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Review

Fermentation Techniques and Biotechnological Applications of Modified Bacterial Cellulose: An Up-to-Date Overview

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Abstract: Bacterial cellulose (BC) is a pure exocellular polysaccharide produced by micro-organisms. It has several properties in comparison with plant-derived cellulose that make it perfectly suitable for many applications, ranging from the food industry to the biomedical area. Different production methods and modification or functionalization procedures have been investigated in response to the many possible attractive applications of BC. This review overviews the different fermentation techniques and functionalization methods together with the main possible biotechnological applications of BC for food industry and biomedical purposes.

Keywords: bacterial cellulose; fermentation processes; functionalization methods; biotechnological applications

Citation: Sayah, I.; Gervasi, C.; Achour, S.; Gervasi, T. Fermentation Techniques and Biotechnological Applications of Modified Bacterial Cellulose: An Up-to-Date Overview. *Fermentation* **2024**, *10*, 100. https://doi.org/10.3390/fermentation10020100

Academic Editor: Mohammad Taherzadeh

Received: 31 December 2023
Revised: 1 February 2024
Accepted: 4 February 2024
Published: 8 February 2024

1. Introduction

In 1886, a British professor named A.J. Brown reported the synthesis of a white, gelatinous pellicle that appeared on a liquid medium surface during an acetic fermentation process [1,2]. Further investigations revealed that this pellicle was composed of pure cellulose, and it was produced by certain types of bacteria, particularly belonging to the genera *Achromobacter*, *Alcaligenes*, *Aerobacter*, *Agrobacterium*, *Azotobacter*, *Komagataeibacter* (formerly known as *Gluconacetobacter*), *Pseudomonas*, *Rhizobium*, *Dickeya*, and *Rhodobacter* among Gram-negative bacteria, as well as by Gram-positive bacteria from the genus *Sarcina* [3].

The species Komagataeibacter europaeus, Komagataeibacter medellinensis, Komagataeibacter rhaeticus, Komagataeibacter xylinus, and Novacetimonas hansenii are recognized as the best cellulose producers among bacteria and are commonly used as model organisms for investigating the synthetic pathways of bacterial cellulose (BC) and its various applications [4,5]. Usually, bacteria produce this layer as a form of protection against desiccation, ultraviolet radiation, or unfavorable pH conditions [6]. BC is not essential for survival, but it gives the micro-organisms that produce it a competitive advantage by supporting their attachment, adherence, and colonization of substrates [7].

BC is a nanoscale porous network biopolymer consisting of linear chains of β-D-glucose linked by β-1,4-glycosidic bonds. During fermentation, micro-organisms metabolize glucose forming linear β-1,4-glucan chains. These chains are secreted extracellularly and crystallized to make cellulose monofilaments. The following aggregation of a certain number of these monofilaments form filamentous fibers and subsequently a three-dimensional and gelatinous structure on the surface of the liquid medium where the bacteria are growing [8].

The biosynthesis of BC includes three stages: the synthesis of uridine diphosphate glucose; the synthesis of the cellulose molecular chain; and the crystallization and polymerization of cellulose (Figure 1). First, glucose is phosphorylated into glucose-6-phosphate (Glc-6-P) through the action of an enzyme called glucokinase. Then, Glc-6-P is isomerized into glucose-1-phosphate (Glc-1-P) by another enzyme known as phosphoglucomutase. Finally, UDP-glucose (UDP-Glc) is synthesized from Glc-1-P by the UDP-glucose pyrophosphorylas. Under the function of cellulose synthase (Bsc) operon, the UDP-Glc monomers are assembled into a sequence of β-1,4-glucan chains, which further aggregate to form fibrils. These fibers are secreted through outer membrane pores and in turn, contribute to the creation of a complex cellulose network structure [9].

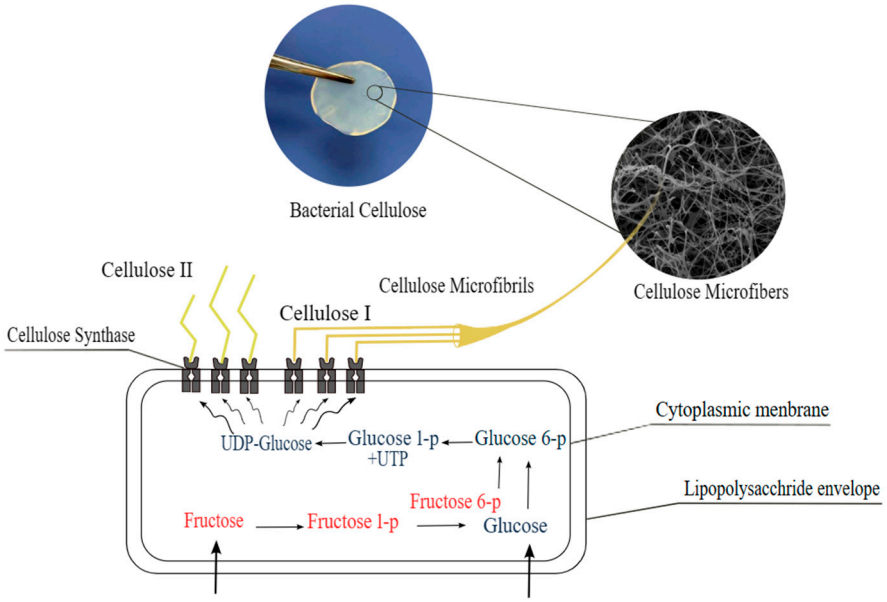

Figure 1. Schematic representation of bacterial cellulose biosynthesis pathways from glucose and fructose in *Acetobacter xylinum*.

There are analogous glucose metabolism and UDP-glucose synthesis pathways in other types of cellulose-producing micro-organisms under anaerobic conditions as well as other non-glucose carbon sources, including sucrose, fructose, as well as ethanol, which are converted into UDP-glucose through TCA, gluconeogenesis, etc. entering the metabolic network [10].

Generally, through the fermentation of sugars, bacteria may produce bacterial cellulose extracellularly in different allomorphic forms, but celluloses I and II are the most widely studied, [11–13]. Cellulose I has a ribbon structure, comprising bundles of microfibrils, whereas cellulose II is an amorphous polymer with a high thermodynamic stability compared to cellulose I [14].

2. BC Production Methods

BC can be produced through several techniques, including static culture, agitated or shaking culture, and bioreactor cultures. The bioreactors design is a fundamental element for the BC structure and yield [15].

Each technique gives BC with different properties in terms of morphology and microstructure. In static culture, BC forms a gelatinous membrane of cellulose at the surface of the culture solution [16]. While agitated or shaking cultures result in various structures, such as asterisk-like, sphere-like, pellet-like, or irregular masses [17]. The choice of the production method depends on the intended applications of BC and the required characteristics for those applications. Based on the various fermentation purposes, to increase the

BC production, different bioreactors have been designed including stirred tank reactors, airlift, aerosol, membrane reactors, and other types [18].

