BACTERIAL NANOCELLULOSE FOR PAPERMAKING AND PACKAGING

Pratima Bajpai

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Bacterial Nanocellulose for Papermaking and Packaging

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FOREWORD

Dr. Pratima Bajpai is one of the most prestigious and renowned authors in the pulp and paper industry. She has a great ability to write extensively yet concisely, using her expertise and the most important bibliographical references. Her books are very well accepted by people interested in improving their knowledge. Several of her texts are related to the important utilization of biotechnology in fields, such as environmental and industrial processing technologies. The utilization of microorganisms as an aid to develop new products and improve traditional (or even new) production processes has deserved great attention at present days. In recent years, bacterial or microbial cellulose has played an important role in some specific areas in terms of product development, mainly those related to medicine. As an extension of this specific biotech area, bacterial nanocellulose is expected to have new and promising industrial potentials, mainly in the paper and packaging industries. In this regard, this book by Dr. Bajpai covers theoretical, practical, and product development approaches to bacterial nanocellulose. The topics being reviewed in the book are at the frontier of technological knowledge, and Dr. Bajpai has the vision to write about them and disclose them to future book readers. These technologies are natural and environment-friendly and may bring benefits to human society. For this reason, I am sure this book will become a kind of knowledge foundation on this topic to help further developments in the industry, paper businesses, and the environment.

Celso Foelkel
Consultant, Professor, Writer, and Researcher

PREFACE

Large-scale biopolymers that may be obtained from various natural sources are the subject of an increasing amount of research. Significant advancements in this sector show how promising it is to create and apply novel biomaterials for a variety of uses. The most prevalent molecule on earth, cellulose, is one of the earliest and most promising biopolymers. The primary sources of cellulose for all goods made or produced from it are wood and cotton. Furthermore, certain bacterial species that may be cultivated in culture are also responsible for the synthesis of cellulose, as are plankton and unicellular algae found in seas. When compared to other naturally occurring or artificially created nanomaterials, bacterial nanocellulose (BNC) is a singular natural nanomaterial. Numerous bacteria have the ability to generate BNC, which helps them survive in various ecological environments. Beyond its potential applications in biology, BNC has also shown promise in the paper sector, as evidenced by recent research. High inter-fiber hydrogen bonding is ensured by its nanoscale fiber size and plenty of free hydroxyl groups. As a result, BNC has a lot of promise for use as a reinforcing material. It works particularly well with recycled and nonwoody cellulose fiber paper. Modified BNC exhibits significant promise for the creation of specialty and fire-resistant sheets in addition to improving the strength and durability of paper. By creating innovative, value-added products that extend the life of paper, BNC has the potential to completely transform the papermaking sector. To make this technique commercially viable, however, the biotechnological components of BNC must be enhanced in order to reduce manufacturing costs. The production of culture techniques, biosynthesis, special structural features, and uses in papermaking and packaging are all covered in this book to highlight the significance of BNC as a highly biocompatible and promising material that can be obtained from sustainable natural resources.

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CHAPTER 1

General Background and Introduction

Abstract: Bacterial nanocellulose (BNC) is a singular natural nanomaterial when compared to other naturally occurring or artificially created nanomaterials. Numerous bacteria have the ability to generate BNC, which helps them survive in various ecological environments. Due to its exceptional physico-chemical and biological properties, it is becoming a biomaterial that is significant in many industrial areas. BNC is a strong contender for usage in papermaking because of its intrinsic nanometric size and strength characteristics. For the manufacture of cellulose, *Gluconacetobacter xylinus*, previously known as *Acetobacter xylinus*, is the species of bacteria that has been investigated the most. These bacteria are confined behind a gelatinous, skin-like BNC membrane, which keeps them at the surface of the culture medium throughout the production of cellulose. Bacterial-derived cellulose nanofibrils have the benefit of having unique characteristics, plus the ability to modify culture conditions to change the way the nanofibrils develop and crystallize. An overview and background information on bacterial nanocellulose are provided in this chapter.

Keywords: Acetobacter xylinus, Bacterial nanocellulose, Cellulose biosynthesis, Cellulose nanofibrils, *Gluconacetobacter xylinus*, Nanomaterial, Papermaking.

INTRODUCTION

Cellulose is one of the most abundant and commercially significant biodegradable polymers on Earth on a worldwide scale (Romling and Galperin, 2015; Kim *et al.*, 2006). With the yearly production of cellulose anticipated to exceed 180 billion tons, the market is seeing an increase in demand for cellulose and its derivatives (Sundarraj and Ranganathan, 2018; Zhang *et al.*, 2021; Hafid *et al.*, 2021). Cellulose is the most prevalent biomaterial derived from renewable resources like fungi, algae, and terrestrial plants (Gupta *et al.*, 2019). It is a homogenous polymer composed of β-(1, 4) connected β-D-glucopyranose units (Moon *et al.*, 2011; Mohite and Patil, 2014). As it is widely accessible, inexpensive, and easy to process, cellulose has long drawn the interest of academics and is frequently used in many different applications (Motaung and Linganiso, 2018). Cellulose is appropriate for numerous industrial uses because of its many attributes, including its low weight, hydrophilic and hygroscopic nature, non-toxicity, mechanical strength, biodegradability, and recyclability (Zhang *et al.*, 2021; Du *et al.*, 2018; Hafid *et al.*, 2021). Cellulose and its derivatives, like microcrystalline cellulose,

cellulose esters, cellulose ethers, cellulose fiber, and nanocellulose, can be used to make a lot of different things. These are widely utilized in the manufacture of paper, textiles, pharmaceuticals, medicines for pets, cosmetics, food, and water treatment products (Lakshmi *et al.*, 2017; Arca *et al.*, 2018; He *et al.*, 2020; Kassab *et al.*, 2020).

