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Bacterial cellulose: a biodesign critical analysis on the artifact and industrial manufacture

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Abstract

The design field encompasses aspects of culture, thought, and, ultimately, can appropriate other disciplines like biology and engineering. One of the potentials of biodesign is the replacement of current materials for more sustainable ones. Bacterial cellulose (BC) is a biopolymer that is produced by microorganisms such as *Komogataeibacter* spp. and has been recently explored for applications in fashion, architecture and material science receiving global media attention. In this impact paper, it is assessed the challenges of producing BC through analysis of its production and chemistry. Through a critical analysis of applied case studies, it is argued that it is yet work to be done to allow a widespread use of BC. In conclusion, the increased understanding of the acetic acid bacteria (AAB) genetic landscape and biochemistry will potentiate the education, research, development, manufacture, and market implementation of more feasible and sustainable cellulose-based products.

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1. Introduction

What if designers and fabricators did too much? Despite the need of an enhanced design practice, specifically in the prototyping and post-making phases, as Song and Paulos (2021) assume, we are in an era of the built environment. Manmade environments are massified and the civilisation is entirely and completely represented by artificial objects accompanied by the domestication of other-than-humans' beings. Makers must go beyond the traditional anthropocentric perspective, and not neglect relevant knowledge by quickly appropriating novel and innovative creations that might not be scientifically rigorous, ecologically sustainable, and ethically sound.

"The access and growing ubiquity of digital fabrication has ushered in a celebration of creativity and 'making'. However, the focus is often on the resulting static artifact or the creative process and tools to design it. We envision a post-making process that extends past these final static objects — not just in their making but in their 'unmaking'." (Song and Paulos, 2021).

To merge the gap between humans and nature and to project other ways of manufacturing, designers and engineers are experimenting with biological materials. They are expanding the materials' arsenal and changing the paradigm from a top-down and humanised creation to a co-creation approach with living organisms (Dade-Robertson & Davies, 2023; Hénaff, 2023; Diniz, 2023). This new paradigm is influencing makers to introduce novel methodologies coming from disciplines usually apart from the design field. Therefore, biodesign is a promising field in this new landscape, where the crossing-over between the biological sciences and the design, creative, and artistic disciplines, happen. Additionally, the biodesign discipline is emerging as a strong educational tool, holding promise to tackle several challenges in architecture – e.g., more resilient materials to reduce the negative ecological impact of construction (Andréen & Goidea, 2022); design – e.g., sustainable manufacture of products (Camere & Karana, 2018); materials science – e.g., reduce waste (Mcmeeking *et al*., 2024); fashion – sustainable raw materials to reduce the negative impact of production processes (Ng & Wang, 2016; Rathimanoorthy & Kiruba 2020); and visual communication – widespread acceptance of new materials (D'Olivo & Karana, 2021).

Specifically, bacterial cellulose (BC) is a biomaterial that has recently grabbing the attention of the mass media and the broader audience (e.g., Suzanne Lee, Modern Synthesis, Polybion-Gani partnership). Research-wise, BC is an interesting material to investigate due to its low-cost and relatively easy production, treatment, and design (Ng *et al*., 2016; Ng, 2017;

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Bastida & Peirano, 2020; Kapsali, 2022; Bell *et* al., 2023a; Bell *et al*., 2023b; Nicolae *et al*., 2023). For its better comprehension a proper investigation of the BC-producing microorganisms and their respective biochemical pathways is required to reach the expectations drawn to this biopolymer.

The aim of this work is to describe why there is a need to better comprehend the biology (taxonomy and genetics) and structure (biochemistry) of BC production. The research goal is to improve the biodesign practicalities calling for more focus on the BC productivity, treatment, and functionalisation. Through a narrative review analysis, an overview and critique of current BC production is performed. Additionally, approaches like genome sequencing are highlighted and discussed to help biodesigners in generating crucial insights for a more sustainable and realistic use of BC (Singhania *et al*., 2021; Manan *et al*., 2022). In the next sections it is provided the biological context of BC, including its genomic and biochemical nuances. Lastly, biodesign educational and professional examples are discussed in order to achieve a successful interdisciplinary approach.

2. Background and related work

2.1 Bacterial cellulose producers

The best studied BC producers are the acetic acid bacteria (AAB). They are gram-negative and obligate aerobic bacteria found in a variety of natural sources that are rich in sugar and alcohols (e.g., fruits and fermented foods) (Yang *et al*., 2022). AAB phenotypes relate to the acetic acid production, nitrogen fixation (Fuentes-Ramírez *et al*., 2001), pigment production (Malimas *et al*., 2009) and exopolysaccharides generation (Tonouchi, 2016; La China *et al*., 2018; Barja *et al.,* 2021). These microorganisms are also known for producing several aldehydes, ketones, and other organic acids through oxidative fermentation (Mamlouk & Gullo, 2013; Lynch *e al*., 2019). Besides producing these compounds AAB can also accumulate a large amount of them extracellularly as happens with BC (Mamlouk & Gullo, 2013; Lynch *et al*., 2019). The most prolific AAB in terms of BC production is *Komagataeibacter xylinus* (Römling & Galperin, 2015; Gullo *et al*., 2017). During the past years, several reviews have been published to elucidate the details of AAB taxonomy (Trček & Barja, 2015; Yamada, 2016), biotechnological applications (Saichana *et al*., 2015), resistance mechanisms (Nakano & Ebisuya, 2016; Qiu *et* al., 2021) and BC production (Gullo *et al*., 2018; De Amorim *et al*., 2020; Barja, 2021), however there are yet research questions

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in need to be answered. These questions relates simultaneously to the diversity of BC producers and their asosicated BC biosynthesis pathways.