2.1. Static Culture Method

Static cultivation is the most used method for BC biosynthesis (Figure 2). In this case, the BC membrane is generated at the air–liquid interface of the culture media. Depending on the shape and size of the container employed, the resulting BC membranes can take various forms usually in the form of a film. The thickness of this membrane is influenced by the incubation time, which typically does not exceed 14 days. In fact, prolonged fermentation times can lead to the accumulation of inhibitory metabolites like glycolic and formic acids, among others [19]. In static cultivation, cellulose is usually produced in layers which are parallel to the film plane, resulting in a remarkably rigid and robust structure. This makes it well-suited for applications in membrane technology, food packaging, or as scaffolds in biomedicine [20–24]. According to Schramm and Hestrin, BC-producing bacteria generate carbon dioxide (CO_2) bubbles during their metabolic activities. These trapped gas bubbles contribute to the pellicle's ability to float on the liquid surface [25].

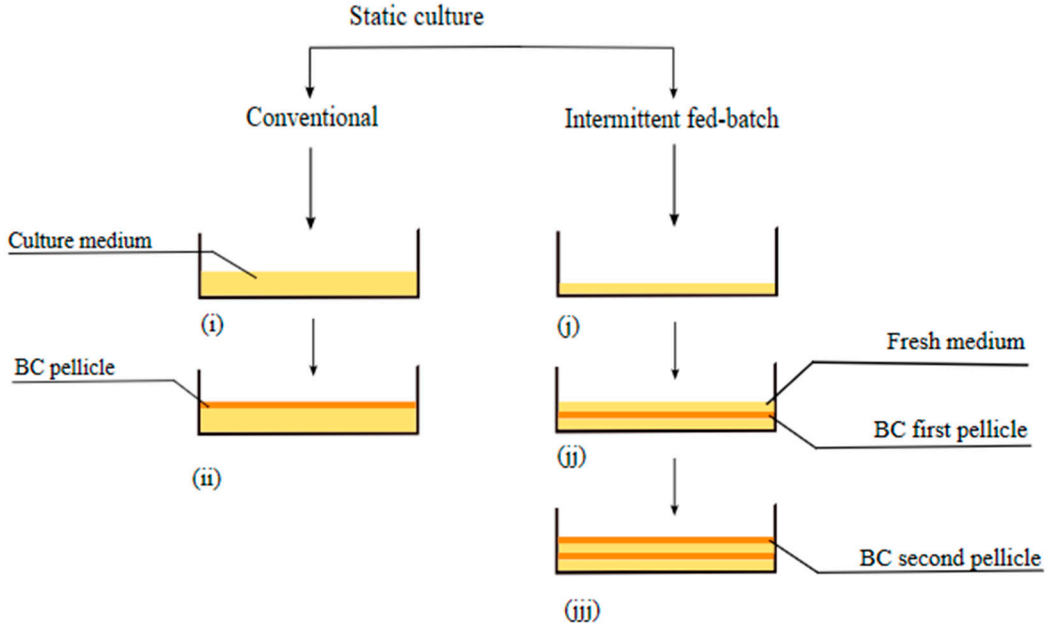

Figure 2. Schematic diagram of BC culture. Conventional static culture: (i) substantial amounts of medium are being fed all at once, (ii) only one layer of a BC sheet can be obtained. Intermittent fed-batch culture: (j) a certain amount of medium being fed gradually, (jj) fresh batch of the medium being fed after first BC layer is formed, (jjj) another layer of BC is formed, and another fresh medium will, subsequently, be fed.

Despite the significant importance of this technique, some drawbacks may limit its industrial application, mainly the long cultivation time, low productivity, larger area, and greater workforce. In this regard, an intermittent feeding strategy, or fed-batch fermentation has been developed to improve BC production yield during static culture. These processes are based on a periodic addition of media. As shown in Figure 2, a new batch of fresh medium is added directly on the top of the pre-existing BC pellicles, typically the first layer. New pellicles then form on the air–liquid surface, with a critical depth of over 1 mm. This procedure is repeated continuously until several layers of pellicles are produced [26].

Compared to the conventional process, the intermittent feeding maintained a constant BC yield of 0.02 g/day for 30 days cultivation, while the production rate in the conventional process is nearly negligible [27]. Many attempts were made to enhance BC production within the fed-batch technique by making modifications to the growth medium. Bae and

Shoda obtained 7.82 g/L of BC through fed-batch fermentation using H_2SO_4 heat-treated molasses as a carbon source, versus 5.3 g/L of BC with the conventional batch fermentation techniques [28]. Further advancements have been achieved by combining the fed-batch technique with various nutrient sources. For example, Shezad et al. demonstrated that beer liquid waste, when coupled with the fed-batch technique, could increase BC production from *G. hansenii* PJK threefold within a 30-day cultivation period [29]. Additionally, Dubey et al. reported that *K. europaeus* SGP37 could produce BC at a rate 1.47 times higher in fed-batch fermentation by modifying the HS media with a hot water extract of sweet lime pulp [30].

2.2. Submerged Fermentation

Due to the limitations associated with static culture, submerged fermentation has been suggested as an alternative approach. It offers several advantages over static culture, including the higher productivity in lesser time and more oxygen supply. Nevertheless, the submerged fermentation the shearing stress generated, which is due to agitation, can cause the revert of bacteria to non-BC producing mutants, thereby inhibiting the BC production [31,32], the formation of irregularly shaped BC granules, and modifications to BC's physical properties [31]. The agitated fermentation process is more appropriate for industrial BC production, thereby for commercial applications in various fields. Additionally, strains mutations are more probable thus influencing BC production [32].

Additionally, at high rotation speeds, bacteria tend to prioritize gluconic acid synthesis over cellulose production, and hydrostatic stresses can lead to the accumulation of self-protection metabolites [33].

Several types of submerged fermentation have been employed for BC production, including stirred tank, rotating disk, and airlift bioreactors. Each of these approaches has its own set of advantages and limitations, and the choice of the method depends on the specific requirements of the BC production process [26].

2.2.1. Stirred Tank Bioreactor

The process involves the use of liquid medium that is vigorously aerated and agitated in large fermenters (Figure 3). These fermenters can be of two types: closed or open, and then the cultivation can be conducted as batch or continuous processes. These bioreactors are constructed from non-corrosive metals or glass-lined materials. In batch fermentation, the micro-organism is cultivated in a predetermined quantity of culture medium for a defined period, after which the cellular mass is separated from the liquid for further processing. On the other hand, in continuous culture, the culture medium is withdrawn based on the rate of product formation and the introduction of fresh medium [34]. This technology exhibits excellent volumetric mass transfer capabilities, and it is already in widespread use.

