BACTERIAL CELLULOSE

New bio-composites based on bacterial cellulose for architectural membrane applications

Master Thesis
by Bastien Damsin
Architectural Engineering

June 2019

Supervision by
Prof. Dr. Ir. Arch. Lars De Laet
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Masther thesis submitted under the supervision of
Prof Dr. ir. arch. Lars De Laet
The co-supervision of
Elise Elsacker
In order to be awarded the masters degree in Architectural Engineering

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Speech of thanks

This thesis came to life through an interesting but challenging year. I would like to thank everyone around me that kept supporting me and could bear my complaints. Especially my girlfriend, Minne, but also my family to adapt themselves to my loaded planning and support me in this. And finally, without the fun my classmates create, I would not have reached where I am now.

I would of course also like to thank Lars for the excellent supervision and Elise for introducing me to this super exciting subject I could work on, as well as for all the continuous confidence, help and motivation. The close follow-up, especially from the beginning of the year, is in my opinion the reason that I came this far in the development of this research.

Furthermore Eveline and everyone else from the microbiology department on the 6th floor deserves a big thanks for letting those architectural engineers in their labs. Especially Karl, to provide the assistance when asked for and Simon, for the help with the bacterial strains. Sorry for the microwave!

At last, thanks to Svetlana from the construction department for the assistance with my tensile tests.

Abstract

Architectural tensile membrane structures are temporary or limited-lifetime structures that are mostly fossil-fuel based and recycling is limited. In this thesis bacterial cellulose, a sheet material grown at the surface of a culture liquid by a bacteria, is explored and assessed for the first time in the light of an application as structural membrane. The aim is to define whether bacterial cellulose could replace or complement today's common tensile membrane structures. This is done through a wide exploration of alterations of the plain material with a focus on post-processing such as soaking, coating, heat pressing, creating composites and mixing. Also the creation of connections between sheets is explored. All experiments are subjected to mechanical tensile tests. Also water absorbency and appearance are described. The approach is quite unusual, as academic literature was combined with discussion in an online DIY forum. The results of the thesis are satisfying. Three alterations of bacterial cellulose improved its strength to a competing level with today's common membrane materials. Furthermore a self-assembled seam connection implying only the drying together of sheets was discovered, a concept to deploy a membrane structure based on this observation was developed.

structural membranes, circular design, bacterial cellulose, post-processing, composites, grown seams, tensile tests

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Introduction

Facing far-reaching environmental damage due to humanity's continuous need for materials, a global transition from a linear (extract resources and discard at the end of life) to a **circular economy** is needed. With a society largely dependent on fossil fuels, multiple sectors have to take up the challenge to search for alternatives. For example, a calculation of the Netherlands' construction sector waste in 1993 accounted for about one-quarter of the total amount of produced waste (Bossink and Brouwers 1996). The building sector is a world making industry, which evolved into a world-breaking industry. As a possible part of the solution, a whole range of **cultivated building biomaterials** could emerge. Grown materials that revolutionise the way architecture is not only created, but that would generate new design perspectives, a new design logic. More precisely so-called 'nutrient-dependent, soil-independent products in biotechnology' are materials that recreate natural processes which could be carried out anywhere and are independent of exhaustible resources. This represents a new vision on conceiving materials, since they could be for example grown on the construction site, maybe from existing local waste streams. Instead of shipping a final product, only an initiator such as a bacterial strain or mycelium spore has to be carried around. Not only the material and architecture will be revolutionised, but also the urban manufacturing process (Hebel and Heisel 2017).

In this thesis the more specific issue of fossil-fuel based temporary **architectural tensile membranes** is addressed by investigating an alternative material: **bacterial cellulose**. For the first time this bacteria-grown sheet material, known in microbiology and already being discovered by (fashion) designers, will be **explored for application in an architectural context** which adds multiple layers of complexity such as mechanical demands, durability in outside conditions, aesthetical factors or practical upscaling needs. This thesis focuses firstly on assessing the tensile characteristics of the material. The question of membrane connections is researched and an exploration of post-processing adaptations such as plasticizer soaking or composites is conducted. This is a quite broad exploration of the material, needed to be able to draw a conclusion but which also allowed to develop a conceptual application protocol for bacterial cellulose as a structural membrane.

At the end of the research the conclusions will define **whether bacterial cellulose can or cannot be used for architectural membrane solutions**, based on the conducted experiments. If the result is positive, there are other aspects of the material that will have to be assessed which are not part of this thesis. Amongst other aspects, a lifecycle assessment (LCA) has to study the environmental impact of the material and compare it to the existing materials, and the durability of the material has to be studied as well.

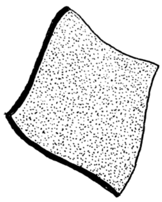
Handling a sheet of wet bacterial cellulose (left page)

1 Goals and delimitation

The goal of this master thesis is to set off a starting point for the research that will **evaluate the use of self-assembling biomaterials for structural applications** in the environmentally polluting construction sector. More specifically to evaluate whether **bacterial cellulose could embody a substitute, complement for or reinvention of fossil-fuel based tensile membrane structures**.

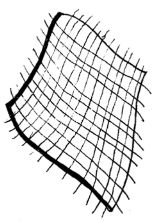
Detail of heat pressed bacterial cellulose (left page)

Tensile strength

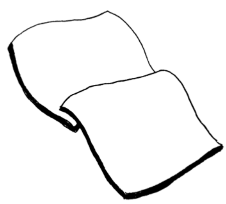


Texture and appearance

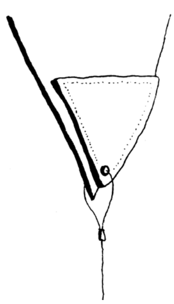
Water repellency



Composites



Seams



Reinforcements

2 Research Questions

Multiple properties of bacterial cellulose are to be studied, optimised, compared and assessed in order to evaluate the central question: *Is bacterial cellulose a valuable replacement of common membrane materials?* The scope of this large study transcends the scale of this master thesis. Therefore some delimitations have to be made on the properties that will be studied and their extent. In order to take BC to a structural level, the first property to be assessed is the **strength**. Therefore researching **mechanical properties such as tensile strength and Young modulus** will form the base of the research. The aim is that this thesis will be able to clearly state whether the strength of the material would permit the use as a structural membrane or not. Mechanical strength tests will be performed on a variety of samples. The BC will be altered to improve its characteristics by means of **post-processing.** A large amount of literature deals with the microbiological structure of BC and how it can be influenced before and during the growing period. The author being an architectural engineering student, this will be considered out of scope. The focus will lay on techniques applied from the moment of harvesting. As mentioned before, the inspiration for those techniques will come from academic literature as well as online communities. Besides the strength, it is important to note the influence on the expression of the material itself.

RQ 1 What is the optimal production process of BC?

RQ 2 What is the strength of bacterial cellulose in existing literature?

RQ 3 What is the strength of self-grown bacterial cellulose?

RQ 4 What post-processing techniques can improve the strength of bacterial cellulose?

RQ 5 What are the mechanical strengths of these post-processing techniques?

RQ 6 What are the influences on the expression of the material (texture, suppleness, colour) of these post-processing techniques?

Additionally, smaller side-experiments will be conducted with the aim of creating a more horizontal overview of possibilities, an exploration into other aspects of the material. The focus will not remain on generating data, but on trying out and gaining knowledge and experience with the material. This applies for example to **watertightness** treatments, the **creation of connection**s (seams) and the **creation of composites**. Wherever possible, they will still be tested mechanically. Watertightness of all samples will then be tested afterwards.

RQ 7 How can seams be created and what is their strength?

RQ 8 What watertightness treatments are there and do they have an influence on strength and appearance?

RQ 9 How can composites be created and what is their strength, water-tightness and appearance?

Additionally to these general research questions that are guiding the research, sub-research questions are defined where relevant. In chapter 7 a series of research questions is drawn in relation with the preliminary experiments carried out and for each quantitative experiment, laid out in chapter 8, specific research questions are defined and answered.

3 Thesis structure

After this introduction, the thesis is organised as follows.

Conducting this thesis which crosses different disciplines and combines DIY and academic methods asked for quite a specific **approach**, which is described in **chapter 4**.

In **chapter 5** the **state of the art** is provided, with a more in-depth explanation of existing literature. The biological functioning, as well as common methods, are described. To conclude, an overview of existing literature on the tensile strength of BC is given.

After the overview of existing knowledge, the methodology is illustrated in **chapter 6** under **materials and methods.** A detailed explanation of the whole production process from inoculation to dried BC sheet is given, mentioning the interpretation of norms. The analysis of data is clarified too.

Chapter 7 provides insight into the different **explorative experiments** conducted. These experiments are necessary to get acquainted with the material, build a base of experience and try out different techniques and ideas. Encountering different challenges, this chapter illustrates very well that the thesis was a strongly non-linear process, where problem-solving thinking proved indispensable in order to generate relevant results. The outcomes of the explorative experiments allowed to draft more precise tests which aim at quantitative results for the next chapter.

The **actual experiments and tests** exerted are described in **chapter 8.** For each sample, the post-processing is explained and the results of the mechanical tests are listed. The outcomes are interpreted in their context and all results are compared and assessed.

More specific as well as large **conclusions** are drafted in **chapter 9**, followed by critical reflections on the thesis and the material.

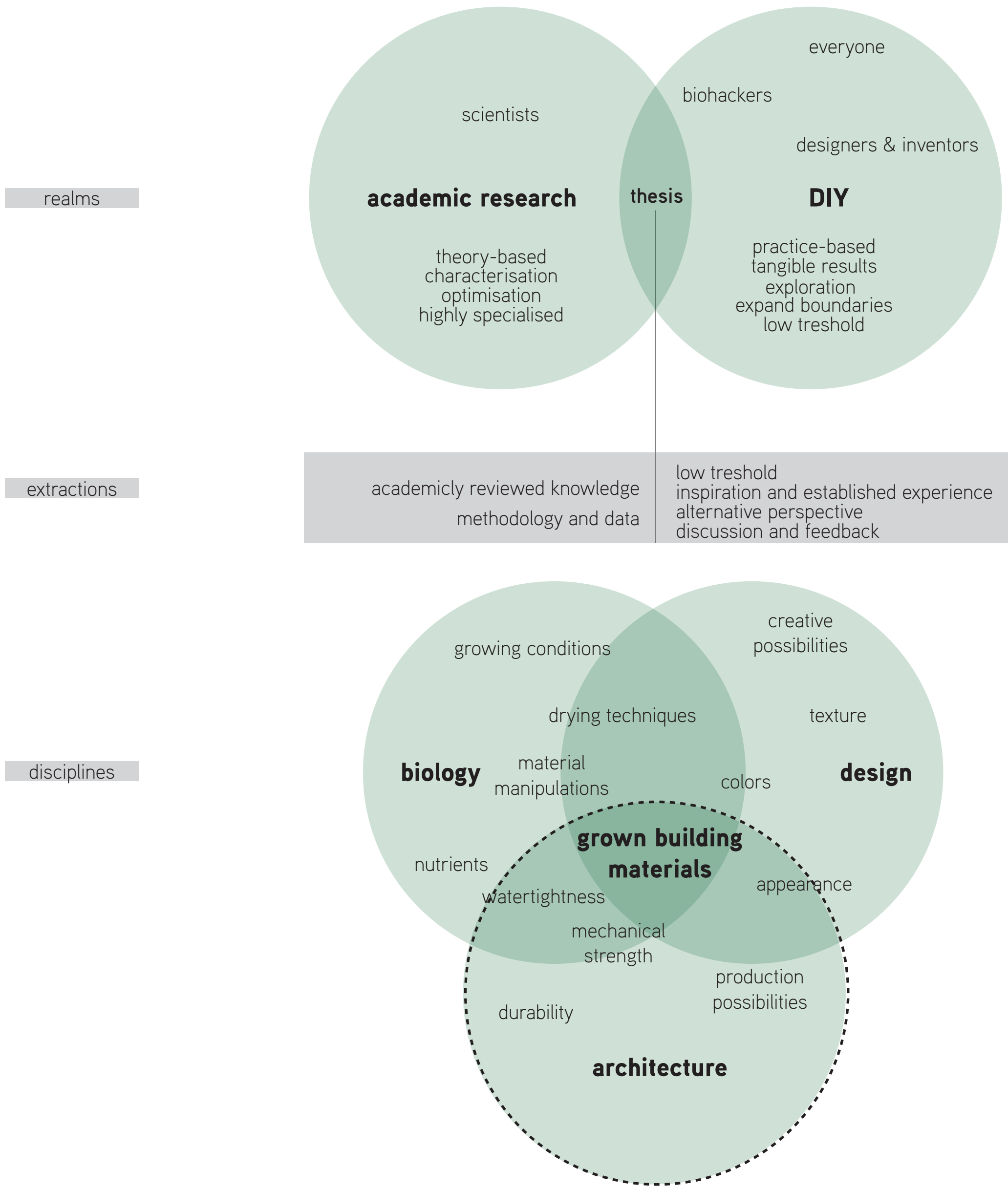


Figure 1
Diagram illustration of the approach

4 Approach

An approach between academics and DIY for a material bridging architecture and biology.

Multiple initiatives **combining biology and design already exist** (see 4.2). The aim is to **extend this material to architecture.** This multidisciplinary approach is a novel way of developing the material. The perception is that of an architectural engineer, combining mechanical research and architectural contexts. The biological discipline is added from two perspectives, by means of the **academic** and the **DIY-realm**. Combining these different disciplines from this unusual perspective and walking this unwalked path asked for a troubleshooting and quick problem-solving thinking mindset.

There exists a substantial amount of research on BC in **academic literature**, mainly in microbiology concerning the development of wound-dressings or in research optimising its production. There a well-defined production process is developed and optimised involving a bacterial strain and a culture medium. It grows a homogeneous and predictable BC sheet. On the other hand, **BC is also very common in DIY** *(Do It Yourself)* **communities**, where it is grown in a symbiotic mixture with bacteria and yeast, transferred from one *SCOBY (Symbiotic Culture Of Bacteria and Yeast)* to a new one. Those two very different approaches both have an important contribution to this thesis as shown in Figure 1, as they helped overcoming different encountered issues. In the next paragraph the approach vis-à-vis this way of working is elaborated.

4.1. Working method and multidisciplinary approach

The academic research about BC will certainly construct the base for this thesis by providing reliable knowledge, methodologies and data of BC to compare my results to. On the other hand, the DIY, artistic and design examples play an important role too, as illustrated in Figure 1. They explore possibilities, how the material can be altered and improved (transformations, drying techniques, dying…), what the material looks like and how it can be grown in different contexts. **A base of experience that outspreads academic knowledge and conditions** is created that proves very useful for interdisciplinary research. More experimental designs also extend the boundaries of what is thought to be possible. The knowledge available from *biohackers* is also easily available as there is a low threshold for interaction through the digital community. For these reasons, the **DIY community** will have an **active participation in this thesis**, as the blog *Biofabforum.org* will be used as a **knowledge sharing platform**. At first, it will serve as a **source of knowledge and inspiration**. Further on the aim is to share my findings in order to **complement the shared knowledg**e, but also to **generate feedback** from the community. Throughout the thesis, it will be clearly stated when there has been interaction with the forum.

This double way of working, academic and DIY, is used as a **tool** to overcome the challenge which is illustrated in the lower scheme of Figure 1. The core of this thesis is multidisciplinary. The existing multidisciplinary bridge between **microbiology** and **design**, as explained further on in 4.2, is extended to **architecture**, adding new layers of complexity such as mechanical strength, water repellency and durability. This overbridging perspective was really explicitly shaping the work on this thesis, as a lot of time was spent in the biology lab to develop and grow the materials, as well as in the construction lab to test the materials.

In the end, an evaluation on this way of working will be drawn. Some research questions relating to this part of the working method will be stated in the conclusion to guide the evaluation.

Interests in the material are extending beyond the lab, since **BC can also be grown in a DIY atmosphere** where it is known as bacterial leather, kombucha leather, SCOBY (=Symbiotic Culture Of Bacteria and Yeast) or vegan leather. There it is created as a pellicle forming on the surface of the fermented *Kombucha*-drink which contains BC-forming bacteria. This process involves adding the bacteria to a culture based on sugar, tea and vinegar, the sheets will produce after a couple of weeks (the longer, the thicker). The techniques are simple and there are infinite alterations possible by investigating parameters like colours, texture, thickness or anything you can invent yourself. **BC creates the opportunity of producing your own sheet material at home**. Applications can go from thin translucent cover sheets to thick textiles, with varying properties. It is even possible to replace the needed sugars with food scraps as shown by Ellen Rykkelid in her experiments (Rykkelid 2015), which closes to loop to a circular system. Since it is so accessible to grow BC at home, one could imagine that a huge range of sheet materials will disappear from classic production lines since they will be homemade. As put forward by *materiom.org,* in the end, **the only thing needed is a recipe** as an initiator in order to start acting. They want to provide a database of recipes for DIY creation from abundant sources of natural ingredients, the aim is to stimulate DIY biofabrication as a social and ecological action. *The Big Bang Project* by Guillian Graves designed the *Growduce* (Figure 2 (d)). This kitchen hardware-style machine **converts food scrap into SCOBY and thus in a usable material**. "*Croisement entre un biocomposteur et une imprimante 3D*." It is exactly the same process as described before, only in an automated way, meant to reach out to the general public as a casual kitchen tool. This narrative of **decentralised manufacturing** was predicted as well in the context of 3D printers. They did not completely change the production chain as was anticipated, but the creation of own custom materials and objects was brought a significant step closer to individuals, unlocking possibilities for creators. It is to be awaited to what extent biohacking will be able to infiltrate in people's homes and interests.

A wide range of interested individuals with different backgrounds– *known as biohackers* - experiment with SCOBY and tons of other biomaterials, forming **communities where knowledge is shared freely** in order to bring the practice to everyone whose curiosity is triggered, without any boundaries. Online platforms such as *Biofabforum* work as a forum. Passionate people share their knowledge and findings through tutorials, questions, examples, ideas and discussions. While some are just intrigued by biomaterials, thanks to this online sharing of knowledge multiple initiatives exist of people creating their own artistic interpretations (Figure 2 (a)), (design) objects such as phone cases by Ellen Rykkelid (Figure 2 (c)) or probably most famously, fashion. Suzanne Lee is the pioneer of Kombucha leather fashion design with for example her famous Bomber jacket shown in Figure 2 (b).

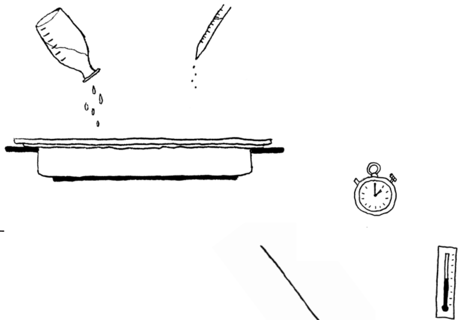
Figure 2
Microbial skin grower, crafted by Naja Ryde Akerfeldt (2014) (a); BioBomber jacket by Suzanne Lee, Biocouture (b); Cellulose Cell phone cover by Ellen Rykkelid (2015) (c); Growduce by Guillian Graves - The Big Bang Project (2015) (d)

4.2. Contributions

In short, these are the main contributions of this thesis to the research concerning bacterial cellulose.

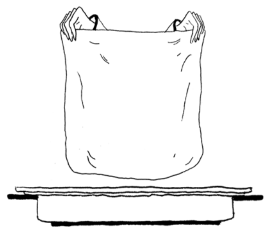
- first evaluation of bacterial cellulose for structural architectural applications
- create a large overview of tensile tests with alterations on bacterial cellulose containing input from academic literature, DIY-communities and own impulses.
- combine tensile test results to a water absorbency assessment
- revelation that alterations on bacterial cellulose can improve strength
- revelation that the strength of heat pressed as well as ethylene glycol and ethylene glycol choline chloride treated bacterial cellulose reach the strength of common tensile membrane materials.
- discovery of self-combining bacterial cellulose connections through drying
- development of a concept to deploy a bacterial cellulose tensile structure, using the self-combining connections
- development of techniques to create composites in different ways, although not satisfactory
- experimentation with a methodology combining input from academic literature and interaction with online DIY communities

Growing bacterial cellulose

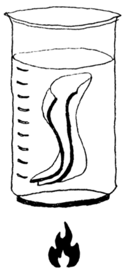


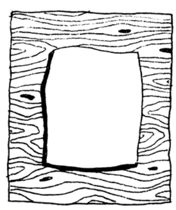
Culture medium and bacterial strain are poured together in the culture recipient

At ideally 30°C a pellicle forms on the surface of the culture over time

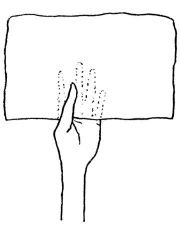


When the desired thickness is reached, the sheet is harvested and cleaned, for example by boiling.





Finally, the sheet is dried



Tensile architecture membranes are until now **fossil-fuel based** (polyester, PTFE, ETFE foils, except for glass fabric) and existing out of different layers, with PVC or PTFE coatings. These are finite resources and result in waste. Tensile materials also have limited lifespans ranging between 10 and +30 years depending on the material and environment and **will eventually be discarded** (Blum, Bögner, and Némoz 2004; Son 2007). Although PVC-coated polyester membranes are now recyclable, they will still need specific processes and production sites to do so (Leadbitter 2002; Blum, Bögner, and Némoz 2004). Developing a membrane with competing characteristics, but without the resource and waste issues would be a revolution.

A potential material is bacterial cellulose (from now on referred to as 'BC'). This is a **sheet biomaterial grown by bacteria** that could be envisaged for this innovation due to its high stiffness and tensile strength, low density (Ramana, Tomar, and Singh 2000), high purity, being biodegradable and its shape being easy to manipulate (Qiu and Netravali 2017). BC is currently researched as a biomaterial for artificial tissues due to its biocompatibility (Shah et al. 2013) and was patented for an acoustic diaphragm in headphones by Sony (Uryu and Kurihara 1993), although antibacterial, antioxidant, conducting and magnetic properties are opposing its potential in these fields (Shah et al. 2013) It is also used for cosmetics, paper, foods (Nata-de-coco), textiles… (Qiu and Netravali 2014)

Other **alternatives in biologically grown materials** exist that could also compete for applications as architectural membranes. For example, mycelium materials are materials where natural reinforcement fibres (jute, flax or straw) are bound together by fungal mycelium, forming a self-assembled lightweight biomaterial (Elsacker et al. 2019). Compressing and plasticizing these materials creates membranes, as elaborated in 6.4. Specimens of **compressed and plasticized mycelium composite**s are provided by Elise Elsacker and will be tested in tensile strength too, in order to be able to compare both materials.

The question will be how suitable BC really is for tensile architecture membranes. Not only in terms of **strength** (in the plane, resistance against punctual forces, tearing strength) but also relating to multiple other properties. **Resistance to external factors** (water, moisture, UV, fire) and **maintenance** of the material properties over time under different conditions, **practical factors** (seams, connections), **density**, **cost**, **visual factors** (colour, texture, transparency) and **production conditions** (for example sterile environments). The **longevity** in outside conditions and **long-term behaviour** of the membrane will define whether it could compete with today's fossil-fuel-based architectural membranes, in short- and/or long-term constructions. Based on the results for the different properties, it is possible that a direct replacement of common membrane materials will not be at stake, but that the material formulates a new kind of use.

left page

illustration of how a sheet of bacterial cellulose is created.

5.1. Biological background

Cellulose is mainly known as the structural component of plant tissues, but some bacteria are also capable of producing cellulose. BC doesn't rely on wood consumption and therefore doesn't contribute to deforestation. Furthermore, BC is much purer. Plant cellulose also contains lignin and hemicellulose, requiring alkali and acid treatments in order to obtain pure cellulose. BC owes its high strength and modulus to its high purity, a high degree of polymerization (improves strength) and high crystallinity. The fibres are 40-70 nanometers thick, each one composed of a bundle of 2 to 4 nm microfibrils. This results in high surface area, explaining its high water holding capacity (Esa, Tasirin, and Rahman 2014) (Qiu and Netravali 2014). Comparing crystalline structure, both native and bacterial cellulose consist of Iα- and Iβ- cellulose. Native cellulose is nevertheless mainly constituted out of Iβ- cellulose whereas BC's Iα-cellulose content reaches 60%, with only 30% in native cellulose. Iα-cellulose would have a smaller unit cell density and undergo a conversion to Iβ- cellulose under hydrothermal treatment, thus being less stable.

5.2. The bacteria and cellulose creation

Komagataeibacter xylinus (from now on referred to as 'KX') is an aerobic bacteria producing BC that has been extensively studied for producing BC in the different fields mentioned before. It is a strain that can produce cellulose from a wide range of carbon/nitrogen sources (K.-Y. Lee et al. 2014). In its original nature, Gluconacetobacter species colonise fruit and produce BC as a biofilm protecting cells from desiccation and UV damage. In the lab under static conditions in liquid culture, the bacteria grow at the air-water interface for aerobic growth. They synthesise and secrete chains of cellulose, attached to the cell surface. Altogether a pellicle of intertwined cellulose fibrils is created wherein the bacteria are embedded (Figure 3a). UDP-glucose monomers are added to the cellulose chain, going from the inner to the outer cell membranes. The result is a cellulose without hemicellulose, pectin and lignin with good mechanical properties thanks to its regular arrangement of glucan chains (Gilbert and Ellis 2018). In Figure 3a a SEM image of BC is shown. A cellulose network is visible, composed of fibrils lying in different directions and containing branching points, presumably at points where cell division occurs, as shown in Figure 3b. A high Young's modulus is found and explained by the fact that all fibrils lay in the plane of the cellulose. Figure 3c shows how different layers of these two-dimensional laying fibrils are ordered (Yamanaka et al. 1989). More in-depth information on the creation of cellulose by KX can be found in the literature but is not of use for this research.