Acetobacter xylinus was first identified in 1886, and since the 1950s, its cellulose synthesis has drawn growing interest (Brown, 1886a,b). The production of bacterial nanocellulose (BNC) from "*Acetobacter xylinum*" has been the focus of many studies since the 1970s, when Malcolm Brown and colleagues at the University of Texas conducted their studies on it (Brown, 1996, Brown *et al.*, 1976a,b). In nature, BNC biofilms are able to promote bacterial colonization of disintegrating substrates and reduce the chances that other species will effectively compete with cellulose-synthesizing bacteria for scarce resources, such as decaying fruit. The purpose of cellulose biofilms is to capture carbon dioxide generated during the tricarboxylic acid cycle, preserve moisture to keep the bacteria from getting dehydrated, and offer buoyancy to the bacteria. Moreover, it has been proposed that bacteria make cellulose to shield themselves from harmful substances and UV radiation as well as to maintain an aerobic environment (Eichhorn *et al.* 2001; Brown 2004; Putra *et al.* 2008; Rajwade *et al.* 2015; Retegi *et al.* 2010; Ross *et al.* 1991; Schramm and Hestrin 1954; Williams and Cannon 1989; Iguchi *et al.* 2000; Somerville 2006; Shoda and Sugano 2005; Glaze, 1956). The cellulose biofilms produced by plant-associated bacteria assist in binding the bacteria to the plant tissue, creating an environment that is more suitable for their growth (Romling, 2002). A special kind of nanocellulose that is used in several sectors is BNC. Despite this, its immense potential as a multifunctional material has been constrained by high production costs.

Fig. (**1**) depicts the chemical structure of BNC (Mensah *et al.*, 2021). It contains several hydroxyl groups (OH), which creates an environment that is conducive to the absorption and assimilation of other hydrophilic compounds and nanoparticles (Portela da Gama and Dourado, 2018; Keshk, 2014; Jozala *et al.*, 2016; Gama *et al.*, 2012).

Bacteria release a viscous gel made of cellulose fibrils with a delicate structure found outside of their cell walls. The BNC fibrils have a width of 20–100 nm and are made up of even smaller cellulose nanofibrils, which have a width of 2-4 nm. BNC shows a higher degree of polymerization, molar mass, crystallinity (60–80%), and purity. BNC usually has a very high mechanical strength, but it is also quite elastic and formable. Due to its extremely porous structure and huge specific surface area, BNC has a great water-retaining capacity when compared to cellulose nanoparticles derived from plants. The structure of BNC is more intricate and sophisticated. It is devoid of lignin and hemicellulose. It demonstrates increased water absorption capacity, Young's modulus, and crystallinity. It may be grown in any thickness and form and generated on a variety of substrates. The quality of the cellulose is determined by the bacterial strain and the growth medium.

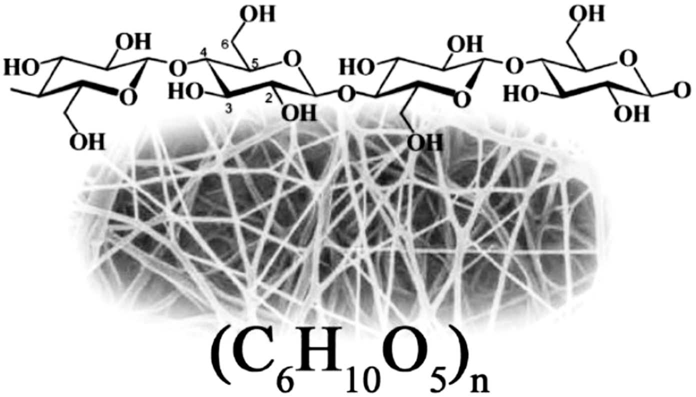

Fig. (1). Chemical structure of bacterial cellulose (Mensah *et al.*, 2021) (distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license).

The cornerstone for the economical use of BNC in the Philippines was formed in 1819 by the fortunate discovery of pineapple peels in Laguna, which were used to bleach pina cloth. In addition, acetic acid bacteria were cultivated on pineapple peels in order to promote their proliferation and cellulose synthesis. The Nata de Coco cottage enterprise has developed into a prosperous conventionally fermented food business in numerous Pacific countries after multiple attempts at fermenting under static culture (Lapuz *et al.*, 1967; Anna Seumahu *et al.*, 2007; Sanchez, 2008; Rahayu and Budhiono, 1996; Setyawaty *et al.*, 2011; Gallardo-de Jesus *et al.*, 1973).

Due to its ability to hold onto water and its nanostructured form, which resembles the shape of the cell protein collagen, BNC is perfect for immobilizing and adhering to cells. BNC is useful for a variety of applications because of its various distinctive qualities as well as the fact that it is a material that is Generally Regarded As Safe (GRAS) (Reshmy *et al.*, 2021; Skočaj, 2019; Lourenço *et al.*, 2023). Biocomposites of BNC are used in wound repair, tissue regeneration, drug delivery, and the creation of artificial blood channels (Biofill is one example) (de Amorim *et al.*, 2020). If the high production costs can be decreased by using state-of-the-art multipurpose culture medium and switching to high-efficiency bioreactors for bioprocessing, BNC could be able to compete more successfully in the biomedical business.

CHAPTER 2

Properties of Bacterial Nanocellulose

Abstract: Bacterial cellulose is recognized as a multifaceted, versatile biomaterial with abundant applications. It is a completely biodegradable, ecological, non-toxic, chemically stable, and biocompatible material. Unlike plant cellulose, it is characterized by high crystallinity, a higher degree of polymerization, and higher tensile strength and Young's modulus. In addition, bacterial cellulose, unlike vegetable cellulose, has a smaller diameter of fibres and hence possesses higher hydrophilicity. The properties of bacterial cellulose depend on multiple factors, such as culture conditions, the type of microorganisms, and nutrients present in the growth medium. These factors have a huge impact on the properties of the polymer, such as strength, crystallinity, degree of polymerization, or hygroscopicity.

