

Microbial cellulose: an alternate source for plant cellulose

L. Sarvananda^{1*}, P.R.M.K. Fernando², P.A.D.S. Palihaderu³, Amal D. Premarathna⁴

¹Department Botany, University of Peradeniya, Sri Lanka, P. O. Box 20400,

²Department of Animal Science, Faculty of Agriculture, University of Peradeniya, P. O. Box 20400, Sri Lanka.

³Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, P.O Box. 20400, Sri Lanka.

⁴School of Natural Sciences and Health, Tallinn University, Narvamnt 29, 10120 Tallinn, Estonia

Corresponding author: L. Sarvananda (sarvacool18@gmail.com)

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Abstract

In the contemporary generation, rapid urbanization, industrialization, and declining woodland lead to global weather modifications. The massive scale of deforestation for firewood, constructions, paper products, textile, and plenty of different packages are steadily enforcing a critical poor impact on the surroundings. Inherently, plant cellulose has restrained utility because of the presence of hemicellulose and lignin. Consequently, studies in the discipline of microbial cellulose display many benefits over plant cellulose. It possesses numerous crucial and unique properties compared to plant cellulose, including high purity, better absorptivity, excellent polymerization, crystallization, in-situ mold potential, biodegradability, biocompatibility, and plenty of others. This assessment looks into a potent cellulose producer to develop an economically feasible manner for huge-scale production of microbial cellulose therefore, it may replace some of the requirements where plant cellulose has been currently in use.

Keywords

deforestation, microbial cellulose, polymerization, global warming

Introduction

“Biopolymers” are natural substances that consist of macromolecules. They are made from monomeric subunits covalently linked in a repeating pattern. (Klemm *et al.*, 2005). Biopolymers are created from renewable sources and are easily bi-

degradable because of the oxygen and nitrogen atoms found in their structure. Biodegradation converts them to CO₂, water, biomass, vapor, and different herbal substances. These biopolymers were mainly recycled naturally through organic processes (Gross and Scholz, 2001). Biopolymers play vital roles in preserving genetic expression data, catalysis of reactions, storage of essential elements such as carbon (C), nitrogen (N), phosphorus (P), and different nutrients, and avert harmful effects of other cells and protecting from harmful environmental factors. Furthermore, biopolymers act as mediators for adhesion to surfaces of other organisms, and communicators with the environment and other organisms. (Steinbuechel, 2003; Steinbuechel and Doi, 2005).

Cellulose (C₆H₁₀O₅)_n is the most abundant naturally occurring carbon-based polymer, which is most commonly found in plants. Out of all constituents in plant structures, approximately 30% is comprised of cellulose making it the most abundant organic substance on earth. Nevertheless, the cellulose content varies significantly depending on the plant species (Klemm *et al.*, 2005). It is the building block of plant cell walls, algae, and oomycetes (fungus-like eukaryotic microorganisms). This polysaccharide which is made of long linear chain of β (1-4) linked D-glucose units (Updegraff, 1969; Crawford, 1981), plays a pivotal role in the modern-day industry. It is used as a structural ingredient for cellulose-based products such as paper, textiles, and construction substances. Moreover, a variety of cellulose derivatives: spinoff (cellophane), rayon, and cellulose acetate are also used at the industrial level (Saxena and Brown, 2005; Peng *et al.*, 2011). However, the presence of lignin and hemicellulose along with cellulose has become a major challenging factor in using plant cellulose to provide the needs in many industrial approaches. Moreover, the plant resources cannot cater to the growing demand for cellulose with the quickly diminishing woodland sources, decreased agricultural land, and other environmental concerns.