Apart from *K. xylinus*, several AAB species are also known to produce BC. Among them are members of the genera *Komagataeibacter*, *Acetobacter*, *Gluconacetobacter*, *Rhizobia*, *Rhodobacter*, *Agrobacterium*, and *Sarcina* (Delmer, 1999; Brown, 2004; Morgan *et al*., 2013, Matsutani *et al*., 2015). Other non-AAB species can also produce BC such as *Achromobacter*, *Alcaligenes*, *Aerobacter*, *Azotobacter*, *Pseudomonas*, *Dickeya*, and *Lactobacillus* (Deinema & Zevenhuizen, 1971; Brown, 2004; Jahn *et al*., 2011; Morgan *et al*., 2013; Khan *et al*., 2020). As an example, Khan and colleagues (2020), characterised a *Lactobacillus hilgardii* strain capable of producing BC in high quantities. Using a fructose-rich medium, they observed that *L. hilgardii* was able to produce immensely pure and crystalline BC with a yield of 7.23 g/ L after 16 days of incubation. Hence, the plethora of organisms able to produce BC represent novel routes of research to detect, engineer, characterise, and standardise the best possible BC producer.

One of the easiest ways of producing BC is through kombucha fermentation. Kombucha is a slightly alcoholic and carbonated beverage resulting from the fermentation of a tea-based aqueous solution and sugar by a symbiotic culture of bacteria and yeast (SCOBY) (Villareal-Soto *et al*., 2018). At the aqueous-air interface there is the deposition of BC that forms a layer at the surface of the liquid. This pellicle can be collected by hand for further treatment without any intricate technique. Microbiologically, kombucha is constituted by the AAB such as *Gluconobacter* sp., *Acetobacter* sp., *Komagataeibacter* sp. (de Roos & de Vuyst, 2018), lactic acid bacteria such as *Lactococcus* sp. and *Lactobacillus* sp. Yeasts such as *Zygosaccharomyces bailii*, *Saccharomyces cerevisae*, *Schizosaccharomyces pombe*, *Saccharomycodes ludigii*, *Kloeckera apiculata*, *Torulaspora delbrueckii*, and *Brettanomyces bruxellensis* have also been detected (Coton *et al*., 2017; Laavanya *et al*., 2021).

A recent study by Keating and colleagues (2023) has argued for a formation of a new taxonomy – *Novacetimonas hansenii* – to incorporate a BC overproducer strain (*N. hansenii* NQ5) due to insights gained from whole genome analysis. In support of this taxonomical rearrangement, Ryngajłło and colleagues (2019) investigated 19 *Komagataeibacter* genomes and concluded that there was sufficient evidence to distinguish between the *K. xylinus* and *K. hansenii* clades. They found variance in the genomic traits related to the carbohydrate uptake and regulation of its metabolism, exopolysaccharide synthesis, plasmid DNA content, and the c-di-GMP signaling network that explain the phenotypic diversity found in these clades. This

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new knowledge represents research routes yet to be explored that directly and indirectly influence BC generation.

2.2 Bacterial cellulose biochemistry and synthesis limiting factors

BC possesses better physico-mechanical properties than the plant-derived cellulose due to its nanofibrous 3D structure (Ul-Islam *et al*., 2012). Additionally, BC has a high purity and crystallinity, mechanical strength, jellified appearance, porous geometry, biocompatibility, and easy mouldability representing a promise material for designers (Khan *et al*., 2022). BC is synthesised through four main sequential enzymatic steps:

i) Phosphorylation of glucose by the glucokinase;
ii) Glucose-6-phosphate isomerises into glucose-1-phosphate by the effect of the phosphoglucomutase,
iii) UDP-glucose is synthesised by the UDP-glucose pyrophosphorylase, and
iv) Cellulose synthase reaction (Yoshinaga *et al*., 1997; Shingania *et al*., 2022).

In the last step, UDP-glucose polymerases into cellulose by the activity of a membrane protein complex called cellulose synthase which is an unstable high molecular mass protein that is also responsible to cellulose secretion to the extracellular matrix (El-Saied *et al*., 2004). The cellulose synthase consists of four core proteins that are encoded by the cellulose synthase operon containing the genes *bcsABCD* (Yoshinaga *et al*., 1997). However, the operon is not equally observable among the *Komagataeibacter* species (Saxena and Brown, 1995; Matsutani *et al*., 2015).

