RESSALVA

Atendendo solicitação da autora,
o texto completo deste
documento será disponibilizado
somente a partir de 27/02/2028.

UNIVERSIDADE ESTADUAL PAULISTA – UNESP
Faculdade de Ciências Farmacêuticas - Campus de Araraquara

GIOVANA MARIA NAVARRO DE MENDONÇA

Utilização de resíduos agroindustriais para a produção de esferas de celulose bacteriana como veículos para probióticos

ARARAQUARA
2026

GIOVANA MARIA NAVARRO DE MENDONÇA

Utilização de resíduos agroindustriais para a produção de esferas de celulose bacteriana como veículos para probióticos

Tese apresentada à Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Araraquara, para obtenção do título de Doutora em Engenharia e Ciência de Alimentos, junto ao Programa de Pós-Graduação em Alimentos, Nutrição e Engenharia de Alimentos, Câmpus de Araraquara.

Área de Concentração: Engenharia de Alimentos

Orientadora: Profª. Dra. Henriette Monteiro Cordeiro de Azeredo

Coorientador: Prof. Dr. Wilton Rogério Lustri

Araraquara

2026

D376u

De Mendonça, Giovana Maria Navarro

Utilização de resíduos agroindustriais para a produção de esferas de celulose bacteriana como veículos para probióticos / Giovana Maria Navarro De Mendonça. -- Araraquara, 2026

144 p.

Tese (doutorado) - Universidade Estadual Paulista (UNESP), Faculdade de Ciências Farmacêuticas, Araraquara

Orientadora: Henriette Monteiro Cordeiro De Azeredo

Coorientadora: Wilton Rogério Lustri

1. Esferas. 2. Melaço. 3. Encapsulação. 4. Probióticos. 5. Heyndrickxia coagulans. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Dados fornecidos pelo autor(a).

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Araraquara

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: Utilização de resíduos agroindustriais para a produção de esferas de celulose bacteriana como veículos para probióticos

AUTORA: GIOVANA MARIA NAVARRO DE MENDONÇA
ORIENTADORA: HENRIETTE MONTEIRO CORDEIRO DE AZEREDO
COORIENTADOR: WILTON ROGÉRIO LUSTRI

Aprovada como parte das exigências para obtenção do Título de Doutora em Alimentos e Nutrição, área: Engenharia de Alimentos pela Comissão Examinadora:

Profa. Dra. HENRIETTE MONTEIRO CORDEIRO DE AZEREDO (Participaçao Virtual)
Programa de Pós-Graduação em Alimentos, Nutrição e Engenharia de Alimentos - UNESP

Profa. Dra. KATIA SIVIERI (Participaçao Presencial)
Programa de Pós-Graduação em Alimentos, Nutrição e Engenharia de Alimentos - UNESP

Profa. Dra. ADRIANE ELISABETE ANTUNES DE MORAES (Participaçao Virtual)
Programa de Pós-Graduação em Ciências da Nutrição, Esporte e Metabolismo - UNICAMP

Prof. Dr. HERNANE DA SILVA BARUD (Participaçao Presencial)
Laboratório de Biopolímeros e Biomateriais / Universidade de Araraquara (UNIARA)

Araraquara, 27 de fevereiro de 2026.

Dedico este trabalho à Deus e aos meus pais, Sônia e Sidney.

AGRADECIMENTOS

A Deus, por sempre me dar uma nova oportunidade todos os dias.

Aos meus pais, meus irmãos, a minha tia e ao meu namorado, por sempre estarem ao meu lado, mesmo quando tudo parecia incerto, obrigada pelas palavras gentis, pelos abraços, por todo suporte e por me permitirem realizar esse sonho.

À minha orientadora, Profª. Drª. Henriette M.C de Azeredo, por aceitar me orientar ao longo destes quatro anos e pela confiança depositada.

Aos meus coorientadores Dr. Wilton Rogerio Lustri e a Prof.ª Drª. Simara Lazarini, sem vocês eu não teria terminado essa jornada, vocês foram para mim muito mais do que "simples" coorientadores, o meu muito obrigada e minha eterna gratidão por todo carinho e ensinamento ao longo destes três anos.

Aos amigos que fiz ao longo desta jornada, aos amigos de longa data.

Aos analistas e técnicos da Embrapa Instrumentação por todo apoio durante minha estadia na unidade, em especial para a Joana, Silviane, Viviane, Paulinho, Alice e Adriana.

À Faculdade de Ciências Farmacêuticas – UNESP.

À Seção de pós-graduação da Inter unidade (Araraquara, São José do Rio Preto).

À Embrapa Instrumentação pelo espaço e estrutura laboratorial.

À UNIARA campus Araraquara, pela estrutura.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

“Não é o que sabemos que nos impede de saber, mas o que pensamos saber”

(Tradição socrática).

RESUMO

Desenvolveu-se e avaliou-se a produção de celulose bacteriana em formato esférico utilizando melaço de cana-de-açúcar e melaço de soja como fontes alternativas e sustentáveis de carbono em substituição à frutose, com o objetivo de reduzir custos e ampliar aplicações tecnológicas. Investigou-se a produção sob cultivo agitado e a influência dos diferentes substratos nas propriedades do material, incluindo rendimento, morfologia, porosidade, organização fibrilar e estabilidade térmica. Verificou-se que os meios à base de melaço proporcionaram rendimento e pureza estrutural comparáveis ao meio convencional, porém promoveram modificações significativas na microestrutura das esferas de celulose bacteriana, incluindo alterações na organização da rede tridimensional de nanofibras, no diâmetro das fibrilas e na arquitetura porosa do material. Observou-se que o melaço de cana favoreceu a formação de redes mais abertas e altamente porosas, enquanto o melaço de soja resultou em matriz com maior microporosidade e volume de poros, evidenciando que a composição do substrato modula a arquitetura tridimensional da celulose bacteriana. Avaliou-se ainda a aplicação do material como matriz carreadora do probiótico *Heyndrickxia coagulans* (ATCC 23769), analisando-se propriedades estruturais, térmicas e funcionais após a incorporação. Constatou-se que a impregnação do probiótico não alterou a estrutura química nem o perfil cristalino da celulose, mantendo-se elevada estabilidade térmica. Observou-se alta viabilidade celular durante armazenamento prolongado e maior proteção sob condições simuladas de digestão gastrointestinal, especialmente nas amostras produzidas com melaços. Concluiu-se que subprodutos agroindustriais constituem alternativa viável e sustentável para a produção de celulose bacteriana esférica com propriedades ajustáveis, além de demonstrarem potencial como sistema eficiente de proteção e liberação de probióticos para aplicações alimentícias e biotecnológicas.

Palavras-chave: Esferas; Melaço; Encapsulação; Probióticos; *Heyndrickxia coagulans*.

ABSTRACT

Sphere-like bacterial cellulose was developed and evaluated using sugarcane and soybean molasses as alternative and sustainable carbon sources to replace fructose, aiming to reduce production costs and expand technological applications. Production under agitated conditions was investigated, as well as the influence of different substrates on yield, morphology, porosity, fibrillar organization, and thermal stability. Molasses-based media provided yield and structural purity comparable to the conventional medium, while promoting significant modifications in microstructure of bacterial cellulose beads, including changes in the organization of the three-dimensional nanofibrillar network, fibril diameter, and the porous architecture of the material. Sugarcane molasses favored the formation of more open and highly porous networks, whereas soybean molasses resulted in a matrix with greater microporosity and pore volume, demonstrating that substrate composition modulates the three-dimensional architecture of bacterial cellulose. The material was further evaluated as a carrier matrix for the probiotic *Heyndrickxia coagulans* (ATCC 23769), and its structural, thermal, and functional properties were analyzed after incorporation. Probiotic impregnation did not alter the chemical structure or crystalline profile of bacterial cellulose, and high thermal stability was maintained. High cell viability was observed during prolonged storage, along with enhanced protection under simulated gastrointestinal conditions, particularly in samples produced with molasses. It was concluded that agro-industrial by-products represent a viable and sustainable alternative for producing sphere-like bacterial cellulose with tunable properties, demonstrating strong potential as an efficient system for probiotic protection and delivery in food and biotechnological applications.

Keywords: Beads; Molasses; Probiotics; *Heyndrickxia coagulans*; Encapsulation.

FIGURE LIST

TABLES LIST

SUMMARY

1. EXPANDED INTRODUCTION

The increasing global demand for food, energy, and materials has significantly driven the expansion of agro-industrial supply chains, resulting in the growing generation of residues throughout the stages of production, processing, and distribution. This scenario constitutes one of the major contemporary environmental challenges, particularly considering the intensification of industrial processes and the need to meet the demands of a continuously growing global population (Maqsood et al., 2025; Sharma, 2024). Residual biomass originating from agro-industrial activities, when not properly managed, can cause significant environmental impacts, including soil and water contamination, greenhouse gas emissions, and overloading of final disposal systems (Sharma 2024).

Agro-industrial residues comprise a wide range of by-products generated throughout agricultural production and food processing, including crop remains, peels, leaves, stems, fruit pulps, and industrial liquid effluents (Reyes-Barquet et al., 2022). These materials present a high organic load and are predominantly composed of carbohydrates, proteins, lipids, and phenolic compounds, in addition to micronutrients essential for microbial metabolism (Sharma 2024). Traditionally, such residues have been considered low-value by-products and are frequently directed toward animal feed, soil fertilization, or discarded into the environment without appropriate treatment (Kaul et al., 2024).

