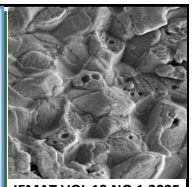
JEMAT, 2025, Vol 13, No 1, 39-46
https://doi.org/10.54987/jemat.v13i1.1110

JOURNAL OF ENVIRONMENTAL MICROBIOLOGY AND TOXICOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JEMAT/index



Enhanced Cellulose Production in Kombucha SCOBY Through Microbial and Genetic Optimization

Hussaini Adib Haslan[1], Murni Halim[1], Fadzlie Wong Faizal Wong[1], Zulfazli M. Sobri[1], Nor'Aini Abdul Rahman[1], Mohd Sabri Pak-Dek[2], Yanty Noorzianna Abdul Manaf[3] and Helmi Wasoh[1,4]*

[1]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[3]Halal Research Group, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia.
[4]UDRP Halal Authentication, Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

*Corresponding author:
Helmi Wasoh,
Department of Bioprocess Technology,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 UPM Serdang,
Selangor,
Malaysia.
Email: helmi_wmi@upm.edu.my

History

Received: 19th May 2025
Received in revised form: 21st June 2025
Accepted: 30th July 2025

Keywords

Cellulose
Kombucha
Biosynthesis
Fermentation
Acetic Acid Bacteria

Abstract

The symbiotic culture of bacteria and yeast (SCOBY) represents a dynamic microbial consortium that plays a fundamental role in kombucha fermentation. This complex system consists of acetic acid bacteria (AAB), lactic acid bacteria (LAB), and various yeast species whose synergistic interactions generate bioactive compounds including organic acids, polyphenols, and bacterial cellulose (BC). Within the SCOBY consortium, *Komagataeibacter* and *Gluconobacter* spp. (AAB) catalyze the oxidative conversion of ethanol to acetic acid, generating an acidic microenvironment that both inhibits competing microorganisms and promotes bacterial cellulose biosynthesis. LAB, including *Lactobacillus* and *Pediococcus*, enhance fermentation stability, probiotic potential, and biofilm structure through exopolysaccharide production and bacteriocin secretion. Yeasts like *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* metabolize sugars into ethanol and CO_2, supporting AAB activity and contributing to flavor complexity. Recent advances in biosynthesis research have identified over 200 microbial species in SCOBY, with high-throughput sequencing revealing key metabolic pathways. Genetic optimization of BC production involves the *bcsABCD* operon, which regulates cellulose synthase activity, with CRISPR and metabolic engineering enhancing yield and crystallinity (84-89%). Engineered strains of *Komagataeibacter xylinus* demonstrate improved BC properties, including nanofibrillar density (2-4 nm) and water retention (>99%). However, SCOBY's industrial application faces challenges, including batch variability, environmental sensitivity, and inconsistent microbial profiles, necessitating precision fermentation with defined consortia for standardized production. Future research should focus on robust clinical validation of health claims and scalable bioprocessing techniques to harness SCOBY's full potential in food, biotechnology, and biomedical applications.

INTRODUCTION

Symbiotic culture of bacteria and yeast (SCOBY) is a dynamic microbial consortium (**Fig. 1**) responsible for the fermentation of kombucha and other biofilm-forming systems [1]. SCOBY typically develops into a floating biofilm at the liquid–air interface of the fermenting tea. This layer creates a transitional zone that allows microbes to access both atmospheric oxygen and dissolved nutrients [2]. The microbial consortium is mainly comprised of acetic acid bacteria (AAB) which are crucial in bacterial cellulose (BC) synthesis and acetic acid production [3,4]. The presence of lactic acid bacteria (LAB) enhances the probiotic potential of kombucha while helping in the prevention of its fermentation deterioration [5]. Meanwhile, yeasts metabolize sugars which further facilitate bacterial conversion [2,6]. The functional and compositional SCOBY properties and its structure are defined by the interactions which constitute its

microbial structure, hence making it valuable in food, biotechnology and biomedicine [7].

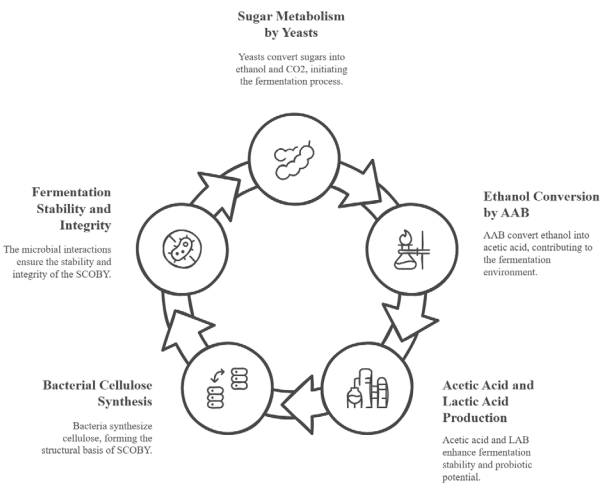


Fig. 1. Overview of SCOBY fermentation cycle driven by synergistic interactions within the microbial consortium.

Understanding the role of each microbial group in SCOBY matrix is essential for optimizing fermentation processes and expanding industrial applications of SCOBY-derived products. Typically, SCOBY's unique microbial consortium contributes to the production of various bioactive compounds, including organic acids, polyphenols, and vitamins that are responsible for various kombucha's claimed health benefits [8]. SCOBY exhibits a unique collaborative relationship among its diverse microorganisms [9] and the consortium forms a cellulosic pellicle, creating an environment that allows microbes to access oxygen and nutrients. The microbial composition includes *Komagataeibacter* and *Gluconobacter*, for bacterial cellulose production and acetic acid generation, *Lactobacillus* and *Pediococcus*, mainly for fermentation stability and probiotic potential and, yeast species such as *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Brettanomyces bruxellensis* for initiating alcohol fermentation, maintaining acidic and high sugar conditions as well as developing complex flavors through phenolic compound production [10]. These microorganisms establish a metabolically active network that synthesizes a broad spectrum of bioactive metabolites with functional and physiological relevance. The functional properties of SCOBY arise from intricate microbial interactions, boosting its versatility in industrial applications [11].