In general, cellulose producers are a relatively well studied group of microbes but the high cost and low yield of BC production makes it necessary to increase the depth of research and characterisation. Specifically, it is necessary to clarify the potential genotype-phenotype dualism related to the BC synthesis, secretion machineries, and other relevant cellular processes (Ryngajłło *et al*., 2019). One example is the high phenotypic variability of *Komagataeibacter* (Gullo *et al*., 2018). Since different strains can be recovered throughout the fermentation and BC production experiments (Valera *et al*., 2014; La China *et al*., 2018) it is possible that a microbial consortium is needed for achieving the best results. The hypothesis is that different strains prefer different growth conditions within the same production cycle, potentiating a “cascade” effect that results in high BC yields. Therefore, BC production is strain-dependent, differing in the yields, structure, and strain stability (Fang &

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Catchmark, 2015; Chen *et al*., 2018; Ryngajłło *et al*., 2019). Moreover, the fermentation substrate, the culture media, and the genetic organisation of the cellulose synthase and its related genes can also account for the detected phenotypic variability (La China *et al*., 2020; Singhania *et al*., 2022).

Depending if BC is produced by a SCOBY or through pure culture of strains, the substrate requirements might differ as well as other factors related to the equipment and post-production treatments (Fernandes *et al*., 2020; Laavanya *et al*., 2022; Rathinamoorthy & Kiruba, 2022; Singhania *et al*., 2022). The leading factors contributing to the BC production are the type and concentration of the nitrogen (e.g., peptone) and carbon source (e.g., glucose, sucrose), the dissolved oxygen in suspension (~10-15%), the pH (~4-6) and temperature (~25-35 °C). Various types of wastes and byproducts (both having complex chemical compositions) have been tried to grow BC, but the best results observed are from the experiments where additional nutrient sources are supplemented (Fernandes *et al*., 2020; Nascimento *et al*., 2021; da Silva *et al*., 2021). Other relevant factors are the proportion (~1:15-1:10) and age (~3-30 days) of the inoculation, and the co-substrate concentration (e.g., ethanol, vitamins) (Fernandes *et al*., 2020; Singhania *et al*., 2022). Alterations in the biochemical pathways for microbial growth and cellulose synthesis differ between strains (Masaoka & Sakota, 1993; Toyosaki *et al*., 1995; Czaja *et al*., 2007; Ochaikul *et al*., 2013; Zeng *et al*., 2014; Fang & Catchmark, 2015). Other soluble exopolysaccharides like acetan and its derivatives, and levan that indirectly affect BC production also vary among BC producing strains (Ryngajłło *et al*., 2019). In summary, the genetic instability of the cellulose synthase, its differential presence in AAB and the paraphernalia of other factors directly and indirectly affecting BC synthesis makes its production an extremely hard experimental setup. This constitutes a major challenge for biodesigners which would benefit from a standardisation of BC producing experimental protocols.

2.3 Bacterial cellulose, genomics, and proteomics

About the genomic features of *Komagataeibacter* genus, Matsutani and colleagues (2015) analysed the whole genome of *Komagataeibacter medellinensis* NBRC 3288 and found the particular genetic conditions that makes this strain lose and regain the ability to synthetise BC. They also found other mutations associated with such phenotypic variance. Together, this genetic instability and easiness to lose and regain abilities related to cellulose production constitute a risk of using this strain in a standard routine. Such risk can be

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extrapolated to other strains belonging to the *Komagataeibacter* genus since these bacteria are known to have transient phenotypes in their essential metabolism (Beppu, 1994; Coucheron, 1991; Takemura *et al*., 1991; Sokollek *et al*., 1998; Azuna *et al*., 2009; Castro *et al*., 2013; La China *et al*., 2020). For instance, Florea and colleagues (2016a) found and described two additional cellulose synthase operons in *Gluconacetobacter hansenii* and several previously unknown genes related to BC production. Recently, Bimmer and colleagues (2023) performed a proteomic analysis on the same strain (*Komagataeibacter hansenii* ATCC 53582) and their characterisation of the regulatory diguanylate cyclases (*dgcA* and *dgcB* deleterious mutants) suggested a new regulatory mechanism of cellulose synthesis in *K. hansenii*.

Recent studies have shown the extensive involvement of the operon *bcsABCD* in the biosynthesis, extracellular transport, and assembly of cellulose (Manan *et al*., 2022). The cellulose synthase enzyme is encoded by two types of operons, and both types consists of four genes:

i) Type I: *bcsA-D* (Matsutani *et al*., 2015), and;

ii) Type II: *bcsABII*, *bcsX*, *bcsY*, and *bcsCII* (Ryngajłło *et al*., 2019).

These two types of operons are subjected to mutations. Specifically, the *bcsC* subunits (related to the cellulose export through the membrane) are prone to disruption, suggesting that cellulose export is subject to evolutionary forces (Ryngajłło *et al*., 2019). However, the cellulose synthase is a complex enzyme and other descriptions have been referred including a third type of operon and the presence of more related genes (Römling & Galperin, 2015; La China *et al*., 2020; Manan *et al*., 2022). Despite the well conserved function of the BcsA and BcsB (responsible for cellulose synthesis activity and β-glucan chain formation, respectively (Ross *et al*., 1991; Yoshinaga *et al*., 1997; Park *et al*., 2009; Römling & Galperin, 2015; Morgan *et al*., 2016)), the function of BcsC and BcsD is still under debate (Saxena *et al*., 1994; Hu *et al*., 2010, Iyer *et al*., 2011). Regarding the genomic instability of the AAB there is also the insertion sequences that cause disruptions in essential biochemical mechanisms and also hamper cellulose synthesis (Asai, 1968; Valla *et al*., 1987; Coucheron, 1991; Takemura *et al*., 1991; Beppu, 1993; Coucheron, 1993; Sokollek *et al*., 1998; Steiner and Sauer, 2001; Matsutani *et al*., 2015; Ryngajłło *et al*., 2019). So, the genomic landscape of AAB represents a plethora of challenges to be addressed to reach a stable and efficient BC production.

