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Chapter

Bacterial Cellulose: Biosynthesis and Applications

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Abstract

Bacterial cellulose (BC) or microbial cellulose (MC) was considered a bioactive material characterized by high absorbed water, high crystalline, high tensile strength, and biodegradability. However, bacterial cellulose has wide applications, such as biomedical, textile, paper industries, food, drug release, and cosmetic applications. So the microbial cellulose production from Acetobacter *xylinum* from different wastes such as carbon and nitrogen sources, for example, pineapple peel juice, sugar cane juice, dry olive mill residue, waste beer yeast, and wheat thin stillage, are characterized by FTIR, XRD, SEM, and TEM. The product yield of bacterial cellulose is affected by different factors such as the concentration of sugar in carbon source, temperature and time of incubator of the strain, and pH of media. So, it must be studied with the enzymatic pathway procedure.

Keywords: bacterial cellulose, microbial cellulose, synthesis, biomedical, applications

1. Introduction

Natural polymer cellulose is synthesized by plants, as well as fungi, algae, and bacteria, while the structure of a bacterial cell has rarely a ratio to cellulose. The cell wall of plants and seed shells, wood, contains the main component is cellulose macromolecules that are constructed from an unbranched D-glucose chain. However, the glucose units are connected with each other by a 1,4-β-glycosidic linkage in a linear form. The length of cellulose polymer chains depends on the nature of the producer and accordingly differs among themselves [1]. The main sources of produced cellulose are of four different types: The first is produced from plants, the second method is the preparation of cellulose from various microorganisms, fungi, and algae, and the remaining two are less common: the first is the synthesis from enzyme *in vitro*, beginning with cellobiose fluoride, and the second is synthesis from glucose chemically by opening the ring polymerization of benzylated and pivaloylated derivatives. The cellulose from plants and bacteria is the same molecular form of $(C_6H_{10}O_5)_n$; however, the chemical and physical properties are different (**Figure 1**). Plant cellulose is different from the bacterial cellulose by its low crystallinity, low water absorbing capacity, and ultrathin structure [3, 4].

There are some components that are often found in plant-derived celluloses, including lignin, hemicelluloses, and pectin, but these components are absent from bacterial cellulose (BC), which is highly purified relative to plant cellulose and hence

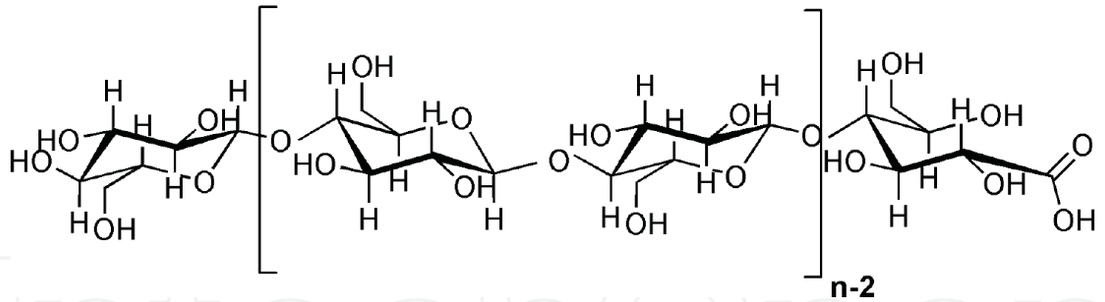


Figure 1.
The 1,4-β glycoside chain of cellulose [2].

requires a low-energy procedure [4]; usually, we use more chemicals in purification of cellulose produced from plants, which is low energy consumption process [5]. Regardless the plant cellulose is constructed from polysaccharide based on glucose units, this is the basic material of plant cell walls, which is utilized as crude materials in paper, pulp, fabric, and textile industry (as 10 hydrogen-bonded chains, each with 500 to 14,000 l,4- β-linked glucoside molecules) [6]. In a cellulose chain, 1, 4-β glycoside bonds are present in order to link the D-glucose pyranose units as a linear polysaccharide **Figure 1**. The length of chain is approximately 0.3 nm wide [7].

Usually, natural polymer must be biodegradable, renewable, and bioactive compound, which is characterized by a high modulus, high mechanical strength, and low density; therefore, during the processing, it is more difficult to damage, and for processing equipment we have some requirements, and cheap raw material [8]. The plant cell wall is used to isolate the cellulose. So, there are different sources of cellulose, including wood, pulp, and cotton. After the long fibers are removed from cotton seed, the short fibers remain. Also, it can produce cellulose from the fibers of plants, the plant that produce the cellulose like bagasse (sugar cane stalks), soybean hulls, oat hulls, rice hulls, corn cobs, wheat straw, bamboo, sugar beet pulp, yarn of jute, ramie, and flax [9].

The natural cellulose polymer has a number of glucose molecules about 10,000 [10]. The cellulose chain includes inter- and intramolecular hydrogen bonds, where the free rotation of ring is hindered and the hydrogen bonds of cellulose chain caused the stiffening of chain, and is insoluble in common solvents. In fact, cellulose is a natural polymer. It has hydrophilic properties and contains two hydroxyl groups; one is secondary and the other hydroxyl is primary. However, due to water adsorption of cellulose, it has these hydroxyl groups in chain [11].

Bacterial cellulose (BC) or microbial cellulose (MC) was considered a bioactive material, which is more characterized by high crystalline, high-absorbed water holding, high tensile strength, and biodegradability. Due to the better aforesaid characteristics of bacterial cellulose, it is supported for many human applications, such as in textile and paper industries, food, drug release, medical fields, and cosmetics.

Compared to the high cost of the commercial culture media, bacterial cellulose (BC) production is more expensive. So, researchers study to change different formulations in the food source of strains such as yeast extract and glucose, to lower the cost of food source, and hence, the cost of the production of BC is reduced.

The high purification of cellulose can be produced from several bacteria. The *Acetobacter xylinum* species is used to produce bacterial cellulose, its nomenclature, the genus *Gluconacetobacter* as *Gluconacetobacter xylinus* [12]. The characterization of bacterial cellulose such as degree of polymerization (Dp) between 2000 and 6000 [13]. The cross-sectional diameter is between 2 and 4 nm [14], and crystallinity is up

Property	Plant cellulose	Bacterial cellulose	References
Fiber width, mm	$1.4–4x10^{-2}$	70–80	[15]
Crystallinity, %	56–65	65–79	[16]
DP	13,000–14,000	2000–6000	[17]
Young's modulus. GPa	Cotton 5.5–13	BC sheet 15–30	[15]
Water content %	Jute-27	BC fiber 120	
	Flax-28	BC crystal 138	
	60	98.5	

Table 1.
Comparison between BC and plant cellulose.

1.	High purity
2.	High degree of crystallinity
3.	Sheet density from 300 to 900 kg/m^3
4.	High tensile strength
5.	High absorbency
6.	High water binding capacity
7.	High elasticity, resilience, and durability
8.	Nontoxicity
9.	Metabolic inertness
10.	Biocompatibility
11.	Susceptibility
12.	Good shape retention
13.	Easy tailoring, physiochemical properties

Table 2.
Characteristics of BC [16].

to 60%. It has excellent shape and strength retention. **Table 1** shows the comparison between plant cellulose and bacterial cellulose BC (**Table 2**) [16].