Although SCOBY is valued for its intricate microbial synergy, a thorough assessment requires acknowledging its constraints and challenges. These microorganisms may interact in ways that can make the fermentation process unpredictable. Generally, SCOBY's efficiency depends on environmental stability, in which minor deviations can compromise its microbial equilibrium. For commercial producers, environmental changes during fermentation process can lead to product defects such as off-flavors, hyperacidity, or spoilage, which may directly affect quality and profitability [12]. While SCOBY is often marketed for its probiotic potential, inconsistent microbial profiles may limit its actual health benefits. Furthermore, certain yeasts and bacteria present in SCOBY may produce byproducts that are not beneficial for everyone [13]. Inadequate monitoring of the fermentation process may compromise product safety, necessitating strict quality control measures [14]. Although fermentation-derived compounds are frequently claimed to confer various health benefits, nevertheless robust scientific evidence has remained limited. Current health claims associated with SCOBY and kombucha principally rely on anecdotal reports rather than empirical validation, underscoring the necessity for rigorous clinical studies to substantiate these assertions.

The inherent complexity of SCOBY's symbiotic microbial consortium presents significant challenges for scaling up production in industrial fermentation processes [12,14]. Mainly, the inherent biological variability of SCOBY's living microbial consortium poses potential challenges in maintaining batch-to-batch consistency that complicating quality assurance in commercial-scale production. These reproducibility limitations constrain its reliable application across many biotechnological sectors, despite its considerable scientific and industrial potential [12,13].

Role of acetic Acid Bacteria in SCOBY Fermentation

Acetic acid bacteria (AAB) acts as the primary driver of ethanolic oxidation in SCOBY. It mediates the biotransformation to acetic acid, generating the characteristic acidic microenvironment (pH 3-4) that functions as biochemical barrier against microbial contaminants, and self-regulating mechanism for maintaining consortium equilibrium in continuous fermentation systems [4,15]. *Komagataeibacter* and *Gluconobacter* spp. dominate the AAB population, exhibiting two industrially relevant metabolic competencies: (i) high-yield BC production through extracellular polysaccharide synthesis, and (ii) robust acidification capacity via membrane-bound alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymatic cascades [16]. *Komagataeibacter xylinus* emerges as a functionally preeminent species within the SCOBY consortium, primarily due to its robust capacity for extracellular biosynthesis of the BC [17,18]. This microbial exopolysaccharide constitutes the primary structural scaffold of the biofilm matrix, conferring mechanical stability and spatial organization to the symbiotic microbial community [19].

In the meantime, *Komagataeibacter rhaeticus* and *K. europaeus* exhibit complementary metabolic functions, synergistically enhancing the SCOBY biofilm through co-acidification via ethanol oxidation, which maintains the low-pH microenvironment, and auxiliary cellulose biosynthesis that reinforces the extracellular matrix [4,9]. Their collective activity modulates critical biophysical parameters including moisture retention (>95% water content), tensile strength (Young's modulus: 15–18 GPa), and fibrillar density which govern the structural integrity and production efficiency [20]. The metabolic efficiency of AAB in ethanol oxidation and acetic acid production is modulated by several key environmental parameters including oxygen concentration, temperature, and pH [15]. The dynamic interplay between AAB and yeast within the SCOBY consortium is fundamental for optimizing kombucha production. This symbiosis drives efficient substrate conversion, prevents fermentation arrest, and ensures reproducible product quality [21-23].

Strategic inoculation with specialized yeast strains (such as *Saccharomyces eubayanus*) can further refine metabolic output for superior organoleptic characteristics [19]. Chong et al., [24] and Harrison & Curtin, [2] also reported that the microbial composition of SCOBY exhibits inherent variability, directly influencing fermentation outcomes, including acetic acid yield and final product quality. Precision fermentation approaches were implemented to mitigate this variability by employing defined microbial consortia. These methods enable standardized

fermentation, ensuring consistent acetic acid production while enhancing functional attributes such as antioxidant capacity and γ-aminobutyric acid (GABA) content [22,25]. As the primary drivers of kombucha fermentation, AAB critically governs both the beverage's organoleptic properties and its potential health benefits.

Role of Lactic Acid Bacteria in SCOBY Fermentation

Lactic acid bacteria (LAB) constitute a metabolically active subset of the kombucha microbial consortium, contributing to ecological structuring through acidogenic flux within the SCOBY matrix. Their fermentative activity produces lactic acid, which reduces environmental pH and imposes a selective pressure on non-acidophilic microorganisms, thus constraining consortial entropy and enhancing microbial succession stability. LAB strains such as *Lactiplantibacillus plantarum* and *Pediococcus pentosaceus* frequently dominate under low-oxygen regimes when glucose, ethanol and thermal parameters favor their metabolic profiles [5,26]. Feng et al., [7] and Revin et al., [27] found that these taxa influence SCOBY integrity through the biosynthesis of exopolysaccharides, which intercalate with cellulose fibrils produced by AAB, modifying hydrogel rheology, tensile strength, and water retention. LAB further secrete bacteriocins that act through cytoplasmic membrane disruption and quorum interference, selectively inhibiting gram-positive contaminants and preserving community composition [5,7].

LAB and AAB metabolic interactions modulate redox gradients and substrate channeling, particularly influencing BC crystallinity, yield, and structural architecture. For example, it was reported that co-metabolism between LAB and *Komagataeibacter* spp. has been associated with enhanced glucose polymerization and accelerated pellicle formation under sustained fermentation [27]. Furthermore, LAB-induced shifts in nutrient availability and acid–base buffering impact both pH regulation and microbial competitive dynamics.

Despite their functional prominence, LAB representation in microbial assessments is frequently distorted by the laminar stratification of SCOBY. Harrison and Curtin, [2] and Li et al., [28] stated that in some cases, microbial quantitation skews toward LAB-rich regions, reducing taxonomic resolution and underestimating less abundant taxa especially if it occurs without standardized radial and vertical sampling. LAB functionality spans microbial inhibition, biofilm structuring, and probiotic modulation, each governed by niche partitioning, metabolite diffusion and cross-kingdom feedback [7,27,29].