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Therefore, it is questionable that BC can be assumed as a definitive solution for more sustainable manufacturing practices, despite the intellectual property protection efforts attempted in the recent years (Da Silva *et al*., 2021). Another relevant limitation regarding the use of *Komagataeibacter* spp. is that only a limited fraction of their already identified proteins possesses assigned functional categories (*ca*. 30% for *K. xylinus* E25 (Ryngajłło *et al*., 2019). Such lack of knowledge regarding protein function represents an opportunity for further exploration of proteomics (Zhang *et al*., 2010).

Such instability represents a risk for prototyping research and the effort to get outside-of-the lab is substantial (Bernstein *et al*., 2017). The only way to mitigate these risks is to increase the effort to decipher the genomic and biochemical details of AAB BC-producers. Ultimately, only after that effort will be possible to obtain a standard framework to be utilised across disciplines and outside-of-the-lab.

2.4 Bacterial cellulose and genetic engineering

Several attempts to genetically engineer AAB to generate higher yields of BC have also been investigated. Jang and colleagues (2019) engineered a *K. xylinus* strain and were able to more than double the yield of BC production (3.15 g/ L) by overexpressing the heterologous *pgi* and *gnd* genes from *Escherichia coli* or *Corynebacterium glutamicum*. To increase the ability of *K. xylinus* to use mannose as a carbon source, Yang and colleagues (2023) engineered a strain capable of better using mannose-rich biomass as a sole carbon source through the expression of the mannose kinase (*mak*) and phosphomannose isomerase (*pgi*) genes from *E. coli*. Their results showed that the yield almost doubled while improving BC tensile strength and elongation potential. Since the yield is not the only feature relevant for BC generation, Huang and colleagues (2020) used the Clustered Regularly Interspaced Short Palindromic Repeats interference (CRISPRi) system to test and control the BC mechanical characteristics such as porosity and crystallinity by overexpressing the *galU* gene (responsible for controlling the carbon metabolic flux between BC synthesis and the pentose phosphate pathway). They found that the *galU* is positively associated with the BC crystallinity and negatively associated with the porosity. To allow a standard genetic engineering approach, Florea and colleagues (2016a) developed a modular toolkit to guide the genetic engineering of *K. rhaeticus* aiming a high BC yield. Their toolkit works twofold, being applied to genetically engineer *K. rhaeticus* and applying extracted proteins to the BC itself. However, the toolkit is tailored specifically to this strain and optimised protocols must

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be tested to every other strain. Additionally, the BC mechanical properties (e.g., tensile, stiffness, viscoelasticity, porosity) have also to be studied to achieve a usable biomaterial (see Chen *et al*., 2018, where they analysed the mechanical properties of six different *Komagataeibacter* strains, five *K. xylinus* and one *K. hansenii*).

3. Real world implications for bacterial cellulose

BC represents an interesting material due to its malleability, biocompatibility, and strength (Florea *et al*., 2016b). Up until now BC has been explored to a varied range of applications such as cosmeceuticals, mining and refinery, textiles, sewage treatment, foods, paper industry, biomedical apparel, electronics, *etc* (Singhania *et al*., 2021). According to Manan and colleagues (2019) and Rathinamoorthy and Kiruba (2022) the main limitations for BC production for mundane and technical applications can be summarised as related to the:

i) Culture media required for production: since different strains show different nutritional needs and phenotypes;
ii) Post-treatment processes: since every treatment is tailored for its application and so a high degree of specialisation is necessary in research, development, and industrial manufacture of every unique appliance, and;
iii) Scaling-up: since it is not trivial how to produce high amounts of BC in a stable, controlled, and cost-effective manner.

The argument is that only possessing a full comprehension of the BC biodesigners can approach BC as an innovative and sustainable polymer. The BC-producers' phenotypic variation and how this plasticity correspond to the different BC chemical and functional features constitute additional challenges for biodesigning.

3.1 Practical and industrial biodesign applications

To address the complex nature of BC, professionals are pushing the boundaries of knowledge, bringing other disciplines to their practice. Neri Oxman's and Suzanne Lee's works represent the next paradigm shift in biotechnological engineering, biofabrication, augmented architecture, and biomaterials.