The difficulty of isolating cellulose from hemicellulose and lignin from plant-based sources has paved the way for a discussion about an alternative source. (Brown, 2004). Similarly, the scale of deforestation with the aim of harvesting plant cellulose has a negative effect on the ecological balance. Therefore, the necessity of locating an alternative for plant cellulose was due. Microbial cellulose (MC) has shown the signs of a feasible substitute for plant cellulose. MC is free from lignin and hemicellulose, and it also has a higher degree of polymerization, gadget-driven capacity, excessive level of crystallinity, high purity, high water-absorbing capacity, in-situ moldability, biodegradability, and biocompatibility (Iguchi *et al.*, 2000; Brown, 2004; Torres *et al.*, 2012).

Due to these physical and chemical properties, MC emerged as a flexible biopolymer in multi-industries including textile, paper, cosmetics, audio products, and the medical area. (Brown *et al.*, 1992; White and Brown *et al.*, 1989). Some microorganisms can produce cellulose where its commercial uses are possible, for instance, *Acetobacter*, *Agrobacterium*, *Rhizobium*, *Pseudomonas*, and *Sarcina* (Chawla *et al.*, 2009). Among those microorganisms, *Sarcina* is one of the most prominent green cellulose producers in the presence of oxygen and glucose (Chawla *et al.*, 2009). Furthermore, this bacterium is a non-photosynthetic organism that can procure glucose, glycerol,

or different natural substrates from others and which could convert into natural cellulose (Brown, 1976).

Overall, the application of this MC as an alternative for plant cellulose can be of utmost importance for both industrial avenues and eco-protective approaches.

Cellulose and its derivatives

Cellulose is often considered the most abundant macromolecule on earth consisting of dozen to several thousands of monosaccharide units (Brown, 2004; Lavanya *et al.*, 2011). The main occurrence of cellulose used to be existing lignocellulosic material in forests which ultimately made the most vital source of cellulose (Ummartyotin and Manuspiya, 2015). Various plant fibers such as cotton and vascular plants have cellulose as an essential constituent (Myasoedova, 2000; Gross and Scholz, 2001). Apart from this main plant cellulose resources, many industries additionally utilize algae, the slime mold *Dictyostelium*, a variety of bacterial species (including the cyanobacteria), and tunicates in the animal kingdom (Saxena and Brown, 2005). Cellulose was first identified in 1839 from green plants by Anselme Payen, a French chemist (Purves, 1946). He found that cellulose has the same structure as starch, but exhibits differences in dimensions with physical and chemical properties. But the amount of cellulose and its extraction varied from plant to plant, the environment, and the life span of the plant.

Cellulose ether derivatives

Cellulose ethers are high molecular weight compounds produced through changing the hydrogen atoms of hydroxyl groups in the anhydrous glucose units of cellulose with alkyl or substituted alkyl groups. Cellulose ether derivatives have their special properties such as solubility, viscosity in solution, surface activity, thermoplastic film characteristics, and stability towards biodegradation, heat, hydrolysis, and oxidation. Examples of the most used cellulose ethers are Methyl Cellulose (MC), Ethyl Cellulose (EC), Hydroxyethyl Cellulose (HEC), Hydroxypropyl Cellulose (HPC), hydroxypropylmethyl cellulose (HPMC), Carboxymethyl Cellulose (CMC) and Sodium Carboxymethyl Cellulose (NaCMC) (Shokri and Adibki, 2013).

Cellulose ester derivatives

Cellulose esters are generally water-insoluble polymers with good film-forming characteristics. Hence, it is widely used in the pharmaceutical industry. Cellulose esters are categorized into two different groups: organic and inorganic groups. Most of the natural cellulose has been used in industrial merchandise or pharmaceutical investigations such as Cellulose acetate (CA), Cellulose acetate phthalate (CAP), Cellulose acetate butyrate (CAB), Cellulose acetate trimelitate (CAT), and Hydroxypropylmethyl cellulose phthalate (HPMCP), (Heinämäki *et al.*, 1994). Cellulose nitrate and Cellulose sulfate are examples of inorganic cellulose esters.

