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Review

Bacterial Cellulose Production in Co-Culture Systems: Opportunities, Challenges, and Future Directions

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Abstract

Bacterial cellulose (BC), a nanostructured biopolymer produced by *Komagateibacter* spp., exhibits remarkable mechanical strength, purity, and biocompatibility, making it highly attractive for applications in biomedicine, food, and sustainable materials. Despite its potential, monoculture fermentation suffers from low yield and limited scalability. This review highlights the innovative application of co-culture fermentations as a novel strategy, where *Komagataeibacter* is paired with complementary microorganisms such as yeasts, lactic acid bacteria, and photosynthetic microbes. This approach has emerged as a promising solution to overcome the limitations of monoculture by enhancing BC productivity, tailoring material properties, and improving sustainability. We explore the synergistic interactions within co-cultures, including metabolic cross-feeding and in situ polymer integration, while also addressing critical challenges such as microbial stability and operational complexity. Unlike previous reviews focused primarily on BC biosynthesis, applications, or genetic engineering, this article emphasizes co-culture fermentation with *Komagataeibacter* as a novel and underexplored strategy to improve the yield, functionality, and scalability of BC production.

Keywords: bacterial cellulose; co-culture; fermentation; *Komagataeibacter*; synthetic ecology; multi-omics

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1. Introduction

Bacterial cellulose (BC) is a highly pure extracellular polysaccharide predominantly synthesized by species of the genus *Komagataeibacter* (formerly classified as *Acetobacter* or *Gluconacetobacter*) [1,2]. Although chemically identical to plant-derived cellulose, composed of linear β-1,4-linked D-glucopyranose units, BC exhibits superior physicochemical and structural characteristics due to its microbial origin and distinct biosynthetic pathway [1–3]. The key distinction lies in its exceptional purity; unlike plant cellulose, which is embedded within a lignocellulosic matrix containing lignin, hemicellulose, and pectin, BC is secreted in an essentially pure form, typically exceeding 99% cellulose content [3,4]. Structurally, BC features an ultrafine three-dimensional nanofiber network with fiber diameters ranging from 20 to 100 nm—approximately 100 times thinner than plant-derived microfibrils—resulting in a very high surface-area-to-volume ratio [2–4]. This nanostructure contributes to its high crystallinity (70–80%), high degree of polymerization

(DP ~2000–6000), and outstanding mechanical properties, including its excellent tensile strength and Young's modulus [3,4]. BC hydrogels can absorb up to 100 times their dry weight in water, a property dramatically enhanced through composite formation. For instance, BC/poly-AEM composites exhibit swelling capacities up to ~6200% by preventing collapse of the BC structure during drying, while BC/acrylic hydrogel composites reach 4000–6000% swelling and, in vivo, significantly promote burn healing via enhanced epithelialization and fibroblast proliferation [3,5–7]. Furthermore, BC is inherently biocompatible, non-toxic, and biodegradable—qualities essential for biomedical applications such as wound dressings, drug delivery systems, and tissue engineering scaffolds [5–12]. The cellulose synthesis process, in which fibers form tightly packed ribbons under standard conditions and become more loosely organized with additive supplementation, is illustrated in Figure 1.

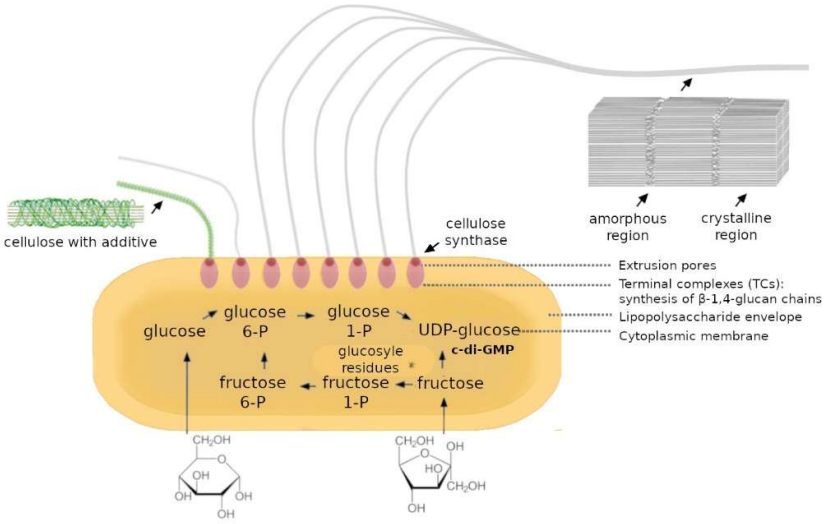

Figure 1. Illustration of BC biosynthesis and nanofiber assembly. Intracellular pathways convert sugars into UDP-glucose, which is polymerized and extruded as cellulose fibers.

BC biosynthesis in *Komagataeibacter xylinus*, a model high-yield strain, is mediated by the cellulose synthase complex encoded by the *bcs* operon. This complex catalyzes glucose polymerization into glucan chains that self-assemble into nanofibrils [6,7,10,11]. While prior reviews have focused on BC's structural and physicochemical features [3,4,9], biomedical applications [5–8,11,12], or advances in nanocellulose and cellulose biosynthesis at the genetic level [9,10,13], few have addressed co-culture fermentation strategies involving *Komagataeibacter*. This review fills that gap by synthesizing emerging evidence on co-culture systems, particularly those pairing *Komagataeibacter* with yeasts, lactic acid bacteria, or photosynthetic organisms, and highlights how such systems offer a promising, underexplored approach for scalable and sustainable BC production.