Role of Yeasts in SCOBY Fermentation

Yeast represents a fundamental functional element within the SCOBY consortium, driving the kombucha fermentation process through two sequential metabolic phases [2]. Initially, they hydrolyze sucrose into glucose and fructose, via extracellular invertase activity. These monosaccharides will undergo alcoholic fermentation to yield ethanol and carbon dioxide as primary metabolites. The ethanol output serves as the essential substrate for AAB, which catalyzes its oxidation to acetic acid that contributes to kombucha's distinctive sour taste and preservative. The SCOBY yeast consortium exhibits substantial taxonomic and functional diversity, with multiple species contributing distinct metabolic capabilities to kombucha fermentation. For example, *Saccharomyces cerevisiae* serves as the primary ethanologenic agent, catalyzing the conversion of hexoses to ethanol and establishing the substrate base for AAB [6]. Kaashyap et al., [15] reported *Zygosaccharomyces bailii* demonstrates exceptional environmental resilience, maintaining fermentative activity under elevated acidity (pH < 3.0) and osmotic stress (>500 g/L sugars). [2] also reported other types of yeast such as *Brettanomyces bruxellensis* generate diverse volatile esters (such as ethyl acetate, phenethyl alcohol) that define kombucha's organoleptic complexity. Meanwhile, complementary contributions from *Candida* spp. expand the consortium's metabolic range, particularly in oligosaccharide utilization [6]. Moreover, yeasts exert dual functional impacts on kombucha, influencing both biochemical and physical properties. The metabolic production of carbon dioxide induces effervescence and surface gloss characteristic of properly fermented kombucha [2].

Yeast-mediated modifications to bacterial cellulose nanostructure (with fibril density in the range of 10-50 nm diameter) can also directly affect beverage viscosity and mouthfeel [15]. This yeast-bacteria synergy is fundamental to achieving kombucha's distinctive organoleptic profile. The composition and dynamics of yeast populations within SCOBY are governed by key environmental parameters such as temperature regimes establish species-specific thermal niches (e.g., *Saccharomyces cerevisiae* thriving at 25-30°C versus *Zygosaccharomyces bailii* at 30-37°C), initial sugar concentration selects for osmotolerant strains (with > 15°Brix favoring *Zygosaccharomyces* spp.), and oxygen availability modulates metabolic pathways (microaerophilic conditions of 0.5-2 mg/L dissolved O_2 promoting fermentative metabolism over respiratory activity), collectively shaping the yeast community structure and its functional interaction with bacterial components during fermentation [3,30].

Role of Other Microorganisms in SCOBY Fermentation

High-resolution microbial characterization of 103 distinct SCOBY specimens identified *Brettanomyces* spp. yeasts and *Komagataeibacter* spp. bacteria as the core functional taxa governing the dynamics of kombucha fermentation [2]. The prokaryotic component additionally comprises *Bacillus* spp., which performs critical structural and catalytic functions through the secretion of extracellular hydrolytic enzymes (including cellulases [EC 3.2.1.4] and amylases [EC 3.2.1.1]) that mediate substrate depolymerization and enhance nutrient bioavailability within the consortium [31,9]. Although *Aspergillus* spp. represents transient constituents of the SCOBY microbiome, their sporadic occurrence is often associated with modified metabolite profiles.

This phenomenon is facilitated by the temperature and pH dependent biosynthesis of fungal secondary metabolites, including mycotoxins and extracellular enzymes [32,33]. Neffe-Skocińska et al., [34] and Mangayil et al., [35] reported that the key microorganisms are complemented by secondary yeast genera (such as *Zygosaccharomyces*, *Lachancea*, and *Starmerella*), which exhibit niche partitioning and contribute to metabolic redundancy, particularly in microbial communities to occupy a non-dominant ecological position (*Brettanomyces*). Furthermore, the strategic introduction of non-conventional yeast species such as *Pichia kluyveri* modulates kombucha fermentation dynamics through two principal mechanisms. First, enhanced acetic acid production via accelerated ethanol oxidation (increased yield by 15-22%), and second, biosynthesis of volatile acetate esters (including isoamyl acetate and ethyl acetate) that collectively contribute to the beverage's organoleptic complexity [30,36]. The summary of the microbial composition of SCOBY and their roles is shown in **Table 1**.

Table 1. Microbial diversity and functional roles in SCOBY.

Microbial Group	Genus & Species	Role in SCOBY	Ref
Acetic Acid Bacteria (AAB)	*Komagataeibacter rhaeticus*	Contributes to cellulose production and acidification	[15,3]
	Komagataeibacter europaeus	Survives in acidic conditions and enhances fermentation	[4]
	Gluconobacter oxydans	Participates in sugar oxidation and organic acid production	[2]
Lactic Acid Bacteria (LAB)	*Lactobacillus plantarum*	Enhances gut health and biofilm stability	[5]
	Lactobacillus casei	Produces lactic acid, aiding microbial balance and flavor	[7]
	Pediococcus pentosaceus	Produces bacteriocins with antimicrobial properties	[2]
Other Bacterial Species	*Bacillus subtilis*	Produces enzymes and improve fermentation	[37]
Yeasts	*Saccharomyces cerevisiae*	Ferments sugars into ethanol and carbon dioxide	[6]
	Zygosaccharomyces bailii	Stress-tolerant yeast that stabilizes fermentation	[3,15]
	Brettanomyces bruxellensis & Candida stellata	Produces flavor compounds, aroma complexity and biofilm formation	[2]
Other Microorganisms	*Aspergillus* (Fungi)	Present in some fermentation condition	[33]

Biosynthesis, Microbial Strains and Genetic Optimization

The latest research into biosynthesis pathways has elucidated many aspects of microbial presence and metabolism taking place in SCOBY during fermentation of kombucha tea. High-throughput sequencing techniques have been applied to characterize the microbial diversity in SCOBY ecosystems. As a result to the existence of multiple microbial communities, 34 genera and 200 species of microbes have been annotated [3,15]. The AABs usually predominate as *Acetobacter*, *Gluconobacter*, and *Komagataeibacter* and followed by yeasts such as *Brettanomyces*, *Saccharomyces* and *Zygosaccharomyces* [8,38]. These microorganisms are believed to play an important role in the biosynthesis of some bioactive materials that can be beneficial for many applications [8]. Studies critically reviewed the role of commensal propionibacteria in biogenesis of propionic acid in synthetic communities. It was found that in structurally designed ecosystems commensal acetic bacteria SAnC35 and C18 produced higher amounts of organic acids when acting together than as separate strains [39].