Figure 3
Scanning electron micrograph of the surface of a freeze-dried BC pellicle (a); Secretion of cellulose by dividing cells creating branchings in the cellulose network (b); Cut through the edge of BC where a pile of thin layers is visible (c). (Yamanaka et al. 1989)

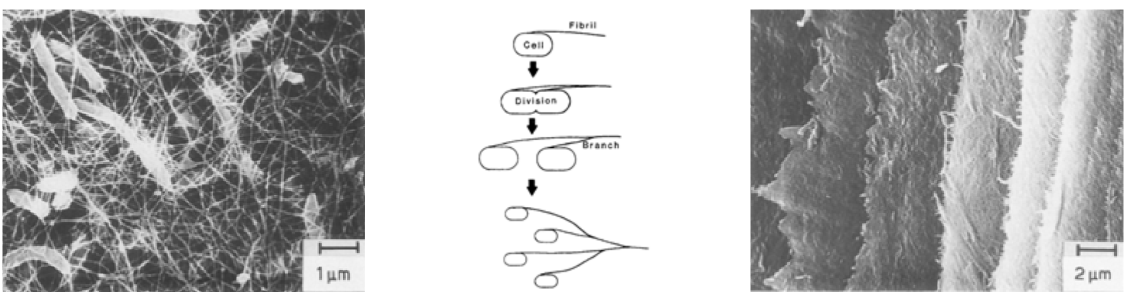


Table 1
Komagataeibacter xylinus culture medium by Hestrin and Schramm (1954)

Compound	Amount
Glucose	20 g
Peptone	5 g
Yeast extract	5 g
Na_2HPO_4	2.7 g
Citric acid	1.15g
Distilled water	1000 mL

5.3. The growing medium

The bacteria create cellulose on the surface of the culture medium. The recipe for this culture medium differs in different sources but is always a version of the Hestrin and Schramm medium developed in 1954 (Hestrin and Schramm 1954). Studies have been carried out in order to optimise the yields of the bacteria, for example on the source of the carbon and nitrogen and on the influence of glycerol as a carbon source (Jung et al. 2010; Ramana, Tomar, and Singh 2000). Optimising the growing medium is certainly an interesting way to increase yield, although it is thought to mainly affect the growth rate. Since this is not primordial for this research, the HS medium is used.

5.4. Growing process

A figurative overview of the production process of BC is figured on page12. Assuming a cellulose-producing bacterial strain is already available, the culture medium has to be prepared. The different ingredients are mixed in distilled water and then sterilised. The recipient in which the BC will grow has to be selected. Its shape will define the shape of the bacterial cellulose sheet, that will grow on the culture medium surface. The recipient has to be able to be sterilised. Once the growing medium and the recipient are sterilised and cooled-down, they can be moved to sterile conditions (laminar flow). If the bacterial strain is available in a previously-grown bacterial cellulose starter, around 5% volume of liquid from this starter has to be transferred into the culture. Being placed at 30° C, the culture will start to grow in a couple of days. A thin layer will develop on top of the culture. After some days to weeks, it can be harvested and retrieved from sterile conditions, cleaned and dried in order to form the end product. Different cleaning and drying techniques have already been tested and will be discussed further on. This process has been adapted from the *Kombucha leather manual* (Poncelet 2018) considering a laboratory environment and the growing medium mentioned before.

5.5. Harvesting and processing

A recurring process (Zeng, Laromaine, and Roig 2014) to clean the bacterial cellulose from its bacteria is the following. The process varies slightly between research but comes down to the same principle.

The method used to dehydrate the freshly harvested wet BC pellicle into a dry sheet varies depending on the research. For example, it can be oven dried until the weight is constant (Yamanaka et al. 1989) or not dried at all and used as a wet pellicle (Qiu and Netravali 2014). In a comparative study air-drying, freeze-drying and supercritical drying are performed to dehydrate the BC (Zeng, Laromaine, and Roig 2014). For another purpose, the pellicles can also be pulped and freeze-dried in order to store the bacteria (Hestrin and Schramm 1954).

	Immerse in ethanol
40min	Place in 90°C deionised water
4 x 20min	Place in 90°C 0.1M NaOH solution, rinse with water between baths.
24h	Neutralise with deionised water

Table 2
alkali cleaning procedure obtained from (Zeng, Laromaine, and Roig 2014)

5.6. Literature on tensile strength

This part deals with general research question 2:

RQ 2 What is the strength of bacterial cellulose in existing literature?

The mechanical characteristics of BC have already been investigated in a variety of contexts (bio-medical, paper production, polymer alternatives). A small overview of relevant research is drawn up below. The main interest is to **gather tensile testing results** in order to already evaluate whether with the current research BC already fulfils mechanical strength needs for architectural membrane structures. Furthermore, the data will then be used **for comparison with the generated results**. Secondly, the **testing methodology** will also be looked at, in order to be able to create a relevant methodology for the tests. The ideas proposed and researched in the papers below are not always relevant to my research. If they are, they will be mentioned further on when appropriate.

First mechanical paper

A 1989 paper (Yamanaka et al. 1989) describes mechanical characteristics of BC, praising a high Young's modulus (>15GPa) and bringing it forward as a potential complement to or an alternative for polymers. For sheets without preferential molecular orientation such as BC, polymers would only reach 10Gpa. Sheet growing and preparation methods were in line with common techniques. Tensile tests and dynamic mechanical tests are performed but a testing methodology is unfortunately not shared. The results are interesting to take into account but will provide fewer interpretation possibilities in comparison with own results.

By means of a hot press, the influence of the drying routine with **applied heat and pressure** is researched. The conclusion was that both **do not have a significant influence** on the mechanical properties, apart **from a certain pressure (196MPa) a negative effect on the tensile strength and elongation is noted.** InTable 3 the best results from this research are shown. Varying cultivation time (which affects cellulose content) and different bacterial strains were used but no significant difference in mechanical properties was found.

The study also experimented with making pulp from the BC sheets by mixing them with water and sieving the mixture afterwards. The remaining pulp was then spread out and turned into a paper-like sheet by means of a heat press. Composite mixtures with cotton lint pulp were tried as well but had significantly less performing results. The results for mixed BC are lower than those of plain BC.

Preparation method	T (°C)	Pressure (MPa)	Young's Modulus (Gpa)	Tensile strength (Mpa)	Elongation (%)
Air dried	20	0	16.9	256	1.7
Hot pressed	150	49	18.0	231	1.8
Hot pressed	150	49	16.9	260	2.1

Composition	Young's Modulus (GPa)	Tensile strength (Mpa)	Density (g/cm³)
Pure BC	4.9	85	0.99

Table 3
Mechanical characteristics in function of temperature and pressure applied with a hot press on BC. Table compiled based on literature (Yamanaka et al. 1989)

Table 4
Mechanical characteristics of mixed BC pulp processed into paper. Table complied based on literature (Yamanaka et al. 1989)

Composites

Experimenting with increasing the strength of BC quickly leads to the idea of creating composites. A study already investigated 'green' hybrid structures, where all constituents are biodegradable, using microfibrillated cellulose (MFC, because of its high tensile properties, 2 to 6 GPa tensile strength, 140 GPa modulus) and sisal fibre (high tensile strength and good biodegradability) by placing them into the culture medium. The strain grows preferably on the surface of these added polymers than freely in the medium. BC composites can thus be created in-situ. Hydroxyl groups on the surface of cellulosic fibres and BC create decent adhesion through hydrogen bonding. BC pellicles have a large number of pores or voids that are uniformly distributed. This will probably be a factor diminishing the tensile strength of BC. The use of MFC in the composite bridges these gaps and eliminates weak points in the structure. At first tensile tests were performed. The tests were carried out according to ASTM D-882-02, which are norms for *Tensile Properties of Thin Plastic Sheeting*. In order to know the interfacial shear strength (IFSS) of the composites, a microbond strength test was performed too. The principle is shown in Figure 4. The results show that Young modulus, tensile strength and fracture strain slightly increased between BC and BC-MFC (Table 5). But despite the pores or voids in the BC network, the mechanical characteristics are not improved substantially. (Qiu and Netravali 2017)

Composition	Young's Modulus (GPa)	Tensile strength (Mpa)	Strain at break (%)
BC	2.490 +/- 9.6	79.1 +/- 13.6	5.6 +/- 17.8
BC-MFC	2.826 +/- 6.9	84.1 +/- 14.7	6.0 +/- 22.3

Table 5
Results of study on in-situ produced composites Table compiled based on literature. (Qiu and Netravali 2017

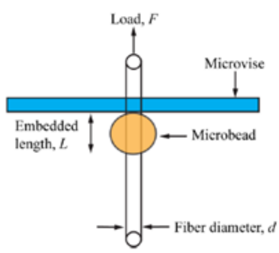


Figure 4
scheme of microbond test

Production in agitated culture

In order to understand the structural functioning of BC, the following literature proved valuable. In this research static and agitated cultures are compared in terms of yield and mechanical characteristics. The influence of some additives such as agar, carboxymethylcellulose (CMC), microcrystalline cellulose, and sodium alginate on yield was compared too. The additive providing the highest yields was carboxymethylcellulose (CMC), which was then submitted to mechanical tests. Additionally, the findings are linked to the crystallinity of the structure too. Disintegrated BC is produced by growing the bacteria in a liquid suspension in agitated cultures. The stable suspension contains smaller, fragmented fibrils of nanocellulose because the continuous movement did not allow the bacteria to build continuous fibres, or the fibres were broken down. The strong binding ability of BC makes it possible for the mixture to recreate a sheet that could have interesting properties (Fumihiro Yoshinaga, Tonouchi, and Watanabe 1997). Another study revealed that agitated cultures lose strength in comparison with BC sheets (Cheng, Catchmark, and Demirci 2009), although the principle remains interesting for the production of composites. Tensile tests were performed by means of a DMA (dynamic mechanical analysis).

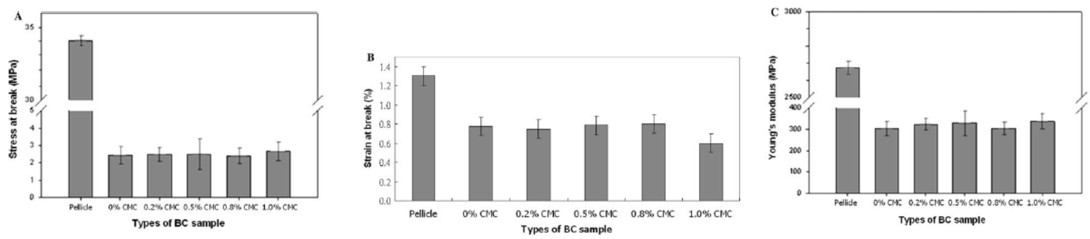


Figure 5
Results of tensile tests of BC, by means of a DMA (Cheng, Catchmark, and Demirci 2009)

Table 6
Data visually retrieved from Figure 5, showing results of tensile tests of BC, comparing static (pellicle) to agitated cultures. Data in the table is interpreted visually from the charts for the purpose of being interpretable with other collected literature data and comparable with future own measurements.

Sample	Yield (g/L)	Young's Modulus (GPa)	Stress at break (MPa)	Strain at break (%)
BC pellicle	1.3 +/- 0.3	2.68 +/- 0.03	34.0 +/- 0.3	1.3 +/- 0.5
0% CMC	1.5 +/- 0.0	0.30 +/- 0.03	2.3 +/- 0.5	0.8 +/- 0.5
0.2% CMC	4.8 +/- 0.1	0.31 +/- 0.02	2.4 +/- 0.4	0.7 +/- 0.5
0.5% CMC	6.5 +/- 0.3	0.31 +/- 0.05	2.4 +/- 1.0	0.8 +/- 0.5
0.8% CMC	8.1 +/- 0.2	0.30 +/- 0.02	2.3 +/- 0.3	0.8 +/- 0.5
1.0% CMC (highest yield)	8.4 +/- 0.2	0.31 +/- 0.03	2.5 +/- 0.5	0.6 +/- 0.5

Physical properties in presence of lignosulfonate (Keshk 2006)

Another study that provides tensile strength data for BC looked at how alterations of the **culture medium** can not only increase yield but also **improve mechanical strength**. **Lignosulfonate** is a by-product of cellulose production. By adding 1% into the culture medium, significant improvements of crystallinity index were found. Higher viscosity means also a higher degree of polymerization. SEM scans also showed that thicker and less branched ribbons are created. These created a better uniplanar orientation, increasing stiffness and strength. Unfortunately only the tensile break load was measured without calculating the stresses. Therefore the tensile strength will not be comparable to measurements from other literature.

The tensile tests were performed on dumbbell-shaped samples measuring 0.16mmx100mmx150mm at 20mm/min strain rate. Unfortunately the break stress and break strain were not mentioned in the data, therefore only the Elasticity modulus will prove interesting for comparison.

Sample	Yield (g/L)	Tensile strength (N)	Young's Modulus (MPa)
HS 10245	4.4	10.0	331.75
HSL 10245	7.2	18.28	606.45
HS 13772	8.7	15.51	514.55
HSL 13772	11.4	18.69	620.05
HS 13773	10.1	15.03	498.62
HSL 13773	16.2	19.23	637.95
HS 13693	7.9	13.81	458.15
HSL 13693	16.3	21.53	714.27

Table 7
Strength measurements of four strains of AX (number code), with (HSL) and without (HS) lignosulfonate. Table compiled based on literature (Keshk 2006).

Comparison of literature data and common membrane strengths

The found literature proves useful to understand methodologies and to compare data already in an early stage. The different tensile strengths and elasticity moduli are listed together with other provided data in Table 8 (Annex 10.1) and plotted in Figure 6 for quick comparison. In order to already settle a perspective on the possibilities of BC as a structural membrane, the **found literature measurements will already be compared to an overview of available data of common structural membranes.** Found data of common membrane strengths are listed in Table 9 (Annex 10.1) and plotted in Figure 7. The comparison that is being made here has to be interpreted with the conscience that not all the retrieved data (of BC and common fabrics) is comparable. For example, the paper handling the influence of lignosulfonate on BC (Keshk 2006) provided only tensile strength loads instead of stresses, rendering the data irrelevant for comparison. Furthermore, the strength of structural fabrics is often characterised as a variation on [load/length] such as kN/m. The comparison is based on stresses in order to remove the dependency on thickness. Therefore only membrane materials for which the thickness is known can be compared, these are marked grey in the table.

The *common membranes* mentioned before are defined as such in the *European design guide for tensile surface structures* (Blum, Bögner, and Némoz 2004) and the general data in table 7 is retrieved from this guide. The common membranes are PVC coated polyester fabrics, PTFE coated glass fabrics, Silicone coated glass fabrics, PTFE coated PTFE fabrics and ETFE foils. Examples for PVC coated polyester fabrics are retrieved from *PAR Group* ("PVC Coated Polyester | PAR Group" n.d.). *Fiberflon* ("Silicone Coated Glass Fabrics" n.d.) provided thicknesses for silicone coated glass fabrics. ETFE foil thicknesses were mentioned in the design guide. For PTFE coated glass fabrics and PTFE coated PTFE fabrics no examples with mentioned thicknesses were found. **It is clear that the represented values for common membranes are thus quite arbitrary and need to be interpreted as such.**

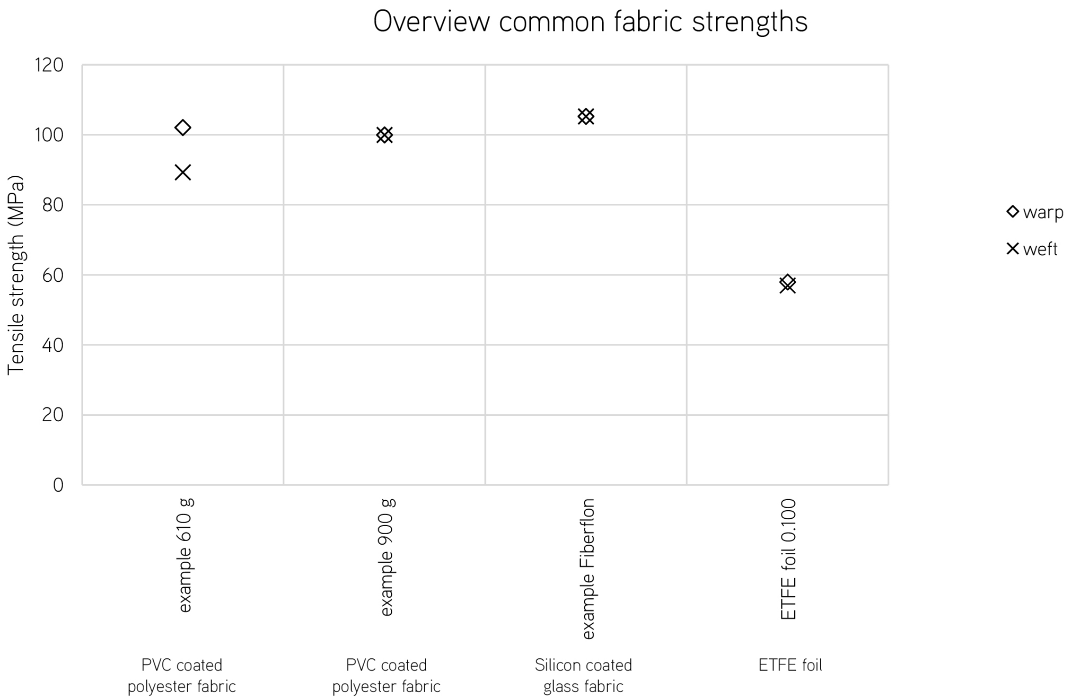
All the retrieved data is listed in the tables (8 and 9), the figures (Figure 6 and Figure 7) show a summary of the comparable data. Values settle **around 100MPa for membranes and 60MPa for ETFE foils**. These strengths could be defined as **the goals for BC strengths**. Depending on the research, the strength of BC actually does come close to these goals (paper by (Qiu and Netravali 2017) has values between 50 and 100 MPa), remains below 50 MPa (paper by (Cheng, Catchmark, and Demirci 2009)) or even widely surpasses the goals (paper by (Yamanaka et al. 1989)) by showing results around 250 MPa. These **very fluctuating results** are difficult to interpret and could be attributed to different causes. **This confirms the need to add to the current research, by creating a larger range of variations on the BC pellicle with a characterisation of its mechanical tensile properties**. The data gathered here will be useful for a new comparison in the conclusion with the gathered measurements. That way they will be able to be drawn up in a context of existing research as well as common architectural materials.

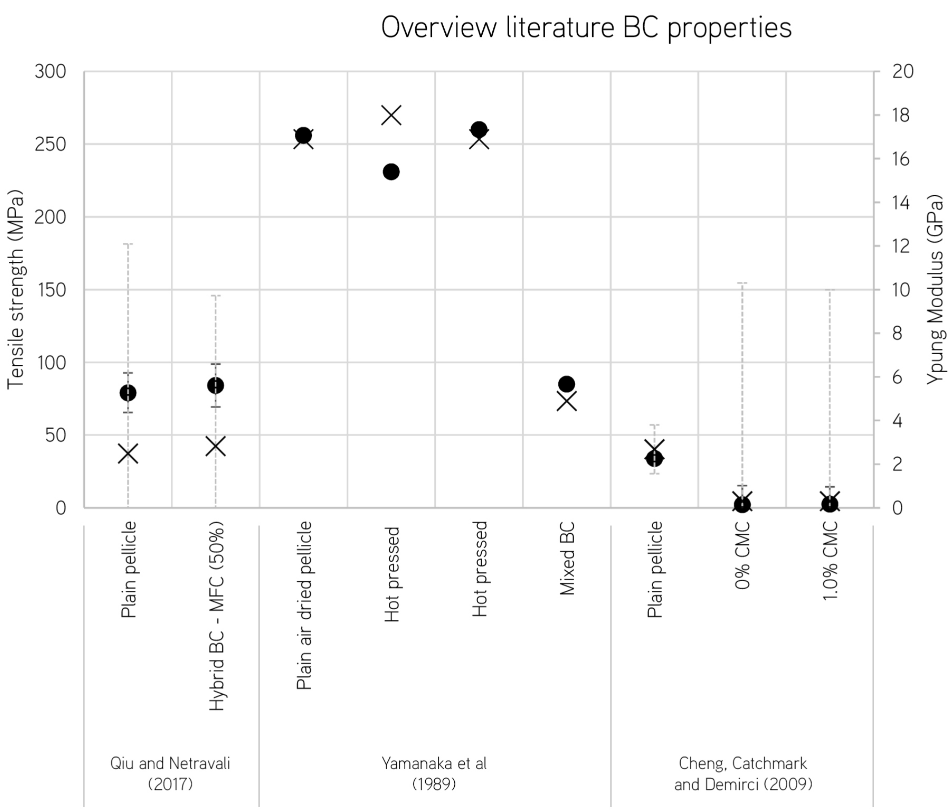
Table 8
Overview of mechanical characteristics found in literature. (Annex 10.1)

Table 9
overview of mechanical characteristics of common membrane structures (Annex 10.1)

Figure 6
Overview of BC strengths found in literature, data from Table 9.

Figure 7
Overview of common (woven) fabric strengths, data from Table 10.





State of the Art

6.1. Determination of samples

The aim of the tests is to **explore post-processing techniques that could enhance BC in favour of architectural applications** allowing to **evaluate the possibilities**. The main aspect that is focused on is **mechanical strength. Appearance and water tightness** will be assessed as well.

The starting point is the plain material. Afterwards, techniques commonly used in academic literature will be reviewed. Furthermore, there is a wide range of ideas and techniques which are experimented with by *biohackers* that are not decently quantified. Interesting ideas are picked out and tried. Finally, some own ideas will be developed too. Before creating the real samples an exploratory phase with preliminary tests is conducted, described in paragraph 7.

All samples will be evaluated on **mechanical strength through tensile testing**. The production process, as well as visual aspects and texture, will be characterised too. Under *Literature on tensile strength* in paragraph 5.6 available data is listed. Own measurements will be compared to this data.

6.2. Preparation of the BC sheets

In the different papers from the state of the art, a variety of production methods is presented. Most of them do come back to the same principles. The used BC production method is described below. The final method was found after a process of failures and research to solve the problems encountered. This itinerary is described in paragraph 7. After having found a growing method which created homogeneous BC sheets and did not produce any contaminations, it was used for all samples. The described method draws the base for each test. Some tests explore variations on this method, the actual method will be explained where relevant.

In short, the strain of cellulose-producing bacteria is placed with a sterile culture medium in a sterile dish. Over a couple of weeks, the BC can be harvested.

Culture medium

The culture medium described in Table 10 is a commonly used medium in scientific literature, established by *Hestrin and Schramm* in 1954. Plenty of alternatives and optimisations of the culture medium exist, but these are considered out of the scope of the thesis. The medium is prepared by heating the distilled water in order to dissolve the different compounds. Then it is placed in 1l PYREX bottles, autoclaved and stored for later use. One large batch was made for the whole thesis, allowing to have constant reliability of the medium.

Figure 8
A bottle of culture medium

Table 10
HS culture medium (Hestrin and Schramm 1954)

Compound	Amount
Glucose	20 g
Peptone	5 g
Yeast extract	5 g
Na_2HPO_4	2.7 g
Citric acid	1.15g
Distilled water	1000 mL

The bacterial strain

Multiple possibilities exist here and have been explored in the preliminary experiments (paragraph 7.3). A strain is isolated which produces BC by means of the HS culture medium, which is designed specifically for the cellulose-producing bacteria. Identification confirmed that indeed Komagataeibacter xylinus (formerly known as Acetobacter xylinum and Gluconacetobacter xylinus) was present in the culture. The strain is stored in Erlenmeyers in growing conditions (30°C, no light) or in the fridge to slow down growth.

Producing BC

The BC sheets are produced in PYREX 40x27cm glass oven dishes (Figure 9a). The glass dishes are covered with aluminium and sealed with tape before being sterilised in an autoclave (Figure 9b) at 121°C.

Figure 9
Used Pyrex dish (a); autoclave (b); inoculating the culture in a laminar flow (picture by Elise Elsacker) (c)

Once the dish has cooled down below 30°C the culture can be inoculated. In a laminar flow unit, the aluminium sheet is carefully cut open in one corner, folded over to open the dish (Figure 9c). The sterile culture medium is poured in slowly. Out of the Erlenmeyer containing the strain, about 1% of the volume of the culture medium is extracted and transferred to the glass dish by means of a 25ml pipette. As opposed to what is often done by *biohackers*, there is no need for transferring a part of the cellulose itself as it may only augment risks of contamination. Now the aluminium is closed again and sealed with tape. The dish is placed in a dark, 30°C room for a couple of weeks until the desired thickness is reached. The bacteria reproduce and develop BC sheets at the water-air interface. Yields can vary between dishes.

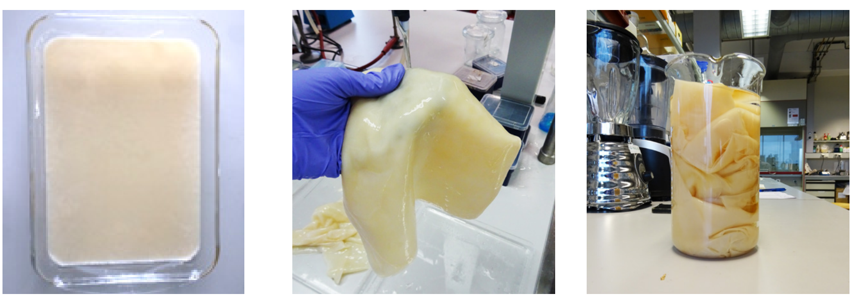


Figure 10
Harvesting of the sheets (a) and (b); placing in 90°C deionised water for alkalic cleaning (c)

6.3. Processing after harvesting

- Cleaning

When harvested, the sheets are removed from the dishes. This can happen out of sterile environments. The sheets are thoroughly washed with running water and immersed in ethanol to remove all apparent slimes and bacteria. For simply cleaned sheets these are now dried. All others go through an alkalic cleaning which kills all the bacteria and renders the material very white and homogeneous.

- Alkali cleaning

As mentioned in the literature, the sheets are always cleaned with distilled water and a base solution to kill bacteria and render the sheets inert. In this case, the sheets will be cleaned as shown in the following way, retrieved from (Zeng, Laromaine, and Roig 2014) because of being extensive and producing pleasing white sheets.

Table 11
Alkali cleaning procedure obtained from (Zeng, Laromaine, and Roig 2014)

	Immerse in ethanol
40min	Place in 90°C deionised water
4x20min	Place in 90°C 0.1M NaOH solution, rinse with water between baths.
24h	Neutralise with deionised water

- Drying

When cleaned sheets are retrieved, they can be dried in several ways. The sheets are dried on water-absorbing textile in some hours or days, depending on environmental conditions and thickness. The sheets are regularly flipped to have equal drying on both sides. Before curling occurs because of uneven drying, the sheets are placed between two layers of absorbing textile and a weight to preserve flatness. In order to know the undergoing changes in mass, the sheets are weighted before drying. Also, the thickness is measured by means of a calliper. The used balance is a Mettler Toledo PB1502-S with a precision of 0.01g.

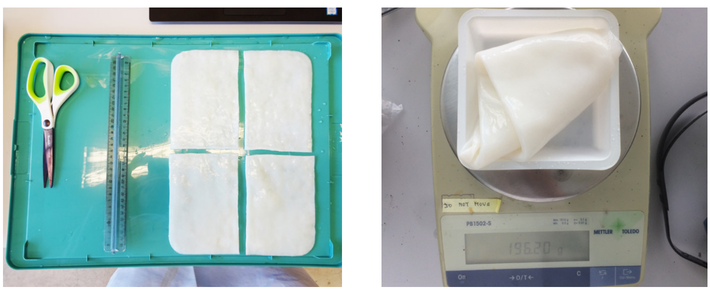


Figure 11
Facultative cutting of specimens and weighing.