2. Synthesis of bacterial cellulose from *Gluconacetobacter swingsii* sp.

2.1 Sugar cane juice and pineapple peel juice were used as food culture source

There are a few animals and some number of bacteria, such as *Gluconacetobacter* (named Acetobacter) [18, 19]. This is a strictly aerobic and gram-negative bacterium; at certain conditions, such as incubator (25 to30^{O}C and pH from 3 to 7) [13, 20], the bacterial cellulose production uses carbon sources such as glucose, fructose, sucrose, mannitol [21, 22]). The bacteria take three processes to synthesize bacterial cellulose. In the first process, the polymerization of glucose molecules forms the cellulose chains, where the molecules are linked by β -1,4- glucosidic linkages each. Nearly, 1.5-nm-wide protofibril consists of 10–15 equal parallel chains. Then, in the second step, 2–4-nm-wide protofibrils have been collected to form microfibrils, and, in step three, the microfibril groups are collected into a 20–100-nm-wide ribbon. After the former steps, the pellicle of bacterial cellulose [13, 23] produces a matrix of interwoven ribbons.

Hestrin and Schramm's medium is used for producing bacterial cellulose [24]. The cellulose microfibrils are synthesized in different media.

From homemade vinegar, culture can isolate *Gluconacetobacter* strain, identified by 16S rRNA method [25], as *Gluconacetobacter swingsii sp.* [26], sucrose, 0.23%,

w/v, sugar cane juice (0.008%, w/v, fructose, 8.57% w/v, glucose, 0.066%, w/v, total nitrogen), pineapple peel juice (2.4%, w/v, fructose,2.14%, w/v, glucose, 2.10%, w/v, total nitrogen, sucrose, 0.31%, w/v) were used as culture media for producing bacterial cellulose, and Hestrin-Schramm (HS) medium (0.5%, w/v, peptone, 2%,

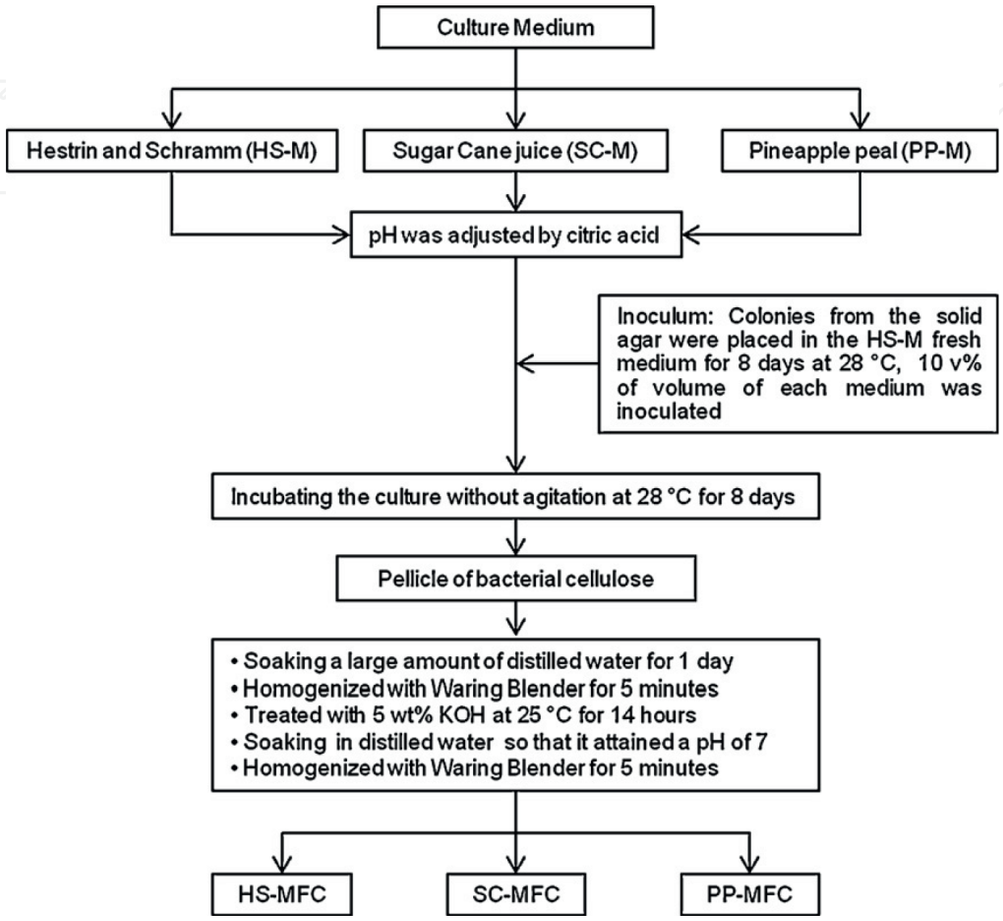


Figure 2.
Steps of BC production using different culture media.

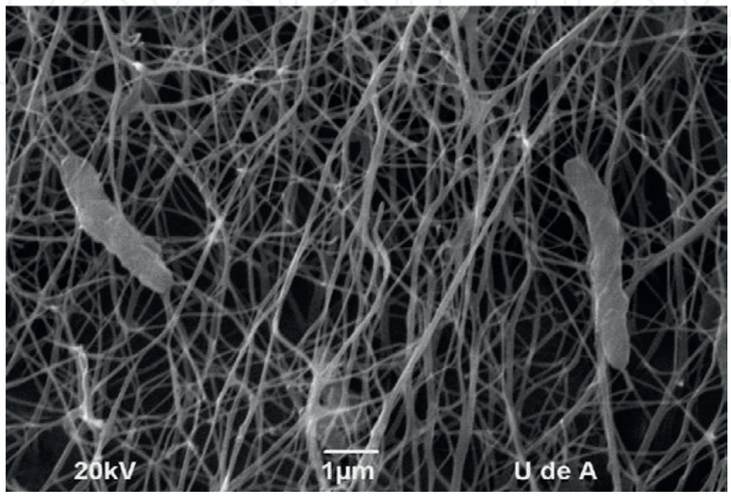


Figure 3.
SEM picture of cellulose ribbons with attached homemade vinegar pellicle and bacteria.

w/v, glucose, 0.5%, w/v yeast extract, 0.27%, w/v, Na_2HPO_4). In the HS medium, the nitrogen source, peptone, and yeast extract are very important [18].

The cellulose obtained from different media is summarized in **Figure 2**. Three culture media will be written as SC-MFC, PP-MFC, and HS-MFC; consequently, after 13 days, 28°C and pH at 7 give bacterial cellulose [18], where the characterized of the bacterial cellulose by SEM and TEM gives this image in **Figures 3** and **4**. The picture of scanning electron microscopy (SEM) has the rode shape of the surface of pellicle formed. TEM picture shows negatively stained specimens of typically 20–70-nm-wide ribbons.

The high yield of bacterial cellulose production using Hestrin and Schramm's medium has similar properties to that produced using pineapple peel juice. The result amount of bacterial cellulose using pineapple peel juice is (2.8 g/L), which is higher than produced by Hestrin and Schramm's medium (2.1 g/L) [18]**.** Thus, it can be produced BC, with low-cost sources in order to increase its production of bacterial cellulose.