Gluconic acid was claimed to be the predominant organic acid in symbiotic microbial community (SMC) of fermented seasoned tea and contributed to the delicious sour taste of the drink. Equally important were the social mechanisms of certain microbes such as *Starmerella* and some AAB. Such understanding has led to the design of specific consortia capable of producing probiotic-dense kombucha with preferred taste attributes and improved shelf-life, as noted by Fabricio et al., [40]. Buldum and Mantalaris [41] developed an innovative method to fabricate functional materials based on biological composites, utilizing a continuous culture of *Saccharomyces cerevisiae* and bacterial cellulose producing bacteria. The BC biosynthetic pathway commences with the phosphoglucomutase (EC 5.4.2.2)-catalyzed isomerization of glucose-6-phosphate to glucose-1-phosphate, followed by UDP-glucose synthesis via UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9). The membrane-embedded cellulose synthase complex (*bcs*AB) subsequently mediates the processive β-1,4-glycosyl transfer of glucosyl units from UDP-glucose to the elongating polymer chain, with nascent polymers extruded through the *bcs*C porin and crystallized into a nanofibrillar network (2-4 nm diameter fibrils) through *bcs*D-facilitated chain association [42]. This microbially derived cellulose exhibits superior structural properties compared to plant cellulose, including enhanced crystallinity (84-89%), porosity (surface area >100 m^2/g), and water retention capacity (>99% wt/wt) [43].

The production of BC was also enhanced through genetic modifications. Römling & Galperin, [44] described the addition of *bcs* operon genes and some of its regulating enzymes as further targets for BC amplification. Amr & Ibrahim, [45] studied the organization of the *bcs* operon and its contribution to the full activation of cellulose production. They focused on the *bcs*ABCD operon regarding BC synthesis controls in *Gluconacetobacter xylinum*. Commencing with *Komagataeibacter xylinus* (formerly known as *Acetobacter xylinus*), the biosynthesis pathway of BC is intricate and largely controlled via the *bcs*ABCD operon. This operon is made up of four genes (*bcs*A, *bcs*B, *bcs*C, and *bcs*D) needed for packaging and crystallization of cellulose. Genera such as *Acetobacter*, *Komagataeibacter*, and *Gluconobacter* exhibit conserved operons for cellulose production yet markedly differ in yield profiles, substrate conversion rates, and morphological characteristics of the cellulose matrix [46]. As reported by Krystynowicz et al., [47] genetic consistency remains a determinant of bioproduction robustness, static culture conditions are associated with greater plasmid stability and reduced genomic drift in *Acetobacter xylinum* E, contrasting with phenotypic heterogeneity observed under agitation.

Strain and system compatibility imposes constraints on bioreactor design and necessitates alignment of cultivation architecture with genotype-specific metabolic bandwidths. Metabolic engineering strategies have emerged to overcome intrinsic bottlenecks in flux partitioning. CRISPR-derived editing tools and rational gene overexpression constructs targeted the redirection of carbon intermediates toward β-1,4-glucan assembly while attenuating enzymatic branches responsible for gluconic acid accumulation and oxygen stress-induced attenuation [48]. Pre-genomic methods based on random mutagenesis introduced unpredictable variability into cellulose yield and enzyme expression profiles, constrained by the nonlinear topology of regulatory feedback loops [49]. Subsequent transition toward site-directed mutagenesis and modular gene cloning enabled locus-specific modulation of key enzymes in the cellulose synthase complex. Functional annotation of cloned genes facilitated delineation of core metabolic nodes, advancing precision strain engineering. Fluxomic models integrated with transcriptomic datasets have delineated regulatory hierarchies underpinning BC synthesis, guiding the rational construction of high-yield strains through systematic metabolic rewiring. These convergent methodologies underscore a shift toward genetically stabilized, process-adaptable strains as central to the refinement of BC manufacturing platforms [50].

In order to enhance the research landscape surrounding the production of bacterial cellulose in symbiotic microbial systems, a Scopus keyword search was conducted using terms related to "bacterial cellulose," "SCOBY," "Komagataeibacter," "genetic engineering," and "bioprocess optimization," yielding 247 journal articles. The search included combinations such as "bacterial cellulose" OR "BC production" OR "microbial cellulose" AND ("SCOBY" OR "symbiotic culture of bacteria and yeast" OR "kombucha") OR ("Komagataeibacter" OR

"Gluconobacter" OR "acetic acid bacteria" OR "lactic acid bacteria" OR "yeast") AND ("genetic engineering" OR "CRISPR" OR "metabolic engineering" OR "gene regulation" OR "bcsABCD" OR "synthetic biology") AND ("cellulose synthase" OR "biofilm formation" OR "exopolysaccharide production") AND ("bioprocess optimization" OR "biotechnology application" OR "industrial fermentation" OR "nanocellulose" OR "fermentation scalability"). The software generated a keyword co-occurrence network, which showed different thematic clusters for microbial composition, metabolic engineering, and bioprocess applications and also a temporal overlay visualization that shows new research trends and how scientific focus areas have changed over time. The analysis was meant to seek and deliver a thorough bibliometric evaluation of contemporary research initiatives, which focused on the microbial and genetic enhancement of bacterial cellulose production via SCOBY-based fermentation systems, and highlights the industrial scalability, synthetic biology instruments, and biotechnology integration.