2. The Co-Culture Paradigm: A Strategy for Enhanced Yield and Functionalization of BC

2.1. Overcoming BC Monoculture Limitations Through Co-Culture Strategies

Conventional monoculture-based BC production, though foundational, faces persistent challenges related to scalability, productivity, and functional customization. The most common method, static cultivation, yields high-quality BC pellicles at the air–liquid interface but is hindered by slow kinetics, poor oxygen diffusion, and labor-intensive operations, thus limiting industrial viability [2,8–10]. Agitated or submerged bioreactor systems improve oxygen and nutrient transfer and offer potential for scale-up [8,14]. However, these

systems may cause shear-induced defects, lower crystallinity, and increased formation of non-cellulose-producing (Cel^-) mutants, ultimately reducing BC quality and yield [2,4,15].

Meanwhile, co-culture fermentation leverages synergistic interactions between *Komagataeibacter* and partner microbes to improve yield, expand substrate range, and functionalize BC [16–20]. Mechanisms include metabolic cross-feeding, pH regulation, and dynamic interspecies interactions [16,19,20]. For example, ethanol produced by yeasts can serve as an additional carbon source for *Komagataeibacter*, while organic acids from lactic acid bacteria (LAB) stimulate BC biosynthesis [20]. Such systems also enhance process robustness and adaptability under industrial conditions.

Nonetheless, uniform pellicle thickness remains a challenge especially in static systems due to oxygen and nutrient gradients that result in heterogeneous structures [14,18,21]. Applications such as biomedical scaffolds or textile membranes require mechanically robust, uniform BC layers [22]. Additionally, co-cultures introduce complexity: differing oxygen requirements and nutrient competition can destabilize microbial balance and hinder BC formation [23]. However, they also enable division of labor and facilitate in situ incorporation of microbial exopolymers—such as pullulan or hyaluronic acid (HA)—into the BC matrix, yielding composites with enhanced functionality and mechanical properties [24–28].

2.2. Bioreactor Configurations for Co-Culture-Based BC Fermentation

Recent innovations in bioreactor design have expanded the applicability of co-culture strategies by addressing oxygenation, shear stress, and microbial spatial organization. The following bioreactor types have been employed in co-culture-based BC production:

1. Static Fermentation

Reported that 181 studies employed a static system for monoculture fermentation [21], whereas its application in co-culture settings has been far less common. Typically, shallow trays where *Komagataeibacter* interacts with yeasts or LAB at the air–liquid interface. Metabolite exchange enhances pellicle formation and crystallinity, though productivity remains low due to surface area limitations and slow kinetics [10,15,29].

2. Agitation Fermentation

Reported in 33 studies [21], this approach uses shaking flasks or stirred-tank reactors to enhance mixing, oxygenation, and substrate dispersion. Shear forces may impair BC structure, favoring amorphous forms. Strategies like intermittent agitation or optimized stirring speeds aim to reduce such effects [10,30,31].

3. Airlift Bioreactor

Gas-lift systems provide high oxygen transfer in low-shear environments—suitable for aerobic co-cultures. Wu and Li (2015) achieved high-yield BC production using a modified airlift design [32]. However, BC pellet formation and excessive sparging may disrupt synthesis [10,29,32].

4. Rotating Biological Contactor (RBC)

RBC systems utilize disks partially submerged in a culture medium, allowing microorganisms to cyclically access air and nutrients, which supports aerobic and facultative anaerobic co-cultures such as *Komagataeibacter* with yeasts or fungi [10,29,33]. These systems offer high oxygen transfer, low shear stress, and continuous biofilm growth. A novel rotating disk bioreactor (RDBR) introduced by Pilafidis et al. (2025) featured perforated horizontal disks, achieving superior oxygenation and nutrient diffusion. The system reported 15.2 g/L BC yield and 2.17 g/L/day productivity with improved fibril architecture [34].

5. Dual-Vessel Co-Culture Reactors

These systems spatially separate the aerobic (e.g., *Komagataeibacter*) and anaerobic/microaerophilic (e.g., LAB or yeast) components into two connected vessels. Nutrient and metabolite exchange is facilitated through a closed-loop circulation system. While highly customizable and effective in maintaining microbial balance, dual-vessel systems introduce higher capital costs and engineering complexity. They are particularly suited for bioprocesses requiring strict metabolic separation [10,29,35].

6. Mesh Dispenser Vessel (MDV) Bioreactor

The MDV integrates static pellicle growth with intermittent nutrient feeding. A mesh scaffold suspends the growing BC at the air–liquid interface, allowing thick, vertically expanding pellicles without submersion. Loh et al. (2025) reported pellicle thicknesses exceeding 80 mm, with a 3.4-fold increase in glucose conversion and improved water-use efficiency [2,36]. This reactor is well-suited for co-culture systems requiring sustained aeration and minimal disturbance, though it demands precise control of feeding regimens and harvesting techniques.

Figure 2 represents the different bioreactor types which have been employed in co-culture-based BC production, followed by a brief overview of each bioreactor type.

