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Growing Bacterial Cellulose: Envisioning a Systematic Procedure to Design This Promising Material

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Abstract: This contribution presents an approach for studying and understanding bacterial cellulose (BC) as a growing material (GM) to be produced, optimized and controlled for potential applications in the design field. The dialogic exchange between the world of design and that of applied sciences led research groups to envision, as a promising environment, the practice of growing materials instead of extracting them. This research has been structured to explore and verify the possibilities offered by design, as an experimental and holistic discipline, in the management of GMs, and specifically of BC. Through a detailed experimental setup and in-depth observation of the materials, a procedure to grow repeatable samples of BC is presented. Several progressive attempts were made and reported to define a precise procedure to grow BC. Potential improvements to the growing techniques and future developments of the work are discussed in the final part of the article, defining possible directions for the research in the design field.

Keywords: bacterial cellulose; growing materials; materials design; sustainable materials

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1. Introduction

Design and Nature have formed a solid relationship over time [1,2]; beginning when designers looked to Nature to find formal and functional inspiration for their projects, it evolved into emulating how Nature works with its principles and, nowadays, how Nature carefully deploys available resources is a topic of great interest. From simple emulation, the design discipline recently started to look at nature not only for specific purposes, but also in a systemic way, striving for a new "more-than-human" way of designing [3,4]. Increasing concern for ongoing climate change, together with the willingness to investigate interconnections in the biosphere—where, as living beings, we all belong—has now driven designers to ask themselves how to work in synergy with Nature when ideating and realizing artifacts [5]. From this perspective, research is exploring innovative strategies to overcome production linearity in favor of circular and holistic practices [6]. At the same time, designers, autonomously and independently, are on the lookout for new materials and material properties [5]. Due to the holistic nature of design and the capacity of designers to guide and face complex problems with a flexible attitude [7,8], design research may offer a way to go beyond simple materials application in designed products and embed practical experiments on materials while designing. This synergy between exploration and practical grounding of research results, enabled by design, may allow for greater consistency, which would easily set the stage for multidisciplinary activities and hands-on practices [9]. Therefore, in this environment, experimentation linked to exploring and understanding raw biomaterials offers a nourishing base ground to produce new knowledge, which can provide an important boost to broaden the manufacturing outlook. Cross-fertilisation between design and biology through bio-fabrication techniques [10] is a promising and interesting research field. Bio-fabrication techniques deploy hybridization between designing and natural processes, understanding how to co-design with living beings to create biomaterials and growing materials [11,12], and contributing to the creation of an emerging research area, already populated by numerous studies [12–15].

Growing materials (GMs) are designed from living organisms like fungi [16], algae [17], and bacteria [18] and characterized by assembly precision at nanometric scale. GMs offer diverse design opportunities: the possibility to influence embed material properties by intervening in the growing environments [19]; to monitor and manage the auto-assembly capability of GMs both at macro and micro scale (through hierarchical structures) [20–22]; to explore the programmability of GMs growing in non-standardized shapes to create materials in a zero-waste perspective. All the cited characteristics show how the practice of growing materials instead of extracting them could efficiently embrace production logics that are sustainable, circular, and low impact, according to the Sustainable Development Goals (SDGs) [23]. Notably, bacterial cellulose (BC) can find wide application in artifact design and production; thus, it is the material on which this contribution focuses. BC can derive, among others, from the fermentation of Kombucha tea supplemented with Symbiotic Colony of Bacteria and Yeast (SCOBY), and, due to its growing behavior, may be a sustainable alternative to traditional materials production lines.

The dialogic exchange between the world of design and that of applied sciences [24,25] led national and international research groups to envision a promising environment for growing materials instead of extracting them. While designers have always been involved in the material selection process [26], today the focus is on creating experimental materials [27–29]. While GMs are inherently sustainable, as they are renewable and biodegradable [30,31], current experiments do not consider the production system in terms of circularity, integration, and optimization within potential applications. Therefore, by considering the applicative and functional evidence of the material's characteristics, the aim is to present a research pathway that approaches the GM research area with a perspective strongly oriented toward defining a repeatable growing procedure. Framing an approach such as this by enhancing the designer's interpretive skills is particularly strategic because, from the moment he/she becomes a creator and connoisseur of the BC-based material, it can be more easily and consciously deployed, and real change may take place. On these premises, a study on the possibilities of BC as a material was conducted from a design perspective and aimed to benefit other designers with information and critical observations in choosing whether to adopt BC within projects.

To understand, monitor and comprehend the growing parameters of BC in Kombucha tea [32], several growing mediums were prepared and studied, with the objectives of identifying the most important growing parameters and setting up a repeatable procedure to obtain specific BC properties. This, in future, can enable control of both the living quality of organisms and, at the same time, provide a well-defined methodology to guarantee replicable materials, making it easier to embed in design applications. This issue was explored through an experimental investigation conducted at the Department of Design of Politecnico di Milano. The resulting conclusions offer a critical reflection on the limitations and opportunities of BC use in product applications towards environmental, social, and cultural sustainability.

2. Literature Review

This contribution presents the approach and evidence gathered to understand the specific macroscopic qualities of BC as a GM to be produced, optimized and fully understood for potential applications in the design field. This purpose is the basis for the DE_Forma project, which is funded by the Department of Design of Politecnico di Milano's Basic Research Fund for Young Investigators.

The focus on GMs, and specifically on BC, has been structured with the intent to explore and verify the possibilities offered by the collaboration between bio-fabrication processes and practices, through the filter of design as a holistic discipline [8]. When exploring the topic of bio-fabricated materials, it is possible to distinguish two different production strategies: engineering processes such as bio-printing [33], and experimental and self-produced processes for the growth and understanding of the material itself [29]. This second strategy is the one referred to in the current contribution. In this context, numerous application studies are developing design and manufacturing approaches that can incorporate living organisms to create biomaterials, hybridizing nature and its growth processes [12]. These biomaterials, or GMs, contain biomass, are biodegradable, and are inherently made through biological processes and/or are biologically derived [34]. Their main properties have been already listed in previous paragraph, but for the presented experimentation it is worth to underlining how the programmability of growth in predetermined shapes will improve consequent limitations of material waste [35].

All these properties directly correspond to potential applications, since one first asks, when approaching a new material, asks "what does it do?" rather than "what is it?" [36]. But when it comes to living materials, which grow and are formed by the process of bio-fabrication, the methods through which the material is obtained become as relevant as an object of design. Although GMs are inherently sustainable, as they are renewable and biodegradable [30,31], current experiments do not consider their production system, integration and optimization with potential applications from a circular perspective. This happens if the replicability of the growing process is not efficiently monitored: common Do it Yourself (DIY) approaches are in fact non-replicability by their own nature, risking producing materials not fully understandable at interaction. Traditional material selection methods are based on the analysis of the technical properties and numerical attributes of the material [37,38] to develop concepts [39]. Therefore, to deploy GMs in artifacts, it is essential to ensure their effective stability and replicability; in this specific study, BC was chosen as candidate for the experiment.

BC is created by placing activator bacteria in a sweetened tea base; the bacteria, nourishing on the glucose contained in the solution, starts a fermentation process generating Kombucha tea, a commercially popular beverage [40]. The fermentation process also stimulates the growth of additional bacteria that distribute themselves on the liquid's surface, whose natural activity produces the formation of macroscopic organic structures that are completely natural and biodegradable [41]. BC is also known as microbial cellulose, and can be defined as a biodegradable natural cellulose, synthesized by bacteria [42].

Compared to synthetic materials and those of animal origin, BC is less impactful from an ethical, social, and environmental point of view because its production process usually requires renewable resources without chemical treatments. Moreover, being completely biodegradable, its end-of-life belongs to the same sphere of organic waste.

On an aesthetic–sensorial level, BC appears very flexible, with versatile properties and characteristics that can be altered during growth. In particular, it is possible to design a wide range of colors, finishes, and textures that can have a rebound effect on the BC surface (consistency and flexibility) [43,44] and optical (transparency and colors) qualities [45]. Such aesthetic–sensorial information can be interpreted and manipulated by designers to enhance the development of the material, favoring its use and defining its own unique features and identities [29]. The role of designers in this context becomes crucial since they move from being a passive "material-deployer" to becoming an active producer of materials [16], capable of associating and transferring aesthetic, physical, and perceptual characteristics of the material directly to the final artifact [46].

Therefore, to enable design activities of selection, interaction, development, and deployment of innovative GMs such as BC, a repeatable procedure to generate "homogeneous" samples are required. As such, two main research questions guided the research path:

1. What are the best environmental conditions for BC growth (in terms of timing, ingredients, and environmental temperature) to reach a good output quality and reduce defects?
2. Once the most appropriate parameters have been chosen, is there a way to standardize and optimize cultivation conditions as much as possible to achieve, without waste and defects, a replicable and stable BC-based material?

The exploration of these questions led to the structuring of a set of parameters to investigate the possibilities (still not fully exploited) of BC, focusing on the issues of production scalability and sustainability starting from its procedural specificities: controlled growth, ingredients dosing, and bacterial culture wellness.

