

BACTERIAL NANOCELLULOSE FOR PAPERMAKING AND PACKAGING



Pratima Bajpai

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Bacterial Nanocellulose for Papermaking and Packaging

Authored by

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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 (Sundarraaj 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 Liganiso, 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.

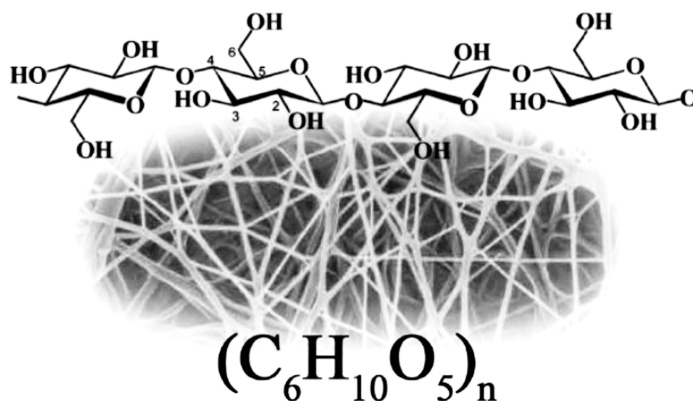


Fig. (1). Chemical structure of bacterial cellulose (Mensah *et al.*, 2021) (distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license).

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.

According to a worldwide projection study, the BNC market will increase three times more quickly in the upcoming years. The main areas to focus on optimizing for a low-cost future BNC market are genetic alterations, instability issues, substrate selection, biosynthetic processes, and manufacturing technology viability. As the biosynthetic process causes fibrils with a variety of physiochemical characteristics to self-assemble, it is becoming more popular. BNC may be produced with exceptional purity using biosynthetic techniques since it is free of lignin, pectin, and hemicellulose. As a result, inefficient purifying procedures can be avoided. The identification of potential microorganisms involved in the manufacture of BNC has been made feasible by the biosynthetic pathways (Barja, 2021). To prevent both an inadequate supply of essential precursors for downstream biosynthesis and an excessive metabolic load brought on by overexpression of the system, it is essential to determine the appropriate level of biosynthetic pathway enzyme expression. For instance, sugar-rich biowaste that is more readily available and includes sugar cane bagasse, fruit and vegetable waste, wood processing waste, and others is becoming increasingly important as a source of nutrients for biosynthesis. Therefore, to fully utilize BNC for commercial applications, new feedstocks must be used for cost-effective BNC synthesis, and upgraded bioprocess methods must be used for scaling up.

The characteristics, the lengthy production time (5 to 20 days), and the poor yield (8 g/L) of the target metabolite are only a few of the difficulties encountered throughout the BNC manufacturing process' scaling up (Shavyrkina *et al.*, 2021). One possible way to raise the yield of BNC manufacturing is to improve fermentation technology (Sil *et al.*, 2024; Bai *et al.*, 2024).

However, because of the microbiological producers, the strict aerobes that make BNC, it is unclear how to control culture, both dynamic and static. To develop a semi-continuous growth strategy, Kralisch *et al.* (2010) coupled static and agitated culture methods with a horizontal lift reactor to harvest BNC biofilms. This method was applied to process scaling up to significantly reduce manufacturing costs. Fig. (2) shows the characteristics of BNC, and Table 1 presents the characteristics of BNC to be used in various applications (Reshmy *et al.*, 2021). Figs. (3a and b) present applications of bacterial cellulose in different fields (Lahiri *et al.*, 2021; Reshmy *et al.*, 2021).

Donini *et al.*, in 2010, conducted a study to evaluate the advantages of producing microbial cellulose in comparison to the synthesis of the same material from plants and microbes. The study made a comparison of the production of cellulose from one hectare of Eucalyptus, which had an average annual increment of 50 m³, resulting in a minimum annual yield of 25 tonnes per hectare per year and a base density of 500 kilograms per cubic meter. This technique was found to yield

approximately 80 tonnes of cellulose per hectare after seven years of cultivation, utilizing a seven-year growth cycle with a cellulose yield of 45%. The scientists discovered that in a bioreactor of 500 m³, it would take around 22 days for bacteria to produce the same amount of product at a hypothetical yield of 15 g/L in 50 hours of growth (on average, 0.3 g/h). BNC produced by this effective approach was pure and environmentally friendly. In contrast to plant cellulose, BNC is created in its purest form, free of any animal byproducts, and manufactured without hemicelluloses, lignin, pectin, or any other ingredient found in plant pulp. In addition, it outperforms plant cellulose in terms of mechanical qualities (Fu *et al.*, 2013). Fibers that are entirely not soluble in water but may be hydrate are compacted as a result of the intimate interaction between the anhydroglucose units and different BNC fibrils to produce a crystalline structure (Conley *et al.* 2016; Lynd *et al.* 2002). Due to the hydrophilic nature of BNC and the huge surface area per unit of thin nanofibers, they are more capable of absorbing water, adhere better, and contain more moisture (Numata *et al.* 2015; Fu *et al.* 2013).