Keywords: Bacterial cellulose, Biodegradable, Biocompatible, Chemically stable, Degree of polymerization, Non-toxic, Renewable.

INTRODUCTION

The use of cellulose nanoparticles, also referred to as cellulose elements with at least one dimension within the range of 1 to 100 nm, has expanded in recent years due to their numerous desirable characteristics, including their reusability, abundance, affordability, low cost of raw materials, the higher surface to volume ratio, stiffness and strength, extremely low thermal expansion coefficient, and low density and weight combined with biological degradation. There are many methods for extracting cellulose nanoparticles from lignocellulose, including acid hydrolysis and extensive mechanical processing. The fact that some bacteria may produce cellulose microfibres as a significant metabolite in a particular fermentation medium and favorable circumstances for fermentation is already well recognized (Barja, 2021). Despite having the same chemical formula as plant cellulose, bacterial nanocellulose (BNC) is fundamentally different from it because of its distinct nanofiber architecture. BNC has become increasingly popular in recent years as a result of the peculiar way in which released fibrils self-assemble into nanostructured biomaterials with extraordinary biophysical properties that are useful for a range of biological applications (Sharma and Bhardwaj, 2019; Reshmy *et al.*, 2001; Lahiri *et al.*, 2021). BNC also falls under the group of substances whose safety has been largely acknowledged.

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Adrian Brown first made reference to BNC in 1886. In fact, when studying the chemical reactions of Bacterium aceti, he utilized an additional acetic ferment known for its ability to yield the “mother of vinegar” (Brown, 1986a,b; 1996). This did not resemble Bacterium aceti in appearance, and the film that formed on the medium's surface was quite durable and felt like an animal membrane to the touch. Additionally, Brown was able to confirm that this “vinegar plant” possessed all the properties of cellulose based on the outcomes of treating this film with various chemical solutions (Hu *et al.*, 2014). The name “Bacterium xylinum” was given to this acetic ferment as a result of this discovery. Nowadays, the bacterium is referred to as *Komagataeibacter xylinus* (or sometimes *Gluconacetobacter xylinus*) and is considered to be the reference acetic acid bacteria for producing cellulose. Although plant cellulose (PC) and BNC are chemically similar, there are significant differences between them in terms of macromolecular characteristics, purity, and physical properties. Since BNC does not include lignin, hemicellulose, or pectin, it has a higher degree of purity than PC and can crystallize and polymerize to a greater extent (Czaja *et al.*, 2007). Thus, lengthy and constrictive purifying operations are avoided by using BNC for PC, thus reducing pollution. BNC contains a network of ultrafine fibers with a diameter of 20–100 nm, or approximately 100 times more slender than cellulose fibers from plants (Fig. **1A-E**). It is very resistant to wet environments, has great elasticity, and has outstanding conformability due to its distinctive nanomorphology, which allows it to hold 200 times its dry weight in water (Ross *et al.*, 1991). Bandages have been made using these unique qualities, among other things, in the medical industry (Moradali and Rehm, 2020). In actuality, BNC is a porous substance that functions as a physical barrier to prevent pathogens from the outside while allowing the passage of antibiotics and other medications. Additionally, because of its exceptional capacity to hold water, it hastens wound healing since re-epithelialization advances while the site is still moist (Ullah *et al.*, 2016; Picheth *et al.*, 2017). BNC can be used in speakers and headphones as a high-frequency diaphragm due to its extraordinary capacity to preserve its form (high Young's modulus) and quick sound transmission over a broad frequency range (Iguchi *et al.*, 2000). The paper industry also utilizes BNC since it can be added to paper pulp in particular amounts to create higher-quality paper with better tensile strength and four to five times stronger folding resistance (Iguchi *et al.*, 2000). Last but not least, the distinct inherent properties of BNC have also been marketed in industries, including food, textiles, agriculture, and cosmetics (Wang *et al.*, 2019; Lin *et al.*, 2013). Considering all of these features, together with the fact that using PC reduces forest resources and thus causes a number of environmental problems, BNC can be used as a viable substitute for plant-based cellulose.

