

Review

Bacterial Nanocellulose Produced by Cost-Effective and Sustainable Methods and Its Applications: A Review

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Abstract: This review discusses the recent advancements in cost-effective fermentation methods for producing bacterial nanocellulose (BC) from food and agro-industrial waste. Achieving economical cell culture media is crucial for large-scale BC production, requiring nutrient-rich media at low cost to maximize cellulose yield. Various pretreatment methods, including chemical, physical, and biological approaches, are stated to break down waste into accessible molecules for cellulose-producing bacteria. Additionally, strategies such as dynamic bioreactors and genetic engineering methods are investigated to enhance BC production. This review also focuses on the environmental impact assessment and updated application challenges of BC such as medical applications, energy storage/electronics, filtration membranes, and food packaging. By providing insights from the recent literature findings, this review highlights the innovative potential and challenges in economically and efficiently producing BC from waste streams.

Keywords: bacterial cellulose; agro-industrial waste; fermentation; zero-waste concept



Citation: Taokaew, S. Bacterial Nanocellulose Produced by Cost-Effective and Sustainable Methods and Its Applications: A Review. *Fermentation* **2024**, *10*, 316. <https://doi.org/10.3390/fermentation10060316>

Academic Editor: Donatella Cimini

Received: 20 March 2024

Revised: 8 June 2024

Accepted: 11 June 2024

Published: 14 June 2024



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1. Introduction

Utilizing biopolymers to manufacture products offers a sustainable approach that avoids increasing the carbon footprint. By doing so, carbon neutrality is maintained, facilitating the natural carbon cycle [1]. This strategy aligns closely with the objectives of the United Nations' Sustainable Development Goals (SDGs), which emphasize the importance of transitioning from petroleum-based polymers to bio-based alternatives. These sustainable alternatives hold significant potential to alleviate the accumulation of single-use products in the environment, primarily due to their non-biodegradability and inadequate waste management practices [1,2].

Cellulose, among various biopolymers, emerges as a particularly promising candidate for widespread adoption in various applications such as textiles [3], packaging [4], functional papers [5], and biomedical applications [6]. Its abundance in nature and versatility makes it an attractive option. Through the utilization of cellulose-based materials, dependency on fossil fuels to the synthesis of petroleum-based polymers can be decreased, carbon emissions can be lowered, and environmental deterioration can be alleviated [7]. Recent advancements in engineering have facilitated the cost-effective production and widespread availability of various cellulose-derived products, positioning it as a viable alternative to conventional plastics. Additionally, cellulose-based plastics offer biodegradable alternatives that can help address concerns regarding waste accumulation and its adverse effects on ecosystems [8].

In plants, cellulose is intricately bound with other biopolymers like hemicellulose, lignin, and pectin. These non-cellulosic biopolymers are typically removed through acid/alkali pretreatments and bleaching processes. Additionally, nanocellulose—a highly sought-after material for its unique properties—can be obtained from plants using nanofibrillation techniques. Moreover, cellulose can be synthesized by bacteria, resulting in BC, produced by species like *Acetobacter* (A.) (also known as *Gluconacetobacter* (G.) or *Komagataeibacter* (K.)) [9–12], *Taonella mepensis* (*T. mepensis*) [13], and *Lactiplantibacillus plantarum*

(*L. plantarum*) [14]. BC, distinguished by its high purity devoid of hemicellulose, lignin, or other natural components, exhibits physicochemical properties that differ from those of plant cellulose. Notably, BC possesses an ultrafine nanoscale mat structure (see Figure 1) with a high degree of polymerization, crystallinity, mechanical strength, water retention capacity, porosity, and surface area [15]. These properties, vital for various applications, can be further tailored through precise fermentation strategies, selection of microbial strains [16], and modifications such as using acid [17] and plasma treatments [18]. Producing cellulose using bacteria eliminates the need for chemical treatments associated with plant cellulose extraction, thereby minimizing the environmental impact [19].

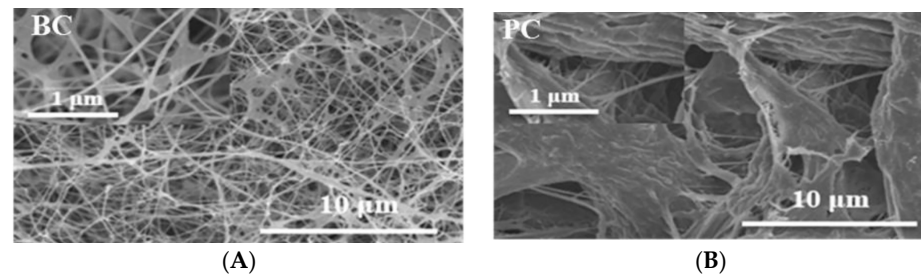


Figure 1. SEM images of BC (A) and plant cellulose (B). The inset images show the high-resolution photographs of the samples (Reprinted with permission from ref. [20] Copyright © 2019 Elsevier).

The Hestrin–Schramm (HS) medium stands as the predominant choice for BC production, yet its synthetic nature contributes to high process expenses [21,22]. The affordability of the culture medium is a decisive factor in the production of BC, making the search for cost-effective alternatives a fundamental aspect of cellulose biosynthesis. The attainment of a substantial BC yield is intricately linked to this endeavor [23]. To effectively address the imperative of cost reduction in BC manufacturing, a substantial focus has been placed on using waste sugars sourced from a variety of outlets [24].

The aggregate count of publications pertaining to BC has demonstrated a consistent annual increase over the past decade, as illustrated in Figure 2A. This trend indicates a growing interest and significance in BC research. Moreover, Figure 2B illustrates the extensive spectrum of applications attributed to BC across diverse fields. This depiction reveals the versatile potential and broad applicability of BC in various domains. In recent decades, an increasing number of studies have acknowledged the importance of large-scale production to accelerate the commercialization of BC. Accordingly, strategies have been proposed for utilizing agro-industrial biomass as cheap culture media [25]. The utilization of waste in BC production aligns with the zero-waste concept, contributing to sustainability and mitigating environmental issues associated with waste disposal [26].

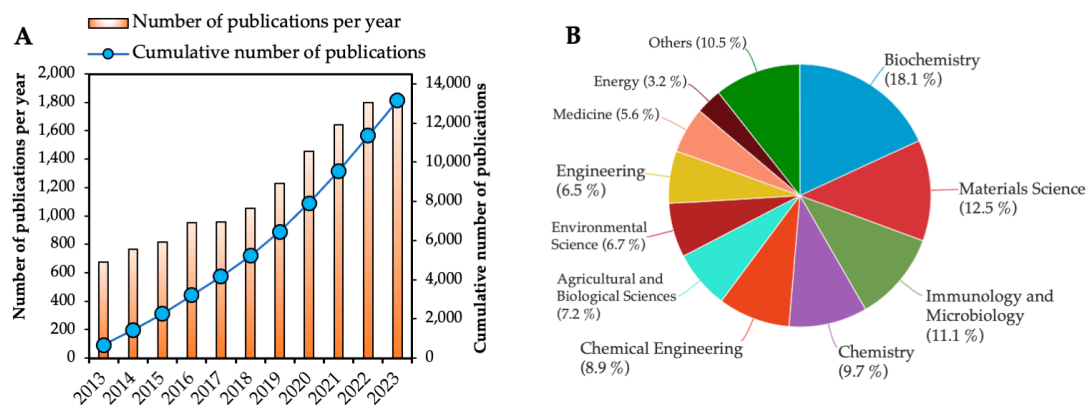


Figure 2. Trend of publications from 2013 to 2023, showing the cumulative number of articles related to BC (A). Additionally, it provides a breakdown of the percentage of publications by subject area (B), obtained through keyword searches for “Bacterial nanocellulose” on the Scopus platform.

Therefore, this review highlights approaches to fermenting waste and other strategies for economic feasibility and environmental sustainability in BC production from agro-industrial waste by summarizing the state of knowledge over the past decade. These findings show the potential for future advancements in utilizing waste streams for sustainable production processes and utilizing BC in various applications.

2. Industrial Wastes Used in Fermentation

Choosing a cost-effective culture medium aligns with the contemporary trend toward a zero-waste economy, emphasizing the importance of repurposing food manufacturing waste [27]. Utilizing waste from the food supply chain to produce chemicals, biopolymers, biofuels, and bioactive compounds reflects a broader transition toward a circular bio-economy, marking a significant shift in industrial practices [28,29]. Organic waste, generated at various stages of agro-industrial processes, is often deemed unsuitable for commercial use due to quality concerns. However, it represents a reservoir rich in sugars such as glucose, fructose, and sucrose, along with nitrogen and vitamins [30]—precisely the components essential for cellulose biosynthesis. Over the last thirty years, molasses and corn steep liquor have emerged as prominent alternative sources of complex carbon and nitrogen for BC production [24,31]. Molasses derived from sugar cane or beets has been found to be effective in BC production as a carbon source [24,31]. In recent years, researchers have increasingly investigated other waste sources for BC production, as shown in Table 1.

The utilization of grapes as an economical carbohydrate source for BC production has been studied [32,33]. Typically, unpressed residues or grape extracts rich in free monosaccharides have been employed as alternative carbon sources in HS media or in alternative media supplemented with corn steep liquor as a nitrogen source [34]. However, the primary waste generated in wineries is grape marc or grape pomace, which, post-fermentation and pressing, contains limited available monosaccharides for fermentation [35]. Conversely, many studies have focused on separating components of the grape marc (such as seeds, stalks, pulp, or skin) and subjecting them to separation processes to utilize mainly the components with the highest monosaccharide content [35]. However, such additional processing steps elevate production costs. The acid hydrolysis process applied to wine bagasse residues offers a straightforward and cost-effective method to degrade cellulose and hemicellulose structures, thereby enhancing the concentration of fermentable monosaccharides and BC production yield [36].

The utilization of potato waste to produce BC at a low cost has been assessed [37]. In this instance, potato tuber juice is used as the culture medium for BC biosynthesis, obtained similarly to industrial starch production methods [38]. The potato juice medium necessitates no pretreatment or supplementation with additional nutrient sources, apart from the inclusion of 1% *v/v* ethanol. Following the dilution of the potato juice with water at a 1:1 ratio to formulate the medium, the produced BC yield is similar to that achieved using a commercial HS medium. Moreover, the BC derived from the potato juice shows no variance from conventionally produced HS-BC concerning its physical, chemical characteristics, and cytotoxicity, affirming its comparable quality [38]. While the juice can be directly incorporated into culture media, macromolecular waste such as cellulose in potato peels necessitates hydrolysis to obtain smaller molecules like monosaccharides and disaccharides before being introduced into the medium [37]. The culture medium solely comprised of sugars derived from the acid hydrolysis of potato peels has the potential to enhance BC production yield or facilitate the creation of functionalized films with antioxidant properties [37].

Table 1. The utilization of waste-derived nutrient sources in the production of BC, based on recent research findings published from 2019 to 2024.