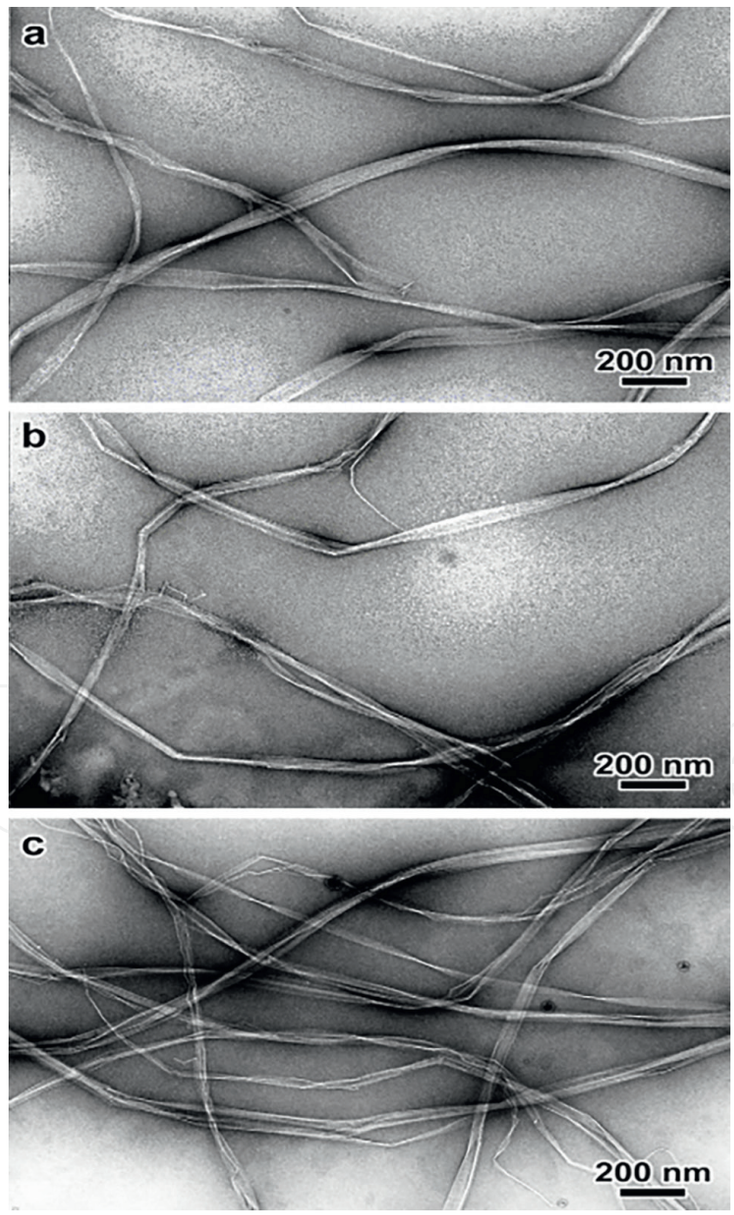


Figure 4.
TEM image of biosynthesized bacterial cellulose ribbons: (a) HS-MFC, (b) SC-MFC, and (c) PP-MFC, prepared by negatively stained specimens of typically 20–70-nm-wide ribbons.

2.2 Characterization of bacterial cellulose produced by *Gluconacetobacter swingsii* sp.

We can see, in **Figure 3**, to show the pellicle surface was formed by *Gluconacetobacter swingsii sp*. from homemade vinegar in rod shape, which is examined by SEM. We have observed the three-dimensional cellulose microfibrils [27], arising from the cell surface and forming bundles. **Figure 4** shows typical 20–70-nm-wide ribbons; this is examined by TEM images recorded from negatively stained species, and a thickness between 6 nm and 8 nm was estimated. Therefore, the cellulose microfibrils consist of 3–11 ribbons [28].

The bacterial cellulose from pineapple peel juice and sugar cane juice with low-cost resources is increasing production to a larger scale [18]. Compared with Hestrin and Schramm's medium, it gives low yield of bacterial cellulose with similar characteristics of these results.

3. *Gluconacetobacter sacchari* using dry olive mill residue produces bacterial cellulose

Wastes from many industries can be used successfully to produce BC, and Japanese pear and grape [29, 30], sugarcane molasses, Konjac powder [31], corn steep liquor [32], many fruit juices, such as apple, orange, pineapple, and beet molasses [33] as well as coconut water [34], are investigated. Due to high cost in the production of BC because it uses quite expensive culture media, the aim of this work is the utilization of residues from the dry olive mill residue production industry as food for *G. sacchari* to evaluate the possible presence of carbon source for the production of BC. On the other hand, it was using conventional HS culture medium to produce BC at nearly 2.5 g/l [29]. So, this study hydrolyzed DOR by acid, after hydrolysis by dilute acid, in order to give compound containing sugars and carbon for food source of BC production.

3.1 Producing bacterial cellulose from extract dry olive mill residue (DOR)

To prepare source of sugar-rich aqueous extracts from dry olive mill residue (DOR40 and DOR100, respectively) to produce BC, it has two water extraction, one at 40°C and second at 100°C. **Figure 5** shows that when lower amount of BC is produced in case of DOR40 and DOR100, the yield of BC is equal to 0.81 and 0.85 g/l compared with conventional HS culture medium (2.5 g/L). This study shows a decreased amount of BC resulting in case of the conventional HS culture medium with a relative ratio from 32 to 34%.

In the step, during hydrolysis of dry olive mill residue (DOR 100H) no BC results, because during the hydrolysis process, the monosaccharide is produced with some of the organic compounds such as furfural, and the phenolic compounds were the results from the degradation of sugar, which could have damaged the metabolism of *G. sacchari*. So the BC is produced during the two aqueous extracts DOR40 and DOR100 but in the aqueous extract at 40°C, low energy is consumed [29].

3.2 Supplemented with N and P sources to produce BC from DOR residues

In order to improve the production of BC, this work can be used as the source of nitrogen and phosphate as supplements with the extract of aqueous DOR 40. These sources were potassium dihydrogen phosphate (KH_2PO_4) and ammonium

sulfate $(NH_4)_2SO_4$, respectively. There is slight decrease of BC at high concentration of ammonium sulfate and increase of BC yield at low concentration of ammonium sulfate $(NH_4)_2\ SO_4$ at 1 g/l of salt. But, after the addition of 1 g/l of KH_2PO_4 there is slight decrease in the production of BC. This study indicates use of ammonium sulfate and potassium dihydrogen phosphate as a source for increased yield of BC. So, these data indicate that phosphorus and nitrogen sources could also play important roles for the production of BC. But this result in lower conventional HS media.