Figure 12
Before and after drying

When dry, the samples are weighted and measured again. This time the thickness is measured with a micrometer. The dry thicknesses are used later on to calculate the sectional area of the samples, needed to convert load measurements to stress.

- Post-processing and sample creation

Depending on the test, the sheets are now cut and labelled into unique rectangular samples measuring 25mm in width and at least 160mm length. The weight and thickness of each sample are measured before and after the processing in order to be able to evaluate the influence of the post-processing on both. For each test, a post-processing protocol is defined. Suiting to the post-processing technique, specific as well as general research questions about the post-processing are drawn up, about tensile strength but also relating to various other aspects.

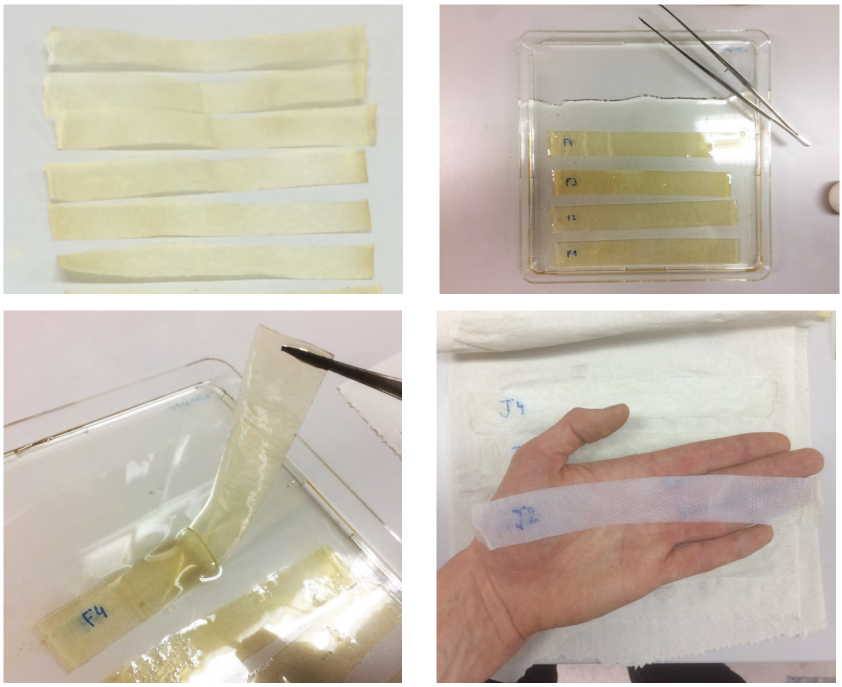


Figure 14

(a) cut sheet into specimens. (b) label and process. (example: glycerol soaking) (c) retrieve (d) dry

6.4. Norms

Since BC is not a common material in a construction context, no specific norms prescribing a testing methodology exist. Resembling materials that do provide normative information on testing can be used as a base for the own used methodology, such as leather or thin plastics. Together with methodologies used in previous work on tensile testing of BC an **own methodology** can be designed. This will then also be influenced by the available set-up and other contextual factors. Further information on the interpretation of the norm to set-up an own methodology is placed in annex 10.2.

6.5. Set-up and testing

Tensile testing is done in the following set-up (see Figure 15 (a)) for all specimens. For composite tests, the load cell was changed to a 100kN load cell as it was expected to exceed the limit of the more precise load cell used for the other tests.

Figure 13

A sheet of undried alkali cleaned BC.

Drawing bench: Instron 5900R; Clamp distance= 60mm; testing speed 2mm/min. Since the reaction of the material is not known yet, a slower displacement-based test than what the norm suggests is used.

Static load cell Instron 500N. The low limit of this load cell allows higher accuracy for the relatively small loads that BC will be able to bear, keeping in mind that a +/- 1% accuracy can be taken into account.

Upper clamp Instron high wycomb with pneumatic pressure set at 4 bar. Weighing 15kg, the remaining load cell range reaches 350N. The clamps have 25x50mm grips, equipped with a ribbed metal surface. Depending on the sample, when a failure occurs in/ because of the clamps, a rubber piece will be added.

Due to the fragile material, an extensometer is not an option, as it would have a too significant influence on the thin material. Therefore displacement will be measured through the bench movement. This is less precise than the extensometer, phenomena like the slip of the samples in the clamps will influence the results. Furthermore, there can always be a small movement in the bench itself. For each test the displacement is reset to 0, the specimen placed, a line drawn on the specimen at the clamps (in order to notify if slip occurs), a picture of the original sample is taken (see Figure 15 (b)), the measured load is calibrated and then the test is started. After the test, a picture of the broken sample is taken (see Figure 15 (c)) and a load-displacement curve is drawn.

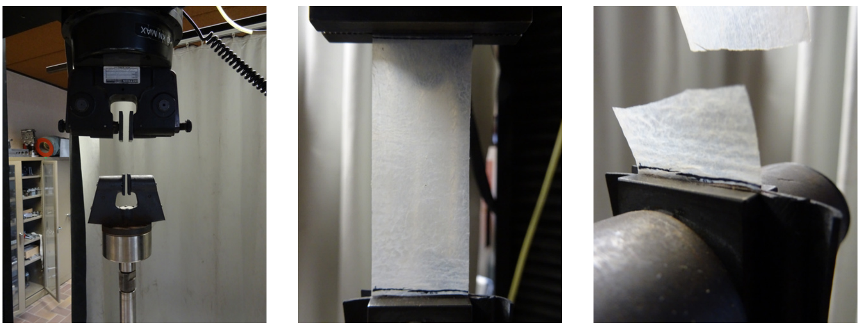


Figure 15*(a) test set-up (b) example of a sample before testing (c) example of a sample after failure.*

6.6. Data processing

Processing of the data follows the standard methods, as is further explained in annex 10.3. Some deviations are listed here.

Strain shift and Young modulus

Because the samples are not stiff, they will not be totally stretched when placed. At first, the samples will sit loose. The first displacement will only be tensioning the sample without any load build-up. This false displacement is deleted from the measurements by means of a 'strain shift'. In Figure 16 a dotted line indicates the moment load starts to develop. A **threshold of 0.10MPa** (corresponding to approximately 0.15N) is chosen and is applied to all samples in order to have a consistent *strain shift*. The displacement corresponding to 0.10MPa stress is reset to 0 before calculating the strain. As mentioned before, for composites a load cell of 100kN was used. Being less precise, a larger threshold value of 3 MPa was used.

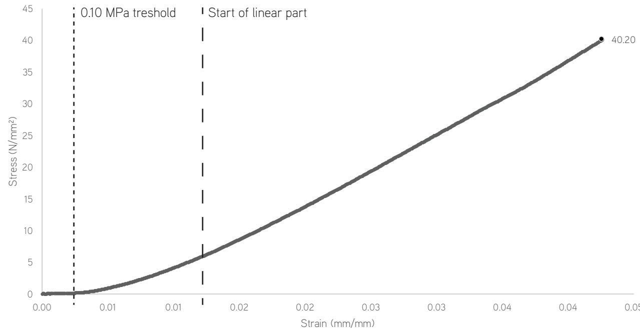


Figure 16
Example of a stress-strain curve with a dotted line illustrating the strain shift value and a dashed line showing the linear part of the curve

After performing the strain shift, a certain displacement will still be needed before full stress development in the sample occurs. The first part of the measured displacement will indicate the arrangement of the sample until a section with homogeneous stress is reached. This is reflected in the graph by a gradually increasing stiffness as can be seen in the example chart in Figure 16 between the dotted and the dashed line. After a certain settling, a constant stiffness develops. Because of this non-linearity, defining the Young's modulus is not straightforward. Ideally, a cyclic loading would induce prestressing and unveil the material's highest and most reliable stiffness, independent from wrinkles, placement or how the material was treated before testing (Capurro and Barberis 2014). Because of the small number of samples available, the tests are limited to non-dynamic testing.

The Young modulus will be calculated for each curve. A **regression analysis** will provide a stiffness value reflecting the whole curve. This will be calculated by means of a linear trendline starting from (0,0) as in Figure 17.

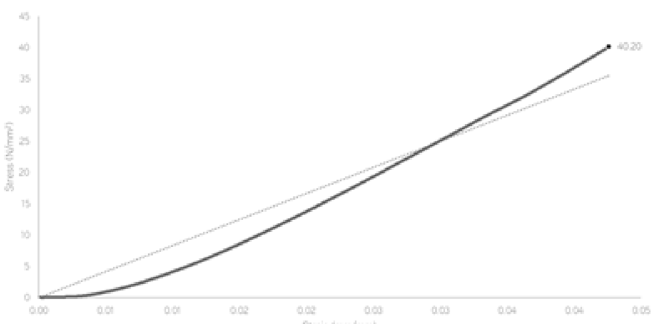


Figure 17
Stress-strain graphs of sample B1 with a trendline, by means of an example

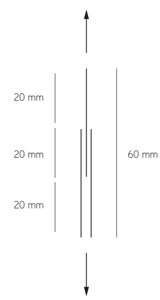


Figure 18
Scheme of double layer connection sample setup

Connection samples

Tests on connections of BC sheets will be performed. The aim is to know the strength of the connection, or whether the connection is stronger than the plain BC or not. A double layer connection, as shown in Figure 18, is created in order to avoid eccentricities and ensure a straight pulling force in the plane of the material. Since actually two connections are made, the resulting strength will have to be divided by two if the break occurs in this connection. If the break occurs in the plain BC, it can be concluded that the plain material is less strong than the double strength of the connection. Because this method does not provide decisive information, tests with purely the connection technique (= double BC dried together) will be performed too.

7 Explorative experiments

This first experiment series **explores different possibilities** for the mechanical testing samples and draws the first conclusions. Furthermore, the aim was also to simply **learn** to generate a desired outcome for the BC-production.

At first, different experiments comparing culture medium recipes and strains were conducted. Preliminary post-processing such as glycerol soaking was tried out, creation of in-situ composites and possibilities for seams were examined. The influence of the heat press was investigated and the possibility of mixed BC studied. The composition of these experiments is very broad and really explores different tracks which can be interesting for BC in general, the ideas emerged out of suggestions, inspiration from other projects or own impulses.

These are some more specific research questions for the explorative experiments. They will be answered at the end of this chapter.

EXP 1 What **growing protocol** will be used for all measurements (culture medium, bacterial strain, protocol)?

(This question also relates to '**RQ 1** *What is the optimal production process of BC?'*)

EXP 1 Can **composites** of natural fibres with a BC matrix be created, and how?

(This question also relates to '**RQ 9** *How can composites be created and what is their strength, water-tightness and appearance?'*)

EXP 1 How could the creation of **connections/seams** be fulfilled? Can these be created by growing the BC together without interference of a glue? If yes, how?

(This question also relates to '**RQ 7** *How can seams be created and what is their strength?'*)

EXP 1 What **post-processing protocols** such as soaking in plasticizers can be performed on BC? What is their influence? What is a decent soaking duration?

EXP 1 What is the influence of a **heat press** treatment on BC?

EXP 1 Can a sheet of **mixed** BC be created?

7.1. First cultures

Before being able to grow and experiment with harvested BC, it was necessary to learn to grow the material and to work in the lab environment. Multiple growing attempts failed and contaminated after a couple of days (Figure 19 c and d).

The first experimental campaign which was meant to gain insight in BC cultures is explained below. All future cultures are extracted from one Kombucha mother culture or SCOBY, kept in the freezer (Figure 19 a). 5 batches of growing culture, which were isolated out of the SCOBY culture with HS culture medium are kept in continuous growing conditions as alive cultures, supplying starting cultures for each experiment (Figure 19 b).

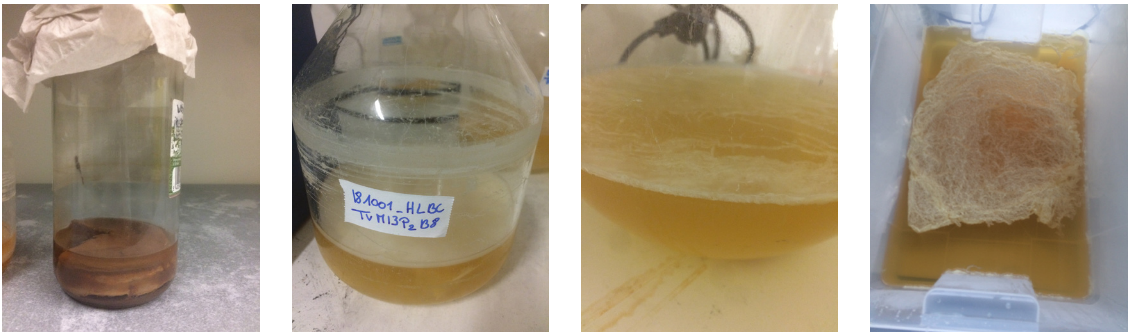


Figure 19
Kombucha mother culture/ SCOBY stored in freezer (a); living culture of AX, isolated out of SCOBY (b); contamination (c) and (d).

7.2. Recipe test

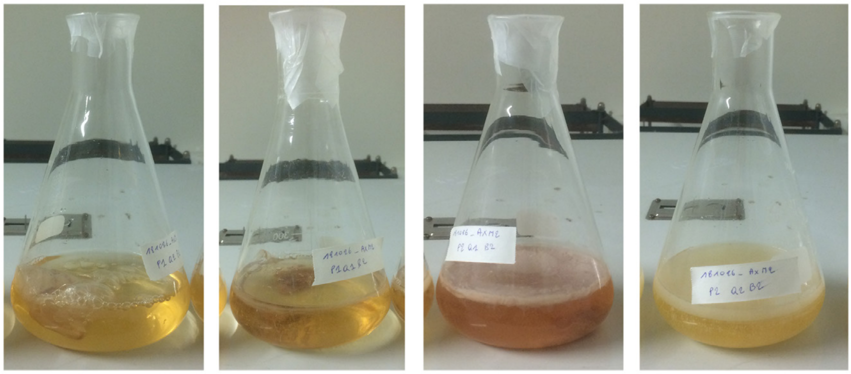
To define the most effective culture recipe in terms of quality and yield of the cellulose sheet, and to gain insight in the origin of the contaminations, a small comparative study was made by looking at **two variables** in the growing process. The first variable is the **growing medium**. Two possibilities exist. At first a recurrent DIY recipe obtained from an online manual published on *Biofabforum.org* on how to grow kombucha leather based on sugar and tea by *Winnie Poncelet (P1)* (Poncelet 2018) and secondly the HS culture medium that was already used in the previous experiments *(P2)*. The other variable is the **source of the AX bacteria**. The first source is the SCOBY mother (Figure 19a) *(Q1)* the second one is the living culture purified out of the SCOBY mother by means of the HS culture which feeds specifically this cellulose-producing bacteria, therefore it can be interpreted as a more pure culture, although this was not proven (Figure 19b) *(Q2)*. Three cultures each were started.

Flasks of 200 ml growing medium were inoculated with pieces of 2-4g from the mother culture's cellulose.

Type	Growing medium	AX source	#	Result after 12 days (Error! Reference source not found.)
P1Q1	DIY culture medium	Kombucha strain	3	- No BC development, only gas bubbles
P1Q2	DIY culture medium	Purified strain	3	+ BC development - not homogeneous, gas bubbles trapped inside
P2Q1	Hestrin-Schramm culture medium	Kombucha strain	3	- 1 flask contaminated + 2 flasks have continuous and homogeneous BC developments (1-3mm)
P2Q2	Hestrin-Schramm culture medium	Purified strain	3	+ very consistent BC development in all flasks + highest thickness (5-8mm)

Table 12
recipe test results

Figure 20
BC development after 12 days of incubation.



P1Q1
DIY culture medium
Kombucha strain

P1Q2
DIY culture medium
purified strain

P2Q1
HS culture medium
Kombucha strain

P2Q2
HS culture medium
purified strain

This small experiment confirms that at this stage the **most effective recipe** to grow cellulose is by using the HS culture medium with an already purified AX culture, which was isolated by using the same medium from the kombucha mother culture.

7.3. Bacterial strain

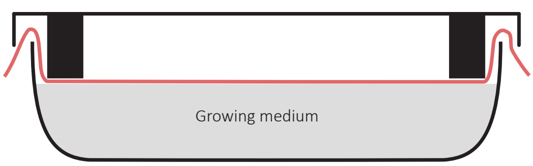
A possible problem causing the contaminations in the growing cultures could lie in the bacterial strain source. Therefore some alternatives were investigated.

A strain was thankfully received from researcher Tom Ellis, active in BC-research. Another one was obtained from the DSMZ library (German Collection of Microorganisms and Cell Cultures). The originally used strain remained unidentified until now. The BC-producing bacteria was identified by determination through amplification of the 16s ribosomal DNA, followed by sequencing and use of the Basic Local Alignment Search Tool (BLAST). This identification was executed by Simon Vandelook. The results confirmed that a BC-producing strain was present in the original Scoby mixture, namely Komagataeibacter xylinus. In small Erlenmeyers the growth of those three strains was compared. It appeared that the originally used strain, which is now identified, remained a good option. Therefore this one is chosen to continue working with.

7.4. In-situ composite test

A preliminary test for developing a composite of cellulose and a fibre net has been performed. The aim was to try a technique that would grow the cellulose as a matrix fixed to a fibre net directly in the sterile culture. The challenge is that the fibre net needs to be placed inside the sterile container, in a tensioned way, at the surface of the culture. Since the cellulose will grow on the surface of the culture, it should interfere with the fibre mat, and in the best case, attach to it, as shown in figure 22.

Figure 21
Scheme of composite exeperiment set-up



Two sheets were grown in a set-up as shown in Figure 21. Unfortunately, contaminations occurred along with the cellulose growth. Figure 22b shows the cleaned sheets, with the cellulose left. It adhered quite strongly on the fibre net, forming a composite. Also when the cellulose was air-dried, it remained as one with the fibre net (Figure 22c). Although the **cellulose adheres to the fibre net**, it is rather laid upon the net than completely embedded around it. This is to be improved in further experiments. Without any testing, it is already clear that **the fibre net in its composite appears to be stiffer** and will probably have a **higher shear strength** than the fibre net on its own.

Figure 22
Composite set-up (a); remaining cellulose adhered to fibre net (b); dried cellulose on fibre net (c)

7.5. Seam tests

Wherever fabrics are used, the seams form an important aspect of the production process. In architectural membranes, depending on the material different techniques are employed. PVC coated polyester fabrics are connected with high-frequency technology, PTFE coated glass fabrics can be connected thermally, silicone coated glass fabrics by means of vulcanisation and PTFE coated PTFE fabrics by stitching. (Blum, Bögner, and Némoz 2004) In order to assess BC as an architectural membrane, apart from its own strength, its connections and seams have to be examined. Multiple possibilities exist. Dried sheets being **stitched** together is an evident option, just as **glueing**.. More interesting would be that sheets can be **'grown together'** or **auto-combine** in some way, without any external intervention or addition, remaining in the same realm of techniques as the material itself. Furthermore, it could potentially be airtight, in comparison to sewn connections. This would enable inflatable structures.

Some preliminary tests were carried out for the 'growing together' or auto-adhesion of sheets without any addition or intervention (glue, heat, sewing). Multiple environment growing conditions for the joining of existing sheets are examined, listed below. The ideal situation would be that a strong bond is created in non-sterile, out-of-culture conditions. Making it possible to combine large sheets out of lab environments.

Sheet manipulation possibilities:

- When working with freshly harvested sheets, which will be done here:

1. the sheets can be superficially cleaned with water and ethanol, or thoroughly cleaned by means of an alkalic cleaning procedure involving boiling, as explained in 5.5.

2. In air conditions (out of culture) or again in culture liquid (wet).

3. Sterile (seams prepared under a laminar flow) or non-sterile (BC retrieved and seams prepared out of sterile conditions)

Air dried sheets have not been considered here. It doesn't seem likely that dry BC would join without intervention. From a **freshly harvested BC sheet** the samples are cut into rectangles and placed by two with an overlap of 2-3cm. The following tests were performed.

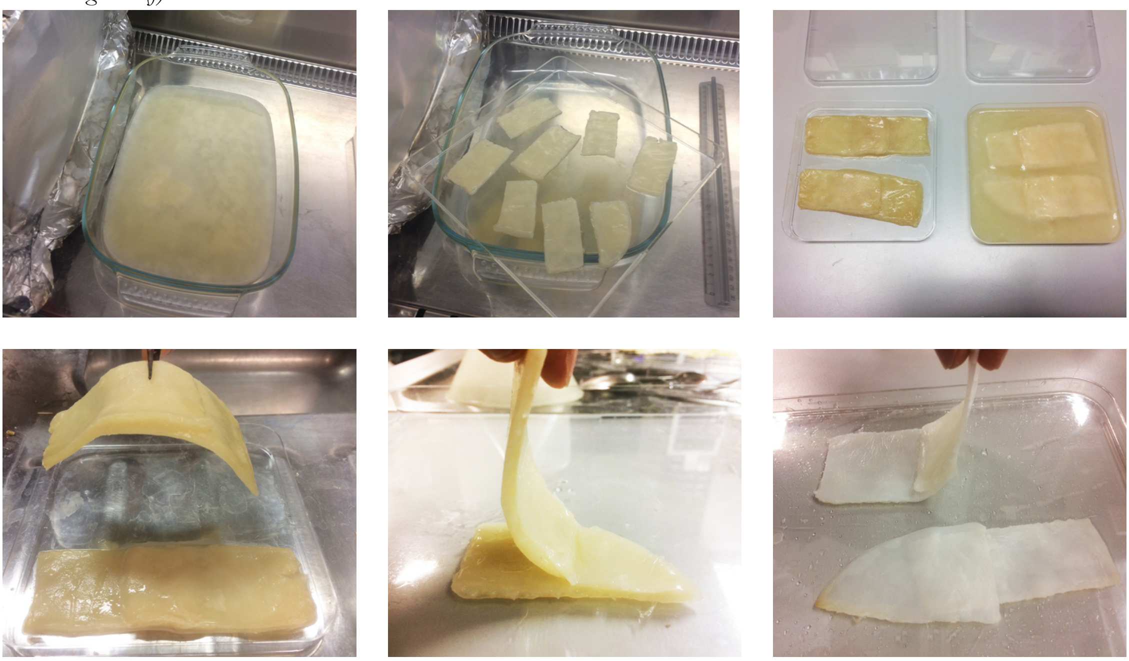
- P1: sterile growing conditions
- P2: non-sterile, sheets cleaned with $H_2 0$ and ethanol
- P3: non-sterile, alkalic boiling cleaning, following the process explained in 5.5.

Each test was carried out in a **wet (in culture liquid) (A)** and **dry (air contact) (B)** environment, with two samples for each, as shown in Figure 23c for P1.

Timeframe:

Grow plain BC sheet	9 days	Achieved thickness of +/- 5mm
Harvest and prepare seam samples		
establish seams, non-drying conditions	5 days	
Retrieve samples dry	1 month	

Figure 23
original sheet (a); sheet cut into rectangles (b); batch P1 after 5 days. Dry (left) and wet (right) conditions. On the right a new film of cellulose formed on the surface of the liquid (c); harvested batch P1 dry - continuous strong bond (d); harvested batch P1 wet - external new BC film connects both parts (e); harvested batch P3 dry - sticks together (f)



Batch	Conditions	Dry/wet	#	Contamination	Bond	
P1	sterile growing conditions	Dry	2	No	**Strong grown bond**	Figure 23 (d)
		Wet	2	No	Adhere to new grown film. No bond.	Figure 23 (e)
P2	non-sterile slightly cleaned	Dry	2	No	Weak grown bond	
		Wet	2	No	Adhere to new grown layer. Very weak.	
P3	non-sterile thoroughly cleaned	Dry	2	No	Sticks together lightly	Figure 23 (f)
		Wet	2	Yes	/	

Table 13
Non-dried seam test result

The strongest bond was created in sterile, dry conditions. The sheet were let to **air-dry completely** by placing them during one month at room temperature on a massive wooden plank, in order to assess the dried samples as well. The qualitative evaluation of the results are described in Table 14.

Batch	Conditions	Dry/wet	#	Description	Figure
P1	sterile growing conditions	Dry	2	- **Bond merged perfectly as if it became one sheet** - Bond itself seems stronger than rest of membrane - Not very flexible, quite brittle - Light brown transparent look	
		Wet	2	Identical to 'P1-dry'	
P2	non-sterile slightly cleaned	Dry	2	- **Bond merged perfectly as if it became one sheet** - Bond itself seems stronger than rest of membrane - More flexible than 'P1', still quite brittle - Dark coloured, not transparent	
		Wet	2	Identical to 'P2-dry' - Except for the transparency: more light brown with darker stains.	
P3	non-sterile thoroughly alkali cleaned	Dry	2	- **Bond merged perfectly as if it became one sheet** - Bond itself seems stronger than rest of membrane - **Ductile** and greasy feeling - **White transparent look**	
		Wet	2	(was dried despite contamination, no bond) - Strength and feeling very comparable to dry specimen - A bit more brittle, nevertheless still ductile - Slightly browner look, almost like 'P1' - Sheets remained more flat, unwrinkled and homogeneous	

Table 14
Dried seam test result description

Ranking in flexibility: P2 < P1 < **P3**

Ranking in transparency and visual look: P2 < P1 < **P3** (dry is best)

This experiment revealed interesting knowledge **on how two sheets can be attached together**, but also on the **plain cellulose** itself. These are the valuable **conclusions**:

Conclusions on the *seams*:

- During the air-drying phase, **all seams combined**. This means that **any cellulose sheet that dries attached to another one will combine** as if it became a continuous sheet with a double layer. A tensile test will have to define what the weakest point will be. The hypothesis is that, if both parts truly combine, the seam will be the strongest part since it is built out of a double layer.
- The **non-dried samples only grew together decently remaining in sterile conditions**, at air contact. Looking at how all samples joined while drying, the joining of sterile samples could be attributed simply to drying.