Waste Source	Bacteria Name	Cultivation Condition	BC Yield (g/L)	Ref.
Grape pomace hydrolysate	<i>K. melomenus</i>	Static culture, 3 days, 30 °C	1.2	[32]
Potato peel	<i>G. xylinus</i>	Static culture, pH 6.0, 4 days, 30 °C	1.27–2.61 HS ^a : 1.21	[37]
Potato juice	<i>K. xylinus</i>	Static culture, pH 6.0, 7 days, 28 °C	2–4 HS ^a : 3–5	[38]
Straw biomass (sugarcane bagasse, bamboo, corn cob, wheat straw, and rice straw)	<i>A. xylinus</i>	Static culture, 6 days, 30 °C	1.9–2.6 HS ^a : 1.7	[39]
Orange peel waste	<i>G. xylinus</i> <i>K. sucrofermentans</i>	Static culture, 8 days, 30 °C Dynamic culture, 30 °C	0.9–6.1 HS ^a : 0.9 1.9–11.6	[40–44]
Pineapple peel waste	<i>G. xylinus</i>	Static culture, pH 4.5, 14 days, 30 °C	3.8 HS ^a : 2.1	[45–47]
Pineapple core	<i>K. xylinus</i>	Static culture, pH 4, 15 days, room temperature	2.4–2.5 HS ^a : 2.5	[48]
Mango waste	<i>K. xylinus</i>	Static culture, 16 days, 30 °C	1–6 HS ^a : 2.0	[49,50]
Pear peel waste	<i>L. plantarum</i>	Static culture, 8 days	0.7–3.5 HS ^a : 1.9	[14]
Watermelon waste	<i>G. xylinus</i>	Static culture, 16 days	5.8	[51]
Asparagus waste	<i>K. rhaeticus</i>	Static culture, pH 4.5, 25 days, 30 °C	1.0–2.5	[52]
Sweet lime pulp waste	<i>A. xylinus</i>	Dynamic culture, pH 5–6, 7 days, 25–35 °C	5.2–7.0	[53]
<i>Jasminum sambac</i> and <i>Camellia sinensis</i>	<i>K. intermedius</i>	Static culture, 7 days, 30 °C	3.7–7.1 HS ^a : 5.6	[54]
Coffee ground	<i>K. rhaeticus</i>	Static culture, 7 days, 30 °C	0.5–11	[55,56]
Liquid wastes from preserved tamarind and preserved mango	<i>A. xylinus</i>	Static culture, 10 days, 30 °C	4.7 and 4.5 HS ^a : 2.5	[57]
Olive oil mill wastewater	<i>K. xylinus</i>	Static culture, pH 4.5, 7 days, 30 °C	1–5 HS ^a : 1	[58]
Sago residue	<i>G. xylinus</i>	Static culture, pH 6.0, 14 days, 30 °C	1.55 HS ^a : 1.57	[59,60]
Pecan nutshell	<i>G. entanii</i>	Static culture, pH 3.5, 28 days, 30 °C	2.8	[61]
Residue of cashew apple juice processing	<i>K. xylinus</i>	Static culture, pH 4.3, 12 days, 30 °C	1–3 HS ^a : 0.5–3	[62]
Brewing by-products	<i>K. rhaeticus</i>	Static culture, pH 6, 10 days, 30 °C	4.0	[63]
Beet molasses, vinasse, and waste beer fermentation broth	<i>K. xylinus</i>	Dynamic culture, pH 5, 7 days, 30 °C	8.2 HS ^a : 1.7	[64]
Mulberry pomace waste extract	<i>K. xylinus</i>	Static culture, pH 8, 10 days, 30 °C	1.5	[65]
Cheese whey	<i>G. xylinus</i>	Static culture, pH 5.5, 14 days, 28 °C	1–3.55 HS ^a : 3.26	[66]
Tofu wastewater	<i>K. xylinus</i>	Static culture, pH 4.5, 15 days, 30 °C	10.6	[67]
Soybean residue	<i>G. xylinus</i>	Static culture, pH 4.5, 15 days, 30 °C	1.5–2.8 HS ^a : 5.1	[68]
Waste and by-product streams from biodiesel and confectionery industries	<i>G. xylinus</i>	Static culture, pH 5.0, 7 days, 30 °C	1–7.32	[69]
Tobacco waste extract	<i>G. xylinus</i>	Static-shaking (150 rpm) cultures, pH 6.5, 7–15 days, 30 °C	0.5–5.2 HS ^a : 3.26	[70]
Rice-washed water	<i>K. xylinus</i>	Static culture, pH 4.5, 15 days, 30 °C	6.57	[67]
Kitchen waste	<i>A. xylinum</i> <i>K. rhaeticus</i>	Static culture, 15 days, 30 °C Static culture, 10 days, 30 °C	2.0 4.7	[71,72]
Paper waste sludge hydrolysate	<i>A. xylinum</i>	Static culture, pH 4.8, 15 days, 30 °C	12.0	[73]
Beeswax recycling wastewater	<i>K. xylinus</i>	Static culture, pH 6, 14 days, 28 °C	2.6 HS ^a : 2.7	[74]
Spent black liquor from cotton pulping	<i>G. xylinus</i>	Static culture, pH 5.5, 2–3 weeks, 30 °C	0.2–3.25 HS ^a : 4.66	[75]
Textile waste	<i>T. mepensis</i>	Static culture, 14 days, 30 °C	1–2.2 HS ^a : 2.2	[76]

^a Yield of BC in conventional HS medium used as a reference.

The obstacle in utilizing lignocellulosic residues lies in their resistant structure, necessitating a pretreatment process to deconstruct their complexity. This pretreatment step aims to enhance the accessibility of cellulose and hemicellulose by disrupting hydrogen bonding within the lignocellulose structure for effective hydrolysis, which releases sugars essential for microbial utilization [77]. The acidic hydrolysis of lignocellulosic and starchy wastes has been found to enhance the release of fermentable sugars, facilitating microbial product production. Different mineral acids such as HCl, HNO₃, and H₂SO₄ employed for the saccharification are studied in the potato peel waste [37]. The BC yield is higher in potato peel waste acid hydrolysate media due to their buffering capacity, particularly in potato peel waste-HNO₃-hydrolysate and potato peel waste-H₂SO₄-hydrolysate (2.2 and 1.8-fold increases compared to the HS medium) [37]. While biomass waste, consisting of cellulose, hemicellulose, and lignin, shows promise as an alternative carbon source, its utilization via acid hydrolysis to convert cellulose into fermentable sugars may introduce fermentation inhibitors like furfural, 5-hydroxymethyl-furfural (HMF), and formic acid into the hydrolysate. These carboxylic acids and furan aldehydes, might exhibit toxicity to the bacteria, potentially impeding their growth and fermentation process [78]. Various studies have recommended the adoption of detoxification procedures prior to fermentation when employing cellulosic acid hydrolysis. Cheng, Yang et al. [79] demonstrated that active carbon adsorption could remove at least 80% of furfural from acid-hydrolyzed corn stalks while retaining 90% of the total sugars. Additionally, they utilized an ion-exchange resin for detoxification and observed that ion exchange was more effective than active carbon in degrading acetic acid, although it resulted in lower removal of furfuran compounds [79]. Despite active carbon's efficient adsorption of furan inhibitors, it may also eliminate other beneficial compounds [79]. For instance, the active carbon effectively removes inhibitory compounds and also adsorbs total phenolics, indicating its non-specific adsorption properties [80]. This trait may lead to a reduction in nutrients in the hydrolysate, thereby restricting its use in fermentation processes. Lin, Huang et al. [81] studied sulfuric acid hydrolysis of sugarcane bagasse, and atmospheric cold plasma (ACP) to eliminate the toxic inhibitors. Under the optimized ACP conditions (argon ACP at 200 W for 25 min), the degradation rates of formic acid, HMF, and furfural reached 25.2, 78.6, and 100%, respectively. In BC production by *K. xylinus*, the ACP-treated sugarcane bagasse hydrolysate group demonstrated high BC production (1.68 g/L), but it was still lower than that of the ACP-untreated sugarcane bagasse hydrolysate (1.88 g/L) [81]. This result is similar to ACP-assisted pineapple peel waste hydrolysate detoxification for the production of BC by *K. xylinus* [82]. The BC produced by *K. xylinus* in both Ar and air-plasma-treated media exhibits yields comparable to BC produced by various other strains. It is noteworthy that BC production in fermentation using ACP plasma-treated media results in higher yields (3.82 g/L) compared to several reported strains. Specifically, *A. xylinum* achieves a BC yield of 2.86 g/L using acetic acid pre-hydrolysis liquor of corn stalks [79], *G. xylinus* yields 2.53 g/L of BC from litchi extract [83], and *A. aceti* yields only 1.73 g/L of BC after fermentation in optimized HS media [84]. Despite this, the BC yield from *K. xylinus* in this study remains lower than that reported using citrus peel and pomace media by enzymolysis for *K. xylinus* (5.7 g/L) [85] and optimized HS media for *K. intermedius* (3.91 g/L) [86]. These reports suggest that detoxification by plasma treatment may also lead to some loss of crucial nutrients in the hydrolysate, resulting in decreased BC production.

The enzymatic hydrolysis process offers a more environmentally friendly alternative to conventional acid treatment, operating at lower temperatures. For example, amylase, e.g., alpha-amylase and glucoamylase, is used to release the reduced sugar from the biomass [87]. This not only reduces the energy consumption and operational costs in biomass conversion but also minimizes the environmental impact [88]. However, a drawback is the use of commercial enzymes, which might increase production costs. One potential solution is to produce hydrolytic enzymes onsite, like crude glucoamylase [89], cellulase [90], and xylanase (hemicellulase) [91], from waste lignocellulose through fermentation. This approach allows for the onsite generation of enzymes, thus reducing the reliance on commercial

enzymes [91]. Those enzymes can be produced by bacteria such as *Streptomyces rochei* (*S. rochei*) [92] or fungi like *Trichoderma reesei* (*T. reesei*) [14,93]. Enzymatic hydrolysis increases the content of simple sugars with the subsequent hydrolysis time showing minimal effects on sugar release [14]. Waste jasmine flowers are utilized to produce BC with antimicrobial properties through a sequential fermentation process involving the fungus *T. reesei* and the bacterium *T. mepensis*. Initially, waste jasmine flowers are employed to generate cellulase and xylanase with *T. reesei*. The waste jasmine flowers are then enzymatically hydrolyzed, and the resulting hydrolysate is utilized for BC production. The highest BC yield from the supplemented jasmine flower hydrolysate is 2.1 times higher than that achieved with the HS medium [93].

