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SYNTHESIS AND DEVELOPMENT OF BACTERIAL NANOCELLULOSE FROM DIFFERENT SOURCES

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ABSTRACT

Bacterial nanocellulose is an innovative material that has the potential to revolutionize various aspects of dentistry. This unique form of cellulose produced by certain bacterial strains possesses exceptional properties that make it a promising candidate for a wide range of dental applications. The synthesis process of BNC plays a pivotal role in determining a material's unique properties making it a fascinating subject to know more about. This concise review provides insights into the key aspects of Bacterial nanocellulose synthesis and production.

Keywords: Bacterial Nanocellulose, Innovative Materials, Bacterial Cellulose, BNC Synthesis, Biomaterials.

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INTRODUCTION

In recent years, the field of biomaterials has witnessed a remarkable evolution as innovative solutions for various biomedical and clinical applications. One such pioneering material is bacterial nanocellulose (BNC) also known as bacterial cellulose (BC), a remarkable nanofibrous structure produced by certain strains of bacteria. With its exceptional mechanical properties, biocompatibility, and potential for tailored modifications, BNC has garnered considerable interest in the realm of dentistry. This versatile material holds promise for a range of dental applications, including tissue engineering, drug delivery, wound healing, and as a scaffold for regenerative therapies. Moreover, its synthesis from diverse sources showcases its adaptability and sustainability, further enhancing its appeal in the dental field.[1] Bacterial nanocellulose stands out due to its unique properties that make it particularly well-suited for dental applications. Its high surface area, porosity, and impressive mechanical strength lend themselves to the development of novel dental materials with enhanced durability and functionality. Additionally, BNC exhibits biocompatibility and a low immune response, minimizing potential adverse reactions when interacting with oral tissues. These attributes make it an attractive candidate for addressing various challenges in dentistry.[1,2] The synthesis of bacterial nanocellulose for dental applications can be achieved through the cultivation of cellulose-producing bacteria, primarily belonging to the Acetobacter and *Komagataeibacter genera*. These microorganisms are cultured in controlled fermentation processes, wherein they metabolize glucose-based substrates to produce nanofibrillar cellulose structures.[3] What sets BNC apart is its adaptability to various cultivation sources, including agricultural by-products, industrial waste, and even unconventional substrates like fruit juices. This versatility ensures a consistent supply of raw materials, driving its feasibility for large-scale dental applications.[2,3] Furthermore, the synthesis of BNC can be fine-tuned to yield specific properties suitable for dental needs. By modulating cultivation parameters such as temperature, pH, and oxygen availability, researchers can manipulate the nanocellulose's physical and chemical characteristics. Surface functionalization through chemical modifications or incorporation of bioactive agents allows for tailoring BNC to fulfill specific requirements, such as antimicrobial properties or enhanced tissue integration.[4] This comprehensive review article aims to provide a thorough understanding of the synthesis and production of bacterial nanocellulose, covering various aspects of microbial sources and synthesis strategies. By exploring the evolving landscape of BNC production, this review seeks to contribute to the broader knowledge base and stimulate further research in this field.

EXPERIMENTAL

Bacterial Nanocellulose: Sources

BNC owes its origin to diverse and intriguing sources. Emerging from the natural cellulose-producing capabilities of specific bacterial strains, BNC can be synthesized from a variety of substrates, ranging from traditional sources to different waste materials. A. J. Brown was the first to describe the production of bacterial cellulose from *Acetobacter xylinum*, also known as *Gluconobacter xylinum* and formerly *Bacterium aceti*, in 1886. In the culture media, this strain created a substantial gelatinous film made entirely of cellulose. Due to its widespread use in the production of acetic acid, this bacterium was once known as "The vinegar plant.[5] In the second half of the 20th century, increased research interest in the production of bacterial cellulose resulted from rigorous work by Hestein *et al.* utilizing *A. Xylinum* as the model bacterium in the presence of glucose and oxygen. [6] Additionally, Colvin found that the cell-free extract of *A. Xylinum* could synthesize cellulose in the presence of glucose and adenosine triphosphate (ATP).[7] Several native and recombinant strains have been identified to generate cellulose efficiently while using a variety of carbon sources, such as various wastes, as their substrates. BC, which comprises both Gram-positive and Gram-negative strains, is said to be produced by bacteria species including *Alcaligenes, Achromobacter, Aerobacter, Agrobacterium, Azotobacter, Gluconacetobacter, Pseudomonas, Rhizobium, Sarcina, Dickeya,* and *Rhodobacter*. However, because of its capacity to metabolize a variety of carbon/nitrogen sources, the species *Komagataeibacter*, formerly known as *Gluconacetobacter,* is the most frequently employed industrially significant strain for the synthesis of BNC. While the aerobic Gram-negative *A. xylinum* has been utilized for commercial production of BNC for some time, the strains *A. hansenii, A. pasteurianus,* and *A. xylinum* are the most efficient sources for the manufacture of bacterial cellulose.[8]

