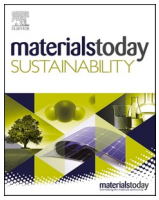
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Production methods for bacterial biomaterials: A review

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ABSTRACT

Alternatives for synthetic materials are necessary to meet climate goals and reduce waste. Biomaterials derived from the extracellular matrix of various bacteria provide a solution to these pressing issues due to their diverse material properties and applications. This review explores a wide range of biofilm producing bacteria, equipping researchers, and manufacturers with information regarding growth conditions and material characteristics to enable sustainable bacteria-derived material innovation. Data on extracellular matrix producing bacteria strains is presented, including information on material properties, production methods, material characteristics, and economic feasibility. This provides a valuable tool kit to help selection of bacteria strains for a variety of products and applications. Currently, there is a notable research need in the range of bacteria that produce extracellular matrix in the field of material science. Through the identification of barriers and the proposition of solutions such as genetic modification, system design, growth medium supplements, and rotating magnetic fields, researchers can sustain their innovation efforts. This, in turn, contributes to the improved quality and accessibility of cost-effective bacterial-produced biomaterials. This review addresses this need and aims to enable the creation of bio-derived materials by leveraging microbiology.

1. Introduction

This paper reviews production methods for bacterial biomaterials, a sustainable alternative to plastics. It demonstrates the broad spectrum of Non-pathogenic (NP) biomaterial-producing bacteria which can be used, each with unique material characteristics. The review highlights methods for accelerating bacterial biomaterial production, which is necessary if these materials are to compete with plastics and make the adoption of biomaterials more attractive to industry.

Plastics have become a major environmental issue despite providing versatility and affordability in many applications. An increasing amount of single-use plastic is discarded annually, with one million plastic drinking bottles purchased every minute and five trillion single-use plastic bags discarded annually [1]. The packaging industry uses 26 % of the total volume of plastic, with poor end-of-life management contributing to the pollution of the environment [2].

The accumulation of plastic waste in nature is a significant concern. Global plastic waste generation is expected to triple by 2060 from 353 million tonnes in 2019 if no action is taken [3]. The growth of plastic waste is expected to be fastest in developing and emerging countries in Africa and Asia. Plastics are derived from fossil hydrocarbons and are not biodegradable, leading to their build-up in the natural environment. Plastic debris is found in all major ocean basins, and contamination of freshwater systems and terrestrial habitats is increasingly reported [4].

The international trade in plastics accounts for 5 % of total trade and is a global issue. In 2017, China produced 28 % of global resin production and 68 % of global polyester, polyamide, and acrylic fibre production [4]. The clothing and fabric manufacturing industries are highly plastic-intensive, representing 45 % of the top 20 plastic-intensive industries and 55 % of the top 20 polluting plastic-intensive industries [5].

As an alternative, several materials derived from fungi, natural fibre-based materials and composites of synthetic and natural materials have been developed. Biomaterials refer to substances that are naturally produced, originating from sources such as insects, plants, or bacteria. These materials possess the potential to serve as fuels or offer an eco-friendly substitute for plastics. Despite limitations in the production of biomaterials, companies such as Adidas have used a combination of ethylene-vinyl acetate (EVA) and hydroponically sourced algae in products, such as the Adidas Yeezy Foam Runner [6]. Although these shoes are popular and are known for their comfort, the composition of the foam is predominantly EVA with an algae filler, meaning it primarily

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relies on fossil fuels for production. The use of hydroponic algae does represent a step towards sustainable production. However, the current technology and infrastructure for algae farming are not yet sufficiently advanced to replace fossil fuel-based materials such as EVA. Nevertheless, the success of the Adidas Yeezy Foam Runner highlights the growing demand for sustainable products and the need for continued innovation in the use of bio-based materials in consumer products [7].

Examples of truly bioderived biomaterials include chitosan, a material derived from crustaceans and insects. This is biocompatible and biodegradable, making it useful in drug delivery and medical implants. Industrial applications, include replacing non-renewable materials in packaging and purifying water by removing heavy metals and organic pollutants [8]. Being derived from a biological source is not synonymous with biodegradability. Just because a material is created from a biological source does not mean it can be effectively decomposed, which is a trait that consumers often mistakenly assume.

Bacterial biomaterials (BB) are abundant and versatile, making them promising candidates for sustainable materials. They are produced from the secretions of bacteria, and microorganisms that are found in every environment on Earth. BB have the potential to serve as a substitute for synthetic materials in a range of applications. Their biocompatible properties make them ideal for use in both medical and industrial fields. They can act as antimicrobial wound wraps and surgical sponges, as well as for water purification and packaging [9–12]. BB can be produced in situ using renewable resources such as sugar or local waste streams. Additionally, they can be broken down by natural processes, making them biodegradable and reducing the need for costly recovery strategies. Unlike synthetic materials, which can be harmful to the environment, BB are part of the natural cycle of life and is not considered a pollutant. Bacterial Nanocellulose (BNC) is a purer form of cellulose than alternatives such as NFCs (nanofibrillated cellulose) and CNCs (cellulose nanocrystals), which require enzymatic, mechanical, or chemical treatment during processing for manufacture [13,14]. Additionally, BNC is over ten times stronger than plant-based versions, making it highly durable [14]. The option of 3D printing using BNC allows the production of custom materials, which increases biomechanical benefits and durability, especially in products such as footwear where a “perfect fit” can improve pressure distribution. Moreover, BNC allows the development of microvascular networks, which could enable the future implementation of self-healing technologies such as self-healing polymers housed in these networks [15]. This alternative form of manufacturing would promote deglobalisation, bringing resources back to local communities, while fostering growth in new technologies and industries, leading to employment and economic growth [16].

Bacteria, such as *Komagataeibacter xylinus* (Kx), secrete pellicles as a means of survival in the natural environment. Biofilms adhere and grow on solid surfaces and are produced by certain species of bacteria, whereas pellicles are formed by bacteria at an air-liquid interface. Biofilms represent microorganism communities that attach to solid surfaces by producing Extracellular Polymeric Substances (EPS). Pellicles specifically form at the interface between air and water, as seen with Kx. In the context of biofilms, EPS serves as a subtype of Extracellular Matrix (ECM), facilitating their formation. EPS comprises a diverse array of substances, including exopolysaccharides, proteins, lipids, and nucleic acids, which are uniformly arranged to form a cohesive matrix. The Extracellular Matrix (ECM) encompasses a collection of biomolecules secreted by cells to offer structural and biochemical support to neighbouring cells. Kx produces an extracellular, chemically pure β-glucan, which forms a protective barrier around the cells. This is known as a cellulose matrix, keeping the cells at the surface of culture media entrapped inside a gelatinous, skin-like membrane consisting of entangled cellulose fibres. This self-immobilisation of the cells promotes the efficient transport of nutrients and oxygen, essential for these aerobic bacteria. Additionally, the high water-holding capacity of the cellulose matrix protects the Kx from desiccation and shields them from other adverse environmental factors, such as Ultra violate radiation. The ability to synthesize cellulose and adhere to its surface allows for the successful colonisation of ecological niches, reducing available space and supply of nutrients for competing microorganisms in the same habitats [17].

The pellicles produced by Kx and related strains are commercially important as the bacterial nanocellulose (BNC) market is growing rapidly. In 2018, the global biopolymer market was valued at £10 billion, with an expected compound annual growth rate (CAGR) of 19 % between 2019 and 2025. This growth is being driven by increasing demand for sustainable and environmentally friendly materials in various industries, such as packaging, healthcare, and food. Major players in the market, including Danimer Scientific (Bainbridge, Georgia, USA), ASF SE (Germany), and Total Corbion (Netherlands), are actively involved in the production and development of BNC-based products [18,19].