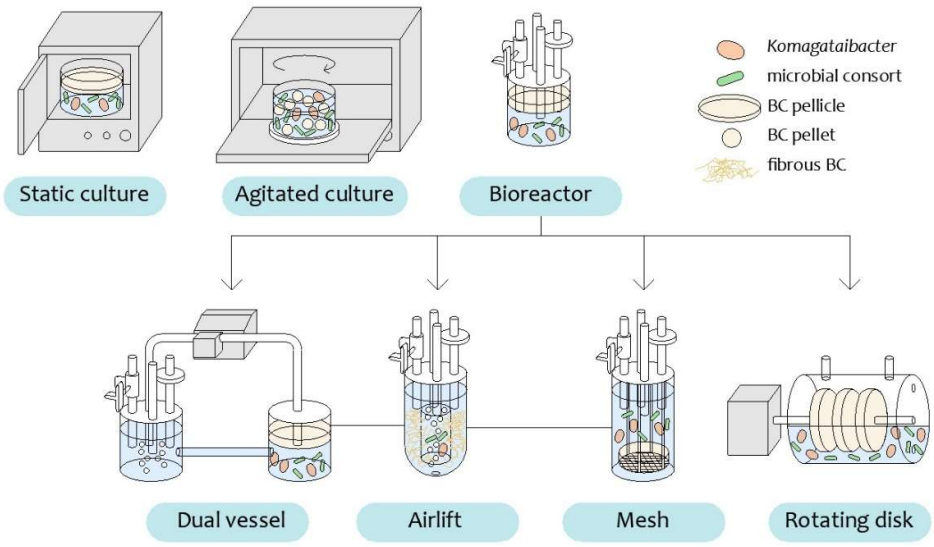

Figure 2. Schematic representation of bioreactor configurations for BC production strategies applicable to co-culture systems.

3. Advancements in BC Production: From Natural Consortia to Co-Culture Fermentation Systems

The conceptual foundation for co-culture strategies in BC production originates from traditional fermented foods, which rely on complex, naturally occurring symbiotic communities of bacteria and yeasts, commonly referred as SCOBY (symbiotic culture of bacteria and yeast), to form cellulosic pellicles. Well-known examples include kombucha (fermented tea), in which yeasts such as *Saccharomyces* and *Zygosaccharomyces* hydrolyze sucrose into glucose and fructose, producing ethanol as a byproduct [16,24]. Acetic acid bacteria (*Komagataeibacter* spp.) subsequently utilize both sugars and ethanol to synthesize BC and organic acids [16,18,37,38]. Similarly, in *nata de coco* (fermented coconut water), *Komagataeibacter nataicola* coexists with lactic acid bacteria (*Lactobacillus* spp.), leading to improved BC yields [18,24].

Early research predominantly focused on isolating *Komagataeibacter* monocultures to optimize BC biosynthesis [38,39]. However, limitations soon emerged, including inefficient

substrate utilization, byproduct inhibition (e.g., gluconic acid accumulation), and restricted yields [28,38]. In recent years, BC production has shifted toward engineered co-culture systems designed to deliberately optimize microbial interactions. A landmark study by Seto et al. [17] demonstrated that co-culturing *Gluconacetobacter xylinus* with *Lactobacillus mali* increased BC yield threefold compared to monoculture. Their work provided one of the first clear validations that a well-designed co-culture can achieve higher performance than a single strain. This transition from relying on undefined natural consortia to utilizing rationally constructed co-cultures marks a significant step forward in BC biotechnology. It enables precise control over microbial interactions, allowing researchers to enhance yields, customize material properties, and expand substrate versatility. The progress of co-culture BC fermentation studies is summarized in Table 1.

Table 1. Progression of co-culture strategies for BC production.

Phase	Conceptual Overview	Key Studies	Ref.
Natural Consortia	Traditional fermented foods such as kombucha and *nata de coco* relying on undefined microbial communities (yeasts and bacteria) that interact synergistically to produce BC.	Kombucha: yeast + *Komagataeibacter* → ethanol → BC; *Nata de coco*: *K. nataicola* + *Lactobacillus* increased BC production.	[16,18]
Defined Dual-Microbe Co-Culture Strategies (2006–2020)	Strategic pairing of BC producers with mutualistic partner microbes (e.g., ethanol producers, acid regulators) to enhance and stabilize yields.	*G. xylinus* + *L. mali* yielded 3× BC yield compared to monoculture; other examples include BC–HA and BC–PHB composite production.	[20,38]
Engineered Co-Cultures and Omics-Guided Design (>2020)	Integration of synthetic biology, signal-responsive gene regulation, and omics-based optimization for fine control of microbial interactions and BC production.	Examples include quorum-sensing-based luxR overexpression in *G. xylinus*, co-cultures with synthetic signaling modules, and omics-informed pathway engineering in *K. nataicola* and *Komagataeibacter* spp.	[39–43]

3.1. Key Advances in Co-Culture BC Production by Komagataeibacter

Numerous studies have demonstrated that pairing *Komagataeibacter* with compatible microbes can substantially enhance BC yield and tailor its physicochemical properties. In metabolic cross-feeding systems, one partner microbe produces metabolites that directly stimulate BC biosynthesis. For example, co-culturing *K. xylinus* with the ethanol-producing yeast *Kluyveromyces marxianus* on brewing yeast waste resulted in a twofold yield increase compared to monoculture [24,35]. In another dual-bacterial system, *Bacillus cereus* metabolized corn stover hydrolysate and secreted acetoin and 2,3-butanediol, both of which stimulated *K. xylinus* to produce more cellulose than in monoculture [17].