The abundant availability of paper sludge, a primary waste product from paper manufacturing, presents both environmental and economic challenges. Fermentation medium costs represent a significant portion of BC production expenses, making the utilization of paper sludge for BC production an attractive prospect to mitigate the environmental impact and reduce costs [94]. Chemical analysis reveals a high sugar content in paper sludge, primarily consisting of cellulose and hemicellulose, but low levels of simple sugars and glucose in crude paper sludge due to the complex nature of its cellulosic materials. This necessitates pre-hydrolysis such as saccharification by cellulase produced by *S. rochei* to support BC production [92]. Utilization of starchy kitchen wastes (SKWs) as a cost-effective substrate is used for BC production [71,95]. By employing a Box–Behnken design, the maximum production of reducing sugars from SKWs through enzymatic hydrolysis using amylase produced by *Bacillus methylotrophicus* SCJ4 is optimized. The study found that optimal conditions yielded 148.0 g/L of reducing sugars and 2.11 g/L of BC [95].

The disposal of waste from the dairy industry is a significant concern due to the high-water usage in factories. Effluents from dairy processing contain lipids, lactose, detergents, and sanitizing agents that require treatment before being discharged into the environment [96,97]. Dairy wastewater typically contains elevated levels of total nitrogen (14–830 mg/L) and total phosphorus (9–280 mg/L). The nitrogen sources also affect BC production [98]. Therefore, utilizing dairy waste offers the dual advantage of wastewater purification and viable BC production [96,99]. Whey, a by-product of cheese or soybean curd manufacturing, contains approximately 55% of the nutrients found in the original milk, including soluble protein, lactose, minerals, and B-group vitamins [66]. The cheese whey can be used to replace half of the expensive yeast extract in the conventional HS medium, facilitating economic BC production [100]. Cultivating *G. xylinus* using untreated or lactose-pretreated cheese whey results in BC yields of 1 g/L and 3 g/L, respectively, after 14 days of static cultivation [66]. Soybean residue, rich in amino acids comparable to commercial yeast extract, can serve as a sole nitrogen source when supplemented with sucrose in the culture medium [101]. After 7 days of cultivation with a 10% *G. xylinus* inoculum, BC yields ranging from 1.5 g/L to 2.8 g/L are obtained, depending on the pretreatment method used to increase the water solubility of the soybean residue [68]. However, when the soybean residue is the sole nitrogen source in the HS-based culture medium, BC production decreases to approximately 1 g/L [68].

3. Strategies to Improve BC Yield

3.1. Dynamic Bioreactor

Traditionally, BC is generated through static culture methods, where cellulose is formed as a gelatinous pellicle at the interface between the air and liquid. However, once BC pellicles reach a certain thickness, production reduces due to nutrient depletion in the aerobic zone [77,102] and oxygen deficiency in the lower regions of the pellicle [103]. Prolonged fermentation under these conditions offers minimal contribution to BC production [104]. The optimization of three key factors—incubation temperature, shaking frequency to maximize oxygen availability, and pH—is performed using response surface analysis [53,105]. This analysis is based on the actual and predicted responses obtained from a Box and Behnken design experiment, which involves three factors each at three

levels [53]. An alternative lies in continuously feeding the fresh medium onto the pre-formed BC pellicle at the air–liquid interface [106]. The activated bacteria can access sufficient nutrients and oxygen from the fresh medium to sustain BC synthesis. Consequently, newly formed BC continuously occurs at the air–liquid interface, regardless of the location of previously existing pellicles. Implementing intermittent feeding in this manner facilitates continuous BC synthesis and enhances overall BC production [106].

Fed-batch or intermittent feeding strategies supplying the culture with fresh media under sterile conditions to sustain BC synthesis are promising strategies to scale-up BC production [106]. By using enzymatically hydrolyzed paper sludge (EHPS) as a culture medium, in fed-batch fermentation, the HS medium outperforms EHPS/HS and EHPS. BC production increases substantially in fed-batch compared to batch fermentation across all media types. The BC production in fed-batch fermentation increases approximately 2.1, 4.6, and 2.9-fold compared to batch fermentation when using HS, EHPS/HS, and EHPS media, respectively [92]. For Alcohol Lees, a by-product of alcohol production, BC is produced using an Intermittent Feeding Strategy [107]. Through the utilization of the respond surface methodology, the synergistic effects between Alcohol Lees dilution, pH, intermittent feeding height, and intermittent feeding interval are analyzed. The optimal conditions identified for maximum BC yield are Alcohol Lees dilution of 86%, pH of 5.6, intermittent feeding height of 1.4 mm, and intermittent feeding interval of 22.2 h, resulting in a BC yield of 4.4 g/L. Comparatively, conventional static cultures of BC yield 2.0 g/L in a one-week culture period [107]. The BC production through *G. xylinus* using glycerol as a carbon source is studied in a 3 L bench-top bioreactor under fill-and-draw and pulse-feed fed-batch cultures at 30 °C [108]. The fill-and-draw fed-batch culture accumulated 24.2 g/L of BC with a yield and productivity of 0.2 g/g and 2.69 g/L/day, respectively, after 9 days. The pulse-feed fed-batch culture yielded 24.38 g/L of BC with a yield of 0.38 g/g and productivity of 2.71 g/L/day. The study suggests that fed-batch cultivation, particularly pulse-feed, holds promise for industrial BC production, potentially reducing costs [108].

A rotating biological contactor (RBC) has been employed as a bioreactor to enhance the production of BC (Figure 3A) [109]. The RBC utilizes mechanical rotations to introduce air to the biofilm, promoting oxygenation to support growth [110]. The increased rotation speed enhances oxygen transfer, while RBC offers advantages such as low energy consumption, minimal maintenance, operational cost efficiency, and a compact design, making it an environmentally friendly option for BC production [109,111]. Factors influencing RBC performance include disc rotation speed, disc immersion, surface roughness, material, spacing, biofilm characteristics, and dissolved oxygen (DO) levels [112]. The impact of disk rotation speed, disk spacing, disk material, surface roughness, and aeration is individually examined using a one-factor-at-a-time approach [112]. The findings reveal that the greatest quantity of BC is generated on the surfaces of integrated polyethylene discs rotating at 13 rpm. Moreover, optimal BC production occurs when the distance between adjacent discs is set at 1 cm, with a total of 16 discs. Using an aquarium pump to aerate the RBC, comprised of 12 integrated polyethylene discs, operating at the optimal rotation speed of 13 rpm, results in an increase in both the wet weight and dry weight of BC. Specifically, this aerated fermentation leads to a more than 64% increase in wet weight and a 47% increase in dry weight compared to non-aerated RBC [112].

In the airlift bioreactor, Vázquez et. al. (2023) [113] investigated the viability of utilizing a culture medium derived from *Garnacha Tintorera* grape bagasse, rich in phenolic compounds, and waste potatoes, a glucose source, for simultaneous production of BC and gluconic acid through *K. xylinus* fermentation (Figure 3B). Acid hydrolysis of the grape bagasse enhances the phenolic content while minimizing growth-inhibiting by-products, e.g., furfural and HMF. Statistical analysis identifies acid concentration and temperature as pivotal variables, suggesting the optimal hydrolysis conditions of 2% sulfuric acid concentration for 60 min at 125 °C. These conditions enable a significant increase in total phenolic content compared to aqueous extraction [113].

Utilizing different force fields, like the magnetic force field, presents a promising alternative to enhance biomass production compared to conventional methods [114–116]. Studies have demonstrated the efficacy of static magnetic fields (SMF) in stimulating microbial growth and enhancing enzyme production. Furthermore, the potential of rotating magnetic fields (RMF) in diverse biotechnological applications, including the biogenesis of BC is studied in a bioreactor equipped with RMF generators (Figure 3C) [116–118]. The utilization of RMF has been shown to enhance the growth and metabolic activity of *K. xylinus* [118]. Furthermore, employing RMF-assisted bioreactors has led to an increase in BC yield compared to conventional static methods because they enhance both the quantity and quality of *K. xylinus* inoculum, consequently leading to a higher yield of the resulting BC [115]. However, the RMF application does not result in a higher incidence of cellulose non-producing mutants within the 72 h exposure period [119].

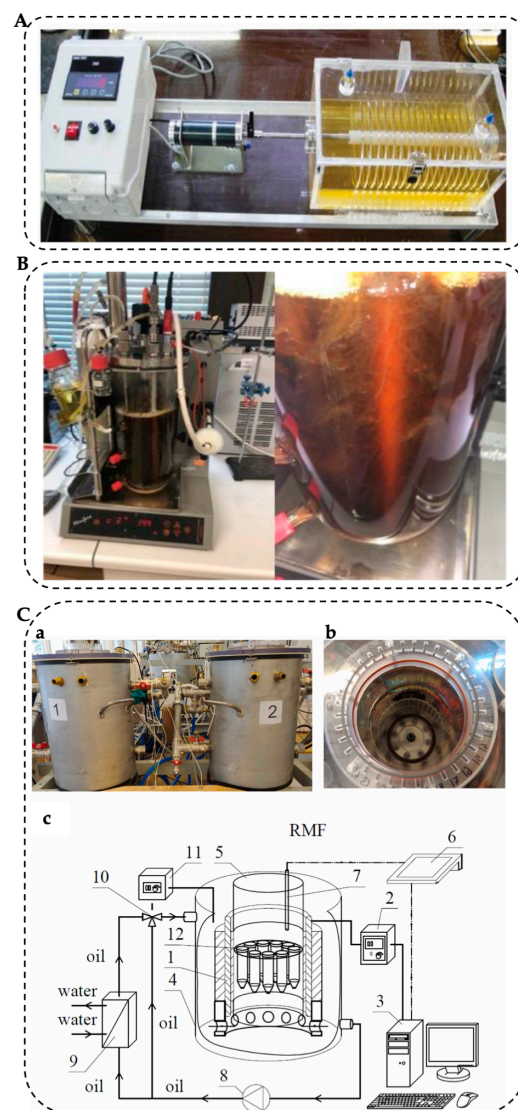


Figure 3. Bioreactors for BC production. Rotating biological contactor (A) (Reprinted with permission from ref. [109] Copyright © 2023 Elsevier). Airlift bioreactor (B) (Reprinted from ref. [113] open access Copyright © 2023 by the authors. Licensee MDPI). Bioreactor equipped with generators of rotating magnetic fields: front view (a), top view (b), schematic of the experimental setup (c). 1—RMF generator, 2—phase inverter, 3—PC, 4—generator tank, 5—inner container, 6—CX-701 multimeter, 7—temperature probe, 8—circulation pump, 9—plate heat exchanger, 10—three-way valve, 11—valve controller, 12—plastic probe (with cells suspension) (C) (Reprinted with permission from ref. [116] open access Copyright © 2022 Elsevier).