However, the contemporary sustainability perspective has promoted a reinterpretation of these materials, which are now recognized as important sources of renewable biomass capable of supplying carbon and nutrients for biotechnological processes aimed at producing high value-added bioproducts (Silva, 2019). It is estimated that approximately 1.3 billion tons of food are lost or wasted annually worldwide, with significant economic impacts in both developed and developing countries (Shukla et al., 2022). These figures highlight the still underexplored potential of agro-industrial residues as strategic resources within more sustainable production chains.

The sugar–ethanol industry exemplifies the magnitude of agro-industrial residue generation. Sugarcane processing results in the formation of several by-products, such as bagasse, straw, vinasse, filter cake, and molasses, the latter being generated in considerable amounts during the sugar crystallization process (Pérez-

Contreras et al., 2025). On a global scale, sugar and ethanol production from sugarcane may lead to the generation of hundreds of millions of solid and liquid residues annually, underscoring the need for effective strategies for their management and valorization (Ungureanu et al., 2022).

In this context, the use of agro-industrial residues as substrates for biotechnological processes, such as bacterial cellulose production, emerges as a promising alternative. In addition to reducing costs associated with raw materials at an industrial scale, this approach contributes to mitigating environmental impacts resulting from the accumulation and improper disposal of these materials, aligning global sustainability and circular economy guidelines (Ghosh et al., 2024; Urbina et al., 2021).

The growing concern regarding the environmental impacts arising from the traditional linear economic model — based on extraction, production, consumption, and disposal — has driven the transition toward more sustainable production systems grounded in the principles of bioeconomy and circular economy (Maqsood et al., 2025). In this context, the valorization of agro-industrial residues plays a central role by enabling the reintegration of residual streams into production chains, thereby promoting the efficient use of renewable biological resources.

Bioeconomy is based on the sustainable use of biomass to produce energy, food, chemicals, and materials, gradually replacing fossil resources with renewable alternatives (Pérez-Contreras et al., 2025). Agro-industrial residues and by-products, previously treated as disposable materials, are now being reinterpreted as strategic raw materials capable of supplying carbon, nitrogen, and micronutrients for fermentative processes and other biotechnological pathways (Silva, 2019).

This paradigm shift contributes not only to reducing the environmental footprint associated with the disposal of such materials but also to the creation of new economic and technological value chains. The transformation of residues into productive inputs enables the development of integrated biorefinery systems, in which different biomass fractions are directed toward the production of multiple bioproducts, such as biofuels, organic acids, enzymes, and biopolymers (KOUADIO et al., 2025).

The production of biomaterials through microbial pathways, such as bacterial cellulose, presents significant environmental advantages when compared to the extraction of plant-derived polymers, as it does not involve complex chemical purification steps and can be conducted under controlled fermentation conditions (Silva, 2019). The sustainability of these processes can be further enhanced using alternative culture media derived from agro-industrial residues, thereby reducing dependence on high-cost synthetic substrates (Quijano et al., 2021).

Furthermore, the incorporation of agro-industrial residues into bioprocesses enables the reduction of reliance on conventional inputs used in synthetic culture media, such as analytical-grade glucose. The replacement of conventional carbon sources with residues rich in fermentable sugars represents an economically viable strategy for the large-scale production of microbial biopolymers (Wang et al., 2025).

Among the different types of agro-industrial residues available, by-products rich in fermentable sugars, such as sugarcane and soybean molasses, stand out due to their high biotechnological potential. Molasses is a by-product generated during the sugar crystallization process, representing the remaining liquid fraction that no longer allows the formation of additional sucrose crystals (Geremew Kassa et al., 2024).

Sugarcane molasses presents a chemical composition characterized by high levels of sucrose, glucose, and fructose, in addition to containing minerals, vitamins, and nitrogenous compounds, making it a highly favorable substrate for microbial growth (Palmonari et al., 2020; Ungureanu et al., 2022). Similarly, soybean molasses, generated during the industrial production of protein concentrates, are rich in sucrose and raffinose family oligosaccharides, and contain small amounts of monosaccharides and bioactive compounds, including proteins, lipids, and phenolic acids (Fernandes et al., 2024; Rodrigues et al., 2021).

The abundance of these compounds provides molasses with suitable nutritional characteristics for their use as an alternative source of carbon and nutrients in fermentative processes. Several studies have demonstrated the feasibility of using molasses as a substrate for microbial growth and to produce bioproducts, including organic acids, biopolymers, and microbial biomass (Bispo et al., 2023; Zhang et al., 2021).

Additionally, the presence of essential micronutrients in these residues may enhance microbial metabolism and reduce the need for supplementation of the culture medium with additional nitrogen sources or vitamins (Pérez-Contreras et al., 2025). This feature represents a significant operational advantage, as it simplifies culture medium formulation and may contribute to reducing the costs associated with the industrial-scale production of microbial biopolymers (Gambarato et al., 2025).

In the context of bacterial cellulose production, the replacement of the standard Hestrin–Schramm culture medium with alternative substrates derived from sugar-rich residues has been widely investigated as a strategy to make the process economically viable at an industrial scale (Quijano et al., 2021; M. Wang et al., 2025). The presence of fermentable sugars and essential micronutrients in molasses may favor the metabolism of cellulose-producing bacteria, such as species of the genus *Komagataeibacter*, promoting cellulose biosynthesis with physicochemical properties comparable to those obtained in synthetic media (Quijano et al., 2021).

In addition to economic advantages, the use of sugar-rich residues in biotechnological processes contributes to reducing the pollutant load associated with their disposal and promotes the full utilization of agricultural resources. Thus, the valorization of by-products such as molasses represents a key strategy for the development of sustainable bioprocesses and for the consolidation of production models based on the circular bioeconomy (Pérez-Contreras et al., 2025).

Bacterial cellulose (BC) has become established as a biopolymer of considerable scientific and technological interest due to its combination of renewable origin, controllable biosynthesis, and structural properties that, in many application scenarios, position it as superior to cellulosic matrices obtained from plant sources. Extracellularly produced by different microorganisms, particularly acetic acid bacteria of the genus *Komagataeibacter* (formerly *Gluconacetobacter* and historically associated with denominations such as *Acetobacter xylinum/xylinus*), BC is chemically identical to plant cellulose at the monomeric level — a linear homopolysaccharide composed of β-D-glucopyranose units linked by β-1,4-glycosidic bonds — but differs decisively in its supramolecular organization and in the degree of purity with which it is obtained directly from cultivation (Diaz-Ramirez et al., 2021a; Urbina et al., 2021). This distinction is not merely descriptive: in BC, the secretion of protofibrils and their self-aggregation culminate in a three-dimensional network of highly entangled nanofibrils, with porosity and high surface area, forming hydrogels in which the water content may be extremely high, approaching a material composed predominantly of an aqueous phase and a minor fraction of structural cellulose (Joseph et al., 2025; Manan et al., 2022). This nanofibrillar architecture, combined with the abundance of hydroxyl groups on its surface, underpins properties such as hydrophilicity, water retention capacity, and a broad window for functionalization and composite formation, aspects that are consistently associated with the value of BC in fields such as biomedicine, food, packaging, materials engineering, and environmental applications (Garavand et al., 2023; Joseph et al., 2025; Manan et al., 2022).

BC can be understood as a “bottom-up biosynthesis product,” in which the microorganism converts metabolic precursors (notably through pathways associated with UDP-glucose) into cellulose chains, polymerized by cellulose synthase enzymatic complexes and exported to the extracellular environment, where they organize into fibrils and ribbons that constitute the final network (Manan et al., 2022; Shahzad et al., 2023a). This network is described as a three-dimensional mesh of nanofibers that confers high structural integrity to the material in its hydrated state and a high specific surface area, contributing to its mechanical performance, porosity, and hydrogel-like behavior (Joseph et al., 2025; Manan et al., 2022; Shahzad et al., 2023b). In dimensional terms, different studies and reviews highlight BC as being composed of nanoscale elements (nanofibrils, bundles, and ribbons), with nanometric diameter values reported in multiple review and application works — often within ranges on the order of tens of nanometers for nanofibers and hierarchical organization extending to microfibrils/ribbons (Gan et al., 2023; Gregory et al., 2021a; Joseph et al., 2025; Urbina et al., 2021). In some descriptions focused on biomedical applications, it is further emphasized that fibril bundles may organize with very small diameters and preferential orientation, forming a matrix with high crystallinity and strength, in addition to remarkable water retention capacity (Infante-Neta et al., 2024; Joseph et al., 2025; Swingler et al., 2021).

The functional superiority frequently attributed to BC, particularly when compared to plant cellulose, derives from a set of mutually reinforcing factors: (i) native purity (absence of lignin, hemicelluloses, and pectins), (ii) a highly ordered micro/nanoarchitecture, and (iii) the potential for control of form and properties through bioprocess engineering (Gregory et al., 2021a; Joseph et al., 2025; Shahzad et al., 2023b; Swingler et al., 2021; Urbina et al., 2021). While plant cellulose is intrinsically associated with a lignocellulosic matrix whose recovery requires separation and purification steps that may be potentially aggressive (with impacts on integrity and processing sustainability), BC is secreted in an already pure form, with post-processing being more related to the removal of cells and culture medium residues than to the extraction of a complex plant matrix (Cazón e Vázquez, 2021; Gregory et al., 2021a; Joseph et al., 2025; Swingler et al., 2021). This purity has direct implications for sensitive applications, such as food contact and biomedical use, as it reduces the need for severe treatments and favors performance reproducibility (Cazón e Vázquez, 2021; Joseph et al., 2025; Swingler et al., 2021). In the field of food packaging, BC has been discussed as a biopolymer with a promising profile, supported by properties such as film-forming ability and water retention capacity, in addition to toxicological safety widely reported in food-oriented reviews (Cazón e Vázquez, 2021). In this regard, BC has been described as classified as “generally recognized as safe” (GRAS) by a United States regulatory agency since 1992, reinforcing its potential for applications in food contact materials and food formulations (Cazón e Vázquez, 2021).