3. Methodology: Experimental Procedure Setup and Objectives

The research and experimentation, lasting about a year, was conducted in the university's makerspace, with non-specific and customized instrumentation. This piloting was structured in two phases: the first phase focused on the study, the knowledge and optimization (time, ingredients, and temperature) of the growing process, while the second phase will focus mainly on the possible BC applications in designed artifacts. In this contribution, the authors are presenting and discussing results emerging from the first phase, since these are already consolidated and compelling. The results will serve to appropriately structure the second phase of the research, which is still ongoing.

In this first phase, research methodologies (similar to that of experimental research) were developed to define an ideal set-up for growing BC [47]. The controlling and monitoring of BC cultures, iteratively repeated, has been supported by applying techniques and processes deriving from design studies (i.e., designing and realizing tools, supports, and prototypes as per research through design and research prototyping [48]).

To formulate repeatable standards for the BC growth, several empirical tests have been carried out to:

- replicate the most popular growth modes;
- identify and understand the most promising strategies; and
- verify the reliability of selected processes.

Following, several set-ups with different combinations of ingredients were designed, tested, and monitored according to quasi-quantitative parameters:

- ingredients and quantity;
- temperature; and
- jar material and diameter.

The aim was to monitor and highlight elements affecting the growth and the quality of the final BC, with particular attention to:

- acidity and pH control;
- sugar/honey quantity control;
- temperature;
- growth (thickness) measurement based on time; and
- assessments of different growth mediums.

The objective of conducting BC hybridization attempts is to explore the programmability of certain BC features at macroscopic level during its growth and ensure the repeatability of the process. Therefore, qualitative analysis of obtained samples has been conducted evaluating:

- aesthetic characteristics of the obtained samples (color, uniformity of surface);
- time taken for growth; and
- thickness obtained.

Samples have been then dried. Between the strategies that involved the use of a dryer and those that used ambient temperature to dry samples, the latter methodology was chosen in order to address potential limitations due to the size of the drying chamber in case of subsequent production of larger materials. After trying to place BC samples to dry in contact with multiple types of materials, the most homogeneous process was obtained by placing the BC sample between two layers of fabric or polymethylmethacrylate (PMMA). To prevent fabric (another equally promising alternative) from imprinting a superficial texture on samples, which could alter their final perception, the samples subjected to this analysis were dried between two layers of PMMA.

4. Results

4.1. Growth Monitoring Results

In this section, we present experiments conducted to discuss the potential and critical issues of BC's planned growth. In response to the questions introduced earlier, it is clear that the priority is to organize progressive and structured setting up of the culture.

In the beginning, three starting solutions were prepared by introducing the bacterial SCOBY starter into brewed black tea, green tea, and karkadè in jars. Aware of the properties of BC [49,50], starting cultures in glass jars, or ceramic or plastic food jars does not affect growth efficiency. In contrast, the use of metal containers may slow or prevent SCOBY formation. It was preferred to start cultures in glass or clear plastic vessels where possible to observe the progress and evolution of the growing stage more effectively. These mediums were chosen because they appeared to be the most popular in the literature reviewed, as well as readily available on the market. Figure 1 shows the first BC cultures started in the above-mentioned three mediums and in glass containers of the same diameter.

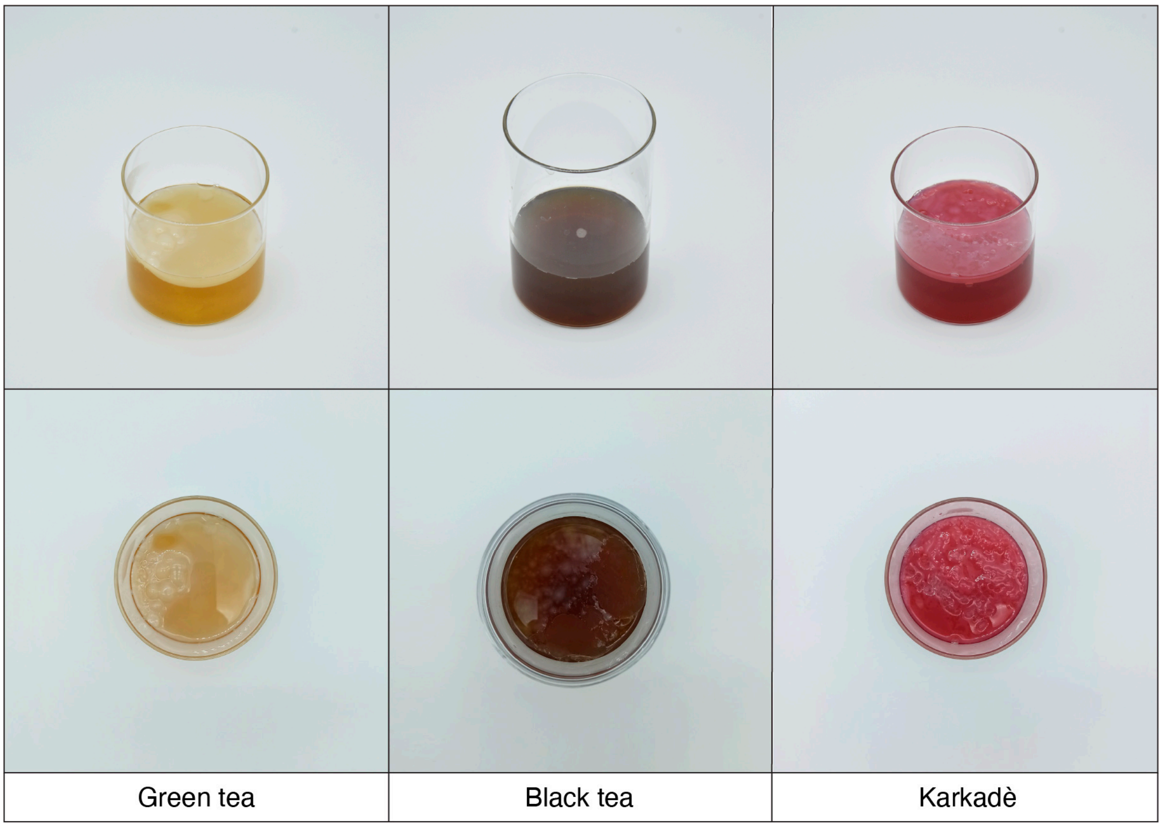


Figure 1. BC cultures in the first three selected medium, left to right: green tea, black tea, karkadè.

The obtained BC samples grew with the following path (Table 1).

According to the evaluation criteria of aesthetic qualities [44] of the obtained samples (color, uniformity of surface), time for growth, and thickness obtained, the karkadè and green tea SCOBY offered the most interesting results. Karkadè offers rapid growth of the sample and an interesting coloring, despite to a slightly non-uniform thickness. Green tea has less rapid growth, but the obtained SCOBY is homogeneous and very clear in color, which offers opportunities for pigmenting it. In contrast, black tea appeared slower in growth and the coloration of the resulting BC layer is very dark, making many possible subsequent colorations ineffective.

By observing the variations in the growth performance of the various mixtures made, it was found that the medium recipe and environmental parameters played a decisive role in the successful creation of the BC layer. Once the most satisfactory "basic recipe" was defined in terms of reduced growth time and surface homogeneity, green tea and karkadé were selected as the most promising mediums. Indeed, green tea ensured obtaining a culture with a light color and a fairly consistent surface, with little probability of having surface defects. Karkadè, on the other hand, generates a deep red colored BC layer, which tends to grow very fast in the early stages, but requires more attention to avoid the formation and entrapment of bubbles in the BC layer, given by the fast fermentation activity.

Table 1. BC growing monitoring for the three starters tea.

Name	Ingredients and Quantity	T (°C)	Jar Material and Diameter	Day	pH	Brix (%)	Thickness at Day 25 (mm)
SCOBY Green Tea	Green tea—330 mL Sugar—33 g Vinegar—10 mL SCOBY—45 g Starter liquid—75 mL	35°	Glass 95 mm	1–25	3.00	9.4	9.90/10.10/ 11.00/12.30 Fairly non-constant
SCOBY Black Tea	Black tea—330 mL Sugar—33 g Vinegar—10 mL SCOBY—45 g Starter liquid—75 mL	35°	Glass 95 mm	1–25	2.27	8.2	2.20/2.80/3.60 Very thin but almost constant
SCOBY Karkadè	Karkadè—330 mL Sugar—33 g Vinegar—0 mL SCOBY—48 g Starter liquid—75 mL	35°	Glass 95 mm	1–25	2.33	9.1	2.80/3.20/4.40/4.60/5.40/6.30 Non-uniform

Subsequently, karkadè and green tea environments have been replicated in parallel with some variations:

- tea-based solutions (green tea)
- water-based solutions
- powder pigmentation (choosing among affordable and edible coloring powders for coloring samples, and metallic powder to investigate eventual change in physical properties; performed only on green tea basic solution, due to its uniform growth)
- honey as a replacement for sugar as the nutrient (performed only on green tea basic solution, due to its uniform growth).

The purpose was to compare the initially defined standards with other variables in order to confirm or refute the choices made. Specifically, in choosing the powders to be added to the mixtures, it was decided to select two edible powders (turmeric, known for its high ability to confer bright coloration; and chili pepper, itself colored but which wasn't incorporated into the BC during growth and also wasn't able to impart coloration by contact) and one metallic powder derived from iron filing waste (to check for possible oxidation and alteration of the pH and brix parameters of the medium). Then, a total of nine different mixtures were created for growing BC (Figure 2).

