

MICROBIAL CELLULOSE BASED FILMS AND COMPOSITES FOR FOOD PACKAGING: A REVIEW

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Abstract

Currently, the production and application of non-biodegradable petroleum-based synthetic polymer (commonly known as plastic) are highly prevalent. As synthetic polymers as mostly non-biodegradable, they adversely affect the environment and result in the generation of excessive solid waste. The increasing awareness about the ill-effects of synthetic polymers among consumers has resulted in a demand for natural, disposable, biodegradable, reusable, or recyclable food packaging materials. Bio-based polymers and biopolymers have been one of the most favorable alternatives to be exploited and developed into eco-friendly food packaging materials. Certain microorganisms, such as *Gluconoacetobacter xylinus*, produce cellulose by a fully green procedure which is called bacterial cellulose. Bacterial cellulose demonstrates exceptional properties such as being a chemically pure material, highly flexible, high water absorbency, great tensile strength, highly crystalline nature, highly moldable, non-toxic nature, and biocompatible. However, there are some limitations such as lack of antibacterial properties, optical transparency, and stress-bearing capability which can be overcome by developing bacterial cellulose composites using hydrocolloids like proteins, starches, pectins, etc. The bacterial cellulose composites are employed to develop packaging films with properties such as high mechanical strength; antimicrobial, transparent, biodegradable, with air, water, and oil resistance properties, thus, making it an appropriate material for packaging.

Keywords: bacterial cellulose, biopolymer, biocomposites, *Gluconoacetobacter xylinus*, food packaging, biodegradable

Introduction

In today's time, a synthetic and non-biodegradable petroleum-based polymer (commonly known as plastic) has found a firm place in our daily life. Due to an increasing demand and flexibility in applications, the production and use of non-

biodegradable materials have significantly increased. It is also one of the most used substances in the packaging industry including food packaging industries. It is a well-known fact that non-biodegradable polymers have an adverse environmental impact because it takes a long time to degrade after getting discarded into the landfill. The durability of synthetic polymer goods was once considered a boon which has now become a curse for our environment. The problem of solid waste came out as a crisis during the 1980s in the United States. The crisis happened because of the increasing amount of municipal solid waste (MSW), decreasing landfill capacity, increasing costs of dumping waste, etc. (Philp *et al.*, 2013; Regan *et al.*, 1990). In the year 1960, plastics were reported to make less than half a percent of United States MSW generation which increased up to 12.4% by the end of 2010 (Philp *et al.*, 2013), out of which only 8.2% was recovered. The crisis did not remain confined to the US only but soon materialized to become a global threat.

Along with the above-mentioned environmental issues, food packaging also depends on prominent alterations in food distribution, globalization of the food supply, consumer's demand for more fresh and convenient foods as well as a need for safe and quality products. Not only these, but there has also been a demand by the consumers to have food packaging materials that are natural, disposable, biodegradable as well as reusable or recyclable (Khan *et al.*, 2014). A growing number of investigations have been directed towards the development of food packaging materials that could rapidly degrade and completely mineralize in the environment (Othman, 2014). Material components like natural fibers and biodegradable polymers have the potential to be used as alternatives to produce new packaging materials.

Given this, bio-based packaging material comes to our rescue. Bio-based packaging is defined as packaging made of raw materials originating from agricultural sources such as starch, cellulose, and bio-derived monomers (for example, polylactic acid and polyhydroxyalkanoates). To date, biodegradable packaging is attaining huge attention and numerous projects are underway in this field. One important reason for this attention is the marketing of environment-friendly packaging materials. Bio-based packaging materials include both edible films and coatings (Ullah *et al.*, 2016). Biopolymers or bio-based polymers have been one of the most favorable alternatives to be exploited and developed into eco-friendly food packaging materials (Li *et al.*, 2015; Tang and Weder, 2010). These are the substances that are obtained from natural, renewable resources, produced by the microorganisms, or synthesized with the help of fossil-based materials. Used food packaging materials produced from biopolymers can be disposed into the bio-waste collection to form compost leaving behind organic end-product i.e. carbon dioxide (CO₂) and water (H₂O). Cellulose, being the most ubiquitous and abundant renewable organic material in the biosphere, with about 2 trillion tons annual production, is hugely used in paper and paperboard packaging. It is the main component of plant cell walls which is made up of unbranched, linear chains of D-glucose molecules, linked to one another by 1,4-β-D glycosidic bonds.

In addition to plant cellulose (PC), cellulose can also be obtained through biosynthesis carried out by various microorganisms, such as algae and fungi (Ullah, 2016) as well as various aerobic non-pathogenic bacteria of the genera *Agrobacterium*, *Sarcina*, *Rhizobium*, and *Acetobacter* (Dufresne, 2017; Shezad et al., 2010). Bacterial cellulose does not have impurities like lignin and hemicellulose (as present in plant cellulose), which makes it a highly pure source of cellulose (Mohite and Patil, 2014). It is also distinguished from its plant equivalent by a high crystallinity index (above 60 %) and different degrees of polymerization (DP), usually between 2000 and 6000 (Jozala et al., 2015; Mohite and Patil, 2014). It also demonstrates high water absorbing and water holding capacity, high tensile strength, strong biological adaptability, and good biocompatibility (Revin et al., 2018; Zhong et al., 2013).

Due to its unique properties, bacterial cellulose has been employed as a new biological material in the food industry, as edible packing material, as wound dressing materials, artificial skin, vascular grafts, scaffolds for tissue engineering, artificial blood vessels, medical pads, and dental implants (Jozala et al., 2016). Depending on the purpose of the application, bacterial cellulose can provide improved mechanical qualities to the biomaterial owing to its biocompatibility, biofunctionality, lack of toxicity, and ease of sterilization (Jozala et al., 2016; Klemm et al., 2011).

