



Exploring the Versatility and Potential of Microbial Cellulose: Applications, Producers, and Sustainable Production Methods

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Abstract

Microbial cellulose (MC) is a versatile biomaterial synthesized by various microorganisms, possessing unique properties such as high tensile strength, biocompatibility, and biodegradability. These characteristics make MC suitable for applications in biomedical, food, and packaging industries. Traditionally, *Acetobacter aceti* has been the primary source for MC production, but recent research has identified other potential microbial producers such as *Komagataeibacter xylinus*, *Sarcina ventriculi*, and *Rhizobium* sp., which offer efficient cellulose synthesis. This study reviews the diverse applications of MC, including wound dressings, edible films, and composites, and discusses cost-effective substrates like sugarcane molasses and fruit waste used in MC production. The paper highlights the importance of sustainable and scalable production methods, exploring the impact of different substrates and environmental conditions on MC yield. Through understanding these factors, the potential for MC-based materials in various industries can be fully realized, promoting environmental sustainability and innovation in material science.

Keywords: Biomaterials; Cost-effective Substrates; Microbial Cellulose; Sustainable Production

Abbreviations

MC: Microbial Cellulose; BC: Bacterial Cellulose; OLED: Organic Light Emitting Diodes.

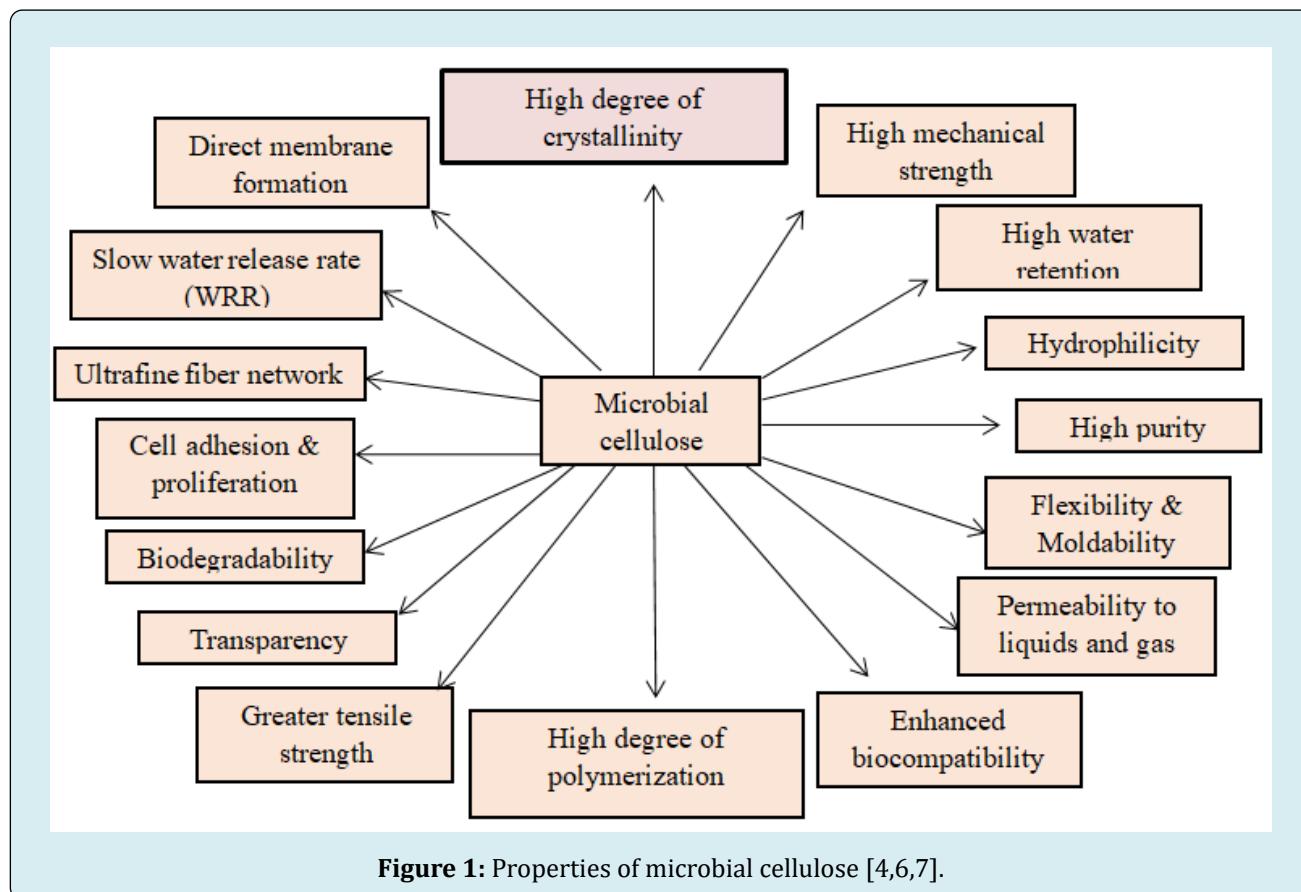
Introduction

Microbial Cellulose

Microbial cellulose (MC), an exopolysaccharide which is produced by bacteria [1]. Though MC is highly pure compared