All data were collected according to the example given in Table 2, while complete details of growth parameters, thicknesses achieved, and any additional observations of the mediums can be found in Appendix A.

Table 2. Example of BC growing monitoring; full tables in Appendix A.

Name	Ingredients and Quantity	T °C	Jar Material and Diameter	Day	pH	Brix (%)	Thickness at Day 25 (mm)
SCOBY Turmeric	SCOBY—45 g Turmeric powder—15 g Starter liquid—75 mL	35°	PP 98 mm	1	3.35	10.2	5.00/6.00/ 6.60 Fairly constant over the surface area
				16	2.23	5.0	
				25	2.30	5.0	

After 25 days of growth, the samples were moved to the drying stage.

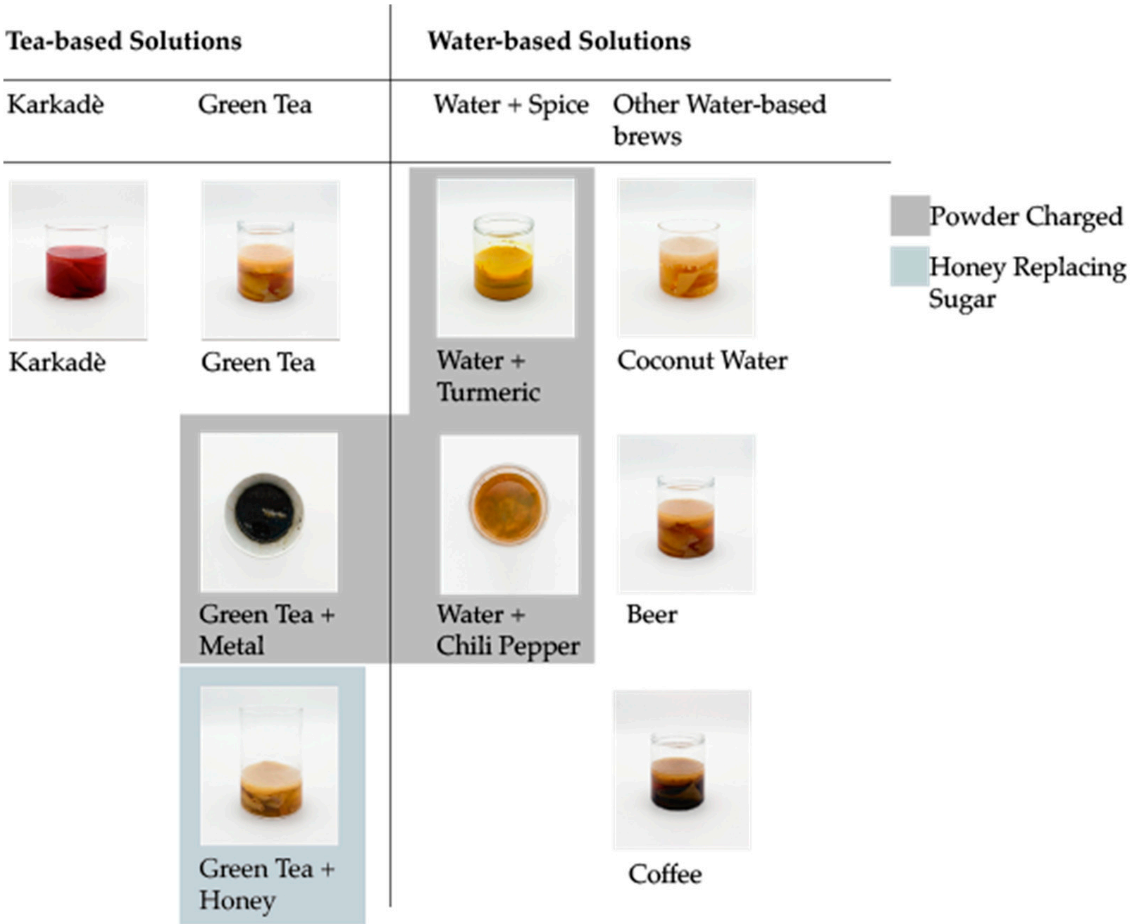


Figure 2. The nine different BC samples grown in different brews and with different charges.

Some cultures, especially those with additional metal powder and chili pepper powder, presented no or very poor growth, invalidating their potential application. Indeed, the related images of these two cultures (Figure 2) have different camera framings because, unlike the other mediums, these were not fruitful, and the final stage of growth was not reached. For this reason, the growth parameters of these two mediums are not shown in Appendix A. Other cultures, obtained from mixes of different fluids also tested in their pure form, did not generate particularly notable results when compared to those offered by BC samples obtained from recipes with only one main ingredient. Therefore, only seven of the materials obtained were found to possess characteristics useful for their evaluation for potential replicability. The selected media composition are:

- water, sugar, and turmeric;
- coconut water and sugar;
- beer;
- coffee and sugar;
- karkadè and sugar;
- green tea and sugar; and
- green tea and honey.

Figure 3 shows the growth stage and results obtained after drying the seven promising samples in air.

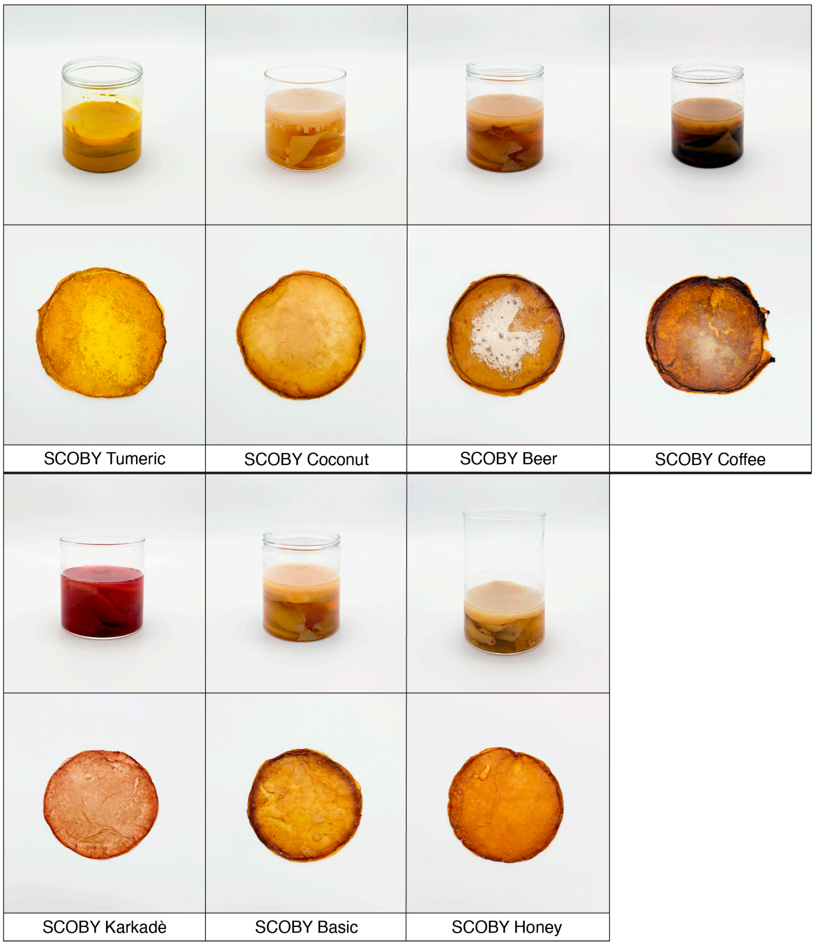


Figure 3. Seven sample's growth stage and post-drying result.

4.2. Results after Drying Phase

After some preliminary tests on the first cultures, the most promising drying procedure was defined, according to which all the various samples were subsequently processed: drying samples at ambient temperature, in contact with two PMMA sheets. By using two layers of rigid plastic material, initial pressure could be applied to facilitate the spill of excess liquid contained in the BC hydrated samples. The pressure exerted led to an increase in the initial diameter of the sample only in some cases. In detail, this dimensional change can be caused by the factors listed below.

A high disc thickness during pre-drying induces a greater expansion of the BC fibers when subjected to pressure during drying. Once dry, the material sample presents an edge extending for a few millimeters. This edge shows a different appearance and texture from the rest of the sample. The regular and effective surface in the case of a material originally of high thickness is therefore the same as in the case of samples of average thickness.

Significant fermentation activity during the growth phase can lead to the creation of samples with very variable thickness. This happens because bubbles grow between the liquid and the BC surface layer, the latter stopping its growth at that point. In this case, the pressure exerted on the entire sample during the drying phase is not evenly distributed over the entire surface, causing uneven sagging. This alters the maintenance of the initial shape given by the container, in this case circular. When BC samples present very low thicknesses, these tend to offer little resistance during drying, and, at the final stage, they appear as very delicate and somewhat fragile films when subjected to manual tensile stress.

Finally, in the case of samples grown in small containers, even a thickness of just over 1 mm causes an increase in diameter. Therefore, a link between the disc's size and its expansion is evident. Regarding diameter, it was noted that the general tendency is to have a shrinkage in the length of up to 5%. However, this phenomenon is reversed when the thickness of the hydrated material exceeds about 15 mm, causing an increase in initial size. In these cases, the samples show thick and irregular edges, which are not aesthetically pleasing and cannot be used directly but need to be finished with post-production processes, for example cutting the edges with the laser cutting machine.