Bacterial Nanocellulose: Culture Methods

The process typically begins with the cultivation of bacterial cells in a growth medium that contains carbon and nitrogen sources, as well as essential nutrients. The bacteria multiply and form a biofilm composed of intertwined cellulose nanofibers. The culture conditions, including temperature, pH, oxygen availability, and agitation, play a crucial role in shaping the characteristics of the resulting BNC. The cultivation process used during BNC's synthesis is one of the main elements affecting the product's quality and traits. BNC's yield and purity as well as its mechanical characteristics, crystallinity, and possible applications are all influenced by the culture procedure. Over the past few years, various culture techniques, including static, shaking/agitated, and bioreactor cultures, have been investigated for the development of BNC production.[9] Among these the static cultivation approach results in the buildup of cellulose pellicle at the interface between air and liquid, whereas the shaking/agitated culture method produces irregular masses characterized by sphere-like, asterisk-like, or pellet-like formation.[8]

Static Method

The static method represents a fundamental approach to the cultivation of BNC. This method is typically employed with strains like *Gluconacetobacter xylinus*, where bacterial cells are inoculated into a liquid growth medium contained within a static, non-agitated environment. As the bacteria proliferate, they synthesize cellulose nanofibers that form a dense, three-dimensional network, often adhering to the container’s surface. The static culture method provides meticulous control over critical culture conditions, including temperature, pH, and nutrient availability. This level of precision enables a consistent and controlled environment conducive to the reliable production of BNC.[10] Despite its tendency to result in lower production rates when compared to dynamic methods, the static approach is preferred in applications where meticulous control over the material's characteristics is paramount. This method allows for a fine-tuned manipulation of key parameters, ensuring the desired properties of the produced BNC. This method involves filling a variety of shapes and sizes of containers with a culture medium that has a pH range of 4.5 to 6.5 and incubating them for 2 to 20 days at an appropriate temperature of 25 to 30 °C. A hydrogel pellicle is a component of BNC made using the static culture technique. It forms at the air-culture media contact. The cellulose subfibrils, which are created by self-assembling, overlapping, and entangled cellulose ribbons in parallel-disorganized planes, are continually crystallized into microfibrils and extruded from linear pores at the bacteria's wall membrane.[5] As a result, the gelatinous cellulose membrane gathers on top of the culture media. The culture time affects the thickness of the produced BNC. Once the membrane has been created,

it must be cleaned by soaking it in a sodium hydroxide solution, neutralizing it with acetic acid, and then rinsing it with deionized water until the BNC is translucid. Membranes can either be dried or moistly kept after purification.[11,12] The pellicle that formed has a 3D network structure with great porosity, elasticity, and stretch resistance. This static culture approach is a widely utilized method for producing BNCs since it is comparatively easy to employ.[11,12,13] The static culture method, deemed impractical for large-scale industrial BNC production due to costly synthetic media and low yield rate, has prompted investigations into alternative approaches. Notably, researchers are exploring the viability of employing fruit juices, and agricultural and industrial-based waste as cost-effective carbon and nutrient sources. This shift not only addresses economic concerns but also aligns with sustainability goals, offering potential improvement in BNC Yield and environment-friendly production process.[13-15]

Agitated/Shaking Method

To address the inherent limitations of the static fermentation process, characterized by low production rates and extended fermentation times, an alternative approach involves adopting an agitated or shaking culture. This modification aims to enhance the production rate of Bacterial Cellulose (BC) by improving oxygen transfer in the fermentation broth through increased agitation. While this strategy proves effective in promoting a better growth rate and increased BC production, it introduces challenges such as the formation of irregular BC granules and elevated viscosity in the fermentation broth when agitation is excessive. These factors impede air diffusion into the medium, creating a micro-aerobic environment. This reduced oxygen supply results in the accumulation of acids, including glucuronic acid, acetic acid, and lactic acid, thereby lowering the pH of the medium below its optimum point. The resultant acidic conditions can foster the growth of cellulose-negative strains, adversely impacting BC yield. Careful consideration and optimization of agitation parameters are essential to strike a balance between increased production rates and avoiding undesirable effects on BC morphology and medium pH[8,15] Moreover, in the submerged process, Bacterial Cellulose is generated in granular or spherical forms, diverging from the pellicle structure observed in the static method. The size and shape of these granules are intricately linked to factors such as agitation speed, culture duration, and the composition of the culture medium. Consequently, precise regulation of these parameters becomes imperative to achieve BC with the targeted properties, emphasizing the need for meticulous control during the production process.[16]

Cell-Free Production

While various strategies, including fermentation approaches, genetic engineering techniques, strain isolation, and modification, have been implemented to enhance the efficiency of the process and boost Bacterial Cellulose productivity, the demand of the current market necessitates the exploration and development of more advanced techniques. Meeting these evolving market demands requires continuous innovation and the adoption of cutting-edge methodologies in BC production.