Microbial cellulose as an alternative

Cellulose exists in the cell components of a notable variety of organisms, from microorganisms (*Cyanobacteria*), prokaryotes (*Acetobacter*, *Rhizobium*, *Agrobacterium*) to eukaryotes (fungus, amoebae, green algae, freshwater, and marine algae, mosses, ferns, angiosperms, gymnosperms). It is additionally produced through some animals, the tunicates (urochordates), individuals of the subphylum *Tunicata* in the *Chordata* phylum (Nobles, 2001; Kimura and Itoh, 1998).

The feature of cellulose in these specific groups of organisms reflects the various roles related to this easy structural polysaccharide. Whereas it is feasible for some of these organisms, specifically bacteria, to live on in the absence of cellulose synthesis, it might also no longer be real for most vascular plant cells to continue to exist in the absence of cellulose synthesis (Saxena and Brown, 2005). Production of cellulose from *Acetobacter xylinum* was once first stated in 1886 by A.J. Brown (Brown, 1886). He observed that the resting cells of *Acetobacter* produced cellulose in the presence of oxygen and glucose. When it comes to the molecular characteristics, MC is equal to that of plant cellulose; however, possesses unique physical and chemical characteristics (Yoshinaga et al, 1997).

Why Microbial cellulose is preferred over plant cellulose?

The benefit of MC used to be associated with the purity of the product as it can be acquired in greater purity and is well-known for its greater degree of polymerization and crystallinity index (Shoda and Sugano, 2005). Cellulose prepared from microorganisms was free from wax, lignin, pectin, and hemicelluloses, which used to be regularly existing in cellulose derived from plants. In addition, microbial cellulose has greater tensile power and water-conserving potential than that of plant cellulose, making it more than appropriate for the production of excessive-constancy acoustic speakers, excellent paper, and dessert meals (Shoda and Sugano, 2005). Moreover, cellulose originating from bacteria should be efficiently managed on its repeating unit and the molecular weight in the fermentation process. However, from the perspective of industrial commercialization, the value of cellulose from bacteria was high. The use of microbial cellulose-based fabric for sustainable power was consequently restricted if any mass manufacturing was to be persisted (Ummartyotin and Manuspiya, 2015).

Characteristics of microbial cellulose (MC)

MC produced by some microbes has unique physical, functional, structural, and chemical properties. Cellulose is an unbranched polymer, β (1 \rightarrow 4) linked to D-glucopyranose residues. The chemical structure of plant and MC is identical. However, the degree of polymerization differs from about, 13,000 to 14,000 for plants and 2,000 to 6,000 for MC (Jonas and Farah, 1998). Additionally, microbial cellulose stands apart from its plant counterpart with the utilization of high crystallinity index (above 60%). Native microbial cellulose takes place in two specific crystalline structures, particularly cellulose Ia and cellulose Ib (Yoshinaga et al., 1997). These two sorts of crys-

talline structures show up to be separately disbursed in the microfibril of cellulose with exception of tunicin (sea squirt cellulose) which is pure Cellulose I β .

Cellulose Ia is dominant in microbial cellulose whilst cellulose I β is dominant in plant cellulose. Moreover, the content material of cellulose Ia is about 60% in microbial cellulose whilst it is solely about 30% in the greater plant cellulose, cotton, and ramie. In contrast, Cellulose I β is the essential element in plant cellulose (Sugiyama et al., 1991).

The structural aspects of microbial cellulose differ by the way of life conditions in which it has been produced and the subcultures used. The crystallinity and cellulose I content are decreased in an agitated culture compared to in a static culture. Similarly, the degree of polymerization in cellulose molecules is additionally decreased in agitated subculture prerequisites (El-Saied et al., 2004).

A xylum cellulose consists of ribbons of microfibrils generated at the surface of the microbial cell. The dimensions of the ribbons are 3–4 nm thick and 70–80 nm wide. The structure of the microbial cellulose sheet looks to be maintained by hydrophobic bonds. It is stated that the inter and intramolecular hydrogen bonds initially take place in every cellulose sheet, and then the cellulose crystalline shape is formed with the improvement of hydrogen bonds between cellulose sheets (Bielecki et al., 2005).