It is also interesting to note that specimens of high and moderate thickness, once dried, show little variation in height. In fact, the average shrinkage in thickness is higher in the samples that have grown the most, and reduced in the samples of lesser height, with percentages ranging from 96% to 65%. All the samples, with the exception of those whose growth led to the formation of thin films, therefore show average thicknesses of between 0.30 mm and 0.60 mm.

Hence, there is evidence of advantages deriving from use of moderately thick hydrated BC samples, which take less time to grow and have faster drying while still maintaining the same final characteristics and properties as thicker hydrated BC discs.

4.3. Electronic Microscope Observations

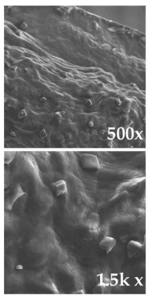
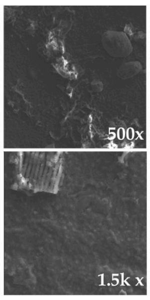
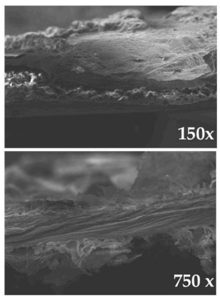
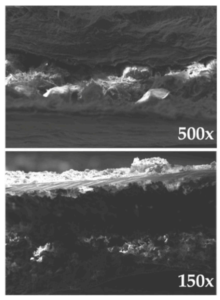
Electronic microscope observations were considered useful for two purposes: first, to envision and study the BC structure at micro scale; secondly to understand through the pictures if the bacterial culture was healthy (almost no presence of bacteria in the BC) or not, and if some of the additives were not perfectly dissolved in the solutions.

They have been processed through environmental scanning electron microscopy (EVO 50VP) and energy dispersive X-ray spectrometry for microanalysis.

The air and compression dried samples were then observed with different magnifications and in different points of the sample:

- on the intact edge (surface);
- in the center (surface);
- in the thickness (section) (See Table 3).

Table 3. Example of electronic microscope observation output. Full table in Appendix B.

Sample	Top View (Border)	Top View (Center)	Section
Water, Sugar, Turmeric	 		

Detailed descriptions of the observations are available in the following sub-section.

4.4. Samples Profiling

Information collected for each sample has been summarized in the following images (Figures 4–10) to offer an overview of the results collected. In doing this work, the authors aim to provide a repeatable methodology to grow samples with a predefined set of aesthetic properties by having full control on the growing process.

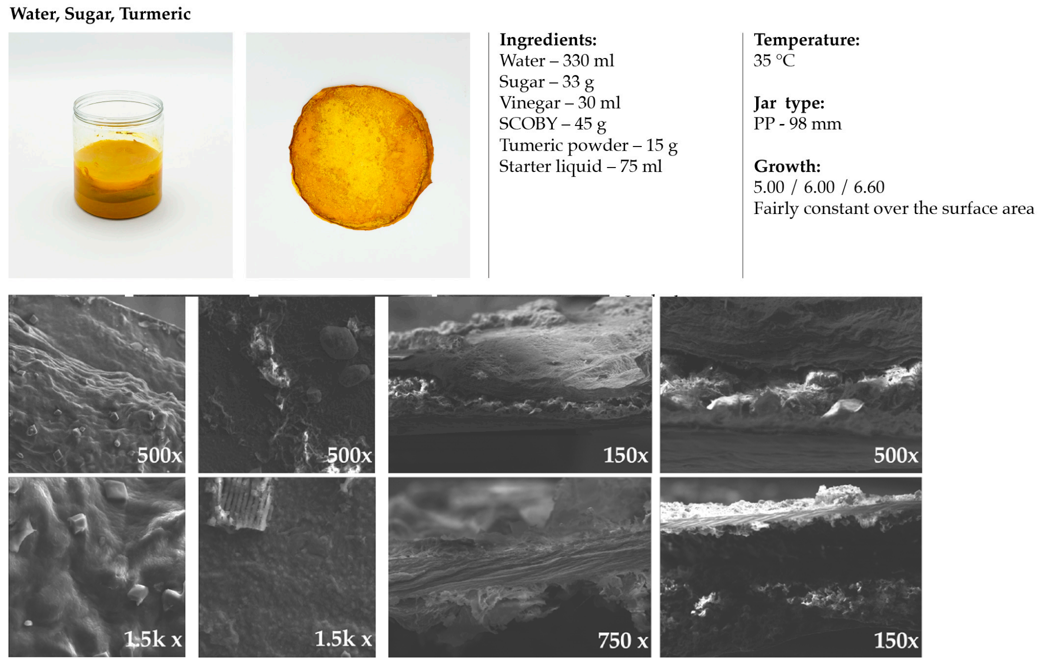


Figure 4. Sample "Water, Turmeric, Sugar" characterization. On the border surface, crystals can be identified, along with a profile abundant in protuberances and agglomerates. Overall, the structure has a distinctly organic feel. Conversely, the central surface of the sample appears homogeneous and smooth, albeit with the inclusion of similar crystals, albeit in fewer quantities and in rounded agglomerates (turmeric aggregates). In the cross-section, a clear inclusion of pulverized elements (turmeric) between two layers of bacterial cellulose is observable, with the cellulose appearing as very thin compacted layers in the section.

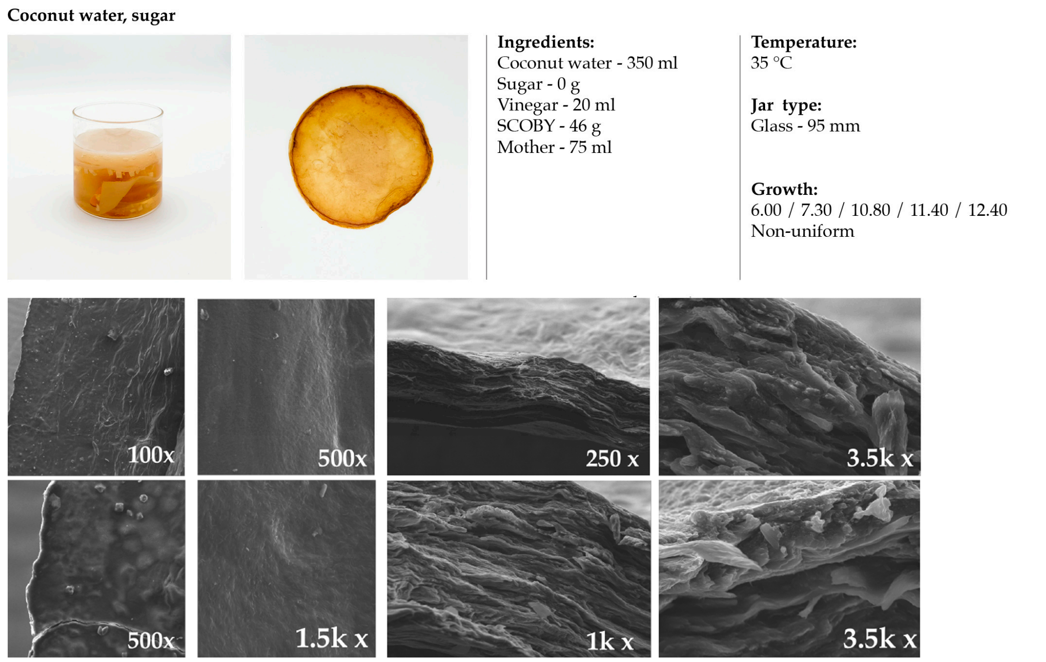


Figure 5. Sample "Coconut water" characterization. The border surface exhibits a flat geometry with visible small bubbles and inclusions. The central surface mirrors these inclusions and maintains the flattened aspect observed at the border. In the cross-section, a compact stratification of bacterial cellulose layers is evident at various scales, indicating a securely adhered structure.