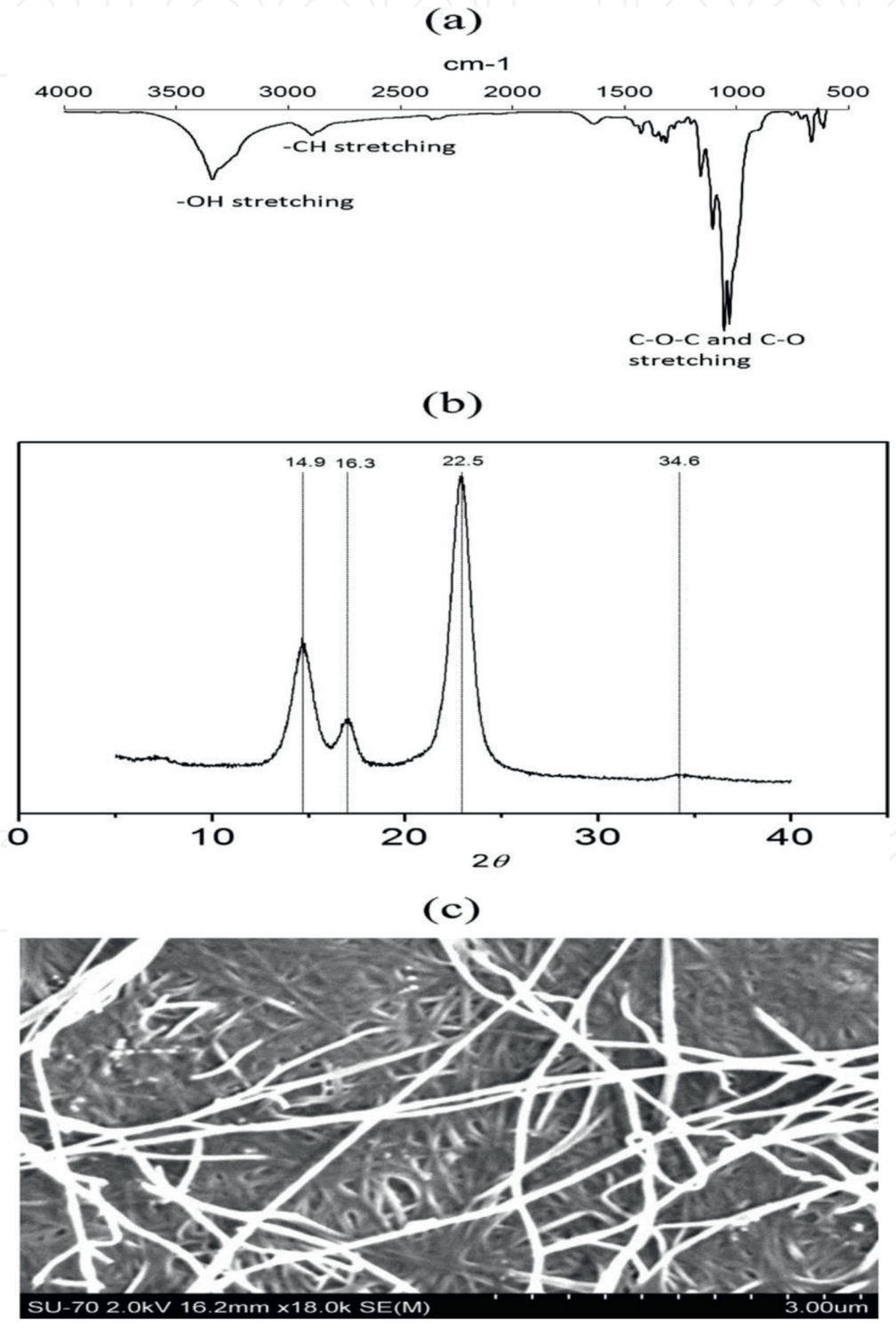


Figure 5.
(a) FTIR spectrum, (b) X-ray diffractogram, and SEM image of the BC membrane obtained with DOR40.

3.3 Characterization of bacterial cellulose (BC)

Production BC is studied when dry olive mill residue water extract is used as nutrients and source of carbon in the presence of nitrogen and phosphate salts. Crystallinity, chemical structure, and morphology of BC can be characterized by XRD, FTIR, and SEM, respectively. **Figure 5a** shows the absorption peak of FTIR for BC appears strong at 2880, 3300, and 1100 cm^{-1}, indicating the vibrations of the CH, hydroxyl group (OH), and C-O-C functional of (BC) and **Figure 5b** indicates the crystallinity, where the presence of the diffraction peaks is at 2 Θ, 14.9, 16.3, 22.5, and 34.6 and crystallinity of these samples is at 80%. The image of SEM is in **Figure 5c** showing homogeneous nano- and microfibrils of cellulose in the tridimensional network.

4. *Gluconacetobacter hansenii* CGMCC 3917 using only waste beer yeast as a nutrient source for biosynthesis of bacterial cellulose

However, using industrial materials waste would not only reduce environmental pollution to a high degree but also improve the production of cellulose by microorganisms. In general, waste beer yeast (WBY) is composed of 23–28% carbohydrate, 48–55% protein, 2% vitamin B, 6–8% RNA, and 1% glutathione; also, it has some elements such as K, Ca, Fe, P, and Mg [35, 36]. Microorganisms could use its sufficient food supply to produce natural green materials. Additionally, large-molecular-weight polymers made of proteins and carbohydrates can be found in the cell walls. So, it is more difficult to utilize it directly as a food source for microorganisms [37].

In this study, it can be produced by reducing sugar yield from waste beer yeast (WBY) by two-process pre-treatments. The first was treated by four methods, including a) high-speed homogenizer, b) 0.1 M NaOH treatment, c) microwave treatment, and d) ultrasonication. The second step is using mild acid condition (pH 2) for hydrolysis at 121°C for 20 min and after this pre-treatment must be evaluated for reducing sugar. While this was modified, the hydrolyzed WBY was directly used as food media culture for *G. hansenii* CGMCC3917 to produce BC. This bacterial cellulose (BC) can be evaluated by 1) water-holding capacity (WHC), 2) water absorption rate, and 3) water release rate (WRR) (WAR) estimated and its microstructure was evaluated using scanning electron microscopy (SEM).

4.1 Production of bacterial cellulose by *G. hansenii* CGMCC 3917 strain

In order to isolate this strain from homemade vinegar, it was recorded as CGMCC3917 at China General Microbiological Culture Collection, Beijing, China, and it was kept on glucose agar slants, including 2% glucose (w/v), 1.5% ethanol (v/v), 0.1% K_2HPO_4 (w/v), 1.7% agar (w/v), 0.5% yeast extract (w/v), and 1.5% $MgSO_4.7H_2O$ (w/v). It was put in a refrigerator at 4°C for every 2 months for inoculum development sub-cultured or deposits at 80°C, and this process must be occurring instead of agar for long-period storage using 20% (v/v) glycerol [38].

4.1.1 Pre-treatments and hydrolysis of waste beer yeast (WBY)

This pre-treatment occurred by taking different concentrations of dry waste beer yeast between 5%, 10%, 15%, and 20% (w/v), respectively, in a 250-ml round-bottom flask by adding 100 ml of distilled water to it, by using unmodified WBY

mixed liquor [35]. Four processes were explained to modify WBY mixed liquor. The modification method was as follows:

Pre-treatment 1: In this process of modification, the different amount of WBY is taken, and then, a certain solution from 0.1 M NaOH is taken at 50°C for different interval periods of 6, 12, 18, 24, 30, and 36 h, respectively.

Pre-treatment 2: mixed liquor for 5, 10, 15, and 20 min, respectively.