Notably, co-culture advantages extend beyond BC yield improvements. They also facilitate the use of low-cost feedstocks (e.g., agricultural residues, brewery waste) by combining *Komagataeibacter* with microbes capable of degrading complex nutrients [44]. Beyond microbial pairing, strain selection within the genus *Komagataeibacter* itself significantly influences co-culture outcomes. While *K. xylinus* is the most commonly engineered strain, less explored species such as *K. rhaeticus*, *K. oboediens*, and *K. medellinensis* may possess adaptive traits, such as acid stress tolerance or oxygen efficiency, that are particularly advantageous under co-culture conditions [14,20]. Incorporating these strains could unlock novel efficiencies in co-culture BC production.

Besides co-culture strategies, in situ BC functionalization has been explored by pairing *Komagataeibacter* with exopolysaccharide-producing microbes. For example, co-culturing *K. hansenii* with *Aureobasidium pullulans* produces BC–pullulan nanocomposites with ~22% higher yield and improved tensile strength and Young's modulus [45]. Similarly, engineered *Lactococcus lactis* secreting hyaluronic acid (HA) can be paired with *G. hansenii* [46,47], producing BC/HA composites with greater fiber width, higher water retention, and improved elasticity, properties valuable for wound dressings [6,7,12,46]. Such in situ integration of functional polymers reduces the need for purified additives, and post-processing fermenta-

tion promotes homogeneous incorporation of functional polymers into the BC structure, as demonstrated in *L. lactis*–HA and *A. pullulans*–pullulan co-culture systems, reducing production costs and simplifying workflows [45–47]. A comprehensive comparison of co-culture systems is presented in Table 2.

Table 2. Summary of co-culture studies involving *Komagataeibacter* spp. for enhanced BC production and improvement in material characteristics.

Primary Producer (BC)	Co-Culture Partner(s)	Fermentation Type	Yield	Core Finding (Properties)	Mechanism	Ref.
K. xylinus	*B. cereus*	Corn stover enzymatic hydrolysate	Fourfold increase in BC yield with lignocellulosic hydrolysate	Robust BC formation under nutrient-rich cultivation	Acetoin and 2,3-butanediol from *B. cereus* enhance BC synthesis	[17]
K. nataicola Q2	*L. fermentum*	Coconut water-based medium	BC yield increased by up to 59.5%	Improved crystallinity, reduced thermal degradation, enhanced water retention and mechanical performance	LAB-derived acids activate Krebs cycle; enhanced β-1,4-glucan formation and cell co-aggregation support fiber assembly	[18]
G. xylinus	*L. mali*	Static	3-fold increase in BC production	Not analyzed	LAB secretes growth-promoting metabolites	[20]
*K. xylinus MS*2530	Various yeasts	Sterilized brewing waste	BC production improved 4–5-fold compared to monoculture; 2–2.5-fold increase with solely brewing waste	Industrial waste enhances productivity and reduces cost	Yeasts provide ethanol and CO_2 that stimulate BC synthesis; waste stream offers nutrients	[24]
Komagataeibacter sp.	*Lactocaseibacillus* sp.	Static	Higher BC yield	Reduced crystallinity, increased fiber size and WHC	In situ incorporation of hyaluronic acid (HA)	[38]
K. hansenii	*A. pullulans*	Molasses medium	Increase of 22.4% over monoculture	Improved Young's modulus and tensile strength	In situ integration of pullulan into BC matrix	[45]
G. hansenii	*L. lactis*	Static (pH-controlled)	Yield varies with pH; best mechanics at pH 4.0	Young's modulus improved to 5029 MPa, altered ribbon width-tuned HA secretion affects BC/HA matrix architecture	Lower pH showed favorable synergy between *G. hansenii* and HA secretion from *L. lactis*	[46]
G. hansenii	*L. lactis* (HA^+)	Two-vessel circulation	Enhanced yield with HA integration	Controlled HA production, improved crystallinity and mechanical strength	Engineered HA secretion and stirrer bioreactor to optimize lactic acid production of LAB circulating into BC vessel	[47]
G. hansenii ATCC 23769	*E. coli* ATCC 700728	Static	Yield increased by 10.8% compared to monoculture	Enhanced mechanical properties	In situ incorporation of mannose-rich exopolysaccharide	[48]
K. hansenii	*C. reinhardtii*	Static	~20% increase	Enhanced 3D BC architecture formation, overcoming oxygen limitation	In situ generation O_2 generation by photosynthetic microalgae	[49]
K. intermedius	*B. bruxellensis, Z. bisporus*	Inoculum ratio optimization (1:10:10)	Maximum dry weight yield of 5.51 g/L under optimized inoculum ratios	Co-culture promoted efficient substrate conversion and BC assembly	Yeasts produced ethanol and growth factors; optimal inoculum ratio also critical for microbes' interaction	[50]