BNC-based products have demonstrated significant market success in the food industry. As environmental concerns become more prevalent in our society, more companies will turn to BNC for their material requirements, feeding the upwards trajectory of the market. To cater to the growing market demand, the continual development of production methods and advances in biomaterials research is critical [20].

Considering the rapidly increasing quantity of plastic waste generated globally, along with the urgent need to adopt sustainable practices, this literature review presents an alternative solution in the form of BB. As a substitute for synthetic materials, BB have the potential to reduce the need for costly recovery strategies and promote deglobalisation, leading to employment, technological development, and economic growth. The topic addressed in this review is timely and significant, given the ongoing repercussions of plastic waste on the environment and human health, as well as contributing to a growing industry.

The review offers designers and researchers a valuable resource to explore the use of a wider range of materials. By providing a toolbox of materials to choose from, each with different properties, the review opens the door to new shapes and forms due to new material properties unlocking new possibilities. This allows designers to push the boundaries of what is possible with BB, creating novel designs that were not previously feasible. For researchers, the literature review provides a stimulus to continue investigating less well-known types of bacteria, unlocking the potential for completely new materials and new applications. In addition, the review addresses the previous limitations of BB, proposing new technologies that could fix these issues. This demonstrates that designers should not be deterred by these materials, as by adapting the production method, BBs can be made to compete with synthetic materials.

2. Research design

The methodology of this paper is based on the PRISMA checklist [21] as seen in Fig. 1, which presents findings and outcomes clearly, and effectively communicates the purpose of the report. To ensure scientific integrity, the literature review for this paper was conducted using Web Of Science and Science Direct, where relevant articles were identified using keywords such as “Bacterial nanocellulose” and “biofilm-producing bacteria”. When searching for relevant research papers, Boolean operators such as “OR” and “AND” were utilised.

A Bibliometric network analysis was conducted using VOSviewer software version 1.6.19 (0). A map was generated composed of nodes of different sizes referring to the frequency of Authors’ keywords in papers (Fig. 2), linked by connections indicating how regularly they co-exist in papers. The proximity of nodes represents a closer relationship between them, and the colour gradient indicates the publication year.

The combination of “bacterial nanocellulose” OR “bacterial cellulose”, and a range of 2008–2022 was used on Web Of Science, this helped to identify 6715 related documents. This information was transferred to VOSviewer, where the co-occurrence setting with Authors Keywords was used, reducing the number of frequently occurring

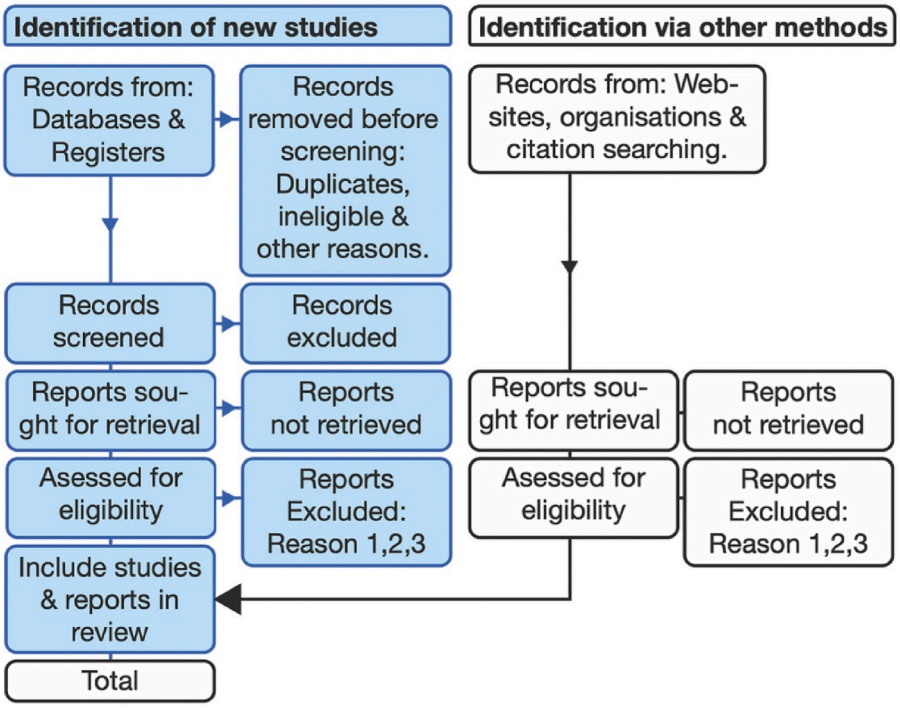


Fig. 1. PRISMA workflow diagram, demonstrating the methodology used when collating research for this review paper.

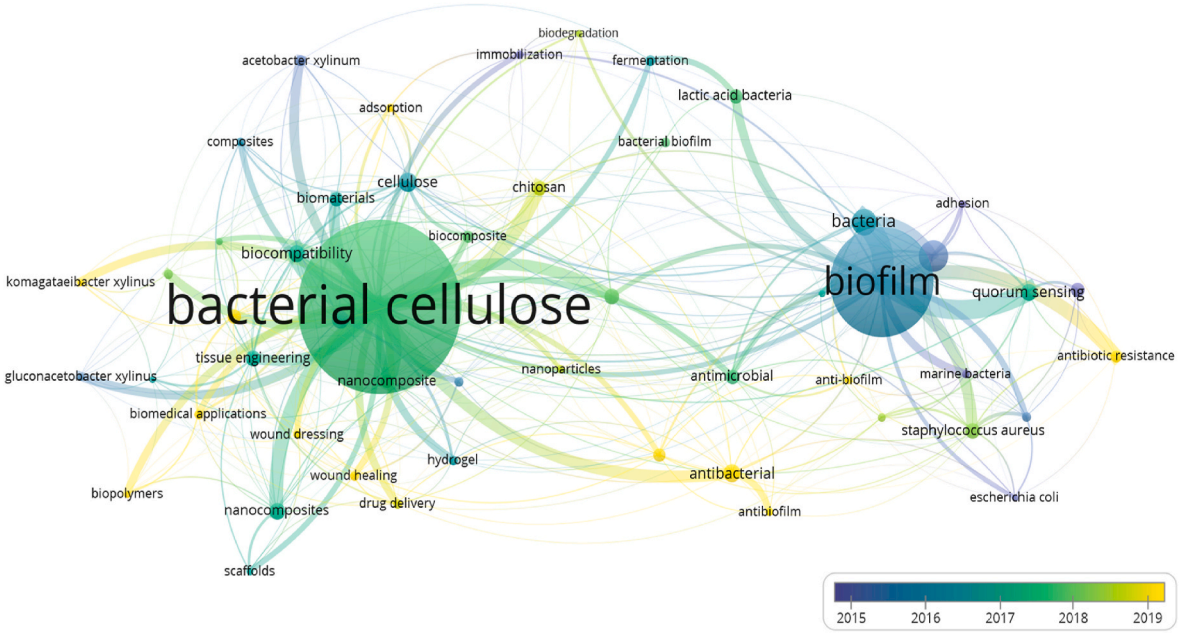


Fig. 2. VOSviewer Map shows authors' keyword connections between papers related to BNC, biomaterials, and biofilms. The variance of colour indicates the year of publishing. Node size indicates frequency, and the thickness of the links between demonstrates how often keywords coexist in papers.

keywords down to 221. This was further reduced by increasing the threshold of occurrences to 13, making the number of keywords decrease to 53.