Conclusions on the *plain material*:

- An important working point is **the strength and ductility of the material**. The P3 sample, which was subjected to alkalic cleaning and already had a satisfying flexible result when wet, proved to be the only sheets not breaking easily after drying. It feels the most flexible and less brittle. A manual pulling force proves a certain strength.
- In terms of **visual look**, the P3 sample also is the most homogeneous, transparent and least wrinkled. The white colour and lack of stains give it a better outlook.
- In order to get to higher strength, a **higher thickness** will have to be reached

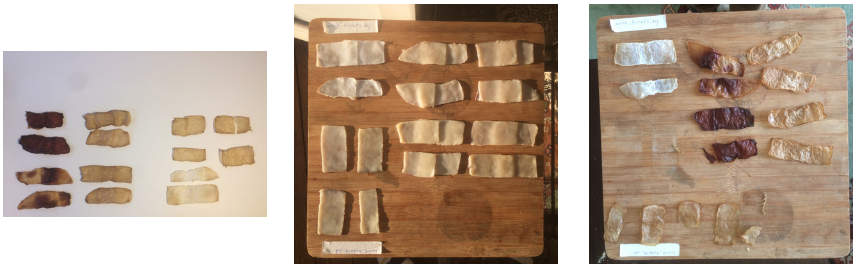


Figure 24
processed seam samples left to dry (a); dried seam samples after one month (b); Dried samples. P2 (left), P1 (middle), P3 (right). Dry (above), wet (below)

7.6. Glycerine soaking test

Dried sheets of bacterial cellulose can become quite brittle, they lose their supple and flexible characteristics while drying. A way to make the dried sheets gain flexibility again could be by placing them in glycerol as a plasticizer. A preliminary test is conducted here to have an idea of the result.

Two round bacterial cellulose sheets were harvested and air-dried. Both were cut in half, one half of each was soaked in glycerine (less pure glycerol) for 24h. After this time the sheets were retrieved and a maximum amount of glycerine was scraped off.

The harvested glycerine feels much more flimsy, thin, white transparent and very flexible. It also has grown in size. The strength seems a bit reduced, although the reduction in brittleness could improve it.

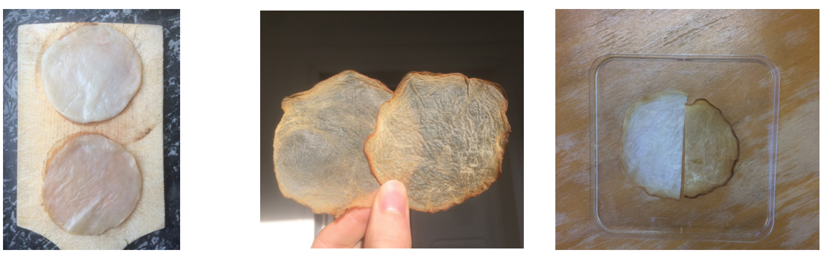


Figure 25
1) freshly harvested BC 2) dried BC 3) glycerine-soaked BC below, reference on top.

7.7. Heat press

A physical way of altering the appearance and properties of BC is by subjecting the material to heat and pressure. By means of a heat press both can be applied simultaneously. Multiple possibilities exist.

- plain wet BC as a way to dry the material.
- on dried BC to simply heat it.
- on mixed, wet BC as a way to dry the material (see 7.8).
- on in-situ grown composites (see 7.4).
- in order to create composites by combining BC and fibres by means of heat and pressure treatment.

An explorative session exploring a wide range of possibilities with the heat press, mixed BC, plain BC and fibres was conducted in order to extract some preliminary knowledge on what could be done further on and define the tests for the tensile tests. The used BC comes from a SCOBY, explaining the brown colour.

Figure 26
Plain heat pressed BC (a); double layer heat pressed BC (b); composite by heat pressing both matrix and fibres (c); heat press of mixed BC (d)

Some results are shown in Figure 26. Not all experiments were very satisfying. The plain BC was pleasing from the moment that an appropriate temperature, pressure and time range was found. Double layers as in (b) always jumped from each other due to the pressure, the adherence between BC and fibres in the composite trial (c) was not satisfactory. At last, treating mixed BC with a heat press made it impossible to remove the formed sheet from the aluminium used for pressing.

Figure 27
The heat press

7.8. Mixed BC

Following an example where mixed and dried BC proved a lot stronger than pure BC, this will be tried as well (Aqualose 2014). By means of preliminary test, SCOBY was mixed using a kitchen mixer and spread out in a rectangle in order to see whether it would combine. Also a composite version is tried

Figure 28
mixing SCOBY BC (1); spread out mixed BC (2); spreat out mixed BC on a fibre mat to create a composite (3)

The mixed BC did recombine in a satisfying sheet thus these examples will be reproduced with BC for tensile testing.

7.9. Conclusions on explorative research

The broad range of explorative experiments proved very important for the whole research. It allowed first and foremost to **get acquainted with the production of BC**. For example, as mentioned at the beginning of this chapter, some difficulties were encountered to achieve homogeneous pellicle growth. Experimenting with different cultures and different strains, varying growing recipients and sterile techniques and combining DIY and academic input made it possible to **build the base of experience that was needed** in order to be able to generate a steady amount of quantifiable results later on. Furthermore, it allowed to define the **optimal methods** of growing the BC.

The experiments also allowed to **try out different ideas and anticipate the outcomes** of some experiments in a preliminary phase. The successful experiments can then be picked up again in the following part, with or without alterations on the original way of working. After this experimental phase, it was possible to answer the research questions set at the beginning of the chapter. Then **an overview could be drawn of what tests would be performed in order to generate an interesting conclusion, how these would be performed and what research questions they raise**.

1. *What* **growing protocol** *will be used for all measurements (culture medium, bacterial strain, protocol)?*

After the culture (7.1), recipe (7.2), and bacterial strain (7.3) test it was concluded to work with the HS-culture medium and the originally used AX bacterial strain retrieved from the Kombucha mother culture. It appeared that the number of contaminations dropped drastically when using glassware and aluminium covering. The eventually used complete protocol is described previously under *Materials and methods (chapter 6).*

2. *Can* **composites** *of natural fibres with a BC matrix be created, and how?*

The growing tests showed that the in-situ technique which was tried can certainly be used, although an improvement on sterile working is necessary. A similar set-up as the one used in test 7.5 will be developed with glass dishes and aluminium covering, also finding a way to improve the uniform pre-tensioning of the fibres where the BC grows on. Due to the satisfactory result of a combined material with natural fibres, more ways of creating a composite will be investigated.

3. *How could the creation of* **connections/seams** *be fulfilled? Can these be created by growing the BC together without interference of a glue? If yes, how and in what conditions?*

The seam tests (7.5) revealed very interesting conclusions. Leaving two pieces of BC together to dry will combine them into one, and the strength of this connection feels already very satisfactory. No sterile conditions or the presence of a culture medium is necessary. Therefore the investigations concerning seam connections will be maintained, a strength comparison with simply sewn BC sheets will be performed too.

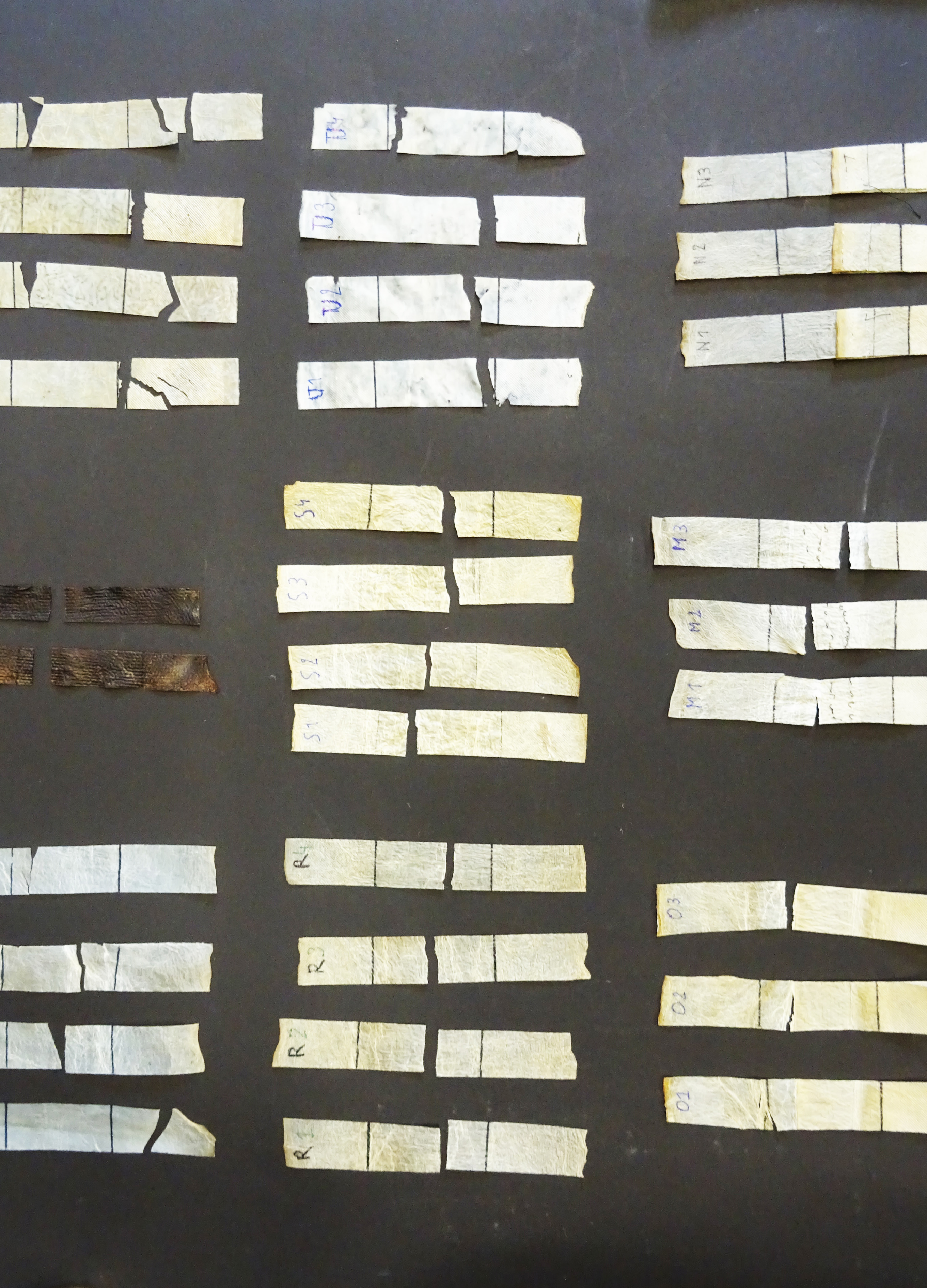
4. *What* **post-processing protocols** *such as soaking in plasticizers can be performed on BC? What is their influence? What is a decent soaking duration?*

Soaking BC in glycerol had a positive influence on the appearance of the material, as it became white and more opaque. The flexibility also drastically increased. The influence on strength but also on watertightness have to be defined. As post-processing soaking generates good results, other soaking liquids will be looked for. The practised duration of 24h was satisfactory and will be maintained.

5. *What is the influence of a* **heat press** *treatment on BC?*
   A heat press dries the BC in a matter of minutes. Different possibilities were tried but to conclude, it is clear that placing a blank sheet of wet BC in the heat press has a good result, its influence on the tensile strength will be assessed. Placing a dried sheet in the heat press will be tested too, just as a test with in-situ grown composites.

6. *Can a sheet of* **mixed** *BC be created?*

Yes, and the result is satisfying. A plain BC mixed sample and a composite of fibres with mixed BC will be created.


N3
N2
N1
S4
S3
S2
S1
M3
M2
M1
R4
R3
R2
R1
O3
O2
O1

8 Quantitative experiments

After a round of explorations on a wide range of aspects dealing with BC, this chapter will take aspirations from the results of the explorative research and develop quantitative results on the material. The explorative research conclusions together with further research enabled the possibility to make a list of all samples which will be created for quantitative testing. All samples eventually produced and tested are listed in Table 16.

The samples can be divided into different **categories** that are also mentioned in Table 16.

- **Reference samples**: different original states of BC are quantified, enabling comparison between these common states of the material, but mainly with the other samples.
- **Plain sheet post-processing**: multiple samples deal with altering the plain BC sheet itself by soaking in different liquids or applying a coating, for strength, ductility or watertightness purposes. Heat pressed and mixed BC are also investigated as plain sheet alterations.
- **Composite:** different variations on composed samples of fibres and BC are investigated.
- **Seams**: samples with a connection of BC are created to measure its strength.
- **Mycelium**: in the introduction another possible biomaterial is mentioned. Three heat pressed and plasticized mycelium samples are provided by *Elise Elsacker* and will be tested too.

In this important chapter, which constitutes the main part of the research, the following general research questions, firstly stated in chapter 2, are dealt with:

> **RQ 3** What is the strength of self-grown bacterial cellulose?
>
> **RQ 4** What post-processing techniques can improve the strength of bacterial cellulose?
>
> **RQ 5** What are the mechanical strengths of these post-processing techniques?
>
> **RQ 6** What are the influences on the expression of the material (texture, suppleness, colour) of these post-processing techniques?

The analysis of each experiment is divided as follows. The **post-processing** is elaborated further on. For each sample a context and background are drawn, specific research questions are listed and answered, the production protocol is explained and the material is already assessed visually. Then the tensile test results are listed.

After the characterisation of each sample, the post processings are evaluated in paragraph 8.2. Then the tensile test results are compared and evaluated in 8.3, followed by a water absorbency overview in 8.4. All of these results are placed in an overview in paragraph 8.5.

Some comments on the shown results

Some samples did not make it to production and testing for diverse reasons, although the research preparation was made. These are listed in *Other samples* at the end of this chapter.

The **measurements of the samples** comprises thickness and weight in different stages of the production process. The **thickness measurement** is needed to calculate stresses from the tensile test. The **weight evolution** of a sample is most relevant when assessing the influence of post-processing soakings (experiments D to J, as listed in Table 16) or to know the shrinkage rates of wet BC (Table 17). In annex 10.2 the complete overview of all measurements is given. The thicknesses are defined as the average of 6 (for sheets) or 4 (for samples) thickness measurements on the same sample.

For the experiments A to J (see Table 16) the four original sheets where they were cut from were measured in wet and dry state in order to **calculate average shrinkage rates from wet to dry of thickness and weight**, listed in Table 17, placed in annex 10.4. On average, the produced cellulose lost 98% of its weight through water evaporation, which can be defined as the moisture content of freshly harvested BC.

Table 15
First sheets: weight and thickness measurements with shrinkage rates, placed in annex 10.4.

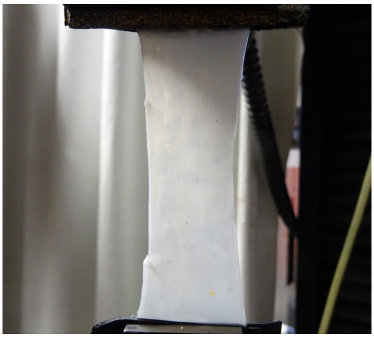
For all tests the weight and thicknesses of the final samples will be mentioned. When available and if relevant, weights of other phases of the experiments are listed.

The tensile tests of each experiment defined in the previous paragraph are performed following the defined methodology in 6.6 and 6.7.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Drying technique		Composite	Seams	Mixed BC	Post processing	Number of samples
					Air dried	Heat Press					
A	Undried, wet	Reference								/	3
B	Only air dried	Reference			x					/	4
L	SCOBY	Reference			x					/	2
C	Alkalic cleaning, air dried	Reference		x	x					before drying	4
K	Alkalic cleaning, air dried	Reference		x	x					before drying	4
D	Ethylene glycol	Soaking	Ethylene Glycol	x	x					after drying	4
E	EGCC	Soaking	EGCC	x	x					after drying	4
F	Glycerol	Soaking	Glycerol	x	x					after drying	4
G	Coconut oil	Soaking	Coconut oil	x	x					after drying	4
H	Beeswax	Soaking	Beeswax	x	x					after drying	4
I	Coconut oil and Beeswax	Soaking	Coconut oil and Beeswax	x	x					after drying	4
J	Citric acid solution	Soaking	Citric acid solution	x	x					after drying	4
M	Sewn wet and dried	Seams		x	x			x		/	3
N	Dried and sewn	Seams		x	x			x		/	3
O	Seam by drying	Seams		x	x			x		/	3
Q	Drying by heat press	Heat press		x		x				/	3
R	Dried and heat pressed	Heat press		x	x	x				/	4
S	Double layer by drying	Seams		x	x					/	4
T	Mixed	Mixed		x	x				x	/	4
U	Mixed, spread on fibers	Composite		x	x		x		x	/	3
V	Composite fibres	Reference		x			x			/	3
W	Grown comp, air dried	Composite		x	x		x			/	5
X	Grown comp, heat press	Composite		x		x	x			/	4
Y	Composite by drying	Composite		x	x		x			/	4
MYC	Plasticised mycelium	Mycelium	Glycerol			x				/	8

Table 16

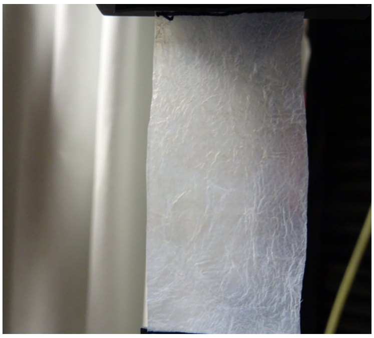
Overview and classification of all performed tests



A
undried, wet

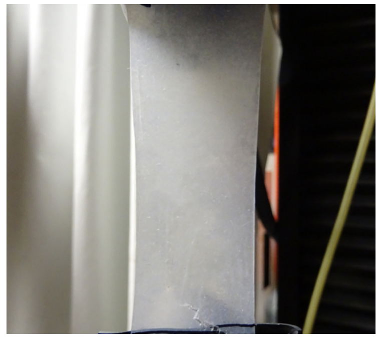
B
air dried

C
alkalic cleaning, air dried

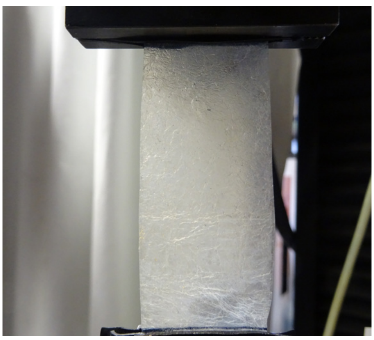


D
ethylene glycol soaking

E
EGCC soaking

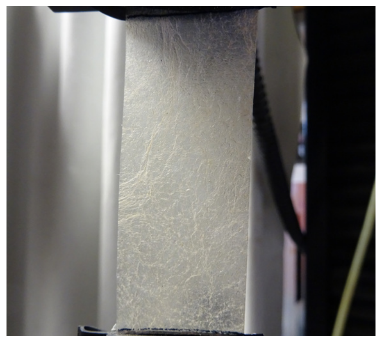


F
glycerol soaking



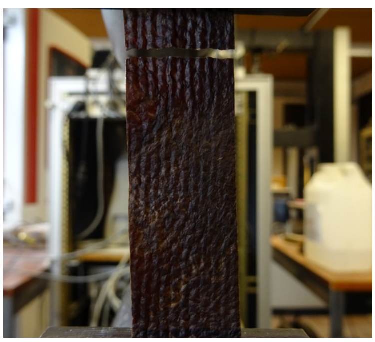
G
coconut oil coating

H
beeswax coating

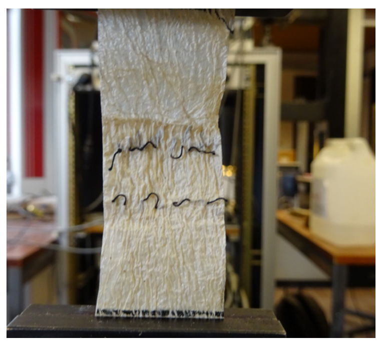


I
coconut oil and beeswax coating

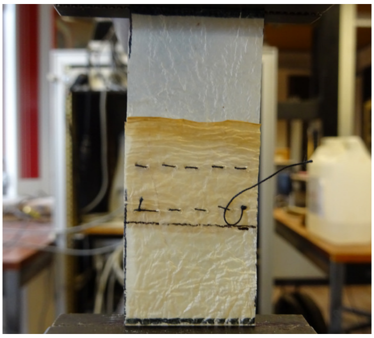
J
citric acid soaking



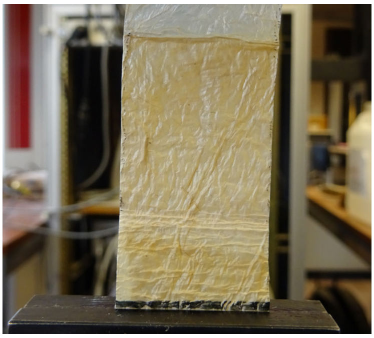
L
SCOBY



M
sewn wet and dried



N
sewn

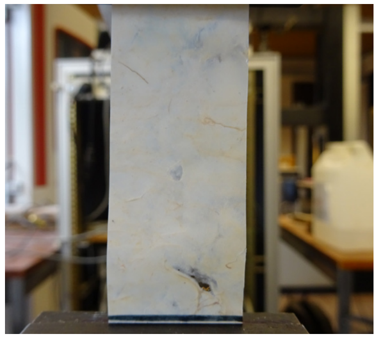


O
combined through drying

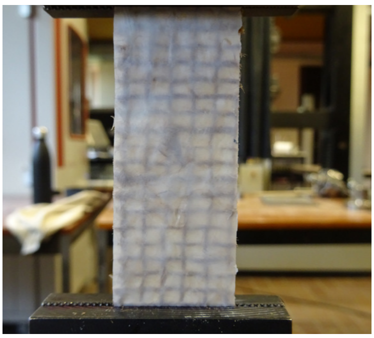
Q
heat press dried

R
heat press on dried sample

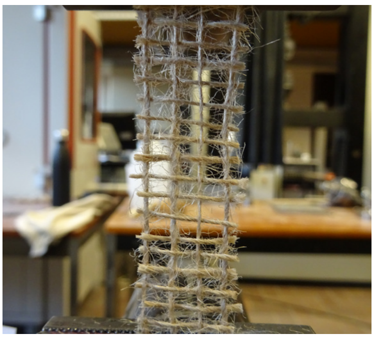
S
combined through drying



T
mixed



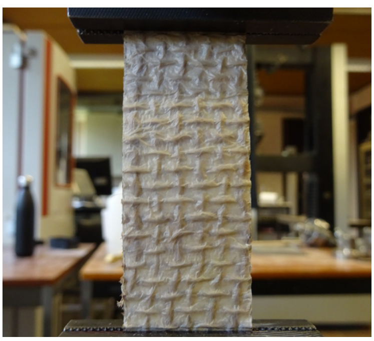
U
mixed, spread on fibres



V
only fibres

W
grown, air dried

X
in-situ grown, air dried



Y
in-situ grown, heat press

MYC
glycerol soaked and heat press

Four reference samples are defined. The most important one is sample C (alkalic cleaning and air-drying) as it is the preparation protocol used for all other samples. Other reference samples reflect different states of the material: non-dried (A), air-dried without alkalic cleaning (B) and air-dried samples of SCOBY cellulose (L).

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
A	Undried, wet	Reference								/	3

As a reference towards sample B and sample C, the plain undried material is tested. This is the state right after harvesting, before drying, containing approximately 98% water (Table 17, annex 10.4). This will be interesting in order to know how the drying process influences the tensile properties. The wet BC feels very strong, sturdy and is impossible to tear apart by hand pulling force. It is also flexible, folding goes without issues. The hypothesis is that freshly harvested BC will have satisfying strength results. Of course wet BC can at first sight not be used in a potential architectural context, as the moisture content would fluctuate, also the strongly increased weight

Preparation protocol

- Harvest the sheets of BC, which were grown as mentioned in 6.2.
- Rinse in distilled water, removing slimes
- Immerse in ethanol.
- Rinse and cut into 25mm wide samples.
- Store in submerged conditions until testing in order to sustain wet conditions

These samples were harvested and cleaned 7 days prior to testing. In order to keep them wet, they were placed into culture liquid in sterile conditions.

Figure 29
harvesting of wet BC

Contaminations occurred, thus they were placed in ethanol for half of the week. It is possible that ethanol has an influence on the results.

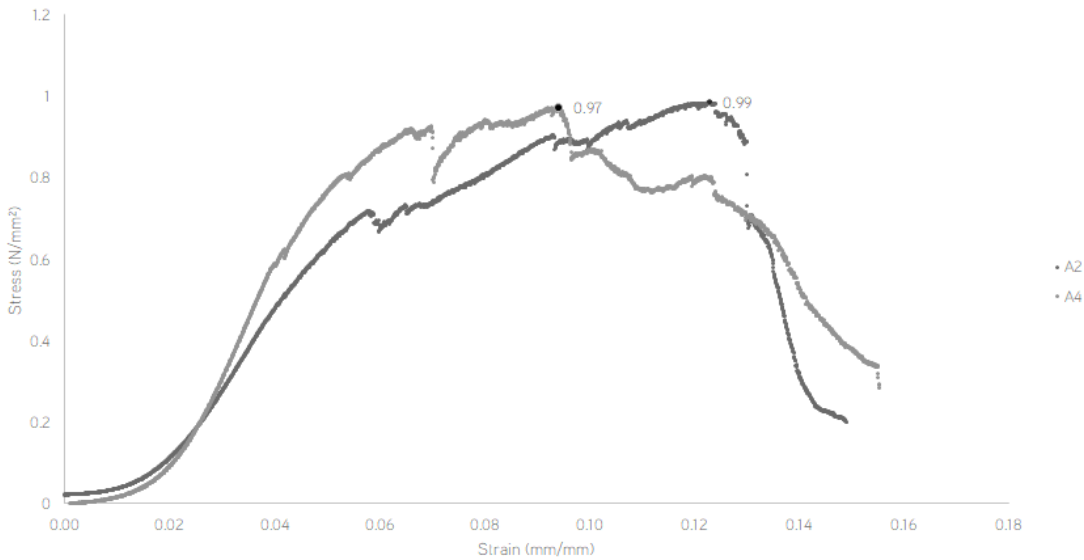
Sample measurements

Unfortunately, thickness and weight measurement for the separate samples were not performed. However the average thickness of the original sheet was 0.7mm, the thinnest of the four sheets used for tests A to J.

What does freshly harvested BC look and feel like?

Wet BC feels very strong and difficult to tear. The colour is yellow-white and slightly translucent. The texture reminds one a lot of squid.

Tensile test results



Average break stress	0.68 +/- 0.42	Mpa
Average break strain	0.09 +/- 0.03	mm/mm
~~Average Young Modulus~~	~~0.01 +/- 0.01~~	~~Gpa~~

Comments on tensile tests

The wet samples were difficult to clamp decently. The water pressure created in the samples, caused in the hydraulic clamps, broke the first sample A1. Sample A3 also broke quickly close to the clamp. sample A2 and A4 did have a gradual break over their length, combined with narrowing (Poisson) of the sample.

In the comparison with other protocols, it is important to note that the thickness where the stress is based upon consists for 95% out of water.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
B	Only air dried	Reference			x					/	4

An easy way to treat the BC is by simply growing it and then leave it to air dry, as is studied here. The alkalic treatment (sample C) which is explained further on is the production methodology for all samples. The air dried samples studied here serve as a comparison in order to understand the influence of the alkalic treatment, which is explained further on.

