PRODUCTION OF BACTERIAL NANOCELLULOSE USING KOMBUCHA: A REVIEW

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ABSTRACT

Bacterial nanocellulose (BNC) has been attracting a lot of interest in the industrial sector because it is a "green" polymer and because of its specific characteristics, such as sustainability, biodegradability, multifunctionality, high modification capacity and biocompatibility with the human organism, among others. With the aim of elucidating the use of BNC originating from the Kombucha SCOBY, the different possibilities for producing existing BNC and the most used purification methods, we carried out this literature review. As a result, we observed that the SCOBY BNC can be used in the food, environmental, biomedical and pharmaceutical industries. The production medium, sugar, pH, temperature, oxygen availability, culture form and purification method can shape the properties of BNC.

Keywords: Bacterial nanocellulose. Kombucha. BNC production. Bacterial cellulose purification. Cellulose.

1 INTRODUCTION

Cellulose is the most abundant "green" biopolymer on the planet[1] that has been widely studied because it is a sustainable, biodegradable polymer with excellent chemical and physical properties, and can be used in a wide range of industrial applications[2]. It can be obtained from plants, tunicates, algae and bacteria[3]. Cellulose of bacterial origin is between 20 – 100 nm in diameter, thus being called nanocellulose[4].

One of the ways to cultivate BNC is using the fermented drink Kombucha as a substrate, which is an infusion of black/green tea (*Camellia sinensis*) sweetened with sucrose in which there is a symbiotic relationship between fungi and bacteria to produce cellulose and nutritional enrichment of the drink[5]. Tea and sugar provide all the macronutrients and micronutrients needed as a complete culture medium, as a source of carbon, nitrogen, phosphorus, among others[5,6]. This mixture forms a biofilm on the surface of the liquid, called SCOBY (Symbiotic Colony of Bacteria and Yeast), white or light brown, containing bacterial nanocellulose (BNC)[7].

Kombucha is a fermented, carbonated and slightly alcoholic drink (0.5 to 2.5%), with a sweet and acidic taste, originating from the Manchuria region, in ancient China, which has beneficial properties for health[8, 9]. The production of kombucha involves the infusion of green or black tea with sugar is added. As it is a "green polymer" of high industrial interest due to its qualities, the methods for obtaining BNC and purification have been studied, and therefore, to better understand this process, we decided to carry out a literature review on the subject. This work aimed to carry out a bibliographical survey to find out the most efficient process for obtaining BNC.

2 MATERIAL & METHODS

With the aim of preparing a bibliographical review, searches were carried out in the Science Direct, CAPES newspaper and Scielo databases. The keywords used were: Kombucha and bacterial nanocellulose, Synthesis of bacterial nanocellulose, production of bacterial nanocellulose, bacterial nanocellulose produced with kombucha and purification of bacterial nanocellulose. Articles were chosen from a period of 10 years (2014-2024), among them 9 articles were selected, covering both the production and purification part at BNC.

3 RESULTS & DISCUSSION

Using Science Direct, when searching all the keywords together we obtained 41 results, but a greater number was found when searching individually. Among all the studies, nine were chosen because they covered all stages from production to purification. BNC can be synthesized in different shapes and sizes, properties, flexibility and functionality, all depending on the culture method, cultivation conditions and the microorganism used[10]. To produce BNC, two culture methods can be employed: static culture and agitated culture[11]. All examples in Table 1 used the static method.

The production of BNC in such low pH conditions is considered by some to be unfeasible, as postulated by El-Gendi et al. (2022)[12], who suggest the impossibility of production below a pH of 4, or 3.5, according to Gupte et al. (2021)[13]. The pH can be adjusted by adding acetic acid, to prevent fungal contamination[6]. Additionally, the pH of the medium after fermentation approaches 3[14], a

phenomenon resulting from glucose metabolism that results in the formation of gluconic acid and the oxidation of ethanol into acid acetic acid by *K.xylinus* bacteria [15]. As indicated in Table 1, the tea concentration used varies between 5 g/L and 11.3 g/L. Sucrose is mostly used in a concentration of 10%. However, Sharma and Bhardwaj (2019)[16] noted that increasing the sucrose concentration from 60 g/L (equivalent to 6%) to 100 g/L (equivalent to 10%) results in decreasing the final weight of the SCOBY produced. A temperature variation between 20°C and 30°C is observed, which can be attributed to the optimization of the bacterial growth rate. The bacteria *K. xylinus* exhibit optimal growth rates between 18°C and 22°C, and other acetic acid-producing bacteria exhibit rates between 25ºC and 30ºC, while the yeasts *Saccharomyces* and *Zygosaccharomyces* exhibit superior growth rates between 30°C and 32°C[17]. The native structure of the BNC formed by the kombucha symbiosis is Type Iα cellulose, however, it can be transformed into Type II cellulose through mercerization, which is obtained by alkaline purification[19], or even when cultivated at a temperature of 4ºC[19]. Type I cellulose is more crystalline and Type II cellulose is more amorphous[20].