The VOSviewer graph was analysed and used as an initial stimulus for this project. Key topics were identified from the selection of bacterial nanocellulose papers, including biofilm, bacteria, nanocomposites, biocomposites, biomaterials, biopolymers, *Staphylococcus aureus*, and *Escherichia coli*. The most prevalent keywords had a general theme relating to materials, indicating a clear area of interest among the scientific community. Additionally, bacteria strains other than just Kx were mentioned, which led to the exploration of the properties of other strains and drove research further. Seven clusters were identified within the data, further emphasising the importance of biomaterials, material composites the bacteria strains utilised.

Yearly, there has been an increase in the number of publications related to bacterial nanocellulose, with China leading research efforts [22]. Using Web of Science, a bar chart showing the gradual increase of Bacterial nanocellulose related publications over time was created (Fig. 3). As this topic becomes more relevant in commercial goods, research interest will increase, making the relevance of this paper, which

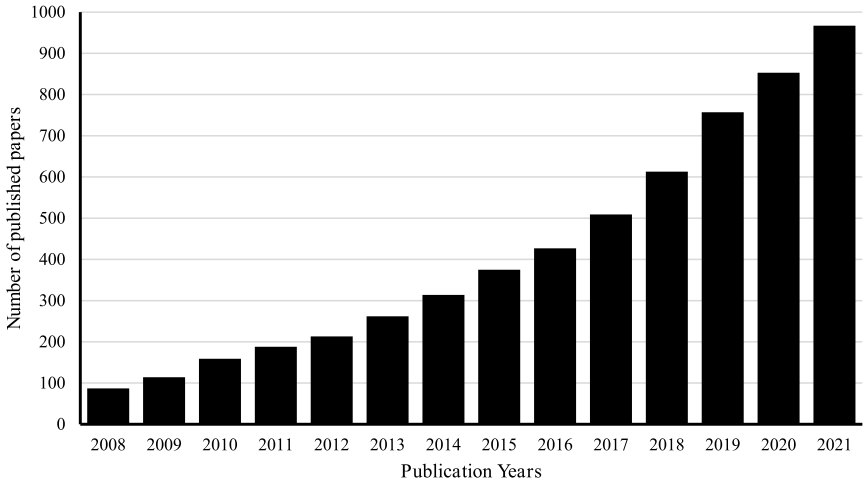


Fig. 3. Graph to show the number of bacterial nanocellulose articles published by year, created using Web of Science.

describes an array of bacterial strains feasible for biomaterials, even more significant.

It is important to recognise the significance of choosing the most suitable bacterial strain for each application. This will ensure the biomaterial's success in a specific use case, leading to greater product longevity and usability. Fig. 4 shows the selection process used to select bacteria with pertinent properties for this table. Firstly, Web of Science was utilised to search for papers relating to biofilm-producing bacteria. Each strain had to have a low-risk level, to ensure it was safe to handle during manufacture. To check each strain was non-pathogenic the BCCM online archive of bacteria was used [23]. This reduces production costs as less infrastructure is required to ensure safety and workers are unlikely to contract related illnesses during manufacture. The bacteria were then checked to ensure their biomaterials material properties were relevant and useable for commercial products. Finally, the financial feasibility of each strain was noted, specifically looking at its ideal growth conditions and required growth medium. Selected NP biomaterial-producing bacteria were then added to Table 1 before looking for further strains.

3. Existing biomaterials and their applications

Currently, due to infrastructure restrictions and economic feasibility, biomaterials such as BNC are made by smaller start-up companies, which often fail to realise consistent and reliable material production at scale [81]. Whereas in the food and cosmetics industries, the production of BNC is well-established and meets mass market demand. The reason for this is that these industries do not require complex post-processing. To create biomaterials with desirable and durable properties, post-processing must occur, however, the current infrastructure is not equipped to handle this [82].

Table 2 provides a comprehensive overview of biomaterials derived from bacterial sources and their diverse range of applications. One notable example is Polybion Celluim, which is claimed to have the world's first industrial-scale facility to produce bacterial cellulose. Polybion utilises kombucha to create luxury leather products, including shoes and handbags, which have been adopted by popular brands like Allbirds.

Kombucha SCOBY is an extremely versatile material, mostly used for filtration, textiles and in the food industry. Researchers recently utilised the Kombuchas' filtration properties for extracting rare earth elements

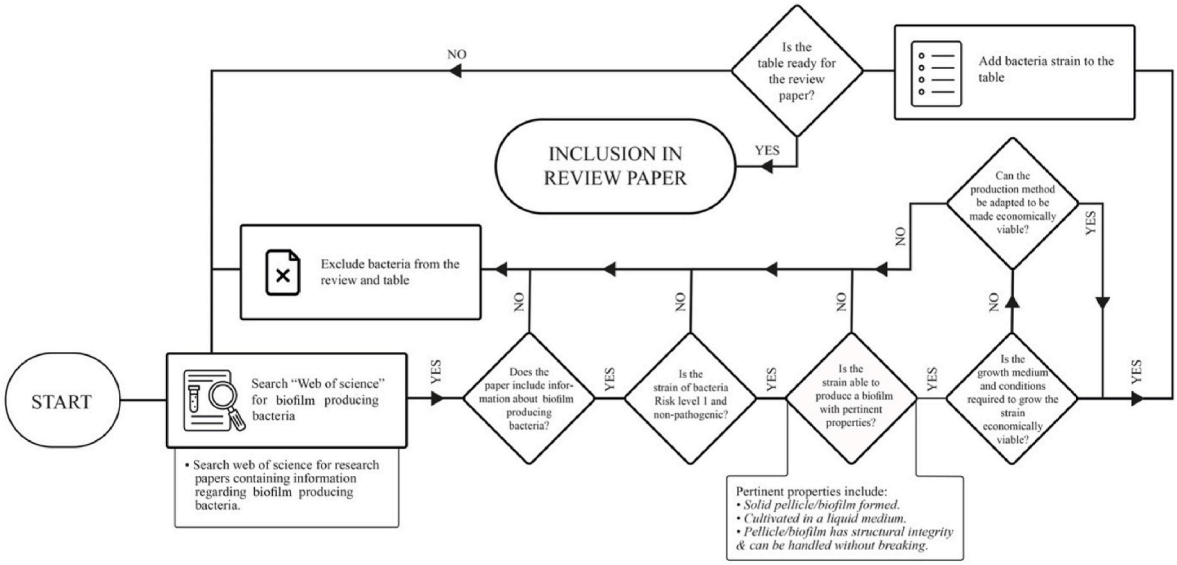


Fig. 4. Selection process for exploring the characteristics of extracellular matrices produced by different bacteria for biomaterial applications.

Table 1
Non-Pathogenic, risk level 1, Biomaterial-producing bacteria.