A certain different weight from WBY is modified by homogenizer at 15,000 rpm (XHF-D, Ningbo Xingzhi Biotechnology Co., Ltd., Zhejiang, China) Pre-treatment 3: by ultrasonication modification for 10, 20, 30, 40, 50, and 60 min respectively, while the ultrasonicator has power of 500 W (YQ-1003A, Ningbo Power Ultrasonic Equipment Co., Ltd., Zhejiang, China). Pre-treatment 4: modified mixed liquor with different concentrations of WBY in microwaves at microwave power of 600 W (Galanz P70D20P-TF, China) for 5, 10, 15, and 20 min, respectively, after each pre-treatment, hydrolysis of mixed liquor samples is carried out with different concentrations of WBY. Pre-treatment is carried out under dilute acid at 121°C for 20 min (pH 2.0).

4.1.2 BC production using WBY hydrolysates with different concentrations

For the production of BC, the highest reducing sugar yield was selected after modifying the waste beer yeast (WBY). After pre-treatment, at 121°C for 20 min, in the presence mild acid condition at (pH 2), WBY was hydrolyzed for 15 min using centrifugation at 4000 g to remove precipitate and the amount of solution was collected and added.

(50%, w/v) glucose solution was prepared using sterilized water in a glass vessel (500 mL) from its initial reducing sugar of 4.38% (w/v) containing 100 mL of WBY hydrolysates at different concentrations of between 1%, 3%, 5%, and 7% (w/v), respectively and after adjusting its pH to 5 using 2 M NaOH, the prepared seed inoculums with (9%, v/v) were stored to cultivate at 30°C for 14 days. The production of bacterial cellulose was from WBY hydrolysates as carbon and food sources without any extra nutrient added. They were directly supplied to G. hansenii CGMCC 3917 to produce high yield of bacterial cellulose [35].

We notice that the samples do not centrifuge after these pre-treatments have a high sugar concentration. So, they see that inhibition of the BC production decreases the supply of oxygen by the liquid medium in case of uncentrifugation. While, in case of using the centrifuge for samples, It reduces sugar by adding water to the supernatant; this is referred to as diluting and gives a better yield for BC production. In contrast, pre-treatments 2 and 4 and unmodified WBY give a lower BC result compared to those not using the centrifuge and centrifuged WBY. Likely, the *G. hansenii* CGMCC 3917 strain type was damaged and does not give bacterial cellulose, because of decrease in sugar concentration present in the centrifuged samples. Additionally, pre-treatment method 3: the WBY is treated by ultrasonication to produce the highest yield of BC (3.89 g/L). Further, the amount of BC from pre-treatment 1 by 0.1 M NaOH is 2.33 g/L [35].

It is clear that the amount of BC in pre-treatment processes 2,4, and 5 was decrease in processes 1 and 3.

4.1.3 Effect of culture time on the production of BC

The concentration of reducing sugar affected BC production. So the ultrasonication method is used to give the highest reducing sugar to improve the BC production, and we must select this to investigate the optimal sugar concentration. The BC weight

utilizing WBY hydrolysates in the presence of 1% sugar was the lowest yield and it reaches a value 2.18 g/L on day 7. When, using the sugar of 3%, the BC weight is 7.02 g/L on day 10 [35].

5. Biosynthesis of bacterial cellulose by *Gluconacetobacter sucrofermentans* B-11267 using wheat thin stillage

The thin stillage of rice (R-TS), the wine distillery of rice, was giving wastewater that has organic acids. Recently, researchers are working to use stillage in order to obtain a high yield of BC at optimal conditions [39–41], and this is achieved using the traditional HS medium for BC production. BC amount is 6.26 g/L after 7 days obtained at static cultivation [41], in the 50/50 R-TS—HS medium. Therefore, the strain *Gluconaceto-bacter sucrofermentans* B-11267 in agitated culture conditions without any pre-treatment or addition nitrogen source and highly acidic by-products of the alcohol in order to, production of bacterial cellulose (BC).

5.1 Isolate of *G. sucrofermentans* B-11267 (bacterial strain) from kombucha tea

Bacterial strain was prepared in a test tube suspension using 1 ml of the suspension tea and then 9 ml of 0.9% sodium chloride (w/v) was added. The groups of different dilutions (10 to $1x10^{-6}$) were synthesized by solution from sterilized saline. About 0.1 ml of each dilute is taken on a media (agar (15 g/L), yeast extract (10 g/L), glucose (100 g/L), and calcium carbonate (20 g/L)). The plates were put in an incubator at 28°C for 3 days. After 3 days, we see a clear zone around with colonies, and they were selected and moved into glass vessel having 10 ml of Hestrin and Schramm (HS) medium [39].

The strain of produced positive cellulose is in the liquid medium, and the media for BC production include the following (g/L): yeast extract (5), citric acid (1.15), glucose (20), peptone (5), and disodium hydrogen phosphate (2.7), at pH 6, which is called "Hestrin and Schramm medium" (HS): thin stillage (TS) without pH adjustment, pH 5 and pH 6, cheese whey without pH adjustment, pH 4.96, pH 3.95; in autoclave for 20 min at 120°C [39].

5.2 Effect of thin stillage (TS) to produce bacterial cellulose

In this work, they investigated *G. sucrofermentans* B-11267 in agitated culture utilizing thin stillage (TS) to result in bacterial cellulose and whey without any modification as food sources to lower the manufacturing costs. For comparison, the HS medium was used. We find that increasing the bacterial cellulose using thin stillage after 3 days the yield is equal (6.19 g/l) and this amount of BC is approximately three times than that produced by HS (2.14 g/l). In the whey medium, the yield of bacterial cellulose is equal (5.45 g/l) after 3 days of cultivation**.** However, the maximum rate of the production of bacterial cellulose is the first day during the growth of the bacterium [39].

5.3 Effect of pH on the production of BC from thin stillage (TS)

The production of bacterial cellulose was affected by pH of the culture medium with thin stillage. So the bacterium *G. sucrofermentans* B-11267 on TS gives BC amount [39] (6.19 g/L) at pH 3.95.

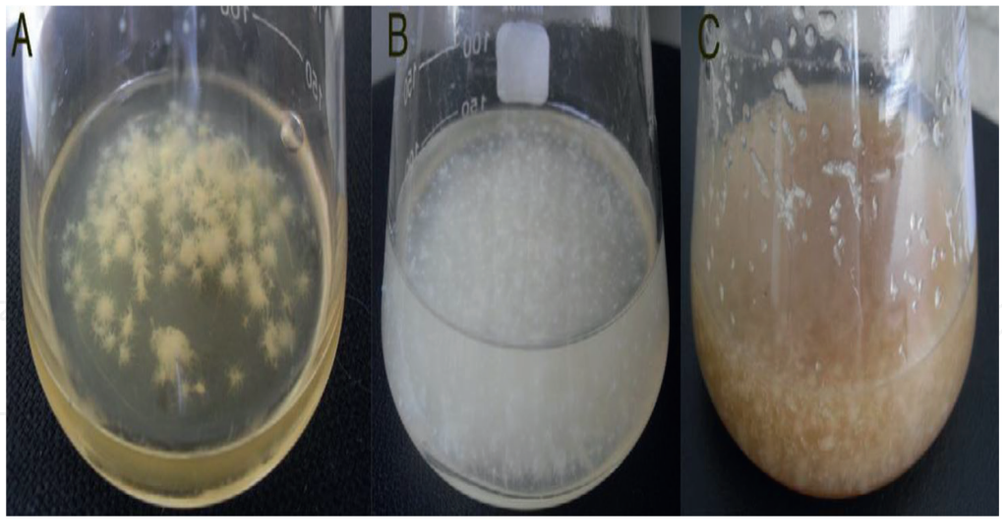


Figure 6.
Gluconacetobacter sucrofermentans B-11267 in agitated culture conditions using HS medium (A), whey (B), and thin stillage (C) to produce BC.