Cellulose biosynthesis is not uniform among microorganisms. Reviews indicate that different bacteria may produce cellulose with varying degrees of supramolecular organization, including amorphous cellulose associated with the extracellular matrix in some strains and crystalline cellulose produced by microorganisms classically associated with the production of high-purity BC (Urbina et al., 2021). In this context, *Komagataeibacter xylinus* remains a widely cited model organism due to its capacity to produce high levels of the polymer, serving both fundamental studies and practical applications (Gregory et al., 2021a; Urbina et al., 2021). Other genera are also referred to as producers (e.g., *Acetobacter*, *Agrobacterium*, *Rhizobium*, *Sarcina*, *Alcaligenes/Achromobacter*, *Gluconacetobacter* in historical nomenclature), and biomedical reviews further expand this list, including several bacterial genera associated with the synthesis of cellulose or extracellular cellulosic materials (Joseph et al., 2025; Swingler et al., 2021; Urbina et al., 2021).

The diversity of strains and species is not merely taxonomic: it is reflected in differences in yield, metabolic robustness, tolerance to cultivation conditions, and the potential for conversion of alternative substrates, which are particularly relevant when production is considered from the perspective of sustainability and scalability (Diaz-Ramirez et al., 2021a; Shahzad et al., 2023a; Urbina et al., 2021). This variability explains why, in the transition from laboratory-scale systems to industrial applications, strain selection is described as a strategic step, capable of simultaneously influencing productivity, morphology, and the quality of the final material (Płoska et al., 2023; Shahzad et al., 2023a).

BC production is intrinsically linked to the composition of the culture medium, as bacterial growth and polymer synthesis depend on carbon and nitrogen sources, micronutrients, and physicochemical conditions that preserve metabolic and enzymatic activity (Cazón e Vázquez, 2021; Gregory et al., 2021a; Urbina et al., 2021). The Hestrin and Schramm (HS) medium is consistently reiterated as a historical and experimental reference, containing a carbon source (often glucose), nitrogen sources such as peptone and yeast extract, as well as components for pH adjustment/stability and salts, and is widely used as a basis for comparisons and optimizations (Diaz-Ramirez et al., 2021a; Gouripriya et al., 2024; Gregory et al., 2021a). At the same time, multiple reviews converge in identifying the cost of the medium, particularly the carbon source—as a bottleneck for commercial viability, driving research into alternative substrates and waste valorization pathways (Cazón e Vázquez, 2021; Diaz-Ramirez et al., 2021a; Płoska et al., 2023; Urbina et al., 2021).

The importance of the carbon source extends beyond its nutritional role: it is presented as a determinant of growth, yield, and, in certain scenarios, final product properties, as it fuels biosynthetic pathways and may influence production rate, fibrillar organization, and characteristics such as crystallinity and rheological behavior of suspensions (Brandes et al., 2018; Deng et al., 2022; Martins et al., 2024a). In reviews addressing sustainable production from agro-industrial residues, the search for “accessible, low-cost, and non-food-competing” sources is emphasized, integrating BC into circular economy approaches (Diaz-Ramirez et al., 2021a; Urbina et al., 2021). This movement includes both lignocellulosic residues and by-products from food and agro-industrial chains, with the recurring caveat that residual materials may require standardization steps, pre-treatments, and variability control to ensure reproducibility and suitability for more demanding applications (Swingler et al., 2021; Urbina et al., 2021).

A particularly illustrative example of integration between residual substrates and process control is the production of BC spheres from winery by-products, in which grape pomace (GP) is used as a nutrient source, associated with pre-treatments (e.g., ultrasonication and hot water hydrolysis) to improve the availability of fermentable components and thus favor biopolymer biosynthesis (Diaz-Ramirez et al., 2021a). In this case, the selection of a strain suited to acidic media and high yield—*Komagataeibacter medellinensis* ID13488—is emphasized as a strategy to reduce operational complexity, including the avoidance of prior neutralization steps when the raw material is naturally acidic (Diaz-Ramirez et al., 2021a). Such an approach is representative of how bioprocess engineering (strain selection, substrate preparation, pH adjustment, agitation regime, and cultivation geometry) can be articulated to simultaneously valorize residues, reduce costs, and direct specific morphologies, such as spheres (Diaz-Ramirez et al., 2021a; Płoska et al., 2023).

BC is well known for its morphological versatility. Under static conditions, the predominant description is the formation of a pellicle (biofilm/mat) at the air–liquid interface of the culture medium, driven by the aerobic nature of cellulose-producing bacteria and the selective advantage of remaining close to atmospheric oxygen (Cazón e Vázquez, 2021; Gouripriya et al., 2024; Gregory et al., 2021a; Urbina et al., 2021).

This pellicle tends to thicken over cultivation time, although reviews point to practical limitations such as metabolite accumulation and time constraints (for example, incubation not exceedingly approximately two weeks under specific laboratory conditions) (Urbina et al., 2021). Pellicles obtained through static cultivation are often associated with greater uniformity and, in several descriptions, superior mechanical properties and crystallinity when compared to materials formed under agitation, reinforcing their suitability for applications such as membranes, packaging, and scaffolds (Diaz-Ramirez et al., 2021a; Gregory et al., 2021a; Urbina et al., 2021).

In contrast, under agitated (dynamic) cultivation, BC assumes morphologies dispersed throughout the liquid volume, including fibrous aggregates, pellets, and globular/spherical structures, frequently described as irregular masses or “sphere-like” forms (Cazón e Vázquez, 2021; Diaz-Ramirez et al., 2021a; Urbina et al., 2021). This morphological transition is central to the present thesis because it materializes the idea that the “product” BC is not merely a chemical composition but an architecture that can be controlled through cultivation conditions. Reviews focused on the production of bacterial biomaterials emphasize that different applications require materials with distinct properties, and that morphology—determined by strain and operational parameters—becomes a design axis rather than a by-product of the process (Mcmeeking et al., 2024). Thus, the production of BC spheres may be interpreted as a morphological engineering strategy to meet demands in which the planar pellicle form is not ideal, such as particulate systems, fluidization, fixed beds, or applications requiring high surface area and dispersibility (Mcmeeking et al., 2024; Płoska et al., 2023; Urbina et al., 2021).

In the food field, for instance, it has been discussed that although film-form BC is attractive for biodegradable packaging, the particulate form (including spheres/pellets) may be functionally advantageous in systems where texture, stability, dispersion, and interactions with liquid phases are decisive (Cazón e Vázquez, 2021; Martins et al., 2024b). In biomedical applications, the spherical form is associated with opportunities in controlled release, 3D models, and cellular support systems, as three-dimensional geometries and high surface area may favor diffusion, active compound loading, and interaction with tissues (Deng et al., 2022; Joseph et al., 2025; Shahzad et al., 2023b). In environmental and separation applications, the transformation of cellulose (including BC as an inducing/structuring component) into porous microspheres directed toward chromatography demonstrates the value of spherical architectures for permeability and mass transfer (Li et al., 2023).

The production of BC spheres stands out for shifting synthesis away from the “interfacial film” paradigm toward a particulate regime, in which polymer nucleation and growth occur at multiple points within the medium, generating discrete bodies with high surface-to-volume ratios and greater potential for handling and incorporation into matrices compared to continuous pellicles (Cazón e Vázquez, 2021; Deng et al., 2022). This innovative character is explicitly discussed in different contexts: (i) as a route to generate natural carriers (for example, for fertilizers) with high water retention capacity, (ii) as a shape-engineering alternative for controlled release systems, and (iii) as an architecture suited to separation and purification processes in columns (porous microspheres) (Brandes et al., 2018; Diaz-Ramirez et al., 2021a; Garavand et al., 2023).

In studies involving winery by-products, BC spheres are described as “superabsorbent”, with water holding capacity (WHC) superior to that of membranes produced under static conditions, attributed to the porosity and spherical shape of the samples (Diaz-Ramirez et al., 2021a). Moreover, the ability to retain urea in high proportions relative to dry weight and release it in aqueous environments highlights their potential use as natural carriers for fertilizers, linking sphere production to the agenda of agricultural sustainability and rational water resource use (Diaz-Ramirez et al., 2021a). In recent reviews focused on food applications, spherical morphology is similarly associated with innovation in texture and functionality, including prospects for use as fat substitutes or carriers of bioactive compounds when protection and controlled release within food matrices are required (Płoska et al., 2023). Although specific applications may vary, the common denominator is the capacity to “design” BC for a given function: porous spheres for absorption and loading; denser spheres for mechanical integrity; and particles with controlled size distribution for reproducible performance in industrial processes (Jayani et al., 2020; Płoska et al., 2023; Swingler et al., 2021).