Strain	Biomaterial type	Growth Medium
Caulobacter crescentus (CB15)	This pellicle is typically thin, composed of a single layer of cells and densely packed, mushroom-shaped structures [24]	Caulobacter medium [25]
Komagataeibacter xylinus	An even, translucent, and medium thickness layer composed mainly of cellulose, along with the possible inclusion of other polysaccharides and proteins (Dependent on growth conditions) [26].	DSMZ Medium 105 [27]
Bacillus subtilis	A thin, heterogeneous, multi-layered, and exhibit complex extracellular matrix and cell-to-cell interactions. Consisting of polysaccharides, proteins, and other molecules [28,29]	MSgg medium at 22 °C.
Shewanella oneidensis	A thin layer comprised of a complex, multi-layered, and structured matrix. This matrix is made from exopolysaccharides, proteins, and other molecules [30,31]	Luria-Bertani (LB) broth [31]
Acetobacter aceti	A structured cellulase-resistant and alkaline-sensitive matrix made up of exopolysaccharides, proteins, and other molecules [32]	YPM MEDIUM (DSMZ Medium 360) [33]
Komagataeibacter saccharivorans	A thin and structured layer composed mainly of cellulose, along with the possible inclusion of other polysaccharides and proteins (Dependent on growth conditions) [34,35]	DSMZ Medium 1 [36]
Komagataeibacter maltaceti	A smooth, thin, multi-layered, and structured matrix made primarily from cellulose (Dependent on growth conditions) [35].	RAE MEDIUM - containing 1 % of ethanol and 1 % of acetic acid [37]
Gluconacetobacter kakiaceti	A thin, smooth, and multi-layered matrix composed primarily of cellulose (Dependent on growth conditions) [38,39]	GYP Glucose-Yeast-Peptone Medium or EM1 agar [38]
Gluconacetobacter swingsii	Comprised of cellulose as a key component, along with exopolysaccharides, proteins, and other molecules. (Dependent on growth conditions) [40,41]	ACE Medium (50 g glucose l^{-1}, 5 g yeast extract l^{-1}), Notes: Agro-industrial residues such as pineapple peel juice and sugar cane juice were used as culture media [40]
Gluconoacetobacter rhaeticus	A structured, and multi-layered matrix composed primarily of cellulose and exopolysaccharides, as well as proteins and other molecules (Dependent on growth conditions) [40,42].	HS medium [43] Alternatively YPD medium can be used.
Komagataeibacter sucrofermentans	A multi-layered matrix composed of cellulose, exopolysaccharides, and proteins (Dependent on growth conditions) [44]	Gluconobacter Oxydans Medium (DSMZ Medium 105) [45]
Komagataeibacter medellinensis	A multi-layered matrix composed of cellulose, exopolysaccharides, and proteins (Dependent on growth conditions) [46]	HS growth media [47]
Komagataeibacter intermedius	A multi-layered matrix composed of cellulose, exopolysaccharides, and proteins (Dependent on growth conditions) [48]	DSMZ 849. AE Medium [49]
Rhizobium leguminosarum	Composed of EPS and varies in thickness and texture [50].	MGYP medium. (Malt extract, Glucose, Yeast extract and Peptone) [51]
Gluconacetobacter sp.	Composed of cellulose and is typically thin, smooth, and highly adherent [52]	10 % citrus juice, 10 % sucrose, 1 % acetic acid, and 1 % ethanol [53]
Komagataeibacter hansenii	A multi-layered matrix composed of cellulose, exopolysaccharides, and proteins (Dependent on growth conditions) [54]	Schramm–Hestrin (SH) medium [55]
Komagataeibacter melomenusus	A multi-layered matrix composed of cellulose, exopolysaccharides, and proteins (Dependent on growth conditions) [37]	RAE medium with 1 % of ethanol and 1 % of acetic acid [37]
Komagataeibacter nataicola	A multi-layered matrix composed of cellulose, exopolysaccharides, and proteins (Dependent on growth conditions) [56]	Medium 1 [36]
Komagataeibacter europaeus	A multi-layered matrix composed of cellulose, exopolysaccharides, and proteins (Dependent on growth conditions) [57]	631. Acetobacter Europaeus Medium [58]
Komagataeibacter oboediens	A multi-layered matrix composed of cellulose, exopolysaccharides, and proteins (Dependent on growth conditions) [59]	848. RAE Medium [60]
Saccharibacter floricola	Composed of extracellular polymeric substances that form a thin, smooth, and adhesive layer on surfaces [61]	YPG medium [1 %(w/v) yeast extract (Difco), 1 %(w/v) peptone (Difco) and 7 %(w/v) glucose, adjusted to pH 6.0 with HCl] [61]
Ralstonia eutropha	Made up of extracellular polymeric substances and has a thin, smooth, and homogeneous appearance. EPS provides structure, protection, and communication among bacterial cells [62,63].	Fructose–Glycerol–Nitrogen (FGN) medium [64]

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Table 1 (*continued*)

Strain	Biomaterial type	Growth Medium
***Escherichia coli* (MG1655)**	Composed of EPS and has a thin, smooth, and uniform structure [65–67]	LB Broth [67]
Saccharomyces cerevisiae	A complex structure that is primarily composed of EPS, and appears thin and smooth under certain growth conditions [68,69]	YEPD (yeast extract, peptone, dextrose) Medium [70]
Oenococcus oeni	Primarily composed of EPS, is thin, and can have a smooth or rough surface [71].	MRS modified (MRSm) medium.
***Asticcacaulis biprosthecum* (YB5408)**	Composed of EPS, making it thin, smooth, and highly adhesive [72]	PYE Medium [73]
***Pseudomonas fluorescens* (WG)**	Biomaterials can vary in thickness and surface morphology, composed of exopolysaccharides, proteins, and extracellular DNA [74,75].	1. Grow culture in 5 mL LB overnight. 2. Transfer to a static culture of the K10 medium [76]
***Asticcacaulis excentricus* (YB258)**	Composed of extracellular polymeric substances (EPS) and is generally reported to exhibit a thin and smooth morphology [77].	PYE medium (0.2 % peptone, 0.1 % yeast extract) [78]
***Hirschia baltica* (YB5842)**	Composed primarily of extracellular polymeric substances (EPS) and appears thin, smooth, and transparent [79].	Marine medium (Difco marine broth/agar) [79]
Caulobacter crescentus* (CB15) – *With holdfast	This biomaterial is thin, smooth, and composed of exopolysaccharides, cellulose, proteins, and lipids, enabling adhesion and survival in various environments [80].	Luria-Bertani (LB) medium [80]

from fluorescent powder using a symbiotic mixed culture comprising of *Zygosaccharomyces lentus* and *Komagataeibacter hansenii* [83]. In addition, another symbiotic relationship has been developed to improve the performance of lithium-sulphur batteries by carbonising the Kombucha and combining this with various additives [84]. Furthermore, Kombucha has proven effective in removing heavy metals from water and can also be used to produce vegan leather as an alternative to animal leather [85]. Individual strains of bacteria seen in kombucha such as *Acetobacter xylinus* have also been combined in a composite with silver-nanoparticles to create a biocompatible and antimicrobial wound dressing [10]. These materials and applications were chosen due to their unique features, including eco-friendliness and biocompatibility.

The process of going from pellicle to the final material involves several steps. Initially, the pellicle is separated from the growth medium. It is then subjected to a cleansing process using distilled water and sodium hydroxide. This step eliminates surface residue, including sugars, while also sterilising the pellicle by eradicating any remaining bacteria. Following this, the extracellular matrix, which primarily comprises cellulose fibres and a mixture of water and sodium hydroxide, is washed with water to remove the sodium hydroxide. Subsequently, it is compressed to remove a large quantity of water content, thereby reducing its weight for transportation. This pellicle undergoes further processing for application in various industries. In the face mask industry, mechanical processes uniformly shape the pellicle, and additional additives are

Table 2
Biomaterials derived from microbes and their applications.