In **Figure 6**, it was shown that the collection of bacterial cellulose is formed in different sizes and shapes. It is clear that thin stillage (TS) and whey have the finer shape and homogeneous structures of BC produce in B, and C images compared to standard HS medium.

From this study, it was indicated that in the alcohol and dairy industries, such whey and thin wheat stillage are used as crude materials for producing bacterial cellulose (BC). So BC can be produced by using thin wheat stillage to give high yields and good quality. Further, the strain *G. sucrofermentans* B-11267 explained here yields a large amount of BC at acidic pH media [39].

6. Effect of lignosulfonate on the produce of bacterial cellulose

The acid-sulfite pulping of wood is the waste product of lignosulfonate. However, this lignosulfonate has more properties, such as high dispersive and adhesive abilities, and can be used as a soil stabilizer [42]. In this work, they study production and structure of bacterial cellulose. However, the presence of several types *of Gluconacetobacter*

Strain parameter	**HS medium**			**HSL medium**			**Yield ratio (HSL/HS)**
	Yield (g/30 ml)	**Gluconic (μg/20 μl)**	**Final pH**	**Yield (g/30 ml)**	**Gluconic (μg/20 μl)**	**Final pH**	
10,245	.1344	5.43	2.98	.2178	2.59	4.03	1.62
13,693	.2376	7.41	3.4	.4896	2.61	4	2.06
13,772	.2628	5.73	3.5	.3432	2.11	4.06	1.31
13,773	.3036	6.84	3.55	.4866	2.7	4.25	1.6
14,815	.114	8.83	3	.234	2.94	4.06	2.0
15,237	.1272	8.94	3.01	.312	2.5	4.11	2.45

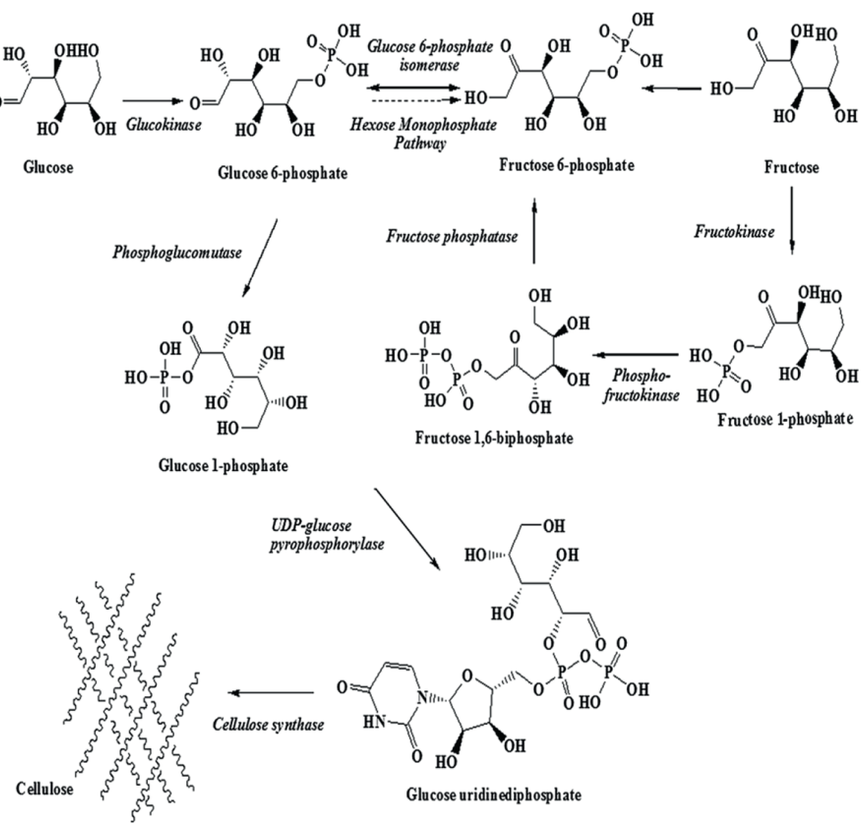
Table 3.
The weight of BC (mg) per 30 ml of culture medium after cultivation for 7 days.

xylinus (=*Acetobacter xylinum*) strain utilizes only 15237, IFO 13693, ATCC 10245, 13772, 14815, and 13773 in the presence of lignosulfonate. This study evidences this all types of strains produced BC with improving nearly 57% with added (1%, w/v) lignosulfonate, and the higher crystallinity index cellulose, which means amorphous region in the presence of lignosulfonate was relatively lower. These data indicate the high BC yield due to the damage of gluconic acid in the presence of lignosulfonate. That lignosulfonate contains (antioxidant) polyphenolic compounds. **Table 3** shows an increase in BC yield in the presence of 1% lignosulfonate using Hestrin-Schramm (HS) medium [42].

7. Biosynthetic pathway of BC in *Gluconacetobacter xylinus*

The BC biosynthesis in the presence of enzymes as catalysts is used by the following steps: (a) Glucose is converted to glucose-6-phosphate using glucokinase as enzyme; (b) the glucose-6-phosphate produced from glucose is isomerized to glucose-1-phosphate; (c) uridine diphosphate glucose (UDP-glucose) is produced from glucose-1- phosphate by UDP-glycose pyrophosphorylase; and (d) finally, glucose uridine diphosphate produced glucan chains [43, 44] (cellulose) using cellulose synthase enzyme.

After this, the chains of glucan were arranged in parallel and crystallized to construct the microfibrils region; then, the microfibrils are aggregated to form bundles of cellulose fibers [13, 45], by complete washing to remove culture medium, and for purification of the yield, colorless, odorless, and tasteless, they have obtained the BC in the form of a gel. So, its presence has several applications in our life for this gel product [45]. The following mechanism shows the bacterial cellulose synthesis pathway in *G. xylinus.*



8. Applications of bacterial cellulose (BC)

Due to its biocompatibility, hydrophilicity, biodegradability, and nontoxicity properties for cellulose, it is the most abundant biodegradable material and has been widely used in medical applications, such as wound dressing, tissue engineering, controllable drug delivery system, and blood purification [46]. The bacterial cellulose has better properties compared to plant cellulose, such as higher crystallinity (80–90%) [47], water absorption capacity [48], and a higher degree of polymerization (8000) [49]. Finally, the characteristic properties of bacterial cellulose included wound care, biosensors, tissue engineering, drug delivery, and diagnostic [50], which are the medical applications of BC composites.

