OPTIMIZATION OF BACTERIAL NANOCELLULOSE PRODUCTION: A REVIEW OF RECENT ADVANCES

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ABSTRACT

The current state-of-the-art related to bacterial nanocellulose production focuses on optimization and challenges in the field. Bacterial nanocellulose (BNC) is a polysaccharide polymer synthesized by some bacterial strains such as *Acetobacter xylinus* and *Komagataeibacter medellinensis*. The industry has been mass-producing BNC for food and cosmetic applications, but companies face economic and systematic challenges in achieving low-cost production lines and scale-up production. The objective of this research was to fill the existing knowledge gap and provide an overview of the latest advancements in the optimization of BNC production, based on the most recent studies published from 2023 to May 2024. The methodology employed in this study involved a systematic search and selection of relevant scientific documents from Scopus and Web of Science databases. These findings indicate that fermentation and bacterial growth conditions can be tailored to achieve the desired aspects of synthetized cellulose, a high BNC yield, cost reduction, and a more environmentally friendly process. Variables such as culture medium composition, SCOBY volumetric ratio, microbial strain, and pH of the medium are key factors for optimizing the process and regulating the nanocellulose obtained. This review observed a scarcity of studies on the optimization of BNC production, environmental impact analysis, and economic feasibility.

Keywords: Bacterial nanocellulose. Fermentation. Production. Optimization.

1 INTRODUCTION

Bacterial nanocellulose (BNC) is a polysaccharide polymer synthesized by some bacterial strains, such as *Acetobacter xylinus* and *Komagataeibacter medellinensis* (formerly *Gluconacetobacter xylinus*) [1]. These molecules often exhibit at least one dimension in the range of 1-100 nm [2], although some studies have reported that the average size of BNC may be 20-100 nm in diameter [3]. The increased interest in BNC for novel sustainable materials in several applications is due to its high polymerization and degree of polymerization; however, the overall chemical structure does not differ from that of nanocellulose from other sources, such as plants [4]. In addition, BNC presents advantageous features such as high water retention capability, biocompatibility, good tensile strength, high aspect ratio, and nontoxicity, to name a few [1,5,6]. The industry has been mass-producing BNC for food and cosmetic applications because it does not demand heavily controlled and standardized nanocellulose as the final product, as opposed to other industries, such as pharmaceuticals and textiles. Unfortunately, companies that provide BNC for the industry are still facing economic and systematic challenges, not only to achieve a low-cost production line but also to scale-up production in such a way that BNC morphology and physicochemical properties are consistent with a high BNC yield that supplies market demand [7]. Therefore, the high costs associated with the routes for BNC production currently restrict its use to small, specific, and high-valued markets[8]. Fermentation and bacterial growth conditions can be tailored to achieve the desired aspects of synthetized cellulose, cost reduction, and a more environmentally friendly process, making the BNC biotechnological route more attractive and competitive than the traditional cellulose production routes [9]. After the authors' extensive bibliometric analysis, it was observed that, in the past couple of years, there have been few studies aiming to optimize BNC production to make it more efficient, low-cost, and eco-friendly, and there was a gap in reporting the newest discoveries in this regard. The aim of this study was to bridge this gap and shed light on the state-of-the-art in the optimization of BNC production brought about by recent studies published between 2023 and May 2024.

2 MATERIAL & METHODS

The methodology employed in this study involved a systematic search and selection of relevant scientific documents from Scopus and Web of Science databases. Data were gathered based on prospecting documents returned from the databases using terms of interest correlated by Boolean operators and wildcards in "Article title, Abstract, Keywords" field. The results were also filtered by publication year within 2023-2024 range. Documents deemed suitable and relevant were chosen based on the latest studies on bacterial nanocellulose focusing on the optimal reaction conditions, which resulted in a higher BNC yield, better economic feasibility, or improved nanocellulose properties. The three combinations of terms specified in the Scopus and Web of Science search fields were (nanocellulose AND bacter* AND ferment*), (nanocellulose AND bacter* AND optim*), and (nanocellulose AND bacter* AND product*). After assessing the results, five articles were found relevant to the topic at hand.

3 RESULTS & DISCUSSION

It is interesting to note the diversity of the variables studied in the articles presented in Table 1, as the culture media composition was the most significant variable affecting BNC yield, regardless of the bacterial strain.

Table 1 - Articles summarization regarding the key parameters evaluated, which affect BNC bacterial cellulose production and their optimal values.