The implementation of a cell-free system emerges as a viable remedy for numerous challenges encountered in traditional Bacterial Cellulose fermentation processes. This system had previously found application in bioethanol production by Butchner.[17] Park and colleagues pioneered the development of a cell-free system utilizing *G. Hansenii PJK*, incorporating bead beating for efficient cell lysis. Park and team pioneered the assessment of cellular lysate from *G. Hansenii PJK* lysis for cellulose production. Operating under static conditions at 30 °C and pH 5.0, their developed cell-free system achieved a noteworthy cellulose output of 3.72 g/L in an in vitro setting, with a corresponding 57.68% yield determined based on the glucose consumption over 15 days. Termed 'bio-cellulose,' This milestone represents a substantial leap forward in the exploration of alternative methods for cellulose production.[18] In summary, the cell-free biosynthesis approach proves to be an efficient, dependable, and viable method, holding significant promise for cost-effective and large-scale cellulose production. Nonetheless, it is advisable to conduct additional research focused on the purification, stability, and reusability of cell-free enzymes, along with ensuring a balanced supply of cofactors for the in vitro biosynthesis of cellulose. [19]

Use of Various Reactors in the Fermentation Process

In pursuit of achieving industrial-scale production of Bacterial Cellulose, numerous endeavors have been undertaken to optimize reactor design. The primary objectives include enhancing productivity, reducing

production costs, and shortening cultivation times. Challenges encountered in agitated cultures, such as the significant proliferation of cellulose-negative mutants, the accumulation of undesirable acids, and the adherence of BC broth to reactor walls, have prompted innovative solutions.[11] To surmount these limitations, various reactor designs have been explored in recent years. These include stirred tank reactors, airlift bioreactors, trickling bed reactors, rotating disk reactors, rotary biofilm contractors, bioreactors with spin filters, and reactors with silicone membranes. Successfully implemented, these diverse reactor configurations have demonstrated efficacy in BC production. An examination of diverse reactors employed in BC production and their applications has been previously documented.[8,20] The bacterial cellulose derived from these bioreactors can take the form of either fibrous structures or pellets. Notably, the airlift bioreactor stands out in its ability to generate a membrane-type BC from *Gluconacetobacter xylinus*. This specific type of BC exhibits a superior water-holding capacity compared to cellulose varieties produced through static cultivation methods. [21]

RESULTS AND DISCUSSION

Production of Bacterial Nanocellulose Using Different Substrates and Their Culture Results Commercial Sugars and Fruit Juices

Fruits serve as a good substrate for the synthesis of several value-added goods, including BNC, due to the abundance of simple sugars present, particularly glucose, fructose, and sucrose. According to reports, a variety of fruit juices were used as a medium for the static fermentation process to produce BNC, with yields ranging from 3.2 to 5.9 g L^{-1} for pineapple juice, orange juice, and rambutan juice. According to the study, adding a nitrogen source to the apple juice improved the BNC output even more.[22] In addition to glucose, sucrose, and fructose, molasses, a by-product of the extraction of sugar from sugar beets or sugarcane, also contains some nitrogen and vitamins. Microorganisms generate BNC rapidly when there is more nitrogen and vitamins around.[23] Molasses are prepared utilizing a variety of techniques, including physical, physicochemical, and chemical pretreatment, to remove the inhibitors before being used in the fermentation medium. The most often used pretreatment method, which produces a high quantity of sugars, is heat pretreatment in the presence of acid (HSO4; 120º C; 20 minutes). This pretreatment process reportedly produced more BC with better mechanical characteristics than the one made from glucose.[8,22]