3.2. Key Challenge of Co-Culture Fermentation-Based BC Production

Despite the promise of co-culture systems, several unresolved issues limit their industrial application. These include long-term stability in continuous fermentation, the controllability of population dynamics, unintended consequences for BC quality, and the genetic instability of *Komagataeibacter* strains [10,14,21,51,52]. Furthermore, the broader scientific debate emphasizes skepticism about the feasibility of co-cultures in industrial contexts. Despite their synergistic potential, co-culture systems often experience population imbalance due to nutrient competition, pH sensitivity, and differences in growth rates [53–55]. This challenge becomes particularly acute in continuous or fed-batch processes at industrial scale, where fluctuating conditions can destabilize mutualistic interactions [54,55]. In addition, co-culture optimization requires careful tuning of inoculum ratios, medium composition, and inoculation timing [4,8,10,15,21]. Nutrient-enriched media often required for partner microbes may also increase the contamination risk, leading to yield variability and altered BC physicochemical properties [53,56]. Addressing these challenges will require system designs, advanced real-time monitoring, and deeper mechanistic understanding

of interspecies interactions [57,58]. Table 3 summarizes the key challenges, representative studies, and potential mitigation strategies.

Table 3. Overview of key challenges, representative studies, and mitigation strategies in co-culture-based bacterial cellulose (BC) fermentation.

Aspect	Key Challenges	Prior Studies	Potential Mitigation Strategies	Ref.
Yield of BC and Material Characteristics	Enhancing BC yield may compromise essential material characteristics such as crystallinity.	*K. nataicola* + *L. fermentum* SR improved yield but reduced mechanical performance.	Real-time monitoring of bacterial cellulose formation combined with genetic modification of strains to tailor material characteristics.	[18,19,57–59]
Stability and Yield Predictability	Co-cultures often lack stability, especially under industrial-scale or prolonged fermentations.	Pre-fermented coconut water became unstable, especially with more than three species.	Designing obligate mutualistic systems and using adaptive laboratory evolution (ALE) to enhance stability.	[19,55]
Contamination and Metabolite Interference	Co-culture partners may produce inhibitory metabolites or alter BC structure.	HA-producing LAB enhanced BC yield but reduced crystallinity.	Comprehensive chemical and structural characterization to meet regulatory standards for food and biomedical applications.	[38,46,47]
Operational Complexity and Cost	Multispecies cultures require advanced infrastructure and pose economic challenges.	Complex systems increase contamination risk and operational costs.	Using simplified two-member systems, employing real-time multispecies sensing, and integrating economic modeling.	[60,61]
Mutualism vs Competition	Microbial interactions may be antagonistic or neutral, not always beneficial.	Some strains outcompete others, leading to reduced BC yield.	Conducting detailed strain screening and utilizing omics-guided metabolic modeling to select true mutualists.	[50,53,54]
Genetic Instability	*Komagataeibacter* spp. exhibit adaptive genetic variation in response to environmental stress and consortial conditions.	Cellulose synthase operon mutations have been observed, affecting production consistency.	Engineering robust *Komagataeibacter* strains through genomic stabilization and enhanced stress-response regulation.	[55,59]
Process Controllability	It is difficult to control population dynamics and metabolic flux in real time.	Population ratios shift dynamically; substrate pulsing helps but is not universal.	Engineering of inducible gene circuits, integrated with feedback control systems utilizing real-time sensor data.	[57,58]

Meanwhile, Figure 3 presents representative studies along with potential mitigation strategies for these challenges and opportunities.

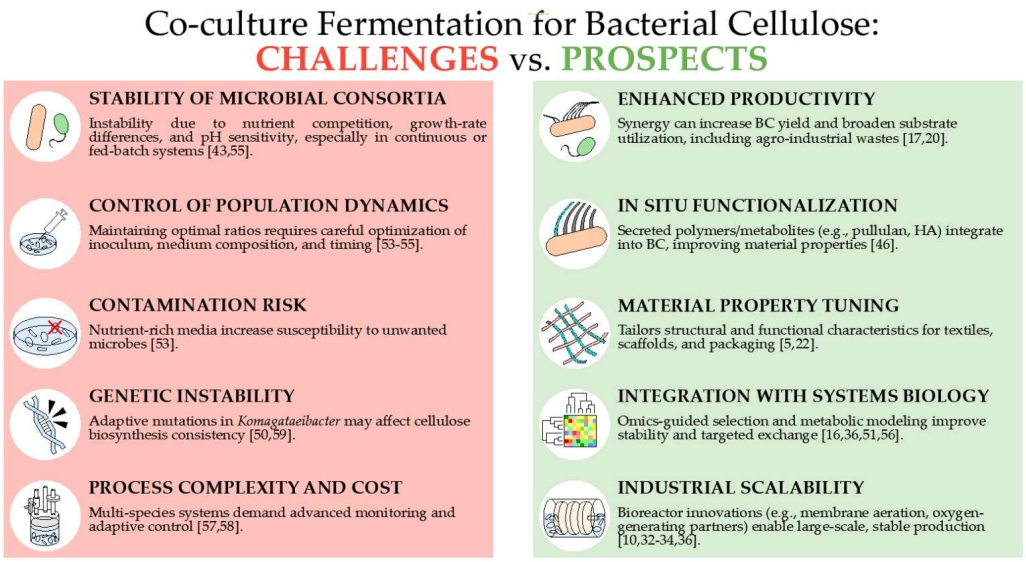

Figure 3. Comparative overview of key challenges and future opportunities in co-culture fermentation for BC production.