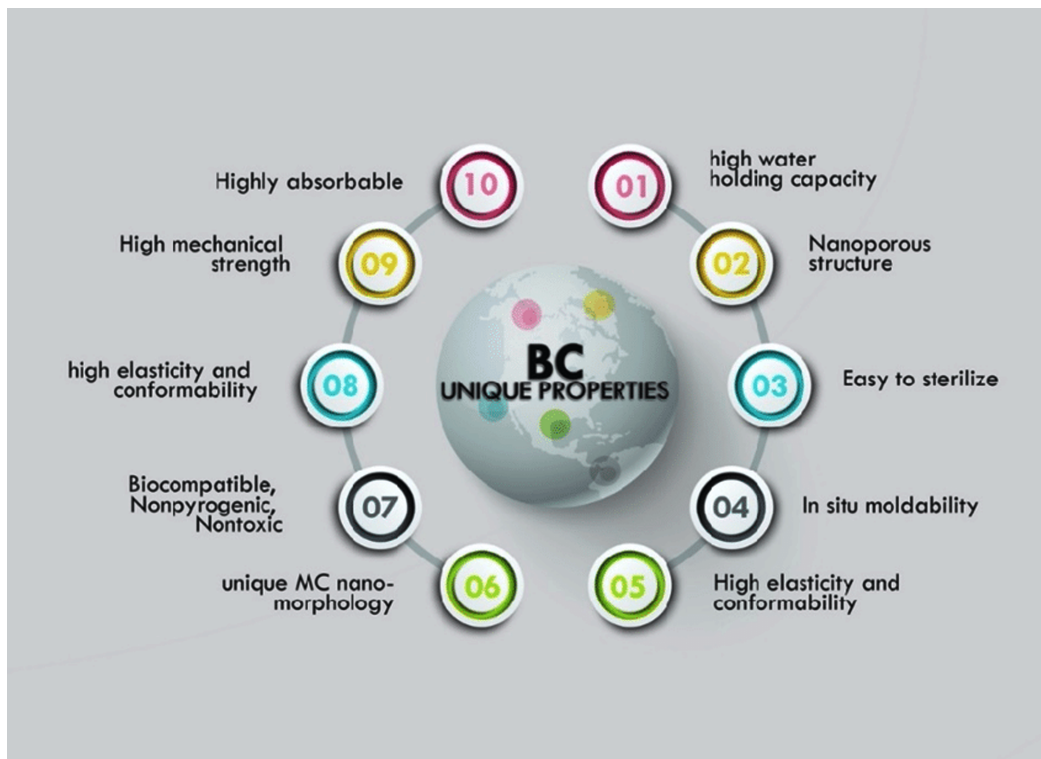


Fig. (2). Characteristics of bacterial nanocellulose (Mensah *et al.*, 2021) (distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license).

Table 1. Characteristics of bacterial nanocellulose for implementation in various applications.

Mechanical strength	BNC's impressive mechanical characteristics make it ideal for use as a load-bearing material in a variety of applications, including food packaging and medicinal implants.	de Amorim <i>et al.</i> (2020); Nimeskern <i>et al.</i> (2013)
Withstand extremely high temperature	Sterilization using Gamma rays or steam is simple; it is ideal for packing sterile. equipment.	Sharma and Bhardwaj (2019).
Absorbency and resistance to fiber lift	For paper-based dressings.	Sharma and Bhardwaj (2019).
Biocompatibility- nontoxic to human cells	BNC-based implants, wound healing dressings, and personal care products can all benefit from this property.	Fernandes <i>et al.</i> (2021).
Wet strength – the capability to withstand extremely high temperature	As labeling adhesive for clinical samples, such as blood, while being kept in deep freezers, which can withstand extremely low temperatures.	Wu <i>et al.</i> (2014); Tusnim <i>et al.</i> (2020)
Application orient processability	Enhanced mechanical and barrier properties, along with oil absorbency, air permeability, and antimicrobial properties upon functionalization, open up easy molding to different shapes for wider applications.	Breuer (2010); Jawaid and Kumar (2018).
Inertness – to avoid any reactions between packed products during storage	For packing food items, medicines, and surgical devices.	Bacakova <i>et al.</i> (2019); Rajwade <i>et al.</i> (2015)
Biodegradability	Scaffolds should disintegrate within the body after the primary extracellular matrix development starts; thus, this is very useful when building them.	Alizadeh-Osgouei <i>et al.</i> (2019); Weng <i>et al.</i> (2013).

Reshmy *et al.* (2021). Distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>).

BNC is the perfect material to use for making a range of high-quality items like synthetic skin that is temporarily substituted with natural skin while treating burns and other cutaneous ailments. This is on top of the molecule's distinct mechanical and physical properties other than its ability to be biodegradable, insoluble, flexible, durable, strong, non-toxic, and non-allergenic (Cakar *et al.* 2014; Rehim *et al.* 2014; Thompson and Hamilton 2001).

BNC is a glucose-based linear polymer that is extremely crystalline and is mostly produced by *Gluconacetobacter xylinus* bacteria, which was previously known as *Acetobacter xylinus*. While most studies on BNC synthesis have focused on *G. xylinus*, other bacteria, including other *Gluconacetobacter* species, *Rhizobium* spp., Gram-positive *Sarcina ventriculi*, and *Agrobacterium tumefaciens*, also