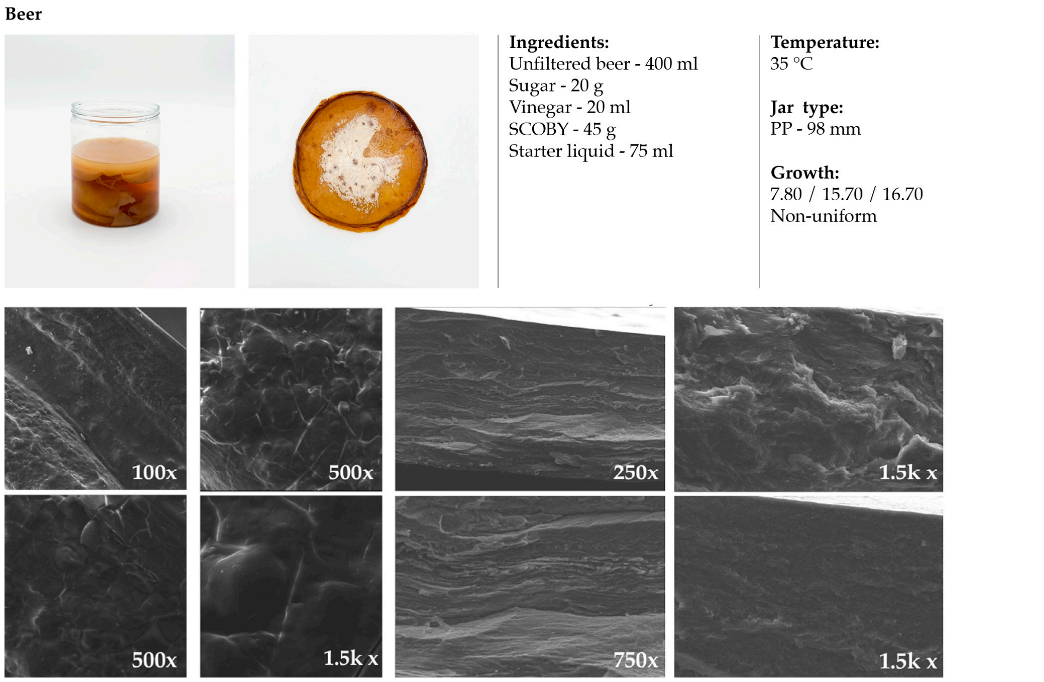


Figure 6. Sample "Beer" characterization. The border surface displays a complex geometry, with an overall organic appearance but covered with noticeable cracks. The central surface exhibits a similar appearance to the border, with cracks appearing even more pronounced and frequent, indicating a fragile nature of the material. These cracks appear superficial, suggesting mechanical defects occurring during the drying phase or sample manipulation. In the cross-section, a compact stratification of bacterial cellulose layers is visible at various scales, with the layers being very thin.

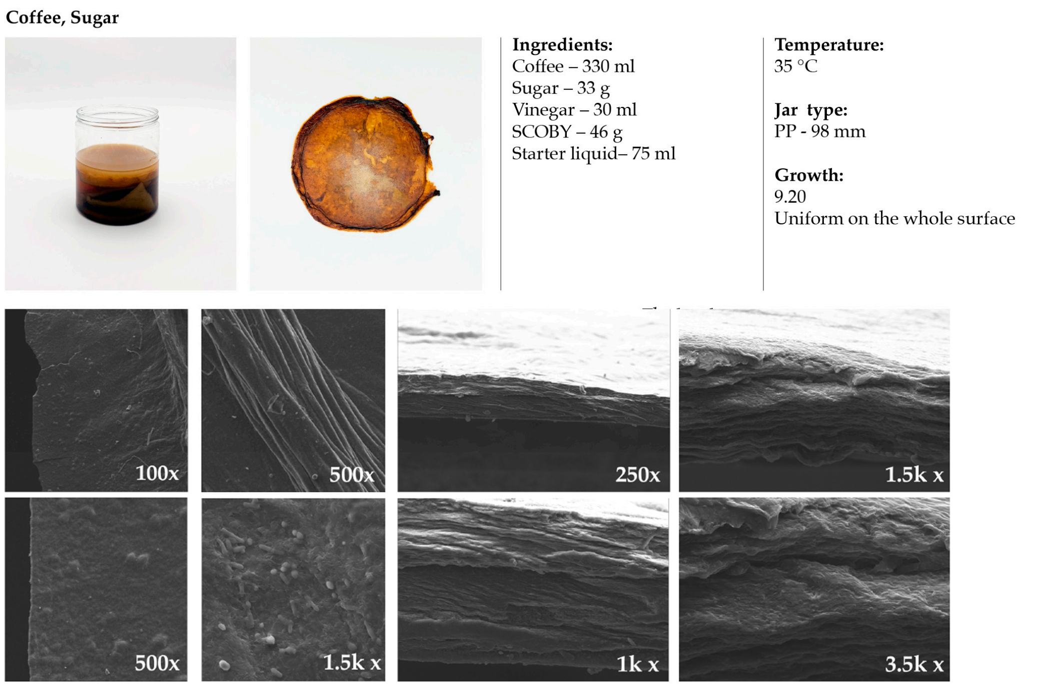


Figure 7. Sample "Coffee, Sugar" characterization. The border surface is flat, featuring some inclusions and small cracks. The central surface exhibits numerous wrinkles. Moreover, a bacterial colony is visible at a magnification of 150 k×. In the cross-section, the stratification of bacterial cellulose layers is apparent, but some sections appear to be overlapped, making it susceptible to delamination. The layers seem less uniform in thickness compared to other samples.

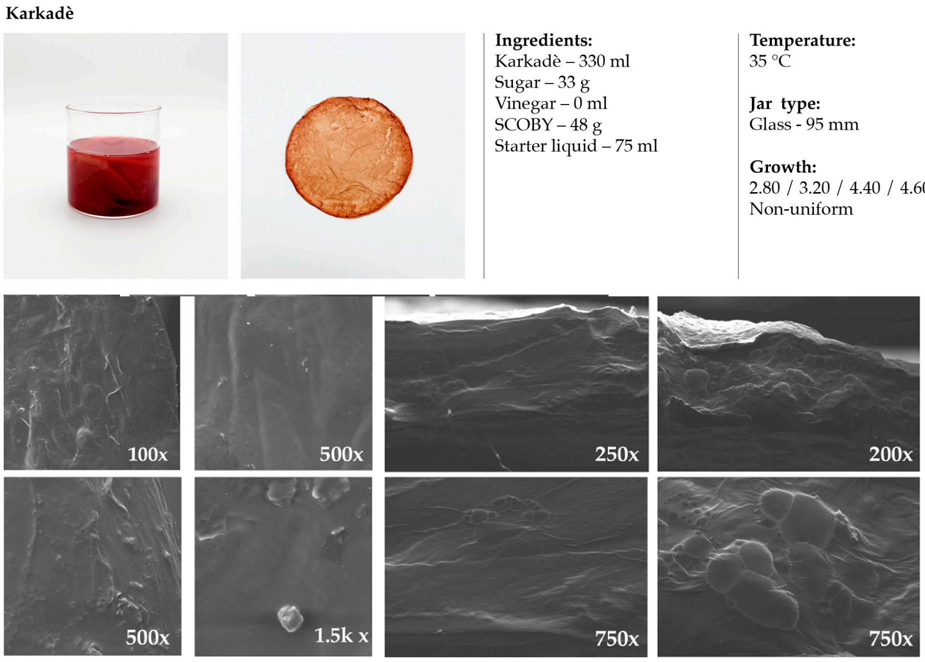


Figure 8. Sample "Karkadè" characterization. At the borders (top view), sugar crystals and a few wrinkles are visible at various magnifications. In the center of the samples, visualizations reveal a very smooth surface and a uniform pattern, suggesting a relatively elastic behavior of the material. In the cross-sections, some trapped bubbles are visible, likely arising from the rapid growth of bacterial cellulose in the karkadè brew.

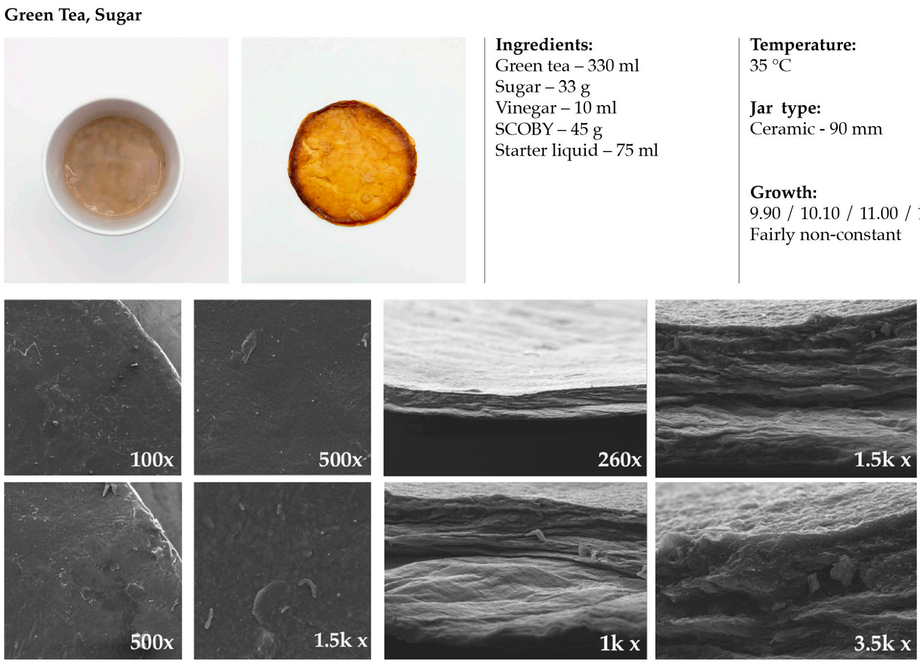


Figure 9. Sample "Green Tea, Sugar" characterization. The green tea and sugar samples display a dense structure at the borders in a top view. Towards the center, the surface appears homogeneous, though slightly less so than karkadè due to some superficial roughness. In the cross-section, the layers appear thin, compact, and well-adhered to each other, indicating a high degree of compactness.