In chromatographic applications, a distinct strategy is observed in which BC is not the final "biosynthesized spherical product", but rather a structural element (in the form of a "slurry") that induces the formation of hyperporous cellulose microspheres in a subsequent manufacturing process (pre-crosslinking, emulsification, and solidification), resulting in convective macropores that mitigate mass transfer limitations (Garavand et al., 2023). Although this work does not describe the bioproduction of BC itself, it reinforces the relevance of spherical and porous architectures for high-performance applications, demonstrating how BC can act as a structuring/nucleating agent and enable hyperporous networks with high surface area and permeability suitable for column-based systems (Garavand et al., 2023). Thus, the notion of "spheres" is not restricted to agitated cultivation; rather, it is embedded within a continuum of strategies in which BC may be produced or employed to generate spherical morphologies with defined functionality (Garavand et al., 2023).

Sphere formation under agitated cultivation is not a random event; rather, it reflects an orchestration of parameters that simultaneously regulate metabolism, mass transfer, and supramolecular organization of the polymer (Cazón e Vázquez, 2021; Gregory et al., 2021a; Swingler et al., 2021). The literature used in this thesis converges on the idea that pH, oxygenation, and agitation constitute the "core" of morphological control, whereas medium volume, flask size, inoculum concentration, temperature, and carbon source modulate yield and quality, often with non-linear interactions (Deng et al., 2022; Jayani et al., 2020; Urbina et al., 2021).

Medium pH: pH is identified as a critical factor due to its influence on growth, enzymatic activity, and synthesis efficiency. In reviews related to packaging and food applications, "adequate" cultivation conditions for pellicle production include slightly acidic pH, explicitly reported in the range of approximately 4–5 in the context of cultivation and film formation (Gregory et al., 2021a). In systems involving *K. medellinensis*, pH adjustment to 3.5 (using citric acid) is described as part of the process design, consistent with a strain that tolerates and thrives in acidic media—an advantageous condition when using naturally acidic raw materials such as grape pomace (Diaz-Ramirez et al., 2021a). In studies dedicated to spherical BC, the use of a specific initial pH (e.g., 6.6) is reported in experimental protocols, and it is discussed that pH variations may affect biosynthesis and even the dispersion of additives in BC matrices (Deng et al., 2022). Collectively, these examples reinforce that pH is not merely a condition for bacterial "comfort": it is integrated into process design and may facilitate sustainability (by avoiding neutralization steps), system stability, and material quality (Gregory et al., 2021a; Swingler et al., 2021).

Oxygenation and aeration: Most BC-producing bacteria are described as strictly aerobic, making oxygen availability a determining factor for productivity (Cazón e Vázquez, 2021; Swingler et al., 2021; Urbina et al., 2021). Under static cultivation, the interfacial pellicle is interpreted as a mechanism that favors access to surface oxygen and functions as a barrier/structure associated with the bacterial lifestyle (Diaz-Ramirez et al., 2021a; Gregory et al., 2021a). Under agitated cultivation, oxygenation becomes dependent on hydrodynamics and the medium volume-to-flask volume ratio, and sphere formation is associated with the maintenance of cells and aggregates throughout the liquid volume, with more distributed mixing and oxygen diffusion (Cazón e Vázquez, 2021; Deng et al., 2022; Swingler et al., 2021). Studies dedicated to spheres discuss that agitation influences oxygen distribution and substrate penetration, and that cellular adhesion to bubbles in an agitated medium has been proposed as a mechanism for the biosynthesis of spherical bodies, directly linking physicochemical phenomena to morphological outcomes (Urbina et al., 2021).

Agitation/rotation: intensity as a morphological selector. The agitation regime is described as the most distinctive parameter between pellicles and spheres, since agitation prevents the formation of a single film at the surface and promotes multiple nucleation events and particle formation(Cazón e Vázquez, 2021; Deng et al., 2022; Gouripriya et al., 2024). In the case of spheres produced from winery by-products, moderate speeds (for example, 130 rpm) are reported as effective for obtaining spheres, whereas variations (120 rpm, 150 rpm) alter shape, purity, and crystallinity, indicating an operational window in which the balance between mixing and shear stress is favorable (Diaz-Ramirez et al., 2021a). In a specific study on processing parameters for spherical BC, it is discussed that lower speeds tend to favor greater dry mass production and improved sphericity, while increases in speed promote aggregation and size reduction, potentially culminating in irregular masses under more intense conditions (Jayani et al., 2020). The same discussion highlights that agitation, although useful for yield and scalability, may result in a more disordered structure, with lower crystallinity and reduced mechanical strength compared to static membranes, reflecting the interference of motion and aeration in the formation of hydrogen bonds and microfibril crystallization (Cazón e Vázquez, 2021; Jayani et al., 2020). Thus, agitation control should be understood as a trade-off: sufficient intensity to avoid pellicle formation and promote spheres, but not so high as to excessively disrupt the material or induce aggregation/fragmentation (Cazón e Vázquez, 2021; Deng et al., 2022).

Medium volume and flask size: Although not all articles address this topic uniformly, the recurring principle is that system geometry affects aeration and hydrodynamics, influencing productivity and morphology (Cazón e Vázquez, 2021; Jayani et al., 2020; Swingler et al., 2021). In protocols involving winery by-products, the use of different medium volumes for static and agitated cultivation in Erlenmeyer flasks is described, evidencing adjustments in filling volume to optimize interaction with agitation and oxygenation conditions (Swingler et al., 2021). In studies on spherical BC, it is observed that medium volume may affect the produced dry mass, although its effect on sphericity may be small under certain conditions, reinforcing that some variables modulate yield more than final geometry depending on the operational regime (Jayani et al., 2020). In any case, the surface-to-volume ratio, mixing pattern, and oxygen transfer efficiency form a design triangle that must be explicitly considered when seeking reproducible sphere control (Deng et al., 2022; Diaz-Ramirez et al., 2021a; Gregory et al., 2021a).

Carbon source and inoculum concentration: In a study dedicated to spherical BC, the carbon source is presented as a high-impact variable, influencing growth, dry mass production, and water absorption capacity, with observations that certain substrates favor higher production and, simultaneously, greater crystallinity and improved relative purification of the material (Jayani et al., 2020). Inoculum concentration is also discussed as a determinant of homogeneity and spherical shape, with the proposition that residual fibers present in the inoculum may act as nucleating structures, favoring cellular aggregation and the initiation of spherical growth (Jayani et al., 2020). These observations connect the “initial state” of the system (cell load, presence of nucleating agents, and carbon availability) to the trajectory of sphere formation, reinforcing that morphological control may begin even before cultivation, during inoculum and medium preparation (Jayani et al., 2020).

Temperature and cultivation time: Temperature is identified as a variable capable of influencing water absorption capacity and mean sphere diameter, with the identification of conditions under which spherical morphology forms with specific diameters and aggregation tendencies over the course of cultivation days, suggesting that not only fixed temperature values but also process time modulate morphological stability and performance (Jayani et al., 2020). In reviews, pellicle thickness under static cultivation is associated with incubation time, and practical limitations due to metabolite accumulation over prolonged periods are discussed, highlighting that even when the focus is on spheres, process kinetics and by-product dynamics are integral elements of bioprocess design (Urbina et al., 2021).

The relationship between morphology and quality is presented in the materials used as complex and dependent on the cultivation regime. Reviews in the packaging field describe that spheres/pellets obtained under agitation tend to exhibit a lower crystallinity index and inferior mechanical properties compared to uniform pellicles obtained under static conditions, reinforcing the notion that agitation may disorder the structure and reduce parameters associated with crystallinity (Gregory et al., 2021a). In texts that explicitly discuss agitation, it is highlighted that movement-induced forces may interfere with hydrogen bond formation and chain packing, resulting in smaller crystallites and a higher amorphous fraction, which impacts strength and degree of polymerization (Urbina et al., 2021). At the same time, spherical morphology may maximize absorption and water retention properties, as exemplified by the significant increase in WHC observed in spheres compared to membranes, attributed to the porosity and three-dimensional architecture of the particulate material (Swingler et al., 2021). In this sense, “quality” should be interpreted contextually: in packaging and mechanical applications, higher crystallinity and integrity may be prioritized; whereas in carriers, hydrogels, and water retention applications, porosity and swelling capacity may be more valuable (Gregory et al., 2021a; Płoska et al., 2023; Swingler et al., 2021).

This reasoning is consistent with reviews that describe nanocellulose (including BC) as an adjustable platform in which key properties—surface area, porosity, mechanical stability, and reactivity—can be modulated through synthesis, processing, and functionalization (Li et al., 2023; Manan et al., 2022). In the context of stimuli-responsive nanocellulose hydrogels, BC is incorporated into a broader discussion of porous polymer networks, water retention, and functionality, reinforcing that its capacity to form hydrogels (or to integrate into hydrogels through physical/chemical crosslinking) makes BC particularly attractive for smart materials (Shahzad et al., 2023b). Although not all texts detail the production of spheres, the conceptual convergence is that morphological engineering—including spheres—

operates as a mechanism to tailor the material's interaction with water, solutes, cells, and end-use environments (Płoska et al., 2023; Shahzad et al., 2023b).

The wide range of applications associated with BC is consistently highlighted in reviews that emphasize the “smart” and multifunctional character of this biomaterial (Brandes et al., 2018; Deng et al., 2022; Diaz-Ramirez et al., 2021a). However, spherical morphology adds an engineering component that enhances specific applications.