Network Visualization: Structural Landscape

The resultant VOSviewer network visualization depicts a complex research landscape on making bacterial cellulose (BC) in kombucha SCOBY systems (**Fig. 2**). The output map shows several tightly linked clusters that represent different thematic directions in the field. The central hubs—cellulose biosynthesis, nanocellulose, and the symbiotic culture of bacteria and yeast—bring together various areas of microbiology, materials science, and bioprocess engineering [4,6,26,32]. Their distinction highlights the merging of research on microbial mechanisms, material innovation, and application-driven process development.

The nanocellulose cluster appears particularly important as it connects research on functionalization, reinforcement, and biomedical uses [32,36,41]. Important keywords related to healing, tissue engineering, and biodegradable polymers show significant translational potential for BC in the areas of wound care and regenerative medicine, as well as packaging and sustainable materials [19,32,35]. This highlights a strong focus on refining functional properties such as biocompatibility, mechanical strength, and sorption capacity [32,41]. Another significant structural motif is based on Komagataeibacter and other acetic acid bacteria, the main bacterial types in kombucha [2,6,36,50]. Their centrality in the network reflects their dual roles: (1) driving cellulose biosynthesis, and (2) influencing fermentation performance through metabolite production [3,5,21]. This links microbial ecology to studies on biofilm structure, static vs. agitated culture conditions, and genome sequencing, which have deepened our understanding of BC biosynthesis [4,30,37].

The assemblage of terms such as biofilm, enzyme pathways, and fermentation parameters further underscores the interdisciplinary overlap between microbial physiology and functional biomaterial synthesis [18,30,41]. On top of this microbial-material axis are keywords associated with sustainability, including "circular economy," "bioremediation," and "low-cost substrates" [27,31,48]. Since these terms are closely related to nanocellulose and biosynthesis, this suggests an emerging research narrative centered on resource recovery, waste valorization, and scale-up strategies [27,31,46]. These thematic links indicate that the field aligns with the broader framework of the circular bioeconomy [19,27, 46]. The network topology, therefore, represents three principal research fundamentals:

1. Microbial consortia and biosynthetic mechanisms (Komagataeibacter metabolism, cellulose synthase genes, static vs. agitated culture) [2-4, 6,37,50].
2. New applications in biomaterials and biomedicine (nanocellulose, tissue engineering, and wound healing) [19, 32,35,41].
3. Sustainability and process optimization (waste-derived substrates, fermentation scalability, and the circular economy) [27,31,46,48].

In addition, peripheral but promising node components—such as composite reinforcement, enzymatic modification, and hybrid material design—point to emerging frontiers that may evolve into key themes in the future [35,41,50]. In conclusion, the structural landscape depicts a research ecosystem built upon microbial–material linkages, offering flexibility for the pursuit of sustainable, application-oriented innovation [41,48].

Overlay Visualization: Changes in Time and Citations

The overlay visualization (**Fig. 3**) illustrates how research on bacterial cellulose (BC) derived from kombucha SCOBY systems has evolved over time, both in terms of thematic focus and publication volume. In this VOSviewer map, node size indicates keyword frequency, while color gradient (from blue to yellow) reflects the average publication year. This dual-coded representation reveals the historical foundation and emerging frontiers of the field. Older nodes—colored blue/green—represent early studies focused on fundamental microbiology and process parameters, including cellulose biosynthesis, cellulose synthase genes, static versus agitated cultures, and Komagataeibacter metabolism [4,6,36,37,50]. These foundational areas have provided key insights into microbial consortia behavior and strategies for enhancing fermentation efficiency [6,36].

In contrast, recent research trends—represented by yellow-colored nodes—show a clear shift toward innovation and application, particularly in the biomedical and sustainability domains. Keywords such as nanocellulose, tissue engineering, wound healing, and biodegradable polymers highlight the expanding focus on material science and regenerative medicine applications of BC [19,32,35,41]. Simultaneously, the rise of terms like “circular economy,” “bioremediation,” and “waste valorization” signals increasing alignment with sustainable development goals (SDGs) and eco-conscious biomanufacturing practices [27,31,46]. The appearance of smaller, newer nodes—such as composite reinforcement, mechanical property optimization, and hybrid material design—suggests novel specializations emerging in the field, with emphasis on tailoring BC properties for industrial applications and as eco-friendly plastic alternatives [32,35,41]. These new directions underscore the growing intersection of material engineering and environmental sustainability, which now shapes much of the field’s trajectory. Citation overlay trends further support this evolution. Initial high-impact work concentrated on microbial growth kinetics, yield improvement, and substrate utilization [6,26,47]. More recently, attention has turned toward life cycle analysis, sustainability metrics, and application-driven functionalization strategies [27,46,48]. This pattern reflects a mature yet dynamic research field—anchored by strong foundational knowledge yet constantly pushing boundaries through interdisciplinary innovation. In the case of kombucha SCOBY-derived BC, this trajectory exemplifies the field’s duality: one axis grounded in microbial and biochemical mechanisms that provide continuity, and another focused on scaling, diversification, and integration into sustainable industrial systems [19,31,50].