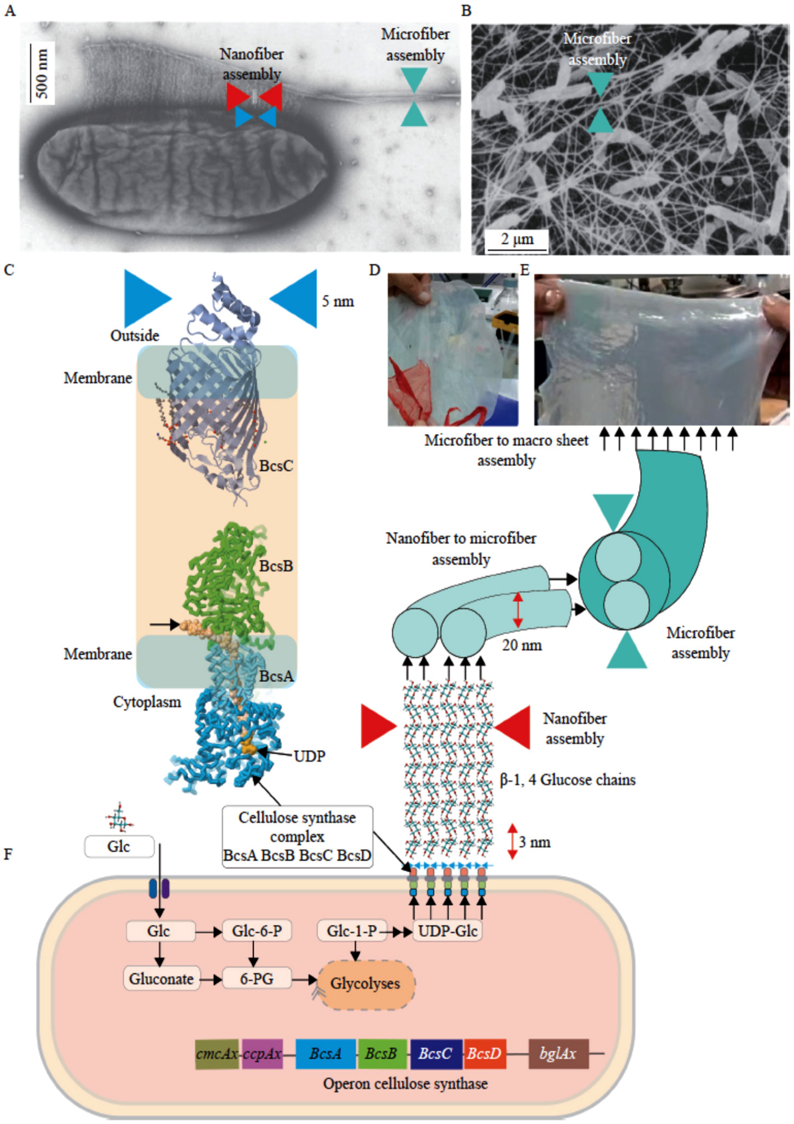

Fig. (1). Bacterial nanocellulose biosynthesis. A and B: Electron microscopy image of bacteria synthesizing cellulose and their assembly into nanofibers and microfibers in the medium. C: Cellulose synthase complex. D: Dry bacterial nanocellulose sheet (red is a dyed piece). E: Wet bacterial nanocellulose sheet. F: Schematic representation of bacterial cell synthesizing cellulose assembling into nanofiber and microfiber structures (Barja *et al.*, (2021); distributed under the Creative Commons Attribution (CC BY 4.0) license).

CHAPTER 3

Biosynthesis of Bacterial Nanocellulose

Abstract: Bacterial nanocellulose (BNC) biosynthesis is a well-organized and strictly controlled process and has two stages: first, the formation of 1,4-glucan linkages, and subsequently the assembly and cellulose crystallization. The process starts with the carbon source, such as glucose and fructose, being transported into the cell, where the cellulose precursor UDPG is produced. Bcs then polymerizes glucose from UDPG into 1,4-glucan strands. Finally, cellulose chains are secreted as sub-fibrils through pores in the cell membrane and then combined into ribbons in a 3D nanofiber network supported by hydrogen bonds.

Keywords: Bacterial cellulose, Bacterial nanocellulose, Cellulose biosynthesis, Glucokinase phosphorylates, Glucose-6-phosphate, Phosphoglucomutase, UDPG-pyrophosphorylase.

INTRODUCTION

Various bacterial genera are involved in the synthesis and metabolism of cellulose, such as *Azotobacter, Rhizobium, Aerobacter, Salmonella, Agrobacterium, Acetobacter, Achromobacter, Escherichia, Pseudomonas, Putida, Burkholderia*, *etc.* Table **1** lists some of the popular bacterial strains that produce bacterial nanocellulose (BNC).

Bacterial cell synthesis is a major contributor to the production of BNC through oxidative fermentation. Glucose chains made inside a bacterial cell are released *via* the cellular pore when a bacterial cell starts BNC production. These glucose chains aggregate to form cellulose strips. The web-like network of cellulose strips creates an extremely porous matrix network, and hydrogen bonds serve to hold the cellulose chain together. These BNC fibrils, when used as biocomposites, are about 100 times smaller in size as compared to plant cellulose. The bacterial species that has been found to produce cellulose in amounts that are commercially viable is *Acetobacter* sp. According to Esa *et al.* (2014), the fibrous patterns give BNC porosity and mechanical strength. In the presence of water and air, BNC develops a white, leathery texture. Although it has a molecular structure that is similar to phytocellulose, it is very different from it in terms of biocompatibility,

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tensile strength, purity, porosity, polymerization, and capability for water retention and reuse (Wu *et al.,* 2014).

Table 1. Bacterial nanocellulose production by some reported bacterial strains.

Bacterial Strain	**Supplement**	**Carbon Source**	**Duration**	**Yield (g/L)**	**References**
Acetobacter xylinum BRC 5	Oxygen ethanol	Glucose	50 h	15.3	Halib *et al.* (2012)
Acetobacter xylinum BPR2001	Oxygen, agar,	Fructose	72 h	14.10	Shi *et al.* (2014)
Acetobacter xylinum BPR2001	—	Molasses	72 h	7.82	Phisalaphong and Chiaoprakobkij (2012)
Acetobacter xylinum BPR2001	Agar	Fructose	56 h	12.00	Han *et al.* (2018)
A. xylinum ssp. sucrofermentans BPR2001	Oxygen, agar	Fructose	44h	8.70	Lin *et al.* (2011)
A. xylinum ssp. sucrofermentans BPR2001	Oxygen	Fructose	52 h	10.40	Khirrudin (2012)
Acetobacter xylinum NUST4.1	Sodium Alginate	Glucose	5 days	6.00	Xiao *et al.* (2012)
Gluconacetobacter xylinus IFO 13773	—	Molasses	7 days	5.76	Keshk and Sameshima, 2006
Gluconacetobacter sp. RKY5	—	Glycerol	144 h	5.63	Khirrudin (2012)
Gluconacetobacter xylinus strain K3	Green tea	Mannitol	7 days	3.34	Wu *et al.* (2013)
Gluconacetobacter xylinus IFO 13773	Lignosulfonate	Glucose	7 days	10.10	Karim *et al.* (2016)
Acetobacter xylinum E25	—	Glucose	7 days	3.50	Urbina *et al.* (2018)
Acetobacter sp. A9	Ethanol	Glucose	8 days	15.20	Shi *et al.* (2014)
Acetobacter sp. V9	Ethanol	Glucose	8 days	4.16	Hussain *et al.* (2017)
Gluconacetobacter hansenii PJK	Ethanol	Glucose	72 h	2.50	Hussain *et al.* (2017)
Gluconacetobacter hansenii PJK	Oxygen	Glucose	48 h	1.72	Lin *et al.* (2011)

Mishra *et al.* (2022) (Distributed under the terms of Creative Commons CC-BY license).