A magnetically assisted external-loop airlift bioreactor (EL-ALB) equipped with RMF generators, facilitating *K. xylinus* inoculum development was studied by Żywicka, Ciecholewska-Juśko et al. [115]. The study introduces the EL-ALB equipped with RMF generators for *K. xylinus* inoculum production in three-cycle repeated fed-batch cultures within a 40 L working volume. Analyses reveal improved fluid hydrodynamics in the RMF-assisted EL-ALB compared to a non-RMF EL-ALB, suggesting enhanced mass transfer rates during fermentation and increased *K. xylinus* cell multiplication [120]. Fermentation in the RMF-assisted EL-ALB yields inoculum with over 200 times higher cellular density compared to traditional methods, maintaining high and stable metabolic activity across repeated batch fermentation cycles. Importantly, RMF-assisted inoculum production does not induce cellulose-deficient mutants, and *K. xylinus* demonstrates consistent BC production regardless of inoculum age, achieving 7.26 g/L of dry mass [115].

3.2. Genetic Engineering

Studies into genes associated with BC synthesis offers valuable insights for the industrial utilization of BC-producing bacteria to generate BC from diverse carbon sources, including agro-waste feedstocks [121]. Based on these studies, an alternative method to improve BC production includes the utilization of synthetic biology tools and the alteration of metabolic pathways responsible for BC synthesis [122,123]. The genetic engineering involves leveraging adaptation mechanisms that bacteria develop in response to specific environmental conditions such as the adaptation ability to various carbon sources (Figure 4A) [124]. The effectiveness of genetic modification in fine-tuning *K. xylinus* is demonstrated. For example, in one study, the *bcsA*, *bcsAB*, and *bcsABCD* genes, which encode a complete cellulose synthase operon, are overexpressed in *K. xylinus* DSM 2325. This modification led to an accelerated production rate and a two to four-fold increase in BC yield [125]. Another study focuses on heterologously expressing the *vgb* gene from *Vitreoscilla* hemoglobin, resulting in a 50–70% boost in BC production under low oxygen conditions [126]. Furthermore, research on the *pgi* gene, responsible for encoding *Escherichia coli* (*E. coli*) phosphoglucose isomerase, demonstrates that its high expression enhances the metabolic pathway of BC synthesis, resulting in a remarkable 115.8% increase in BC production (Figure 4B) [127].

A recombinant strain for BC production in mannose-rich media is developed (Figure 4C) [128]. The introduction of the *mak* and *pmi* genes from *E. coli* into *K. xylinus* ATCC 23770 enhances the metabolic efficiency of mannose to fructose 6-phosphate. Comparing wild-type and recombinant strains reveals a 1.6-fold increase in the mannose utilization rate and an 84% boost in BC yield in the recombinant strain. Moreover, BC produced by the recombinant strain exhibits improved mechanical properties, with a 1.7-fold increase in tensile strength and elongation, and a 1.3-fold increase in Young's modulus. The results suggest that the expression of the *mak* and *pmi* genes leads to better adaptation of the recombinant strain to the mannose carbon source and enhances the mechanical properties of BC. However, while the pBBR1MCS-2 plasmid carrying the exogenous gene confers kanamycin resistance, only *K. xylinus* ATCC 23770 exhibits tolerance to kanamycin upon transformation, suggesting potential limitations for strain transformation in other *K. xylinus* strains [128]. A study focuses on the characterization of *Komagataeibacter* sp. nov. CGMCC 17276 [129]. Whole-genome analysis reveals four BC synthase operons, particularly emphasizing the significance of *bcs* II and *bcs* III in BC synthesis under static and agitated culture conditions. Metabolic pathway analysis identifies glycerol as the most effective carbon source for BC production among eight potential options including glucose, sucrose, fructose, trehalose, maltose, mannitol, and ethanol. Glycerol is as an effective carbon source for BC production, with waste glycerol also presenting eco-friendly strategies for industrial BC production [129].

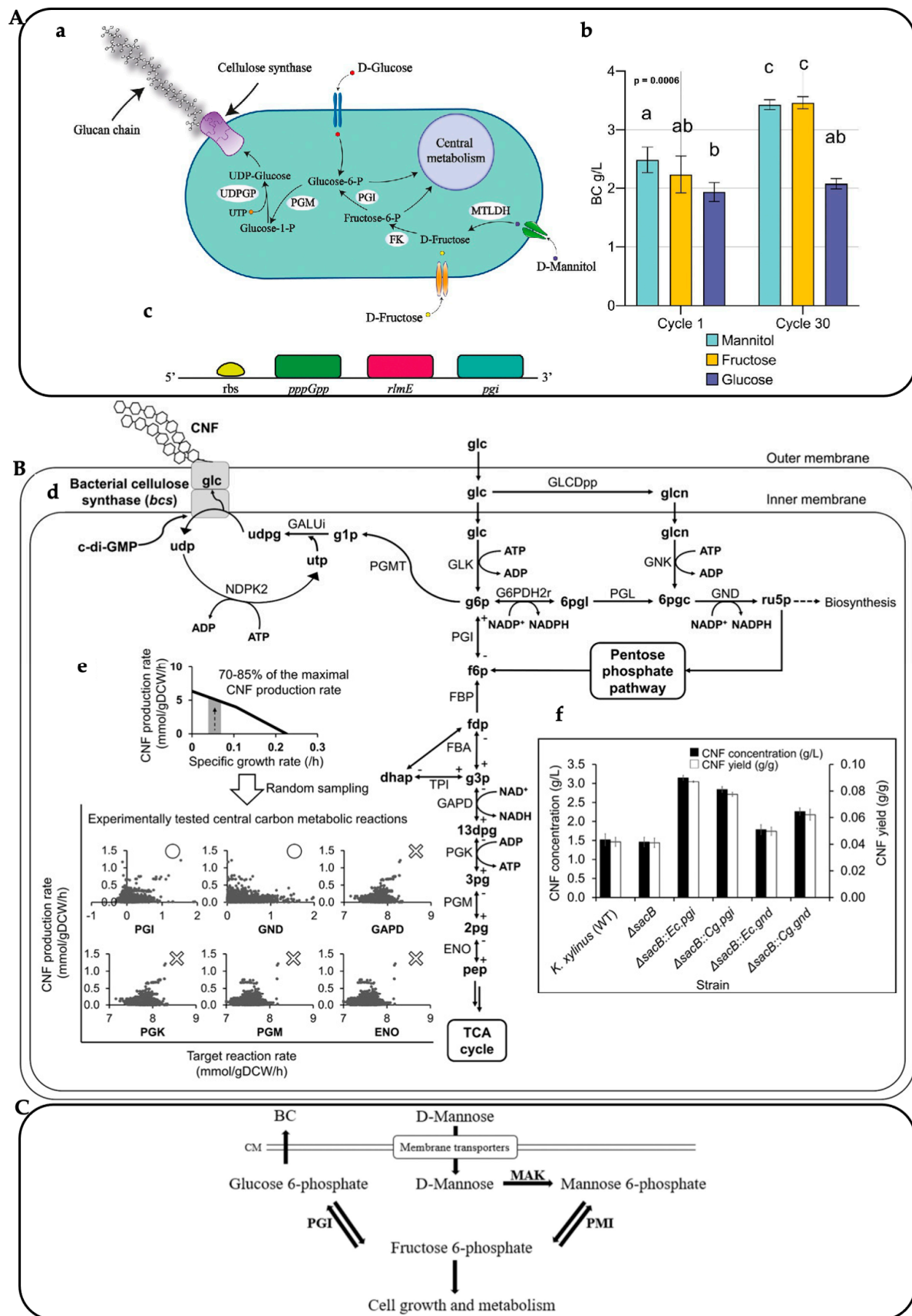


Figure 4. BC production strategies by genetic engineering of *K. xylinus* strains under different conditions. Adaptive laboratory evolution stress conditions in strain K2G30 (UMCC 2756) (A): schematic

representation of the metabolic pathway for fructose and mannose (a), BC production using fructose and a comparison with mannitol and glucose (b), and schematic depiction of the arrangement of the *pgi*-related gene in the genome of *K. xylinus* K2G30 (c) (Reprinted from ref. [124] open access Copyright © 2022 Frontiers). Genomic and metabolic engineering of strain DSM 2325 (B): central carbon metabolism and BC biosynthetic pathway of *K. xylinus* (d), Systems Biology Markup Language file depiction of reversible reactions in a genome-scale metabolic model KxyMBEL1810 (e), random sampling and correlation analysis results for glycolysis and pentose phosphate pathway reactions, identifying overexpression targets PGI and GND (f). BC concentration and yield comparisons among wild-type and genetically modified strains (Reprinted with permission from ref. [127] Copyright © 2019 Wiley). Recombination of strain ATCC 23770 to use mannose-rich source (C) (Reprinted with permission from ref. [128] Copyright © 2023 Elsevier).

4. Environmental Impact Assessment

Global industrial focus is shifting toward more efficient production methods that promote sustainability, aiming to minimize the adverse environmental effects of manufacturing processes. The recent report from the Intergovernmental Panel on Climate Change emphasized the significance of decreasing net anthropogenic greenhouse gas emissions [130]. Life cycle assessment (LCA) has been utilized to evaluate the environmental implications of processes used to manufacture bio-based products, whether on a laboratory or industrial scale [131]. Currently, LCA studies have primarily focused on assessing the influence of various fermentation media on BC production. Overall, the BC production process has a relatively small impact on resource consumption and environmental factors in the global life cycle. The carbon footprint of BC production is often lower compared to cellulose extraction from plant biomass. Microcrystalline cellulose obtained from sugarcane bagasse, following acid–alkali pretreatment and depolymerization, exhibits the estimated carbon footprint of 261 kg CO₂-eq/kg of cellulose [132] and nanocellulose derived from wood pulp by acid–alkali treatment and homogenization causes 80 kg CO₂-eq/kg of nanocellulose [133]. The carbon footprint of BC typically falls within the range of 34–296 kg CO₂-eq/kg BC [131,134,135]. These considerable variations can be attributed to differences in production methods, product yields, equipment, the specific LCA software used, inventory databases, and the system boundaries considered [131,134,135]. The lowest carbon footprint at 39 kg CO₂-eq/kg BC is reported when utilizing nata-de-fique agro-waste for BC production [135].

A basic, small-scale LCA of BC production was conducted by using the SIMAPRO[®] software [136]. The study investigated the use of corn steep liquor (CSL) as a replacement for yeast extract and peptone in BC production [136]. The LCA compared two scenarios: one using CSL in the culture medium and one using the conventional HS medium. The results showed reductions in environmental impacts with the CSL-containing medium, including decreases in global warming, freshwater eutrophication, freshwater ecotoxicity, and human carcinogenic toxicity. The study also noted an increased environmental impact from the energy-intensive electric greenhouse used during fermentation, highlighting the importance of energy efficiency in minimizing environmental effects [136]. From the ReCiPe 2016 methodology, the production of BC requires significant resources, with water being the primary component, accounting for 36.1 tons per kilogram of BC produced [131]. Most of this water (98%) is returned to fresh waters after treatment. Raw material production contributes significantly to resource consumption, with 17.8 tons of water per kilogram of BC used, primarily for carton packaging, culture medium raw materials, and sodium hydroxide for BC washing [131]. Fermentation and downstream processes also utilize substantial amounts of water. However, substituting petroleum-derived plastics with bio-based alternatives could potentially decrease CO₂-equivalent emissions by 30%, thereby reducing the global warming potential [131].