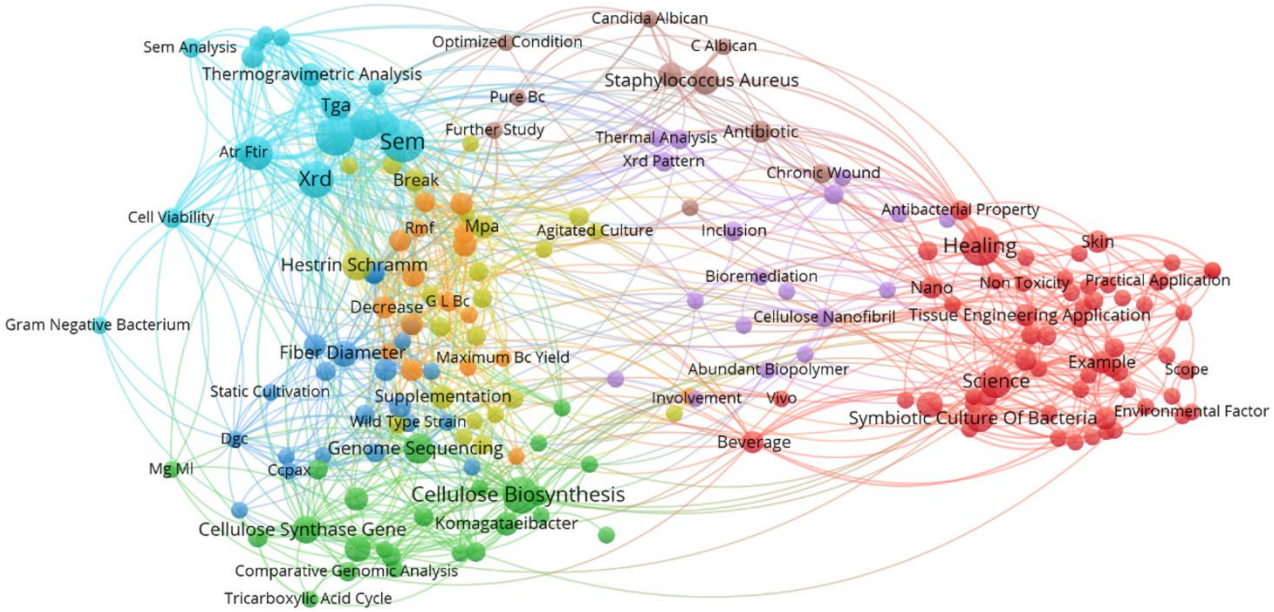


Fig. 2. A keyword co-occurrence network map made with VOSviewer from a Scopus bibliometric search on making bacterial cellulose in kombucha SCOBY systems. The cleaned dataset highlights the most important thematic clusters in the field. Some important areas of research are: how different types of microbes work together (like *Komagataeibacter, Gluconobacter,* acetic acid bacteria, and yeast), new biomaterials (like nanocellulose, cellulose synthase genes, and composite applications), and bioprocess engineering (like fermentation optimization, low-cost substrates, and scale-up strategies). The nodes' size shows how often a keyword appears, and the lines connecting them show how topics are interrelated and how they assemble together to form research clusters. The resultant image makes it clear how the field is organized around the fields of biotechnology, microbial ecology, and material applications. The results also indicate possible methods or approaches to improve yield, functionalization, and integration into the circular bioeconomy.

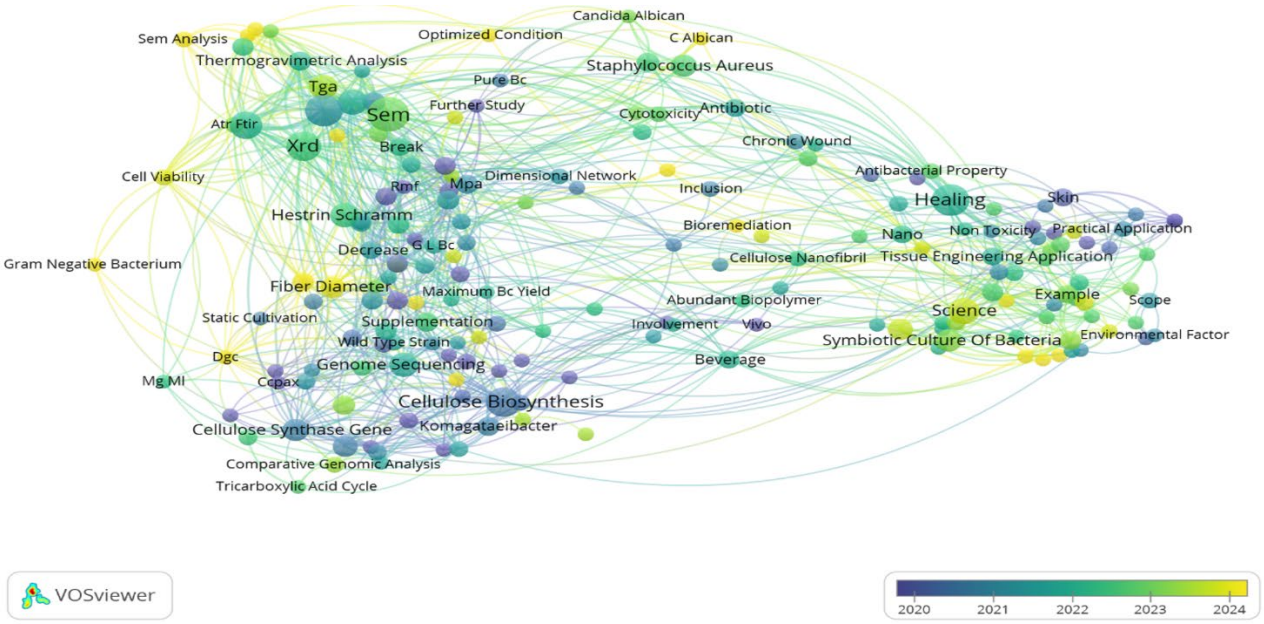


Fig. 3. An overlay visualization of keyword co-occurrence that is produced by VOSviewer, which utilizes the identical Scopus dataset above concerning bacterial cellulose in kombucha SCOBY systems. The size of the node shows how often the keyword appears, and the color gradient from blue to yellow shows the average year of publication. Previous studies (blue) focused on basic microbiology and static culture techniques, including cellulose synthase operons, *Komagataeibacter hansenii*, and *Gluconobacter* metabolism. Recent studies (yellow) focus on new uses and sustainability, such as nanocellulose functionalization, composite reinforcement, circular economy integration, and fermentation on an industrial scale. This temporal perspective underscores both established research domains and emerging trajectories that are gaining traction, demonstrating the transition from fundamental biological investigations to sophisticated biotechnological applications.