The trend for better quality, fresh and convenient food products has intensified among consumers during the last decades. Therefore, a variety of active packaging technologies have been developed to provide better quality, wholesome, and safe foods, and also to limit package-related environmental pollution and disposal problems. Due to the adverse environmental effect of non-biodegradable packaging (such as plastic), bio-based packaging materials are now preferred in the packaging industries. The application of bacterial cellulose composites in packaging may open a new possibility to solve these problems. Biocomposite packaging materials have great potential for enhanced food quality, safety, and stability. The unique advantage of the natural biopolymer packaging may lead to new product development in the food industry, such as individual packaging of particulate foods, carriers for functionally active substances, and nutritional supplements.

The present article has been written with an objective of providing extensive yet compiled information regarding biocellulose structure, morphology, properties, production methods etc. Development of bacterial cellulose composites and properties that extend their functionality have been highlighted. The potential benefits and future prospects of utilization of biocellulose in food packaging industry have also been discussed.

Bacterial cellulose

In the year 1886, Adrian Brown reported the presence of bacterial cellulose (BC) for the very first time whilst working on *Bacterium aceti*. Unexpectedly, he observed the formation of a solid mass on the outer surface of the fermentation

medium. Initially, the so-formed solid mass was called the ‘vinegar plant’ or ‘mother’ and was employed in the production of home-made vinegar. That solid mass was later recognized as cellulose and the name *Bacterium xylinum* was allocated to the microbe accountable for its production. It was then named again in 1925 as *Acetobacter xylinum* (Lin et al., 2013) which became its official name according to the International Code of Nomenclature of Bacteria (Lin et al., 2013; Lapage et al., 1992). In the present time, these Gram-negative, aerobic bacteria are referred to as *Gluconacetobacter xylinus* which is considered as a subspecies of *Acetobacter aceti* (Cannon and Anderson, 1991).

Plant cellulose contains unwanted impurities and contaminants such as hemicelluloses, lignin, and pectin (Figure 1). On the other hand, bacterial cellulose is a pure form of cellulose and does not demand extra processing methods to get rid of these impurities and also helps in retaining a higher degree of polymerization. Bacterial cellulose demonstrates exceptional properties such as a highly crystalline nature, huge water retention capacity, great tensile strength, and easily moldable property (Lin et al., 2013). It can be claimed that bacterial cellulose is a unique biomaterial that has properties like being a chemically pure material, great mechanical property, highly flexible, high water absorbency, highly moldable, inertness, non-toxic nature, biocompatible, biofunctional, and hypoallergenic (Stanislawski, 2016).

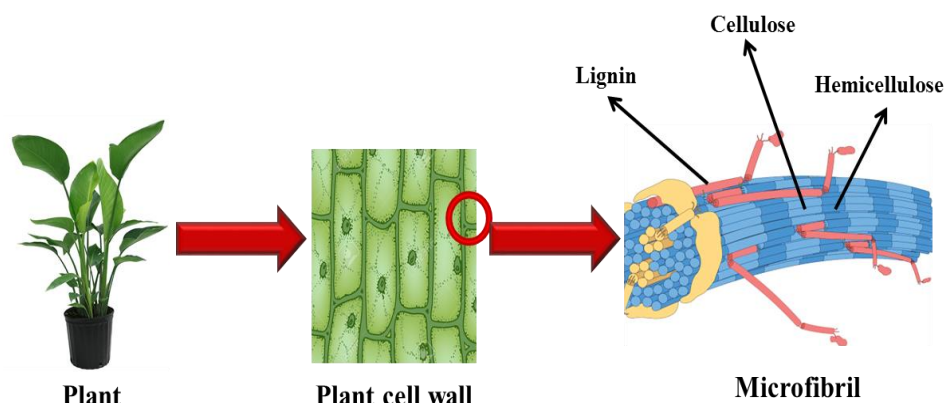


Figure 1. Structure of plant cell wall (after Phanthong et al., 2018 with modifications).

General Properties and Morphology of Bacterial Cellulose

Bacterial cellulose (BC), a homopolymer produced by some bacterial strains, has the same chemical structure of linear β -1,4-glucan chains as plant-derived cellulose. Plant-derived cellulose chains are closely associated with hemicelluloses, lignin, and pectin, BC is free of other polymers.

During the biosynthesis of bacterial cellulose, the polymerization of cellulosic chains occurred from activated glucose with the help of cellulose synthases A (CesA). The rosette terminal complexes then extrude the single chains through the cell of bacteria. A linear line of around 50-80 extrusion sites is situated along the

cell axis which releases the glucan chains (Sulaeva *et al.*, 2015). The macromolecules accumulate into hierarchically systematized units in the form of a complex, predominantly forming subfibrils of 10 to 15 glucan chains that congregate together to form microfibrils, and finally microfibrillar bundles (Bodin *et al.*, 2011). The loosely assembled microfibril bundles then form cellulose ribbons comprising about one thousand polyglucan chains. The continuous spinning of cellulose ribbons by bacteria escorts the formation of a highly pure 3-D structure of nanofibers stabilized by inter-and intra-fibrillar hydrogen bonds (Figure 2). This marvelous fibrillated structure of bacterial cellulose results in developing unique mechanical characteristics, such as a high degree of crystallinity (60–80%) and Young's modulus of 15–30 GPa (Sulaeva *et al.*, 2015). A high surface area resulting from the high aspect ratio of the fibers provides a great fluid loading capacity of up to 99 wt%. In the case of water, about 90% of the water molecules are tightly bound to a large number of hydroxyl groups within the cellulose molecules. Bacterial cellulose fibers have a greater specific area in comparison to plant-derived cellulose. Water absorbency of bacterial cellulose was found to be 30% greater than that of cotton gauze, and the drying time was 33% longer (Suleva *et al.*, 2015; Meftahi *et al.*, 2010).