Microbial cellulose is water-insoluble and due to its giant community of fibers, it has a massive surface area. MC fibers have around 200 times the surface region of fibers compared to that of plant cellulose. Due to the special nano-morphology coupled with its capability to structure hydrogen bonds which debts for their special interactions with water, microbial cellulose can take in up to 200 times its dry mass of water. When microbial cellulose is used in suspension, it shows pseudoplastic thickening properties. Moreover, microbial cellulose shows amazing elasticity, and conformability (Czaja et al., 2006; US Congress, 1993).

Mechanism of cellulose synthesis and purification

Acetobacter xylinum (*A. xylinum*) has been substantially used as a model for the investigations of cellulose due to its functionality to synthesize high numbers of polymers from a huge range of carbon and nitrogen resources. Two techniques are usually employed for the manufacturing of microbial cellulose; particularly the stationary subculture and the agitation (Watanabe et al., 1998). In the static cultivation, also known as the stationary subculture method, the microbial cellulose is produced as a gelatinous membrane on the surface of the medium, whilst in the agitated culture method, the microbial cellulose is gathered in dispersed suspension as irregular masses, such as granule, stellate, and fibrous strand. The agitated culture technique is commonly utilized for industrial manufacturing of microbial cellulose (El-Saied et al., 2004).

Acetobacter or *Gluconacetobacter xylinus* require glucose or sucrose as their major carbon sources since the precursor in cellulose synthesis is uridine diphosphoglucose. The biosynthesis of cellulose from different carbon sources, such as 5- or 6-carbon

monosaccharides, oligosaccharides, starch, alcohol, and natural acid has additionally been reported. Moreover, fructose and glycerol are additionally used as carbon sources and result in nearly comparable yields of microbial cellulose as that from glucose whilst the usage of galactose and xylose yields smaller. The microbial cellulose yield from sucrose is half the yield from glucose. The use of D-arabitol as the carbon source was six times more effective than that of the D-glucose. A nitrogen source is also required for the cellulose-producing strain. Most of the media used for the manufacturing of microbial cellulose utilizes yeast extract and peptone as nitrogen sources. A few amino acids, e.g., methionine and glutamate, have also been used for this purpose. Vitamins such as pyridoxine, nicotinic acid, p-aminobenzoic acid, and biotin stimulate cell growth and cellulose production (El-Saied et al., 2004).

The microbial cell development and cellulose manufacturing are extensively affected by the pH of the culture broth; therefore, the management of the pH is crucial. The conversion of glucose to gluconic acid leads to a sizable drop in the pH of the medium in the batch culture. The ideal pH varies for cellulose production with the species. Provided, the *A. xylinum* requires a pH of 4 ± 6 , whilst some researchers (Oikawa et al., 1995; Delmer and Amor, 1995) confirmed pH 4 ± 7 as optimum. In addition to the pH of the nutrient broth, the yield of microbial cellulose is temperature-dependent. The optimal temperature for cellulose production is 25 ± 30 °C. The cellulose synthesis generally takes place at the air/cellulose pellicle interface, and hence oxygen is a vital element for cellulose production. The manufacturing rate and the yield of microbial cellulose are proportional to the oxygen transfer rate (OTR) and oxygen transfer coefficient (KLa). The providence of excessive oxygen is stated to result in the reduction of the MC productiveness due to a loss of substrate by direct oxygen (El-Saied et al., 2004). After fermentation, the microbial cellulose is typically harvested from the culture medium through centrifugation or filtration. Then those are observed through washing with distilled water and undergoing re-centrifugation or filtration again. At some stage the microbial cells are eliminated from the microbial cellulose in a warm caustic treatment that destroys them. The suspension is then filtered, and the filter cake is washed completely with distilled water to eliminate the traces of sodium hydroxide. The microbial cellulose is ultimately freeze-dried.