The LCA of the developed bioprocess demonstrates that using brewer's spent grain extract (BSGE) and brewer's spent yeast (BSY) in the culture medium can reduce the carbon footprint of 1 kg dry BC by 76% compared to commercial-based media [63]. The

highest carbon footprint is observed with the HS medium, while the BSGE- and BSGE + BSYE-derived media resulted in 4.2 to 4.4-fold lower carbon footprints. This reduction is attributed to the higher BC concentration achieved and the substitution of commercial carbon and nitrogen sources with BSGE and BSYE [63]. Substituting raw materials in the HS medium with brewery-derived side-streams led to further reductions in total carbon footprint, with medium sterilization and inoculation accounting for 1–3% of the total carbon footprint in all cases [63].

5. Applications

The versatility and sustainability of cellulose and its derivatives make them promising candidates for addressing contemporary challenges in materials science, ranging from eco-friendly packaging solutions to advanced biomedical applications [137,138]. As research in this field progresses, leveraging the unique properties of BC holds immense potential for creating innovative, sustainable materials with diverse applications across industries.

5.1. Medical Application

The increasing demand for biodegradable and biocompatible biopolymers manufactured through environmentally friendly methods has surged owing to their minimal greenhouse gas emissions [139]. Especially, the COVID-19 pandemic has led to a notable increase in plastic pollution due to the use of single-use plastics for personal protective equipment and packaging. With increased awareness about the origins of medical products, there is a shift away from plastic-based materials [140]. Consequently, numerous researchers have focused on developing biodegradable polymers for medical applications [141]. Hence, BC is one of the suitable options to meet these criteria due to not only its animal-free and human-free sourcing, but also its biodegradability [142].

Several findings support the development of BC composites for targeted biomedical applications, particularly as antimicrobial wound dressings. The development of BC-based composites incorporating bactericidal nanoparticles like Se [142], Cu [143], Ag [144], ZnO [145], Au [146], and TiO₂ [138] have been extensively researched. Nevertheless, the potential toxicity of these nanoparticles to healthy mammalian cells, resulting from the production of reactive oxygen species, cannot be overlooked despite their antibacterial properties [147,148]. There has been growing interest in utilizing green plant-based antimicrobials to create antimicrobial BC-based composites due to their potent antimicrobial properties and minimal side effects [149]. Studies have explored incorporating animal-based products such as propolis [150] and chitosan [151] or extracts from various plants into BC-based composites as summarized in Table 2. Various bioactive compounds such as curcumin [152,153], lignin [154], quercetin [155], carrageenan [156], and other plant extracts [157,158] have been employed in creating BC-based composites for wound healing and delivery of bioactive compounds. In particular, the addition of the natural extracts not only enhances antimicrobial activity [157] but also improves mechanical and other properties [159]. The plant extracts contain functional groups that serve as active antimicrobial agents, including polyphenols (flavonoids), chalcones, and other nitrogen-containing moieties (alkaloids, amines, etc.), which confer biological activity [157,158].

Research into the applications of pomegranate (*Punica granatum* L.) in medicine and antimicrobial treatments dates back centuries and continues in modern research [160]. Recent investigations suggest that pomegranate containing several phenolic compounds can serve as a natural alternative to synthetic antibacterial agents against various microbial pathogens [160,161]. Studies have also highlighted the potential of the pomegranate in treating conditions such as cancer, bacterial wound infections, among others, owing to its reported anti-carcinogenic, anti-inflammatory, and antioxidant properties [162]. An antibacterial composite using BC infused with pomegranate peel extract (PGPE) for potential biomedical uses was developed by Ul-Islam, Alhajaim et al. [163]. The BC was efficiently derived from food waste, and PGPE was incorporated into a BC hydrogel. The resulting BC-PGPE composite exhibited notable antibacterial properties against both *Staphylococcus*

aureus (*S. aureus*) and *E. coli* strains. The composite could retain 97% of its dry weight in water and maintained this retention for over 48 h. Additionally, it showed superior reswelling ability compared to pure BC after three consecutive cycles of re-wetting [163]. A wound dressing that contains active compounds from mangosteen peel was developed by Marisca Evalina, Yulanda et al. [164]. The extract derived from mangosteen (*Garcinia mangostana*), primarily cultivated in tropical regions of Southeast Asia, offers a range of advantageous biological properties including antibacterial, antioxidant, anti-inflammatory, and anticancer effects attributed to its bioactive constituents such as xanthenes, mangostin, tannins, and other polyphenolic compounds [165,166]. The wound dressing made from BC loaded with mangosteen peel extract exhibits flexibility and suitable thickness [164]. The incorporation of mangosteen peel extract into the dressing enhances wound healing after 15 days, as evidenced by improvements in wound closure. Furthermore, the optimal concentration of mangosteen peel extract for the dressing is determined to be 10%, suggesting its potential application in the development of treatments for diabetic ulcers [164].

Table 2. Natural products incorporated in BC for biomedical applications.

Natutal Products	Incoporation Methods	Improved Properties	Potential Applications	Ref.
Propolis	Impregnation of BC into propolis/ZnO solution	Antimicrobial activity against <i>E. coli</i> , <i>Bacillus subtilis</i> (<i>B. subtilis</i>), and <i>Candida albicans</i> (<i>C. albicans</i>)	Antimicrobial film	[150]
Chitosan	Impregnation of BC into chitosan solution	Antimicrobial activity against <i>S. aureus</i> , <i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>), and <i>C. albicans</i>	Antimicrobial dressing	[151]
Curcumin	Impregnation of BC into curcumin solution	Antimicrobial activity against <i>E. coli</i> , <i>P. aeruginosa</i> , <i>Salmonella typhimurium</i> (<i>S. typhimurium</i>), and <i>S. aureus</i> , wound healing property (accelerated wound closure up to 64% after 15 days)	Antimicrobial wound dressing	[152,153]
Lignin	Impregnation of BC into lignin solution	Antimicrobial activity against <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>Serratia</i> sp., <i>Listeria monocytogenes</i> (<i>L. monocytogenes</i>), and <i>Salmonella typhimurium</i> (<i>S. typhimurium</i>)	Antimicrobial chronic wound dressing	[154]
Quercetin	Phase inversion of mixed BC and quercetin solution	Drug loading capacity and controlled drug release properties	Controlled-release drug delivery	[155]
Carrageenan	Impregnation of BC into carrageenan/gelatin solution	Antimicrobial activity against <i>E. coli</i> , <i>S. aureus</i> , and <i>Klebsiella pneumonia</i> , drug loading capacity and controlled drug release properties	Wound dressing and tissue regeneration	[156]
Green tea leaf extract, roselle flower petals, and <i>Hibiscus rosa-sinensis</i> L. flower extract	In situ biosynthesis with BC	Antimicrobial activity against <i>P. aeruginosa</i> and <i>E. coli</i> and antioxidant property	Wound dressing and face mask	[157]
Combretaceae and Solanaceae extract	Impregnation of BC into the plant extract solution	Antimicrobial activity against <i>E. coli</i> and <i>S. aureus</i>	Biomedical materials	[158]
Pomegranate peel extract	Impregnation of BC into the plant extract solution	Antimicrobial activity against <i>E. coli</i> and <i>S. aureus</i>	Antimicrobial wound dressing	[163]
Mangosteen peel extract	Impregnation of BC into the plant extract solution	Wound healing property (accelerated wound closure up to >90% after 15 days)	Wound dressing	[164]
<i>Euclea schimperi</i> extract	Impregnation of BC into the plant extract solution	Antimicrobial activity against <i>E. coli</i> and <i>S. aureus</i>	Antimicrobial wound dressing	[167]
Asparagaceae leave extract	Impregnation of BC into the plant extract solution	Antimicrobial activity against <i>E. coli</i> and <i>S. aureus</i>	Antimicrobial wound dressing	[168]

5.2. Energy Storage and Electronics

The focus on renewable green energy presents a crucial avenue for addressing the contemporary energy crisis. Solar energy, as a widely accessible clean energy source, holds significant promise and has garnered extensive attention for its utilization [169]. Particularly, photothermal conversion emerges as a key method for harnessing solar energy, prompting intensive research efforts [170]. Various photothermal materials, including polymer materials, carbon-based materials, plasma metals, and semiconductor materials, have been explored [171]. A multifunctional photothermal phase change material (PCM), composed of silylated BC, hydroxylated carbon nanotubes (HCNT), and polyethylene glycol (PEG), has been developed for solar energy utilization (Figure 5A) [172]. This PCM features a cross-linked network structure, enhancing support, dispersion, and interlocking within the material. It demonstrates a high output voltage of 423 mV and power density of 30.26 W/m² under solar irradiation, suitable for solar–thermal–electric energy conversion. Moreover, it finds applications in thermal management for solar cells and light-emitting diode (LED) chips, showing its versatility in waste heat conversion into electricity [172].

Rechargeable lithium-ion batteries (LIBs) are recognized as one of the most promising energy storage solutions for contemporary mobile electronics and hybrid electric vehicles due to their outstanding attributes, including high energy densities, extended cycling life, and minimal self-discharge rates [173]. One crucial element of LIBs is the separator membrane, a porous membrane located between the anode and the cathode. Its primary function is to prevent internal short circuits by ensuring that the two electrodes do not make direct contact with each other [174]. The study of Ajkidkarn and Manuspiya [175] explored the use of BC nanocrystals (BCNCs) derived from nata de coco waste as a bio-filler to enhance polyether block amide (PEBAX) separator membranes for LIBs. The BCNCs are obtained through sulfuric acid hydrolysis, yielding rod-like/needle-like structures with negatively charged surfaces. Non-solvent-induced phase separation and film casting methods are employed to fabricate BCNCs/PEBAX membranes, which exhibit improved thermal and dimensional stability up to 150 °C without shrinkage. The addition of BCNCs enhanced the ionic conductivity and porosity of the membrane, making them promising candidates for replacing commercial polyolefin-based separator membranes in LIBs due to their superior electrochemical performance and reinforcement effect [175].