Material	Description	Application	Production process
***Zygosaccharomyces lentus* and *Komagataeibacter hansenii* pellicle.**	*Zygosaccharomyces lentus* and *Komagataeibacter hansenii* are used to extract rare earth elements (REE) from fluorescent powder using a symbiotic mixed culture called Kombucha, made of yeasts and acetic acid bacteria. The process leaches REE at a significant rate and is eco-friendly compared to current methods using strong inorganic acids or toxic chemicals [83]	Extraction of Rare Earth Elements from fluorescent powder.	The Kombucha SCOBY used is cultivated in a green tea and sucrose Growth medium [83]
Kombucha SCOBY-based carbon and graphene oxide wrapped sulphur/ polyacrylonitrile.	A combination of Kombucha SCOBY-based carbon and graphene oxide with sulphur/ polyacrylonitrile is utilised as a cathode in lithium-sulphur batteries, offering improved performance. The hierarchical-porous carbon sheets provide effective electron transfer and quick electrochemical reactions, leading to a high initial discharge capacity and long-lasting performance [84].	Cathode within a lithium-sulphur battery.	Kombucha tea is made by fermenting sweetened black tea with a SCOBY for 15 days. The resulting SCOBY is washed, carbonized, and activated with KOH. S/PAN/ KC, S/PAN/GO and S/PAN/ KC/GO composites are prepared by mixing and heating the ingredients [84]
Kombucha SCOBY.	Kombucha SCOBY can effectively remove heavy metals from water. Optimal conditions were found through a study using a central composite design and response surface methodology. Results showed high removal efficiencies for Hg^{2+}, As^{3+}, Pb^{2+}, Cd^{2+}, and Cr^{6+} [11]	Removal of heavy metals from water.	Tea fungus was obtained from a company in Iran, washed, and cut. Dominant bacterial strain and yeast components were identified. Metal ions were prepared in deionized water [11].
Kombucha Vegan Leather.	A versatile, durable, and sustainable alternative to commercial animal leather created by Iowa State researchers [85]	Textile alternative to synthetic materials.	This is produced using the SCOBY from fermented kombucha tea. This SCOBY is then dried and used to make leather products [85]

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Table 2 (*continued*)

Material	Description	Application	Production process
Bio-ink.	Cartilage tissue engineering – eg: the creation of a human ear for transplant. This is possible due to BNC's biocompatibility [86]	Biocompatible human ear cartilage transplant.	Additive manufacture: extrusion of a grid-like structure in the form of a human ear [86]
Brown algae – *(Laminaria hyperborea, Ascophyllum nodosum, & Macrocystis pyrifera)*.	This biomaterial is utilised in regenerative medicine for its unique porous structure. The material allows for improved blood flow and nutrient delivery to cells, promoting their growth in the treatment area [18,87]	Nutrient and medicine delivery for patient recovery.	Its ability to form stable microgels allows for consistent extrusion using a controlled injection process. Resulting in the formation of monodispersed droplets, ensuring stability and accuracy in the manufacturing process [18,87]
Guar gum/ carboxymethyl cellulose.	The study focuses on creating bioactive antioxidant films using a combination of guar gum/ carboxymethyl cellulose and halloysite-nanotubes along with litchi shell extract. This combination shows promise as a green solution for active food packaging [88]	Biodegradable food packaging.	Fresh litchi fruit shells are combined with additives and dried to create films which are folded to create packaging [88]
Silver-nanoparticles/ bacterial cellulose composite.	A novel method was developed to synthesize and impregnate silver nanoparticles onto bacterial cellulose nanofibers (AgNP-BC) for use as a wound dressing. The AgNP-BC has antimicrobial activity with a 99 % reduction of common bacteria and promotes wound healing with no cytotoxicity. It is a stable, evenly distributed nanostructure that minimizes toxicity while promoting cell growth [10].	Biocompatible and antimicrobial wound dressing.	The silver-nanoparticle/ bacterial cellulose composite is made by first washing the cellulose membranes and removing impurities. Silver ammonia solution is prepared and diluted, then the purified cellulose is soaked in the solution. The resulting membrane is heated and washed, creating the AgNP-BC hybrid gel membrane [10]
Nanocellulose-CNT Nanocomposite Films.	Supercapacitor/ sensors: Flexible, conductive films made of nanocellulose and carbon nanotubes. They are optimised for conductivity through a series of processing steps including sonication and centrifugal cast moulding. The study found that the surfactant type and sonication energy greatly affect the formation and conductivity of the films [89,90]	Conductive films within supercapacitors/ sensors.	Carbon nanotubes are dispersed into a solution with nanocellulose using sonication and surfactant, then using centrifugal cast moulding to form the films [89,90]
Bacterial Cellulose: Composite modifier for cathode catalysts.	Bacterial cellulose, doped with phosphorus and copper, was used as a composite modifier in the preparation of microbial fuel cell (MFC) cathode catalysts. This preparation method improved bioelectrical generation in MFC and offered an alternative to commercial Pt catalysts, showing a three-dimensional network and higher output power and current density compared to Pt [91].	Cathode catalyst within a Microbial fuel cell.	Bacterial cellulose was treated with phosphoric acid and copper chloride solution, frozen and dried, and then heated in a microwave tube furnace under a nitrogen atmosphere [91]
Bacterial Cellulose.	Bacterial cellulose, a material created by the cellulose-producing bacterium *Acetobacter xylinus*, is becoming a popular choice for interior design due to its environmentally friendly properties. With high mechanical strength and stability when exposed to high temperatures, it is well-suited to produce lampshades [92]	Biodegradable lampshade.	The BC pellicle is grown in a liquid growth solution and draped over a male mould until dried [92]
Polybion Celium.	BNC vegan leather composite is used as an alternative to leather in luxury handbags and footwear [93]	Shoe leather alternative.	The BC pellicles are generated from agricultural fruit waste.

incorporated before packaging them for distribution.

4. Characteristics of biomaterials produced by different bacteria for varying applications

The previous table shows the current utilisation of biomaterials derived from microbial origin, with the majority being sourced from kombucha. However, different applications require materials with differing properties. This presents a problem that needs to be addressed, just as we use a diverse range of synthetic materials for creating commercial products due to their distinct properties. Therefore, if a rigid material is needed, an alternative strain of bacteria must be utilised for the purpose. To tackle this issue, Table 1 presents descriptions of various biomaterials.

These biomaterials are created using the secretions of a variety of bacteria in the form of biofilms. Most biofilms are created through a process called microbial adhesion. Microorganisms, such as bacteria, first attach to a solid surface [94]. Once attached, depending on the strain, the microorganisms produce a matrix which surrounds and protects them. This matrix helps the microorganisms adhere to the surface and aids in their communication with one another. As more microorganisms join the biofilm, the matrix thickens, leading to the development of a mature biofilm [94]. The biofilm can continue to grow if there are enough nutrients and favourable conditions present. Eventually, the biofilm can detach and form new biofilms elsewhere [94]. Alternatively, bacteria such as Kx, cultivated in a liquid medium, will secrete a floating pellicle at the air-water interface [95]. As with biofilms, pellicles act as protection, whilst also aiding the bacteria to compete with other microorganisms.