To carry out fermentation, a symbiotic culture of bacteria and yeast (Start) is inoculated. The most common inoculum concentration was 10%, although there are studies that used up to 50%. It is possible to add between 2% or 3% of SCOBY [5, 9] or, alternatively, exclusively use SCOBY as an inoculum. Documented fermentation periods range from 3 to 30 days; however, for applications involving both SCOBY and tea, a fermentation period of 7 to 21 days is recommended[21].The symbiosis of bacteria and yeast turns the pH of the medium into acid (+3) and produces ethanol and acetic acid, which inhibits contamination by other microorganisms and mold, making the use of Start advisable. Tea also exerts an antibacterial, antiviral and antioxidant action as it contains polyphenols (caffeine and theophylline) which, together with proteins, stimulate the production of cellulose[6, 22, 23].

Kombucha can be inoculated with a symbiotic consortium composed of acetic bacteria, including the *Bacterium xylinum* strain, also called *Acetobacter xylinum* or *Gluconacetobacter xylinus*, in which the genus was subdivided into two, one of them called *Komagataeibacter*, and can be found in the works as *G .xylinus* or *K. xylinus*. The bacteria *Acetobacter ketogenum*, *Bacterium gluconicum*, which can have several species present in a single fermentation. Yeasts are also part of the symbiosis, of which the species *Saccharomycodes ludwigii*, *Schizosaccharomyces pombe, Saccharomyces cerevisiae,* and *Saccharomyces inconspicus*, and the genera *Zygosaccharomyces* and *Brettanomyces*[5,8,24]. Yeasts carry out alcoholic fermentation and hydrolyze polysaccharides into monosaccharides, at the same time, bacteria use monosaccharides to produce cellulose, produce acids and carry out aerobic oxidation of ethanol into acetate[5,8].

Table 1 Characteristics of kombucha produced and purification in 1 liter of water.

Production							Purification			Reference
BNC g/L	Type of Tea	Tea (g)	Sacarose (g)	Start (%)	SCOBY (%)	Production condition	Methods	Reagent	Condition	
-	Black	5	100	10	-	28ºC, 7 days	Alkaline	H2O2 3% NaOH 1M	1:20 pH9.5	Li, Z. et al.2023
-	Green	7	150	12.5	Few	25-27ºC, 30 days, dark	Autoclave	-	121ºC, 100 kPA, 15 min	Agüero et al, 2023
-	Black	11.3	100	-	Few	10 days, dark	Alkaline	NaOH1M	-	El-Shall et al., 2023
-	Green	4	100	50	-	30ºC, 15 days, dark	Alkaline	NaOH1M acetic acid	-	He et al., 2023
3.61	Black	7.5	60	10	-	30ºC, 3 days, dark	Alkaline	NaOH 1M	80ºC, 1h	Avcioglu et al., 2021
-	Green	8	100	-	20	14 days, dark	Acid	H2SO4 30% NaOH0.2M	70ºC, 4h 1h	Pillai et al., 2021
13.3 7.2	Black	5	60 100	10	2	pH3, 30ºC, 20 days	Alkaline	NaOH0.5N	1h	Sharma & Bhardwaj, 2019
29.2	Black	-	60	-	2	pH3.2, 30ºC, 20 days, dark	Alkaline	NaOH0.5N	100ºC, 1h	Sharma et al., 2021
-	Black	8	80	-	Few	7 days, dark	Alkaline	NaOH 1M H2O2 1.5%	1h, room temperature, 2h	Tang et al., 2021

Static culture is the most used method, considered standard. The film floats due to carbon dioxide bubbles trapped in the nanocellulose matrix. Its hydroxyl groups on the inner surface are highly hydrophilic, leading to a high-water retention capacity[15, 14,11].

In agitated culture, BNCs are formed in small clusters or suspended fibers, like curved, disordered and overlapping ribbons, spread out irregularly. As there is a greater possibility of mutations in bacteria, it has a lower yield[20,1,25]. Therefore, it is not the best method of producing and obtaining BNC.

After formation, the SCOBY is subjected to multiple washes with distilled water and purification processes, as shown in Table 1. Predominantly, purifications are carried out in an alkaline medium using 1 M NaOH, although potassium hydroxide can also be used[26]. Alternatively, methods such as acid purification, reverse osmosis, mild enzymatic hydrolysis, mechanical homogenization[20] or autoclaving[27] are used. Purification aims to sterilize the SCOBYs for their subsequent application[5]. Confirmation of nanocellulose production is obtained when the membrane exhibits resistance to the process and a bleached appearance[16]. The procedure is completed with neutralization, which is carried out through successive washes with distilled or deionized water until reaching a neutral pH[28, 29]. The use of NaOH during treatment can help eliminate proteins, bacteria, yeast

cells and tannins that may be present in small amounts in the Kombucha skin[30]. Despite being sterilized with the purification method, Pigaleva et al. (2019)[31] report that cell remains, endotoxins and even purification residues were found in BNC, which could be harmful for medical applications, requiring new searches for a better result.

4 CONCLUSION

As a result, we observed that kombucha is a viable and green means to produce BNC, its properties can be changed depending on the production characteristics such as the production medium, sugar, pH, temperature, oxygen availability, the culture form and the purification method. The best production method will depend on the desired application. However, some steps can be improved, such as purification.

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