A proton exchange membrane requires high conductivity, thermal stability, absorbency, and cost-effectiveness [176]. BC shows promise as a sustainable material for these electronic applications and conducting materials [5,177,178]. However, pure BC lacks optical transparency, electrical conductivity, magnetism, hydrophobicity, and antimicrobial properties [176]. To enhance its capabilities for use in conducting materials and electrical devices, BC must undergo modification. This involves incorporating conductive materials like nano carbon fillers [5,179] and doping with acid and conducting polymers in nanoparticle or nanowire form [180]. Gadim et al. [181] fabricated proton-conducting electrolytes using a composite of BC and poly(4-styrene sulfonic acid) at room temperature (Figure 5B). A yield of 40 mW/cm² at 125 mA/cm² could be achieved. Following this, Naumi et al. [182] developed a polymer electrolyte membrane fuel cell utilizing BC with sulfonated polystyrene and phosphoric acid (Figure 5C). The addition of phosphoric acid was found to enhance membrane proton conductivity [182]. Phosphorylation has an impact on ion exchange capacity, ionic conductivity, swelling index, and contact angle of the membrane [176]. The phosphorylated BC produced from cassava liquid waste and doped with 20 mmol of H₃PO₄ leads to an enhancement in proton conductivity, with a maximum conductivity reaching 7.9 × 10^{−2} S/cm at 80 °C. Furthermore, a membrane electrode assembly was constructed using this membrane, with 60 %w/w Pt/C loading of 0.5 mg/cm² for both the anode and cathode. A single-cell performance achieves a high power density of 25 mW/cm² at an operating temperature of 40 °C [176]. A carbon-fiber-embedded BC/polyaniline nanocomposite electrode was developed for microbial fuel cell (MFC) applications, that are promising renewable energy sources to simultaneously treat organic waste and generate electricity [183]. The MFC performance has a maximum current density of 0.009

mA/cm^2 . The adaptation period of microorganisms using *E. coli* and biofilm development influenced the initial current density, with an increase observed in the nanocomposite compared to carbon fiber alone. The nanocomposite facilitates electron adhesion and conduction, leading to higher conductivity and improved MFC performance compared to the other electrodes tested, including carbon fiber/BC and BC/polyaniline membranes. The enhanced conductivity of this nanocomposite is attributed to the incorporation of carbon fibers and the doping of BC with polyaniline, promoting electron transfer to the conductive material [183]. The in situ fermentation BC with graphene, reduced graphene oxide, polyaniline, and carbon nanofibers using a novel layer-by-layer self-assembly approach was proposed by Luo et al. [5]. Introduction of graphene dispersion into the BC layers was achieved via a repetitive spraying method, resulting in the formation of robust three-dimensional hydrogels [5,184]. Morphological and Raman spectroscopy analyses confirmed the presence of few-layered graphene nanosheets, enhancing hydrogen bonding interactions within the composite [185]. The incorporation of polyaniline leads to the fabrication of freestanding BC-based electrodes exhibiting good electrochemical properties and high charge storage capacity with the aim of developing BC-based nanocomposites with enhanced properties for potential applications in electrodes and supercapacitors [186,187]. The strategic integration of graphene via sustainable in situ fermentation routes leads to the production of composites with properties comparable to those achieved through chemical-based ex situ modifications [188]. Graphene/BC nanocomposites produced via fermentation exhibited a high capacitance of approximately 215 F/g, with exceptional stability over nearly 5000 cycles [189]. However, the inclusion of carbon-based nanomaterials as fermentation additives in the fermentation media affected microbial growth kinetics and process behavior, which are crucial during scaled-up operations [190].

The development of organic light-emitting diodes (OLEDs) typically involves a multilayered structure with an organic semiconductor, placed between two electrodes on a transparent rigid substrate [191]. However, a significant challenge lies in replacing glass with a more flexible substrate to enable OLEDs for use on curved surfaces, which is crucial for the design of wearable devices and real-time communication [192]. As a result, there has been a growing interest in developing flexible and conformable substrates for OLED fabrication [193]. Highly transparent BC (HTBC) biocompatible membranes are developed for use as substrates in OLEDs [194]. These membranes, composed of BC and an organic-inorganic sol containing boehmite nanoparticles and epoxy-modified siloxane, are enhanced with SiO_2 and indium tin oxide (ITO) thin films deposited via radio frequency magnetron sputtering. The HTBC/ SiO_2 /ITO substrate exhibited improved visible light transmission by 88%. Electrical characterization reveals that the ITO-deposited films are n-type doped semiconductors with properties comparable to commercial ITO on glass substrates [194]. The blending of polystyrene with BC presents an appealing option for OLED applications due to the comparable refractive indices of these polymers [195]. Utilizing d-limonene, a renewable solvent capable of dissolving polystyrene, has been explored to enhance the transparency and hydrophobic properties of BC, aiming to achieve a flexible substrate for OLED applications [195].

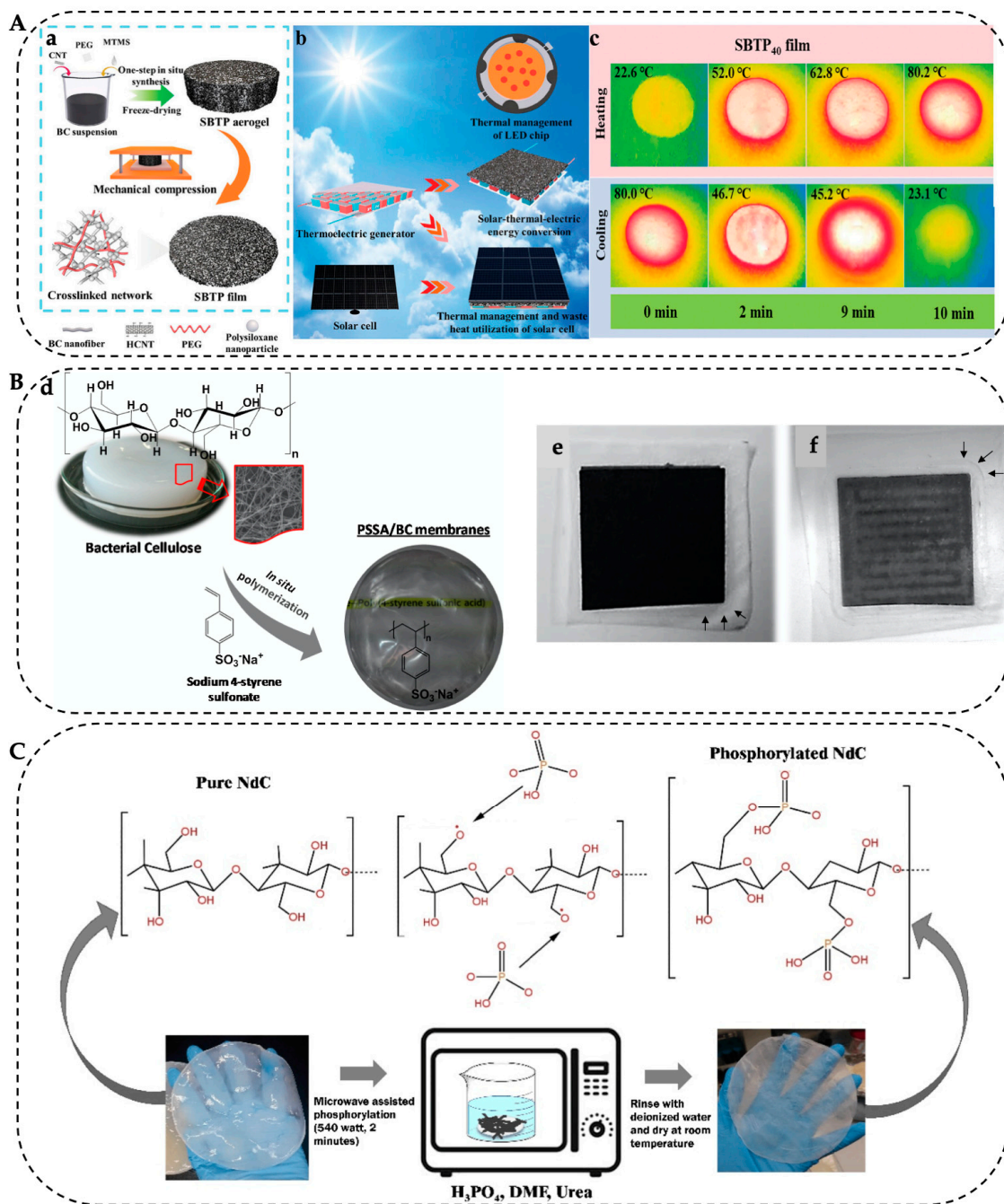


Figure 5. Energy storage application of chemically modified BC nanocomposites. Silylated BC/hydroxylated carbon nanotube/polyethylene glycol (SBTP) film (A): synthesis of the SBTP film (a), mechanism of solar-thermal energy storage, conversion of solar-thermal energy to electricity (b), and infrared thermograph depicting the phase change storage process of SBTP film under solar irradiation (c) (Reprinted with permission from ref. [172] Copyright © 2023 Wiley). Poly(4-styrene sulfonic acid) (PSSA)/BC membrane electrode (B): fabrication scheme (d) and image showing the pristine BC (e) and PSSA/BC after fuel cell testing with no evident signs of degradation (f), except for the groove indicated by the arrow, possibly caused by the rubber seal (Reprinted with permission from ref. [181] Copyright © 2017 Elsevier). Phosphorylated BC (C): phosphorylation process of BC (Reprinted from ref. [172] open access Copyright © 2023 by the authors. Licensee MDPI).

5.3. Filtration Membranes

The utilization of membrane technology has been recognized as an efficient and energy-saving approach for separation processes [196]. However, its widespread adoption on a large scale is impeded by various challenges, encompassing complex operational procedures, high operational costs, concerns regarding toxicity, limited recyclability, and the generation of hazardous by-products. Hence, there is a critical imperative to explore materials that offer cost-effectiveness and environmental compatibility [197]. Addressing some of these challenges, BC-based separation membranes have shown promise. For the separation of emulsions, previous efforts have leveraged the hydrophilicity of inorganic coatings like SiO₂ to enhance membrane performance [198]. For instance, Hou et al. demonstrated the deposition of modified SiO₂ on dried BC membranes using chlorodimethyloctadecylsilane through hydrolysis and condensation processes (Figure 6A) [198]. These nanofiber network membranes exhibit pore sizes of 0.5–1 µm and show underwater superoleophobicity and under-oil superhydrophobicity properties, enabling the separation of water-in-oil (W/O) emulsions. Similarly, Wahid et al. developed superhydrophilic membranes based on BC by blending commercial dopamine hydrochloride with micro-sized SiO₂ particles to fabricate BC-SiO₂ composite membranes for oil-in-water (O/W) emulsion separation (Figure 6B) [199]. However, the use of microparticles may lead to increased accumulation of micro-sized particles, potentially posing challenges for waste disposal in terrestrial and aquatic environments, including freshwater, sediments, and soil [200]. Spherical SiO₂ nanoparticles are produced through the hydrolysis of tetraethoxysilane in the presence of ammonia and subsequently applied onto the surface of BC membranes [200]. The surface roughness and wettability are enhanced, facilitating the separation of oil-in-water (O/W) emulsions. The straightforward deposition of SiO₂ nanoparticles offers a practical method for creating superhydrophilic membranes capable of efficiently separating emulsified oil/water mixtures, achieving separation rates of up to 99% [200]. Moreover, composite of BC produced from banana peel waste and SiO₂ nanoparticles was also applied for desalination [201]. The flexible paper-like composite membranes are produced with varying proportions of BC, microcellulose, and SiO₂. The membrane demonstrated the high salt rejection value of 4.89% initially, although this decreased over time due to membrane surface clogging [201].