In agriculture, the production of superabsorbent spheres from winery by-products exemplifies a high-impact pathway: an abundant residue is used as a substrate, resulting in spheres with high water retention capacity and the ability to load and release fertilizers, thereby proposing a solution aligned with challenges related to water scarcity and excessive agrochemical use (Swingler et al., 2021). The process design itself—including control of acidic pH, selection of a robust strain, and optimization of agitation—is presented as an integral part of sustainability and final performance (Swingler et al., 2021).

In the food and packaging sectors, BC is discussed both as a biodegradable packaging material (primarily in film format) and as a functional food ingredient (where particulate morphologies may favor dispersibility and textural effects) (Gregory et al., 2021a; Płoska et al., 2023; Ruiz-Palomero et al., 2017). Discussions of BC in dairy products reinforce interest in properties such as water retention capacity, stability, and potential as a stabilizer/emulsifier, as well as the relevance of alternative substrates such as whey to reduce production costs and integrate food chains into circular processes (Ruiz-Palomero et al., 2017). In studies on the foaming properties of proteins (egg white and soy), BC is described as a material capable of influencing the stability and behavior of dispersed systems, indicating the importance of particle size/morphology control and suggesting, by extension, that controlled particulate formats may be relevant for functional food applications (Jayani et al., 2020).

In biomedicine, reviews highlight BC as a highly pure, biocompatible biomaterial with strong potential for wound dressings, tissue engineering, delivery systems, and other regenerative medicine applications (Deng et al., 2022; Diaz-Ramirez et al., 2021a; Gouripriya et al., 2024).Production in spherical form aligns with the need for three-dimensional geometry and with applications in which uniform particles offer advantages in terms of dispersion, loading capacity, and interaction with biological environments (Deng et al., 2022; Jayani et al., 2020; Swingler et al., 2021).

In perspectives related to 3D printing, it is discussed that BC can be incorporated into bioinks and that agitated fermentative systems may produce irregular “sphere-like” particles, reinforcing the connection between particulate morphology and processability in complex formulations [16]. Although the spheres mentioned are not always perfectly monodisperse, the underlying principle is that the shift toward granular morphologies expands the design space for biomaterials with defined architecture [16].

In separation and purification, hyperporous microspheres induced by BC demonstrate that spherical architectures with controlled macroporosity can reduce mass transfer resistance and decrease column backpressure, qualifying them for anion-exchange chromatography and other operations in which permeability and dimensional stability are crucial (Garavand et al., 2023). Even though this route involves subsequent fabrication rather than direct biosynthesis in spherical form, it reinforces the centrality of “shape” as a functional attribute, highlighting how BC can act as a structuring agent to create high-performance spherical materials (Garavand et al., 2023).

The literature used in this thesis converges on a central point: BC should not be treated as a “fixed” material, but rather as a platform whose final expression—in terms of yield, purity, crystallinity, porosity, and shape—depends on the interaction between biology (strain, metabolism, tolerances) and engineering (medium, substrate, pH, oxygen, hydrodynamics, reactor geometry, and process time) (Cazón e Vázquez, 2021; Infante-Neta et al., 2024; Jayani et al., 2020; Martins et al., 2024a; Swingler et al., 2021). Sphere production constitutes a particularly representative frontier of this integration, as it shifts gresynthesis from the interfacial environment to a three-dimensional regime in suspension, requiring more refined control of mass transfer phenomena and mechanical stress on cells and nascent fibrils (Cazón e Vázquez, 2021; Deng et al., 2022; Jayani et al., 2020).

At the same time, spherical morphology makes explicit the “tailor-made” nature of BC: spheres can be designed to maximize water retention and nutrient loading (agriculture), to enable dispersion and texture modulation (food systems), to facilitate active compound loading and three-dimensional interaction (biomedicine), and to optimize mass transfer and permeability (separation processes) (Garavand et al., 2023; Gregory et al., 2021a; Płoska et al., 2023; Swingler et al., 2021). In all these cases, the conceptual core remains the same: BC combines native purity, a nanofibrillar network, and adjustability through bioprocessing; and spheres represent a particularly fertile expression of this adjustability, expanding the range of applications and concretely linking sustainability, performance, and innovation (Diaz-Ramirez et al., 2021a; Płoska et al., 2023; Urbina et al., 2021).

Probiotics play a central role in maintaining gastrointestinal health by contributing to the restoration of microbial balance and the strengthening of intestinal barrier function, constituting a widely explored strategy for promoting physiological homeostasis and preventing dysfunctions associated with dysbiosis (Sarita et al., 2025). However, probiotic efficacy may be substantially reduced throughout the digestive tract due to multiple physicochemical and biological barriers, including gastric acidity, enzymatic degradation, and the presence of bile salts, in addition to technological factors related to processing, storage, and transportation (Xu et al., 2022; Zhong et al., 2025). Therefore, for a microorganism to be considered probiotic in food and nutraceutical applications, the demonstration of viability and functionality at the target site is as critical as the characterization of safety and genetic stability, requiring rigorous selection criteria that encompass the absence of pathogenicity, compatibility with the oral route, and performance under adverse conditions of the gastrointestinal tract (GIT) (Bhattacharya e Baidya, 2025; Nicolas e Ma, 2025).

In the contemporary field, the definition of probiotics as live microorganisms that, when administered in adequate amounts, confer health benefits to the host, remains the conceptual basis for technological development and regulatory evaluation (Bhattacharya e Baidya, 2025; Nicolas e Ma, 2025). However, the attribution of the term “probiotic” implies the demonstration of functional and safety properties at the strain level, as well as evidence of genetic stability, absence of undesirable effects, and the ability to remain viable during the storage period and throughout gastrointestinal transit (Bhattacharya e Baidya, 2025). Recent reviews describe that probiotic activity is associated with multifactorial mechanisms, including modulation of microbial composition, competition for nutrients and adhesion sites, production of bioactive metabolites, and interaction with host immune responses, with reported impacts on gastrointestinal and systemic disorders (Bhattacharya e Baidya, 2025; Nicolas e Ma, 2025). Nevertheless, the functionality observed in studies depends on the integrity of the administered dose and the fraction effectively capable of resisting gastrointestinal tract conditions, which reinforces the relevance of protection and controlled release strategies (Ji et al., 2025; Sheikh, 2011; Yao et al., 2025).

The maintenance of viable counts at levels considered adequate for therapeutic activity is a critical point in the development of probiotic formulations. It is widely reported that probiotics should be consumed in sufficient amounts, typically in the range of 10^6 to 10^7 CFU per gram or milliliter at the time of ingestion to exert beneficial effects. However, processing steps such as lyophilization and gastrointestinal transit can significantly reduce microbial viability, meaning that the number of viable cells reaching the intestine may be substantially lower (Sheikh, 2011). In addition, even after consumption, the gastrointestinal environment imposes substantial challenges, as the low pH of the stomach and the action of hydrolytic enzymes may compromise cellular survival before reaching the intestine, and exposure to bile salts and mechanical stresses may further reduce the active fraction (Sheikh, 2011; Yao et al., 2025). In this context, the literature indicates increasing investment in technologies capable of protecting live microorganisms throughout the production chain and gastrointestinal transit, including encapsulation strategies and delivery systems that ensure viability up to the site of action (Ji et al., 2025; Rufino Vieira et al., 2024; Yao et al., 2025).

Among recent approaches, the development of synbiotic systems that associate probiotics with components that enhance their survival and performance stands out, including polymeric matrices with protective and/or prebiotic properties (Ji et al., 2025). In targeted release studies, the association with prebiotics is described as capable of increasing resistance to gastrointestinal stress and supporting colonic release, suggesting that combined systems may increase the probability of maintaining functionality under adverse conditions (Yao et al., 2025). In parallel, it is observed that the diversification of probiotic carrier matrices—extending beyond dairy foods to alternative vehicles—broadens application opportunities but also intensifies the need for stabilization technologies, since different products may impose additional constraints due to thermal processing, moisture variations, and shelf life (Perotti et al., 2024a; Rufino Vieira et al., 2024). Thus, the engineering of delivery systems becomes a determining step in transforming biological potential into reproducible and scalable performance.

Among the microorganisms of interest, spore-forming probiotics have received particular attention due to their superior resistance to adverse environmental conditions and, consequently, greater technological stability, a characteristic particularly attractive for functional foods and nutraceuticals [18,19]. Among them, *Heyndrickxia coagulans* (formerly *Bacillus coagulans*) have emerged as a species of increasing scientific and industrial relevance, being described as a Gram-positive, rod-shaped, lactic acid-producing bacterium capable of growing under aerobic or facultatively anaerobic conditions (Konuray Altun e Erginkaya, 2021; Li et al., 2023; Sheikh, 2011). The gathered literature indicates that the most distinctive property of *H. coagulans* is its ability to form endospores, which confers high tolerance to stresses typical of industrial processing and the gastrointestinal tract, including pH variations, desiccation, and exposure to bile salts (Konuray Altun e Erginkaya, 2021; Li et al., 2023; Sheikh, 2011). This intrinsic resistance favors the maintenance of viability during technological steps and, additionally, supports its ability to pass through the stomach and germinate in the intestine, where it may exert beneficial effects (Duncan et al., 2024; García et al., 2024).

The taxonomic trajectory of *H. coagulans* is presented as an example of scientific refinement based on methodological advances, with successive reclassifications that reflected greater phylogenetic resolution (García et al., 2024). Although part of the older literature uses the designation *Bacillus coagulans* and mentions historical confusion with *Lactobacillus sporogenes*, the functional identity described remains linked to lactic acid production and, above all, to sporulation (García et al., 2024; Sheikh, 2011).