The process of BNC synthesis is a complex, highly specific, and highly controlled process that involves several steps (Barja 2021; Lee *et al.,* 2014; Tonouchi, 2016; Acheson *et al.,* 2019a, b; Morgan *et al.,* 2013). Numerous genes encode for distinct enzymes found in catalytic complexes in addition to regulatory proteins

(Fig. **1F**, Chapter 2) (Barja, 2021) When using glucose as a source of carbon, BNC is broken down by four primary metabolic pathways (Barja, 2021):

- Glucokinase phosphorylates glucose to glucose-6-phosphate (Glc-6-P).
- Phosphoglucomutase isomerises Glc-6-P to Glc-1-P.
- UDPG-pyrophosphorylase produces uridine diphosphate glucose (UDP-glucose), a direct precursor of cellulose.
- The cellulose synthase complex polymerizes UDP.

BcsA, BcsB, BcsC, and BcsD are the four proteins that make up cellulose synthase in *K. europaeus* and *K. xylinus* (Fig. **1C**, Chapter 2) (Barja, 2021). The genes that produce these proteins come together to create an operon known as “bacterial cellulose synthase” (bcs), which is controlled by a single promoter (Ross *et al.,* 1987). The catalytic role of the inner membrane in producing BNC and enlarging the transmembrane hole is played by the membrane protein BcsA (Fig. **1C** and **F**, Chapter 2) (Barja, 2021) Eight transmembrane segments make up its structure, along with two cytoplasmic domains: a catalytic β-1,--glycosyltransferase domain that is preserved between transmembrane helices four and five and a C-terminal fragment with a PilZ domain that binds the cyclic secondary messenger diguanosine monophosphate (c-di-GMP). The secondary messenger cdi- GMP stimulates BcsA activity (Ross *et al.,* 1987). C-Di-GMP binds to PilZ in BcsA, causing a change in conformation that enables UDP-glucose to pass through the catalyst site. Cellulose synthase, therefore, does not work or function poorly without cdi-GMP (Ross *et al.,* 1991).

β-1,4-glucan chains are formed when the UDP-glycoprotein disassembles due to the activity of the catalytic domain. A solitary TM helix that interacts with BcsA holds the BcsB protein on the inner membrane (Fig. **1C** and **F**, Chapter 2) (Barja, 2021).

It is believed that BcsB has two carbohydrate-binding domains that it uses to direct the polymer over the periplasm and in the direction of the outer membrane (Morgan *et al.,* 2013). BcsB is essential for the catalytic action of BcsA under all conditions due to their interaction, which enables the stability of the BcsA TM region and the synthesis of catalytically active synthase. BcsA and BcsB are combined into a single polypeptide in several species. The BcsC protein's structure, which includes a beta-barrel inside the outer membrane and a periplasmic domain that contains a tetratricopeptide repeat, suggests that this protein is involved in the construction of the complex. Moreover, pore-forming the outer membrane using BcsC would make the release of periplasmic cellulose from the cell easier. In contrast to *in vitro* research, BcsC is necessary for producing cellulose *in vivo* (Saxena *et al.,* 1994).

CHAPTER 4

Methods for the Production of Bacterial Nanocellulose

Abstract: Bacterial nanocellulose (BNC) has been produced utilizing a range of techniques, which include continuous culture techniques employing common bioprocesses like bioreactors, as well as batch and fed-batch growth techniques. The final application of BNC dictates the manufacturing strategy since the procedure directly affects the supramolecular structure and mechanical and physical characteristics of BNC. Techniques for the production of bacterial nanocellulose are described in this chapter.

Keywords: Agitated fermentation, Bacterial nanocellulose, Batch culture, Continuous culture, Fed-batch culture, Industrial-scale production, Static fermentation.

INTRODUCTION

Over the past 10 years, researchers have become interested in bacterial nanocellulose (BNC) because of its excellent physical properties, which include biocompatibility, thermal stability, crystallinity, and good tensile capabilities (Zhang *et al.,* 2020; Abol-Fotouh *et al.,* 2019; Reshmy *et al.* 2021; Al-Hagar and Abol-Fotouh, 2022). Even though its molecular structure resembles plant cellulose (C6H10O5), BNC is formed as a network of three-dimensional nanofibers, is naturally clean and devoid of hemicellulose and lignin, and has far superior properties in terms of water-holding capacity, surface area, and polymerization (Cacicedo *et al.,* 2016; Chen, 2016). BNC has been utilized in a number of industries, including food packaging, food industry, waste-water treatment, textiles, pharmaceutical and biomedical industries, as well as electroconductive composites (Abol-Fotouh *et al.,* 2019; Reshmy *et al.,* 2021; Gregory *et al.,* 2021). Despite the wide variability in production output, it has been demonstrated that several taxa, including *Agrobacterium, Komagataeibacter, Agrobacterium, Azotobacter, Rhizobium, Salmonella, Sarcina, and Achromobacter*, among others, synthesize BNC (Rahman, *et al.,* 2021). However, *Komagataeibacter* (formerly *Acetobacter*) members were found to be the most productive producers of BNC. *Komagataeibacter xylinus* is used as the model bacterial strain for BNC synthesis (Singhania *et al.,* 2021). Although BNC is becoming more important on a global basis, there are still a number of obstacles in the way of its mass production and use, such as lengthy propagation times, limited yields, and thin cellulose layers (Blanco Parte *et al.* 2020). Researchers have worked hard to improve the BNC yield independent of production circumstances, medium composition, or strain efficacy. The medium makes up around 30% of the overall production expenses (Zhang *et al.,* 2020; Abol-Fotouh *et al.,* 2020). Researchers have explored less costly sources of carbon and nitrogen, such as food leftovers, paper industry effluents, wastes from the textile industry, and agro-industrial wastes. Optimizing the production parameters is another way to increase the BNC yield. Furthermore, there is a need to examine several bioreactors to identify which ones would provide the ideal circumstances for the used bacteria, whether they are in a static or agitated form (Gregory, *et al.,* 2021).