Recent research has examined the fermentation parameters of *K. xylinus* in stirred tank reactors [35]. The findings indicated that higher agitation rates led to increased cell densities and greater production of BC. The study reported that at an agitation speed of 500 rpm, a BC yield of 0.59 g/L and a productivity of 0.01 g/L h were achieved. Under a high agitation of 700 rpm, the BC yield increased to 1.13 g/L with a productivity of 0.02 g/L h. The volumetric oxygen transfer coefficients, which are closely tied to BC production, are influenced by the agitation speed. However, given that this approach requires a significant amount of energy the airlift bioreactor may represent an excellent alternative for this fermentation process.

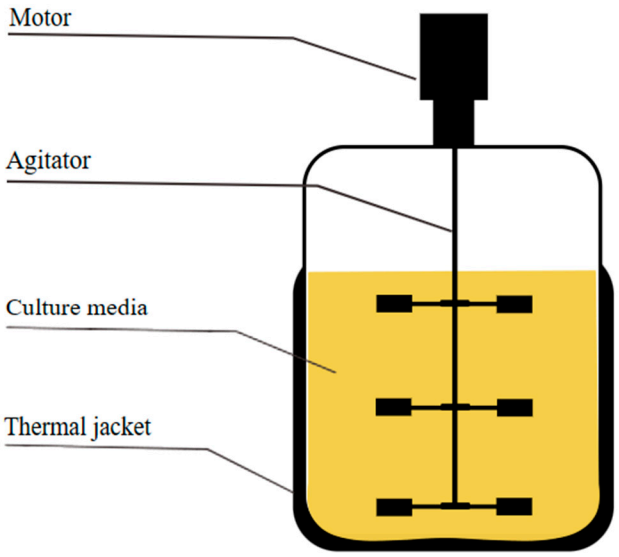

Figure 3. Stirred tank bioreactor design.

2.2.2. Airlift Bioreactor

An airlift reactor generates lower shear stress than stirred tank bioreactors, and it guarantees less energy consumption (Figure 4). However, it exhibits a lower oxygen transfer rate, which is a critical factor for BC production. In this regard, Wu et al. developed a modified airlift reactor, in which the wire mesh tube was substituted with net plates to produce BC membranes [36]. These are more suitable for biomedical applications than BC pellets. The study found a correlation between the number of plates and dissolved oxygen levels, indicating that the increase in the number of plates enhanced the oxygen transfer rate [36]. The characteristics of BC could be controlled by adjusting the number of net plates. For instance, BC exhibited an increased water holding capacity and achieved the highest Young's modulus when six net plates were employed.

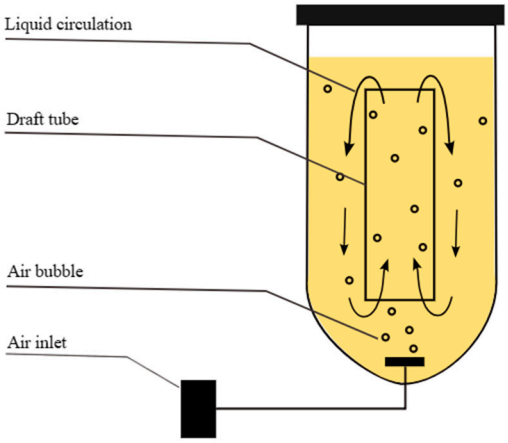

Figure 4. Airlift bioreactor design.

In the same context, Chao and his team [37] enhanced the effectiveness of the airlift reactor by introducing an internal loop. This modification led to obtaining a yield of 10.4 g/L. However, the resulting BC was in a pellet shape, and it exhibited relatively low mechanical strength. In the airlift bioreactor, BC formation typically takes place within the medium itself [37]. Another adapted bubble column bioreactor reported by Choi et al. [38] and Song et al. [39] achieved 5.6 g/L and 5.8 g/L of BC, respectively.

This bioreactor offered low shear stress and a high rate of oxygen supply. However, the BC produced through this method exhibited low mechanical properties, low crystallinity, a low molecular weight, and a low degree of polymerization [38,39].

2.2.3. Rotating Disc Bioreactor

The system comprises multiple flat circular disks, which are installed on a central rotating shaft with an inlet for inoculation (Figure 5). The cells attach to the surface of the disks and synthesize a pellicle on the disk surface [34,40]. In the rotating disc bioreactor, circular discs maintain their rotation, while their surfaces alternatively meet both air and liquid media. This process results in BC with a homogenous structure; the overall yield is not substantially higher than that obtained through static culture methods. A wide range of solid materials and fibers can be introduced directly into the growth medium, where they integrate into the cellulose structure to enhance the characteristics of both BC and BC-based composite materials [41,42].

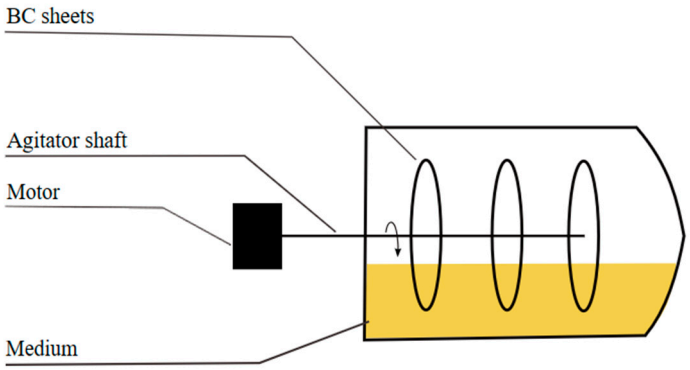

Figure 5. Rotating disk bioreactor design.

Typically, bioreactors were designed with half of the disk's surface submerged in the medium while the other half was exposed to the atmosphere. Consequently, they require a new inoculation after each BC collection. The use of plastic composites to support a rotating disk bioreactor (PCS-RDB) allowed full immersion into the culture media, providing a rough surface for bacterial attachment, resulting in a high BC yield. Notably, the PCS-RDB can continuously produce BC without the need for re-inoculation, maintaining its productivity for at least five cycles. This bioreactor can thus produce BC in a semi-continuous manner and can be easily scaled up for commercial production [3].

3. BC Modification and Functionalization

Cellulose is one of the most extensively used natural polymers; in particular, BC obtained using the previously described fermentative processes represents an example product for sustainable production and consumption. BC has the same molecular structure as that of plant cellulose but compared to the plant one; however, the space required for fermentation is also smaller than that for plant growth. BC possesses unique properties including high purity and crystallinity (60–90%), high water capacity (about 100 times their own weight), ultrafine and porous 3D network, remarkable tensile strength, non-allergenicity, transparency, and moldability [43–45].

Its three-dimensional nanostructure, hydrophilicity, biodegradability, and interesting mechanical properties consisting of a conformability high elasticity, low density, high degree of polymerization, high specific surface area, good permeability, high porosity and water content, and high mechanical strength in a wet state [46,47] allow its biotechnological applications in various fields.

