface area and active sites to enhance the photocatalytic degradation of dyes (Yang et al. 2020). Studies have shown that BC can serve as a supporting material for the Cu and Ni nanoparticles, used for the reduction of 4-nitophenol, a hazardous pollutant in wastewater originating from dye, paper, and pharmaceutical industries (Song et al. 2020).

BC membranes have been combined with chelating agents or absorbent materials such as EDTA, magnetic nanoparticles, graphene oxide, and chitosan for the removal of toxic heavy metals, released to the environment due to industrial activities (ex. factories producing fertilizers, batteries, or tanneries) and causing toxicity to organisms including humans. Zhu and his colleagues have used spherical BC combined with Fe_3O_4 (Zhu et al. 2011) to remove heavy metal ions such as Cr^{3+}, Mn^{2+}, and Pb^{2+}. BC has also proved a good efficiency template for the removal of other heavy metals from contaminated environments such as Sr, Pb, Sb, Cu, Cr, Fe, and As in other studies (Cheng et al. 2019; Hassan et al. 2019; Meng et al. 2019; Mensah et al. 2019; Stoica-Guzun et al. 2016) (Fig. 3).

Oil-contaminated wastewater from industrial activities have a potential hazard for marine life, local organisms, and humans. Sphere-like BC/graphene composite, exhibiting a honeycomb-like surface with 3D interconnected porous structure, showed a perfect ability to absorb oils and organic solvents from contaminated water (Wang et al. 2019). SiO_2 functionalized BC membrane has shown a high separation efficiency in mixed water and oil emulsions with an oil recovery percentage of 88% (He et al. 2018; Hou et al. 2019). Galdino and his team also proved the feasibility of BC membrane as a filter for oil removal (Galdino Jr et al. 2020).

Electronic applications

Scientists have been searching for glass-alternative materials in electronic devices, that have transparency, flexibility and strength (Amorim et al. 2020). Recent studies reported the synthesis of cellulose nanofibers that are optically clear and can be used in electronic devices such as organic light-emitting diodes, antennas and transistors, flexible displays, and solar cells (Nogi et al. 2009; Zhu et al. 2014). The nanocellulose has also been successfully applied in the development of triboelectric nanogenerator which is known for its ability to convert mechanical energy into electric energy. With further enhancement in electronic technologies, there is now a need for electronic devices that can store energy without reducing their performance such as the biodegradable polymers that can replace the presently used, non-renewable resources (Kotatha et al. 2018). BC gel electrolyte coated with chitosan and alginate and containing 1-ethyl-3-methylimidazolium tetrafluoroborate was optimized for use in electric capacitors (Kotatha et al. 2018). BC/graphene nanosheets were coated on their surface with polyaniline to form an electrically conductive nanocomposite for successful use in electromagnetic shielding and flexible electrode materials (Wan et al. 2018). BC can also be used as the membranes of loudspeakers because it can maintain the speed and the frequency of sounds, and effectively respond to the sound power (Phruksaphithak et al. 2019; Shah et al. 2013) although at an escalated production cost.

Using BC as a base material in a real fractional-order element device was first reported by Caponetto and his team (Caponetto et al. 2019). These devices are used to approximate the fractional differential and integral equations in capacitors and inductor circuits. BC has also been incorporated with silver nanoparticles and polyaniline and used to fabricate a highly flexible ternary system, with a strong energy density, as an electrode for supercapacitors (Hosseini et al. 2019). In general, the characteristics of BC membranes allow them to be a promising candidate for use

in electronics and optoelectronic devices. These electronic devices exploit flexibility, transparency, thermal stability, good mechanical performance, and surface morphology of BC (Urbina et al. 2021b). Other reported applications of BC are mentioned in Table 3.

Cosmetic applications

Scientists have identified cosmetics as materials that can enhance the physical appearance and visible aspects of a person, by incorporating these into vehicles that facilitate their skin penetration (Amorim et al. 2020). These cause enhancement of the skin, hair, nails, eyes, and face in order to promote attractiveness, cleansing and beatifying without any negative effects on the body structures of functions (Hasan et al. 2012). Multiple advantages of BC have supported its wide applications in the cosmetics field.