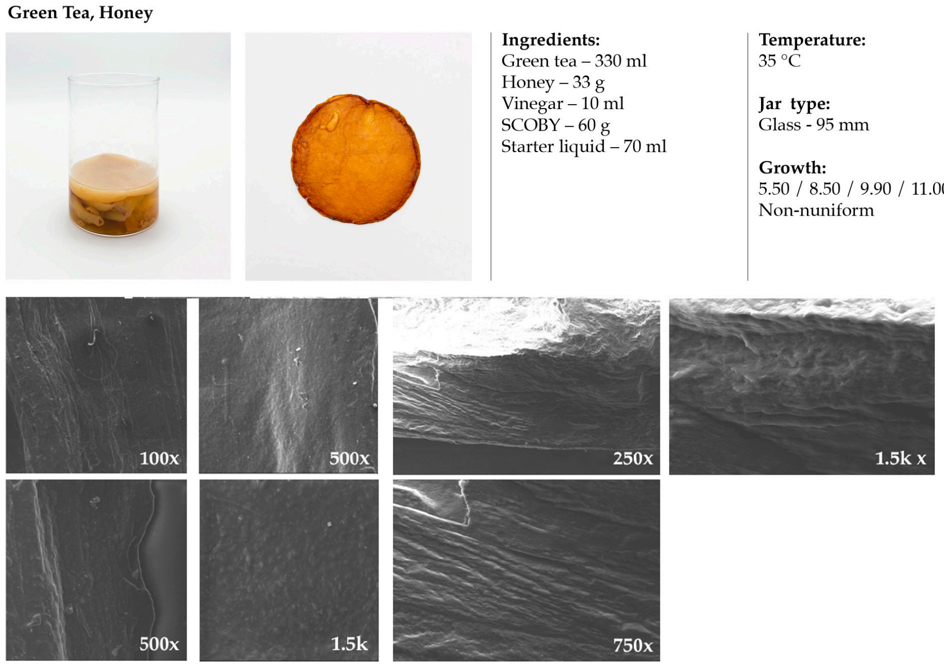


Figure 10. Sample "Green Tea, Honey" characterization. The latest sample closely resembles the preceding one, indicating a comparable structure and identical characteristics.

The medium made of water, sugar and turmeric, while containing no theine, generated a BC film with a fairly constant thickness of about 6 mm, thus adequate for later use in design outputs. During growth, fermentation was slow and bubble-free, probably due to the presence of the turmeric powder in suspension, which prevented their formation. The same powder was incorporated into the BC film, creating a material with a bright yellow coloration. Once dried, the powder remaining in the most superficial part gave the material a visually opaque appearance and was satiny to the touch. More turmeric powder accumulated on the edges so, in order to have a material with a homogeneous composition, it is necessary to remove the edge with post-production processes. In post-production, it is also difficult to alter the yellow color.

This medium could be a viable option, although it is not particularly versatile. In terms of sustainability, one can imagine the use of filtered reclaimed water. Turmeric powder has the advantage of counteracting the acrid smell of BC, although it possesses a characteristic aroma that may not always be pleasant.

The coconut water solution efficiently promoted the creation of a thick and compact BC film. Fermentation peaked after the seventh day, so bubbles were created on the surface of the medium, resulting in a film of uneven thickness, varying between 6.00 mm and 12.40 mm. Coconut fragments were present in the medium, which settled on the underside of the BC layer but were not incorporated into the growth phase. The color of the resulting BC was very light when hydrated, and when dried it took on a rather neutral hue. In the drying phase, the variation in thickness is no longer so recognizable, except from a chromatic point of view; in the thicker areas, the coloration is darker. In both the growing and post-production phases, chromatic alteration can be expected through liquid dyes or powders. The resulting material is smooth, not very sticky, and it is therefore possible to effectively imprint a texture during drying or in post-production. However, this growth medium is not easily available in large quantities in Europe, and this is a possible stumbling block in beginning sustainable production through coconut water. It cannot be ruled out that, in other geographies, this medium is more economically and environmentally sustainable.

The creation of BC in a sweetened beer solution generated an extremely fragile material. Fermentation in the growth phase was quite overpowering, and many bubbles formed, so a very uneven film with a thickness ranging from 7.80 mm to 16.70 mm was created. The edge, after drying, was very thick, inflexible, and with several cracks. A sugar patina formed on the surface. The coloration of the material is in shades of brown. The result, when compared with the use of an edible liquid, does not appear sufficiently attractive to justify its use for the creation of materials for the purpose of artifact production. In particular, the fragility of the resulting BC is to be regarded as a negative aspect.

In contrast, the coffee-sugar medium produced a BC film of uniform and consistent thickness (about 9.00 mm after day 25). During growth, fermentation was steady and sustained, with no bubble formation. However, the material obtained tended to delaminate. This was particularly evident downstream of the drying stage when the obtained material presented itself with a decomposed geometry and surface defects by uncovering some of its constituent layers. The edge is very thick and jagged, so its removal is necessary to have a homogeneous material to use. The material is not sticky, but the coffee coloring is very dark, and any growing or post-production coloring processes may be difficult to implement. The sugar did not completely dissolve in the solution and formed a slight surface patina in some places. This medium may be a viable option, although not particularly versatile due to the dark coloration of the resulting BC. However, in terms of sustainability, choosing to use an edible medium may not be particularly cost effective. Coffee has the advantage of counteracting the acrid smell of BC. One might prefer to use waste coffee powders, but with the risk of obtaining an even darker and rougher material, as in the case of turmeric-based BC.

The sugar-added karkadè maintained a behavior in line with that already observed in the initial cultures, generating a BC film that was not completely uniform and varied in thickness (ranging from 5.00 mm to 6.30 mm over 80% of the area). This thickness variation occurred in only part of the area due to a concentration of bubbles in a particular sector of the container. Specifically, this depended on the geometry of the SCOBY activator inserted into the medium. Fermentation activity was slow at first and then proceeded with great intensity. This led to the creation of a sample with a tendency to delaminate. Such delamination is no longer detectable after drying. At the hydrated stage, the material possesses a deep red color, which decreases in intensity downstream of the drying stage, bringing out the yellow tones typical of BC. This makes it suitable for creating brightly colored films without the need to add other dyes during growth or in post-production. At the same time, the red base makes it easy to achieve all shades of purples and some blues. The material is smooth and not very sticky. The acidity of karkadè allows crops to be started without the use of vinegar, resulting in a more pleasant-smelling material when compared to most other samples.

The green tea and sugar medium is one of the most classic ones when starting BC cultures. The sample obtained in this solution has little consistent thickness in one area specifically, due to the rise of the activator SCOBY. That problem was not readily solved because the culture was done inside a ceramic jar, preventing the problem from being readily visualized. Nevertheless, the resulting film has an important thickness (between 9.90 mm and 12.30 mm) and shows good flexibility even near the edges, although the edges appear thicker downstream of the drying step. This shows how taking the cellulose film to too great a thickness is not interesting. The color is overall quite light and in warm yellow tones. In general, this medium is particularly functional for starting BC cultures for artifact production because it has excellent compactness and color that lends itself well to color alteration in growth or post-production. In addition, it is possible to obtain tea from filtered reclaimed water by perfecting the recipe, with a view to greater sustainability.

This latest sample starts from the same considerations as the previous one but with some variations. Using honey instead of sugar promoted growth from the first days. Nevertheless, after 14 days, the brix measured in the solution dropped sharply, greatly slowing down growth over the total 25-day period. Hence, honey can be considered a viable alternative to sugar when adequate thicknesses need to be achieved in a short time. Despite this advantage, the use of honey generates a negative effect on the final material.

After drying, the BC film is extremely sticky, so much so that it prevents its use in fields other than creating adhesive coatings on other objects. In addition, honey increases the intensity of BC's typical yellow color.

Given the same structural characteristics (Figure 11), in most possible applications it is preferable to start with a material created without the use of honey.

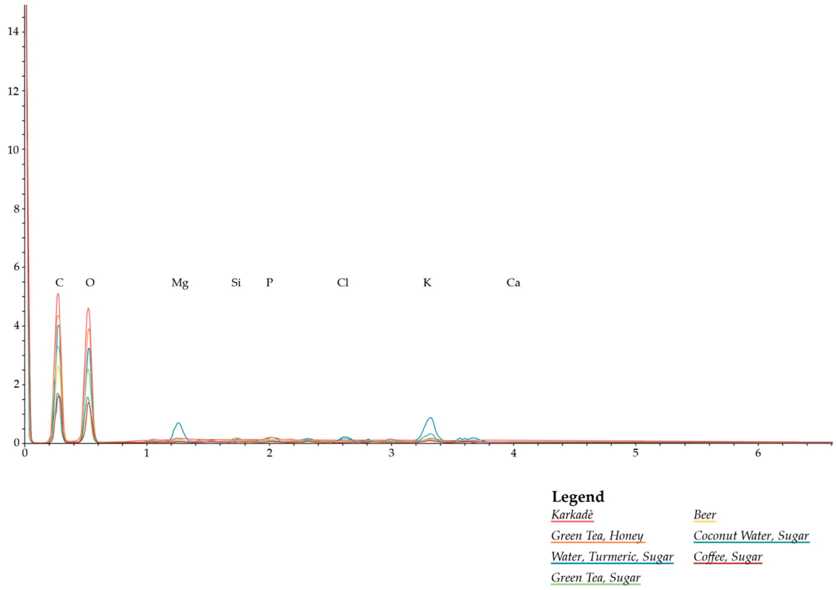


Figure 11. Comparison of different samples' organic composition obtained through environmental scanning electron microscopy (EVO 50VP) and energy dispersive X-ray spectrometry for microanalysis.