Owing to its unique properties, BC could be modified through different approaches to fit several applications. In the food industry, it serves as a novel biological material and edible packaging. In the medical field, BC finds use as a wound dressing material, artificial

skin, vascular grafts, scaffolds for tissue engineering, artificial blood vessels, medical pads, and dental implants [48]. Moreover, BC has industrial applications, such as acting as sponges to collect leaking oil and as materials for absorbing toxins. It also finds use in optoelectronic applications, including liquid crystal displays [49].

Anyway, although BC shows significant basic properties that make it suitable for a wide range of applications, several approaches have been used to adapt and improve its physicochemical and functional properties such as changes in porosity, crystallinity, chemical structure, and functions. The resulting BC polymers have been evaluated for their structural and property characteristics, including macroscopic appearance, microstructure, crystallinity level, chemical composition, polymerization degree, purity, water retention capacity, porosity, and thermogravimetric properties [50].

Likewise, also in terms of modification and functionalization, BC is more efficient in comparison to the plant one.

Modifications are mainly performed during the fermentation process (in situ) or after the BC is formed (ex situ). In situ modifications are conducted by varying the culture media, carbon source, or adding other materials during the fermentation process, while ex situ modifications are carried out after the BC is formed, using chemical or physical treatments [51,52].

3.1. BC In Situ Modification

In situ modifications involve the addition of reinforcement materials like chitosan, gelatin, poly-3-hydroxybutyrate, nanomaterials, clays, and silica to the bacterial culture medium, usually at the beginning of BC production. During the process, the materials were incorporated within BC fibrils, leading to an improvement in physical and mechanical properties, with the potential for introducing new functionalities [51].

In situ modification is an easy and fast-handling process and permits the homogeneous distribution of modified materials, changing the original BC biophysical features, conferring unique properties. The advantage of this process is the encapsulation of materials that become part of the fibrils, and that enhance BC by modifying especially their physical–mechanical properties [50]. Despite the many advantages, there are several limitations related to the use of the in situ modification approach. Some supplementation components in the culture media can inhibit BC synthesis; some of them may have antibacterial activity or may be insoluble in culture media [51–53]. Other characteristics which did not allow the use of a supplement for in situ modification may be the high surface tension towards hydrophobic materials, the lack of control of the BC nanofibers structure, and the introduction of particles with low suspension stability within BC growing media [50].

The in situ modification of BC has been widely used, although strict microbial fermentation conditions limit the supplementation of several external additives. Moreover, interactions among the added compound, the BC fiber growth, and BC nanofibers structure controls still need to be addressed [54]. The compounds, added into culture media during the BC synthesis, can interfere with nanofibers during their assembly and produce novel BC-based materials with new properties. The exogenic material, interacting with the BC–OH moieties, become a part of the nanofibers and form novel hydrogen bonds [55].

Among the supplemented material added into the culture medium to modify the intrinsic properties of BC, water-soluble, hydrophilic, and water-dispersible materials are the most used but the addition of hydrophobic materials has also been reported.

Water-soluble materials, including methylcellulose and carboxymethyl cellulose (CMC), affect the pore size, crystallinity, and thermal stability of BC [56,57].

Paraffin microspheres [58] and hydroxyapatite nanoparticles (HA—a5(PO4)3(OH)) [59] were added in BC culture medium to produce BC scaffolds with enhanced porosities for bone regeneration. In addition, several in situ modifications have been used to produce wound dressings and temporary artificial skin application tools, including potato starch [60], cotton gauze [61], and aloe vera [62] composites.

Butchosa et al. [57] included in the cell culture during BC production deacetylated chitin nanocrystals (D-ChNCs) to add into BC antibacterial properties. The modification resulted in a homogenous distribution of D-ChNCs in the BC matrix, which showed a tensile strength of 377–449 MPa. The authors performed the same modification using also an ex situ modification approach. Gea et al. used BC medium supplement PVOH [63], which does not affect the crystal structure and dimension but modifies BC fibers orientation resulting in a significant higher elasticity, without changing the breaking strength relative to unmodified BC [63].

Among the water-insoluble polymers, cellulose microfibers, sisal fibers [64], poly-3-hydroxybutyrate [65], or polycaprolactone [66] have been used.

For the in situ modification, various alternative techniques have been used, such as the encapsulation of the bacteria in gelatin-based microspheres for the production of BC microspheres, which have potential applications in biochemical engineering, in cell delivery systems. as well as food packaging [67]; the use of an electric field to control the BC fibers orientation; [68,69] or the bacterial cultivation inside a polydimethylsiloxane tube [70] or in a complex-shaped 3D printed architecture [71].

3.2. BC Ex Situ Modification

Ex situ modification of BC is carried out after the BC has been formed, and it is produced by either physical or chemical methods [54].

The physical ex situ modification can be performed through physical absorption. In this application, the porous BC matrix is filled with solutions or particle suspensions and, thanks to the hydroxyl groups of cellulose chains, strong hydrogen bonding between the BC molecules and absorbed molecules are often formed [25,59,72].

Regarding chemical ex situ modification, chemical reagents are used to modify the chemical composition of BC. For example, it can first be phosphorylated and then modified by graft copolymerization or crosslinking reactions [73].

Among the various chemical reagents commonly used to modify the chemical structure of BC and to add additional functionalities, NaOH and H_2SO_4.are the most used.

While 2% NaOH solution is normally used for the purification of BC to wash out by-products and cell debris, higher concentrations of NaOH can convert cellulose type I to cellulose type II and 6% NaOH solution was often reported for structural changes [74,75].

H_2SO_4 promotes the fragmentation of BC and the formation of nanocrystals.

H_2SO_4, hydrolyzing the amorphous region and leaving the acid-resistant crystalline region, generates a negative charge by sulfonation of the OH group on the surface of cellulose, which reduce the nanocrystals' thermal stability [76].

Although the chemical modification of BC is not very studied, the most common chemical modification of BC is oxidation and others include acetylation [77], benzoylation [78], succinylation [79], and phosphorylation [80].

A restricted number of solvents, including N, Ndimethylacetamide/LiCl, dimethyl sulfoxide/tetrabutylammonium fluoride, N-methylmorpholine-N-oxides, and ionic liquids have been used for the chemical modification of cellulose-based materials [8,81–83]. However, these chemical agents make the obtained product unsafe and complex for the discharge treatment resulting in negative and dangerous effects on the environment [82].

Furthermore, BC is often used to produce nano-sized particles, cellulose nanocrystals (CNCs), with high crystallinity through acidic or enzymatic and ionic liquid [84].

Generally, the isolation of bacterial cellulose nanocrystals (BCNC) from BC is based on acid hydrolysis. Briefly, the amorphous regions are hydrolyzed. The hydrolytic cleavage of their glycosidic bonds liberates the individual crystals from the crystalline regions with high acid resistance [84,85].

Among the various materials used for ex-situ modification, natural plasticizers play a key role to improve the flexibility and processability of polymers.