Recent cosmetic preparations are more inclined towards using natural products from botanical sources, to avoid using chemicals (ex. parabens) that may have side effects such as skin allergies (Darbre and Harvey 2008; Hasan et al. 2012). BC has been applied in cosmetics as a non-allergic biopolymer that has been extensively used to stabilize oil-in-water emulsions, without the need for addition of other skin irritating surfactants. A recent study has successfully prepared cosmetic creams of oil-in-water emulsions using BC and carboxymethyl cellulose that replaced two of the commonly used chemical surfactants (Martins et al. 2021). BC has also been used in facial masks and scrubs, cleansing formulations, and contact lenses (Ullah et al. 2016). BC is currently preferred over the botanical cellulose as it is more chemically pure lacking hemicellulose or lignin, has a higher crystalline structure, more porous, and has better water holding capacity with elevated tensile strength (Chawla et al. 2009; Jonas and Farah 1998; Klemm et al. 2001; Mbituyimana et al. 2021).

The general properties of BC in cosmetic field have been improved by the incorporation of other natural materials. For instance, propolis extract has been incorporated into BC films in order to increase its hydrating and anti-inflammatory properties especially for sheet masks used for the treatment and healing of skin prone to acne and inflammations (Amorim et al. 2020). BC has also been used for skin pigmentation through the transfer of 1,3-dihydroxy-2-propanone in the corneum extract causing skin color changes. This application showed an actual skin color that was considered as the closest to the effect of natural tan and can probably be applied as an alternative for patients suffering from vitiligo (Stasiak-Różańska and Płoska 2018). In another study, both in situ and ex situ methods were applied for the successful functionalization of BC with hyaluronic acid and silk sericin, to be used in cosmetic industry, for moisture retaining and improving skin texture (Wang et al. 2020).

In general, BC has many advantages that intensively support its participation in the cosmetics field. It has a high absorbance capacity that helps in retaining liquids nearly ten times more than nonwoven masks and hundred times more than its dry weight (Trovatti et al. 2012). Its thin thickness gives it more flexibility for good adhesion to irregular surfaces of the treated skin, thus allowing it to reach every contour, fine lines, and wrinkles of the face and other locations that traditional masks otherwise fail to reach (Wei et al. 2011). Moreover, its soft touch helps in supporting the skin through its hydration qualities (Bianchet et al. 2020). To further widen its application in the cosmetics field, further research needs to be done to add active components to BC, to enhance its anti-aging, cleansing, or whitening properties (Mbituyimana et al. 2021).

Current stand

Exploring alternative media originating from different environmental wastes is an imperative study for the cost-effective production of BC. Moreover, the determination of the optimized nutritional requirements and the cultivation conditions would enhance the quantity and quality of the produced BC, leading to its even wider applications. For example, suitable physico-mechanical properties, high water absorption capacity and tensile strength, good permeability, biocompatibility, and having randomly aligned nanometer-size distribution, all make BC suitable for wound dressing and tissue engineering purposes. BC also has a gelatinous consistency and a smooth texture in addition to having a strong network and barrier properties thereby making it a good candidate for food packaging applications. Being a green bio-based material, biodegradable, and sustainable, BC is a good choice for other environmental applications as well. BC can also be used in electronic devices, being transparent,

Table 3 Additional applications of bacterial cellulose membranes that have been mentioned by other scientists

Field	Application	Highlight	Reference
Biomedical application	Wound healing	BC-vaccarin (BC-Vac) membranes prepared by immersing BC in vaccarin solution to increase malleability	Qiu et al. (2016)
		Silver sulfadiazine (SSD) particles with narrow size distribution impregnated with BC to produce BC–SSD composite membrane. Used as burn wound dressing which increases healing rate	Wen et al. (2015)
	Antimicrobial activity	BC sheet oxidized by hydrogen peroxide for 6 h to obtain hydrogen-peroxide-oxidized BC which inhibit *Escherichia coli*	Tabaii and Emtiazi (2016)
		Antibiotic drug tetracycline hydrochloride (TCH)-loaded BC composite membranes and the results demonstrate that thecdeveloped BC TCH composites displayed excellent antibacterial activity	Shao et al. (2016)
	Tissue engineering	Designed BC sponges composed of reined nanofibrils with hier-archical pore structure (including large pores and nanopores), were fabricated through the emulsion freeze-drying technique. Results indicated BC sponges as a promising biomaterial for tissue engineering applications	Frone et al. (2020)
		BC oxidized by perio-date oxidation to give rise to 2,3-dialdehyde BC (DABC) with $60.3 \pm 0.5\%$ aldehyde content, further reacted with gelatin (Gel) for Gel immobilization to form DABC/Gel nano-composites. Results indicate DABC/Gel's use as scaffold material in tissue engineering	Gao et al. (2012)
Food applications	Drinking agent	A static fermentation of black or green tea containing sucrose by cellulose-producers microbial consortium named Kombucha. Liquid phase ready to drinkn after surface cellulose membrane removal post 2 weeks	Shi et al. (2014)
	Food additives	BC is useful as a functional food additive: a thickener, texturizer, and/or calorie reducer (ice cream, salad dressing, and weight-reduction base)	Azeredo et al. (2019)
	Food packaging	BC sheets are immersed in poly(L-lactic acid) (PLLA) dissolved in chloroform transparent films have two fold of tensile strength of pure PLLA and increased nano composites crystallinity	Kim et al. (2009)
		Wet BC hydrolyzed with HCl and boiled for 4 h. Different concentrations of BC nanocrystals (BCNCs) are added to gelatin solutions. Results demonstrated the use of BCNCs in the fabrication of edible, biodegradable and high-performance nanocomposite films for food packaging applications at relatively low cost	George (2012)