The industrial production

Industrial scale production of microbial cellulose

Culture medium plays a vital role in MC production. It provides essential nutrients for microbial growth and extensively influences the structure and yield of the MC as well (Jozala et al., 2016). At the very least, a general growth medium consists of a carbon source, a nitrogen source, and other nutrient elements such as phosphorus, potassium, sulfur, and magnesium (Andriani et al., 2020). The major drawback is the high production cost of the commercial process of MC. A techno-economic analysis (TEA) of industrial-scale production of MC has been performed using SuperPro Designer software (Dourado et al., 2016). The software estimated the manufacturing cost

of MC is around US\$ 7.4 million per year whereas, the net profit is US\$ 3.3 million per year. However, producers and research scientists have been working to find the new ways to reduce the production cost through increasing the production efficiency of MC (isolation of high strain yield and optimization of fermentation reactors) and discovering the economical sound nutrient sources as the substrate (Rivas et al., 2004; Ul-Islam et al., 2020). In recent years, the possibility of using alternative culture media was heavily explored as the bacteria can be fed with diverse array of carbon and nitrogen sources. Coconut water has been used as the prominent nutrient source for commercial production of MC (Hainan Yeguo Foods Co., Ltd, 2020). Nevertheless, the huge market for coconut water makes it scarce, consequently causing its price to increase. The isolation of cellulose-producing bacteria was carried out from rotten fruits and vegetables (Rangaswamy et al., 2015). Specifically, from pomegranate, sweet potato and potato. Based on the biochemical properties, bacterial strains were identified as *Gluconacetobacter* sp. RV28, *Pseudomonas* sp. RV14, and *Enterobacter* sp. RV11. The findings of the study conclude that the improvement of cellulose synthesis by these strains, with the involvement of bioengineering to produce cellulose at an industrial scale, is possible. Furthermore, other agricultural and industrial wastes such as molasses i.e. sugarcane and beet (Bae and Shoda, 2005; Premjet et al., 2007; Kusano Sakko Inc, 2020), dairy waste i.e. sour whey waste (Nguyen et al., 2021), waste beer yeast (Lin et al., 2014), dry oil mill residue (Gomes et al., 2013), corncob, alcohol waste liquor, pineapple peel, citrus juice, and apple juice (Zhong, 2008), achieve a comparable MC yield with the fermentation of nitrogen and phosphorous supplements. In addition, MC production through agricultural waste alleviates environmental pollution associated with improper disposal of industrial wastes (Gomes et al., 2013).

Potential applications of microbial cellulose

Microbial cellulose is a new useful material for a broad variety of purposes even in areas where the use of plant cellulose is limited. Since microbial cellulose has traits like excessive purity, an excessive degree of crystallinity, excessive density, suitable form retention, excessive water binding capacity, and a greater surface region in contrast to the plant cellulose, it can be utilized in distinct industries such as the cloth, paper, food and pharmaceutical industries, waste treatment, broadcasting, mining, and refinery (Czaja et al, 2006; Legge, 1990; Shah and Brown, 2005). The essential industrial uses of microbial cellulose can be concluded as follows.

Uses in the food industry

Microbial cellulose has essential functions in diverse meal formulations due to its structure. The excessive stage of purity, change in color, alternate in flavor, and vast possibility to strengthen a range of shapes and textures, makes MC a suitable candidate for the food industry (Gallegos et al., 2016). MC has been categorized as “generally identified as safe” (GRAS) (Badel et al. 2011). The first use of MC in the

food industries used to be in nata de coco (fermentation product of the bacteria, *Acetobacter xylinum*) in the Philippines. The gel-like properties of MC, blended with its entire indigestibility in the human intestinal tract, made this a fascinating food base (Budhiono et al., 1999).