These materials can enter into the polymer structure acting as a lubricant. It decreases the frictional forces between the chains and break polymer–polymer interactions, such as hydrogen bonds or van der Waals forces, and form new polymer-plasticizer bonds [52].

Among water soluble plasticizers, glycerol [52,86], poly(ethylene glicol) [87,88] as well as chitosan [86], which are usually incorporated into the structure of the BC by dipping, gave to the BC structure both a plasticizer and reinforcing effects. In addition, thanks to the PVOH effect the transparency and visual appearance of the films was increased.

BC nanofibers were incorporated in the starch plasticized with glycerol via a solution impregnation and applied as biodegradable reinforcement [89]. The immersion of BC in other reinforcing agents such as gelatin and enzymatically modified gelatin promoted an enhancement in the rehydration abilities properties of pure BC. Immersion of BC in polycaprolactone [90] and poly(3-hydroxybutyrate) [91,92] resulted in transparent membrane [90] with a denser structure and improved mechanical strength [92] (Figure 6).

Barud et al. [93] used a physical ex situ modification method by soaking BC membranes into different silk fibroin solutions obtaining a well inter-connected porous network structure with improved cell permissiveness in comparison with pure BC for medical applications.

In other studies, benzalkonium chloride and polyvinyl alcohol (PVA) with potassium sorbate were absorbed into BC film. The obtained BC was characterized by an antimicrobial [94,95].

Ex situ modification has been used to incorporate various additives in BC matrix, such as antimicrobial and antioxidant agents for the production of active packaging usable in the food industry.

Among the antibacterial agents that have been successfully used, there are polylysine [96,97] sodium alginate, silver sulfadiazine (AgSD) [46,98], and silver nanoparticles (AgNPs) [99].

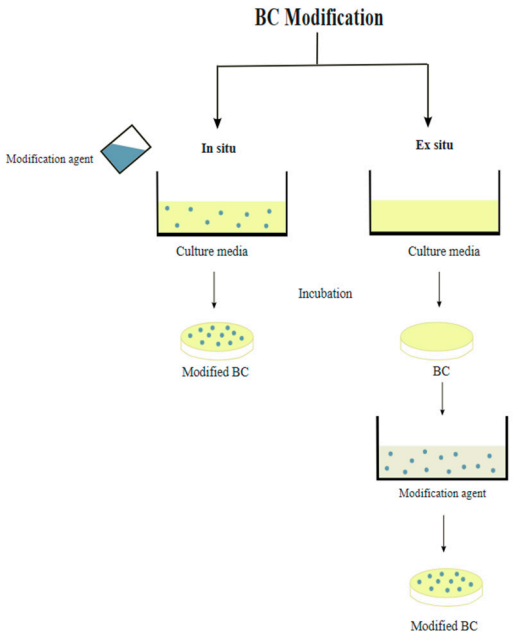

Figure 6. In situ and ex situ BC modifications.

4. Biotechnological Application of Modified Bacterial Cellulose

The vast literature reports on the numerous biological applications of BC.

Today, the innovative strategy used for the modification initiates new perspectives for future applications of this material. In the following paragraphs, the main application of modified BC in the food and biomedical fields are reported.

4.1. BC and Food Industry

Being a dietary fiber, the USA Food and Drug Administration has classified BC as generally recognized as safe (GRAS) since 1992 [100,101].

Several animals and in vitro studies clearly suggest that BC is not genotoxic, carcinogenic, a tumor promoter, pyrogenic, or a developmental or reproductive poisonous substance. Furthermore, available evidence makes clear that BC is non-toxic on ingestion, skin contact, and on inhalation, and that it does not cause any other inflammatory or oxidative stress responses at the cellular level [101,102].

In the food sector, given its suspending, water retention, thickening, and emulsifying stability properties, BC has many potential applications ranging from its use as a raw food material and supplemental ingredients to its application as a packing biopolymer delivery system, as well as enzyme and cell immobilizers.

4.1.1. BC as Food Component

Many studies report the potential of BC, as well as generally of cellulose, to enhance the excretion of total lipids, cholesterol, and bile acids in feces [101]. In a hamster model, the hypocholesterolemic and hypolipidemic properties of BC were significantly higher than those of plant cellulose [101]. Using a mice model, during a high-fat diet the use of BC as dietary fiber supplementation was reported to inhibit obesity [103]. Furthermore, BC could efficiently increase the length of villus cells, and the thickness of colonic mucosa and muscle, alleviating constipation and regulating short-chain fatty acids and gut microbiota [104,105].

The first commercial BC product was the Nata de coco, originated from the Philippines, made through the fermentation of coconut water with the acetic bacteria *K. xylinus*. Nata de coco is generally cut into cubes and pickled into different flavors. Nata de coco represents a good source for dietary fibers, mainly known for their potential in reducing the risk of chronic diseases such as diabetes, obesity, and cardiovascular disease, etc. [106]. BC has also been widely used as a raw material in the manufacturing of Kombucha, as well as other similar products [107,108]. The latter, thanks to its low calories together with the high fiber content and antioxidant ingredients, attenuates lipid accumulation and protects the liver from damage promoting liver restoration in mice [109].

BC was also used as a filling material for the fortification of fragile food hydrogels [110], as tool for improving the mouthfeel of pasty foods, preventing the cocoa precipitation in a chocolate drink, and increasing the strength of tofu [111].

Likewise, BC supplementation can give modified food greater heat stability as the viscosity remained unchanged after heat sterilization and had better functional characteristics [111].

BC and BCNC, thanks to their amphiphilic properties due to their hydrophilic nature given by the high density of hydroxyl groups on their surfaces [112] and hydrophobic properties given by the interactions resulting from the crystalline organization and extensive hydrogen bonding of chains, [113–115], have been applied to stabilize surfactant-free Pickering emulsions [114,115].

BC pellets obtained by agitated fermentation have been applied as new suspension agents thanks to their characterized excellent dispersion stability and low viscosity. Their unique nanofiber-woven 3D structure allowed the suspension stability to be maintained within various pH values, salt concentrations, and high temperatures up to 80 °C [116,117].

Some studies investigated the potential of BC as an ice cream ingredient, in terms of structure/texture modifier, emulsion and foam stabilizer, and fat replacer [110,118–121] However, no study has investigated all these aspects together [122].

BC has been used as a fat replacer. Although the complete replacing of the fat in meatballs with BC used at 20% negatively affected the product acceptance, the use of half the added fat content together with 10% BC resulted in similar sensory properties and shelf stability to control regular meatballs [123].

The encapsulation of probiotics using biocompatible and antibacterial materials such as BC has been proven to increase the stability and the shelf life of these products.

Fijałkowski et al. confirmed the protective effect of BC over probiotic *Lactobacillus* spp. from the harsh conditions of gastric acid and bile salts [124]. Similarly, Oliveira-Alcântara et al. produced BC/cashew gum films incorporating probiotic Bacillus coagulans and prebiotic fructooligosaccharides, resulting in excellent storage stability for the probiotics [125].