The most straightforward production process of BC is to harvest the wet sheet and leave it to air-dry. This is also what is often carried out by designers or in the DIY community. Depending on the conditions, in a couple of days the BC can achieve complete dry states, visually perceivable. Different methods of air-drying exist in DIY realms. A common one is to spread the wet sheet on a massive wooden board. The wood also absorbs part of the water, but since the air-touching side dries quicker, regular flips of the sheet are advised to have equal drying. Another method is to stretch the wet BC in a frame ("Turning Kombucha SCOBY into Leather - YouTube" n.d.). Similar to the wooden plank technique, in the following experiments the sheets were dried between layers of absorbing tissues.

Preparation protocol

- Harvest the sheets of BC, which were grown as mentioned before.
- Rinse in distilled water, removing slimes.
- Immerse in ethanol.
- Rinse
- Place at air contact on a wooden plank or absorbing sheet of tissue until dry, with regular flips.

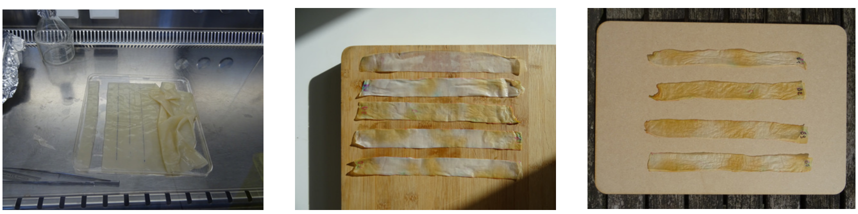


Figure 30
Simply air dried BC samples

For these tests, the samples were cut before drying. This has the disadvantage that **shrinking** alters the width of the samples. It is more preferable for the tensile stress calculation to have precise knowledge about the section of the sample, therefore from now on samples will be cut after drying, to achieve constant width over the length of the sample.

Sample measurements

DRY SAMPLE	**dry weight** (0.01g)	**thickness** (0.01mm)
B1	21	7
B2	24	6
B3	23	7
B4	21	6

What does air-dried BC look and feel like?

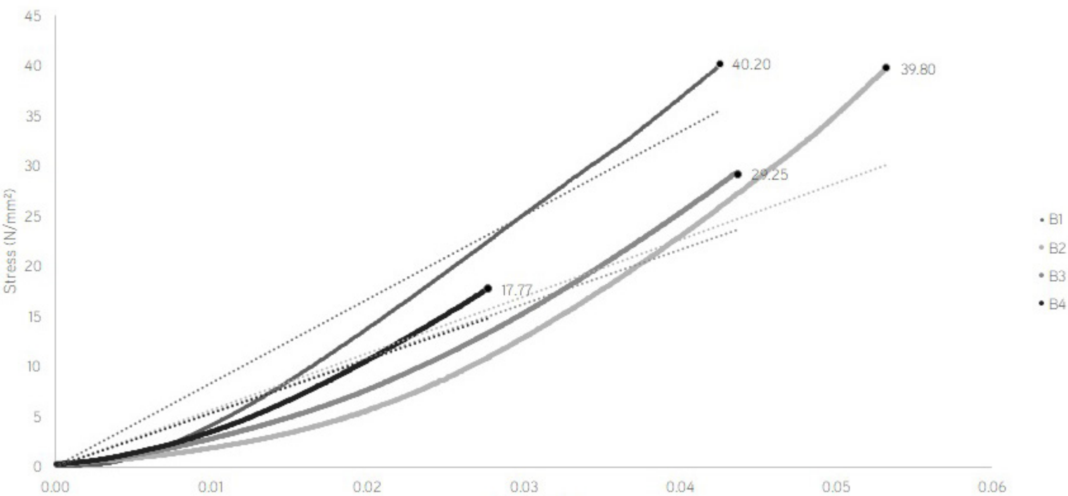
The dried BC was in this case quite thin, as it also came from the thinnest sheet (sheet four in Table 15). A white brownish colour was achieved together with a flexible appearance, the material does not break upon folding.

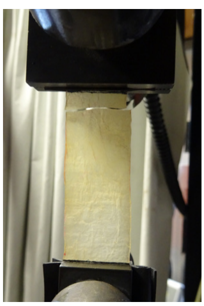
Is the air-drying technique between absorbing tissues satisfactory? Is it efficient, providing good and reliable results?

As mentioned higher up, the samples shrunk and thus have developed uneven widths. Because simple air-drying was carried out, wrinkles and warping also occurred in the samples. Therefore the next samples will need a way to keep the BC flat and unwrinkled.

In comparison to the undried freshly harvested BC, the material does feel quite brittle and less sturdy. The tensile tests will quantitatively asses this hypothesis.

Tensile test results





Average break stress	31.75	+/- 9.19	Mpa
Average break strain	0.04	+/- 0.01	mm/mm
Average Young Modulus	97.59	+/- 168.25	Gpa

Comments on tensile tests

The reference samples were cut into specimens before drying. They had a width of 25mm before drying and shrunk afterwards. Therefore the width was remeasured before testing.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
L	SCOBY	Reference			x					/	2

While all used BC material in this thesis was produced in controlled lab environment in order to ensure the reliability of the measurements, the whole DIY community relies on home-grown experiments with SCOBY, as explained in 4.2. The production of this version of BC seems to be varying a lot because of different techniques, conditions, ingredients etc. Also the results are very diverse, as can be seen on forums such as Biofabforum.org. The aim of this sample is to have an indicative insight in the difference between lab-grown and home-grown BC.

Both brewings differ in the following ways. Lab-grown BC, as elaborated in 6.2, should only contain the desired bacteria in order to develop reproduceable results. The culture medium specifically interacts with this bacteria. Environment conditions are controlled and sterility is ensured throughout the process, from the autoclave through the laminar flow to the sealed containers. On the other hand, SCOBY consists of a mixture of bacteria and yeasts which interact with each other and form a symbiotic culture. Therefore a SCOBY culture should be more resistant to contaminations, hence its application for DIY. Also the yield could be higher since the yeasts can help to convert the carbohydrates, although this is only an assumption. The downside is that identifying the culture and understanding its functioning is more difficult. For this experiment a SCOBY was obtained as a by-product of kombucha brewing, growing conditions and production protocol are unknown.

This experiment also relates more specifically to

RQ 3 *What is the strength of self-grown bacterial cellulose?*

Preparation protocol

The SCOBY was retrieved from a Kombucha brewing. Alternatively, it can be grown following a recipe by *Winnie Poncelet* found on the *Biofabforum* (Poncelet 2018). A **short summary of a SCOBY production protocol** is listed in annex 10.5 For further information the website can be consulted.

Figure 31 *SCOBY recipe proportions. Image retrieved from Biofabforum.org, created by Open Biofabrics. Placed in annex 10.5*

Only two samples were possible to cut out of the SCOBY noting that strongly varying thicknesses were tried to be avoided.

Figure 32 *The texture of freshly harvested SCOBY (a); dried BC (b) (c).*

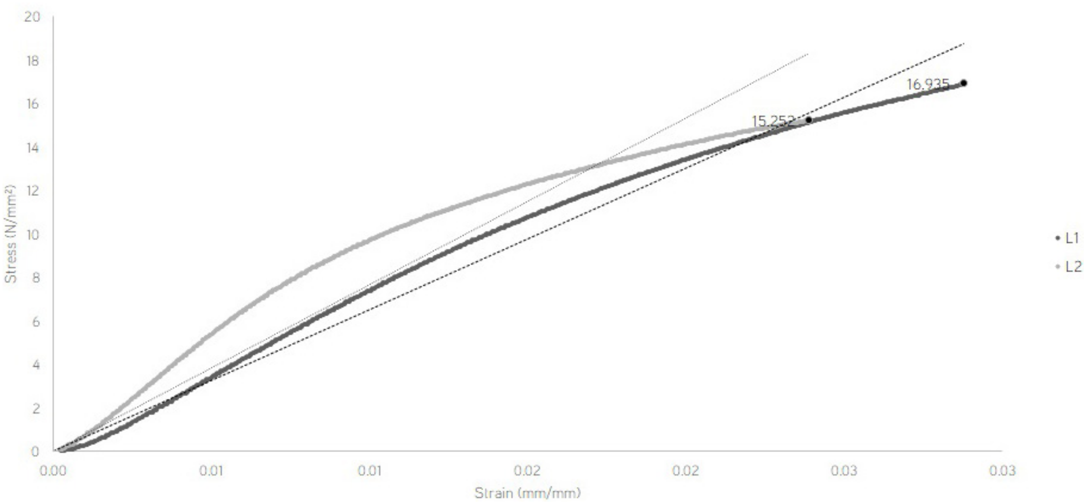
DRY SAMPLE	**dry weight** (0.01g)	**thickness** (0.01mm)
L1	59	48
L2	63	48

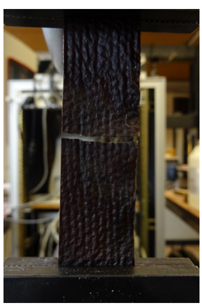
What does air-dried SCOBY look and feel like? How does it compare to lab-grown bacterial cellulose?

As shown in Figure 31 (a), the **wet SCOBY is a lot less clean and homogeneous** compared to lab-grown BC in terms of colour, thickness, texture. Multiple layers of cellulose are not always perfectly combined and can be teared apart from each other. The thickness variations are probably due to the presence of gas in the fermentation liquid. This is a lot less present in the lab-grown BC as yeasts are not active. The irregular outcome of SCOBY is an important shortcoming as it will have an import influence on strength, since a constant thickness can difficultly be achieved. Further research could develop a recipe of a symbiotic culture with increased resistance but without the fermentation. The material itself has the same squid-like feeling as the lab-grown variant. It is not clear whether the composition of the BC differs.

When **dried** the sheets became very dark as can be seen in Figure 31 (b) and (c). The material feels strong but brittle. It has lost all flexibility and could break upon folding. The reason for this is unclear and not further researched. This already shows that the results for comparison might not be reliable at all, since very flexible SCOBY materials have been created commonly, like the examples listed in 4.2.

Tensile test results





Average break stress	16.09	+/- 0.84	Mpa
Average break strain	0.03	+/- 0.00	mm/r
Average Young Modulus	0.71	+/- 0.06	Gpa

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
C	Alkalic cleaning, air dried	Reference		x	x					before drying	4

As previously elaborated in 6.3, alkalic cleaning of the harvested sheets is common in literature. It kills all present bacteria and renders the sheets very white, slightly translucent and clean. The preliminary comparative tests in 7.5 also showed the most satisfying results for this alteration. Because of this neutral and pleasing outcome this technique is used as the base material for all further BC experiments. This is thus the **main reference material** to be compared to.

Preparation protocol

See 6.3

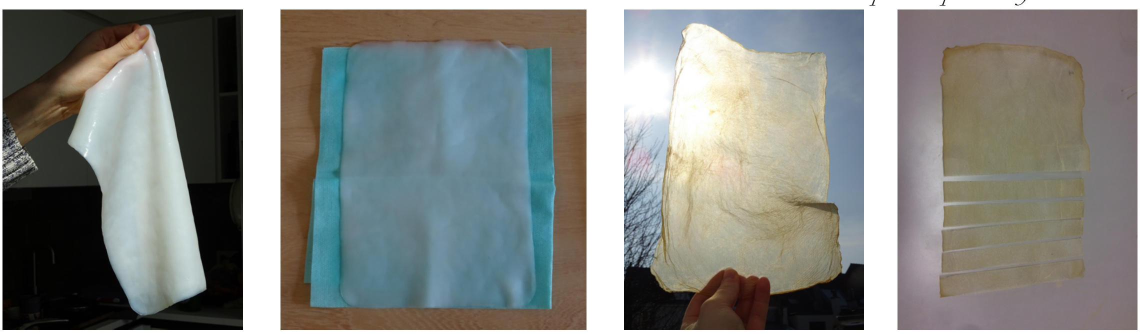


Figure 33
Overview of protocol explained previously in 6.3.

Sample measurements

DRY SAMPLE	dry weight (0.01g)	thickness (0.01mm)
C1	18	17
C2	26	22
C3	32	22
C4	35	21

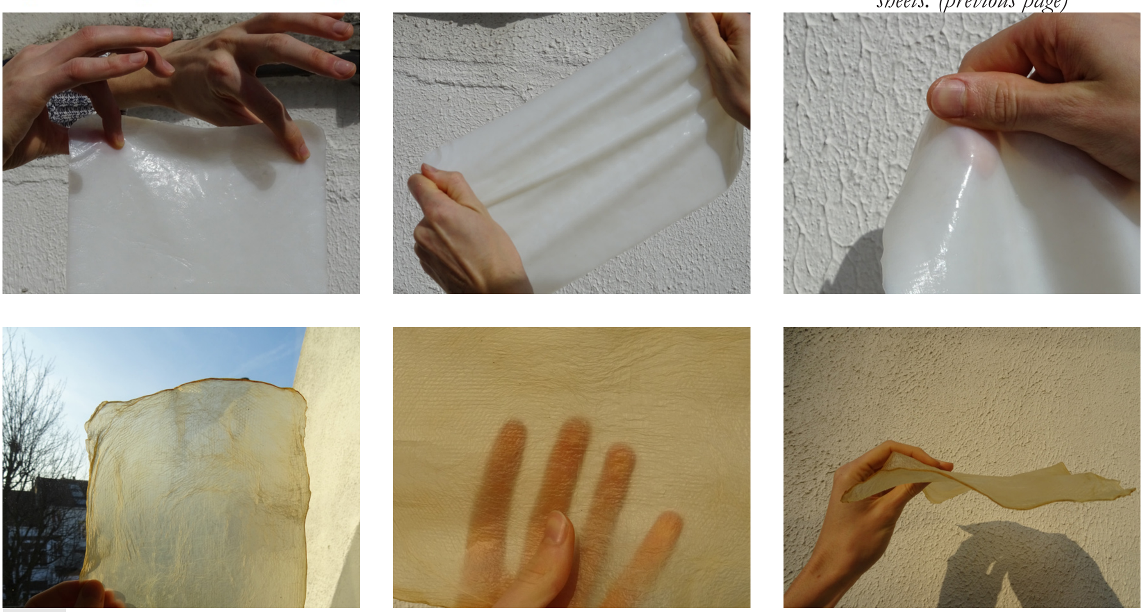
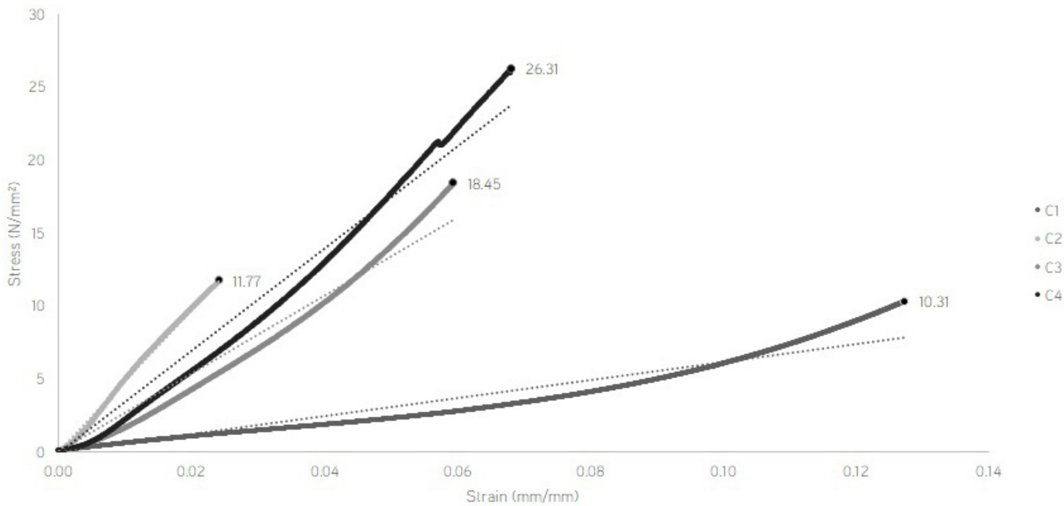


Figure 34
Freshly harvested (above) and dried (below) BC sheets. (previous page)

C2 *What does air-dried BC look and feel like?*

As mentioned before, the material has a satisfying white appearance when wet and dries also into a quite homogeneous sheet as shown in Figure 34. The flexibility is promising as it can be gently moved and folded, but it still feels too brittle to fold without any deterioration.

Tensile test results



Average break stress	16.71 +/- 6.34	Mpa
Average break strain	0.07 +/- 0.04	mm/mm
Average Young Modulus	0.18 +/- 0.10	Gpa

Comments on tensile tests

All samples had brittle failures without any necking. Slip did not occur. The stress-strain lines do show quite varying results. sample C1 had a very long strain in comparison to the other samples. Overall, the results are quite reliable.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
K	Alkalic cleaning, air dried	Reference		x	x					before drying	4

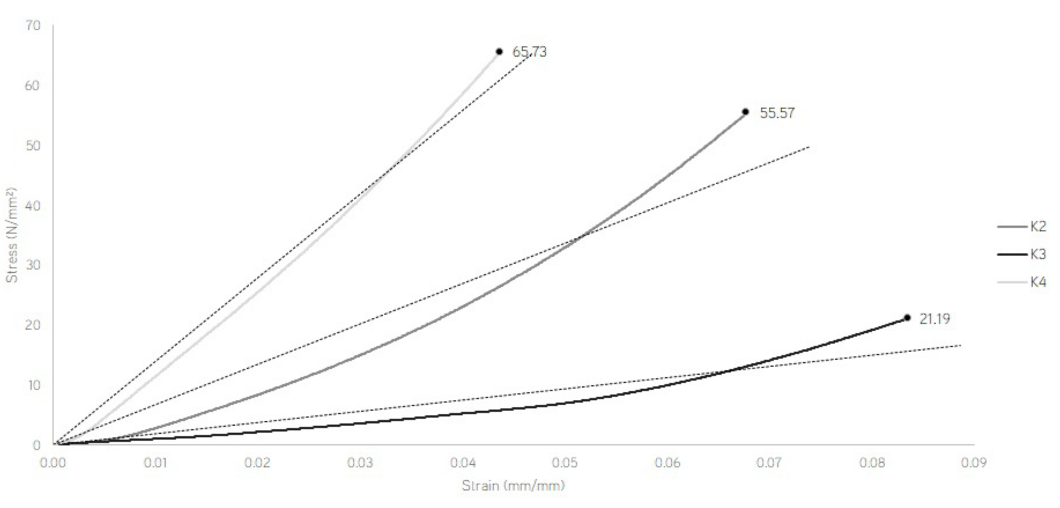
The execution of all tests was divided in two rounds. The reference samples C are used as reference for the first round. For the second round reference samples K are used. Both protocols are identical but to avoid any irregularities between both rounds, a separate reference material that is made at the same time and out of the same BC as the sample itself is used.

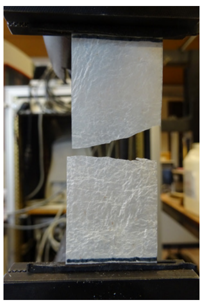
The measurements are the following:

DRY SAMPLE	dry weight (0.01g)	thickness (0.01mm)
K1	27	9
K2	27	13
K3	28	15
K4	27	11

In comparison with reference samples C, a lower thickness was reached but a more consistent weight was achieved.

Tensile test results





Average break stress	47.50	+/- 19.06	Mpa
Average break strain	0.06	+/- 0.02	mm/m
Average Young Modulus	0.62	+/- 0.48	Gpa

Comments on tensile tests

In comparison with C, which has an identical protocol but was produced at a different time, the results also vary quite a lot. Looking at the data, the stress and stiffness are much higher. The reason for this is unknown, but it proves the need of having more reference samples from different growing batches.

This series of **soaking experiments** deals with an attempt to alter the properties of the plain BC, to render it more flexible or make it water repellent. The influence on other factors is also assessed.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
D	Ethylene glycol	Soaking	Ethylene Glycol	x	x					after drying	4

Bacterial cellulose can be influenced on its molecular level to influence the structural functioning. In this case Ethylene Glycol $(CH_2OH)_2$ is used to induce crosslinking between the cellulose fibrils (S. Lee, Tong, and Yang 2016). **Crosslinking** creates a network of the cellulose chains by inducing connections through the addition of a catalyser. A study reported a multiplication factor of 3-4 in terms of tensile strength and a decreased strain with an approximate factor 4 for crosslinking of native cellulose with another compound, epichlorohydrin. Furthermore this crosslinking would inhibit thermal expansion (Guo, Chen, and Yan 2013)flexible, transparent, and very strong cross-linked celluloses have been prepared by cross-linking free cellulose chains with epichlorohydrin (ECH).

From an environmental perspective, ethylene glycol is an organic compound often used in antifreeze and often reaches ecosystems at airports due to airplane de-icing. Ethylene glycol is not harmless for organisms and plants. Adverse effects are reported for aquatic and terrestrial plants and microorganisms, although the compound breaks down in a short timespan (Health Canada 2000). This mainly applies to the substance spread in the environment. Therefore, if the results of this experiment are satisfying, a more profound assessment of risks will have to be conducted concerning toxicity.

The hypothesis for this crosslinking treatment on BC is that strength will increase while becoming more brittle. Due to the crosslinking some hydrophobicity could be introduced. The influence on the visual outlook is not known. The ethylene glycol treatment will be performed on the standard base BC material: alkalic air dried samples.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C)
- Dried sheets are cut into specimen rectangles, labelled, weighed and measured.
- Submerge during 24h in ethylene glycol.
- Retrieve and remove excess liquid.
- Weigh and measure thickness again.

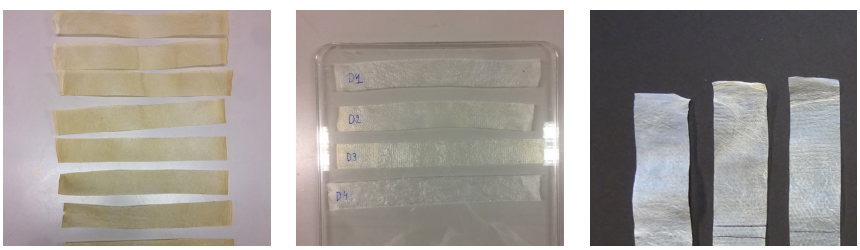


Figure 35
Alkalic cleaned and air dried samples (a); D-experiments submerged in ethylene glycol for 24h (b); resulting samples (c).

Sample measurements

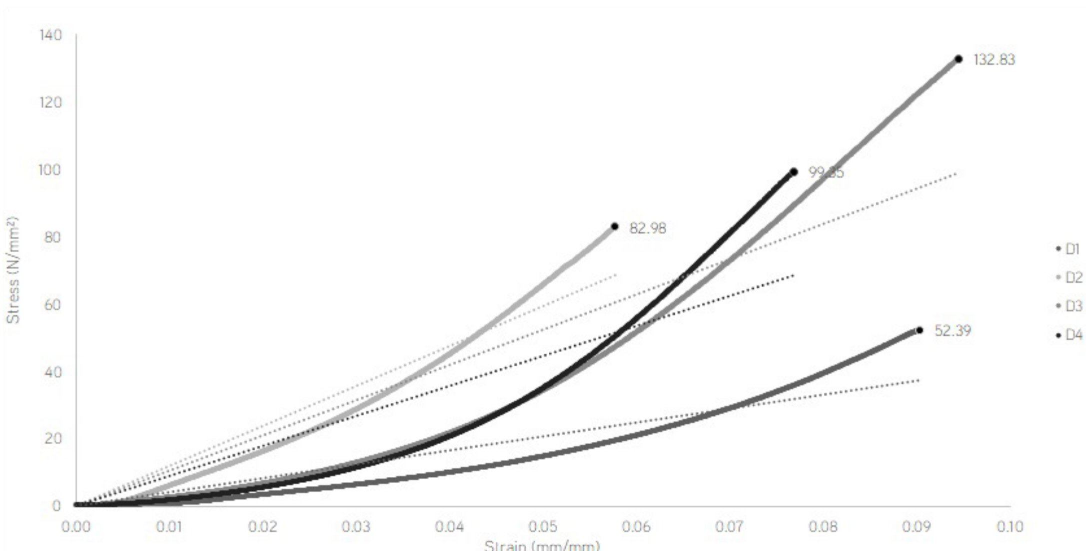
For these post-processing experiments dealing with molecular influences on BC, it is important to measure weight and thickness variations before and after processing. These are shown in the table below. The **ethylene glycol soaking diminished the thickness of BC by more than half**, while **weight increased by 20% on average**. A possible explanation is that molecules of ethylene glycol have been introduced in the BC, possibly to create cross-linking.

	before processing		*after processing*		*processing variation*			
DRY SAMPLE	**dry weight** (0.01g)	**thickness** (0.01mm)	**dry weight** (0.01g)	**thickness** (0.01mm)	**Δ thickness** (0.01mm)	**Δ thickness** (%)	**Δ weight** (0.01g)	**Δ weight** (%)
D1	18	17	22	4.75	-11.75	-71%	4	22%
D2	30	19	37	6.75	-12.5	-65%	7	23%
D3	34	20	43	7.75	-12.5	-62%	9	26%
D4	15	9	16	4.5	-4.25	-49%	1	7%
				average	*-10.25*	***-62%***	*5.25*	***20%***

What is the influence of ethylene glycol on the look and feel of BC?

In Figure 35 (c) the eventual material is pictured. It became a lot whiter and more transparent. Being thinner, it feels a bit flimsy. Also the flexible feeling increased. Overall the appearance is very satisfying. It could be interpreted as less strong, but a tensile test will have to define its tensile strength.

Tensile test results



Average break stress	91.89 +/- 29.03	Mpa
Average break strain	0.08 +/- 0.01	mm/mm
Average Young Modulus	0.89 +/- 0.29	Gpa

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
E	EGCC	Soaking	EGCC	x	x					after drying	4

Choline Chloride (CH3)3NCH2CH2OH]Cl is a salt which is mass produced for animal feed or as a clay stabilizer ("FracFocus Chemical Disclosure Registry" n.d.). Mixed with the previously used organic compound ethylene glycol, a deep eutectic solvent (DES) is formed, ethylene glycol choline chloride (EGCC). These exist out of a hydrogen bond donor (ethylene glycol) and hydrogen bond acceptor (choline chloride). (Ren et al. 2016). The influence of this **crosslinking agent** on air-dried BC will be assessed. The same possibilities for cross linking as in the previous experiment reside.