to plant cellulose it has unique nano structure and mechanical properties [2]. MC has several unique properties and those are listed below (Figure 1). MC could serve as ideal biomass for the development of various industrial products with these extraordinary properties [3]. MC do not require undergoing delignification and other energy and resource intensive processes, thus it is able to retain structural integrity [4]. The

production of eco-friendly products is becoming increasingly important; in this context, the production of nanocellulose through microbial pathways is advantageous. Indeed, MC production using microorganisms is industrially important because such microorganisms exhibit rapid growth, allowing for high yields and year-round availability of product [5].



Methods for Producing MC

There are two main methods for producing MC using microorganisms (Table 1).

Method	Description
1. Static culture	Accumulation of a thick, leather-like white Bacterial Cellulose pellicle at the air-liquid interface [8]
2. Stirred culture	Cellulose is synthesized in a dispersed manner in the culture medium, forming irregular pellets or suspended fibres [6,9]

Table 1: Methods for producing MC.

Standardized Characteristics and Cellulose Production Potential of Various Microorganisms

This Table 2 provides a comprehensive overview of the standardized characteristics and cellulose production potential of various microorganisms. It includes information

on their Gram stain classification, morphology, respiratory metabolism, and a brief description highlighting their unique attributes and relevance to cellulose production. This standardization helps in understanding and comparing the capabilities of different microorganisms in the context of industrial and scientific applications.