4. Industrial Co-Culture Fermentation: Lessons and Comparison with BC System

In industrial biotechnology, co-culture systems are extensively employed in processes such as biohydrogen production, ethanol fermentation, and bioplastic synthesis to improve substrate utilization and product yields. For example, in biofuel production, facultative anaerobes (e.g., *Klebsiella*, *Streptococcus*) are co-cultured with obligate anaerobes (*Clostridium* spp.), enabling the degradation of complex substrates and the removal of inhibitory byproducts (e.g., H_2, formate), thereby enhancing overall metabolic performance and process stability [30,62]. These consortia exemplify metabolic division of labor, where each organism specializes in a specific step of a pathway. A similar approach could be adapted for BC fermentation, for instance, by pairing cellulolytic *Clostridium* species with *Komagataeibacter* to valorize lignocellulosic waste streams into BC [63].

For instance, Li et al. (2023) reported that co-culturing *K. xylinus* with *B. cereus* on corn stover hydrolysate significantly increased BC yield. *B. cereus* fermented complex sugars into acetoin and 2,3-butanediol, which stimulated BC biosynthesis [17]. This illustrates how co-culture can broaden substrate scope (e.g., agro-industrial residues) and enhance productivity through metabolic complementarity.

Additional insights come from traditional fermented foods that rely on stable mixed cultures. In yogurt production, defined LAB co-cultures (e.g., *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) co-metabolize lactose and synergistically produce flavor compounds. Kefir represents a complex microbial ecosystem, comprising diverse LAB (e.g., *L. kefiranofaciens*, *L. kefiri*, *Lactiplantibacillus plantarum*) and yeasts (e.g., *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*) embedded in kefiran-rich grains. These microbes cooperate metabolically by converting lactose and other sugars into organic acids, ethanol, and bioactive compounds, stabilizing the community and enhancing functionality [64,65].

In nata de coco, a traditional fermented coconut water product, BC is synthesized through synergistic interactions between *K. nataicola* and *Lactobacillus* spp. [18,43]. This bacterial–bacterial co-culture exemplifies metabolic complementarity, where lactic acid bacteria produce metabolites that modulate pH and stimulate cellulose biosynthesis [17,18,46,53,54,66]. A similar but distinct consortium appears in kombucha fermented on *Sargassum fusiforme* (brown algae), where *Zygosaccharomyces* dominates throughout, and the bacterial profile shifts from *Acetobacter* to *Komagataeibacter* over time [52]. This shift correlates with acid accumulation (final pH ~2.77), increased antioxidant activity, and volatile metabolite production (e.g., ethanol, acetone, ethyl acetate).

Kombucha, a fermented tea, demonstrates another example of well-orchestrated microbial synergy: yeasts such as *Saccharomyces* and *Zygosaccharomyces* hydrolyze sucrose into glucose and fructose and ferment them into ethanol and CO_2, which are subsequently utilized by *Komagataeibacter* spp. to produce acetic acid and bacterial cellulose, forming the characteristic SCOBY biofilm [16,24,45,66]. High-throughput sequencing of commercial SCOBYs revealed *Brettanomyces* and *Komagataeibacter* as predominant members, with *Zygosaccharomyces*, *Lachancea*, and *Starmerella* functionally compensating when *Brettanomyces* is scarce [45]. These communities cluster into four reproducible "archetypes," each representing a distinct yeast–bacteria balance that maintains cellulose production [51,66].

5. Techno-Economic Analysis of BC Production: Monoculture vs. Co-Culture

5.1. Cost Considerations in BC Production: Monoculture vs. Co-Culture

The economic feasibility of BC production remains a key barrier to industrial adoption, primarily due to the high cost of culture media and fermentation infrastructure. Zhong

et al. (2020) identified two principal strategies to lower costs: enhancing BC yield and utilizing cost-effective nutrient sources, as media can constitute up to 30% of total production expenses [10,14]. Conventional monoculture systems using *Komagataeibacter* spp. in a Hestrin–Schramm (HS) medium yield high-purity BC but are hindered by low yields and costly inputs, particularly refined sugars, yeast extract, and peptones [8,10,14,60,61]. To overcome these limitations, considerable research has focused on replacing costly substrates with agro-industrial byproducts. Promising alternatives such as glycerol [21], corn steep liquor [67], citrus pulp water [68], molasses and cashew apple juice [69], vinegar residue [70], coconut water [61,71], and biodiesel-derived residues [31] have demonstrated cost reduction of up to 97% while promoting circular bioeconomy goals [31,71]. However, scalability remains limited by feedstock variability and seasonal availability.

Henry et al. (2024) reported that substituting an HS medium with solid-state fermentation (SSF)-treated rice bran (RB) and cereal dust reduced BC production costs by up to 90%, from AUD 17.08/g to AUD 2.51/g [72]. Recent findings further support the feasibility of low-cost substrates. Nóvak et al. (2024) reported that a black tea and sugar medium yielded the lowest cost (USD 0.10/L), compared to HS medium (USD 1.36/L), while enzymatically treated substrates reached up to USD 5.58/L [73]. These findings reinforce the viability of SSF-treated agro-waste as a scalable, economical solution for BC production.