According to the above-mentioned properties, several applications of the BC, such as cosmetics, foods, and drug delivery, are present.

8.1 Bacterial cellulose in medical applications

BC-based materials are used in biomedical applications [51]. Due to the ideal structure, biocompatibility and sustainability of BC have led to many studies and prompted its application in a variety of fields, such as medicine [47, 52–54]. Nowadays, BC-based materials are mostly utilized in the medical field, including in wound healing materials, artificial skin and blood vessels, scaffolds for tissue engineering, and drug delivery [23, 55–58].

8.1.1 Bacterial cellulose for wound dressing

In medicine, field dressing material is used as a band aid or as a large bandage. By properties such as biocompatible, sterile, porous, and flexible, it is also used as a protective surface for firefighters, who are often exposed to burns (**Figure 7**) [59]. Such material allows the breathability of wounds, to prevent the formation of scabs and scars that must make different treatments. Also, this reduces pain, protects the skin from various infections, and does not cause loss of body fluid. Advantages of bacterial nanocellulose produced by Biofill® are rapid pain relief, close adhesion to the wound, noticeably rapid dressing, reduction in wound size after surgery, wound

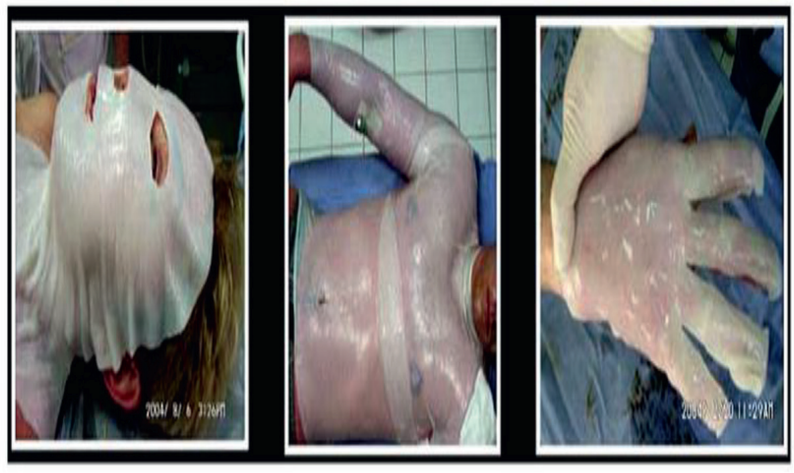


Figure 7.
Dressings made with bacterial cellulose that is imposed on burned tissue [59].

control (transparency), reduction in infection rate, cost reduction, and treatment time. The only drawback of such bacterial nanocellulose is that it cannot be used in the more mobile parts of the body since it is less elastic [60].

Whatever the reason, the high hydrophilic properties of bacterial cellulose and the fact that it never dries make these good properties because they indicate that better wounds heal faster and must be moisturized. Bacterial cellulose has a favorable property for use as skin tissue scaffolds and wound dressings [60].

On the market today, few BC-based commercial products are available for wound dressings of cavities, abrasions, and also as chronic venous ulcers and healing for burns. The structure of BC has porosity. So, it is possible to impregnate drugs in its structure, and this property can develop bacterial cellulose properties, for example, antimicrobial activity. But also, it is incorporates some elements, such as copper and silver [61].

8.1.2 Bacterial cellulose applications in tissue engineering and scaffold

Currently, the bacterial cellulose (BC) has full potentials to select as substrate material in tissue engineering. There are still many challenges to overcome the control porosities of BC scaffold by optimizing culture conditions, which is challenging, increasing BC degradation rate for specific applications and introducing functional groups to BC matrix [56, 62, 63]. So BC has physical modification, such as change porosities, crystallinities, and fiber densities and chemical modification in chemical structure to achieve this goal (TE, scaffold) and the chemical modification can occur by changing the carbon source of BC. It can modify BC done by either chemical or physical processes to evaluate the effectiveness of the composite scaffolds in construct bone regeneration.

In order to evaluate the biological properties of the new BC scaffold for bone regeneration, some materials must be added, such as hydroxyapatite nanoparticles (HA - Ca_5 $(PO4)_3(OH)$), to utilize an additive in the BC culture medium [56], the new bone tissue takes 4-weeks post-implantation [64], and the bone defects were completely fill.

Table 4 shows the added *in situ* treatment of BC for TE applications, and additional materials additives must be controlled to prevent the microbial fermentation limit. Also, using *in situ* modification method, till now, the structure of BC nanofibers still needs to be addressed such as overlapping between BC fibril growth and the externally introduced additives.

Formula G. A: Gluconacetobacter; Acetobacter.

There are two methods for *ex situ* modification of bacterial cellulose (BC), which are as follows:

(i) Chemical modification: the chemical structure of BC polymer is cellulose. So, BC could be modified by phosphorylated compounds and then treated by grafting or reactions with crosslinking to give BC modification [80]. In this method, the bacterial cellulose has a hydroxyl group that can make a strong hydrogen bond between the BC. The films and tubes from bacterial cellulose (BC) are prepared with calcium-deficient hydroxyapatite (BC/Cd HA) for application in bone tissue engineering [81].
(ii) Physical modification: the porous of BC can be filled with solution or particle of suspension of absorbed molecules additives; in this study, impregnated of AgNPs (BC/AgNPs) by immersing into BC membrane, the silver prepared a BC membrane nitrate as precursor ($AgNO_3$) solution after which the impregnated silver ions (Ag^+) were reduced to Ag^0 particles [82], and the inhibition zone of using BC/AgNPs was

Application	Bacteria	Additive material	Modified properties	References
Scaffold for TE	*A. xylinum*	Collagen	Roughness, stiffness color, thickness, crystallinity, and porosity	[65]
Bone regeneration	*A. xylinum*	Polystyrene and optical fibers	Porosity, and swelling behavior, fiber network, crystallinity, and mechanical property	[66]
Wound dressing/artificial Skin	*G. hansenii*	Sand dollar skeleton	Density, porosity, mechanical property, and thickness	[67]
Vascular grafts	*G. hansenii*	Hydroxyapatite	Fiber diameter and orientation, thermal property, mechanical property, porosity, crystallinity, and biocompatibility	[64, 68, 69]
Biofiltration	*G. hansenii*/*Gluconacetobacter xylinus*	Paraffin	Porosity, biocompatibility, and mechanical property	[70–72]
Urinary reconstruction	*G. hansenii*	Glucose or dextrin	Rehydration rate, surface area, water-holding capacity, biocompatibility, and porosity	[73]
	G. xylinus	Potato starch	Crystallinity, thickness, density, porosity, mechanical property, biocompatibility, and rheological	[74]
	A. xylinium	Cotton gauze	Wicking ability, water absorbency, drying time, and porosity	[75]
	A. xylinium	Aloe vera	Porosity, mechanical property, surface area, water vapor permeability, water absorption capacity, and crystallinity	[48]
	A. aceti	Deacetylated chitin	Antimicrobial activity and mechanical property	[76]
	A. xylinum	Nanocrystals	Porosity, roughness, surface area, and biocompatibility	[77]
	G. medellinensis	Chitosan and heparin	Thickness, porosity, water retention, charge, and composition	[78]
	A. xylinum	Carboxymethyl cellulose	Porosity, biocompatibility, and mechanical property	[79]
	Paraffin			

Table 4.
Summary of bacterial cellulose for TE applications for in situ modifications.

tested against *Staphylococcus aureus* and *Escherichia coli*. It can see the inhibition zone around the sample.