Oxman's *Aguahoja* project focused on developing a robotic platform for 3D printing biomaterials, including cellulose (Duro-Royo *et al*., 2018). It is a five-meters tall biocomposite structure, composed by several biopolymers such as BC. *Aquahoja* was

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developed through a computationally driven approach through additive manufacturing (Guzzi & Tibbitt, 2020), and its design was intended to allow temporality, being able to sense, inform the user of, and adapt to changes in the surrounding ecosystem. The team behind *Aquahoja* found that shape and materiality are directly informed by physical properties (e.g., stiffness and opacity), environmental conditions (e.g., temperature and relative humidity), and fabrication technical constraints (e.g., arm speed and nozzle pressure). Such structure aims at optimised structural stability, flexibility, and visual connectivity. Designed for biodegradability, *Aquahoja*'s exposure to environmental conditions like rainwater, will disassembly its structure until disappearance, giving back the biological building blocks to the natural nutrient cycle.

As a pioneer in merging biology and design, Suzanne Lee has been working on removing the boundaries within the two disciplines. As the CEO of Biofabricate[1] (hosting, consulting, and education company for biology-led innovation), she argues that "we have the tools to make the same things [as Nature] – without killing the animal, without cutting down the tree. We can programme biology to do it in a much more efficient way using minimal and renewable resources"[2]. Suzanne Lee's prediction is that the fourth industrial revolution will be a material one, led by biology. Developing BC-based fashion prototypes for 20 years she recalls that "the technology was absolutely right [20 years ago] but people just weren't ready"[1].

Biodesigners have also explored BC as a design material. Carolina De Lara (2024) developed BC-based composite textiles to be applied in footwear designs while defining the work methods tailored for designers with a non-biological background. Fiona Bell and colleagues developed an interactive breastplate biofabricated by SCOBY (2023a), and a non-invasive bio-digital calendar that focus on the SCOBY's wellbeing (2024). Ofer & Alistar (2023), created an immersive learning experience for biodesigning with kombucha. They focused on the sensory experience of designing with livingness, and reporting through an autoethnographic research method. In practice, a lab journal was used for documentation, including writings on the reflective sensory engagement experience through the in-person contact with kombucha and SCOBY (sewing and embroidering, layering, laser cutting and engraving, and molding). Interestingly, Netta Ofer (2023) offers a personal and non-scientifically take on growing BC while there is enough knowledge to grow it with more confidence: "how and when to feed it, what a healthy layer looks like, when a new layer

[1] https://www.biofabricate.co/about
[2] https://www.sleek-mag.com/article/the-material-revolution-with-suzanne-lee/

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should be expected, etc. All these nuances in the Scoby's growth were difficult to predict reliably, as each microbial culture and each grown layer had different behaviour and timelime. However, within that uncertainty, during the research team's meetings, [Netta Ofer] would describe the growth from her own sensory point of view." Despite the relevance of reflecting on the experience of designing with living microorganisms, the rigor of the current knowledge on growing and AAB and SCOBY and produce BC cannot be neglected. Both can be achieved together. When not performed simultaneously, it constitutes an exemple of the need for a more robust interdisciplinarity approach.

Regarding the industrial and commercial applications Polybion™[3] is a company that aims to source bio-based materials to the market, and Celium™ is their first biomaterial, formed by "premium cultivated cellulose". Despite Polybion's promises and media attention in the leather-alternatives' sector, Celium's features still requires further development before being presented as a more sustainable solution. This occurs because the material still requires a polyurethane coating for durability, and it works in combination with synthetic polymers. To achieve a more sustainable biomaterial, the approach must deviate from the reliance on petroleum-based plastics. It is urgent to explore other materials that do not hinders the biodegradability potential.

Consequently, the manufacture of BC is questionable and must be challenged in terms of its sustainable promises. The company assumes the compromise of durability in detriment of the biodegradability and sustainability by arguing that a long-lasting feature reduces frequent replacements, minimising waste generation and the associated environmental footprint (personal communication). The dilemma deserves a more critical view and justify the continue research and development of better solutions to assure the sustainability and durability of biomaterials. Interestingly, the product is already being marketed as a whole solution through a partnership with Gani[4], while there are several questions to be answered. Therefore, the research purpose is to continue to elucidate, clarify, and further explore the potential of novel solutions in addressing world problems (Popper, 1959). Thus, biodesigners must take these cautionary notions into account when performing industrial-led briefings.

3.2 New curricula for biodesign

Questioning the participation of non-human organisms in research, Chen and colleagues (2024) argues for a "microbial revolt". To activate it they developed a workshop that "invites

[3] https://www.polybion.bio
[4] https://www.vogue.co.uk/article/ganni-bacteria-leather-celium-aw24

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designers and biologists to reflect upon the invisible labour of lab organisms that support their research." As often seen in BC experiments, contaminations hinder the laboratory work and increases the challenges for research. According to Chen and colleagues (2024), microbial cell death and contaminations constitute microbial forms of resistance, refusal, and non-cooperation to human activities. The workshop designs comprise the following steps:

i) Microbial embodiment and role-play;
ii) Journaling and group sharing;
iii) Artifacts/ revolts creation ("Chindōgus");
iv) Sketching/ illustrating the results; and
v) Group sharing of the results.