One specific type of bacteria that creates pellicles is Kx. This bacterium follows a specific pathway during the oxidative fermentation process to produce unbranched cellulose pellicle extracellularly. The protofibrils of the glucan chains, which are approximately 2–4 nm in diameter, are formed outside the cytoplasm and are secreted out of the cell wall of the bacteria through the pores present on the surface [94]. Electron micrographs showed that there are 50–80 pores of 3.5 nm diameter, present on the cell surface, called complex terminals or "cellulose export component" which help in extruding these protofibrils. The fibrils are then arranged in a bundle to form a microfibril cellulose ribbon of dimensions approximately 804 nm [94]. The network structure of the pellicle is created through the interconnection of these ribbons, resulting in high porosity and surface area. X-ray crystallography observations of a BNC revealed a planar orientation in the dried form, with a single unit cell comprising two parallel cellobiose units linked by b-1-4 linkage [94]. This was categorised under type I cellulose which resembled a ribbon and had a crystalline structure. Two allomorphs of cellulose (Ia and Ib) were categorised which are structurally similar [96]. Type II cellulose is also formed which is amorphous and thermodynamically more stable. This type II cellulose is produced from pores where the export component is missing on the outer bacterial coat. Both types of cellulose are arranged differently on the surface of the bacterial cell membrane. Sometimes, type II cellulose is formed instead of type I cellulose in the presence of a few physical or chemical factors like increased aeration and chemicals like carboxymethyl cellulose and calcofluor white [94]. BNC is a unique biomaterial synthesised by certain types of bacteria. It is composed of a form-stable water--containing nanofiber network, consisting of 1 % cellulose and 99 % water. The fibres have a diameter of 20–100 nm and are created extracellularly by the enzyme cellulose synthase which is present on the cytoplasmic membrane of the bacterium [11].

Considerable research has been conducted on the pellicles generated by *Komagataeibacter xylinus* and *Komagataeibacter rhaeticus*. However, the material properties of biomaterials produced by other bacteria have not received adequate attention with respect to biomaterials. Table 1 presents a range of NP biomaterial-producing bacteria that generate various pellicles with distinct material properties. Additionally, Figs. 5 and 6 show the ideal growth temperature and pH levels for biomaterial formation within these strains and their origins.

These biofilms consist of cellulose, exopolysaccharides, or proteins, indicating that different strains may be advantageous for different applications. Additionally, diverse bacteria necessitate different growth conditions, meaning specific strains may be more suitable for large-scale production based on the required growth medium, or the time required for the biofilms to mature.

Many studies address material development using *Komagataeibacter xylinus*, *Bacillus subtilis*, *Gluconoacetobacter rhaeticus* and *Escherichia coli*. *Komagataeibacter xylinus* is often researched for its ability to produce cellulose and is utilised as a reference organism for investigating the genetic and biochemical pathways involved in the biosynthesis of bacterial cellulose [97]. Closely related to Kx, *Gluconoacetobacter rhaeticus* is also a producer of cellulose pellicles. Researchers have found *E. coli* to be particularly advantageous due to its comparative simplicity and the convenience with which it can be cultured and examined within a laboratory setting [98]. Additionally, the ability of *E. coli* (Non-pathogenic lab-modified strains) to produce biofilms consisting of EPS designed to protect the microbes makes it an organism of great interest [99]. Finally, pellicle producer *Bacillus subtilis* is predominantly composed of exopolysaccharides. Unlike other strains, *B. subtilis* exhibits a distinctively wrinkled surface texture, which results from localized cell death and the stiffness of the extracellular matrix. The textured surface, combined with its unique material composition, results in a hydrophobic surface, which is an attractive characteristic for potential textile applications [29].

5. Industrial production methods for BNC

A key issue with research conducted into biomaterials is a disconnect between lab-scale and industrial-scale production. Most innovative technologies relating to yield increase and improved material properties cannot be translated into commercial use due to financial infeasibility and lack of infrastructure. For bacterial biomaterials to have the largest economic impact, current industries and techniques must be studied for mass market adoption.

Kombucha has a long history dating back to over 2000 years ago in China. Since then, it has spread to other parts of the world and production techniques have evolved [100]. Similarly, other strains of bacteria have seen advancements in their cultivation methods. Currently, many companies use laboratory conditions to cultivate strains. However, this practice has not been adopted in factories in Southeast Asia, where costs are kept to a minimum. Instead, it is mainly confined to the food and beverage market, where sanitary conditions are a requirement.

The methods currently used for kombucha fermentation mainly consist of static and agitated cultures, as well as various types of bioreactors. The biosynthesis process is performed through semi-continuous, continuous, or fed-batch fermentation, and it must always be preceded by inoculum preparation. Inoculum preparation involves the introduction of dormant microbial cells from a stock culture to a favourable environment where they can grow and form a metabolically active microbial population. This process is crucial to the efficiency of fermentation and directly affects the outcome. Manufacturers commonly use this method, as evidenced by Table 3 which lists those currently utilising biomaterials in their products.

Despite the advantages of the agitated culture method, it is not without its limitations, particularly when it comes to the large-scale production of several thousand litres of the fermentation medium. The static culture method results in the accumulation of a gelatinous membrane produced at the air-liquid interface, while the stirred method can cause unwanted mutations and negatively impact the material properties of the pellicle [106].

BNC can achieve a comparable production efficiency to that of plant cellulose, especially when the yield of BNC reaches up to 15 g/L in 50 h [20]. Additionally, the production area required for fermentation is much smaller than that needed for plant growth, making it a more

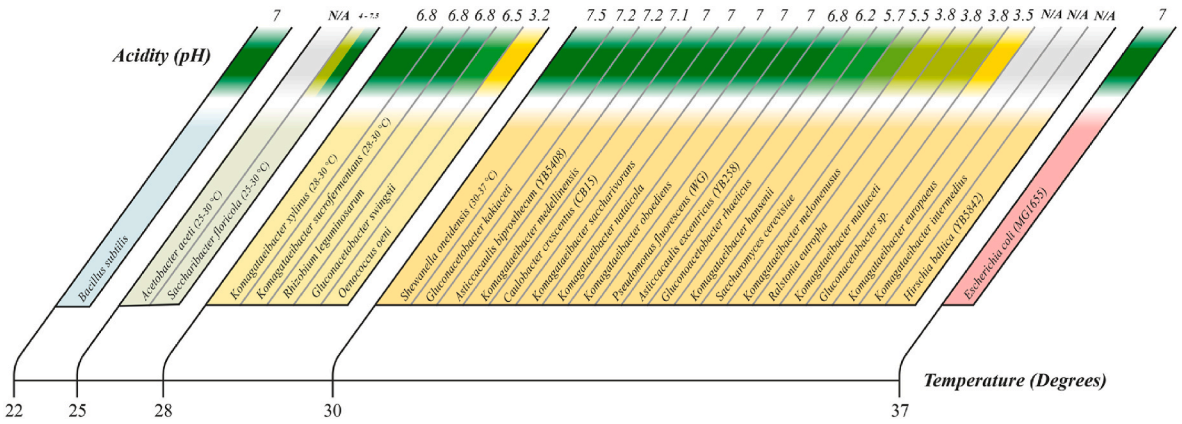


Fig. 5. Diagram to show the ideal growth temperatures and pH levels for the bacteria strains seen in Table 1 to encourage biomaterial formation.