5. Discussions and Conclusions

This article provides an overview of the different configurations that BC can take in relation to the medium in which it was grown. These variations have implications for the samples themselves once they have been subject to the drying process, making them more or less suitable for use in design applications.

At the end of the exploratory path, it is possible to find that, net of any requirements relating to specific coloring obtainable directly in the growth phase with certain mediums, the best performing liquids for the creation of BC samples are green tea and karkadè. Green tea allows researchers to grow samples that are homogeneous in structure and without relevant defects at macroscale (i.e., bubbles), resulting in samples with smooth surfaces and clear coloring. On the contrary, karkadè samples present an interesting natural coloring and a rapid growth timespan, allowing researchers to obtain samples faster. On the other hand, the high speed in growth is coupled with the presence of visible bubbles between the BC layers. However, both mediums make it possible to obtain pieces—even rather large ones—in a short time and with the few surface defects. In these mediums, the choice of using honey instead of sugar as nutrient does not lead to substantial changes in growth time or aesthetic qualities. For this reason, it was chosen to replace sugar with honey only in green tea cultivation and thus verify any reductions in growth times. However, honey has altered the tactile perception of the BC, which was too sticky for possible uses in the production of functional artifacts.

The BC production process requires a large amount of water, though with the advantage of not requiring any purification processes. In the experimental processes of this work, the authors used strictly controlled liquids; one could conceivably reuse the water resources either during (e.g., rainwater or liquids derived from food waste) or at the end of the growth phase. The resulting liquid could be disposed of in the environment as a completely natural compound, or alternatively could be reused as an activator in new crops or even as a by-product for fertilization in agriculture.

In this sense, BC materials facilitate the transition to a non-linear and sustainable cross-sectoral bio-economy model through materials obtained from renewable sources or the reuse of waste [51]. In light of these considerations, the current phase of experimentation regarding this material pushed authors to identify alternative growth and drying strategies, which further experimental setups will help to define.

5.1. Alternative Growing Technique

The experimentations concerning the growth phase, so far described, have been based on the introduction of a part of SCOBY and starter liquid into the various cultures. This choice was made in order to be able to have a constant and comparable amount of culture activator at any time, without altering the results inherent to the growth time. While this strategy allowed for mutually homogeneous and comparable results, in certain cases, it also led to the formation of some surface defects in the samples. This occurs especially in the initial days of liquid fermentation when the SCOBY activator may gradually tend to rise until it makes direct contact with the newly formed BC layer on the surface.

After further trials and small-scale testing, it was decided to not use SCOBY activators in each individual culture, in favor of creating a new format, named SCOBY Hotel (SH); in this configuration, the same SCOBY was always present in a specific brew, and from this culture an activator "starter liquid" was withdrawn for starting new cultures of bacteria, all derived from the same starting solution and so maintaining a similar starting profile. This strategy is the most widely used by kombucha tea producers for food and beverages.

In the case of non-edible BC production, the parameters of the SH vary from those in the food-related literature. Maintaining a solution with very acidic pH values (between 1.3 and 1.8) and Brix values between 7 and 4 is essential for the proper functioning of this SH. The medium used for the SH is green tea, because it is the best choice to have no color alterations in case of specific colorations to be imparted to the growing BC. In addition, as learned from various experiments conducted, using green tea for SH allows for optimal fermentation without excessive spikes that can alter the parameters of the medium too rapidly. The use of SH, moreover, involves the constant stratification of different layers of BC within it, which must be gradually removed. This material, considered as waste in the food industry, from the design point of view represents an interesting opportunity because it can be processed to be made into chopped compound and give rise to further applications [35].

The advantages brought by the adoption of the new strategy can be summarized as follows: (i). achievement of constant thicknesses and almost complete absence of bubbles on the entire surface, thanks to a more homogeneous fermentation activity with fewer peaks; (ii). absence of defects caused by the possible cohesion of the SCOBY activator to the newly formed BC layer; (iii). constant growth speed, even with a 5 °C reduction in operating temperature, with interesting findings on large formats already after 7 days, as opposed to the previous 10 days minimum required.

5.2. Alternative Drying Technique

The drying phase, even if necessary to make BC suitable for use in applications, does not completely stabilize it. Indeed, if placed in a liquid for a prolonged period, BC tends to rehydrate, increasing in volume and decreasing in consistency, before returning to a malleable and flexible state.

To stabilize this material, synthetic coatings can be applied to prevent its rehydration. However, their use would change BC biodegradability characteristics, turning it into a material on a par with other composite materials of a mixed nature.

After observations of the sample under the electronic microscope, it was possible to understand how the different layers of BC are in cohesion with each other. However, as anticipated, the specific conformation displayed is clearly affected by the drying method chosen to produce the samples. With a view to the development of materials that meet the SDGs [23], it was decided to start experimenting with other BC drying techniques.

For this reason, two thin and two thick samples of BC grown in green tea and in karkadè solutions were made to test their behavior when dried by lyophilization drying. The lyophilization-drying process allows the internal structures of BC to be fixed by removing the water inside, without altering its geometry (except for the natural shrinkage of the material when dehydrated).

As it can be seen in Figure 12, the lyophilized structures exhibit thicknesses and surface deformations quite different from the previous BC samples, obtained with the same medium but dried differently. In particular, it can be seen that the thinner samples are subject to the creation of curvatures, since the growth pattern of the BC creates surface tension that is amplified during the drying process. In contrast, the thicker samples, while remaining flatter, show only surface bubbles. This suggests that the structures also present themselves differently internally, which is why one of the next steps in the research activity will also be to analyze the samples obtained through this drying process under magnification analysis.

Figure 12. Thick and thin lyophilized samples of BC obtained by green tea and karkadè brews.

5.3. Future Directions

At the end of the first experimental phase of this design research, in which the focus was placed on understanding and developing the best conditions for the creation of a BC-based material with a design-driven approach, some issues emerged. The authors believe that further exploration with additional steps of experimental and applicative development should investigate:

- Could the same conditions be replicated in semi-automated growth tanks capable of autonomously controlling GM characteristics and fermentation activity in the medium for BC culture?
- Can the amount of medium needed to start BC cultures be predetermined, ensuring that in reaching the desired thickness the entire liquid has been consumed, avoiding wasted resources?
- Are there any alternative drying techniques or natural additives that can temporally stabilize the material and make it biodegradable only when desired?

The first two questions open up discussion for developments in the effective integration of digital fabrication, one of the basic elements of the project De_Forma that initiated the entire experiment. Indeed, the creation of a facility in which the entire production process is structured and controlled, from SH to semi-automated growth tanks, can be envisioned. On the other hand, the last question opens up less defined research perspectives. In order to proceed with knowledge development in this direction, it is necessary to draw on multidisciplinary expertise. In this sense, designers, by their very nature, are prone to turn to other domains to generate transfers that can lead to application innovations.

Another possible direction may be to become aware of the current ephemeral nature of the material in order to apply it, in a reasoned way, in contexts where this limitation can become an opportunity. BC may prove to be an interesting material for applications in speculative or critical design, where its limited duration may offer insights or be a protest expression.

In conclusion, the experimental research procedure described in this contribution is preparatory to the consolidation of knowledge about managing the growth of BC under different levels:

- identification of the best medium to obtain BC samples that can be deployed in design activities;
- set-up of procedural steps to monitor and control the growth of BC samples at macroscale;
- identification of the best techniques to obtain samples that have similar initial configurations to improve the repeatability of the BC samples growth.

This part of experimental research, intentionally carried out through non-specialized biology or materials engineering equipment and instrumentation, is to be considered the foundational basis upon which the applied research around BC material has been structured. Thanks to this work, it was possible to move on to the second phase of the research, where the objective is the optimization of the BC material production strategy on a scalable level, to facilitate the creation of design artifacts optimized in a dialogical manner for this new supply chain.

For these reasons, the authors believe that BC may soon be transformed from material for niche experimentation to material for the creation of functional and commercial artifacts.

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Appendix A

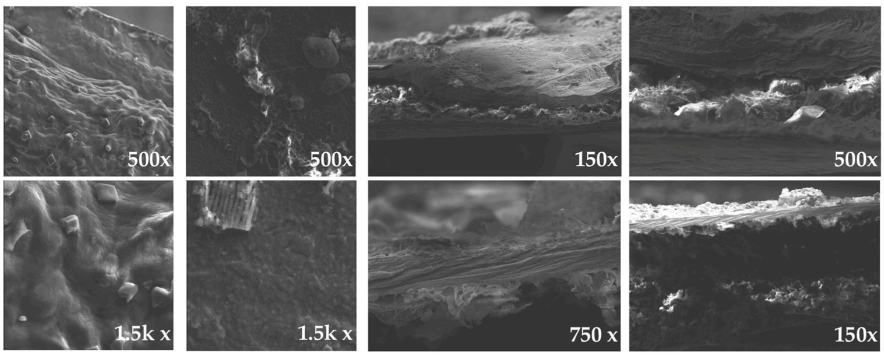
Complete BC growing monitoring.