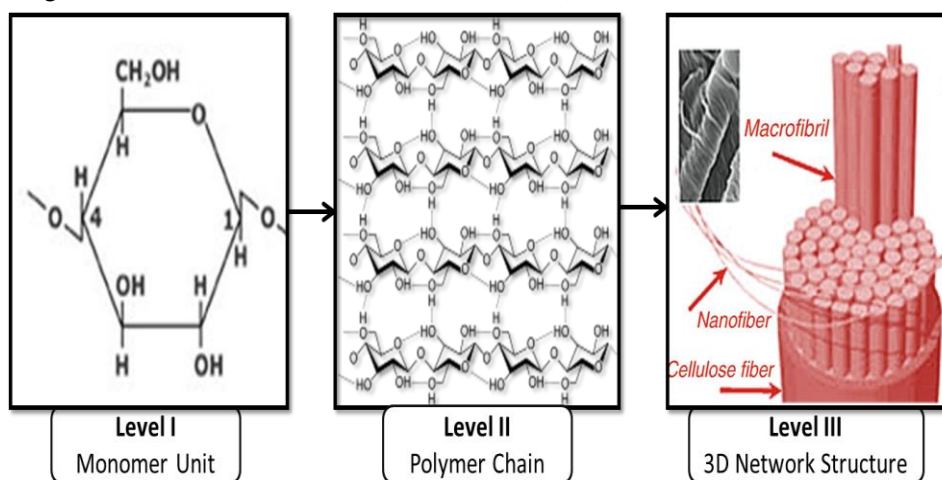


Figure 2. Structural Organization of Bacterial Cellulose (after Sulaeva *et al.*, 2015 with modifications).

Production of bacterial cellulose

Bacterial cellulose can be produced by several bacterial strains by providing the culture conditions desired for its production (Table 1). However, mostly *Gluconacetobacter xylinus* is used to produce bacterial cellulose due to its high yield.

The culture is placed in a medium containing nutrients such as glucose as a carbon source (Revin *et al.*, 2018; Moosavi and Yousefi, 2011). Glucose, along with many other organic compounds (such as peptone, citric acid, etc.) can be transfigured to

form cellulose within few days with the help of this aerobic and non-photosynthetic bacterial strain. The observation of various studies indicated that a single bacterium of this particular strain is capable of converting one hundred and eight molecules of glucose ($C_6H_{12}O_6$) into cellulose $[(C_6H_{10}O_5)_n]$ every hour. The medium producing cellulose must be oxygenated, with a pH in the range of 3-5 as reported by some authors (Kim *et al.*, 2017; Jozala *et al.*, 2015) while pH 6 has also been observed (Auta *et al.*, 2017; Rangaswamy *et al.*, 2015). The ideal temperature for the manufacturing of cellulose was found to be in-between 25-30° C (Kim *et al.*, 2017; Rangaswamy *et al.*, 2015). Under these ideal conditions, the bacteria produce cellulose after 24-48 hours of the incubation period (Figure 3). These distinct parameters determine the competency in the production of bacterial cellulose, which is produced and secreted as EPS or exopolysaccharide while being in contact with the air or oxygen. Fibers formed in bacterial cellulose have a diameter in the range of nanometers. Soon after passing in the aqueous medium, the bacterial cellulose gets combined to form ribbons thereby creating a characteristic three-dimensional (3D) structure. The synthesized bacterial cellulose is white-colored artificial leather, which is highly hydrated and forms a flexible film (Andrade *et al.*, 2010). The bacteria and other residues from the culture medium are removed by heating the film in sodium hydroxide (NaOH) solution (Auta *et al.*, 2017) at boiling point for a period of about 10 to 120 minutes (depending on the thickness of the formed cellulose films) or by washing it repeatedly at an appropriate pressure. The final step in the production of cellulose is film compressing, packaging, and sterilization.

Table 1. Selected Bacterial Cellulose producing strains and the culture conditions desired for its production (Lin *et al.*, 2013; Chawla *et al.*, 2009; Ramana *et al.*, 2000).

Microorganism	Carbon source	Additive	Cultivation time	Efficiency (g/L)
<i>G. xylinus</i> ATCC 23770	Fructose, glucose	Ca(OH) ₂	-	15.40
<i>A.xylinum</i> BRCS	Glucose	Ethanol, oxygen	50 h	15.30
<i>Acetobacter sp.</i> A9	Glucose	Ethanol	8 d	15.20
<i>A. xylinum</i> BPR2001	Fructose	Agar, oxygen	72 h	14.10
<i>A.xylinum</i> BPR2001	Fructose	Agar	56 h	12.00
<i>Gluconoacetobacterxylinus</i> IFO 13773	Glucose	Lignosulphonate	7 d	10.10
<i>Acetobacter xylinum</i> NUST4.1	Glucose	Sodium alginate	5 d	6.0
<i>Lactobacillus mali</i> JCM1116	Saccharose	-	72 h	4.20
<i>Acetobacter sp.</i> V6	Glucose	Ethanol	8 d	4.16
<i>G. hansenii</i> PJK	Glucose	Oxygen	48 h	1.72

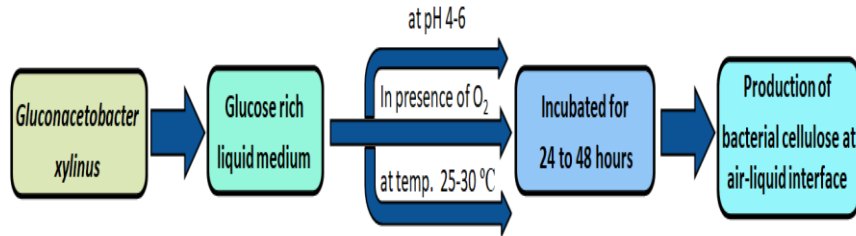


Figure 3. The schematic diagram for bacterial cellulose synthesis.