A study covered biodegradability and toxicity of choline chloride-based DESs, more precisely with glucose, glycerol and oxalic acids. The conclusion stated that these DESs have satisfying results for use in ecological-oriented applications (Radošević et al. 2015). They are seen as 'a new class of green solvents' due to low toxicity and biodegradability (Zdanowicz, Wilpiszewska, and Spychaj 2018)? This already compares better to the toxicity of ethylene glycol mentioned before.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C)
- Dried sheets are cut into specimen rectangles, labelled, weighed and measured.
- Prepare the EGCC mixture with 2:1 molar mass ratios by means of heating and stirring.
- Submerge during 24h in the EGCC mixture
- Retrieve and remove excess liquid.
- Weigh and measure thickness again.

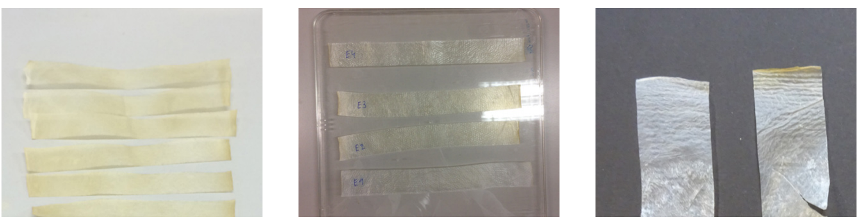


Figure 36
Alkalic cleaned and air dried samples (a); E-experiments submerged in EGCC for 24h (b); resulting samples (c).

Sample measurements

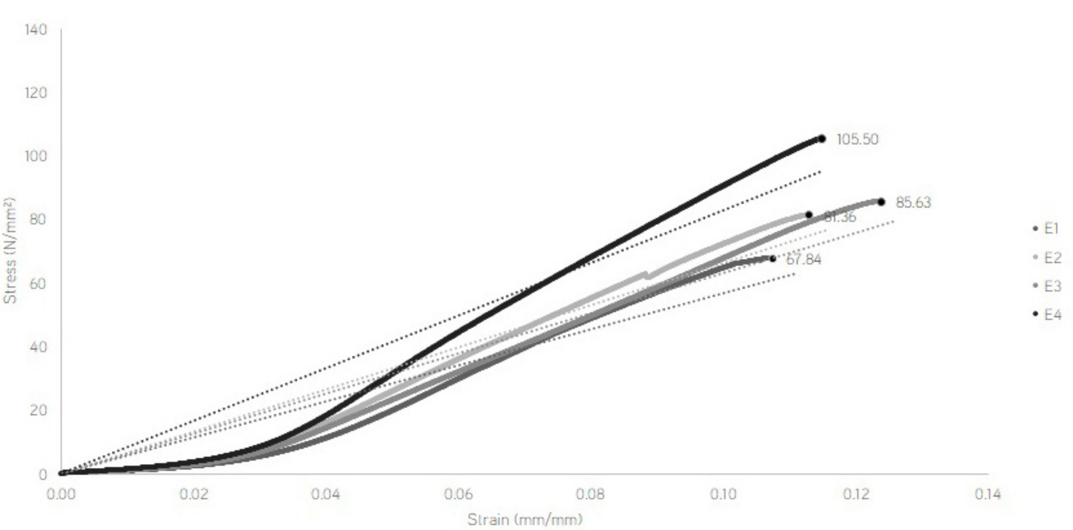
DRY SAMPLE	*before processing* dry weight (0.01g)	thickness (0.01mm)	*after processing* dry weight (0.01g)	thickness (0.01mm)	*processing variation* Δ thickness (0.01mm)	Δ thickness (%)	Δ weight (0.01g)	Δ weight (%)
E1	19	15	30	5.00	-9.50	-66%	11.00	58%
E2	31	21	50	9.00	-11.50	-56%	19.00	61%
E3	34	24	60	10.00	-14.25	-59%	26.00	76%
E4	35	23	60	9.25	-13.75	-60%	25.00	71%
				average	*-12.25*	***-60%***	*20.25*	***67%***

Just as for the previous ethylene glycol experiment, thickness decreased for about 60%, as shown in the table of the measurements. More differing is the weight difference. The samples increased by 67% in weight on average after EGCC soaking, comparing to only 20% with the ethylene glycol soaking. This could mean that more of the soaking compound is absorbed, potentially for cross linking. If the tensile results also show higher strengths, then this hypothesis is reinforced.

What is the influence of ethylene glycol on the look and feel of BC?

As is visible in Figure 36 (c), the result is quite comparable to experiment D. A thin, white translucent material is achieved. More than with ethylene glycol, EGCC rendered the material very flimsy and flexible. The visual results are satisfying.

Tensile test results



Average break stress	85.08	+/- 13.49	Mpa
Average break strain	0.11	+/- 0.01	mm/mm
Average Young Modulus	0.67	+/- 0.10	Gpa

Comments on tensile tests

The stress at break has a quite high result for this and the previous sample. It also has to be mentioned that due to the slightly irregular graph, the trendline does not reflect the right Young Modulus. A more specific stiffness calculation should be performed.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
F	Glycerol	Soaking	Glycerol	x	x					after drying	4

Untreated BC sheets can have a brittle feeling and feel fragile. In the search for a solution for this, glycerol soaking is being used as a **plasticizer** in order to render the material more flexible. Pre-tests (see 7.6) already showed promising results, that glycerol soaking makes the BC very flexible, white trans- parent and less brittle. This test will now show its real influence on the tensile properties of the material.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C)
- Dried sheets are cut into specimen rectangles, labelled, weighed and measured.
- Submerge during 24h in glycerol
- Retrieve and remove excess liquid.
- Weigh and measure thickness again.

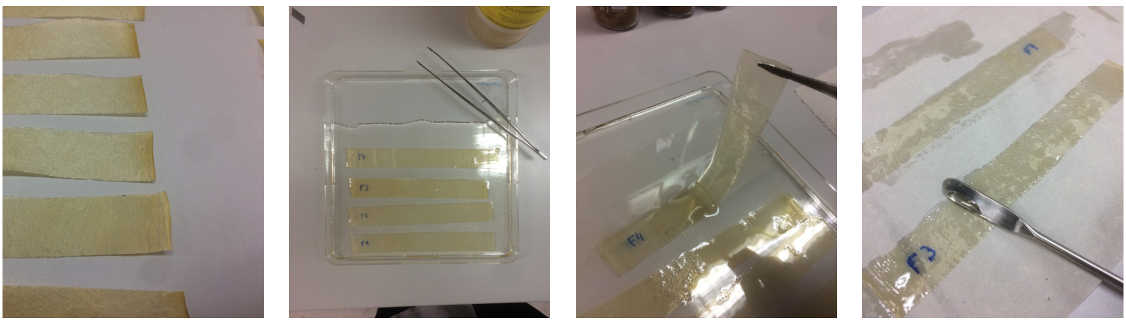


Figure 37
Alkalic cleaned and air dried samples (a); F-experiments submerged in glycerol for 24h (b); retrieve samples from glycerol (c); removal of excess glycerol (d).

Sample measurements

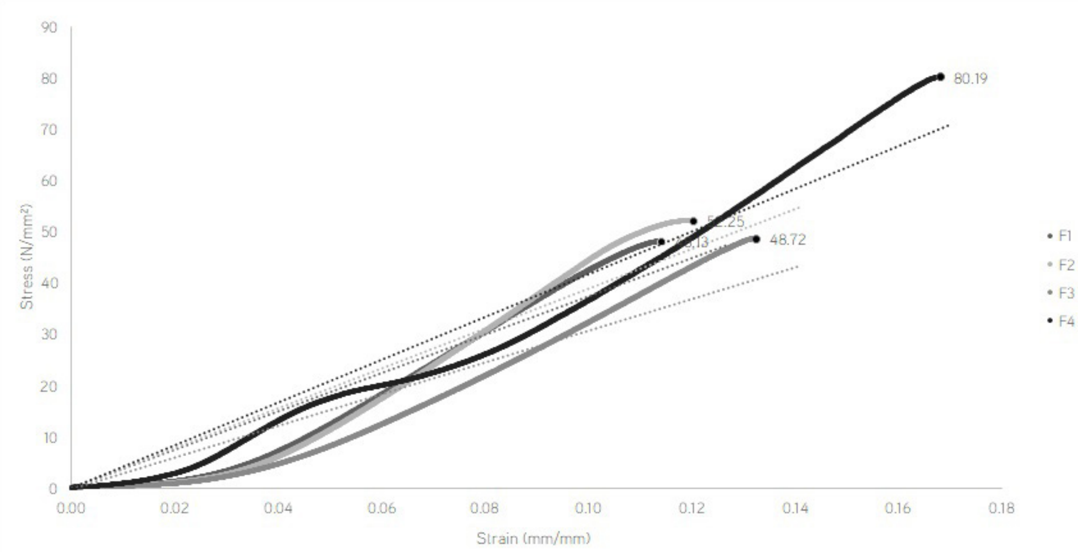
The same trend as for crosslinking agent experiments D and E is noticeable. After soaking, the thickness decreases approximately by half of the original thicknesses or more. On the other hand, the weight difference is much higher, reaching to a double of the original weight. This implies that part of the glycerol has been absorbed by the samples.

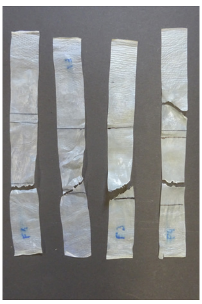
	before processing		*after processing*		*processing variation*			
DRY SAMPLE	dry weight (0.01g)	thickness (0.01mm)	dry weight (0.01g)	thickness (0.01mm)	Δ thickness (0.01mm)	Δ thickness (%)	Δ weight (0.01g)	Δ weight (%)
F1	20	13	39	5.75	-6.75	-54%	19.00	95%
F2	20	13	38	6.00	-6.75	-53%	18.00	90%
F3	35	28	72	10.50	-17.50	-63%	37.00	106%
F4	36	19	73	9.50	-9.50	-50%	37.00	103%
				average	*-10.13*	***-55%***	*27.75*	***98%***

What is the influence of ethylene glycol on the look and feel of BC?

The figures below show the glycerol-treated BC. The result is in the same direction as for sample D and E: white, transparent, flimsy and flexible.

Tensile test results





Average break stress	57.32 +/- 13.30	Mpa
Average break strain	0.13 +/- 0.02	mm/mm
Average Young Modulus	0.37 +/- 0.04	Gpa

Comments on tensile tests

Breaks are more ductile, some deformation in the horizontal dimension is visible (Poisson), as shown in the failure image above.

Slip occured at samples F1, F3 and F4. Twice the failure occured at lower clamp. This could point out that the slip induced the failure because of a small friction. The slip is also visible in graph F4, where suddenly strain increases again between 0.04 and 0.08. This diminishes the Young modulus value. In general, the four samples show quite comparable results, a quite high break stress and high strain.

The following experiments G, H and I deal with applying **greasy coatings** as **waterproofing agents**, also as a means to **plasticise**. The inspiration for these experiments came from the DIY community where some trials with coconut oil and beeswax were conducted (The Thought Emporium 2017). It appeared that a mixture of both would allow the right consistency.

Greasy coatings do not bind with water and could therefore provide a good solution. The challenge of these types of coatings will be to make them last on the material. Scratchings could remove parts of it, while heat from the sun could melt the oils or waxes, removing them from the surface. Therefore a decent coating compound should have a high melting temperature (meaning long chains), but still be easily applicable and long-lasting. In the example cited above from *The Thought Emporium*, beeswax and coconut oil are used in a mixture. This mixture is reproduced in sample I, to understand the behaviour of each of the components a separate test for each will be performed as well.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
G	Coconut oil	Soaking	Coconut oil	x	x					after drying	4

Coconut oil is a compound which is liquid at room temperature, and therefore supposedly will not remain on the BC for long. Because of being liquid it can easily be applied by means of a brush.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C)
- Dried sheets are cut into specimen rectangles, labelled, weighed and measured.
- With a brush, apply coconut oil evenly over the surface and leave to sit for 24H.
- Retrieve and dip dry, remove excess oil.
- Weigh and measure thickness again.

Figure 38
Alkalic cleaned and air dried samples (a); application of coconut oil with a brush (b); processed samples (c); zoom on samples (d).

Sample measurements

	before processing		*after processing*		*processing variation*			
DRY SAMPLE	**dry weight** (0.01g)	**thickness** (0.01mm)	**dry weight** (0.01g)	**thickness** (0.01mm)	**Δ thickness** (0.01mm)	**Δ thickness** (%)	**Δ weight** (0.01g)	**Δ weight** (%)
G1	19	12	28	9.50	-2.00	-17%	9.00	47%
G2	20	12	27	9.25	-2.75	-23%	7.00	35%
G3	37	32	50	33.25	1.25	4%	13.00	35%
G4	36	21	49	17.00	-3.50	-17%	13.00	36%
				average	*-1.75*	***-13%***	*10.50*	***38%***

Also in these samples thickness decreased, be it a lot less (13%). The decrease of thickness in the different samples could be attributed that the soaking and coating renders the samples less wrinkled, therefore thickness is measured a lot thicker when wrinkles are still present (before processing). The fact that the difference is smaller with these coatings could be because the coconut oil coating has had **a smaller contribution to the plasticisation** than the soakings of sample D, E and F. The weight increased by 38% on average, which is normal since a layer of oil has been added. This does allow to quantify the added oil to about 10.5g per sample.

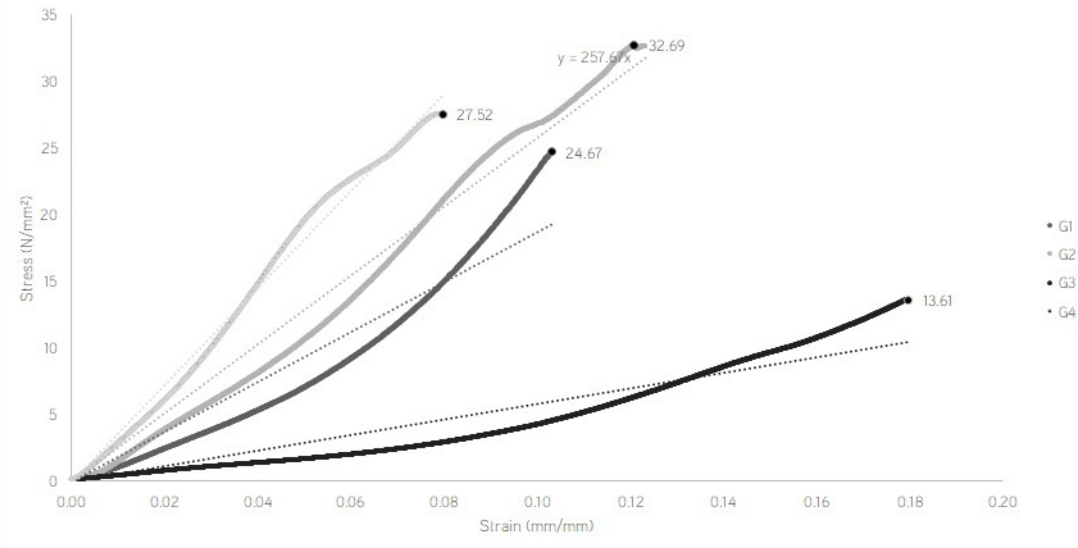
What is the influence of the coconut oil on the look and feel of BC?

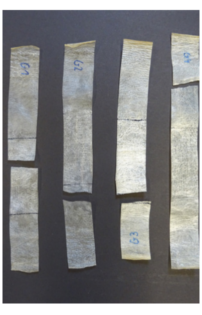
Figure 38 (c) shows the final material. The BC samples are visually still very similar to the untreated samples in terms of colour and translucency. Only the shimmering layer of oil changes. The material did not plasticise as samples D, E and F did. They are still quite wrinkled, although folding goes more easily. Although not impressive, the influence is noticeable and positive.

How is the application of the compound? Does it feel easy to remove?

The coconut oil feels as if it merged a bit with the BC, but also remains very superficial and is easily wiped off. When held with bare hands, fingers remain greasy. But this does not mean that the oil will be wiped off easily, the oil seems to adhere quite well.

Tensile test results





Average break stress	24.62	+/- 6.98	Mpa
Average break strain	0.12	+/- 0.04	mm/mm
Average Young Modulus	0.22	+/- 0.11	Gpa

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
H	Beeswax	Soaking	Beeswax	x	x					after drying	4

Beeswax is a very different greasy material than coconut oil. It is solid at room temperature with a melting temperature of 62.5°C (fisher scientific n.d.) and thus needs to be melted to be applied, but this also means that it will be solid in its applied form and thus should remain in place on the material more easily or for longer than coconut oil.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C)
- Dried sheets are cut into specimen rectangles, labelled, weighed and measured.
- Melt beeswax
- Apply beeswax evenly over the surface.
- Weigh and measure thickness again.

The application of the beeswax proved quite difficult. The beeswax solidified quickly meaning that it could not be spread out on the BC as a liquid but had to be actively spread with a tissue. This resulted in a slightly uneven spreading with thicker and thinner layers of beeswax.

Figure 39
Alkalic cleaned and air dried samples (a); application of melted beeswax by spreading it out (b); processed samples (c); zoom on samples (d).

Sample measurements

A very different result than the previous experiments is found here. The applied beeswax coating is quite thick, this results in an almost doubling of the thickness and more than tripling of the weight. Since the composition of the samples changes so drastically, this will have to be taken into account for the interpretation of the tensile test results.

	before processing		*after processing*		*processing variation*			
DRY SAMPLE	dry weight (0.01g)	thickness (0.01mm)	dry weight (0.01g)	thickness (0.01mm)	Δ thickness (0.01mm)	Δ thickness (%)	Δ weight (0.01g)	Δ weight (%)
H1	19	10	81	29.50	19.50	195%	62.00	326%
H2	39	31	113	38.00	6.75	22%	74.00	190%
H3	37	20	94	30.25	10.50	53%	57.00	154%
H4	37	18	123	30.00	11.75	64%	86.00	232%
				average	*12.13*	***84%***	*69.75*	***226%***

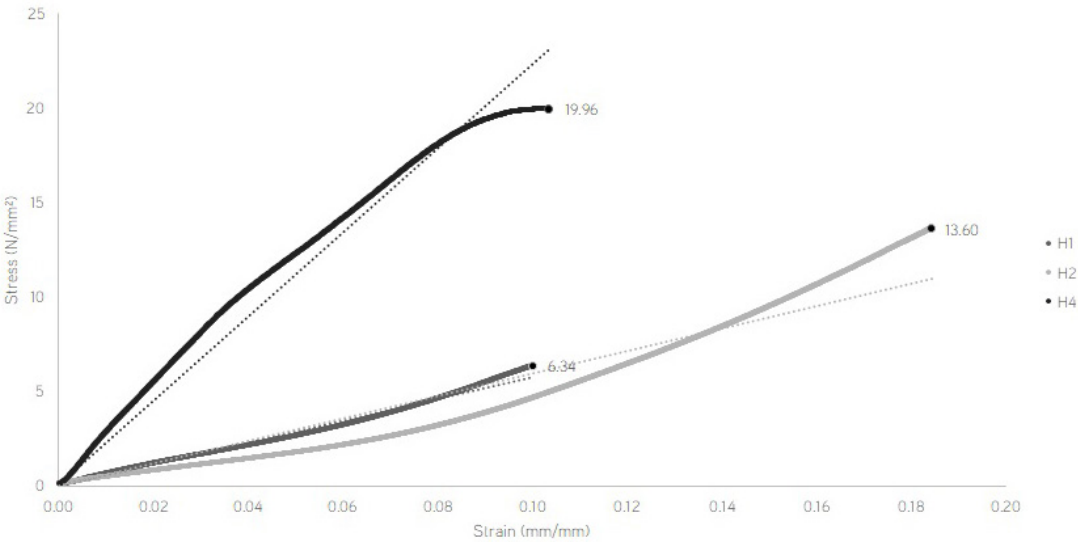
H2 *What is the influence of beeswax on the look and feel of BC?*

The thick applied layer of beeswax renders the BC less translucent. It feels thicker but not too greasy.

H5 *How is the application of the compound? Does it feel easy to remove?*

As mentioned higher, applying it did not prove easy.

Tensile test results



Average break stress	10.84	+/- 6.43	Mpa
Average break strain	0.12	+/- 0.04	mm/mm
Average Young Modulus	0.11	+/- 0.08	Gpa

Comments on tensile tests

The beeswax created less problems with slip compared to the coconut oil. H3 failed partially at the clamps, explaining the gradual graph: from a certain point the sample's cross-section decreased, resulting in an equally decreasing needed load to displace the sample. Sample H4 showed high slip in the clamps from a certain point. At the maximum stress, the pulling force exceed the clamp's grip force. The resulting maximum stress is thus probably not the real maximum stress. In general the greasy experiment results are less reliable, a better testing protocol should be developed in order to gain consistent results. This accounts for samples G, H and I.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
I	Coconut oil and Beeswax	Soaking	Coconut oil and Beeswax	x	x					after drying	4

Coconut oil as a coating proved satisfactory but the result is very greasy and the low melting temperatures are problematic. The beeswax was difficult to apply because of rapid solidification. Properties in between both could be reached by mixing both, such as was done in the DIY example of *The Thought Emporium.*

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C)
- Dried sheets are cut into specimen rectangles, labelled, weighed and measured.
- Mix 10wt% of beeswax with coconut oil and heat the mixture until fluid, mix well.
- Apply coconut oil-beeswax mixture evenly over the surface by means of a brush.
- Leave to dry for 24H
- Remove excess coating
- Weigh and measure thickness again

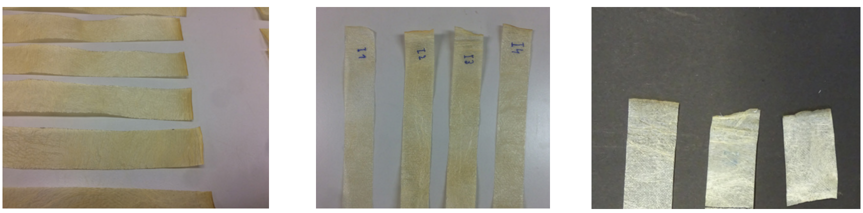


Figure 40
Alkalic cleaned and air dried samples (a); processed samples (b); zoom on samples (c).

Sample measurements

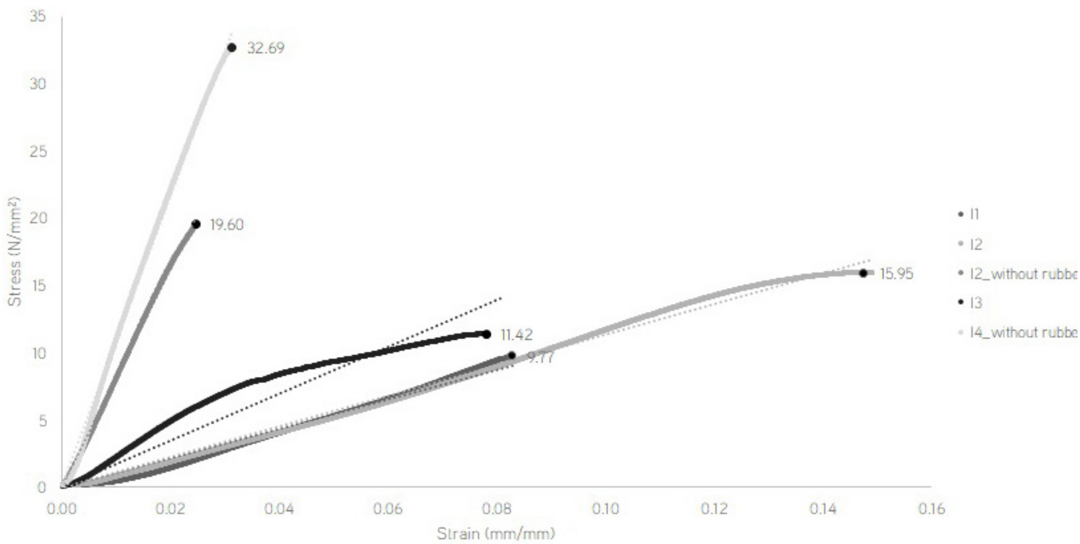
The results of the dimensional measurements are close to the coconut oil coating results. This is logic since the mixture exists out of 90wt% coconut oil.

	before processing		*after processing*		*processing variation*			
DRY SAMPLE	**dry weight** (0.01g)	**thickness** (0.01mm)	**dry weight** (0.01g)	**thickness** (0.01mm)	**Δ thickness** (0.01mm)	**Δ thickness** (%)	**Δ weight** (0.01g)	**Δ weight** (%)
I1	19	16	26	12.75	-2.75	-18%	7.00	37%
I2	41	37	52	29.00	-7.75	-21%	11.00	27%
I3	39	28	50	24.00	-4.25	-15%	11.00	28%
I4	36	19	45	14.75	-3.75	-20%	9.00	25%
				average	*-4.63*	***-19%***	*9.50*	***29%***

What is the influence of the coconut oil-beeswax coating on the look and feel of BC?

This greasy variation is the most pleasing. The coconut oil and beeswax coating is evenly distributed and it seems that it is coming off less easily in comparison with the coocnut oil.

Tensile test results



Average break stress	14.18 +/- 3.86	Mpa
Average break strain	0.08 +/- 0.04	mm/mm
Average Young Modulus	0.29 +/- 0.29	Gpa

Comments on tensile tests

Also in this case, the results vary a lot. The graphs show totally different behaviours, sample I2 was tested again as it did not break, showing a much higher stiffness once 'pre-strained'. Again, these results are not very reliable.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
J	Citric acid solution	Soaking	Citric acid solution	x	x					after drying	4

As mentioned before, crosslinking improves strength in the polymer. Another known **crosslinking agent** in cellulose is citric acid (Raucci et al. 2015). Furthermore a DIY experiment on *Biofabforum.org* was also reporting that citric acid treatment resulted in a BC with a satisfying outcome. There also a small concentration comparison of citric acid was conducted, resulting in 2wt% as a good start value ("Bacterial Leather Crosslinking Treatments" 2018). Therefore a treatment with citric acid is performed in order to assess its influence on flexibility and strength. Citric acid is a naturally occurring completely harmless compound. The treatment is performed in analogy with samples D, E and F, as a post-processing soaking of 24h. Citric acid is a solid and is diluted in water with 2wt% concentration.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C)
- Dried sheets are cut into specimen rectangles, labelled, weighed and measured.
- Prepare the citric acid mixture with 2wt% citric acid in distilled water by means of stirring.
- Submerge during 24h in the citric acid solution.
- Retrieve and remove excess liquid by means of dry-dipping
- Weigh and measure thickness again.