5.2. Techno-Economic Analysis and Industrial Feasibility of Co-Culture-Based BC Production

Techno-economic analysis (TEA) and life cycle assessment (LCA) are essential tools for evaluating feasibility of scaling BC production. These models assess parameters including capital expenditure, operating costs, resource efficiency, and profitability, offering strategic insight into process optimization and cost reduction pathways [61,71]. Although current TEA studies remain limited particularly for co-culture systems, existing analyses reveal promising economic scenarios when alternative substrates, reactor innovations, and microbial consortia are integrated. A notable example is the kombucha-based BC production system, involving a symbiotic co-culture of *Komagataeibacter* and osmophilic yeasts. Behera et al. (2022) simulated a 60-ton/year production facility using SuperPro Designer®, estimating a total capital investment of USD 13.72 million and annual operating costs of USD 3.8 million [61]. A comprehensive techno-economic assessment estimated the production cost of dry bacterial cellulose at USD 63.8 per kilogram, with a capital recovery period of 4.2 years, a return on investment (ROI) of 23.64%, and an internal rate of return (IRR) of 16.48%. A SuperPro Designer® simulation by Dourado et al. (2016) modeled a large-scale facility producing 504 tons/year, showing that use of beet molasses instead of refined sugars reduced the production cost to USD 14.8/kg. This model estimated a capital investment of USD 13 million, with operating costs at USD 7.4 million/year and a projected net profit of USD 3.3 million/year [71]. These results emphasize the significant impact of media composition, particularly carbon and nitrogen sources, which may account for 30–40% of total production costs [71].

Notably, approximately 89% of the total operating costs were attributed to infrastructure and labor, highlighting the relatively minor economic contribution of raw materials such as a sweet tea medium [31]. These data highlight advancements in automated, modular bioreactor systems capable of continuous or semi-continuous operation. Automation not only reduces labor costs but also ensures product consistency. Real-time monitoring tools with dynamic feedback controls for pH, dissolved oxygen, and substrate concentration are essential to optimize fermentation conditions and scale-up potential [21,31,71,73].

El-Gendi et al. (2022) emphasized that effective bioprocess design integrating media optimization, advanced reactor engineering, and microbial performance enhancement is central to achieving economically and environmentally sustainable BC production [15].

Furthermore, Islam et al. (2017) underscored that bioreactor design directly influences BC yield and cost-effectiveness [31].

Market dynamics further influence TEA outcomes. High-purity BC can command prices up to USD 120/kg in biomedical markets, while bulk food-grade BC (e.g., *nata de coco*) is sold for USD 0.2–1.0/kg in Southeast Asia [61]. These low-cost fermentations often rely on open-vat methods with coconut water under non-sterile conditions. However, Sundaram et al. (2023) demonstrated that glycerol–sesame seed meal hydrolysate significantly increased economic productivity and cost efficiency compared to an HS medium (USD 6.98 vs. 4.34 per dollar of nutrient per day), highlighting the value of waste valorization in co-culture fermentation [29]. Key techno-economic indicators for alternative media cultivation are summarized in Table 4.

Table 4. Comparative techno-economic indicators of BC production. (**a**) Production cost and key economic metrics for kombucha-based and beet molasses-based systems. (**b**) Substrate cost comparison. (**c**) Cost reduction from replacing HS medium with SSF-treated rice bran and cereal dust. Values are extracted from SuperPro Designer® simulations reported by Behera et al. (2022) [61] and Dourado et al. (2016) [71].

(a) Production cost comparison (USD/kg)		
Parameter	**Kombucha-based BC**	**Beet molasses-based BC**
Capital Investment (USD)	13.72	13
Operating Cost (USD/year)	3.8	7.4
Production Cost (USD/kg)	63.8	14.8
ROI (%)	23.64	-
IRR (%)	16.48	-
Payback Period (years)	4.2	-
Net Profit (M USD/year)	-	3.3
(b) Substrate cost comparison (USD/L)		
Substrate	**Cost (USD/L)**	
HS medium	1.36	
Black tea + sugar	0.1	
Enzyme-treated substrate	5.58	
(c) SSF vs. HS *medium cost (AUD/g)*		
Medium type	**Cost (AUD/g)**	
HS medium	17.08	
SSF-treated RB and cereal dust	2.51	

6. Omics Integration for Co-Culture Engineering

Omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, are increasingly applied in co-culture systems to unravel microbial interactions and their impact on metabolism, phenotypes, and product quality.

Transcriptomic profiling facilitates omics integration in co-culture systems, allowing researchers to reveal dynamic gene expression changes in mixed cultures compared to monocultures. By analyzing global mRNA expression patterns, it becomes possible to determine which genes in *Komagataeibacter* are up- or downregulated in response to metabolites secreted by partner strains or environmental modifications. An omics-based study by Wang et al. (2018) identified distinct gene expression profiles in *Komagataeibacter* under different carbon sources and co-substrate conditions [74]. In a related study, Fei et al. (2023) found that adding exopolysaccharides from *E. coli* into *Gluconacetobacter* culture significantly improved the tensile strength of the cellulose pellicle [43]. Proteomic studies revealed upregulation of enzymes and stress-related pathways under ethanol-enriched co-culture, linking microbial interaction to enhanced BC synthesis [43,55]. The end-to-end workflow for sample preparation, MS acquisition, and multivariate analysis is summarized in Figure 4.