The recent methodologies for synthesizing bacterial cellulose comprise static culture, shaking/agitated culture and using bioreactors for the production of cellulose. The macroscopic arrangement, microscopic structure, and various other properties of the formed bacterial cellulose vary with the varying synthesizing methods. In the static method of bacterial cellulose production, a gel-like membrane is formed at the air-water interface of the nutrient media. While in shaking/agitated method, small spheres, asterisks, pellets or irregular masses of bacterial cellulose are formed. The methodology for the synthesis of bacterial cellulose is selected on the basis of its final use, and the requirement of the physical, morphological, as well as mechanical properties. Nowadays, many researchers are focusing on using waste products generated by agricultural and industrial sectors as a nutrient medium for producing cellulose to make the production cost-effective. Little or no difference is observed in the physicochemical properties of bacterial cellulose when produced with the help of commercially available selective nutrient medium or by using a variety of industrial or agricultural waste products. These novel research ideas are providing a broad spectrum for the usage of various waste products for the production of cost-effective and eco-friendly bacterial cellulose (Revin *et al.*, 2018).

The bacterial growth curve and the production process of bacterial cellulose are almost same for different nutrient media. As the process begins, the nutrients are sufficient for the bacterial culture but the formation of film is slow due to the lower concentration of bacteria. With the passage of time, the growth and reproduction of bacteria increases rapidly while the supply of nutrients still remains sufficient, thereby increased production of bacterial cellulose films is observed. But when all the nutrient media is consumed by the bacterial culture, the thickness of bacterial cellulose causes inverse effect on the availability of oxygen for the bacteria and hence, the synthesis of bacterial cellulose ceases. Glucose and acetic acid are found to be critical nutrient for the bacterial culture. At the beginning of the incubation, the amount of acetic acid consumed and the glucose acid produced is constant regarding fermentation and pH. But if the concentration of glucose is more in growth media than the acetic acid then the bacterial culture will be able to form gluconic acid from glucose continuously and actively during the entire process of fermentation.

Bacterial cellulose composite

Bacterial cellulose has some unique properties but there are limitations too that restrict its applications such as lack of antibacterial properties, optical transparency, and stress-bearing capability. To overcome these limitations and improve the functionality, the composites of bacterial cellulose were developed which consists of matrix and reinforcement materials. The bacterial cellulose is usually employed as a matrix and a mixture of polymers with nano-sized inorganic or organic fillers, geometry, and surface chemistry properties are used as reinforcing materials. The polymers used are normally hydrocolloids, such as proteins, starches, pectins, and other polysaccharides. Bacterial cellulose owns a porous nature due to which it acts as a matrix for housing a variety of particles from different reinforcement materials. The anchored reinforcement materials provide additional properties to bacterial cellulose that imparts its biological and physiochemical properties (Shah *et al.*, 2013). Various bacterial cellulose composites have been previously synthesized through either in-situ or ex-situ methods. For the in-situ method, reinforcement materials are added to the polymer during its synthesis (Saibuatong and Philsalaphong, 2010) while in the ex-situ process, BC is impregnated with reinforcement materials (Ul-Islam *et al.*, 2012). Some of the polymers used for synthesizing bacterial cellulose composites are chitosan, polylactic acid, starch, polyvinyl alcohol, polyhydroxyalkanoates, alginate, and gelatin. Several types of research based on the use of cellulose composites are summarized in Table 2.

Table 2. Summary of treatments and observations of various cellulose composites.

Treatments/Methods	Observations	References
BC/PLA films were prepared by solvent casting method. Mechanical, optical, and barrier properties were investigated.	The transparency of BC-PLA film was higher than that of neat BC. The incorporation of PLA to BC significantly enhanced the water vapor barrier properties of the BC membranes. The excellent mechanical properties of BC were also maintained.	Urbina <i>et al.</i> , 2016
The preparation of collagen and BC (bacterial cellulose) composite was done for evaluating its thermal, mechanical, and morphological characteristics. The effect of BC was also evaluated for detecting collagen's thermal stability.	BC synergistically interacted with the collagen and acted as its heat resistant, thereby improving the properties of the formed composite. A significant improvement of the thermal stability was observed in collagen-infused BC films. The lyophilized BC films also improved mechanically by the infusion of collagen.	Albu <i>et al.</i> , 2014
The synthesis of BC/PCL membranes was done by impregnating polycaprolactone	The preservation of type I cellulose structure in the newly formed films was observed in the as a result of X-	Barud <i>et al.</i> , 2013