Microorganism	Gram Stain	Morphology	Respiratory Metabolism	Description
<i>Komagataeibacter xylinus</i>	Negative	Rod	Aerobic	Efficiently produces high-quality cellulose with a high degree of polymerization, making it a promising source for cellulose-based materials [10].
<i>Komagataeibacter hansenii</i>	Negative	Rod	Aerobic	Synthesizes bacterial nanocellulose with an improved ability to stretch [11].
<i>Sarcina ventriculi</i>	Positive	Coccus	Anaerobic	Capable of growing on sugars within a wide pH range (2 to 10) and produces cellulose with a degree of polymerization similar to that produced by <i>Acetobacter aceti</i> , making it a potential alternative source of microbial cellulose [12,13].
<i>Rhizobium</i> sp.	Negative	Rod	Aerobic	Found in roots of leguminous plants, these symbiotic nitrogen-fixing bacteria produce cellulose fibrils during growth in the absence of plant cells. The fibrils are 5-6 nm in diameter and up to 10 μ m in length, with a surface pellicle forming in standing cultures [14,15].
<i>Pseudomonas</i> sp.	Negative	Rod	Aerobic	Produces cellulose with a lower degree of polymerization than <i>Acetobacter aceti</i> , but with relatively high efficiency, making it a potential alternative source for industrial-scale microbial cellulose production [16].
<i>Agrobacterium tumefaciens</i>	Negative	Rod	Aerobic	A plant pathogen and free-living soil organism that synthesizes cellulose from various substrates. Cellulose synthesis is a constitutive feature, with cells maintaining high synthesis capacity even in the absence of plant cells [17,18].
<i>Achromobacter</i> sp.	Negative	Rod	Aerobic	The most potent producer isolated from strawberry, identified as <i>Achromobacter</i> S3 using 16S rRNA techniques. Mango peel waste hydrolysate is a significant inducible medium for maximum productivity without extra nutrients [19].
<i>Pichia kudriavzevii</i> (Yeast)	Not applicable (fungus)	Yeast	Facultative anaerobe	Isolated from rotten pineapple, characterized using 18S rDNA analyses. SEM analysis shows pellicles as an interwoven network of fibres [20].
<i>Alcaligenes</i> sp.	Negative	Rod	Aerobic	Strain KT201, a microbial-flocculant-producing bacterium isolated from soil, secretes flocculant in a sucrose-containing culture broth [21].
<i>Aerobacter</i> sp.	Negative	Rod	Facultative anaerobe	Strain 322 produces homogeneous floc and cellulose fibrils visible under an electron microscope, with significant dispersion action of cellulase [22].
<i>Salmonella enteritidis</i>	Negative	Rod	Facultative anaerobe	Cellulose production and biofilm formation are crucial for survival on surface environments. Cellulose is a major constituent of its biofilm matrix [23,24].
<i>Escherichia coli</i>	Negative	Rod	Facultative anaerobe	More than 50% of tested strains can produce cellulose, with alternative cellulose pathways being common [24].

Table 2: Comprehensive overview of the standardized characteristics and cellulose production potential of various microorganisms.

One important and challenging aspect of the MC Production is identification of a new cost-effective culture medium that can facilitate the production of high yields

within short periods of time and also it is essential for scaling up the production process, thereby improving MC production and permitting application of MC in various fields [25]. The

substrates used should be cheap, readily available, and capable of supporting bacterial growth and MC production. The most commonly used substrates for MC production are sugarcane molasses, fruit and vegetable waste, and hemicellulose [25] (Table 3). Sugarcane molasses is a byproduct of the sugar industry that is rich in glucose, which is the primary substrate for MC production. Fruit and vegetable waste, such as pineapple peel and apple pomace, are also rich in glucose and have been found to be suitable substrates for MC production. Hemicellulose is a polysaccharide that is found in plant cell walls and can be hydrolyzed to release glucose, which can be used for MC production. Hemicellulose has been extracted from various plant sources, such as corn cob and wheat straw, and used as a substrate for MC production [26]. Tons of wastes produced from the agro, food, brewery and sugar industries, lignocellulosic biorefineries, textile and pulp mills are ideal raw materials for BC production [27]. Acetic acid pre-hydrolysis liquor of agricultural corn stalk was utilized as a low-cost carbon source for the green synthesis of bacterial cellulose (BC) [28]. This method of microbial cellulose (MC) production using such substrates has been shown to be scalable and cost-effective, making it a promising approach for large-scale production. Additionally, the use of waste materials, such as fruit and vegetable waste, can further enhance the sustainability of the production process.

Substrate	Source
Sugarcane molasses	Byproduct of the sugar industry
Fruit and vegetable waste	Pineapple peel, apple pomace
Hemicellulose	Corn cob, wheat straw

Table 3: Substrates used for MC production and their sources.

The substrates used in MC production vary depending on the microorganism used for the production. Some of the commonly used substrates are listed below:

Glucose: Glucose is the most commonly used substrate for MC production, and it is used in the production of MC by *Acetobacter aceti*, *Komagataeibacter xylinus*, and other microorganisms. Glucose is readily available and is relatively cheap, making it an ideal substrate for industrial-scale production [26].