Parameter	Martínez et al. (2023)[10]	Lima et al. (2023)[11]	El-Naggar et al. (2023)[12]	Jančič et al. (2024)[13]	Dáger-López et al. (2024)[14]
Independent variables	Production cost (HS vs. fique juice media)	coffee ground (g/L); green tea (g/L); vitamin complex (mg/L); sucrose (g/L); SCOBY (m/V); incubation time (day); kombucha (V/V)	carbon source; cantaloupe juice (% v/v); pH; peptone (g/L); incubation time (day); inoculum size (% v/v); citric acid (g/L); yeast extract (g/L); temperature (°C); Na_2HPO_4 (g/L)	CMC; Br; EDC	mixture composition (green tea, SCOBY and BMJ)
Significant independent variables	not applicable	sucrose; SCOBY; kombucha	pH; peptone; cantaloupe juice	CMC; Br	all three variables
Bacteria strain	*Komagataeibacter medellinensis*	SCOBY	*Bacillus* sp. SEE-3	*Komagataeibacter melomenusus*	SCOBY
Medium	Fique juice	Kombucha	Cantaloupe juice	RAE	Optimized culture medium
Main carbon source	Glucose; fructose; sucrose	Sucrose	Glucose; fructose; sucrose	Glucose	Glucose
Temperature (°C)	not mentioned	28	37	30	30
Medium / Reactor Volume	not mentioned	150 mL of culture medium (BC production step); 40 mL in a beaker (kinectic evaluation in the optimal medium)	100 mL in a 250 mL conical flask	in a 250 mL Erlenmeyer flask (medium volume not mentioned)	100 mL in 500 mL wide-mouth glass bottle (medium optimization step); in a 10 L reactor (scale-up step)
pH	3.6	2.38-2.69	5	Not mentioned	3.0-6.0
BNC yield (g/L)	2.2	6.4	20.3	2.0	6.8
Incubation time (day)	15	7	14	5	14
Other optimized factors	not applicable	not applicable	cantaloupe juice (81.27% v/v); peptone (11.22 g/L)	CMC (8.8 mg/mL); Br (10 mg/mL); specific proteolytic activity (2.3 U/mg); immobilization efficiency (39.1 %)	not applicable
Bioreactor Agitation	static	not mentioned	static	agitated at 120 rpm (1st day); static (2nd-4th day)	static
Methods for optimization	SCC; Monte Carlo method	Plackett-Burman; Box-Behnken; ANOVA	Plackett-Burman; FCCCD; ANOVA	Plackett-Burman; CCD; ANOVA	Simplex-centroid; ANOVA

Note: ANOVA = Analysis of variance; Br = bromelain; BMJ = banana midrib juice; CCD = Central composite design; CMC = carboxymethyl cellulose; EDC = N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride; FCCCD = Face centered central composite design; HS = Hestrin-Schramm; RAE = reinforced acetic acid ethanol medium; SCC = Sperman correlation coefficient; SCOBY = symbiotic culture of bacteria and yeast.

Furthermore, it is worth noting that other factors, such as pH and SCOBY volumetric ratio, also play crucial roles in affecting BNC yield, and their impact should not be overlooked when optimizing the production process. In terms of experimental and mathematical approaches, all authors have used tools for process improvement, with a focus on the methodology of the design of experiments. In all cases, statistical analysis was performed when reporting the results and conclusions, increasing the reliability of the outcomes.

Only one study was found to be related to financial concerns in BNC production to evaluate a low-cost solution and the environmental impact (carbon footprint and water consumption estimations)[10], although optimization of the fermentation conditions was not carried out for a higher BNC yield. A study with the main goal of reactional optimization for a high BNC yield in conjunction with financial, environmental, and even life-cycle analyses would be greatly appreciated in the literature and valuable for the industry.

A scale-up article is another topic of great interest for the industry as BNC production still faces challenges in mass production and to assure quality control and uniformity according to market demands [8]. Most of the articles in Table 1 executed their proposed experiments in laboratory-scale, except one that revolved around optimization in a scaled-up 10 L bioreactor [14]. The success of this type of experiment holds a significant contribution to mass-production BNC plant projects suitable for current and future market demands. The major limitations of these studies are the requirement of costly equipment and a large laboratory space to simulate the process to properly understand the optimization synergy among the variables close to the industry settings. This may be the reason there are few articles on this matter because not many research centers have adequate infrastructure to implement such an experimental approach, thus slowing down the advancement in the engineering aspect of the topic.

It is crucial to acknowledge that several articles found during the bibliometric prospection phase, claiming to focus on optimization studies, have not employed any experimental design methods or statistical analyses to verify the influence of the chosen variables on the production of nanocellulose via bacterial fermentation. The absence of a proper experimental design method and data analysis in many studies has resulted in conclusions that are not well founded and are challenging to interpret. Hence, it is critical to develop and apply robust experimental design methods and data analysis procedures to ensure reliable and valid research outcomes. The scarcity of elaborate optimization-related studies is apparent from bibliometric analysis, which suggests that this area is not well represented in the literature. This limited representation hinders the further exploration of optimization and scale-up to make the process attainable for the industry, which is a rare topic in the field.

4 CONCLUSION

This review emphasizes the importance and value of optimizing BNC production. Using alternative culture media for a determined bacterial strain alone or in symbiosis, along with a set of optimal fermentation conditions, BNC yield enhancement, cost, and environmental impact reduction can be addressed, making the production process more cost-effective and greener, aiding future studies in scale-up experiments for the industry in general. Furthermore, the bibliometric review showed that the current state-of-the-art regarding the optimization of BNC synthesis is still scarce in the literature in recent years, to the detriment of the substantial growth of research groups regarding the overall topic of BNC production across the globe.

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