Treatments/Methods	Observations	References
(PCL) acetone in bacterial cellulose (BC) films. Structural, mechanical, and optical properties were evaluated.	Ray Diffraction (XRD). The mechanical evaluation demonstrated the role of PCL as a plasticizing agent for the bacterial cellulose films. UV-visible spectroscopy revealed that the transparency of PCL/BC films increased in comparison to the pure bacterial cellulose.	
Bacterial cellulose (BC)-chitosan films were produced by impregnating BC into a chitosan solution. Mechanical properties and antimicrobial activity were evaluated.	The resulting composite films act against both gram-positive and gram-negative bacteria cultures. Also, as compared to the pure BC membranes, it demonstrated a significant increment in the coefficient of elasticity.	Lin <i>et al.</i> , 2013
Thin nanocomposite films of thermoplastic starch, chitosan, and bacterial cellulose were prepared by the solvent casting of water-based suspensions of the three polysaccharides. Optical, antibacterial, thermal, and mechanical properties were evaluated.	The addition of chitosan improves the transparency (up to 50 % transmittance). At least 25 % of chitosan and no more than 10 % of cellulose are required to observe antibacterial activity but it was found to decrease the thermal stability. The incorporation of cellulose nanofibers had the strongest positive impact on the mechanical properties of the materials (increments of up to 15 and 30 MPa on the young's modulus and tensile strength, respectively).	Tomé <i>et al.</i> , 2013
For evaluating the biodegradability, the preparation of two composites was done. One composite was prepared by mixing polyvinyl alcohol with bacterial cellulose (BC/PVA) and the other consists of bacterial cellulose, polyvinyl alcohol, along with chitosan BC/PVA/CTS.	A high degradation rate was observed in BC/PVA/CTS sheets as compared to the BC/PVA films. The SEM and spectral analysis confirmed the results. The photographs from SEM also demonstrated that the degradation of the films occur both on the surface as well as inside the composite films.	Kibédi-Kibédi-Szabó <i>et al.</i> , 2012
<i>Acetobacter xylinum</i> was employed to produce cellulose by using static culture conditions. Sodium alginate was added in the media used for producing bacterial cellulose-alginate (BCA) films.	The novel BCA film was denser, hydrophilic and had comparatively smaller size of the pore. SEM results revealed the deposition of alginate on the BCA sheet surface. The amount of alginate	Kanjanamosit <i>et al.</i> , 2010

Treatments/Methods	Observations	References
	added directly affects the Young's modulus, tensile strength, and elastic modulus of the newly-formed films. The capacity to absorb water was also increased in the newly-formed films. By adding alginate, the rate of diffusion of water vapor also increased slightly and a significant reduction was observed in the rate of oxygen transmission through the films.	
The BC/starch biocomposite samples were prepared by the solution impregnation method by adding glycerine as a plasticizer. Tensile properties of the BC/starch biocomposites were tested and compared with those of the unreinforced starch.	The obtained composite sheets were found to have a thickness of 0.5 mm and varying fiber loadings of 7.8, 15.1, and 22.0 wt.%. The BC/starch biocomposites possess much higher tensile strength and modulus than the unreinforced starch but show lower elongation at break.	Wan <i>et al.</i> , 2009
A blend of bacterial cellulose was prepared with chitosan (CHBC) by using a conventional drying method for 16 hours. The characterization of the film was done by AFM, SEM, TGA, XRD, tensile strength, etc.	The films were found to have higher flexibility, transparency, and demonstrated an increased mechanical strength in comparison with the pure chitosan membranes. These films were also thermally stable and do not permeate much oxygen.	Fernandes <i>et al.</i> , 2009
Various acrylic resins were reinforced with bacterial cellulose at loadings up to 70 wt% by reducing the average fiber size.	Highly transparent films were obtained.	Nogi <i>et al.</i> , (2006)

Loading Active Compounds on Bacterial Cellulose

Bacterial cellulose can be employed as a medium to slowly transfer the antimicrobial substance on the surface of the food, thereby extending its microbial stability. Dos Santos *et al.* (2018) obtained the antimicrobial membrane by loading bacterial cellulose films into nisin. The results of FTIR demonstrated the immobilized nisin into bacterial cellulose. This was attributed to the linkage of amino groups present in nisin into carboxylic acid groups (C-6) of bacterial cellulose. The immobilization leads to the susceptibility of the films against both gram-negative as well as gram-positive bacteria. The tubes of bacterial cellulose were impregnated with positive and negative polylysine (+-PL) to produce active cases for the sausage (Zhu *et al.*, 2010). The newly-synthesized cases demonstrated excellent barrier properties, tensile strength, and antimicrobial characteristics,

which in turn helps in increasing the product's shelf-life. The cases were also found to be thermally stable when tested in opposition to *Staphylococcus aureus* by keeping it in an autoclave for thirty minutes at 121 °C. As compared to bacteriocins alone, the bacteriocins infused with bacterial cellulose were proved to be more competent in controlling the proliferation of *L. monocytogenes* (Malheiros et al., 2018). This suggests that bacterial cellulose demonstrates a protective action on the polypeptides. Jebel and Almasi (2016) formulated a three-layered membrane in which the antimicrobial layer was placed in between the two outer layers (sandwich form). This membrane was used to monitor the rate of release of ZnO nanoparticles as an antimicrobial material. The active membrane was loaded with nanoparticles by dispersion method. It was then dehydrated and coating of wet bacterial cellulose films was done on both sides. The ZnO nanoparticles resulted in a significant decrease in the permeability of water vapor. The tensile strength of the films was increased, and ZnO nanoparticles also provided antibacterial nature to the films. Bacterial cellulose films can also be mixed with a wide range of active compounds (apart from antimicrobial agents) such as antioxidants, ethylene absorbers, oxygen scavengers, and thus, working as a transportation medium for substances that are found to promote the stability of foods.

Characteristics of bacterial cellulose and its composites

The main factors that affect food quality and safety are chemical contaminants, microorganisms, sunlight, oxygen, water vapor, perpetual force, etc. Due to these factors, it is important to preserve the quality and safety of the food items during transportation and storage conditions. To control the affecting factors of food quality and safety, the packaging materials are required to generate proper physicochemical conditions and deliver physical protection for the products obtaining enhancement of shelf life and preserving quality and safety. The tensile strength, young's modulus, and elongation of bacterial cellulose and various other synthetic polymers were compared by Gatenholm and Klemm (2010). It was found that bacterial cellulose has outstanding mechanical properties and is significantly much better than synthetic petroleum-based polymers.