CONCLUSION

The symbiotic culture of bacteria and yeast (SCOBY) has been widely studied for its role in kombucha fermentation, where diverse microbial interactions produce bioactive compounds such as organic acids, polyphenols, and bacterial cellulose. AAB, primarily *Komagataeibacter* and *Gluconobacter*, were found to drive ethanol oxidation, creating an acidic environment that prevents contamination while synthesizing cellulose. LAB contributed to fermentation stability and probiotic effects, whereas yeasts like *Saccharomyces* and *Zygosaccharomyces* metabolized sugars into ethanol, supporting bacterial activity and flavor development. Advances in genetic optimization have enhanced bacterial cellulose production through targeted modifications of the *bcs* operon, improving yield and structural properties. However, challenges such as microbial variability and environmental sensitivity remain, requiring controlled fermentation approaches for industrial scalability. Further research is needed to validate health claims and optimize production, ensuring SCOBY's potential is fully realized in food, biotechnology, and biomedical applications.

ACKNOWLEDGEMENT

This research was funded by the Ministry of Higher Education Malaysia, under the Fundamental Research Grant Scheme (FRGS/1/2020/STG01/UPM/02/14; Vote: 5540357; Code: 01-01-20-2225FR), and the essential facilities were provided by UDRP Halal Authentication, IPPH, and Faculty Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.

CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to this publication.

REFERENCES

1. Adnan A, Sudin ASNZ. The state of play: symbiotic culture of bacteria and yeasts (SCOBY) in textile industry. J Teknologi (Sci Eng). 2024;86(3):175-86.
2. Harrison K, Curtin C. Microbial composition of SCOBY starter cultures used by commercial kombucha brewers in North America. Microorganisms. 2021;9(5):1060.
3. Liao T, Li XR, Fan L, Zhang B, Zheng WM, Hua JJ, et al. Nature of back slopping kombucha fermentation process: insights from the microbial succession, metabolites composition changes and their correlations. Front Microbiol. 2024;15.
4. Laavanya D, Shirkole S, Balasubramanian P. Current challenges, applications, and future perspectives of SCOBY cellulose of kombucha fermentation. J Clean Prod. 2021;295:126454.
5. Su J, Tan Q, Tang Q, Tong Z, Yang M. Research progress on alternative kombucha substrate transformation and the resulting active components. Front Microbiol. 2023;14(14):1254014.
6. Molina-Ramírez C, Rojas O, Gómez C, Zuluaga R, Castro C, Osorio M, et al. Effect of different carbon sources on bacterial nanocellulose production and structure using the low pH resistant strain *Komagataeibacter medellinensis*. Materials (Basel). 2017;10(6):639.
7. Feng X, Ge Z, Wang Y, Xia X, Zhao B, Dong M. Production and characterization of bacterial cellulose from kombucha-fermented soy whey. Food Prod Process Nutr. 2024;6:20.
8. Prajapati K, Prajapati J, Patel D, Patel R, Varshnei A, Saraf M, et al. Multidisciplinary advances in kombucha fermentation, health efficacy, and market evolution. Arch Microbiol. 2024;206(9).
9. May A, Narayanan S, Alcock J, Varsani A, Maley C, Aktipis A. Kombucha: a novel model system for cooperation and conflict in a complex multi-species microbial ecosystem. PeerJ. 2019;7:e7565.
10. Yuliana E, Permana AH. Isolation and characterization of microorganisms from SCOBY (Symbiotic Culture of Bacteria and Yeast) during kombucha fermentation. WARTA AKAB. 2022;46(2):18-22
11. Yang S, Bai M, Kwok LY, Zhong Z, Sun Z. The intricate symbiotic relationship between lactic acid bacterial starters in the milk fermentation ecosystem. Crit Rev Food Sci Nutr. 2023;65(4):728-45.
12. Biswas S, Biswas S, Sarkar U, Mitra A. Insights into the challenges and resolutions in the bacterial fermentation process. Amsterdam: Elsevier; 2024.
13. Rafay R, Allegue T, Fowler SJ, Rodríguez J. Exploring the limits of carbohydrate conversion and product formation in open mixed culture fermentation. J Environ Chem Eng. 2022;10(3):107513.
14. Healy LE, Zhu X, Kakagianni M, Poojary MM, Sullivan C, Tiwari U, et al. Fermentation of brown seaweeds *Alaria esculenta* and Saccharina latissima for new product development using *Lactiplantbacillus plantarum*, *Saccharomyces cerevisiae* and kombucha SCOBY. Algal Res. 2023;76:103322.
15. Kaashyap M, Mantri N, Cohen M. Microbial diversity and characteristics of kombucha as revealed by metagenomic and physicochemical analysis. Nutrients. 2021;13(12):4446.
16. Laureys D, Aerts M, Vandamme P, De Vuyst L. The buffer capacity and calcium concentration of water influence the microbial species diversity, grain growth, and metabolite production during water kefir fermentation. Front Microbiol. 2021;12:628599.
17. Chen C, Liu BY. Changes in major components of tea fungus metabolites during prolonged fermentation. J Appl Microbiol. 2000;89(5):834-9.
18. Girard M, Gullo M, Ren H. A systematic, complexity-reduction approach to dissect the microbial and functional diversity of kombucha. eLife. 2023;12:e76401.
19. Cruz MA, Flor-Unda O, Avila A, Garcia MD, Cerda-Mejía L. Advances in bacterial cellulose production: a scoping review. Coatings. 2024;14(11):1401.
20. Wang B, Naren N, Mutukumira AN, Rutherfurd-Markwick K, Zhang XX. Microbiological and physico-chemical characteristics of black tea kombucha fermented with a New Zealand starter culture. Foods. 2023;12(12):2314.
21. Dartora B, Sant'Anna V, Hickert LR, Fensterseifer M, Ayub MAZ, Flôres SH, et al. Factors influencing kombucha production: effects of tea composition, sugar, and SCOBY. Food Sci Technol. 2023.43, e8123,
22. Van TP, Pham GB, Quang HP, Do AD, Thi HTT, Phan QK, et al. Multi-strain probiotics enhance the bioactivity of cascara kombucha during microbial composition-controlled fermentation. Prev Nutr Food Sci. 2023;28(4):502-13.
23. Venegas C, Saona L, Urbina K, Quintrel P, Peña T, Mardones W, et al. Addition of *Saccharomyces eubayanus* to SCOBY fermentations modulates the chemical and volatile compound profiles in kombucha. Food Microbiol. 2023;116:104357.
24. Chong A, Chin N, Talib R, Basha R. Modelling pH dynamics, SCOBY biomass formation, and acetic acid production of kombucha fermentation using black, green, and oolong teas. Processes. 2024;12(7):1301.
25. Kim G, Baek K, Lee G, Lee J, Moon J, Seo S. Development of starter cultures for precision fermentation of kombucha with enriched gamma-aminobutyric acid (GABA) content. Fermentation. 2025;11(1):17.
26. Lin SP, Calvar IL, Catchmark JM, Liu JR, Demirci A, Cheng KC. Biosynthesis, production and applications of bacterial cellulose. Cellulose. 2013;20(5):2191-219.
27. Revin V, Liyaskina E, Nazarkina M, Bogatyreva A, Shchankin M. Cost-effective production of bacterial cellulose using acidic food industry by-products. Braz J Microbiol. 2018;49:151-9.
28. Costa AFS, Almeida FCG, Vinhas GM, Sarubbo LA. Production of bacterial cellulose by *Gluconacetobacter hansenii* using corn steep liquor as nutrient source. Front Microbiol. 2017;8:2027.
29. Li Z, Wang L, Hua J, Jia S, Zhang J, Liu H. Production of nano bacterial cellulose from wastewater of candied jujube-processing industry using Acetobacter xylinum. Carbohydr Polym. 2015;120:115-9.
30. Yao S, Hao L, Zhou R, Jin Y, Huang J, Wu C. Multispecies biofilms in fermentation: biofilm formation, microbial interactions, and communication. Compr Rev Food Sci Food Saf. 2022;21(4):3346-75.