Sucrose: Sucrose has also been used as a substrate in MC production, and it has been found to be more efficient than glucose in the production of MC by some microorganisms. For instance, it has been found to be more efficient than glucose in the production of MC by *Rhizobium* sp.

Fructose: Fructose has also been used as a substrate in MC production, and it has been found to be more efficient than glucose in the production of MC by some microorganisms. For instance, it has been found to be more efficient than glucose in the production of MC by *Pseudomonas* sp [26].

Lactose: Lactose has also been used as a substrate in MC production, and it has been found to be more efficient than glucose in the production of MC by some microorganisms. For instance, it has been found to be more efficient than glucose in the production of MC by *Sarcina ventriculi* [26].

Factors Affecting the Production of Microbial Cellulose

Growth Medium: The composition of the growth medium plays a crucial role in microbial cellulose (MC) production. A medium with a high carbon to limiting nutrient ratio, particularly nitrogen, is typically favorable for polysaccharide production. Optimal medium design is essential for the growth of microorganisms and the stimulation of cellulose synthesis. Essential nutrients for microbial growth include carbon, nitrogen, phosphorus, sulfur, potassium, and magnesium salts. Efficient conversion of 60-80% of the carbon source into crude polymer is often observed in high-yield polysaccharide fermentations [29].

Environmental Conditions: Environmental factors such as temperature, pH, and oxygen availability significantly influence MC production. Each microbial strain has specific environmental requirements for optimal cellulose synthesis. For example, *Komagataeibacter xylinus*, maintaining an optimal temperature of 28-30°C, a pH range of 5.0-6.5, and ensuring aerobic conditions through proper aeration are critical factors for maximizing bacterial cellulose production. These environmental conditions help ensure that the microbial strain can efficiently produce high yields of cellulose with desirable quality attributes, aligning with the general principles highlighted by Chawla, et al. [29], while others thrive in anaerobic environments. While *Clostridium thermocellum* itself does not produce cellulose, it is an excellent model for studying cellulose metabolism under anaerobic conditions due to its ability to break down cellulose efficiently. Maintaining an optimal temperature of 60-65°C, a pH of 6.5-7.0, and ensuring strictly anaerobic conditions are crucial for its metabolic activities [30].

For actual cellulose production in anaerobic conditions, researchers often look at other anaerobic bacteria or engineered strains in mixed cultures, but specific strains that produce significant quantities of cellulose anaerobically are less common compared to aerobic cellulose producers like *Komagataeibacter xylinus*.

Formation of Byproducts: The formation of byproducts during fermentation can impact the efficiency of MC production. Byproducts may inhibit microbial growth or interfere with cellulose synthesis. Therefore, controlling the fermentation process to minimize byproduct formation is essential. This can be achieved through careful selection of microbial strains, optimization of the growth medium, and fine-tuning of environmental conditions [29].

Substrate Utilization: The type of substrate used in MC production also affects the yield and quality of the cellulose. Common substrates include glucose, sucrose, fructose, and lactose. The choice of substrate can influence the efficiency of microbial cellulose synthesis, as some microorganisms may preferentially utilize specific carbon sources. Additionally, using cost-effective and readily available substrates, such as agricultural waste products, can enhance the sustainability

and economic viability of the production process [29].

Microbial Strain Selection: Different microbial strains exhibit varying efficiencies in cellulose production. Strain selection is a critical factor in optimizing MC yield. Research has shown that certain strains, such as *Komagataeibacter xylinus* and *Sarcina ventriculi*, produce high-quality cellulose with desirable properties. Genetic engineering and strain improvement techniques can further enhance the cellulose production capabilities of selected microorganisms. By understanding and optimizing these factors, the production of microbial cellulose can be scaled up efficiently, making it a viable alternative for various industrial applications [29].