The sporulation capacity also supports a logistical and industrial argument: the resistance of spores allows for reduced dependence on refrigeration and greater compatibility with different products, facilitating transport, storage, and incorporation into matrices in which non-spore-forming probiotics frequently lose viability (Konuray Altun e Erginkaya, 2021; Zhang et al., 2025). Studies in humans and recent reviews, in turn, reinforce that *H. coagulans* spores may survive gastrointestinal transit and germinate in the intestine, which provides a basis for their use as a functional probiotic in different delivery forms (Duncan et al., 2024; Chen et al., 2026). In the context of delivery in alternative food matrices, the *in vivo* assessment of survival and germination of spores administered via gummies exemplifies the possibility of expanding consumption vehicles while maintaining biological performance, reinforcing the strategic role of the spore form (Duncan et al., 2024). Complementarily, clinical trials and *in vitro* safety assessments describe the investigation of specific strains as candidates for probiotic supplements, reinforcing the need to demonstrate the absence of undesirable effects and adequate tolerance to simulated gastrointestinal tract conditions (García et al., 2024).

Although sporulation confers an evident advantage, the gathered literature indicates that challenges persist when seeking to maximize stability and control release in a reproducible manner, particularly at industrial scale and under variable processing and storage conditions (Khongkool et al., 2025; Sheikh, 2011). In this regard, encapsulation strategies and incorporation into biopolymeric matrices have been investigated to increase the viable fraction and optimize retention at the target site, integrating physicochemical protection and prolonged release mechanisms (Sheikh, 2011; Yao et al., 2025). A relevant example is the formulation of mucoadhesive hypromellose (HPMC) microspheres loaded with *B. coagulans* (nomenclature used in the study), with subsequent enteric coating by hypromellose phthalate, aiming to protect the microorganism against gastric conditions and promote sustained release in the intestine (Sheikh, 2011).

The presented rationale includes the limited residence time of conventional systems and the advantage of mucoadhesion in prolonging retention and directing the delivery system to the gastrointestinal mucosa, potentially enhancing probiotic performance (Sheikh, 2011). This body of evidence supports the premise that carrier engineering can amplify benefits even when the species already exhibits natural resistance. Within the same innovation axis, the immobilization of *H. coagulans* in bacterial cellulose is described as a synbiotic strategy aimed at increasing probiotic stability and overcoming limitations associated with the industrial production of spores in liquid media (Khongkool et al., 2025). In this context, bacterial cellulose is presented as a high-purity biopolymer with a porous nanofibrous structure, capable of forming matrices with high water retention and mechanical strength, which favors its application as a support for the entrapment and protection of microorganisms (Khongkool et al., 2025). The immobilization approach in bacterial cellulose is described as capable of increasing the survival of *H. coagulans* under stresses such as gastric acidity, bile salts, and temperature, promoting maintenance of viability and potentially contributing to a synbiotic system if the matrix acts as an indigestible fiber associated with prebiotic effects (Khongkool et al., 2025). Although spore resistance already provides protection, incorporation into an additional matrix may modulate the diffusion of aggressive agents, create a more stable microenvironment, and favor more controlled release during gastrointestinal transit, aligning with the objective of ensuring that the effective dose reaches the intestine under functional conditions (Khongkool et al., 2025; Yao et al., 2025).

The selection of matrices based on bacterial cellulose is also integrated into a sustainability and waste valorization agenda. Reviews and studies on bacterial nanocellulose indicate sustainable production from alternative substrates as a consolidated trend, aiming to reduce costs and increase industrial feasibility [(Núñez et al., 2024; Quijano et al., 2024). In particular, the literature mentions production routes of bacterial cellulose in alternative media and the production of spheroids/spherical hydrogels with tunable dimensions, which provides a conceptual and experimental basis to produce spheres intended for delivery applications (Sun et al., 2024; Urbina et al., 2021). The production of superabsorbent spheres from agro-industrial by-products reinforces the possibility of structuring matrices with suitable absorption and porosity properties, useful for the impregnation of biological agents (Diaz-Ramirez et al., 2021a). Comprehensive (“cookbook”) reviews on sustainable and cost-effective substrates for bacterial cellulose production reinforce the interest in the use of agricultural and agro-industrial residues as carbon sources, evidencing the alignment of the topic with circular economy and low-cost bioprocesses (Quijano et al., 2024b).

The gathered literature supports that probiotics, although promising, require protection to survive processing and gastrointestinal barriers (Rufino et al., 2024; Sheikh, 2011; Xu et al., 2022; Zhong et al., 2025), and that spore-forming probiotics such as *H. coagulans* offer a relevant technological advantage due to resistance and the ability to germinate in the intestine, with evidence of safety and performance in specific studies (Ke et al., 2026; Konuray Altun e Erginkaya, 2021; Sheikh, 2011). In parallel, bacterial cellulose is described as a robust and promising matrix for immobilization and for sustainable production from residues, with the potential to form spheres suitable for impregnation and release (Diaz-Ramirez et al., 2021a; Khongkool et al., 2025).

2. REFERENCES

Bhattacharya, S.; Baidya, S. (2025). Viability Preservation of Probiotic Bacteria Encapsulated in Pectin-Reinforced pH-Responsive Composite Alginate Hydrogel Matrix as the Synbiotic Delivery System. ***Probiotics and Antimicrobial Proteins***. https://doi.org/10.1007/s12602-025-10661-9

Bispo, R. L. B., Ceccato-Antonini, S. R., Tosta, C. D., Fontanetti, A., Prado, V. S., Takita, M. A., & Rosa-Magri, M. M. (2023). Sugarcane molasses as substrate to soil yeasts: Indole-3-acetic acid production and maize initial growth promotion. *Biocatalysis and Agricultural Biotechnology*, *47*, 102618. https://doi.org/10.1016/j.bcab.2023.102618

Brandes, R., de Souza, L., Vanin, D. V. F., Carminatti, C. A., Oliveira, E. M., Antônio, R. V., & Recouvreux, D. O. S. (2018). Influence of the Processing Parameters on the Characteristics of Spherical Bacterial Cellulose. *Fibers and Polymers*, *19*(2), 297–306. https://doi.org/10.1007/s12221-018-7679-5

Cazón, P., & Vázquez, M. (2021). Bacterial cellulose as a biodegradable food packaging material: A review. *Food Hydrocolloids*, *113*, 106530. https://doi.org/10.1016/j.foodhyd.2020.106530

D A, G., Adhikari, J., Debnath, P., Ghosh, S., Ghosh, P., Thomas, S., Ghandilyan, E., Gorbatov, P., Kuchukyan, E., Gasparyan, S., & Saha, P. (2024). 3D printing of bacterial cellulose for potential wound healing applications: Current trends and prospects. *International Journal of Biological Macromolecules*, *279*, 135213. https://doi.org/10.1016/j.ijbiomac.2024.135213

Deng, Y., Xi, J., Meng, L., Lou, Y., Seidi, F., Wu, W., & Xiao, H. (2022). Stimuli-Responsive nanocellulose Hydrogels: An overview. *European Polymer Journal*, *180*, 111591. https://doi.org/10.1016/j.eurpolymj.2022.111591

Diaz-Ramirez, J., Urbina, L., Eceiza, A., Retegi, A., & Gabilondo, N. (2021). Superabsorbent bacterial cellulose spheres biosynthesized from winery by-products as natural carriers for fertilizers. *International Journal of Biological Macromolecules*, *191*, 1212–1220. https://doi.org/10.1016/j.ijbiomac.2021.09.203

Duncan, R., Mantegazza, G., Gargari, G., Pierallini, E., Russo, R., & Guglielmetti, S. (2024). Heyndrickxia coagulans LMG S-24828 Is a Safe Probiotic Strain Capable of Germinating in the Human Gut. *Probiotics and Antimicrobial Proteins*. https://doi.org/10.1007/s12602-024-10383-4

Fernandes, S., Dias, B., Belo, I., & Lopes, M. (2024). Soybean molasses as a new and low-cost substrate for gluconic acid production by Aspergillus niger. *Process Biochemistry*, *146*, 241–249. https://doi.org/10.1016/j.procbio.2024.07.034

Gambarato, B. C., Carvalho, A. K. F., De Oliveira, F., da Silva, S. S., da Silva, M. L., & Bento, H. B. S. (2025). Soy Molasses: A Sustainable Resource for Industrial Biotechnology. *Sustainability*, *17*(12), 5667. https://doi.org/10.3390/su17125667

Gan, M., Guo, C., Liao, W., Liu, X., & Wang, Q. (2023). Development and characterization of chitosan/bacterial cellulose/pullulan bilayer film with sustained release curcumin. *International Journal of Biological Macromolecules*, *226*, 301–311. https://doi.org/10.1016/j.ijbiomac.2022.12.034

Garavand, F., Nooshkam, M., Khodaei, D., Yousefi, S., Cacciotti, I., & Ghasemlou, M. (2023). Recent advances in qualitative and quantitative characterization of nanocellulose-reinforced nanocomposites: A review. *Advances in Colloid and Interface Science*, *318*, 102961. https://doi.org/10.1016/j.cis.2023.102961