There is also the significant importance of the basic properties of selected material to make sure whether the packed food is safe to eat. Besides, the packaging material should also provide a barrier against oxygen permeability, moisture, carbon dioxide, and other unwanted compounds (Reddy et al., 2016; Rhim et al., 2013). Following are some of the major properties of bacterial cellulose films:

Mechanical properties

While developing the cellulosic materials, the consideration is given more to the higher tensile strength and various other mechanical properties that could be easily achieved by drying such materials. The characteristic elastic modulus and breaking strength (per unit mass) of each and every crystal of cellulose are found to be among the highest in the list of common materials (Hubbe et al., 2017). Furthermore, due to their highly dense structure and substantial hydrogen bonds, the tensile modulus of cellulose-based films can attain values near to metals and

other advanced synthetic polymers. Bacterial cellulose was produced by the bacterium *Acetobacter xylinum* (a gram-negative bacteria) at the interface of air and liquid in a sugar-loaded medium by Gayathry and Gopaldaswamy (2014). They used Hestrin and Schramm medium for producing bacterial cellulose by employing proficient cellulose-producing bacteria. They isolated the culture from carbohydrate-rich sugarcane juice under the conditions of static batch fermentation. The results obtained for the mechanical strength of bacterial cellulose were Tensile strength 120 MPa, Young's modulus 4.9 GPa, Viscosity 127.4 cP, and Degree of polymerization 2074.

Oxygen barrier

The first and foremost concern regarding the application of packaging is to cut off the permeability of oxygen gas and various other gaseous compounds, including VOCs (volatile organic compounds) to penetrate inside the sheets of packaging materials. Regarding this, the films related to bacterial cellulose have been found to have the potential for increasing the shelf life of food products. To check the resistance of air, Gayathry and Gopaldaswamy (2014) performed the air resistance test on bacterial cellulose by measuring the time (s/50 mL) required by the air to penetrate a circular area (about 6.452 cm²) of cellulosic layer by using a pressure differential of 1.22 kPa. It was confirmed that air was not able to penetrate through the cellulosic layer. Fernandes *et al.* (2009) also studied the oxygen permeability of bacterial cellulose and its composites. They synthesized the novel composite films by using bacterial cellulose with various chitosan matrices; one high molecular weight chitosan, one low molecular weight chitosan, and one other derivative that is soluble in water. They also found similar results and confirmed that the new biocomposite materials synthesized presented low oxygen permeability.

Oil and grease resistance

The hindrance for oils and greases is directly associated with the hindrance of non-polar gases like oxygen. Basically, along with oxygen (O₂), oils and grease also does not bound firmly with the hydrogen bonds of the bacterial cellulose films and coatings. As it is important from the viewpoint of food packaging applications, various researches have focused on oil resistance. Interestingly, many kinds of research showed that the systems offering superior oil and grease holding capacity also provide an excellent barrier for the permeability of oxygen. Gayathry and Gopaldaswamy (2014) conducted a castor oil penetration test on bacterial cellulose films. They measured the time required by castor oil (a single drop) to permeate (min/0.05 mL) through the film and form a spot on the underside of the test sample. With the results of the castor oil penetration test, it was found that bacterial cellulose offers complete resistance against oil penetration.

Water vapor resistance

A thin and eco-friendly packaging material providing full hindrance to humidity and moisture would be of great advantage to the food packaging sectors. Cellulose-based materials are found to be inherently sensitive to the presence of water (both in gaseous and liquid form) (Rojo *et al.*, 2015). Also, there have been attempts to

transform bacterial cellulose into materials having improved barrier functions in specific areas. Preventing water vapor permeability is indeed a challenge. Under the conditions of high humidity and wetness, cellulose films lose their capability to avoid oxygen permeability. It is assumed that the beneficial effects of material-reinforcements for limiting the permeability of water vapor can be because of improved film integrity (like resistance to swelling in a damp environment) or due to the vapor-impermeable characteristic of crystalline cellulose.

Liquid barrier

Various studies have focused on providing barrier against liquid penetration through the packaging materials which is a vital property for various purposes. Water is a challenging liquid from the viewpoint of cellulose-based films because of its potentiality to raid and substitute hydrogen-bonding present on the surface of the cellulose films. One should note that it is just not sufficient enough to focus on attaining densely-layered bacterial film having no large pores. Rather, efforts are needed to reduce the penetration of water by focusing on minimizing the wettability of the bacterial cellulose films. Determining the contact angle of liquid is used as a criterion to recognize potential formulations for achieving resistance against liquid water (Rojo *et al.*, 2015).

Antimicrobial properties

Various strategies were evaluated by the researchers to inoculate antimicrobial properties in the bacterial cellulose-based packaging materials. This can be achieved by treating the cellulose films with food-grade chemical compounds like sorbic acid (Hubbe *et al.*, 2017; Dobre *et al.*, 2012) or chitosan which is a naturally occurring cationic polymer (Reddy *et al.*, 2016). Chitosan can increase the capability of the packaging material to protect the food against decay. Apart from this, researchers have also shown interest in using materials like colloidal zinc/silver nanoparticles combined with bio-cellulose for enhancing antimicrobial properties of packaging materials (Jebel and Almasi, 2016). Tome *et al.* (2013) studied the antibacterial activity of synthesized multi-component films against *S. aureus*. The results, as found by them, indicated that Chitosan and WCH (a water-soluble chitosan derivative) composite films represented antimicrobial activities. It was concluded that the biocidal property of the nanocomposite films was dependent on the percentage of different polysaccharides present in them. They also concluded that the starch films having 25 and 50% of the polysaccharide chitosan had a partial antibacterial effect against the pathogen *S. aureus*.