Applications of Microbial Cellulose: MC has numerous applications, and its use has been extensively studied in recent years. Some of its applications are discussed below (Table 4):

Area	Application
Cosmetics	Stabilizer of emulsions like creams, tonics, conditioners, nail polishes [31]
	Face masks [32]
Textile industry	Clothing items [33]
	Jackets and shoes, thin leather [34]
Mining and refinery	Sponges to collect leaking oil, materials for absorbing toxins [35]
Waste treatment	Removal of heavy metals and organic pollutants from wastewater
	Separation of emulsified oily wastewater [36]
Sewage purification	Urban sewage purification, ultra-filtration water [37]
Communications	Diaphragms for microphones and stereo headphones [37]
Food industry	Pourable and spoonable dressings, sauces, and gravies; frostings and icings; sour cream and cultured dairy products; whipped toppings and aerated desserts, and frozen dairy products [38].
	Low-calorie additive, thickener, stabilizer [39]
	Edible cellulose (nata de coco)
	Gluten-Free Bakery item Production [40]
	Edible Film and Coating Applications [41]
Paper industry	Packaging [42]
	special papers
	more durable banknotes; diapers; napkins [43]
Forestry	Artificial wood replacer
	multi-layer plywood
	heavy-duty containers
Machine industry	Car bodies; airplane parts; sealing of cracks in rocket casings

Medicine/ biomedical	Temporary artificial skin for burns and ulcers [44]	
	Skin substitutes [45]	
	Dental implant components [46]	
	Antimicrobial wound dressing [6]	
	Drug Delivery [44]	
	Covers in experimental micro nerve surgery and as artificial blood vessel [47]	
	Scaffold for tissue engineering of cartilage [48]	
	Hydroxyapatite bioactivated bacterial cellulose promotes osteoblast growth and the formation of bone nodules [49].	
	Collagen for bone regeneration [50]	
Laboratories	Protein immobilization, chromatographic techniques, tissue culture medium	
	Electronics	Opto-electronics materials (liquid crystal displays) [51]
		Flexible substrates for the fabrication of Organic Light Emitting Diodes (OLED) [52]
Polypyrrole for conducting nanocomposites [53]		
Polyurethane to improve light emitting diode [54]		
Energy	Membrane fuel cell (palladium) [55]	
New applications	Cellulose thin films for documents and book recovery; bulletproof materials; luminescent materials; liquid crystal display; biodegradable plastic, etc. [56]	
Biosensor	As biosensor to detect bioreaction	

Table 4: Application of MC in different fields.

Conclusion

In conclusion, microbial cellulose (MC) is a versatile type of cellulose synthesized by various microorganisms, including *Acetobacter aceti*, *Komagataeibacter xylinus*, *Sarcina ventriculi*, *Rhizobium* sp., and *Pseudomonas* sp. These microorganisms present promising alternative sources for industrial-scale MC production. MC exhibits numerous applications across different sectors, such as biomedical (e.g., wound dressings and artificial blood vessels), food industry (e.g., low-fat foods), and environmental technologies (e.g., adsorbents for pollutant removal).

The incorporation of MC in composite materials has been extensively studied, demonstrating significant improvements in mechanical properties, thermal stability, and water resistance. These enhancements make MC-based composites highly desirable for various industrial applications. The production of MC utilizes various substrates, with glucose, sucrose, fructose, and lactose being commonly employed. The choice of substrate can influence the efficiency and yield of MC production, thus playing a critical role in optimizing the overall process.

The ongoing development and application of MC-based materials hold tremendous potential for numerous industries. Continuous research into alternative MC-producing microorganisms and the creation of novel composites promise to expand the utility and benefits of MC-based materials further. As research progresses, the scope of MC applications are likely to broaden, contributing to advancements in material science, sustainability, and industrial innovation. The future of MC-based materials appears promising, with the potential to revolutionize various fields through their unique properties and versatility.

Consent for Publication

We certify this manuscript has not been published elsewhere and submitted to another Journal.

Competing Interests

The author(s) declare that they have no competing interests.

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