BNC has been produced utilizing a range of techniques, including fed-batch and batch culture as well as continuous cultivation in bioreactors and other common bioprocesses (Costa *et al.,* 2017; Raiszadeh-Jahromi *et al.,* 2020; Ye *et al.,* 2019; Tsouko *et al.,* 2020; Kumar *et al.,* 2019; Revin *et al.,* 2018; Zhang *et al.,* 2018; Yang *et al.,* 2019; Mangayil *et al.,* 2021; Lee *et al.,* 2021; Wu *et al.,* 2021; Khan *et al.,* 2020; Gao *et al.,* 2021; Sharma and Bhardwaj, 2019).

Numerous experiments comparing the static and agitated modes of BNC manufacturing have been conducted to determine which is the most effective technique (Czaja *et al.,* 2004; Zywicka *et al.,* 2015). The discovery of new strains producing BNC emerged (Castro *et al.,* 2012). The ultimate use of BNC dictates the manufacturing approach, as the process has a direct effect on the material's supramolecular structure and also its physical and mechanical properties. In place of traditional sugar sources, novel substrates like wastewater from various industries, many of which are rich in sugars, such as the wastewater from hot water wood extract, candied jujube-processing industry, and agroindustrial wastes like sugarcane scum during production of jaggery, coconut water, and pineapple waste, are becoming more important (Li *et al.,* 2015; Kiziltas *et al.,* 2015; Adnan, 2015; Khattak *et al.,* 2015; Vazquez *et al.,* 2013).

The final use of BNC determines whether these two production methods, stirred culture or static culture, should be used as the technique of culture that affects the physical, mechanical, and morphological properties of the resultant polymer. For instance, cellulose generated in agitated culture is less strong mechanically than cellulose produced in static culture. Contrary to static cultures, agitated cultures yield lower yields and are more susceptible to microbial mutation, which may influence BNC generation. Greater culture space and more time are needed for static cultures (Jeon *et al.* 2014; Chawla *et al.* 2009; Keshk, 2014; Lee *et al.* 2014; Cakar *et al.* 2014; Reshmy *et al.,* 2021; Singhania *et al.,* 2022; Tyagi and Suresh, 2015).

Fig. (**1**) shows bacterial cellulose production (de Souza *et al.,* 2023). At the air-liquid contact of the culture medium, a gelatinous pellicle develops during static fermentation (Figs. **2a** and **b**) (Zhong, 2020). The culture fluid contains little, uneven pellets that are suspended entirely during an agitated fermentation (Figs. **2c** and **d**) (Zhong, 2020).

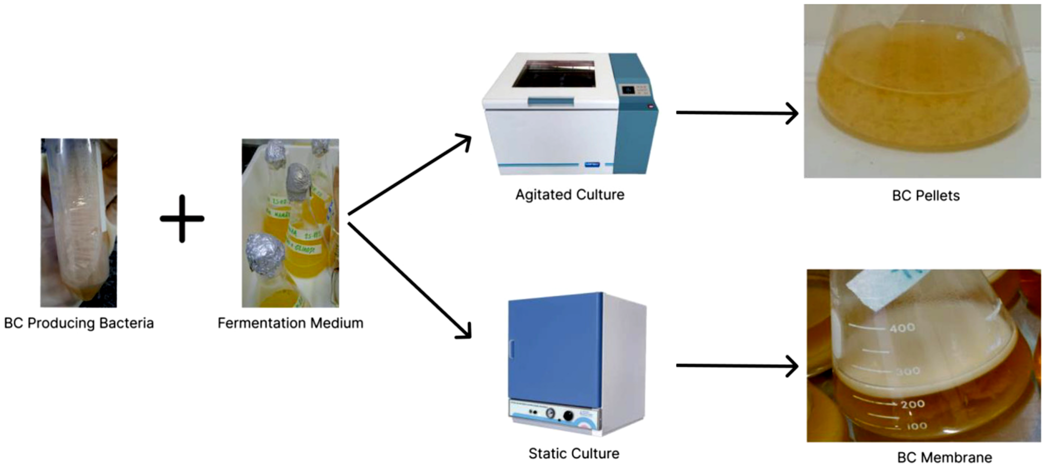

Fig. (1). Bacterial cellulose production. de Souza *et al.* (2023). Distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

Static Fermentation

The most common way of making BNC is by static cultivation, which results in the formation of a dense layer of white, leather-like pellicle BCP at the interface between the air and the liquid (Kuo *et al.* 2015b). The creation of flat BNC with very pure structures and properties is advantageous for the application for which it is designed. It produces pellicles that are utilized in wound treatment and have a lamellar structure and little branching.

One well-known and often utilized technique for creating BNC is static culture. The method is well-liked for fabricating BNC in a lab setting as it is simple to use and necessitates little shear force. Incubation takes place in different shapes and sizes of containers using a culture media that has a pH range of 4.5 to 6.5. BNC, made using the static technique, has the same form as the container holding the nutrition media. Based on several studies, this approach is utilized to yield the most uniform supramolecular BNC structure and very stable substance. Due to its

CHAPTER 5

Application of Bacterial Nanocellulose in Papermaking and Packaging

Abstract: Bacterial nanocellulose (BNC) has several intriguing potential uses and is now employed in various industries because of its remarkable mechanical qualities. The application of bacterial nanocellulose in papermaking and packaging is discussed. A brief description of papermaking and packaging is also presented. BNC is a preferred material for the paper manufacturing sector. The active and intelligent food packaging of BNC offers a new and innovative approach to extending the shelf life and maintaining, improving, or monitoring product quality and safety.