It has a plasma cholesterol-lowering impact and has many different fitness advantages such as the protection against bowel cancer, atherosclerosis, and coronary thrombosis, and prevents the increment of glucose in the urine. In 1992, Chinese Kombucha or Manchurian Tea used to be produced via developing yeast and *Acetobacter* in a medium containing tea extract and sugar, is additionally a famous microbial cellulose-containing food product. (El-Saied et al., 2004).

Future potential uses consist of pourable and spoon-able dressings, sauces, and gravies; frostings and icings; bitter cream and cultured dairy products; whipped toppings and aerated desserts, and frozen dairy products. The use of microbial cellulose, mixing with different agents such as sucrose and carboxymethyl cellulose improves the dispersion of the product. It is also a low-calorie additive, thickener, stabilizer, and texture modifier, and can be used in pasty condiments and ice cream (Khan et al., 2007).

Uses in paper and paper products

The pulp and paper enterprise procedures demand huge portions of cellulosic substances each year (Manda et al. 2012). With an increasing demand for paper and improvements in processing science (Singh et al. 2012), paper can be produced from many unique cellulosic materials, including MC. Microbial cellulose consists of very small clusters of cellulose microfibrils. Therefore, it increases tremendously the energy and durability of pulp when converted into paper. Microbial cellulose is also a precious factor of artificial paper since nonpolar polypropylene and polyethylene fibers offer insulation, warmth resistance, fire-retarding properties, and the inability to form hydrogen bonds. The quantity of wooden pulp in this kind of paper is typically from 20% to 50% to obtain excellent quality (El-Saied et al., 2004). According to Shah and Brown, (2005), much of the research has been directed to produce a digital paper that consists of MC with a digital dye between electrodes. Furthermore, this technology could be a basis for electronic books, wallpapers with modifications patterns, bendy digital newspapers, and dynamic paper.

Application in the biomedical industry

Microbial cellulose is a prominent constituent that finds a range of biomedical applications, from usual wound dressing to tissue engineering. The biomedical purposes of MC have already been reviewed and documented in the latest literature (Fu et al. 2013; Rajwade et al. 2015). The technique of burn tissue recovery includes each generation of the dermis and restoration of the dermis resulting in the formation of scar tissue (Balasubramani et al., 2001). There is still a need for improvement of wound care dressing material, which should sufficiently defend wounds from contamination or excessive loss of fluid. Due to the latest advances in the subject of biomedical ma-

terials, scientists have developed a range of herbal and artificial polymers that can be used for wound closure, drug transport systems, novel vascular grafts, or as scaffolds for the advent of tissue-engineered constructs. As microbial cellulose is a highly porous material, it facilitates the practicable transfer of antibiotics or different drugs into the wound, whilst at the same time serving as an eco-friendly bodily barrier towards any exterior infection. It satisfies the necessities of current wound dressing material (Czaja et al., 2006). Furthermore, due to its special nanostructure and unique bodily and chemical properties, MC has been identified as a key for many scientific functions such as synthetic blood vessels, scaffolding for tissue engineering of cartilage, and a wound dressing fabric for chronic wounds (Ring et al., 1986; Fontana et al. 1990; Klemm et al. 2001; Alvarez et al. 2004; Svensson et al. 2005).

Conclusion

There is a necessity for the conservation of forests that reduce the emission of environmental pollutants because of the rapid industrialization, declining forests, and global climate changes, by using microbial cellulose. Microbial cellulose has the potential to provide a solution for the issues associated with the usage of plant cellulose. Also, microbial cellulose can be used as a superior alternative with extra sturdiness and better performance than plant cellulose. However, packages of biopolymers are reliant on the cost and scale of manufacturing. It would not be feasible to directly shift to microbial cellulose in the current arena. Therefore, studies should be carried out on economically possible processes for large-scale production of microbial cellulose where it would be able to replace at least a portion of the cellulose requirements. Knowing the significance of microbial cellulose, it is advisable to put more focus and effort into manufacturing and developing commercially feasible techniques for cellulose production.

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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