The presence of non-biodegradable, toxic, carcinogenic, mutagenic, and potentially teratogenic dyes and heavy metals poses significant threats to both human health and aquatic ecosystems [202]. Therefore, there is an urgent need to remove metal ions and dyes from wastewater to mitigate their harmful effects [203]. This shows the critical importance and high demand for effective wastewater treatment methods that can address these contaminants and protect environmental and public health. Biosurfactants (BS) derived from *Candida lipolytica* fermentation and BC, both cultivated in media supplemented with industrial waste, have been applied for treating textile effluents [204]. Using solely BC as a filtration agent achieved up to 45% removal efficiency of navy blue dye, while treatment with BS prior to filtration with BC resulted in 65% color removal [204]. Using coffee as a natural dye to produce eco-friendly coffee-dyed BC (BC-COF) bio-leather, aiming for sustainable textile applications was investigated [205]. The optimal dyeing and conditions were determined through various parameters such as temperature and time. The analysis confirmed the colorization of BC with coffee without altering its chemical and crystalline structures, with coffee molecules effectively incorporating into the BC fiber structures. The BC-COF bio-leather exhibits efficient adsorption of methylene blue dye with multiple reusability, suggesting its potential as a sustainable dye adsorbent [205]. A biocomposite of dialdehyde cellulose nanocrystals (DCNC) and wool keratin is fabricated for the purpose of removing Cd²⁺ and Crystal violet from water. The removal efficiency of Cd²⁺ and Crystal violet by the Keratin/DCNC adsorbent slightly decreases after five cycles of recycling [74]. These results show the potential of BC combination for wastewater treatment, offering a cost-effective and sustainable filtration solution.

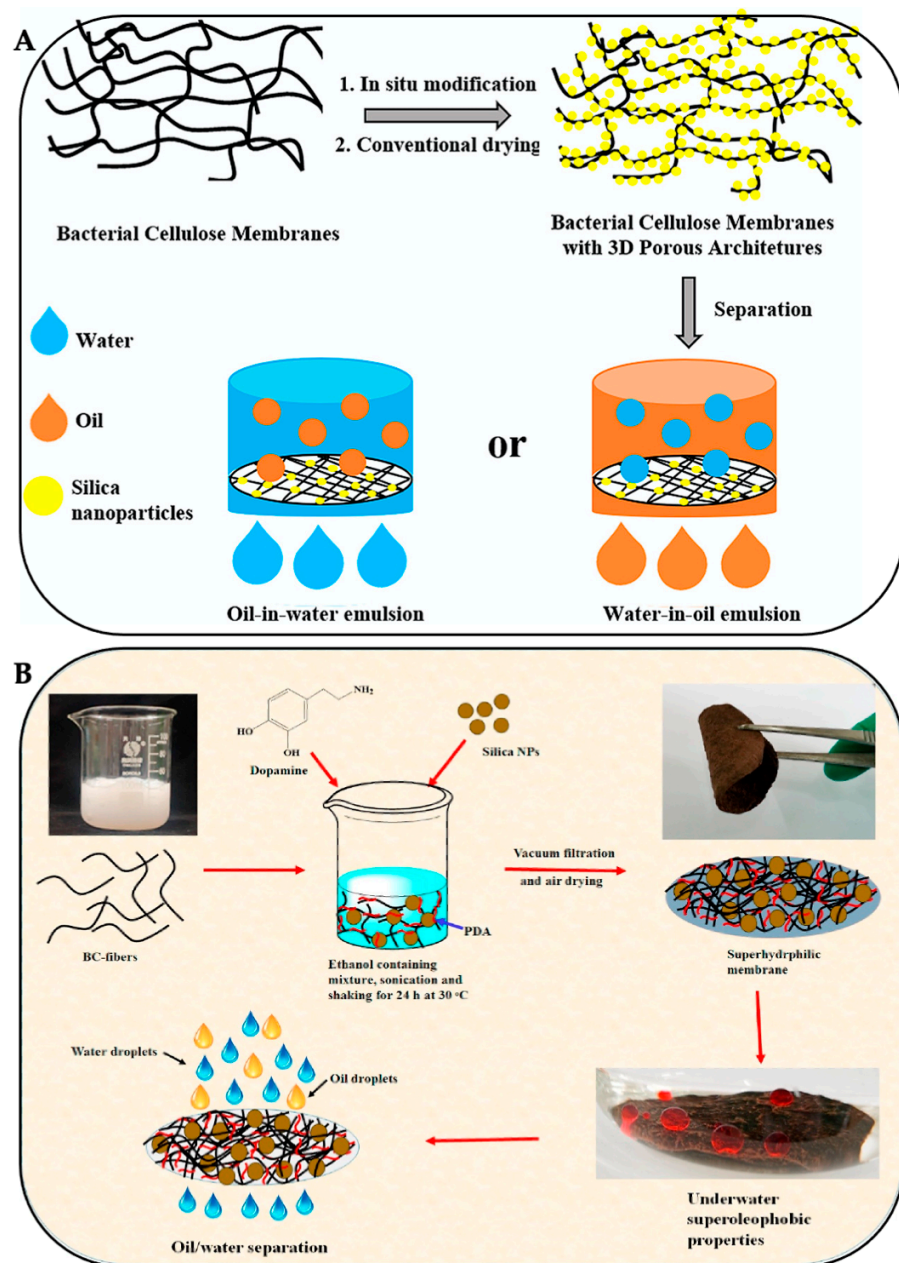


Figure 6. Filtration membrane application of BC loaded with SiO₂ nanoparticles (A) (Reprinted with permission from ref. [198] Copyright © 2019 Elsevier) and SiO₂ microparticles (B) (Reprinted with permission from ref. [199] Copyright © 2021 Elsevier) for emulsion separation.

Metal oxides, particularly magnetite, have been recognized for their ability to improve the adsorption of various contaminants such as metals, textile dyes, organic substances, nitrogen, and phosphorus, while also exhibiting antibacterial properties. Syukri et al. [206] conducted a study to assess the effectiveness of incorporating Fe₃O₄ into nanocellulose membranes derived from pineapple peel waste for reducing bacteria and dye contaminants in wastewater. The inclusion of Fe₃O₄ showed promising results in reducing microbial counts and anionic dye concentrations in water samples obtained from local rivers in Padang city, adjacent to industries such as rubber, cement, and tofu production [206]. Similar observations are noted in the utilization of membranes for removing dyes and bacteria carrying negative charges [207]. The impact of incorporating TiO₂ nanoparticles and graphene into BC acetate-based nanocomposite membranes designed for bacterial filtration in water was investigated. BC was derived from pineapple peel waste through a fermenta-

tion process, followed by a high-pressure homogenization step to reduce nanocellulose size, and an esterification process to produce cellulose acetate. Nanocomposite membranes were then synthesized, reinforced with 1% TiO₂ nanoparticles and 1% graphene nanopowder. The addition of TiO₂ nanoparticles and graphene to the cellulose acetate membrane resulted in improved filtration efficiency against coliform bacteria in water [207].

5.4. Food Packaging Materials

It is crucial to develop bio-based primary packaging to enhance food safety and quality while also addressing environmental pollution from the growing accumulation of packaging waste [208,209]. Bioplastic food packaging can be made from BC, driven by its biodegradability. BC offers a promising alternative to synthetic plastics commonly used in various industries, including food packaging, due to its lignin-free cellulose, high mechanical strength, and environmentally friendly nature [208,209]. To meet the requirements of food packaging standards, modifications are necessary for pristine BC film due to its non-transparency, lack of antibacterial properties, and typically thicker consistency compared to plastic film [210].

The BC films with enhanced properties are developed through surface modification using cyclic anhydrides and dimethyl sulfoxide as a solvent [211]. Different anhydrides have been explored to optimize the film characteristics. The modification with dodecenyl succinic anhydride increases the dry and wet tensile strength, while octadecenyl succinic anhydride reduces water vapor permeability and exhibits strong antimicrobial effects. Films modified with maleic anhydride have improved oxygen barrier properties and facilitate the effective preservation of strawberries (Figure 7A). The films display complete biodegradability in soil within one month. Additionally, surface grafting with different alkyl moieties (C0–C18) leads to films with varied properties, including mechanical strength, water vapor permeability, and antimicrobial activity [211]. The regenerated BC films are fabricated utilizing citric acid as the crosslinking agent to enhance the mechanical properties of the resulting film [210]. The film exhibits a fracture strength of 93.40 MPa and a Young's modulus of 4.2 GPa, surpassing the performance of commercial polyvinyl chloride (PVC) plastic film. Moreover, the modified film demonstrated biodegradability in soil within a mere 2 weeks. Furthermore, the citric-acid-crosslinked film exhibits heat resistance and food preservation capabilities compared to PVC plastic wrap [210]. Modified BC films are investigated, incorporating carboxymethyl cellulose (CMC) as a stabilizer and glycerol as a plasticizer [212]. Various composite films were prepared with different concentrations of CMC and glycerol, and their physicochemical and mechanical properties were assessed. A tensile strength of 17.47 MPa, elongation at break of 25.60%, and Young's modulus of 6.54 GPa were found. The modified BC films were applied as packaging for meat sausages, demonstrating their ability to maintain sausage quality during a 6-day storage period at room temperature (Figure 7B) [212]. Antimicrobial nanocomposite films were developed using polyvinyl alcohol reinforced with glycerol, BC nanocrystals, and boric acid. The film composition optimized by the response surface methodology exhibited reduced water solubility and moisture content, along with enhanced water vapor permeability and water vapor transmission rate. Additionally, the antimicrobial activity of the film against *B. subtilis* and *C. albicans* was observed. The optimized film also demonstrated increased biodegradability compared to neat polyvinyl alcohol film. The addition of BCNCs and boric acid enhanced the thermal stability of the film [213]. The BC-based food packaging material that is biocompatible, biodegradable, bioactive, and non-toxic is also developed by reducing silver nitrate with sodium chloride to prepare a BC/Ag nanocomposite [214]. The nanocomposite exhibits improved water solubility (4.6%) and tensile strength (25.7 MPa). It shows no toxicity toward NIH-3T3 fibroblasts and displays antimicrobial activity against various bacterial and fungal strains. BC/Ag-nanocomposite-film-coated oranges maintain acceptable sensory qualities during storage for up to 9 weeks [214].