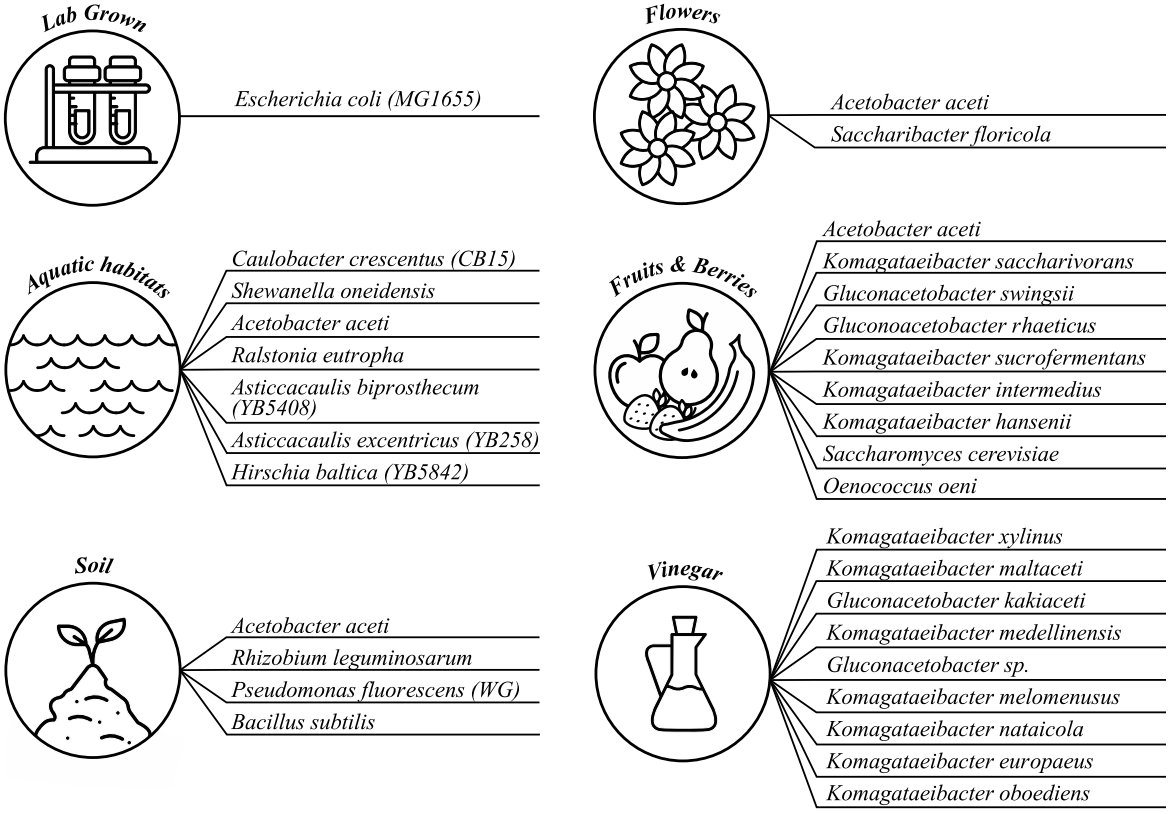


Fig. 6. Diagram to show the origins of biomaterial-producing bacteria strains seen in Table 1.

space-efficient option. The purification of BNC is also simpler and generates less pollution than the cellulose extraction procedure from trees [20].

In addition to traditional growth media, BNC can also be produced from agricultural and industrial waste residuals, making it a cost-effective and environmentally friendly alternative. For example, one study used citrus peel and pomace as a growth medium for BC production and found that it was a novel and environmentally friendly method. With a worldwide total of 27.1 million tonnes of citrus produced each year, of which 40–60 % is peel and pomace, using this waste as a medium for BNC production not only reduces cost but also helps remove it from the environment [107].

6. Enhancing bacterial nanocellulose production

The bacterial growth rate is a constraining factor for bacterial biomaterial production. This slow growth rate makes the adoption of biomaterials less attractive, compared to synthetic counterparts, despite their significant environmental benefits. The amount of biomaterial produced is entirely dependent on the bacteria's ability to metabolise and secrete the material, which limits its scalability and controllability. Although supplements and technologies have been developed to increase yield, they are not yet applicable for mass-scale manufacturing, and the required infrastructure is costly. Table 4 displays the state-of-the-art research to improve the yield of biomaterial production.

In conclusion, technology offers several methods to increase the yield of NP biomaterial-producing bacteria. Supplementations such as ethanol and vegetable oil are cheap and easy to implement but have limited

Table 3
Comparison of different production methods, conditions and additives for scaled biomaterial production.

Production type	Cost	Conditions	Feed chemicals	Industry	Process output
Bacterial cultivation factory – Think Bioscience (Boulder, Colorado, USA).	high-cost	Cultivation in controlled temperature industrial-scale bioreactors, utilising genetically engineered bacteria such as *Escherichia coli*, for pharmaceutical biosynthesis.	Luria-Bertani broth and other laboratory-grade growth media [101,102]	Medical industry.	Generating adaptable bacterial strains for the synthesis of pharmaceuticals.
BNC Cultivation factory (General).	low-cost	30 °C – Commonly seen in Southeast Asia (Vietnam, Philippines, Indonesia etc), as the climate is ideal for bacteria cultivation – This method is more cost-effective compared to the heating required in cooler countries, making it a popular choice in the region [20]	These factories primarily use agro-industrial waste for the growth medium in BNC production, including corncob, alcohol waste liquor, pineapple peel, coconut water, citrus juice, and apple juice. These waste residuals can serve as a cost-effective and environmentally friendly alternative to traditional growth media and can help reduce waste-induced pollution [20]	Food and Cosmetic.	Nata De Coco and BNC hydration facemasks.
BNC Cultivation factory – Processum (Sweden).	High-cost	Process controlled using industrial-scale bioreactors and cutting-edge technology designed to increase biomaterial yield (E.g. genetic modification) [103]	Laboratory-grade optimised growth media [103]	Cellulose production consultancy.	Systems for growing large quantities of cellulose.
BNC Cultivation factory – Polybion (Spain/Mexico).	High-cost	Process controlled using industrial-scale bioreactors and cutting-edge technology designed to increase biomaterial yield - "the first industrial-scale, bacterial cellulose textile, biomanufacturing facility in the world" [104]	Laboratory-grade optimised growth media [93]	Textiles.	Bio-materials – industrial scale.
BNC Cultivation factory – XUJOHN (China).	High-cost	The conditions in this factory are monitored using state-of-the-art technologies. Conditions in the area are also not stable throughout the year, therefore heating must be used in the winter months. This factory is in China [105]	Laboratory-grade optimised growth media [105]	Cosmetic.	BNC hydration facemasks.

potential for yield improvement [108]. Genetic modification has the potential for dramatic yield increase but may face public resistance and high initial costs [111]. Methods such as rotating magnetic fields (RMF) are also promising, but the need for scaling up infrastructure exponentially can make them financially unfeasible. It is important to note that RMF face the additional challenge of the inverse square law, where each time the size of the bacteria yield increases (surface area), the size of the RMF must increase exponentially. This can make it even more financially challenging than other methods that require new infrastructure, as seen by the complex setup in F1ig. 8 [113]. A balance between cost, feasibility, and public acceptance must be considered when choosing the most effective method to increase BNC yield.

7. Potential future applications and challenges for the adoption of bacterial biomaterials

With continued research and development, biomaterials from bacterial origin, have the potential to replace synthetic counterparts, paving the way for a more sustainable and environmentally friendly future. While the two most researched cellulose-based NP biomaterial-producing bacterium are Kx and Gluconoacetobacter rhaeticus, other strains of bacteria produce pellicles composed of different compositions of EPS and proteins; These offer a range of material properties and could unlock unlimited applications for biomaterials.