Keywords: Active packaging, Bacterial nanocellulose, Barrier properties, Coating, Cellulose nanofibrils, Fire-resistant paper, Intelligent packaging, Mineral filler, Papermaking, Packaging, Printing quality, Specialty papers.

INTRODUCTION

It is crucial to familiarize readers with stock preparation, papermaking, and packaging procedures before delving into the use of bacterial nanocellulose in these processes.

Brief Description of Stock Preparation, Papermaking, and Packaging Process

A number of procedures are used in stock preparation to adjust pulp qualities so they match the final product. Optimizing stock preparation requires striking a balance between the dependability and efficiency of the papermaking process and the final product's quality. Fiber stock preparation systems aim to adjust various raw materials so that the final stock provided to the paper machine meets both the equipment's specifications and the quality standards set for the paper or board that is produced. Both recovered paper grades and other kinds of virgin pulps are utilized as raw materials (Biermann, 1996; Paulapuro, 2000). They are available for purchase as bundles, loose stuff, or suspensions in integrated mill scenarios. Regarding the fibers, additives, and contaminants, the end product is a suspension with predefined quality in terms of composition and attributes. This quality essen-

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tially determines the runnability of paper machines. It serves as the foundation for both the board's and the finished paper's quality.

The manufacturing of paper and paperboard involves many processes (Table **1**). Similarly, in stock preparation, several steps are involved (Table **2**). These procedures differ significantly depending on the required supply quality and the raw stock used. For example, the slushing and deflaking processes are skipped when the pulp is pushed straight out of the pulp mill. The following processes are carried out at paper mills: metering, mixing, and dispersion of fiber and additives. Regarding the manufacture of virgin pulp stock, Kadant Lamort has presented some novel ideas.

Table 1. Steps involved in the manufacturing of pulp and paper.

Operation	Processes
Raw Material Preparation	Debarking Chipping and Conveying
Pulping	Chemical Pulping Semichemical Pulping Mechanical Pulping Recycled Paper Pulping
Chemical Recovery	Evaporation Recovery Boiler Recausticizing Calcining
Bleaching	Mechanical or Chemical Pulp Bleaching
Stock Preparation and Papermaking	Preparation of stock Dewatering, Pressing and drying Finishing

Table 2. Unit processes in stock preparation.

Unit Process	Objective
Slushing and deflaking	To break down the fiber raw material into a suspension of individual fibers. Slushing should at least result in a pumpable suspension, enabling coarse separation and deflaking if required. In the case of recovered paper, ink particles and other nonpaper particles should be detached from the fibers.
Screening	To separate particles from the suspension, which differ in size, shape, and deformability from the fibers.
Fractionation	To separate fiber fractions from each other according to defined criteria, such as size or deformability of the fibers.

(Table 2) cont.....

Unit Process	Objective
Centrifugal cleaning	To separate particles from the suspension, which differ in specific gravity, size, and shape from the fibers.
Refining	To modify the morphology and surface characteristics of the fibers.
Selective flotation	To separate particles from the suspension, which differ in surface properties (hydrophobicity) from the fibers.
Nonselective flotation	To separate fine and dissolved solids from water.
Bleaching	To impart yellowed or brown fibers with the required brightness and luminance.
Washing	To separate fine solid particles from suspension (solid/solid separation).
Dewatering	To separate water and solids.
Dispersing	To reduce the size of dirt specks and stickies (visibility, floatability), to detach ink particles from fibers.

Based on Holik (2006).

To create a slush or slurry, dry pulp is mixed with water using pulpers. The pulper's stock undergoes frequent acceleration and deceleration, and the steep velocity gradients create hydrodynamic shear forces that follow work to separate any flakes into individual fibers and relax the fibers.

For the majority of paper grades, pulp from a mill that does not undergo mechanical treatment is inappropriate. Unbeaten virgin pulp produces bulky, rough-surfaced paper with low strength. Strong bonds must form at the places of contact, and the fibers need to be evenly matted into a sheet of high-quality paper. The techniques that alter the undesired traits include beating and refining (Baker, 2000). One of the most crucial steps in preparing fibers for papermaking is mechanical treatment. "Beating" refers to batch processing stock in a Hollander beater or similar device. When pulps are constantly run through one or more refiners, either in parallel or in series, the process is referred to as refining. For particular paper grades, refining generates distinct fiber characteristics in different ways. Generally, the goal is to minimize the formation of drainage resistance while maximizing the bonding capacity of the fibers without unduly weakening each one individually. Therefore, the qualities needed for the finished paper serve as the basis for the refining process. Due to variations in their physical characteristics, various fiber types respond in multiple ways (Baker, 2000, 2005). The kind of fibers must be considered throughout the refining process. Table **3** presents typical refining conditions for a few hardwood pulps. Refining is affected by a number of elements, including the kind of pulps, equipment characteristics, and process parameters (Table **4**).

CHAPTER 6

Challenges and Future Perspectives

Abstract: Bacterial nanocellulose (BNC) is a material of enormous industrial concern and is known to have applicability in versatile fields. Therefore, the additional impetus is obligatory to make this greener material a competitive product while at the same time being economically viable. BNC is widely used in different technological applications. Thus, there are constant efforts for feasible procurement of BNC by bringing down the production costs and yield augmentation and overall improving its performance by tailoring the physical, mechanical and biological properties. BNC has great potential as a reinforcing material and is especially applicable for recycled paper and for paper made of nonwoody cellulose fiber. By enhancing the strength and durability of paper, modified BNC shows great potential for the production of fire-resistant and specialized papers. However, the biotechnological aspects of BNC need to be improved to minimize the cost of its production and, thus, make this process economically feasible.