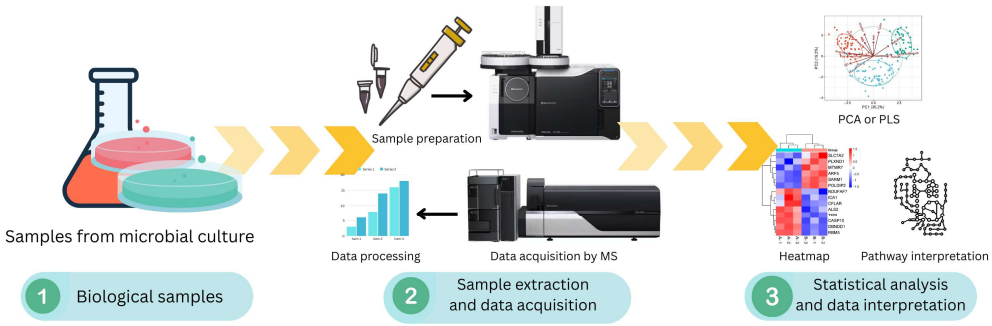

Figure 4. Schematic representation of omics integration in co-culture fermentation.

These findings underscore omics as essential tools for optimizing fermentation, improving material properties, and guiding the development of next-generation BC-based products. For instance, natural consortia such as kefir grains composed of LAB, acetic acid bacteria, and yeasts embedded in exopolysaccharide matrices efficiently coordinate metabolic pathways [64]. Comparative genomics by Ryngajłło et al. (2019) revealed that certain *Komagataeibacter* strains possess multiple *bcs* operons and adaptive stress genes, e.g., *K. medellinensis* carries four copies of the type II operon, offering genetic plasticity [75].

Synthetic systems mimic such consortia. Liu et al. (2021) engineered a co-culture of *Synechococcus elongatus* (photoautotroph) and *E. coli* (heterotroph), where *S. elongatus* fixed CO_2 and light, while *E. coli* produced isoprene. The system extended fermentation from 100 to 400 h, and increased isoprene yield eightfold. Multi-omics revealed oxidative stress responses in *E. coli*, triggered by reactive oxygen species (ROS), which activated protective pathways and extended viability [76].

Co-cultures have also boosted industrial enzyme production. For example, co-culturing *Bacillus amyloliquefaciens* with UV/ARTP-mutated TGase-producing strains enhanced enzyme activity to 16.91 U/mL—a 22.71% increase—through improved protein secretion and stress adaptation. Genomic analysis identified 95 mutations affecting codon usage, while transcriptomics revealed 470 differentially expressed genes related to metabolism, sporulation, and enzyme activation [77].

Omics also uncover cryptic biosynthetic gene clusters (BGCs). Co-culturing *Micromonospora* with *Rhodococcus* activated the silent keyicin gene cluster via interspecies signaling, revealed through transcriptomic and proteomic analyses [78]. Metabolomics similarly exposes latent biosynthetic capacity: *Eurotium amstelodami* co-cultured with *Bacillus* spp. enhanced anti-*S. aureus* activity through altered metabolic pathways and novel secondary metabolites [79].

In continuous microbial cultures, metabolomics-guided co-culture strategies offer solutions to key bottlenecks like contamination, strain degeneration, and metabolic instability. Co-culturing photoautotrophs with heterotrophs can establish stable micro-ecological systems that support prolonged and efficient biosynthesis. For instance, *Chlorella vulgaris* and *E. coli* co-cultivation for isoprene production yielded a tenfold increase (0.6 g/L) and extended fermentation from 100 to 350 h. *C. vulgaris* supported *E. coli* growth and isoprene yield, while *E. coli* modulated algal physiology, forming a mutualistic relationship driven by oxidative stress. Transcriptomic data showed elevated expression of antioxidant (*CysK, CysE, SerA, AhpC, AhpF*) and repair system genes (*YtfE, NfuA, YebG*) in *E. coli*. Interestingly, *C. vulgaris* upregulated cysteine biosynthesis, suggesting cross-feeding to mitigate ROS [80]. Collectively, multi-omics strategies enable precise engineering of microbial consortia for consistent, high-yield BC production, unlocking their full industrial potential.

7. Conclusions and Future Perspectives

This review highlights the emerging potential of *Komagataeibacter*-based co-culture systems as a sustainable alternative to monoculture fermentation for BC production. Through engineered microbial interactions, co-cultures enhance BC yield, enable the utilization of diverse agro-industrial residues, and facilitate in situ functionalization of the cellulose matrix. Advances in bioreactor configurations, including dual-vessel systems and rotating disk bioreactors, have improved oxygenation and scalability, addressing critical limitations of static cultures. Multi-omics approaches have further elucidated the metabolic networks and regulatory pathways underpinning co-culture performance, providing a foundation for rational strain engineering and process optimization. Collectively, these developments position co-culture fermentation as a scalable and resource-efficient platform for BC biomanufacturing.

Future efforts should focus on the rational design of defined microbial consortia guided by systems biology and omics-integrated modeling to enhance metabolic complementarity and process stability. Underexplored *Komagataeibacter* species with stress-resilient phenotypes warrant further investigation for their compatibility in co-culture systems. From a techno-economic perspective, the adoption of modular, automated bioreactors and the integration of low-cost, regionally available substrates are essential to reduce production costs and improve economic viability. Moreover, adaptive laboratory evolution and synthetic regulatory circuits offer promising avenues to enhance co-culture robustness under industrial conditions. Establishing standardized cultivation protocols, quality control metrics, and regulatory frameworks will be critical to support the commercialization of co-culture-derived BC across biomedical, food, and sustainable material sectors.

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