By carrying out interviews to workshop participants, Chen and colleagues (2024) were able to find bottlenecks to interdisciplinarity related to main themes such as the power dynamics inside the lab, care ecologies, and research creative freedom. Such creative freedom can be tackled by blurring the boundaries between the learning, the making, and the growing (Correa & Holbert 2021). Correa and Holbert (2021) proposed the concept of "interspecies creative learning" that aims to foster the work with more-than-humans. So, their Myco-kit represents a biodesign toolkit to allow the learning and prototyping explorations for a more ecologically conscious practice. Despite being developed for young children, it may be potentially useful for older audiences. Therefore, by creating liminal spaces where those boundaries can be contested (Chen *et al*., 2024), "interspecies creative learning" (Correa & Holbert, 2021), and creative discovery that respects the more-than-humans' agency can potentially give rise to a more robust interdisciplinarity. Additionally, it can also be considered incorporating themes in the more-than-humans' agenda like their temporalities (Oktay *et al*., 2023), representation as participatory decision makers[5], values and perspectives (Bekker *et al*., 2023), and engagement and embodying (Light, 2024).

Light (2024) argues for "approaches that involve people in being-with, designing-with and participating-as non-humans" (p. 2). These three aspects should be inserted in the biodesign curriculum, to allow a more non-anthropogenic curriculum. This calls for the more creative tools to be explored, such as imagination – "imagination is invoked to bring in non-human actors; the humans 'becoming' other beings to do tasks." (Light, 2024, p. 3). Assuming that it is challenging to speak of or from the more-than-humans' experience, the

[5] https://www.diva-portal.org/smash/record.jsf?pid=diva2%3A1826944

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exercise stimulates different non-human perspectives and phenomenological possibilities. At least, biodesigners need improved and complete design representations, that allow the development of more representative biological metaphors encompassing the appropriate more-than-humans' agency (Dade-Robertson *et al*., 2024).

Bekker and colleagues (2023) defined challenges in teaching more-than-human perspectives in the field of Human-Computer Interactions. Despite not be directly aimed to biodesigners, the focus on practitioners coming from non-biological backgrounds can relate to design as well. They defined three main themes relevant to the more-than-humans' perspectives: species, things, and designers, and from their experience, the identified challenges are (Bekker *et al*., 2023, p. 57):

i) *Representation*: "who might speak on behalf of whom";
ii) *Inclusion*: "how can students make sure to include all the relevant perspectives – including the more-than-human";
iii) *Human and non-human designers*: "if the designer is a non-human (…) how might this influence the design process";
iv) *Outcome and effect*: "what are the success criteria for working on a project with more-than-human players";
v) *Role of* (bio) *technology*: "if/ how/ when technologies are necessary, or whether it is more fruitful to develop tools with no technologies involved";
vi) *Bias*: how to go beyond "western thinking, and the hegemony of modernist paradigms (…) to bring in perspectives from other cultures that are more aligned with a more-than-human ecological worldview" (p. 57).

The use of biological probes can facilitate and enhance the experience of teaching and learning biodesign maintaining the care for the more-than-perspectives. Briefly, biological probes "are intended to provide the setting in which it is possible to engage with biological systems from a design perspective" (Ramirez-Figueroa, 2017, p. 8). They allow the engagement with other organisms and their phenotypes. Therefore they:

i) Enable open-ended, non-deterministic design outcomes;
ii) Operate within rigorous domains and objectives;
iii) Articulate throughout direct engagements with living systems and;
iv) Operate as inspirations for critical thinking.

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Ultimately, deployment of biological probes can extend the biodesign teaching practice to outside of the lab and formal educational spaces. As Chappell and colleagues (2023) observed, "informal learning spaces can empower multidirectional and multigenerational knowledge exchange and advance a more diverse, inclusive, and innovative biodesign enterprise" (p. 1). Their work shows the benefits for biodesign education of bringing other actors. Artists, teachers, activists, and researchers can activate creativity, playfulness, storytelling, and ancestral scientific knowledge to informal learning spaces such as community bio-labs, summer camps, art-based maker spaces (Chappell, Perez, & Takara, 2023).

4. Interdisciplinarity for biodesign bacterial cellulose

The weak link between the genetics and the biochemistry surrounding BC production and the design setup is hindering the proper transfer of knowledge between microbiologists, engineers, designers, and manufacturers (Bernstein *et al*., 2017; Chen *et al*., 2018; Zhou *et al*., 2020; Da Silva *et al*., 2021; Kapsali, 2022; Pereira *et al*., 2022).

Taking advantage of interdisciplinary approaches, such ambitions can be achieved through research combining different disciplines. Importantly, it is urgent that genomic analysis, and other 'omics' approaches are included in the biodesign practice (Zhang *et al*., 2010; Misra *et al*., 2019; Ryngajłło *et al*., 2020). This effort has the potential to allow the development and use of standard design and bioengineering prototyping protocols (Table 1). These recommendations are aligned with the activities proposed by Chappell and colleagues (2023) for community biodesign and can complement them in the particular case of studying BC as a biological probe.

Recently Brooks and Alper (2021) argued that synthetic biology needs to step outside of the lab. They pointed the challenges of storage and stability of the biological and computational resources for use in other-than-research contexts. Therefore, they suggest the development of platforms suitable for three main outside-the-lab scenarios:

i) bioproduction on remote and non-conventional contexts;

ii) biosensing, and;

iii) closed-loop systems (e.g., therapeutics and drug delivery).