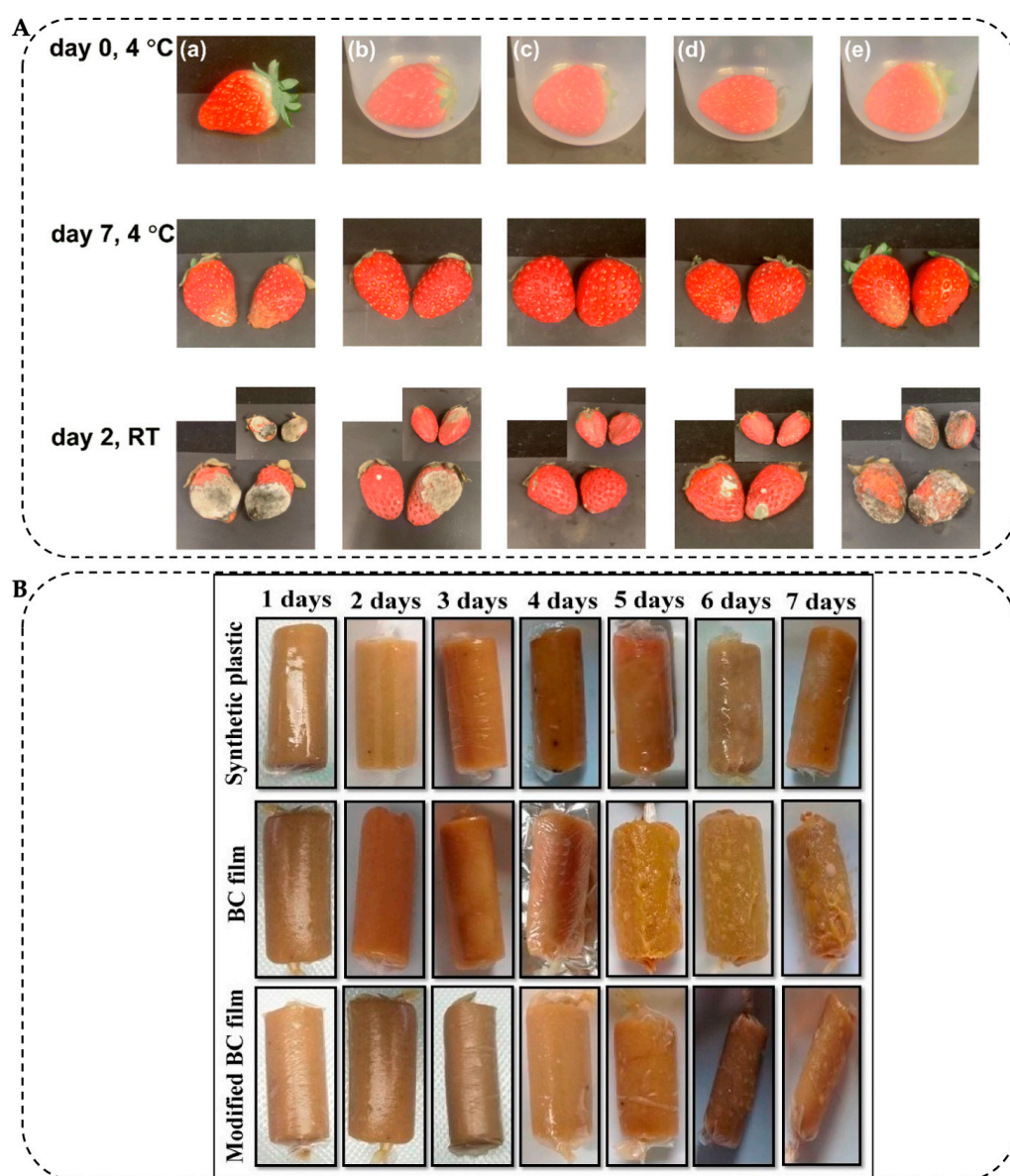


Figure 7. Food packaging application of modified BC films. Cyclic-anhydride-modified BC (A): the surfaces and inner structure of strawberries left uncovered (a) or covered with the pristine BC film (b), BC films modified with maleic anhydride (c), BC films modified with 2-Octenylsuccinic anhydride (d), and low-density polyethylene film (e) for storage durations of 0 and 7 days at 4 °C, 40% relative humidity, followed by storage at room temperature (24 °C, 55% relative humidity) for 2 days (Reprinted from ref. [211] open access Copyright © 2024 by Royal Society of Chemistry). BC/carboxymethyl cellulose/glycerol modified film (B): meat sausage packaged by low-density polyethylene film, native BC film, and modified BC film for storage durations of 1 to 7 days at room temperature (Reprinted from ref. [212] open access Copyright © 2021 by the authors. Licensee MDPI).

The incorporation of natural antimicrobial agents into matrix formulations for food packaging was studied. Biodegradable food packaging films are created using potato peel powder, with BC added as a reinforcement agent [215]. Natural extracts from food processing wastes, such as curcumin, were utilized as antioxidant additives. BC interacts with biopolymers in the potato peel matrix primarily through inter- and intra-molecular bonding interactions, leading to enhancements in mechanical properties, as well as reductions in oxygen permeability, water vapor permeability, and light transparency of the films. The addition of curcumin imparts good antioxidant capacity to the potato peel/BC

films, effectively inhibiting lipid oxidation in fresh pork during storage [215]. Whey protein concentrate and BC nanowhiskers were investigated as raw materials for edible film production, with a focus on modifying these films with oregano essential oils to impart antimicrobial properties against various foodborne pathogens [40]. The inclusion of BC nanowhiskers in whey protein concentrate/oregano essential oil films improves the mechanical properties of the films [216] and increases their antimicrobial effectiveness, with up to 80% inhibition [40]. Structural analysis confirms the homogenous distribution of the oregano essential oils and BC within the film matrix, resulting in rough films with enhanced release capacity for the oregano essential oils [40]. A dual-action method was used by impregnating BC with citrus pectin (CP) and then functionalizing it with an active emulsion coating of thyme essential oil (TEO) encapsulated in CP-TEO [217]. This film exhibits good physicochemical properties, a continuous and dense microstructure, optimal mechanical strength, and the lowest water vapor permeability. The BC composite film demonstrates antibacterial activity against *S. aureus* and *E. coli*, antibiofilm properties, effective UV-blocking, and improves visible light transmittance. It shows superior potential in preserving the quality of grapes over 9 days of storage compared to pristine BC and conventional plastic packaging. During the 9-day storage period, the film maintains the freshness, color, and overall structural integrity of the grape samples while exhibiting minimal weight loss [217]. Moreover, agar/foamed BC incorporating carvacrol, the thyme essential oils, exhibits a gradual release of carvacrol, making it suitable for food packaging for fish. The agar/BC/carvacrol combination inhibits microbial growth and minimizes nitrogen compounds resulting from protein decomposition when microbial spoilage occurs in sea bass fillets after day 6. Additionally, it decelerates lipid peroxidation, thereby prolonging the shelf life of the fish [218]. Since BC possesses unique characteristics such as fibrillated organization, porous structure, and abundant hydrogen bonding sites, BC is also identified as an effective biopolymer for delivering various bioactive compounds such as catechin [219] and cinnamon essential oil [220].

Films based on chitosan/BC have the potential to serve as antimicrobial packaging, enhancing the preservation of packaged foods. An antimicrobial film using dialdehyde BC, chitosan, and ϵ -polylysine (ϵ -PL), a cationic natural antimicrobial peptide derived from microbial metabolites, was studied [221]. The antibacterial activity against *S. aureus* and *E. coli* was enhanced with increasing ϵ -PL content. However, the excessive ϵ -PL tended to aggregate, disrupting intermolecular interactions among polymer chains and consequently reducing tensile strength and water solubility. The optimal formulation was found to exhibit a maximum tensile strength of 36.77 MPa, along with low water vapor permeability and oxygen permeability values. Furthermore, the films effectively extended the shelf life of tilapia fillets during refrigerated storage [221]. A biocomposite consisting of BC nanofibers incorporated into tapioca starch/chitosan-based films was studied [222]. Through ultrasonication, the fillers were uniformly dispersed throughout the film matrix. The addition of the dried BC resulted in an improved tensile strength (4.7 MPa), thermal resistance, moisture resistance (8.9% increase after 8 h), and water vapor barrier (27% improvement after 24 h). Additionally, all the chitosan/BC films exhibited antibacterial activity [222]. The BC and silver nanoparticles were incorporated into chitosan to develop nanocomposite films [223]. The addition of BC and silver nanoparticles enhanced the strength of the chitosan matrix while reducing moisture content, water solubility, and water vapor permeability. Incorporating glycerol and grape bagasse extract (GE) led to higher moisture content due to their hydrophilic nature. The addition of GE increased water vapor permeability. Glycerol and GE incorporation significantly enhanced the swelling capacity and solubility. Using the multilayer method, BC and chitosan compounds exhibited greater swelling capacity and lower solubility compared to compounds developed using the immersion method, where a BC film was immersed in a chitosan solution [224]. The nanocomposite films demonstrated potent antibacterial effects against foodborne pathogens [223] and antioxidant properties [224], which held promise as active packaging materials, capable of extending the shelf life of packaged foods.

6. Conclusions and Challenges

Utilizing biopolymers like cellulose offers a sustainable alternative to petroleum-based polymers, aligning with the UN's Sustainable Development Goals and addressing environmental concerns. Cellulose-based materials present diverse applications and the potential to reduce carbon emissions. They can be sourced from plants or synthesized by bacteria, with BC offering unique properties. Cost-effective BC production is crucial, with efforts focusing on mainly utilizing waste sugars as culture media. Research interest in BC has grown, highlighting its versatile applications and potential for large-scale production using agro-industrial waste. To improve BC yield, dynamic bioreactor systems have been developed, alongside genetic engineering approaches to modify metabolic pathways. These strategies offer promising avenues for maximizing BC production while minimizing energy consumption and environmental impact. The versatility and sustainability of BC make it promising for addressing contemporary materials science challenges in many applications such as medical materials, energy storage/electronic devices, filtration, and food packaging.

As research in this field progresses, harnessing waste-derived resources for BC production holds significant promise for advancing sustainable bioprocessing practices in cellulose biosynthesis. However, there are some challenges related to efficient and economical production. Understanding the intricate interplay between waste utilization, enzymatic processes, and chemical composition is pivotal for devising efficient strategies to enhance BC production while minimizing costs and environmental impacts. The enhancement of BC production yield and its effective application necessitate further exploration through foundational research, particularly focusing on the bacterial cells employed in BC production. The mechanisms underlying the growth of strains of BC-producing bacteria and the synthesis of BC remain incompletely understood. In-depth investigations into the physiology and metabolic pathways of the BC-producing bacterial strains can offer valuable insights into the optimization of culture conditions, substrate utilization, and the regulation of BC biosynthesis. Additionally, elucidating the genetic determinants and regulatory networks governing BC production can pave the way for genetic engineering approaches aimed at enhancing BC yield and modifying its properties for tailored applications. Moreover, understanding the interactions between the BC-producing bacteria and their environment, including nutrient availability, pH, and temperature, is crucial for optimizing BC production processes and scaling them up for industrial applications. Therefore, further fundamental research efforts are warranted to unlock the full potential of BC as a sustainable biomaterial in diverse fields.

Funding: This review received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created.

Conflicts of Interest: The author declares no conflict of interest.

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