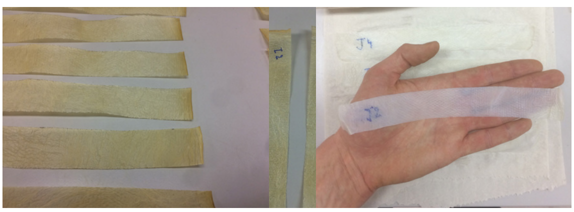


Figure 41
Alkalic cleaned and air dried samples (a); citric acid-treated samples (b)

Sample measurements

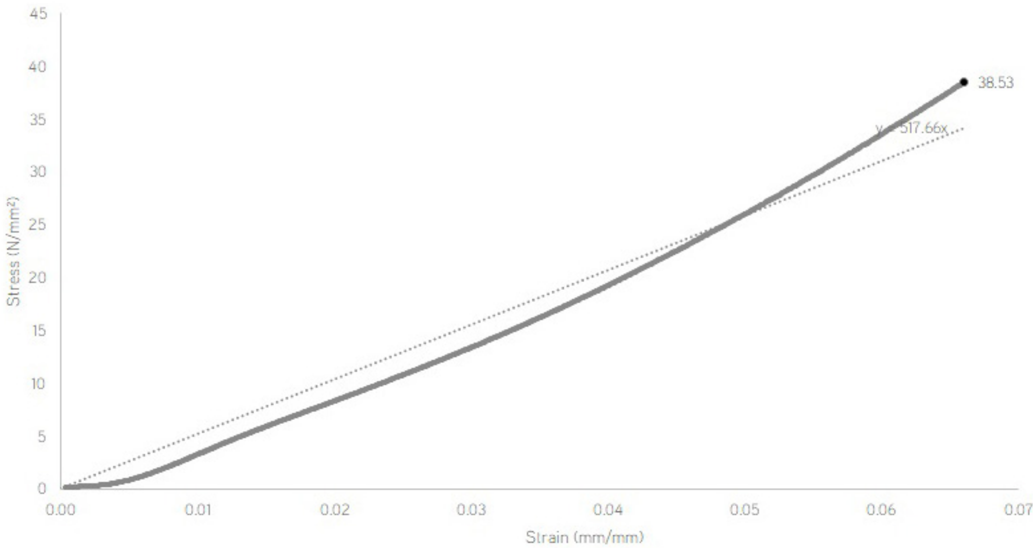
	before processing		*after processing*		*processing variation*			
DRY SAMPLE	**dry weight** (0.01g)	**thickness** (0.01mm)	**dry weight** (0.01g)	**thickness** (0.01mm)	**Δ thickness** (0.01mm)	**Δ thickness** (%)	**Δ weight** (0.01g)	**Δ weight** (%)
J1	19	12	18	9.00	-3.00	-25%	-1.00	-5%
J2	37	27	33	22.75	-3.75	-14%	-4.00	-11%
J3	36	18	34	13.25	-5.00	-27%	-2.00	-6%
J4	34	22	32	19.50	-2.00	-9%	-2.00	-6%
				average	*-3.44*	***-19%***	*-2.25*	***-7%***

For the first time, next to a decrease in thickness, a decrease in weight is measured. This is remarkable, since the completely dry samples were left in the aqueous solution for 24h, leaving them time to absorb, and they were measured upon retrieval, being dipped dry but without leaving a drying time. It was assumed that weight would have increased due to some absorbed water remaining. Instead, mass diminished. This can be explained by the fact that citric acid intervened in the material and matter has been taken out. It is not clear whether this process could have induced cross-linking

What is the influence of citric acid on the look and feel of BC?

The resulting material is very pleasing and became very flimsy, white and translucent. The thin BC is easily foldable and malleable.

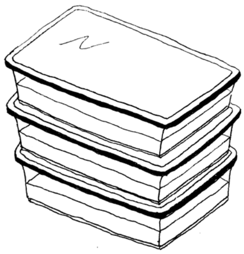
Tensile test results



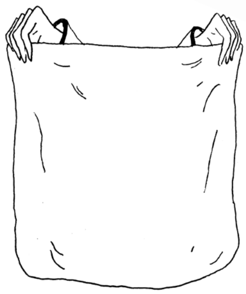
Average break stress	17.00	+/- 12.60	Mpa
Average break strain	0.08	+/- 0.01	mm/mm
Average Young Modulus	0.24	+/- 0.20	Gpa

Comments on tensile test

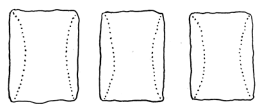
The results for this test were not completely reliable. Sample J1 and J2 have shown some slip, J2 also failed in the clamps. J3 had a brittle break in the sample and also achieved a much higher stress. Only sample J3 is picturede hereabove, the other ones are defined as unreliable.



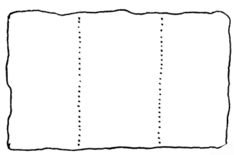
Time and temperature are needed for the bacterial cellulose to grow.

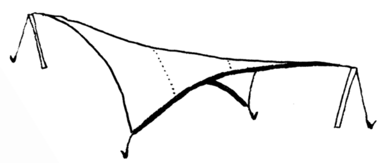


When harvested, cutting patterns are cut from the sheets.



The sheets are sewn together. Because of the cutting patterns they form a three dimensional shape.





In order to keep its shape while drying, the membrane can already be hung. The sewn connections keep the sheets together.

Over a short timespan, the sheets will dry. The seams now completely combined into a continuous sheet.

In the next series of tests some experiments concerning **seams** were developed and tested. The creation of connections in a membrane is an important part of assessing an architectural membrane. In the pre-tests some possibilities were already investigated, where the interesting conclusion was that all wet BC samples dried together combined into one. A first experiment will thus be to evaluate the strength of a simply dried seam (sample O). A possibility is that the strength of the seam is higher than the plain BC itself. Therefore a separate test (sample S) will be performed of a double BC layer, combined such as it would be in a connection, by air drying. This enables to test the strength of solely the connection itself.

Tests M and N relate to a specifically worked out concept, resulting out of the **discovery of the successfully drying together of BC**. A workflow was conceived based on this principle, as **a first pitch on how it could be implemented in architectural membranes**. The idea of two sheets of BC that can be connected by simply leaving them to dry on each other creates the opportunity to connect without interference of any exterior material such as glue or demanding techniques. However, tensile structures are built out of plane cutting patterns that form a 3D shape when combined. It is thus not so easy to simply hold two sheets together over a small connection overlap, in a 3D shape and for a prolonged period of time. A solution to this challenge is developed.

The conceptual process is pictured on the left page. When freshly harvested, the two sheets can be sewn together to just keep them in place. In order to set the 3D shape, the whole membrane with stitched seams can be hung in its final position while still being wet. The weight (which will be approximately 98% higher than the final BC) has to be taken into account here. Over a couple of days the membrane will then dry completely and the seams will develop.

This idea starts from the assumption that a sewn connection is less strong than the dried connection, this will be evaluated by means of test M and N. Test M reflects the concept of sewing wet BC sheets and leaving them to dry afterwards. Test N is made to evaluate the strength of a simple sewn dried material, to enable comparison. Test O focuses purely on the dried seam, cancelling any interference of stitching holes.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
M	Sewn wet and dried	Seams		x	x			x		/	3

As framed higher, in this experiment freshly harvested BC will be sewn together and then air dried. The hypothesis is that the strength is developed by the dried connection and not so much by the stitches. The question will also be to compare with sample O, in order to assess whether the holes decrease its strength, and with sample N, to compare to simply stitched dry sheets.

For the testing of seams a double seam connection is used, as elaborated in 6.7 and shown in Figure 43.

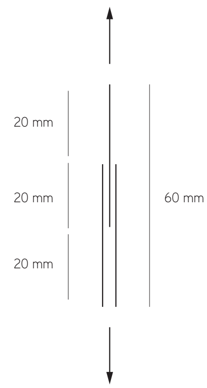


Figure 43

Scheme of double layer connection

Preparation protocol

- After harvesting, follow alkalic cleaning process
- without drying (see sample C)
- Cut the sheets to the right dimensions to allow an overlap of 20mm, as shown in Figure 42 (a).
- Stitch the connections (Figure 42 (b)).
- Leave to dry for a couple of days with a plastic sheet between the double layers to avoid that these combine as well. Exert regular flipping to encourage homogeneous drying.
- When dry, cut into samples.

Figure 42

Creation of the 20mm overlap (a); stitching of the seam by hand (b); stitched seam (c); dried stitched seam (d); dried BC (e); BC cut into samples (f)

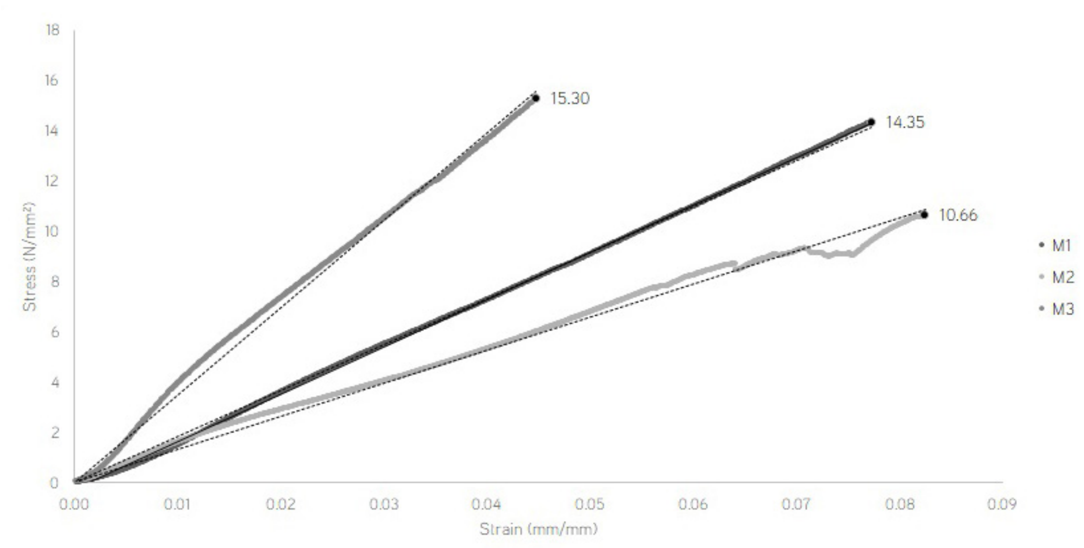
Sample measurements

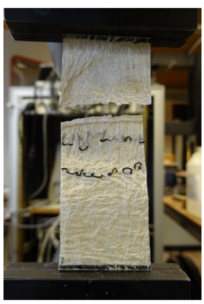
DRY SAMPLE	dry weight (0.01g)	thickness (0.01mm)
M1	86	32
M2	81	28
M3	80	18

In terms of conception, was the process of stitching and placing together successful?

The stitching was performed by hand and is less regular and tight than what a machinal stitch would do. This can be taken into account when assessing the strength of the stitch. Also it is interesting to note that due to the shrinkage of the BC, the stiches became loose, as can be seen in Figure 42 (d). Drying these samples was less easy than a normal sample, due to the double thickness and the fact that part of it had to combine and another part not. This was solved by placing a plastic or non-water absorbing paper in between the layers that should not combine.

Tensile test results





Average break stress	13.44	+/- 2.00	Mpa
Average break strain	0.07	+/- 0.02	mm/m
Average Young Modulus	0.22	+/- 0.09	Gpa

Comments on tensile tests

As the samples broke in the plain BC instead of the connection, the connection strength is not known. Furthermore the connection is made up of a double layer, thus it is only to be concluded that the double connection strength is larger than the plain BC. Further on, sample S will handle the strength of a plain double layer in order to know the connection strength.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
N	Dried and sewn	Seams		x	x			x		/	3

This experiment is to be compared to the previous one. Here the strength of a simply sewn (dry) connection is assessed. The stitching is done simply by hand. A machinal technique should already enable higher strengths.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C).
- Cut the sheets to the right dimensions to allow an overlap of 20mm (see Figure 44 (a)).
- Leave to dry the three separate sheets.
- When dry, stich the sheets to each other.
- Cut the stitched sheets in samples (Figure 44 (b) and (c)).

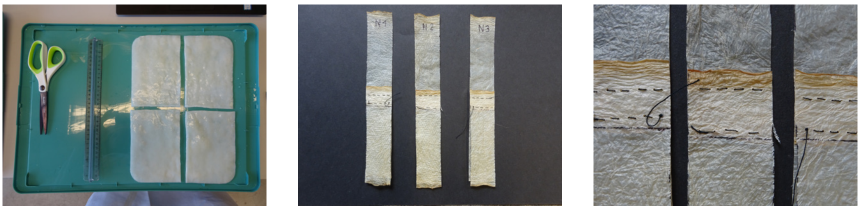


Figure 44
Freshly harvested and cleaned sheets cut into rectangles (a); dried, stitched and cut samples of BC (b) and (c)

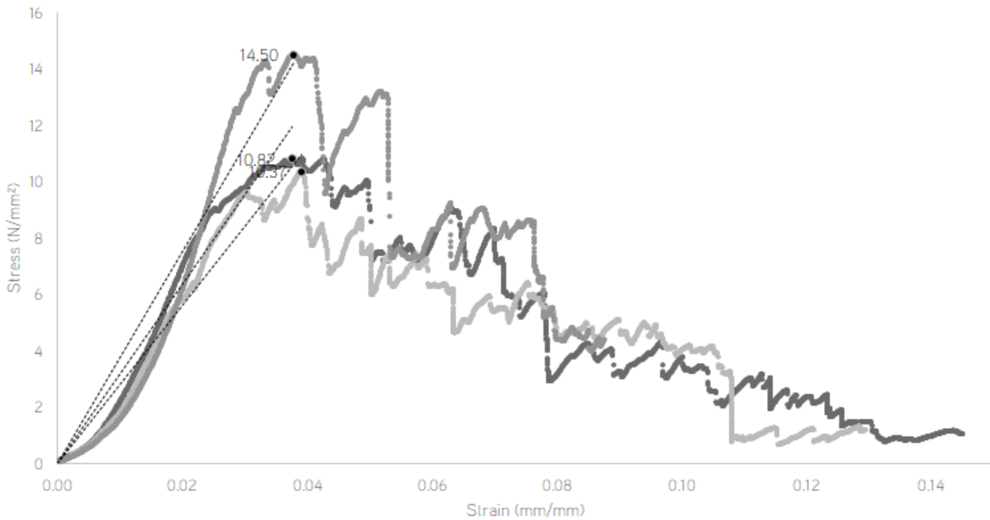
Sample measurements

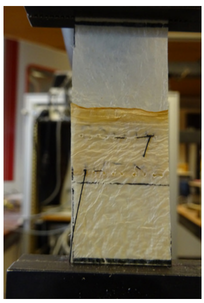
DRY SAMPLE	thickness (0.01mm)
N1	16
N2	63
N3	14

In terms of conception, was the process of stitching and placing together successful?

This protocol went very fluent. Important to notice is that the stitching was performed in one long stitch, then by cutting the samples the thread was cut too. This has to be taken into account in the interpretation of tensile test results.

Tensile test results





Average break stress	11.90	+/- 1.85	Mpa
Average break strain	0.04	+/- 0.00	mm/m
Average Young Modulus	0.32	+/- 0.04	Gpa

Comments on tensile tests

The stress-strain graph illustrates well how the strength of the connection decreases slowly with the unwinding of the threads in the seam. In this experiment the sewn connection causes the failure. It is clearly visible how the threads disconnected and did not exert any force on the material. It can be concluded that the BC resists the thread holes, it is the unfirling of the seam that fails. Therefore, a decent, machine-made sewn seam could improve the strength considerably.

The thickness of the BC is used to calculate the stress, although it did not take part in the strength calculation. Expressed as such, the strength of the connection can be compared to the other seam techniques. The results are not to be interpreted as the strength of the BC.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
0	Seam by drying	Seams		x	x			x		/	3

As introduced before, this experiment focuses on the strength of a connection which is only dried, without intervention of stitching. The hypothesis is that this is the strongest connection, as there are no holes introduced by the stitching.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C).
- Cut the sheets to the right dimensions and place them together, allowing an overlap of 20mm.
- Leave to dry for a couple of days with a plastic sheet between the double layers to avoid that these combine as well. Exert regular flipping to encourage homogeneous drying on all sides.

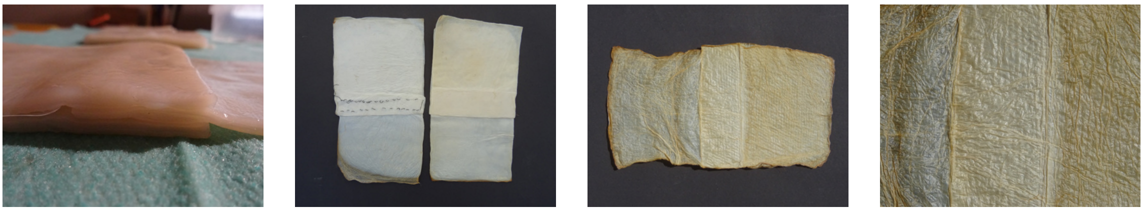


Figure 45
wet sheets placed together (a); completely combined dried sheets (b) and (c); zoom on samples (d)

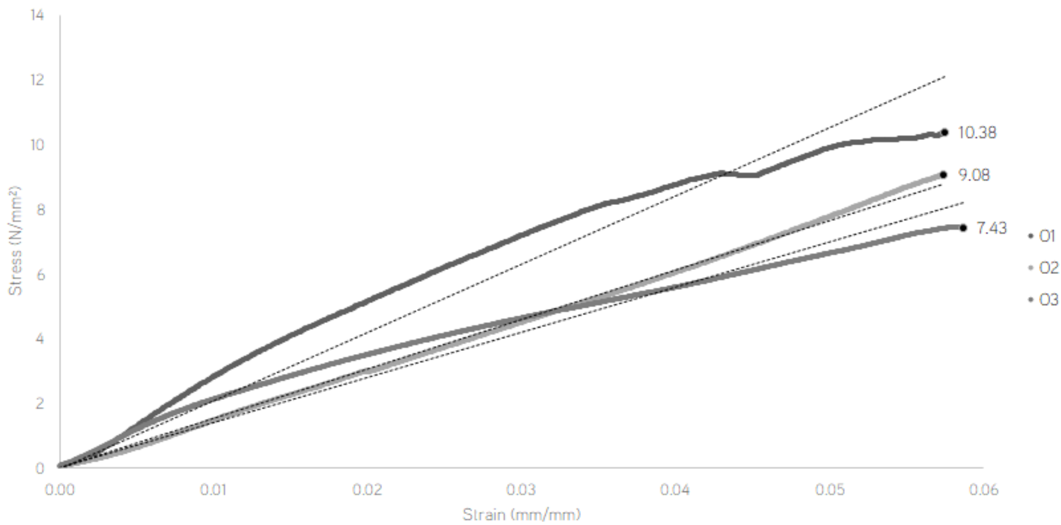
Sample measurements

DRY SAMPLE	dry weight (0.01g)	thickness (0.01mm)
01	89	21
02	101	26
03	92	37

n terms of conception, was the process of drying together successful?

All was fluent, but since the connection is not fixed until dry, one has to carefully handle the samples as long as they are not fully dried.

Tensile strength test



Average break stress	8.97	+/- 1.21	Mpa
Average break strain	0.06	+/- 0.00	mm/m
Average Young Modulus	0.17	+/- 0.03	Gpa

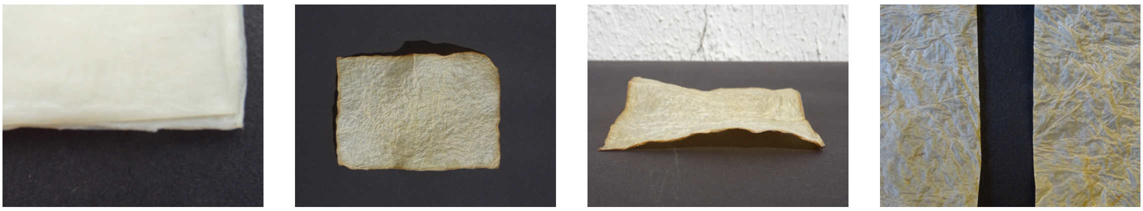
As expected, the samples failed in the plain material, not in the connection. As in test M, it is only to be concluded that the double connection strength is larger than the plain BC. In sample S the strength of a double layer of BC will be tested.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
S	Double layer by drying	Seams		x	x					/	4

This test is part of the seam tests. As it is assumed that the connection in test O will be stronger than the plain material, test O will not provide information on the strength of the seam connection. Therefore a continuous double layer, combined by drying is created, such as the connection itself in test O, is created. The aim is to know the strength of the connection itself.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C).
- Cut the sheets to the right dimensions and compile the two sheets.
- Leave to dry for a couple of days



Sample measurements

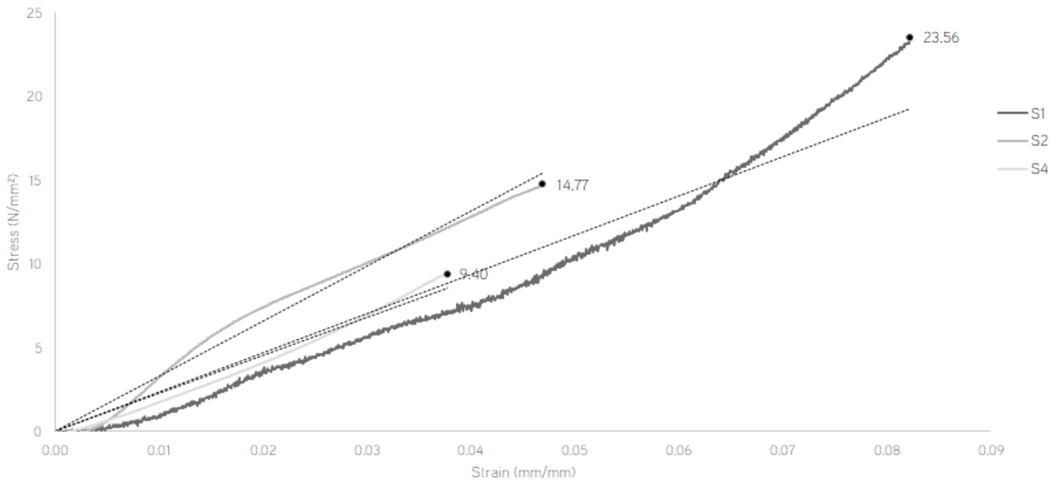
DRY SAMPLE	dry weight (0.01g)	thickness (0.01mm)
S1	76	31
S2	73	34
S3	81	36

Figure 46
wet sheets placed together (a); completely combined dried sheets (b) and (c); zoom on samples (d)

In terms of conception, was the process of drying together successful?

All went fluent, some curling occurred but not problematic. The results feels very though and strong due to its thickness

Tensile test results



Average break stress	15.91	+/- 5.83	Mpa
Average break strain	0.06	+/- 0.02	mm/m
Average Young Modulus	0.16	+/- 0.13	Gpa

The results are quite consistent, sample S1 reached a higher strength. The comparison with the seams tests will be drawn at the end of this chapter.

The following range of tests apply to experiments with **mixing**, a **heat press** and further on with **composites**, as they have been explored in 7.4, 7.7 and 7.8.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
Q	Drying by heat press	Heat press		x		x				/	3

Drying the BC by means of heat pressing dries the material in a short time span. Furthermore the heat treatment could have an influence on the appearance, texture, flexibility and tensile strength. Cross-linking could be promoted.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C).
- Place the sheet in the heat press at 190°C until fully dried.

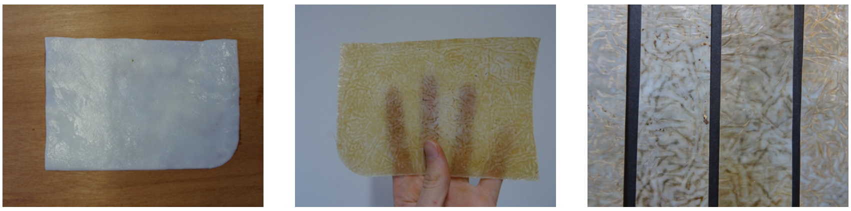


Figure 47
before (a) and after (b) drying through the heat press; samples (c)

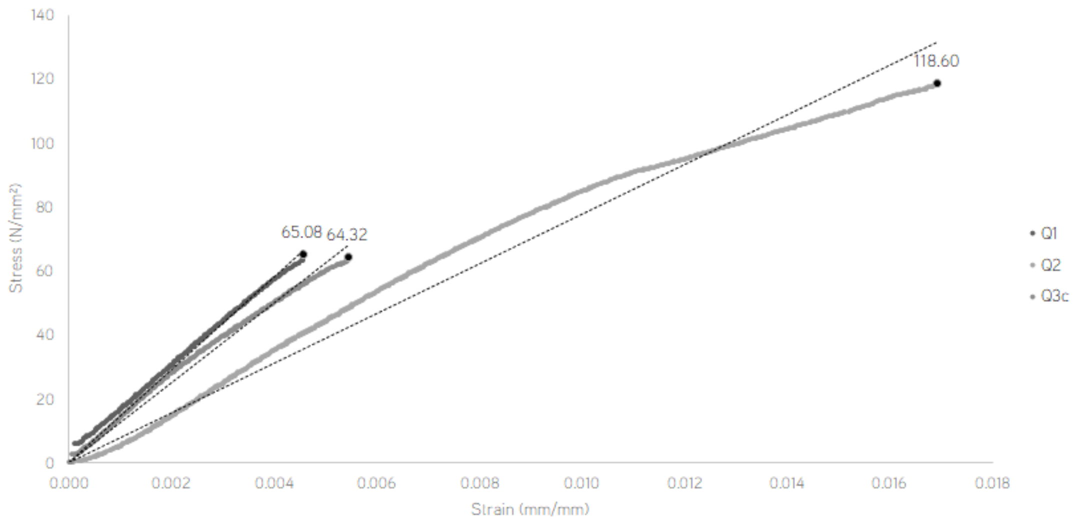
Sample measurements

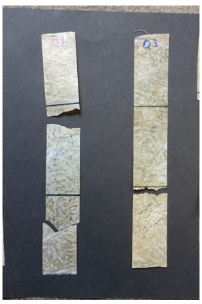
DRY SAMPLE	dry weight (0.01g)	thickness (0.01mm)
Q1	37	8
Q2	39	10
Q3	39	9
Q4	35	9

What is the influence on appearance, texture, flexibility?

In this case, the visual outcome is very satisfying. As can be seen in Figure 47, a pleasing aesthetic was achieved. Also the texture became very smooth and the sheets feels homogeneous and strong, although brittle. In terms of appearance, this sample is a highlight.