Such scenarios would potentially help to mitigate the technical challenges occurring outside-of-the-lab like genetic stability of the biological material, economics related to resources and infrastructure, and feasibility of the technical operations. Still, the multiple

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disciplines and competences needed to the proper transfer of knowledge for outside-of-the-lab constitute a barrier. Additionally, these barriers potentiate the appearance of a "inside-the-lab-syndrome" (Bernstein *et al*., 2016; Flink & Rüffin, 2019; Zhou *et al*., 2020; Pereira *et al*., 2021). Therefore, such boundaries must be removed to increase interdisciplinarity and allow a more robust research and prototyping of innovative, sustainable, and attainable solutions. Still, such effort must be accomplished taking into consideration the rigorous knowledge coming from the involved disciplines. As an example, the "Microbial Revolt" workshop attempted by Chen and colleagues (2024) allowed the observation of "key epistemic differences between designers and biologists, mapped different approaches to more-than-human care and ecologies, and revealed the potential for design to challenge the secluded and productionist culture in biological laboratories." So, a revolt can be seen simultaneously as a creative method and for more-than-human designs and a enacting tool for interdisciplinarity.

Since the synthetic biology possesses several tools for interdisciplinary projects between biologists and engineers, a stronger connection and share of data, and tools and frameworks are essential. According to Tang and colleagues (2020), "synthetic biology applies genetic tools to engineer living cells and organisms analogous to the programming of machines (…) [it] aims to program biological systems to perform user-defined functions." Its engineering principle has paved the way for its establishment as a proper engineering field. To meet this end, Florea and colleagues (2016b) reported a genetic engineering toolkit for *Komagataeibacter* consisting of experimental protocols, modular plasmids, promoters to target, reporter proteins, and inducible constructs that allow external gene expression control. Singhania and colleagues (2021) reviewed and presented the mechanisms of and for genetic engineering aiming at BC production. They included the "heterologous overexpression of glucose 6-phosphate isomerase pgi gene from *Escherichia coli*", the "*gdh* knock down", and "crdS gene introduction and expression to simultaneous synthesise cellulose/ curdlan" (Singhania *et* al., 2021, p. 6798).

Led by the example of biomineralisation, Dade-Robertson and colleagues (2015) questioned the synthetic biology approaches in the design realm. They argued that synthetic biology can be employed as a design approach to: simplify the engineering design cycle; describe DNA sequences and their products as design building blocks; and overcome complex laboratory practices of recombinant DNA. Additionally, biodesigners can engage with the biological media settings, the working strain itself through genetic manipulation, or a combination of both (Dade-Robertson *et al*., 2024). However, it is also important to assure

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that the more-than-human agency is taken into consideration during synthetic biology experiments.

Incorporating knowledge from the biological sciences into the design practice expands the idea of making and prototyping. Biopolymers like BC are alive, unpredictable and they can be programmable if a deep understanding is set beforehand and tried thereafter. Hence, the skillset of biodesigners deserves a shift towards a mediating and open-minded approach of the design process to allow a co-performance with nature (Camere & Karana, 2018; Dade-Robertson & Davies, 2023; Diniz, 2023; Hénaff, 2023).

The non-anthropogenic concerns are becoming increasingly important in this new design approach. They relate to the more-than-humans agency and embodiment (Light, 2024), and temporalities (Oktay *et al*., 2023). Therefore, this change potentially allows the development of a wealthier society and a more balanced interpretation of being. Ultimately, an enhanced awareness about the nature interests promotes the construction of a society that goes beyond humans, relying in post-humanist theories interpretation of being (Camere & Karana, 2018; Neimanis, 2017). Hence, to promote proper interdisciplinarity setups in the biodesign realm the general and practical biology of the organisms under study cannot be neglected. To erase the barriers of interdisciplinarity, other disciplines and more creative approaches surrounding the design process of prototyping with living materials must be included in the education and practice of biodesign at a regular basis (Parkes & Dickie, 2013; Gome *et al*., 2019; Da Silva *et al*, 2021; Kim *et al*., 2021; Andréen and Goidea, 2022).

Quoting Suzanne Lee, the future for interdisciplinarity in biodesign will be a space "where empirical data-based, evidence-tested, hypothesis-focused science meets hunch-driven, intangible and tacit ideation"[1]. There a just collaboration between humans and nature can be achieved, with time, investment, and multistakeholders' acceptance.

5. Limitations and future steps

The dynamism of the biodesign field is pushing the boundaries of disciplines that now are merging. The updated educational curricula are empowering biodesigners to get comfortable with more technical approaches coming from genomics and computational technology. In this study it was highlighted key aspects for BC production specifically in the perspective of its biological and biochemical features.

The work from Huang *et al*. (2020), Jang *et al*. (2019), and Yang *et al*. (2023) exemplifies that BC synthesis is not trivial, and an interdisciplinary effort needs to be seriously

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implemented. However, there is still challenges to be addressed that were not included in this paper such as the effects of epigenetics on BC production (Dade-Robertson *et al*., 2024; Orlovska *et al*., 2021).