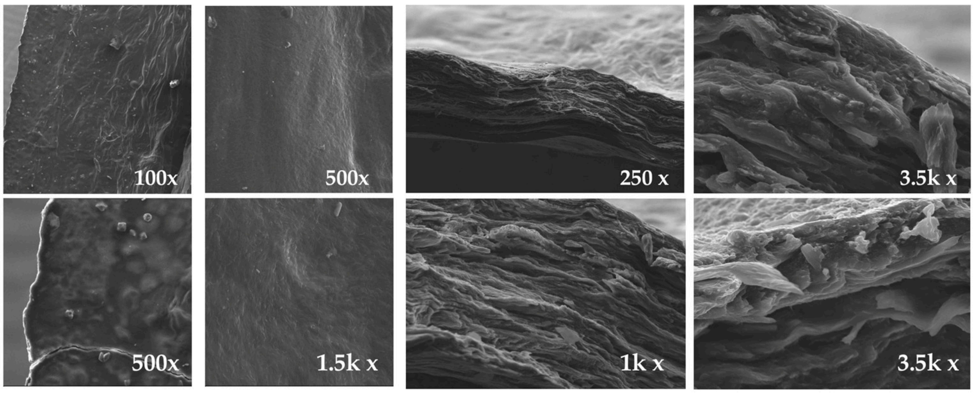
Name	Ingredients and Quantity	Temp. (°C)	Jar Material and Diameter	Day	pH	Brix (%)	Thickness at Day 25 (mm)
SCOBY Turmeric	Water—330 mL Sugar—33 g Vinegar—30 mL SCOBY—45 g Turmeric powder—15 g Starter liquid—75 mL	35°	PP 98 mm	1 16 25	3.35 2.23 2.30	10.2 5.0 5.0	5.00/6.00/6.60 Fairly constant over the surface area
SCOBY Coconut	Coconut water—350 mL Sugar—0 g Vinegar—20 mL SCOBY—46 g Starter liquid—75 mL	35°	Glass 95 mm	1 16 25	3.15 2.31 2.30	8.2 3.6 3.5	6.00/7.30/10.80/11.40/12.40 Non-uniform
SCOBY Beer	Unfiltered beer—400 mL Sugar—20 g Vinegar—20 mL SCOBY—45 g Starter liquid—75 mL	35°	PP 98 mm	1 16 25	3.10 2.36 2.06	9.0 5.6 5.0	7.80/15.70/16.70 Non-uniform
SCOBY Coffee	Coffee—330 mL Sugar—33 g Vinegar—30 mL SCOBY—46 g Starter liquid–75 mL	35°	PP 98 mm	1 16 25	3.19 2.34 2.30	9.8 4.8 4.9	9.20 Uniform on the whole surface
SCOBY Karkadè	Karkadè—330 mL Sugar—33 g Vinegar—0 mL SCOBY—48 g Starter liquid—75 mL	35°	Glass 95 mm	1 16 25	2.33 2.14 2.08	9.1 9.4 9.6	2.80/3.20/4.40/4.60/5.40/6.30 Non-uniform
SCOBY Green Tea	Green tea—330 mL Sugar—33 g Vinegar—10 mL SCOBY—45 g Starter liquid—75 mL	35°	Ceramic 90 mm	1 16 25	3.00 2.11 2.14	9.4 7.8 8.4	9.90/10.10/11.00/12.30 Fairly non-constant
SCOBY Honey	Green tea—330 mL Honey—33 g Vinegar—10 mL SCOBY—60 g Starter liquid—70 mL	35°	Glass 95 mm	1 16 25	3.00 2.23 2.17	7.8 6.8 6.9	5.50/8.50/9.90/11.00 Non-uniform

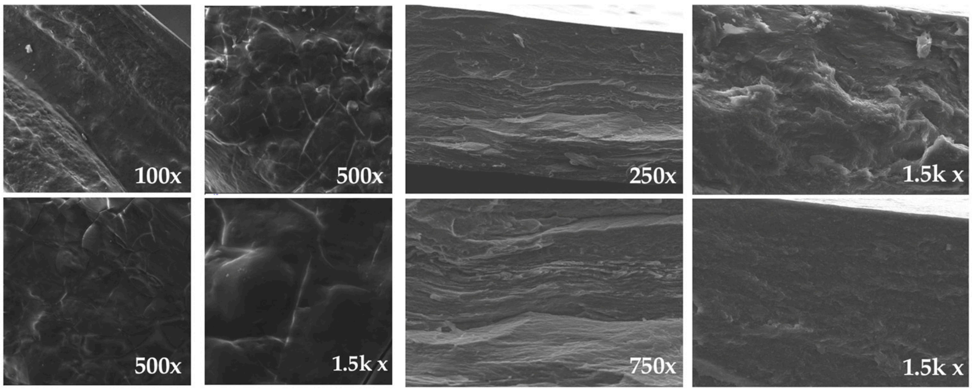
Appendix B

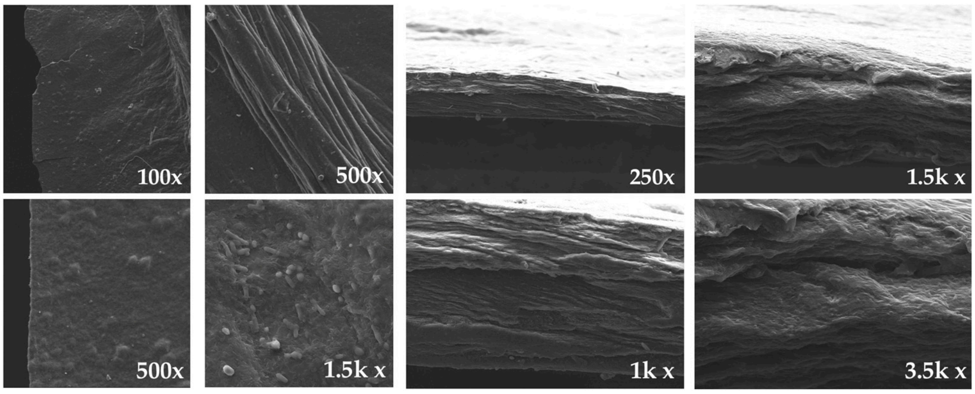
Complete BC electronic microscope visualization. Images have been chosen by the authors from a wider selection of images for their relevance in the discussion.

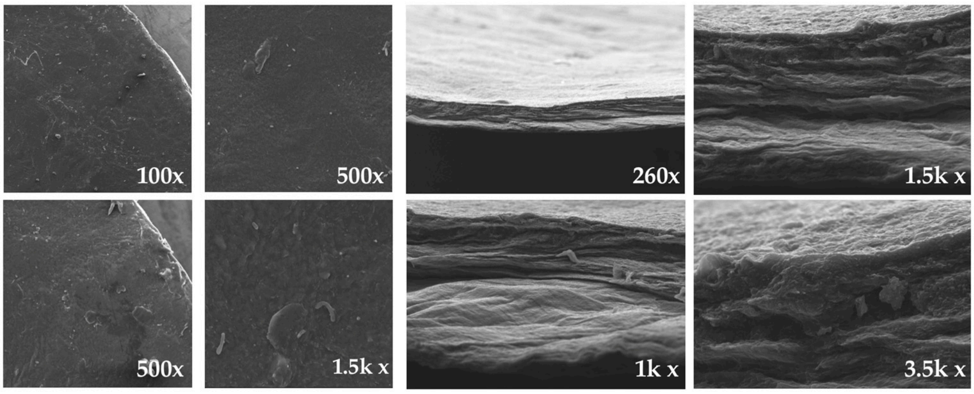
Variations in zooming magnitude have been adapted to the peculiar configuration of certain samples, to better focus on details (e.g., inclusions, bubbles, etc.).

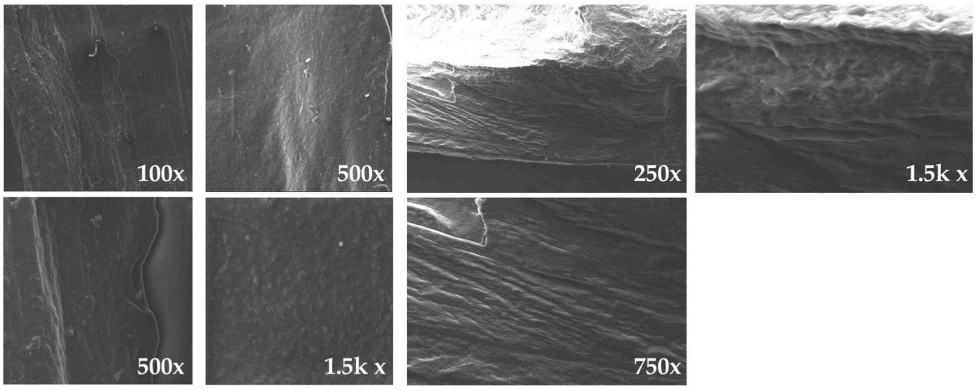
Sample	Top View (Border)	Top View (Center)	Section
Water, Sugar, Turmeric			

Sample	Top View (Border)	Top View (Center)	Section
Coconut water, sugar			

Sample	Top View (Border)	Top View (Center)	Section
Beer			

Sample	Top View (Border)	Top View (Center)	Section
Coffee, Sugar			

Sample	Top View (Border)	Top View (Center)	Section
Green Tea, Sugar			

Sample	Top View (Border)	Top View (Center)	Section
Green Tea, Honey			

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