In addition, BC has been used for the immobilization of enzymes and cells. This technology is widely used in the food fermentation industry. Li et al. reported the proficient use of BC for the immobilization of enzymes, including horse radish peroxidase and laccase for biosensors and bioanalysis [126–128] as well as for yeast [129] for cycling batch fermentation, promoting a reduction of the cost due to the inoculum preparation within the wine manufacture process.

4.1.2. BC and Food Packaging

To date, the global market is oriented to the exploration of new food packaging which may be safe and ecofriendly [130].

Generally, many polymers, including PHA [131], oil seed cake [132], alginate [132], chitosan [133], cellulose, carrageenan [134], or pectin [135] and their derivatives are used as biofilm-forming materials [122,134–136].

Among those, BC, being food-grade, could be used as a matrix for films and coatings, which may be also edible.

This material, which represents a protective tool for packed food, is also an interesting delivery system for bioactive compounds, which may exert a positive function not only for the packed food but also for human health.

BC biobased packaging can be synthesized into two main forms, films and coating. Films are produced using the conventional lab casting, where a solvent is poured into a dispersion that forms a film and then is evaporated on a suitable surface. For coating, electrospinning is widely used; it involves the spinning of polymer solutions using a high-voltage electrical field applied to needle tips connected to syringes [122].

Recent studies report the BC films and coatings capacity of reducing moisture loss and the absorbed oil by fried foods [137], of controlling the water, O_2, and CO_2 permeability and of increasing the mechanical strength of the food packing [138].

To date, the most used BC-based packaging applies the mixture of the natural characteristics of the BNC matrix with biological and physicochemical properties of different reinforcing compounds. By using this approach, the BC characteristics are improved and novel microbial cellulose films with specific characteristics necessary for specific applications can be obtained. The composite materials consist of a BNC matrix acting as a scaffold and reinforcing bioactive compounds, which impart their specific physicochemical and biological properties to the obtained packaging material. Generally, the synthesis of BC composites is possible towards its functionalization, which allows packaging materials to be obtained with improved or new functional features compared with the native material, in terms of physicochemical properties (including mechanical, thermal, chemical, and surface features, rheological characteristics and degradation abilities) or functional characteristics (including the compounds that may preserve, extend, or monitor foodstuff quality and shelf life) [122].

A wide range of additives, including antimicrobial and antioxidative agents, nutrients, plasticizers, stabilizers, and oxygen scavengers, may be applied to modify BC and to develop active packaging. Furthermore, several molecules and additives have been used to produce intelligent packaging material including freshness indicators (pH indicators, humidity sensors, or conductometric nano biosensor) [139].

Thanks to its gelling properties and high specific surface area [140], BC has excellent potential in the delivery of bioactive agents. Several studies report BC loading or delivery capacity for various bioactive agents [139]. A wide range of additives, including antimicrobial and antioxidative agents, nutrients, plasticizers, stabilizers, and oxygen scavengers, may be applied to modify BC and to develop active packaging. Furthermore, several molecules and additives have been used to produce intelligent packaging material

including freshness indicators (pH indicators, humidity sensors, or conductometric nano biosensor [139].

Mono- and multi-layer films obtained supplementing BC with asorbic acid possess promising antimicrobial properties [141,142]. Jebel and Almasi used multi-layer films containing an antimicrobial layer between two outer layers to control the antimicrobial agent ZnO nanoparticles' release rate. In addition to the antibacterial activity, the ZnO nanoparticles improved the tensile strength and decreased the water vapor permeability of the films [143]. Malherios et al. reported the antimicrobial activity against *Listeria monocytogenes* of BC membranes produced by *Gluconacetobacter xylinus* in which bacteriocins isolated from *Lactobacillus sakei* were immobilized by physical entrapment [144]. Santos obtained antimicrobial BC membranes by immobilizing nisin into BC sheets [145]. Zhu et al. produced active sausage casings by impregnating BC tubes with +-polylysine (+-PL). The film showed good tensile barrier properties, good thermal stability, and antibacterial activity, promoting an extension of the shelf life of sausages [96].

Moreover, several antioxidant or oxygen scavenger agents, including spherical nanoparticles of flavonoid silymarin (SMN) and zein [146], herbal extract [147], or laccase [148] were used to prepare functional BC films for food packaging.

Although impregnation has not been widely used to incorporate fortification agents into BC membranes, Ul-Islam et al. by using this approach produced BC nanocomposites with montmorillonite, which were characterized by an increased tensile strength and enhanced thermal stability [149].

In addition to the impregnation method, many BC applications for films and coatings employ disassembled BC, which may be found in suspension or powder form. By using this approach, the coatings are directly applied on food surfaces by using a conventional lab casting [150] or the electrospinning technique, which consist of a high voltage which is applied to create an electrically charged jet of a polymer solution, producing the polymer fibers [151].

BC has been also used in combination with other bio polymers as a reinforcing agent. BCNC have been applied as a reinforcement tool in chitosan matrix, in which thanks to the addition of silver nanoparticles (AgNPs), barrier and tensile properties as well as the antibacterial activity were increased [152] in thermoplastic corn starch films [153] and nanocomposite PVA [154].

Several studies report the application of in situ techniques to produce nanocomposite films. Pectin, gelatin, or carboxymethylcellulose (CMC) have been added in the culture media to enhanced mechanical properties of BC. These polysaccharides, binding to BC, change its network structure water-binding capacity [155]. Gea et al. [63], comparing the in situ process of growing BC in a medium with 5% polyvinyl alcohol (PVA) with the impregnation technique, observed that the in situ method did not change BC fibril arrangement, improved transparency and elongation, and maintained the other tensile properties, whereas the impregnation process caused the formation of aggregates which decreased the tensile properties of the films [63]. Fontes et al. [156] using an in situ approach produced BC with the addition of carboxymethylcellulose (CM). The presence of CMC increased the viscosity of the medium and decreased the porosity of the resulting material.

4.2. BC and Biomedical Applications

Given its characteristics, BC represents a non-immunogenic interesting material [157] for various biomedical applications including wound dressing, antibacterial material, scaffold for tissue engineering, blood vessels, dental implants, bone, and drug delivery [140,158–160].

4.2.1. BC and Wound Dressings

BC given its wide number of properties including its flexibility, ability to retain water, great mechanical strength, and permeability to gas and liquids and compatibility with living tissues represent a good natural hydrogel for wound healing applications [161,162].

In addition to its use for replacing the burnt skin or for different wounds such as pressure sores, skin grafts, and diabetic wounds, its derivatives and composites have also been employed for wound healing [163,164]. BC/gelatin [165] and BC/collagen [166] nanocomposite, BC/kaolin composites [167], BC/dextran hydrogel, and BC/AA hydrogel prepared and loaded with human dermal fibroblast and human epidermal keratinocytes cells [168] show important functional properties as wound dressing materials.