The first limitation of this study is that the BC producers' biodiversity and biochemistry was not fully detailed. The second regards the limited review of the design and industrial whole body of work performed in this study. However, the objective was not to perform an in-depth literature review, but to inform biodesigners about the complexity of experimenting with BC.

The challenge to map, detail, and standardise concepts and tools in biodesign is clear and it would be interesting if the biodesign community could join forces to address such tasks. One way would be to create regional networks of interdisciplinary biodesigners. One of the first activities delivered by such networks could be the creation of biodesign experimental guidelines, as the ones developed by Florea and colleagues (2016b) for guiding genetic modifications in *K. rhaeticus*. Guidelines for selecting the right BC producing strains, delineate their growth conditions, and post-treatments protocols for several artistic and applicable uses are also crucial. Additionally, it is also urgent refocus on guaranteeing scientific rigour and safety procedures for laboratory work. Performing it at the regional level would increase the locally anchored robustness of local communities.

Interdisciplinarity involves different actors to negotiate and agree. However, to transfer the knowledge and results coming from interdisciplinary projects requires a communication effort and biodesigners need to practice it. The "inside-the-lab-syndrome" is an issue, and biodesign schools should expose students to real-world scenarios, bridging the gap between theory, prototyping, and artistic and industrial applications and challenges.

Conclusions and Impact statement

One of the issues of the BC production is the reporting of experiments without taking a rigorous stance on the complexity related to the biology (e.g., investigating the *bcsABCD* operon (Wong *et al*., 1990; Yoshinaga *et al*., 1997) and biochemistry of its production (e.g., Ng and Wang, 2016)). Practitioners in general, and biodesigners in particular, need to have a greater understanding in terms of the supplies necessary to grow microorganisms such as BC-producers, their genetic background, and the post-treatment methodologies available to produce, treat, and prototype cellulose and other biopolymers at reasonable yields. Since

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prototyping is one of the last stages of a design setup, it is essential to expand the boundaries of research to implement the interdisciplinary mindset.

Still, the genetic landscape of BC producers needs to be further studied, catalogued, and experimented, to allow a robust design practice (Singhania *et al*., 2021). The diversity of strains like *K. hansenii*, *K. xylinum*, and *K. ucaveti*, confirms the urgent need for the full comprehension of the complete array of factors that affects BC production.

Finally, more effort must be put into the exploration of appropriate cultivation methods, including the optimised and cost-effective substrates and tailored equipment to increase the productivity of BC. Then, it is necessary to develop and disseminate micro and large-scale protocols to allow the fine tuning and the proper transfer of knowledge and results associated with BC production across fields and organisations. However, these recommendations might not be enough to fully deploy sustainable and widespread solutions to the market. Interdisciplinarity and frequent discussions inside and outside the lab can be key.

Consequently, the ethical compromise towards a more sustainable future must be taken seriously for all biomaterials since it is not an exclusive feature of BC. Lastly, only adding an enhanced design practice, together with the application of quality and safety standards to grow target microorganisms and handle cellulose, biodesigners can expect to have a say in researching, applying, and deploying solutions to the environmental, industrial, and artistic challenges where BC can be applied.

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Conflict of interest

The author declares no conflict of interest.

Ethics statement

Ethical approval and consent are not relevant to this article type.

Data availability statement

Data availability is not applicable to this article as no new data were created or analysed in this study.

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Table 1 – Main challenges for biodesigning BC. Referred current laboratory norm, and recommended actions.

Challenge	Current practice	Recommendation
BC producer selection	Neglecting strains' specificities by using SCOBY.	Include microbial taxonomy into the biodesign research. This can be accomplished by having a microbiologist as a team member, or by studying the AAB taxonomy and biochemistry.
Growth conditions	Using general recipes sourced from unrigorous references.	Perform literature reviews on what type of growth conditions are suitable for the selected BC-producer strain. Be rigorous on quantities and quality controls to check effective microbial growth. This can be accomplished by analysing growth curves (e.g., at time intervals, count growing colonies on solid culture medium or measuring optical density).
Genetic instability (cellulose synthase, acetan and levan variations)	Not addressed.	To stabilise a bacterial strain, it may be necessary to genetically engineering it. As seen in this work, this challenge is not easily solved and so the recommendation is to consult an experienced synthetic biologist for advice. It may include working with a particular known strain or pursuing the work despite the genetic instability.
Equipment	Directly coupled with the end use (e.g., BC-sheets, or more intricate molds).	Simpler the better. Biodesigners should decouple the BC growing from the intended application. This means that BC yields can be increased by optimising growth conditions and equipment. The recommendation is to check in the literature for the best equipment to grow BC using the bacterial strain under study.
Post-treatment	Exploratory and not fully addressed (e.g., impermeabilisation, adding technological feature).	Seeking advice from chemical engineers can provide insights for treat the biomaterial (e.g., clean, purify, composite). If more creative uses are intended, artists can also be called in.
Scaling-up	Usually out of scope of biodesign.	Assemble an interdisciplinary team, including designers, biotechnologists (microbiologists, synthetic biologists), chemical and biological engineers, managers, and supply chain specialists, to delineate a scaling-up plan.

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