Agricultural Waste, Industrial Waste, and Food Waste

Crude glycerol, thin stillage, whey, wastewater, vinasse, wheat straw, cotton cloth, mandarin peel, and red maple extract were just a few of the industrial and agricultural wastes/byproducts that were successfully used in the fermentation process to create BNC. The most often used carbon source for the synthesis of BNC is crude glycerol, a by-product of the biodiesel manufacturing process. After 14 days of static incubation with crude glycerol as the carbon source, the *G. xylinus NRRL B-42* strain produced a maximum BNC production of up to 10 g L^{-1}. Additionally, the impact of crude-glycerol-generated inhibitors on the BNC production by *Gluconobacter xylinus* was examined. High concentrations of glycerol (above 40 g L^{-1}) and salts (NaCl and KCl) were found to be inhibitors of BNC production. Using *G. xylinus* strains in static fermentation, H2SO4-pretreated wastewater from the industry that processes candied jujubes and acetone-butanol-ethanol (ABE) fermentation wastewater produced BC at rates of 2.25 g L^{-1} (in 6 days) and 1.34 g L^{-1} (in 7 days) respectively.[23,24,25] An environmentally beneficial method that is resource-efficient and sustainable is bacterial cellulose synthesis from lignocellulosic waste. Lignocellulosic waste comprises significant amounts of cellulose, hemicellulose, and lignin and is obtained from sources such as agricultural wastes, wood, and paper industries.[9] These waste products can be converted into useful bacterial cellulose by using bacterial strains, such as *Gluconacetobacter xylinus,* in a controlled fermentation process. In addition to reducing the environmental impact of waste disposal, this technique also produces a high-quality, biocompatible material with a variety of applications Bacterial cellulose produced from lignocellulosic waste shows promise in industries including tissue engineering, biodegradable packaging, and bandages for cuts and wounds. It also demonstrates the possibility of repurposing waste materials to create novel solutions for a future that is less polluting.[25,26]

Future Prospects and Challenges

Delving into the functionalization and targeted applications of BNC, particularly within the biomedical realm, presents significant potential. The emphasis on sustainability has propelled investigations into

alternative carbon sources and eco-friendly production techniques. While there have been notable advancements, persistent challenges include cost implications, standardization concerns, and limited awareness within the market.

Table-1: BNC Production from Commercial Sugars and Fruit Juices[8]

Carbon Source/Waste Feedstock	Microorganism	Culture duration (days)	Culture Yield (gL^{-1})	Culture mode
COMMERCIAL SUGARS				
Glucose	*Gluconacetobacter intermedius SNT–1*	6	10.0	static
Sucrose	*Gluconacetobacter xylinus*	7	15.60	Static
Glycerol	*Gluconacetobacter xylinus ATCC 53524*	4	3.83	Static
	Gluconobacter xylinus KCCM 41431	7	3.40	Static
	Komagataeibacter rhaeticusstrain PG2	10-15	8.70	Static
	Gluconacetobactersp.RKY5	6	5.63	Agitated
FRUIT JUICES				
Orange juice	*A. xylinum NBRC19693*	14	5.90	Static
Rambutan juice	*A. xylinumTISTR 107*	5	5.89	Static
Pineapple waste	*Gluconacetobacter medellinensis*	13	3.24	Static

Table-2: BNC Production from Industrial, Agricultural, and Food Wastes[8]

Carbon source/waste feedstock	Microorganism	Culture duration	BC Yield ($g\ L^{-1}$)	Culture mode
INDUSTRIAL WASTE				
Crude glycerol	*Gluconobacterxylinus KCCM 41431*	7	2.93	Static
Whey	*G.xylinus BCRC 12334*	7	6.95	Static
AGRICULTURAL WASTE				
Corn stalk	*Acetobacter xylinum*	7	2.86	Static
Wheat straw	*Acetobacter aceti subsp. xylinus*	11	15.40	Static
Cotton cloth	*G. xylinus ATCC 23770*	7–14	10.80	Static
Cashew tree exudates	*Komagataeibacter rhaeticus*	7	6.0	Static
FOOD WASTE				
Potato peel	*G.xylinus ATCC 10245*	6	4.70	Static
Kitchen waste	*Acetobacter xylinum ATCC 23767*	15	2.07	Static

Addressing these obstacles necessitates optimizing bioprocessing methods and fostering collaborative initiatives between academic and industrial sectors. It is through such collective endeavors that we can truly unleash the practical, sustainable, and impactful applications of BNC synthesis.

CONCLUSION

In conclusion, bacterial nano cellulose (BNC) synthesis from various sources represents a dynamic and promising field with broad applications and opens doors to advancements in wound healing, tissue

regeneration, and dental implant integration. The diverse origins of BNC, whether derived from traditional strains or genetically modified organisms, provide a rich landscape for exploration and innovation. The synthesis methods, including static and agitated cultures, as well as emerging cell-free systems, contribute to the versatility of BNC production. As we navigate the future of BNC synthesis, advancements in biotechnological tools, sustainable sourcing, and scalable production methods are poised to redefine its potential. Overcoming challenges related to cost, standardization, and market awareness will be crucial for the successful integration of BNC into mainstream applications. With ongoing collaborative efforts and continuous research, the synthesis of bacterial nano cellulose holds the promise of revolutionizing materials science, offering sustainable solutions for diverse industries, and paving the way for environmentally friendly and technologically advanced products.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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