To make the adoption of biomaterials into the mass market feasible, the properties of the biomaterials are important, but it's equally important to produce them at scale. Technologies such as supplements are crucial for bringing this closer to reality. Southeast Asia and other hot and humid countries are ideal to produce BNC, as the ideal growth conditions eliminate the need for heaters during incubation [20]. However, several barriers need to be overcome before BNC can become a viable replacement for synthetic materials. The production of BNC must be scaled up from a laboratory setting to an industrial level, which necessitates a substantial investment in infrastructure and the development of efficient production methods. Presently, the production of BNC is too slow to meet industry demands, and the existing infrastructure is insufficient to handle the required production methods.

Furthermore, the lack of fully developed technology is another significant barrier to the widespread use of BNC. In some cases, the material properties of this biomaterial are not yet comparable to those of their fossil fuel-derived counterparts. Despite this, the potential applications of BNC are undeniable. BNC has a tightly compact nanofibril structure that provides incredible filtering properties, making it a promising candidate for use in filtration systems [115]. It also has excellent material properties such as high strength and biodegradability, making it a promising candidate for use in the textile industry. The use of biomaterials derived from bacteria in design is an emerging field and requires further development. This paper presents a catalogue of potential bacterial strains, expanding the possibilities not only for material design but also for the creation of new products.

8. Conclusions

This review has identified many NP biomaterial-producing bacteria and their material properties based on a range of production-related criteria, as well as acknowledging the weaknesses of these materials in comparison to synthetic versions. With a greater selection of NP biomaterial-producing bacteria strains to choose from, designers will be able to create novel products that possess unique properties, whilst not damaging the environment. For researchers, this list will enable them to broaden their scope of material research beyond the commonly studied strains like Kx. It will provide them with access to a range of NP biomaterial-producing bacteria strains with distinctive characteristics, helping to drive a wave of material research that will unlock the potential of bacterial-based biomaterials to revolutionise numerous industries. Further to this, there are immense possibilities for the applications of these biomaterials due to their range of material properties and compact fibre network. Current manufacturing methods for bacterial-based biomaterials have also been examined, analysing their costing and industry type to understand their strengths and weaknesses. Designers can utilise this information to select appropriate manufacturing methods for the biomaterials they create, ensuring their

Table 4
State-of-the-art research to increase the yields of BNC production.

Method	Yield increase	Description
Vegetable Oil	500 % [108]	Researchers have examined the potential of utilising industrial and food by-products, such as discarded fruit and vegetable matter, as substitutes for traditional culture media. The addition of vegetable oil to bacterial culture media is an effective way to enhance cellulose pellicle production. Research has shown that by supplementing just 1 % vegetable oil into the culture medium, BC yield can increase by over 500 % compared to standard media. As there is a growing demand for more cost-effective methods to produce BC, vegetable oil represents a low-cost alternative to traditional methods that use expensive substances like caffeine or certain ions [108]. It is thought that the 1 % volume of oil does not fully cover the surface of the culture medium, thereby allowing bacteria to produce cellulose more efficiently at the start of the process. This simplistic economical approach has demonstrated immense potential in elevating BC pellicle output and is worthy of more in-depth examination [108].
Ethanol	750 % [109]	The symbiotic relationship seen in Kombucha SCOBY involves the yeast and bacterial communities working together, converting sucrose in the growth medium into glucose and fructose, and then converting the glucose into ethanol. This ethanol functions as an antimicrobial agent, preventing contamination by harmful microbes [94]. Furthermore, it helps to inhibit spontaneous mutations in BNC and enhances ATP production, resulting in an increased yield of cellulose. Similarly, artificially adding 1–2% of ethanol has been shown to boost the BNC yield by as much as 7.5 times. These findings have implications for optimising and manipulating BNC produced by *Komagataeibacter rhaeticus* and other cellulose-producing strains [109].
Genetic Modification	140 % [110]	Genetic modification is a powerful method to improve the yield and properties of BNC and other biomaterial producing cultures. BNC-producing bacteria have been the focus of many researchers, who have utilised numerous modification techniques to enhance desired traits. In a recent study, researchers modified the flagella of the bacteria to improve the material properties of the BNC pellicle. It was found that most motile bacteria move using flagella and that the flagellar motor proteins MotA/MotB play a crucial role in biomaterial formation and are involved in energy conversion that generates rotation from the flow of hydrogen ions. Disrupting the flagella resulted in a reduction in motility and higher compaction of nanocellulose fibres, leading to improvement in their mechanical properties [111]. Similarly, a research group has increased the yield of cellulose production in *Komagataeibacter xylinum* by genetically modifying the mechanisms involved in its biosynthesis. They aimed to understand the involvement of regulatory proteins in the BC production process and targeted these genes for genetic manipulation. By altering the genes involved in cellulose biosynthesis, the scientists managed to control the synthesis of BC in the bacterium and improved its yield and properties. The researchers focused on the genetic toolkits and the limited information available on the whole genome of the bacterium to achieve the desired changes in BC production. Through their studies, they presented genetic modification as a tool for introducing changes at the genetic level, leading to improved yield and properties of BC [110]. More research has been conducted enhancing the production of *Komagataeibacter rhaeticus*, resulting in a strain that generates significantly higher yields under nitrogen-deprived growth conditions [14]. In addition, the same lab has recently created a self-dyeing bacterial cellulose strain, which eliminates the need for textiles to be dyed during post-processing. This procedure is notoriously harmful to the environment, making the development of such a strain a critical step towards more sustainable textile production methods [112].
Rotating Magnetic fields	130 % [113]	Rotating magnetic fields (RMF) can be used to increase the yield of bacterial cultures producing BNC. This is because RMF can create micro-mixing effects that agitate elements of the cultivation liquid, stimulating the growth of cells and bacteria metabolic activity. This is achieved by the enhancement of transport phenomena and relatively small shear stress, which are beneficial for cells and BNC structure [113]. The RMF also affects the inter-cellular transport process, especially for the Gram-negative bacteria (Such as Kx) which have a thinner cell wall than the Gram-positive bacteria. Additionally, this technique can create an electroporation phenomenon, which increases the conductivity and permeability of the cell membrane, hence benefiting metabolic processes and activity as observed in experiments [111]. Another way RMF can influence the BNC production process is by affecting the water particles inside EMF, which can lower surface tension and disturb the creation of hydrogen bonds. These hydrogen bonds help stabilize the crystalline structure and the establishment of the BNC membranes at the cultivation medium surface. RMF can also be used to change the morphology of BNC by regulating bacterial cell movement using different types of electromagnetic fields. Electrokinetic force can be utilised to control bacterial motion, allowing for the differentiation of the orientation and organisation of fibres. Research has also shown that the application of 50 Hz RMF significantly increased the rate of growth in *E. coli* and *S. aureus*, with the largest effect seen within the exponential phase. It has also been found that 50 Hz RMF applications can modulate a variety of bacterial functions in various species, such as metabolic activity, biomaterial formation, and overall growth dynamics [114].

products can be produced affordably, at a large scale. Moreover, for researchers, this will enable them to identify the areas where current manufacturing methods can be improved and innovated to increase the efficiency, sustainability, and effectiveness of the production process. This review has also identified methods to increase the yield of biomaterials, which can bridge the gap between research and commercial goods, ultimately bringing bacterial-based biomaterials closer to mass production feasibility. By addressing the main limitations of bacterial-based biomaterials, this research can promote the wider adoption of sustainable materials and contribute to a more sustainable future.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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