Another important approach used to improve BC characteristics for its application as a wound dressing material is the addition of antibacterial agents. Several antibiotic molecules [169–171] and nanoparticles [172–177] have been successfully used.

In this context, Jalili Tabaii and Emtiazi, incorporated silver nanoparticles into bacterial cellulose (BC) by immersing it in an AgNO3 solution. They observed a significant 100% increase in antimicrobial effectiveness against *Staphylococcus aureus* spp. and *Escherichia coli* spp. [178]. Copper ions were also found to cause cell membrane damage [179]. When copper nanoparticles were integrated into BC, they exhibited prolonged bactericidal efficacy against *S. aureus* spp. and *E. coli* spp. for up to 90 days [180].

Additionally, essential oils represent a good source for antimicrobial agents. Dudek-Wicher et al. demonstrated anti-biofilm efficacy (80–100%) of three essential oils: tea tree, geranium, and frankincense incorporated into bacterial cellulose (BC) against both Gram-positive (*S. aureus* spp., *Enterococcus faecalis* spp.) and Gram-negative (*Klebsiella pneumoniae* spp., *P. aeruginosa* spp., *E. coli* spp.) strains, as well as one fungal strain (*C. albicans* spp.) [162]. Bacterial cellulose treated with thymol, an essential oil component, was effectively used for the healing of third-degree burn wounds due to its minimal cytotoxicity to fibroblasts and enhanced cell viability [181].

Furthermore, antibiotics including ceftriaxone, gentamycin, vancomycin, ciprofloxacin, and tetracycline were used in wound treatments. Previously, compresses soaked in antibiotic solutions were employed for treating wound infections [182]. However, this technique led to an increase in the prevalence of resistant strains due to challenges in determining the drug concentration retained and controlling the rate of drug release onto the infected skin. Incorporating antibiotics into bacterial cellulose addresses these issues by allowing the precise measurement of drug quantity and controlled release rates from the BC membrane [183].

BC with gentamycin at a concentration of 2 g/L was developed by Junka et al., equivalent to the commercially available collagen gentamycin sponge. BC exhibited a slower release of gentamycin than the collagen sponge, maintaining effectiveness against *S. aureus* spp. and inhibiting biofilm development [184].

In addition to metals, essential oils, and antibiotics, polymers were introduced into the BC structure to enhance its properties as a wound dressing material; among them, collagen [185,186], gelatin [171,187,188], chitosan [55,189], silk sericin [190], and polyethylene glycol [88] are the most studied. Collagen, widely used in biomedical applications, closely resembles living tissues, promoting cell adhesion and proliferation [191]. A composite material of BC/collagen type I developed by Wiegand et al. shows a decrease in the secretion of proteases, interleukins, and reactive oxygen species, indicating its potential as an effective dressing for chronic and burn wounds [192].

The second polymer is known for its antimicrobial activity, mechanical stability properties, biodegradability, and biocompatibility [189]). Chitosan and chitooligosaccharide were separately incorporated into bacterial cellulose (BC), resulting in composites with reduced porosity compared to native BC. Both composites demonstrated excellent antibacterial activity, with BC/chitosan inhibiting *S. aureus* and *E. coli* by 99.99%, and BC/chitooligosaccharide inhibiting *S. aureus* by 99.64% and *E. coli* by 90.56% [191].

Oliveira et al. [193] reported the potential of BC dressings for the treatment of burn wounds in the ears, face, joint area, hands, feet, and genitals. Czaja et al. [194] reported the positive effect on burn wound infection promoted by the dressing material with antiseptics, including silver nitrate, hydroxyquinoline sulphate or boric acid. Other clinical trials

showed good clinical results obtained by using BC as a dressing in a patient with thermal burns [195].

4.2.2. BC as Drug Delivery Systems

BC-based materials have been largely used as carriers for various drug deliveries [196] including topical and transdermal delivery [197–199], dental drug delivery [200,201], oral and controlled release delivery for different antibiotics, proteins, as well as hydrophilic and hydrophobic drugs [202–205]. The more used method to load drugs into BC is by immersion of the polymer into the drug suspension.

BC-based polymers for delivery applications have been prepared by the in situ modification or by various methods such as absorption, pointpipetting, and spraying.

Yet, the majority of the studies revealed that the in vitro and in vivo tests for the evaluation of the drug release is still partial [74,206].

A study explored the diffusion capabilities of BC membranes using tetracycline-loaded samples, revealing faster drug movement in non-irradiated BC compared to irradiated BC. Therefore, BC membranes could serve as a model for drug adsorption. Moreover, bacterial cellulose can be combined with a conducting polymer like polyaniline, known for its electroconductivity, to serve as an electrically stimulated drug delivery device. The composite was synthesized by polymerizing aniline on one side of the BC membrane, forming a product that functions as a super capacitor [207]. Another study suggests the efficiency of using BC as a delivery system for optimizing the delivery and release of checkpoint-blocking antibodies, which was considered as a safe approach for cancer treatment [208].

BC blended with hydroxyapatite, bone marrow mesenchymal stem cells (BMSC), growth factors, extracellular matrix protein, and estrogen, which represent some bone repair components have been used as a promising biocompatible material for bone repair [209]. These materials figured to be promising biocompatible materials usable in bone tissue engineering applications thanks to their high mechanical properties, which for some BC composites are similar to cortical and cancellous bones [210], easy to fabricate, and characterized by microporosity [211].

Furthermore, BC has been used for the reparation of hyaline cartilage which represent the most abundant cartilage covering the joint surface elastic cartilage which is present in the outer ear, epiglottis and larynx, and auricular deformity and nose skew [211].

4.2.3. BC in Skin and Bone Tissue Engineering Applications

In tissue engineering, BC enters in scaffold designs to facilitate tissue regeneration and repair. Selecting a suitable material is one of the most fundamental challenges in this field [212–215]. The main categories of materials used in tissue engineering are inorganic materials (tricalcium phosphate (TCP), hydroxyapatite (HA), bioactive glass, and metals), synthetic polymers (poly-l-lactic acid (PLLA), polyglycolic acid (PGA)), and natural polymers (collagen, gelatin, silk, cellulose, dextran, and chitin) [214–216]. The main objective in tissue engineering is to provide scaffolds that boost cell attachment, proliferation, differentiation, spreading, and migration [211,217,218].