31. Abol-Fotouh D, Hassan MA, Shokry H, Roig A, Azab MS, Kashyout AEHB. Bacterial nanocellulose from agro-industrial wastes: low-cost and enhanced production by *Komagataeibacter saccharivorans* MD1. Sci Rep. 2020;10(1):3491.
32. Gorgieva S, Trček J. Bacterial cellulose: production, modification and perspectives in biomedical applications. Nanomaterials (Basel). 2019;9(10):1352.
33. Jayalakshmi T, Gayathry G, Kumutha K, Sabarinathan KG, Amutha R, Veeramani P. Plausible avenues and applications of bioformulations from symbiotic culture of bacteria and yeast. J Pure Appl Microbiol. 2024;18(3):1489-501.
34. Neffe-Skocińska K, Sionek B, Ścibisz I, Kołożyn-Krajewska D. Acid contents and the effect of fermentation condition of kombucha tea beverages on physicochemical, microbiological and sensory properties. CyTA J Food. 2017;15(4):601-7.
35. Mangayil R, Rajala S, Pammo A, Sarlin E, Luo J, Santala V, et al. Engineering and characterization of bacterial nanocellulose films as low cost and flexible sensor material. ACS Appl Mater Interfaces. 2017;9(22):19048-56.
36. Gullo M, La China S, Falcone PM, Giudici P. Biotechnological production of cellulose by acetic acid bacteria: current state and perspectives. Appl Microbiol Biotechnol. 2018;102(16):6885-98.
37. Semjonovs P, Ruklisha M, Paegle L. Cellulose synthesis by *Komagataeibacter rhaeticus* strain P 1463 isolated from kombucha. Appl Microbiol Biotechnol. 2016;100(13):5565-76.
38. Içen H, Corbo MR, Sinigaglia M, Korkmaz BIO, Bevilacqua A. Microbiology and antimicrobial effects of kombucha, a short overview. Food Biosci. 2023;56:103270.
39. Wang S, Li S, Ji C, Lin X, Liang H, Qi L, et al. Effect of synthetic microbial community on nutraceutical and sensory qualities of kombucha. Int J Food Sci Technol. 2020;55(10):3327-33.
40. Fabricio MF, Vargas BK, Tischer B, Wagner R, Ribeiro SR, Cordeiro N, et al. Revamping kombucha production: achieving consistency and probiotic potential through a tailor-made microbial consortium. Int J Gastron Food Sci. 2023;34:100844.
41. Buldum G, Mantalaris A. Systematic understanding of recent developments in bacterial cellulose biosynthesis at genetic, bioprocess and product levels. Int J Mol Sci. 2022;22(13):7192.
42. Abidi W, Torres-Sánchez L, Siroy A, Krasteva PV. Weaving of bacterial cellulose by the Bcs secretion systems. FEMS Microbiol Rev. 2021;46(2):fuab051.
43. Serra DO, Hengge R. Cellulose in bacterial biofilms. Berlin: Springer; 2019.
44. Römling U, Galperin MY. Bacterial cellulose biosynthesis: diversity of operons, subunits, products, and functions. Trends Microbiol. 2015;23(9):545-57.
45. Amr A, Ibrahim H. Bacterial cellulose: biosynthesis and applications. London: IntechOpen; 2023.
46. Avcioglu NH. Bacterial cellulose: recent progress in production and industrial application. World J Microbiol Biotechnol. 2022;38(5).
47. Krystynowicz A, Czaja W, Wiktorowska-Jezierska A, Gonçalves-Miśkiewicz M, Turkiewicz M, Bielecki S. Factors affecting the yield and properties of bacterial cellulose. J Ind Microbiol Biotechnol. 2002;29(4):189-95.
48. El-Gendi H, Taha HT, Ray JB, Saleh AK. Recent advances in bacterial cellulose: a low-cost effective polymer. Cellulose. 2022;29(5):7495-7533.
49. Kumar R, Singh OV, Singh S. Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. J Ind Microbiol Biotechnol. 2008;35(5):377-91.
50. Ryngajłło M, Jędrzejczak-Krzepkowska M, Kubiak K, Ludwicka K, Bielecki S. Towards control of cellulose biosynthesis by Komagataeibacter using systems-level and strain engineering strategies: current progress and perspectives. Appl Microbiol Biotechnol. 2020;104(15):6565-85.