García, G., Soto, J., Díaz, A., Barreto, J., Soto, C., Pérez, A. B., Boffill, S., Gutiérrez, Á., & Cano, R. de J. (2024). Clinical and In Vitro Safety of Heyndrickxia coagulans AO 1167B: A Double-Blind, Placebo-Controlled Trial. *Microorganisms*, *12*(12), 2584. https://doi.org/10.3390/microorganisms12122584

Geremew Kassa, M., Asemu, A. M., Belachew, M. T., Satheesh, N., Abera, B. D., & Alemu Teferi, D. (2024). Review on the application, health usage, and negative effects of molasses. *CyTA - Journal of Food*, *22*(1). https://doi.org/10.1080/19476337.2024.2321984

Ghosh, T., Roy, S., Khan, A., Mondal, K., Ezati, P., & Rhim, J.-W. (2024). Agricultural waste-derived cellulose nanocrystals for sustainable active food packaging applications. *Food Hydrocolloids*, *154*, 110141. https://doi.org/10.1016/j.foodhyd.2024.110141

Gregory, D. A., Tripathi, L., Fricker, A. T. R., Asare, E., Orlando, I., Raghavendran, V., & Roy, I. (2021). Bacterial cellulose: A smart biomaterial with diverse applications. *Materials Science and Engineering: R: Reports*, *145*, 100623. https://doi.org/10.1016/j.mser.2021.100623

Infante-Neta, A. A., D'Almeida, A. P., & Albuquerque, T. L. de. (2024). Bacterial Cellulose in Food Packaging: A Bibliometric Analysis and Review of Sustainable Innovations and Prospects. *Processes*, *12*(9), 1975. https://doi.org/10.3390/pr12091975

Jayani, T., Sanjeev, B., Marimuthu, S., & Uthandi, S. (2020). Bacterial Cellulose Nano Fiber (BCNF) as carrier support for the immobilization of probiotic, Lactobacillus acidophilus 016. *Carbohydrate Polymers*, *250*. https://doi.org/10.1016/j.carbpol.2020.116965

Ji, H., Yan, X., Zhang, L., Yang, L., Xie, P., Gu, F., Bian, S., Wan, H., & Nie, S. (2025). Prebiotics empower probiotics with gastrointestinal stress resistance for colon-targeted release to synergistically alleviate colitis. *Journal of Controlled Release*, *380*, 297–316. https://doi.org/10.1016/j.jconrel.2025.01.059

Joseph, A., Umamaheswari, S., & Vassou, M. C. (2025). Bacterial cellulose: A versatile biomaterial for biomedical application. *Carbohydrate Research*, *552*, 109350. https://doi.org/10.1016/j.carres.2024.109350

Kaul, K., Rajauria, G., & Singh, R. (2024). Valorization of agro-industrial waste for pectinase production and its influence on circular economy. *Food and Bioproducts Processing*, *148*, 141–153. https://doi.org/10.1016/j.fbp.2024.09.008

Ke, S., Chen, Z., Qi, Y., Zhang, J., Chen, Q., Chen, J., & Bo, H. (2026). *Heyndrickxia coagulans* as a next-generation probiotic: current evidence and future perspectives. *Food & Function*, *17*(1), 15–40. https://doi.org/10.1039/D5FO03559G

Khongkool, K., Chamnankit, W., Thongnim, K., Tonklang, C., & Lertworapreecha, M. (2025). Enhancing probiotic stability of Heyndrickxia coagulans through synbiotic immobilization in bacterial cellulose. *World Journal of Microbiology and Biotechnology*, *41*(8), 271. https://doi.org/10.1007/s11274-025-04487-4

Konuray Altun, G., & Erginkaya, Z. (2021). Identification and characterization of Bacillus coagulans strains for probiotic activity and safety. *LWT*, *151*, 112233. https://doi.org/10.1016/j.lwt.2021.112233

KOUADIO, N. E., N'GORAN-AW, E. B. Z., BEKE, F. H., & SORO, D. (2025). Evaluation of Physico-chemical and Biochemical Parameters of Sugarcane Molasses and Cashew Apple Juice from Six Locations in Côte d'Ivoire. *European Journal of Nutrition & Food Safety*, *17*(4), 74–84. https://doi.org/10.9734/ejnfs/2025/v17i41680

Li, S., Shi, C., Ai, H., & Du, K. (2023). Bacterial cellulose-based submicrobundle framework-induced hyperporous cellulose microspheres functionalized by ionic liquids for anion exchange chromatography. *Chemical Engineering Journal*, *475*, 146030. https://doi.org/10.1016/j.cej.2023.146030

Li, Z., Zheng, M., Zheng, J., & Gänzle, M. G. (2023). Bacillus species in food fermentations: an underappreciated group of organisms for safe use in food fermentations. *Current Opinion in Food Science*, *50*, 101007. https://doi.org/10.1016/j.cofs.2023.101007

Manan, S., Ullah, M. W., Ul-Islam, M., Shi, Z., Gauthier, M., & Yang, G. (2022). Bacterial cellulose: Molecular regulation of biosynthesis, supramolecular assembly, and tailored structural and functional properties. *Progress in Materials Science*, *129*, 100972. https://doi.org/10.1016/j.pmatsci.2022.100972

Maqsood, S., Khalid, W., Kumar, P., Benmebarek, I. E., Rasool, I. F. U., Trif, M., Moreno, A., & Esatbeyoglu, T. (2025). Valorization of plant-based agro-industrial waste and by-products for the production of polysaccharides: Towards a more circular economy. *Applied Food Research*, *5*(1), 100954. https://doi.org/10.1016/j.afres.2025.100954

Martins, D., Khodamoradi, N., Silva-Carvalho, R., Gama, M., Moradi, M., & Dourado, F. (2024a). Effect of bacterial cellulose on the foaming properties of egg white and soy proteins. *Carbohydrate Polymer Technologies and Applications*, *7*, 100517. https://doi.org/10.1016/j.carpta.2024.100517

Martins, D., Khodamoradi, N., Silva-Carvalho, R., Gama, M., Moradi, M., & Dourado, F. (2024b). Effect of bacterial cellulose on the foaming properties of egg white and soy proteins. *Carbohydrate Polymer Technologies and Applications*, *7*, 100517. https://doi.org/10.1016/j.carpta.2024.100517

Mcmeeking, A., Dieckmann, E., & Cheeseman, C. (2024). Production methods for bacterial biomaterials: A review. *Materials Today Sustainability*, *25*, 100623. https://doi.org/10.1016/j.mtsust.2023.100623

Nicolas, G. M., & Ma, S. (2025). Effects of Heyndrickxia coagulans probiotics in the human gut microbiota and their health implications: A review. *Food Wellness*, *1*(1), 100003. https://doi.org/10.1016/j.foodw.2025.100003

Núñez, D., Oyarzún, P., González, S., & Martínez, I. (2024). Toward biomanufacturing of next-generation bacterial nanocellulose (BNC)-based materials with tailored properties: A review on genetic engineering approaches. *Biotechnology Advances*, *74*, 108390. https://doi.org/10.1016/j.biotechadv.2024.108390

Palmonari, A., Cavallini, D., Sniffen, C. J., Fernandes, L., Holder, P., Fagioli, L., Fusaro, I., Biagi, G., Formigoni, A., & Mammi, L. (2020). Short communication: Characterization of molasses chemical composition. *Journal of Dairy Science*, *103*(7), 6244–6249. https://doi.org/10.3168/jds.2019-17644

Pérez-Contreras, S., Hernández-Rosas, F., Lizardi-Jiménez, M. A., Herrera-Corredor, J. A., Baltazar-Bernal, O., Avalos-de la Cruz, D. A., & Hernández-Martínez, R. (2025). Sugarcane Industry By-Products: A Decade of Research Using Biotechnological Approaches. *Recycling*, *10*(4), 154. https://doi.org/10.3390/recycling10040154

Perotti, S., Mantegazza, G., Pierallini, E., Kirika, N., Duncan, R., Telesca, N., Sarrica, A., & Guglielmetti, S. (2024). Human in vivo assessment of the survival and germination of Heyndrickxia coagulans SNZ1969 spores delivered via gummy candies. *Current Research in Food Science*, *9*, 100793. https://doi.org/10.1016/j.crfs.2024.100793

Płoska, J., Garbowska, M., Pluta, A., & Stasiak-Różańska, L. (2023). Bacterial cellulose – Innovative biopolymer and possibilities of its applications in dairy industry. *International Dairy Journal*, *140*, 105586. https://doi.org/10.1016/j.idairyj.2023.105586

Quijano, L., Rodrigues, R., Fischer, D., Tovar-Castro, J. D., Payne, A., Navone, L., Hu, Y., Yan, H., Pinmanee, P., Poon, E., Yang, J., & Barro, E. (2024a). Bacterial cellulose cookbook: A systematic review on sustainable and cost-effective substrates. *Journal of Bioresources and Bioproducts*, *9*(4), 379–409. https://doi.org/10.1016/j.jobab.2024.05.003

Quijano, L., Rodrigues, R., Fischer, D., Tovar-Castro, J. D., Payne, A., Navone, L., Hu, Y., Yan, H., Pinmanee, P., Poon, E., Yang, J., & Barro, E. (2024b). Bacterial cellulose cookbook: A systematic review on sustainable and cost-effective substrates. *Journal of Bioresources and Bioproducts*, *9*(4), 379–409. https://doi.org/10.1016/j.jobab.2024.05.003

Quijano, L., Speight, R., & Payne, A. (2021). Future fashion, biotechnology and the living world: microbial cell factories and forming new 'oddkins.' *Continuum*, *35*(6), 897–913. https://doi.org/10.1080/10304312.2021.1993574