Table 3 (continued)

Field	Application	Highlight	Reference
Electronic application	Optically transparent films	BC sulfate (BCS) has been rarely reported. This study highlights using BCS to synthesize a highly transparant film by drop casting method	Palaninathan et al. (2014)
	Magnetic materials	Flexible magnetic membranes based on BC with amphiphobicity were prepared by the in situ synthesis of the Fe_3O_4 nanoparticles on the BC nanofibers followed by fluoro alkyl silane (FAS) modification. Magnetically responsive BC membrane with hydrophobic and lipophobic surface would have potential applications in electronic actuators, magneto graphic printing, information storage, electromagnetic shielding coating and anticounterfeit	Zhang et al. (2011)
	Electric conductors	Free-standing films of BC and polyaniline (BC/PA) composites with high electrical conductivity values (0.9 S cm-1) and good mechanical properties (40 MPa) were prepared through in situ oxidative chemical polymerization of aniline (A) on the surface of synthesized BC nanofibers by using $FeCl_3.6H_2O$, as oxidant	Müller et al. (2012)
	Electronic paper	BC converted to an electrically conducting (or semi-conducting) sheet by depositing ions around the microfibrils to provide conducting pathways and then immobilizing electrochromic dyes within the microstructure. The whole system is then cased between transparent electrodes, and switching potentials (2 –5 V), a reversible color change can be demonstrated to a standard pixel-sized area (ca. 100 µm2)	Shah and Brown (2005)
Industrial application	Paper making	Nanopaper was prepared using ground cellulose nanofibers (GC) from canola straw and BC nanofibers. BC nanopaper had the highest tensile strength (185 MPa) and Young's modulus (17.3 GPa)	Yousefi et al. (2013)
Environmental applications	Dye decolorization	The hydroxyl groups of BC were oxidized into aldehyde groups that served as anchors for covalent immobilization of laccaseto the newly developed oxidized BC (OBC) membrane. TiO_2 was additionally coimmobilized to OBC to produce a novel material in which dye degradation was carried out under specific conditions	Li et al. (2017)
	Removal of heavy metals	Mercury, copper, chrome, lead and cadmium	Min et al. (2011), Suárez-Avendaño et al. (2022)
Agriculture applications	Improvement of soil	The addition of BC to soil increases the maximum water-holding capacity (MWHC) of sandy loam soil compared with untreated control soil, while porosity of soil also increased signiicantly. There is need for a more focused study to explore the use of BC for soil fertility improvement	Chawla et al. (2009)

flexible, strong and thereby a suitable glass-alternative material.

Present challenges and future prospects of bacterial cellulose research

In the last few years, integration modern molecular biology tools and different fermentation strategies has resulted in considerable improvement in the BC production, although some areas remain unexplored and hence demand deeper research. These include: (1) implementation of novel and low-cost wastes as alternative carbon/nitrogen substrates to diminish the fermentation cost and time and thereby enhance the BC yield, to fulfill the large- scale commercial production requirements, (2) exploring the Gram-positive bacteria and extremophiles for BC production along with BC produced from the commonly used Gram-negative strains. In this regard, application of modern gene-editing tools like CRISPR and metagenomics analysis of various strains could expand the profiles of BC-producers, (3) intensive research should be conducted in optimizing the cultivation conditions for BC production, such as the effect of oxygen tensions, especially at hyper and hypoxic conditions. In the same line, co-culturing is a recently applied technique for enhanced yield and requires further studies, and (4) expanding the scope of applying BC as a green and sustainable biopolymer to overcome the pressing environmental challenges.

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Declarations

Conflict of interest The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this review.

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