Several research groups have shown interest in utilizing bacterial cellulose (BC) for creating biocomposite bone scaffolds. Wan et al. were among the pioneers, finding that a three-dimensional network of hydroxyapatite–BC nanocomposites closely resembled the structure of the native bone [216], while Cakmak et al. have introduced a novel composite scaffold composed of polycaprolactone (PCL), gelatin (Gel), bacterial cellulose (BC), and hydroxyapatite (HA) using 3D-printing technology. This scaffold was designed to enhance cell proliferation and attachment while improving the mechanical properties of BC through the incorporation of PCL and gel [219]. Moreover, Maia et al. developed a hybrid scaffold of BC/HA that promotes the internal migration, proliferation, and differentiation of bone-forming cells and angiogenesis more effectively than BC alone, due to the biological activity of HA [220]. BC has been studied not only for soft tissue scaffolds but also for drug-eluting bone cements. Adding BC to traditional bone cement with vancomycin hydrochloride

and gentamicin sulfate improved sustained drug release compared to standard cements, addressing issues of limited release and reduced mechanical strength [221].

4.2.4. BC as Artificial Blood Vessels

The distinctive properties of BC, and its resistance, due to the fact that no enzyme or strong acid in the human body can decompose it [222], make this biomaterial suitabile for replacing the artificial vein which is usually derived from synthetic materials such as ePTFE (expanded polytetrafuoroethylene) [223], PGA [224], and PLLA (poly(l-lactic acid)) [225].

Therefore, various methods have been used to produce synthetic vascular grafts using bacterial cellulose (BC) polymers including tubular BC production, surface modification, and grafting anticoagulant factors on the inner surface of a vascular graft [226].

Zang et al. [222] demonstrated, through cytotoxicity and cell compatibility assays, that BC promotes the attachment, proliferation, and growth of endothelial cells, smooth muscle cells and fbroblasts. In addition, the BC tube biocompatibility test shows the absence of obvious signs of inflammation around the implants and complete endothelialisation with a confluent endothelial layer.

Klemm et al. synthesized BC tubes using cylindrical glass molds, indicating the potential use of BC in replacing atherosclerotic coronaries as artificial vessels [227]. Bodin et al. found that BC tubes properties can be modulated by the fermentation techniques and culture conditions [228].

One of the main challenges in vascular tissue engineering is the material complications at the blood–material interface [229], including nonspecific adhesion of proteins and cells, endothelial cell removal, and the occurrence of restenosis and thrombus formation. To address these drawbacks, surface modifications of vascular grafts using hydrophilic polyethylene glycol, zwitterionic polymers, bioactive molecules (such as heparin, fibronectin, and Von Willebrand factor), and active peptides (including RGD, CAG, REDV, and YIGSR) has emerged [230,231]. In this context, Yizao Wan et al. developed a hybrid nanofiber by co-synthesizing heparin and bacterial cellulose (Hep/BC) [232]. The results showed no structural difference between BC/Hep nanofibers and BC nanofibers. Further evaluations confirmed distinct anticoagulant sulfate groups in BC/Hep nanofibers and significant differences in their crystallographic structure compared to BC nanofibers [233]. Furthermore, Klemm et al. conducted the first vascular implantation in small animals, utilizing a bacterial-synthesized cellulose (BASYC) tube with an inner diameter of 1 mm and a length of approximately 5 mm. The results revealed no stimulation of the immune system after four weeks of implantation into the right carotid artery [227].

Another study revealed the development of 3D porous bacterial cellulose/gelatin (BC/Gel) scaffolds loaded with VEGF were modified with heparin, referred to as V-BC/Gel/H, aiming to enhance blood vessel formation by minimizing the initial burst release of VEGF. The findings indicated that the heparinized BC/Gel scaffold significantly reduced the initial burst release and demonstrated sustained delivery of VEGF. According to these studies, BC seems to be a promising material for the development of devices with a wide range of applications in the field of biomedicine [234]. Regardless of its versatile properties, BC biopolymer should be evaluated in terms of environmental impact for a sustainable economy.

5. Evaluation of the Environmental Impacts of Bacterial Cellulose Production

Bacterial cellulose (BC) is a promising material with a wide range of applications. While efforts to industrialize its production are increasing, a more comprehensive understanding of the environmental impact associated with the BC production process is still needed. The life cycle assessment (LCA) is a method used to evaluate the environmental impact of the entire production process. It involves analyzing the inputs, outputs, and associated effects. It helps in comparing recycled products with new materials, determining the environmental viability of waste reduction methods, and evaluating energy consumption, raw materials, and waste in each production step. Applied to bacterial cellulose production,

LCA highlights its environmental implications, affirming its potential as a sustainable material [235,236]. Bacterial cellulose (BC) production offers environmental benefits compared to traditional cellulose production processes. BC is a biodegradable biomaterial that serves as an eco-friendly alternative to synthetic fibers and microplastics. It can help reduce water pollution by utilizing alternative substrates from the agro industry, beverage industry, and sugar industry. However, BC production requires significant amounts of water, energy, and other resources. Furthermore, the fermentation process for BC production consumes energy, which can contribute to greenhouse gas emissions [107,237,238].

BC synthesis is susceptible to contamination from bacteria or fungi. To minimize this risk, production facilities can adopt stringent hygiene measures, employ sterile equipment, and monitor the production process. On the other hand, ethical concerns arise with the utilization of bacterial cellulose, particularly regarding the use of genetically modified bacteria. To address this, companies can opt for non-GMO bacteria or explore sustainable production approaches that eliminate the need for genetically modified organisms [239].

In conclusion, while BC production offers some environmental benefits compared to traditional processes, it also has negative impacts on resource consumption and energy usage. To minimize these impacts, it is essential to optimize production conditions, use alternative substrates, and implement strict hygiene protocols.

6. Conclusions and Future Perspectives

In summary, cellulose secreted by bacteria has high purity and crystallinity and is a sustainable and highly competitive alternative to plant-derived cellulose nanofibers.

This material has a unique structure with a three-dimensional lattice network and, being free charge, has brilliant mechanical properties, including a high water-holding ability, excellent gas permeability, suspension stability, low viscosity, and excellent tolerance to acids, salts, ethanol as well as biocompatible, renewable, and biodegradable. In addition, the modifications and functionalization of BC generate cellulose polymers with different morphologies and physiochemical properties. Commercial applications of BC have spread to various fields, such as the food and biomedical industries. However, despite the advantages of BC, its current applications are still limited, and further attempts should be made to promote new BC uses.

Structurally, BC has an exclusive structure with a 3D reticulated network, and it is uncharged, which donates it extra advantages such as mechanical properties, high water-holding capability, gas permeability, suspension stability, low viscosity, and tolerance to acid, salt, and ethanol. However, numerous concerns must be evaluated and improved for BC industrial production and application development. Among the main strategies for static fermentation the isolation of a high yield of BC-producing strains can be annumerated, the enhancement of new culture media or the construction of innovative fermentation reactors, together with the utilization of automated equipment. For which, concern for the agitated fermentation should be considered as the non-cellulose mutation of bacteria. Although this fermentation system can produce BC at a large scale, the production efficiency and the yield of BC must be improved.

Author Contributions: Conceptualization, T.G.; writing—original draft preparation, I.S., C.G. and T.G.; writing—review and editing, T.G. and I.S.; supervision, T.G. and S.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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