Keywords: Bacterial nanocellulose, Green material, Low-cost substrates, Natural biopolymer, Nonwoody cellulose fiber, Specialty papers.

INTRODUCTION

BNC has caught the attention of researchers and industrialists from a wide range of fields because of its unique properties. BNC has nano-structures that are highly purified and highly crystalline. The high production of BNC at a high yield is comparable to the yield produced by photosynthesis in plant cellulose. Additionally, a lot less area is needed for fermentation than for plant development. Fermentation also uses industrial and agricultural waste as nutrients. This lowers the expense as well as the incorrect disposal of waste-related environmental contaminants. From a structural standpoint, BNC is distinguished by its distinct structure, which includes an uncharged 3D reticulated network. As a result, BNC has additional benefits, such as superior mechanical qualities and a large water-holding capacity. It also has high gas permeability and excellent suspension stability. Furthermore, BNC has low viscosities and is highly tolerant to acids, salts, and ethanol. Moreover, BNC is renewable, bio-compatible, and biodegradable. Furthermore, BNC with various morphologies and physico-chemical properties can be easily produced by the addition of polymers and nanoparticles in the culture medium. As a result, BNC is an environmentally

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friendly and highly competitive substitute for plant-based cellulose nano-fibers (Zhong, 2020; Sharma and Bhardwaj, 2019).

Up to now, both static and agitated fermentations have been used in industry to create BNC. BNC has found widespread commercial use in a variety of industries, including food processing, personal hygiene products, home chemicals, biomedical fields, textiles, pulp and paper, and composite materials. The applications of BNC will grow in number in the future. However, there are also a number of areas that need to be improved for further industrial production and application development of BNC. Static fermentation has a restricted capacity for output since it takes more time and work. A number of strategies may be used to increase production efficiency, such as isolating strains that produce large amounts of BNC, creating novel culture medium and fermentation reactors, and using automated machinery. Large amounts of BNC may be produced *via* agitated fermentation, although the yield of BNC is decreased by bacterial mutations that do not make cellulose. As a result, there is a constant need to increase the yield and production efficiency of BNC. The price of BNC is more than that of cellulose nanofibers obtained from plants, and as marketing needs rise, industrial wastes, such as coconut water, become more costly and insufficient. Thus, novel low-cost nutrient sources for BNC production may also be utilized, including fruit juices, liquid fermentation effluent, and beet molasses. There are still very few uses for BNC nowadays. Exploiting these new uses for BNC should receive more attention. These firms should be releasing well-developed items from their ongoing development. Plant-based cellulose nanofibers are produced more quickly in the industrial process. Plant-based cellulose nanofibers and BNC will have a competitive relationship in some sectors. As a result, BNC may continue to be competitive in the business sector by effectively utilizing its advantages (Zhong, 2020).

Mass production of BNC is currently not possible despite several studies exploring the viability of low-cost industrial-scale manufacturing procedures for BNC. Nonetheless, as BNC is attracting industrial attention and is seen as a potential commodity with multi-field applications, efforts are being made to convert this biotechnological product into a commercially viable and competitive component.

Numerous studies have also explored the potential applications of BNC, but a significant amount of studies are needed to look into all of the possibilities related to its biotechnological production, especially with regard to the culture medium's cost-effectiveness. This will enable further uses for BNC, particularly in the areas of ecological (*e.g.,* decreased usage of organic solvents and metals) and nanotechnology (*e.g.,* nanoparticles as a delivery system for food, cosmetics, and pharmaceutical items). Thus, research on better fermentation methods for increased output ought to go on.

The use of mother vinegar, an agro-industrial waste product of conventional vinegar manufacture, is one potential remedy that would be appropriate for the papermaking sector (Lavrič Gregor *et al.,* 2020). This agro-industrial waste is almost the same as BNC made in a static environment. It is not clear, unlike BNC made in a lab, but the stain from the fermented fruit is much lessened when it is bathed in sodium hydroxide and dried. However, as acetic acid is always a fermentation byproduct of acetic bacteria fermented in a static culture with BNC as a byproduct, the smell of the mother vinegar, or acetic acid, is not a problem. During the mother vinegar's lyophilization process, the acetic acid is removed and becomes undetectable. Furthermore, no complaints of an acetic odor in the finished product, that is, BNC, have been made when BNC was made under static fermentation conditions. In fact, few studies have demonstrated that mother vinegar is a more cost-effective and preferred raw material for the manufacture of BNC than commercially available plant-derived nanocellulose. The paper sheets that have been enhanced with BNC from mother vinegar exhibit properties similar to those that are being presented here. Although this residue might not be appropriate for use in biological applications, it could be an excellent alternative to the costly industrially manufactured nanocellulose, or BNC, used in the papermaking sector (Lavrič Gregor *et al.,* 2020). The objectives of a circular economy are met by the recycling of residuals, which reduces waste and encourages its reuse in a new industry.

Increasing the cost-effectiveness of cultural media can likewise boost BNC production. Static fermentation limits production capacity since it takes more time and effort. Finding high-yielding BNC-producing strains, introducing better culture media and bioreactors, and utilizing automated equipment are some strategies for improving production efficiency. BNC can be produced by large-scale agitated fermentation. However, its productivity is limited by bacterial mutations that do not produce cellulose. BNC production capacity is, therefore, continuously in need of development. High-end nanocellulose production may surely be enhanced by biotechnological approaches, including low-cost substrates combined with high-yielding microbial species and innovative bioreactor designs with optimized process parameters.

Despite a lot of research being done on BNC manufacturing, the objective of producing BNC in an economically viable manner has not been satisfactorily attained. The problems in achieving effective manufacture and continued use of BNC are mentioned in Table **1**. These obstacles fall into four main categories:

SUBJECT INDEX