Transparency

In addition to the barrier properties and other functional attributes of bio cellulose-based films, much study has also been done on desirable characteristics such as film transparency. Generally, the researchers have found that better transparency of the film can be achieved by keeping the cellulose small, which can easily make the embedded matrices wet and it should not get tightly packed or entwined. Fernandes *et al.* (2009) also evaluated the transparency of the synthesized composite films. They prepared composite films by using bacterial cellulose with various chitosan matrices; one high

molecular weight chitosan, one low molecular weight chitosan, and one other derivative that is soluble in water through a fully green procedure. They measured the transmittance of the sheets with the help of UV visible spectrophotometer (400-700 nm) and the results demonstrated the films to be highly transparent.

Biodegradability

Biodegradability of the packaging films is regarded as the most important issue because of the day-to-day increase in the generation of solid waste. The burden on petroleum-derived synthetic plastics reduces to a greater strength when biodegradable substances are employed as packaging materials. Generally, cellulose is said to be biodegradable because of two reasons; firstly, it is liable to cellulase-producing microorganisms, and secondly, the small dimensions of cellulose avail increased exposure to the surroundings. The biodegradation problem has been highlighted in various studies where the authors used bio-cellulose with other naturally occurring film-forming substances. Urbina *et al.* (2016) impregnated the poly-lactic acid (PLA) in bacterial cellulose (BC) films. They performed the soil degradation test by putting the samples under the soil and incubating them by supplying the air every alternate day to provide aerobic conditions. Water was also added to it for ensuring a stable damp condition. The results obtained showed that PLA (Poly Lactic Acid) and PLA-PEG (Poly Lactic Acid- Poly Ethylene Glycol) films were totally disintegrated after forty-two days.

Similarly, Wan *et al.* (2009) also did the soil degradation test. They incorporated the bacterial cellulose nanofibres with starch and plasticized it with glycerol by using the method of solution impregnation. They buried the samples (30 x 10 mm) 100 mm below the soil surface and moistened them regularly with distilled water. The specimens were withdrawn at a predetermined point of time, washed with distilled water several times, dried at room temperature, and then stored in the dark for further testing. After that, the weight of test specimens was measured for determining the weight loss. They found the average degradation rate to be nearly 1% per day as for starch and 0.9% per day, for the starch-cellulose composite. The researchers observed that the loss in the average weight of the bacterial cellulose-starch composite is lesser when compared with the starch alone.

Impregnation of Bacterial Cellulose with Reinforcing Materials

The method of impregnation is not yet used as a common technique for incorporating reinforcing materials into bacterial cellulose films, but Ul-Islam *et al.* (2012) have utilized this method to form bacterial cellulose composites with a clay called montmorillonite (MMT). The bacterial cellulose films adsorbed the MMT clay. This resulted in enhanced thermal stability and increased tensile strength of the nanocomposite material.

Advantages of biocomposites

When a combination of bacterial cellulose and other materials are formed, the produced composites demonstrate enhanced dimensional stability, mechanical

properties, and air-liquid barrier when compared with the BC in its raw and pure form. These newly-formed composites may demonstrate many benefits such as being biodegradable in nature, improving sensory attributes of food, reducing the packaging volume, increasing shelf-life of packed products, individual packaging of small foods (for example; nuts, dates, and raisins), demonstrating antimicrobial properties, etc. Bacterial cellulose composites represent additional advantages such as low density, optically transparent, improved surface properties, and recyclability. The improvement of many characteristics exists in the elementary length scales controlling the morphology and characteristics of these newly formed materials. The presence of a huge interface between organic and inorganic molecules changes the mobility and the relaxation activity, enhances the mechanical characteristics, and also stabilizes the thermal capacity and melting point of the composite material. Apart from that, biocomposites are also biodegradable in nature. The decomposition of the composite polymer can be due to microbial action, macro-organisms, degradation with the help of light (photodegradation), or chemical degradation.

Applications

Packaging plays an essential role in protecting and preserving food items. The use of proper packaging materials and methods to minimize food losses and provide safe and wholesome food products have always been the focus of food packaging. Several properties are required to be fulfilled by a material to be used in food packaging. Bacterial cellulose has been recognized as an appropriate material for packaging (Esa *et al.*, 2014; Tang *et al.*, 2012) because of its fine network structure, biodegradability, and high resistivity for water. Bacterial cellulose is considered an excellent alternative for the packaging of food materials, but it lacks anti-bacterial and antioxidant properties which are essentially required to prevent food contamination. Therefore, composites of bacterial cellulose are manufactured and used to inculcate these properties in the cellulose films (Gao *et al.*, 2014). Bacterial cellulose coatings and films are mainly manufactured for its application in food packing materials, but edible coatings and films needs an extra packet on the outer side to make it hygienic. As bacterial cellulose is safe to eat, it is widely used as a material for producing coatings and films used in food business including edible coatings and films. In addition, it also found its application in the production of durable nanocrystals made up of bacterial cellulose. Combining bacterial cellulose with various other biopolymers can bring positive properties of that polymer into the bacterial cellulose films.

Barud *et al.* (2013) prepared optically transparent films by impregnating acetone solution of polycaprolactone (PCL) into dried bacterial cellulose membranes. Their UV-Vis measurements results indicated an increment in the transparency of the BC/PCL membrane when a comparison was made with pristine bacterial cellulose. They concluded that the excellent transparency of the BC/PCL was because of the presence of bacterial cellulose nanofibers which were smaller than the visible light wavelength and practically free from the scattering of the light. X-Ray Diffraction

(XRD) results of this study showed the preservation of first type of cellulosic configuration in between the PCL/BC sheets, whilst the strength attributes indicated the action of PCL as a plasticizer for the cellulose membrane. Hence, they concluded that the novel BC/PCL films can be used for synthesizing a biocompatible and flexible substance that can be used as a biodegradable packaging film for food.