Reyes-Barquet, L. M., Rico-Contreras, J. O., Azzaro-Pantel, C., Moras-Sánchez, C. G., González-Huerta, M. A., Villanueva-Vásquez, D., & Aguilar-Lasserre, A. A. (2022). Multi-Objective Optimal Design of a Hydrogen Supply Chain Powered with Agro-Industrial Wastes from the Sugarcane Industry: A Mexican Case Study. *Mathematics*, *10*(3), 437. https://doi.org/10.3390/math10030437

Rodrigues, B. C. G., de Mello, B. S., Gonsales da Costa Araujo, M. L., Ribeiro da Silva, G. H., & Sarti, A. (2021). Soybean molasses as feedstock for sustainable generation of biomethane using high-rate anaerobic reactor. *Journal of Environmental Chemical Engineering*, *9*(4), 105226. https://doi.org/10.1016/j.jece.2021.105226

Rufino Vieira, É. N., Caroline de Oliveira, V., Gomes, A. T., Lourenço, M. T., do Amaral e Paiva, M. J., Santos, T. C., Guerra, D. J. R., & Saldaña, M. D. A. (2024). Perspectives of high-pressure technology in probiotic food production: A comprehensive review. *Food Bioscience*, *62*, 105179. https://doi.org/10.1016/j.fbio.2024.105179

Ruiz-Palomero, C., Soriano, M. L., & Valcárcel, M. (2017). Nanocellulose as analyte and analytical tool: Opportunities and challenges. *TrAC Trends in Analytical Chemistry*, *87*, 1–18. https://doi.org/10.1016/j.trac.2016.11.007

Sarita, B., Samadhan, D., Hassan, M. Z., & Kovaleva, E. G. (2025). A comprehensive review of probiotics and human health-current prospective and applications. *Frontiers in Microbiology*, *15*. https://doi.org/10.3389/fmicb.2024.1487641

Shahzad, A., Ullah, M. W., Ali, J., Aziz, K., Javed, M. A., Shi, Z., Manan, S., Ul-Islam, M., Nazar, M., & Yang, G. (2023a). The versatility of nanocellulose, modification strategies, and its current progress in wastewater treatment and environmental remediation. *Science of The Total Environment*, *858*, 159937. https://doi.org/10.1016/j.scitotenv.2022.159937

Shahzad, A., Ullah, M. W., Ali, J., Aziz, K., Javed, M. A., Shi, Z., Manan, S., Ul-Islam, M., Nazar, M., & Yang, G. (2023b). The versatility of nanocellulose, modification strategies, and its current progress in wastewater treatment and environmental remediation. *Science of The Total Environment*, *858*, 159937. https://doi.org/10.1016/j.scitotenv.2022.159937

Sharma, R. (2024). Agro-industrial waste to energy - Sustainable management. *Sustainable Materials and Technologies*, *41*, e01117. https://doi.org/10.1016/j.susmat.2024.e01117

Sheikh, M. A. A. (2011). Formulation and evaluation of Bacillus coagulans-loaded hypromellose mucoadhesive microspheres. *International Journal of Nanomedicine*, 619. https://doi.org/10.2147/IJN.S14621

Shukla, P., Anand, S., Srivastava, P., & Mishra, A. (2022). Hyaluronic acid production by utilizing agro-industrial waste cane molasses. *3 Biotech*, *12*(9), 208. https://doi.org/10.1007/s13205-022-03265-5

Silva, R. (2019). *Avaliação do ciclo de vida da produção de celulose bacteriana em diferentes meios de cultivo*. Universidade Estadual do Ceará.

Sun, B., Wang, P., Zhang, J., Lin, J., Sun, L., Wang, X., Chen, C., & Sun, D. (2024). In situ biosynthesis of bacterial cellulose hydrogel spheroids with tunable dimensions. *Journal of Bioresources and Bioproducts*, *9*(1), 90–101. https://doi.org/10.1016/j.jobab.2023.12.003

Swingler, S., Gupta, A., Gibson, H., Kowalczuk, M., Heaselgrave, W., & Radecka, I. (2021). Recent Advances and Applications of Bacterial Cellulose in Biomedicine. *Polymers*, *13*(3), 412. https://doi.org/10.3390/polym13030412

Ungureanu, N., Vlăduț, V., & Biriș, S.-Ștefan. (2022). Sustainable Valorization of Waste and By-Products from Sugarcane Processing. *Sustainability*, *14*(17), 11089. https://doi.org/10.3390/su141711089

Urbina, L., Corcuera, M. Á., Gabilondo, N., Eceiza, A., & Retegi, A. (2021). A review of bacterial cellulose: sustainable production from agricultural waste and applications in various fields. *Cellulose*, *28*(13), 8229–8253. https://doi.org/10.1007/s10570-021-04020-4

Wang, M., Zhao, L., Wang, Y., Zhang, C., & Li, H. (2025). Sugarcane Molasses Polyphenol Extract Attenuates Alcohol-Induced Chronic Liver Damage via Antioxidant, Anti-Inflammatory, and CYP2E1/Keap1/NF-κB Pathway Modulation. *Nutrients*, *17*(9), 1589. https://doi.org/10.3390/nu17091589

Xu, C., Ban, Q., Wang, W., Hou, J., & Jiang, Z. (2022). Novel nano-encapsulated probiotic agents: Encapsulate materials, delivery, and encapsulation systems. *Journal of Controlled Release*, *349*, 184–205. https://doi.org/10.1016/j.jconrel.2022.06.061

Yao, T., Wang, J., Yao, Y., Fu, L., & Li, L. (2025). Enhanced Survival of Probiotics by Encapsulating in Hydrogel Beads Based on Sodium Alginate/Pectin/Tannic Acid. *ACS Food Science & Technology*, *5*(5), 1979–1989. https://doi.org/10.1021/acsfoodscitech.5c00223

Zhang, S., Wang, J., & Jiang, H. (2021). Microbial production of value-added bioproducts and enzymes from molasses, a by-product of sugar industry. *Food Chemistry*, *346*, 128860. https://doi.org/10.1016/j.foodchem.2020.128860

Zhang, Y., Overbeck, T. J., Skebba, V. L. P., & Gandhi, N. N. (2025). Genomic and Phenotypic Safety Assessment of Probiotic Bacillus coagulans Strain JBI-YZ6.3. *Probiotics and Antimicrobial Proteins*, *17*(5), 3440–3450. https://doi.org/10.1007/s12602-024-10305-4

Zhong, H., Jiang, J., Hussain, M., Zhang, H., Chen, L., & Guan, R. (2025). The Encapsulation Strategies for Targeted Delivery of Probiotics in Preventing and Treating Colorectal Cancer: A Review. *Advanced Science*, *12*(18). https://doi.org/10.1002/advs.202500304

17. FINAL CONCLUSIONS

This thesis investigated the production of sphere-like BC in agro-industrial residues and its application as a matrix for the immobilization and protection of the probiotic *Heyndrickxia coagulans*, with a focus on the development of sustainable delivery systems for application in food and biotechnological systems. The results obtained in the first study demonstrated that the use of sugarcane molasses and soybean molasses as alternative carbon sources was able to replace chemically defined conventional media, such as fructose-based medium, without significant impairment to the yield of BC produced under agitated conditions. Furthermore, it was evidenced that the chemical composition of the substrates directly influenced the fibrillar organization, porosity, and three-dimensional architecture of the formed spheres, enabling the production of matrices with distinct structural properties that can potentially be modulated according to the desired application.

Structural, thermal, and morphological analyses confirmed that the use of agro-industrial residues did not compromise the purity or stability of the produced bacterial cellulose, while promoting relevant modifications in the microstructure of the material, such as increased specific surface area and pore volume, particularly in spheres produced using soybean molasses. These results indicate that the choice of cultivation substrate can act as a structural engineering tool for the cellulosic matrix, allowing the tuning of physicochemical properties that are important for applications involving encapsulation and controlled release of bioactive compounds.

In the second study, sphere-like BC obtained from different culture media were impregnated with *K. coagulans* and evaluated in terms of matrix structural integrity, storage stability, and probiotic protection capacity under simulated gastrointestinal tract conditions. FTIR and X-ray diffraction analyses demonstrated that the impregnation process did not promote significant chemical or crystalline changes in the BC structure, evidencing the compatibility between the polymeric matrix and the incorporated microorganism. Additionally, thermal analyses indicated high stability of the developed systems, regardless of the medium used for sphere production.

Viability assays demonstrated that all formulations were able to maintain probiotic survival during prolonged storage, with negligible losses even after 150 days at room temperature. During in vitro simulated gastrointestinal digestion, spheres produced from soybean and sugarcane molasses provided greater protection to the probiotic, maintaining counts above 9 log CFU g^{-1} after the intestinal phase, thereby evidencing the effectiveness of these matrices in preserving microbial viability under adverse digestive conditions.

Overall, the results demonstrate that the production of sphere-like bacterial cellulose from agro-industrial residues constitutes a viable, sustainable, and economically promising strategy for the development of probiotic immobilization and delivery systems. The use of molasses as a substrate not only contributes to reducing production costs but also allows modulation of the structural properties of the matrix, favoring its application as a protection and delivery system for live microorganisms. Therefore, the developed system presents potential for application in functional foods and other products aimed at promoting intestinal health, while aligning with the principles of circular economy and the valorization of agro-industrial by-products.