As an example of a scaffold, BC is loaded by bone morphogenetic protein-2 (BMP-2) to study the possibility of utilizing BC as a scaffold for bone tissue engineering. The localized growth factor delivery system has more bone formation and higher calcium concentration than blank BC scaffolds from 2 and 4-weeks post-implantations [83].

8.1.3 Bacterial cellulose in blood vessel and cartilage

The blood vessel is also field using bacterial cellulose in medicine application. However, bacterial cellulose can be prepared as nanocellulose film or sheet compared with organic sheets, such as polypropylene or cellophane, and has high mechanical strength, such as tear resistance and shape retention. These properties are better for artificial materials than other materials. Consequently, it can be made the prototype of blood vessels have a tube of 5–25 cm long [84].

Where the cartilage is wider in adults and children, this cartilage is made from bioactive material, such as bacterial cellulose. The high wide in this topic is the nose, an ear, and intervertebral discs using reconstructive surgery.

8.1.4 Bacterial cellulose as drug release

As the structure of bacterial cellulose contains hydroxyl groups and other good properties such as purity, crystallinity, porosity, and water-holding capacity, some polymeric compounds and BC have been studied for controlled drug delivery. The synthesis of nanocomposite of BC to optimize the controlled drug delivery is an important strategy for pharmaceuticals in order to achieve the drug-delayed release effects of BC. In some studies, the matrix of BC and polyacrylic acid (PAA) (BC-PAA) has been synthesized by polymerization initiated through electron-beam irradiation using various doses of radiation [85, 86]. In this case, pH, swelling rate, gel fraction, and gelling time of prepared hydrogel must be controlled. The composite hydrogels from BC-PAA are utilized for drug delivery with different contents of bovine serum albumin (BSA) as a model compound [85].

8.2 Bacterial cellulose in textile applications

The textile industry must be concerned with quality and environmentally friendly products. Various research studies are required for the development of this industry, including preparation, finishing, and dyeing as important factors for sustainable development and economically feasible for the population. So, a hydrophobic cellulosic finishing is needed because it has a wide range of applications, not only in conventional applications but also in functional applications, such as clothing, waterproof textile, stain resistant (oils), antimicrobial, soil release, and self-cleaning. The ideal cellulose fabrics for water repellency are hydrophobic fiber surfaces because they resist water, with some porosity that allows moisture transport for user comfort [87].

8.2.1 Bacterial cellulose as water repellent finishing of textile

The main goal of this study was to use water repellent bacterial cellulose (BC) to create a material with potential application in design, specifically in the textile industry.

Some additives, including the incorporation of a softener, were used to improve BC flexibility. This finishing bath contains a certain ratio of nearly 60% of commercial hydrophobic product, finishing agent, and six samples of bacterial cellulose (BC) in different concentrations to soluble in 0.5 ml of softener and after treatment, the samples of fabrics are then dried and cured in oven at 120°C for 1 minute. The contact angle can be measured by using bacterial cellulose in finishing bath, which means the more hydrophobic of samples in the presence of bacterial cellulose (BC). This is due to the presence of coating layers on the surface of textile to decrease the surface energy [88].

8.2.2 Bacterial cellulose applied as comfortable textile

In this study, bacterial cellulose as breathable and water impermeable depends in the preparation of nanocomposites, using two commercial hydrophobic polymers to treat as water/oil repellency and comfortable fabrics in textile, and Persoftal MS (polydimethylsiloxane) and Baygard EFN (perfluorocarbon) are used on bacterial cellulose (BC) membranes, by an exhaustion technique [89]. However, by incorporating the commercial hydrophobic material with porous of bacterial cellulose as network, the contact angle measured in the presence of finishing by bacterial cellulose alone, in the presence of softener polydimethylsiloxane, (S) and in the presence of hydrophobic compound (perfluorinated) (H), where the contact angle in the presence of bacterial cellulose alone is equal to (63.8°).

By evaluating water vapor permeability (WVP) and static water absorption (SWA), this property is the most important for the comfort textile. When the concentration of S and H composites in BC is increased, the WVP decreases when compared to BC alone.

8.3 Bacterial cellulose in food application

The bacterial cellulose is utilized in food because it is a dietary fiber and is known as a "generally recognized as safe" (GRAS) food by the US Food and Drug Administration (FDA) [90]. BC is widely applied in the food industry [91]. The nata-de-coco, a juicy and chewy dessert from the Philippines, is made from the material of bacterial cellulose. The raw material produced from BC is grown from coconut water with many carbohydrates and amino acids. After manufacturing, it can be cut into cubes and put in sugar syrup [13]. The Monascus-produced meat analog—the BC complex has the best property, such as dietary fiber, and the lowering of the cholesterol from Monascus as well as the non-animal origin. This BC makes the product that is a suitable substitute for meat products for humans with dietary restrictions [43].

8.4 Bacterial cellulose in cosmetic applications

The human body must use cosmetics substances to enhance some of the organoleptic characters [92]. Alternating the appearance of the person's body utilizes cosmetic products that are cleansing or beautifying the body parts and enhancing the attraction, without affecting the normal body functions or structure [92]. Bacterial cellulose has biodegradability, low toxicity, and ability to hydrate the skin, so this BC treats dry skin. The cosmetics are applied in the case of oil-in-water emulsion without any addition of surfactant. The bacterial cellulose has high water-holding capacity and good gas permeability, so it is used as an appropriate carrier for the active ingredients for cosmetic materials. The cosmetics materials can be used as moisturizers

and have whitening ingredients, for example, hyaluronic acid and salicylic acid, kojic acid or ursolic acid, anti-wrinkling agents (e.g., exfoliator and polypeptides), growth factors, and a combination thereof or enzymes [93].

The mechanical properties of bacterial cellulose result in the adhesion force of the mask; therefore, the structure of the formulation includes hydrophilic and hydrophobic chemicals. So, these properties provide a better interface between the skin and the mask. Images of the masks adhered to the face and hand skin indicate the good attractive of the mask to the skin [94].

9. Conclusion

Bacterial cellulose (BC) or microbial cellulose (MC) is a bioactive substance with high water absorption, crystalline structure, high tensile strength, and biodegradability. However, bacterial cellulose has a wide range of uses, including biomedical, textile, paper, food, medication release, and cosmetics. So FTIR, XRD, SEM, and TEM are used to characterize the microbial cellulose production from *Acetobacter xylinum* using diverse waste as carbon and nitrogen sources, such as pineapple peel juice, sugar cane juice, dry olive mill residue, waste beer yeast, and wheat thin stillage. The concentration of sugar in the carbon source, the temperature and time of the strain's incubator, and the pH of the media all influence the production of bacterial cellulose.

10. Future of perspectives

Production of the bacterial cellulose industrially with high yield and green synthesis uses an industrial fermenter design to grow *A. xylinum* culture.

Production of bacterial cellulose from the environment wastes uses less water and energy.

The applications of bacterial cellulose are widen to meet various trends of the modern world.

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