Khan *et al.* (2010) also prepared biodegradable methyl cellulose-based films by solution casting method. Before casting, they also added crystalline nanocellulose into the film matrix for investigating its effect on the mechanical, thermal, and structural properties of methyl cellulose-based films. They measured the mechanical properties and water vapor resistivity of the formed films. They found that the contribution of nanocellulose fibers helped in improving the mechanical properties and resistivity of methylcellulose-based films. From their results, they concluded nanocellulose as a satisfactory reinforcing material in biodegradable packaging films (Khan *et al.*, 2010).

Bacterial cellulose works as a food packing to confirm safety and increase the shelf-life of the products. Antimicrobial ingredients, ethylene and oxygen scavengers, and moisture and taint removers are all used in active bacterial cellulose-based packaging systems (Tome *et al.*, 2010). Moreover, modified bacterial cellulose films with improved barrier and surface characteristics have been produced by esterification of hexanoyl chloride in a heterogeneously controlled environment. The bacterial cellulose formed by esterification showed an increment in the hydrophobicity while maintaining the overall configuration of the original bacterial cellulose. The barrier properties were measured by knowing whether it is permeable to moisture at various concentrations of relative humidity along with humidified nitrogen, oxygen, and carbon dioxide. Near about 50 % reduction was observed in gas as well as water permeability for the modified bacterial cellulose (Tome *et al.*, 2010).

Furthermore, Jipa *et al.* (2012) designed biodegradable bacterial cellulose (BC) and sorbic acid (SA) based monolayer and multilayer films by incorporation of SA as an antibacterial agent. The study showed that the concentration of both BC and SA affected the sensitivity to water, rate of SA release, and antibacterial activity of BC-SA mono and multilayer film. There was no SA degradation during film preparation. The faster SA release rate was observed at a lower concentration, but it became significantly slower at higher SA concentration due to the slower dissolution rate of the formed SA crystals. Moreover, the SA release rate was faster from the monolayer films compared to the multilayer films. The antibacterial susceptibility test of BC-SA was done for *E. coli*, which demonstrated that the newly formed BC sheets possess favorable antimicrobial properties.

Similarly, composite materials with antimicrobial activities were designed, where polyvinyl alcohol (PVOH) acted as a polymeric ground material and for the reinforcement, fibers of bacterial cellulose were used (Dobre *et al.* 2012). In the food processing industries, sorbic acid is widely used as a preservative, therefore, in this study it is employed as an antibacterial agent. The designed film showed an

antibacterial effect against *Escherichia coli* which revealed that new composite film could be a promising antimicrobial material for food packaging. Bandyopadhyay *et al.* (2019) formulated and evaluated the films made up of galactomannan (guar gum) mixed with bacterial cellulose which was based on PVP-CMC as a substitute material for food packaging, where PVP-CMC stands for polyvinyl pyrrolidone carboxymethyl cellulose. The evaluation was done on the basis of the structural properties, mechanical properties, color, bioadhesive nature, rheological features, glossy effect, surface hydrophobicity, water solubility, water permeability, biodegradation behavior, and enhancement of the shelf-life of blue berries. The obtained results demonstrated an improved load-bearing tendency and elasticity of the BC-CMC-PVP membranes when incorporated with guar gum. The guar gum infused films also showed enhanced hydrophobic and barrier properties as compared to the other composites used.

Future prospects

The large and economical availability of raw materials, the advanced knowledge on the chemistry of cellulosic biopolymers and the excellent properties achievable with nanocellulose particles are the key factors that make actually predictable the growing use of bio-cellulose for the design of novel and high-performance packaging materials, as well as improving the conventional ones. The real and substantial exploit of bio cellulose-based materials, however, needs some issues to be more focused on and technological solutions still have to be tested and implemented. An important point is certainly the moisture sensitivity of cellulose, a serious issue in many packaging applications, especially in the food sector. Two pathways seem nevertheless feasible: the chemical route, to reduce the hydrophilicity of the biopolymer, and the technological route related to the chance of trapping bio-cellulose as a core between more hydrophobic substances. It is even obvious to remind that packaging technology has already faced this issue while making possible use of barrier polymers like ethylene vinyl alcohol, polyvinyl alcohol, and polyamides.

Although several studies specifically focused on the safety issue of modified and unmodified nanocellulose, as many researchers suggested, further investigations yet should be carried out in near future. Nowadays, bio-cellulose is under much consideration for having a potential to develop bio-based materials, which can be realized soon at an industrial scale and applied to different fields. Thus, the safety issues of bio and nanocellulose should be precisely monitored and controlled to confirm its harmful effects on human health and on our environment. Nevertheless, there are a limited number of available studies in the field of food and packaging, and there still exists adequate scope for advanced research in these areas in more detail.

Conclusion

In present times, bio-based packing films are generally being used for packing products having short shelf-life, such as fresh fruits and vegetables, and long shelf

life like pasta and chips, which does not require very high oxygen and/or water resistance properties. Nevertheless, the array of such films demonstrates a broad spectrum of beneficial properties, which can help in making them pertinent as packing material for various other foodstuffs having strict conditions, such as Modified Atmospheric Packaging (MAP). Tests on storage conditions and on the industrial level packaging types of machinery are supposed to be carried out for confirming the use of these films at the commercial level. It could be concluded that bio cellulose-based substances have immense potential for the food packaging industry. However, it is also noteworthy to realize that a systematic assessment of the efficient properties of the cellulose-based material is crucial before its use as a substitute for conventional film materials.

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