Tensile test results





Average break stress	82.67	+/- 25.41	Mpa
Average break strain	0.01	+/- 0.01	mm/m
Average Young Modulus	11.58	+/- 2.81	Gpa

The achieved results are quite satisfactory, as the tests went really fluent without any slip, a high strength but mainly a very high stiffness, in comparison with the other materials, was found. As can be seen in the image above although, due to the brittleness of the material, sample Q3 broke at the clamp. It is thus possible that it could even have reached higher strengths.v

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
R	Dried and heat pressed	Heat press		x	x	x				/	4

The aim of this test is to see whether a heat treatment on an already dried BC sheet has any effect.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C) and leave to air-dry.
- Place the sheet in the heat press at 170°C for three minutes.

Figure 48
samples before testing (a); zoom on samples (b)

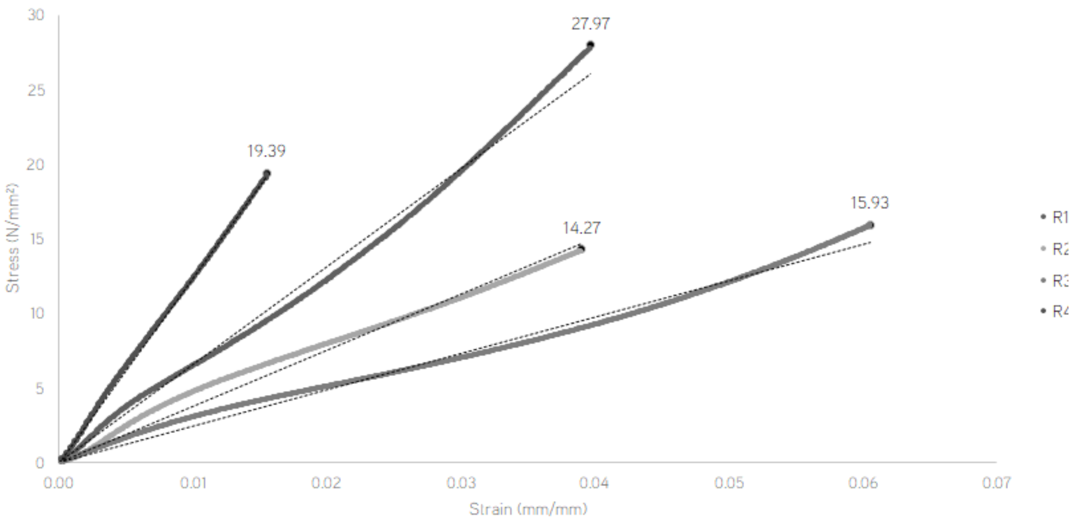
Sample measurements

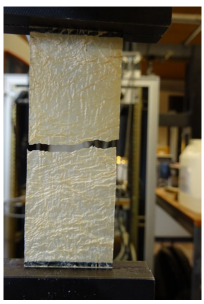
DRY SAMPLE	thickness (0.01mm)
R1	22
R2	23
R3	18
R4	16

What is the influence on appearance, texture, flexibility?

Heat pressing the dried samples had no significant influence on the physical appearance of the BC.

Tensile test results





Average break stress	19.39	+/- 5.29	Mpa
Average break strain	0.04	+/- 0.02	mm/m
Average Young Modulus	0.63	+/- 0.38	Gpa

Comments on tensile tests

It is difficult to compare these results to reference samples C and K, as they lie so far apart. More samples are needed in order to gain more consistent information.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
T	Mixed	Mixed		x	x				x	/	4

Inspired by a water filter test with mixed BC (Aqualose 2014), the strength and appearance of this recombined sheet is to be assessed.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C)
- Blend the equivalent of four samples until smooth.
- Spread out the obtained mixture in a rectangle
- Leave to dry. From the moment it combines, gently flip it regularly to promote homogeneous drying.
- When dry, cut in samples.

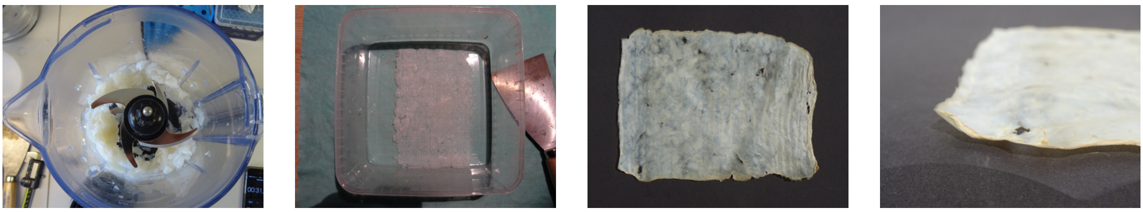


Figure 49
mixing of BC (a); spreading of mixed BC in a rectangle (b); the dried mixed BC (c) and (d)

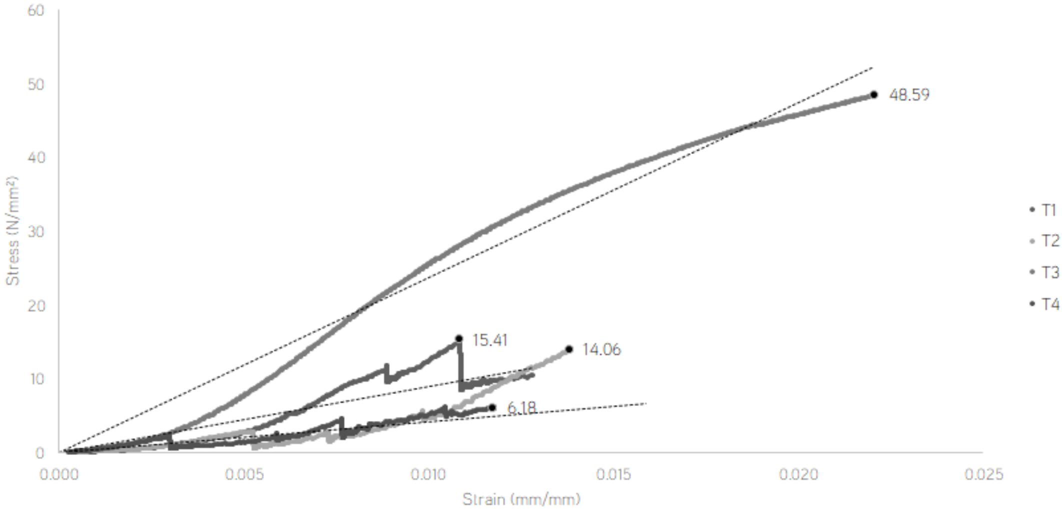
Sample measurements

DRY SAMPLE	dry weight (0.01g)	thickness (0.01mm)
T1	37	14
T2	38	12
T3	42	14
T4	26	12

What is the result in terms of appearance, texture, flexibility?

The mixing of the BC did not go as fluent as for the explorative tests, causing that chunks of BC were present in the mixture. This made it more difficult to spread the BC evenly. The result is that the final sample does have some holes and uneven distributions, which will have an influence on tensile strength. But on the overall, the material does look quite nice and the texture is very smooth because of being dried on plastic.

Tensile test results



Average break stress	21.06	+/- 16.28	Mpa
Average break strain	0.01	+/- 0.00	mm/m
Average Young Modulus	1.02	+/- 0.81	Gpa

Comments on tensile tests

As can be seen in Figure 49 (c) and (d), the material is not totally continuous. This diminished the reached strengths. Especially for sample T4, the failure occured through a hole. In the graphs it is visible how the drops in strength occured, these are measurements of gradual breaks. This is a phenomenon not seen with any other samples. The hypothesis is that the mixed BC consists out of 'grains' which was then recombined by spreading it into a sheet. If the material breaks gradually, it is because the recombined connection between them is stronger than the plan BC grain itself. Each drop in stress is a failure of a grain.

If this hypothesis is right, it is a very interesting property. Often the other tensile membranes simply break at once, since nothing is available to inhibit continuation of the break (except for the composites). In reality, this is a dangerous property for tensile membranes. If the failure spreading can be inhibited by introducing grains, this would increase the safety of membrane failure.

The following tests all concern composites, thus where natural fibres (flax fibres in this case) are combined with BC which functions as a matrix. Different techniques are tried out, such as working with a mixed BC spread on the fibres (test U), in-situ grown composite such as was pre-tried in 7.4 in air-dried (test W) or heat pressed (test X) version and finally a composite created by drying the different layers together, assuming they would combine.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
U	Mixed, spread on fibers	Composite		x	x		x		x	/	3

The mixed BC assessed in test T is used here in order to create a composite, by spreading the wet mixture below and on top of the fibres.

Preparation protocol

- After harvesting, follow alkalic cleaning process (see sample C)
- Blend the equivalent of four samples until smooth.
- Spread out half of the obtained mixture in a rectangle
- Place the fibre net on top and cover with the remaining half of mixed BC.
- Leave to dry. From the moment it combines, gently flip it regularly to promote homogeneous drying.
- When dry, cut in samples.

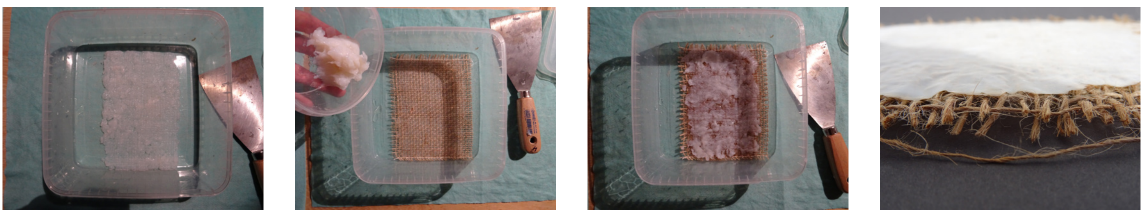


Figure 50
spreading of first layer of BC (a); add fibre layer (b): add BC top layer (c); dried result (d)

Sample measurements

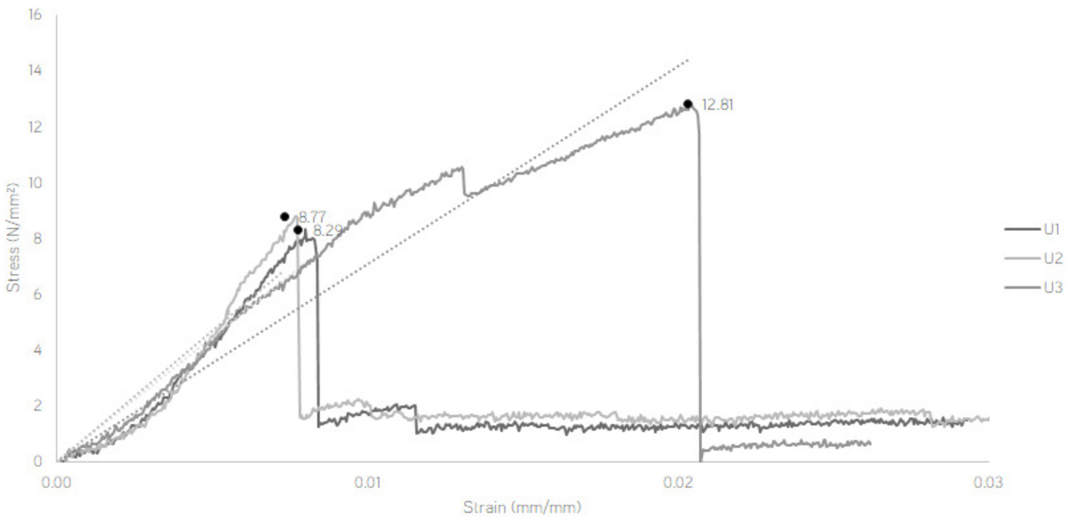
DRY SAMPLE	dry weight (0.01g)
U1	98
U2	97
U3	96

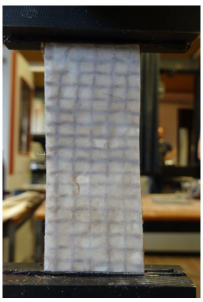
Direct thickness measurement of composites is less relevant.

What is the result in terms of appearance, texture, flexibility?

The BC itself is very comparable to previous sample T. Important to note is that it is already clearly visible in the dried sample how the BC matrix did not truly combine with the fibres. The BC form separate layers on top and below that stick to the fibres but the connection feels very weak.v

Tensile test results





Average break stress	9.96	+/- 2.03	Mpa
Average break strain	0.01	+/- 0.01	mm/m
Average Young Modulus	0.78	+/- 0.14	Gpa

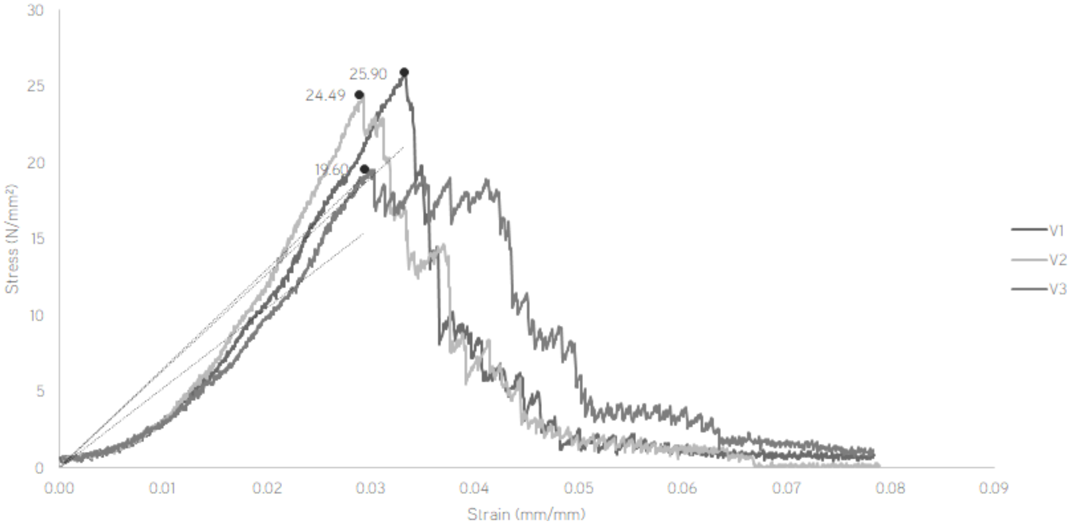
Comments on tensile tests

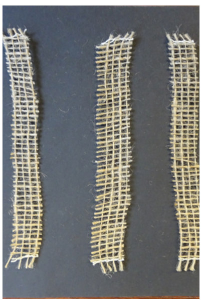
The tests have been stopped manually before the strength on the fibres could develop, as they can be seen in experiments W and X. Therefore the results are unfortunately incomplete. Although the most relevant part is measured. The development of the curve shows how from a certain strength the matrix breaks (defined here as the maximum stress) and a low amount of stress remains upon increasing strain. This corresponds to the fibres moving within the BC matrix. The (low) adherence between both probably keeps the strength above 0. Because the fibres have such a high strain capacity in comparison to the brittle mixed BC, they do not combine and work together as a composite.

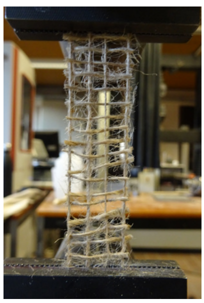
Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
V	Composite fibres	Reference		x			x			/	3

This test is meant to only define the strength of the fibres to be able to place the composite strength into perspective.

Tensile test results







Average break stress	23.33	+/- 2.70	Mpa
Average break strain	0.03	+/- 0.00	mm/m
Average Young Modulus	0.60	+/- 0.06	Gpa

Comments on tensile test

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
W	Grown comp, air dried	Composite		x	x		x			/	5

In-situ grown composites proved successful in the pre-tests (see 7.4). The technique is reproduced and improved here and subjected to tensile tests. To have a sort of pretensioning, the flax fibre net is tensioned at the height of the culture surface, so that the pellicle would embed the fibre net, as shown in the first figure below. This test is then air-dried, in the next experiment this in-situ grown sheet is subjected to a heat press drying.

Preparation protocol

- After harvesting the in-situ grown composites, follow alkalic cleaning process (see sample C)
- Leave to dry

Figure 51
Tensioned fibre net with grown BC adhering to it (a); freshly harvested in-situ grown composite (b); dried material (c); zoom on dried composite (d)

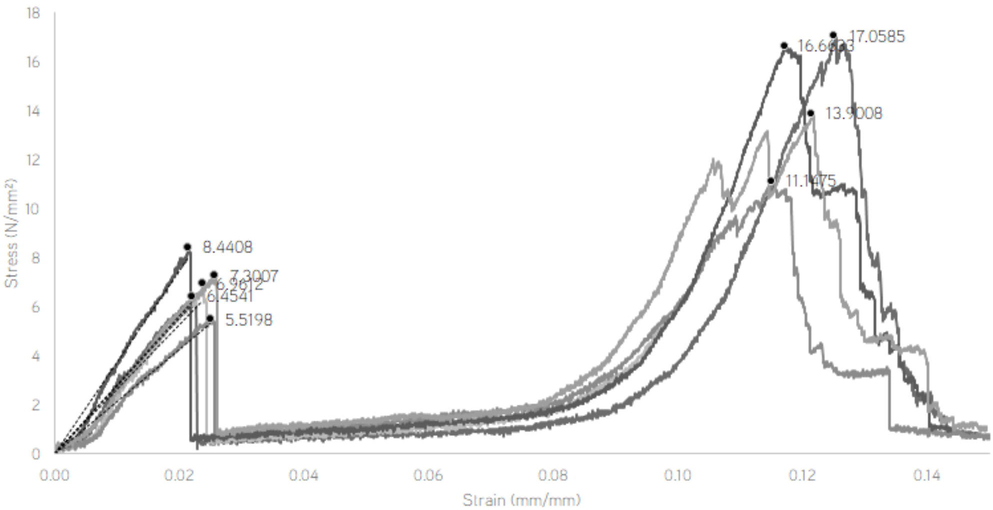
Sample measurements

DRY SAMPLE	dry weight (0.01g)
W1	96
W2	85
W3	83
W4	88

What is the result in terms of appearance, texture, flexibility?

The in-situ growth was successful as both materials really combined, but unfortunately the BC only adhered to one side of the fibres. This is probably due to the set height of the tensioned fibres in the liquid. After drying the adherence remained. Ideally, the fibres would be completely embedded in the BC matrix to have a full adhesion on all sides of the fibres.

Tensile test results



Average break stress C	6.84	+/- 1.06	Mpa
Average break stress F	28.65	+/- 4.64	Mpa
Average break strain	0.02	+/- 0.00	mm/m
Average Young Modulus	0.28	+/- 0.06	Gpa

Comments on tensile tests

A first maximum is reached when the BC matrix reaches its maximum strain and fails. Then only the fibres develop their strength due to a high strain. The fact that the fibres can develop such a large amount of strain after matrix failure is flaw that could be solved by prestressing the fibres. Then both matrix and fibre could develop load increase in a coordinated way, where the matrix redistributes the loads within the material. Also the bad cohesion between matrix and fibres is an important flaw that needs further attention. In the data table the stress for the composite (first failure) and for the remaining fibres is shown (second failure). If compared to test V (only fibres), it is clear that the long strain shown here is because the fibres were not tensioned when the matrix failed, since test V has a similar strain at break as the matrix (0.02). The stress break of the fibres is a bit higher than for the fibres alone. This could be attributed to the matrix, playing a redistributing role (even after failure), but this is only a hypothesis.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
X	Grown comp, heat press	Composite		x		x	x			/	4

The approach is identical to the previous one (sample W) where in-situ grown composites are used. In this case the drying technique will be heat pressing of the whole composite, hoping that it promotes bindings between matrix and fibres.

Preparation protocol

- After harvesting the in-situ grown composites, follow alkalic cleaning process (see sample C)
- Place in heat press at 190°C until completely dry.

Figure 52

Tensioned fibre net with grown BC adhering to it (a); freshly harvested in-situ grown composite (b); dried material (c); zoom on dried composite (d)

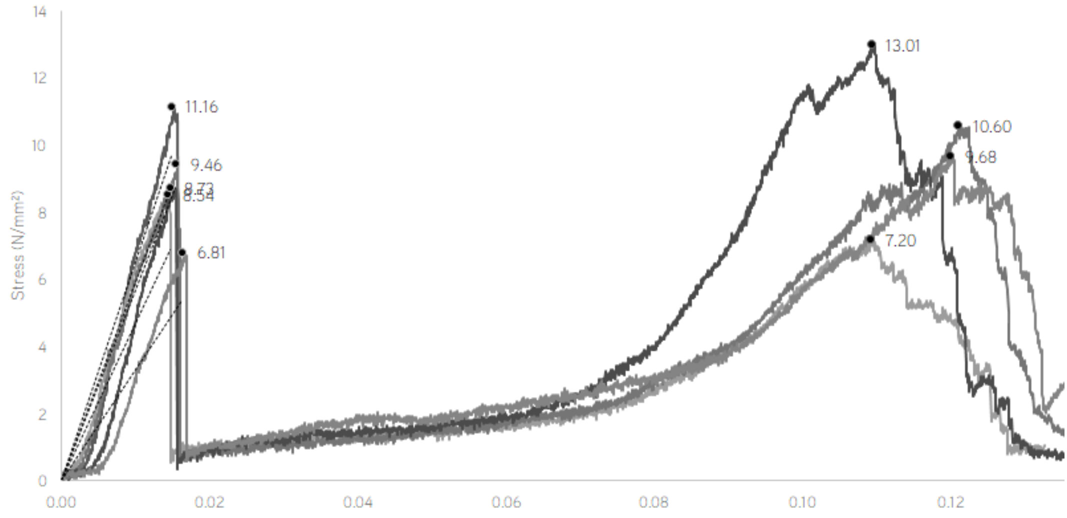
Sample measurements

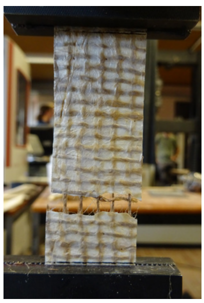
DRY SAMPLE	dry weight (0.01g)
X1	88
X2	76
X3	81
X4	80

What is the result in terms of appearance, texture, flexibility?

The in-situ growth was successful as both materials really combined, but unfortunately the BC only adhered to one side of the fibres. This is probably due to the set height of the tensioned fibres in the liquid. The heat pressing rendered the BC the same appearance as the other heat pressed samples (sample Q).

Tensile test results





Average break stress C	8.94	+/- 1.41	Mpa
Average break stress F	15.69	+/- 3.22	Mpa
Average break strain	0.01	+/- 0.00	mm/m
Average Young Modulus	0.52	+/- 0.11	Gpa

Comments on tensile test

A higher average stress (8.94MPa), stiffness (0.52) and a lower strain (0.01) than in the air-dried test are measured. This probably solely relates to the fact that the BC is heat pressed. The combining of fibres and matrix still is far from what is needed.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
Y	Composite by drying	Composite		x	x		x			/	4

In this approach is investigated whether the fibres and matrix combine into a composite by simple drying. Starting from the knowledge that two sheets of BC combine, this could potentially be extended to combining composites.

Preparation protocol

- After harvesting the in-situ grown composites, follow alkalic cleaning process (see sample C)
- Place a layer of BC, followed by a fibre net, followed by another layer of BC.
- Let to dry, with regular flips to ensure homogeneous drying.

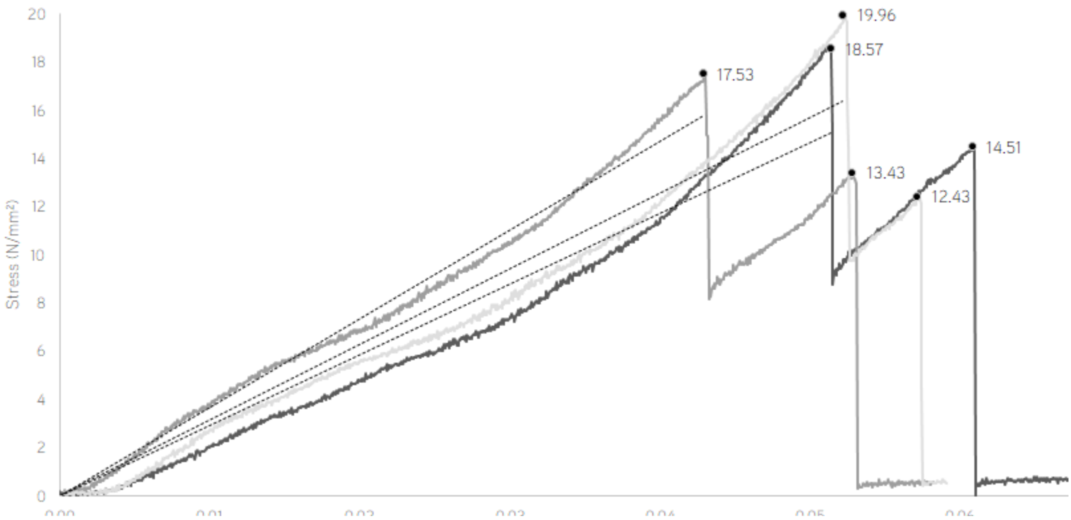
Figure 53
The components that constitute the test (a); BC and fibres placed together (b); the resulting dried composite (c)

Sample measurements

DRY SAMPLE	dry weight (0.01g)
Y1	139
Y2	139
Y3	141
Y4	142

The combined composite proved successful at first sight. The tensile tests will have to prove the strength and adherence.

Tensile test results



Average break stress	18.28	+/- 1.12	Mpa
Average break strain	0.05	+/- 0.00	mm/m
Average Young Modulus	0.32	+/- 0.03	Gpa

Comments on tensile tests

The samples are built out of a layer of fibres in between two layers of BC, simply air-dried. The failures clearly show how both sheets of the BC failed after eachother, with the first one showing a higher failure strength than the second one. Unfortunately this also illustrates how, again, the matrix and fibres are not embedded and do not work together.

A totally different biomaterial is **mycelium composites.** This biologically augmented congregate of organic fibres, where mycelium fibres grow through the material to combine it, is subjected to glycerol soaking and heat press treatment as post processing. Whereas mycelium composites are often categorised as solid materials, this material gains a certain flexibility and its thinner appearance reminds one of a thick membrane. For these reasons, this material will be subjected to the same tests as the BC in order to be able to set up a comparison.

Label	Sample	Category	Involved compounds	Alkalic cleaning	Air dried	Heat Press	Composite	Seams	Mixed BC	Post processing	Number of samples
MYC	Plasticised mycelium	Mycelium	Glycerol			x				/	8

Three identical square mycelium samples are grown. (B1, B2 and B3). These are subjected to a heat press treatment during 20min. Then the samples were not completely dry yet, thus oven drying was conducted, as in the standard mycelium protocol. When dry, the samples were soaked in glycerol for different timespans. An overview is listed in Table 17. *Further information on the creation of those samples can be retrieved from Elise Elsacker, who conceived, fabricated and provided them.*

Sample	Heat press temp	Heat press time	Glycerol soaking
B1	100°C	2x600s	24h
B2	150°C	2x600s	48h
B3	200°C	2x600s	120h

Table 17
Plasticised mycelium samples preparation

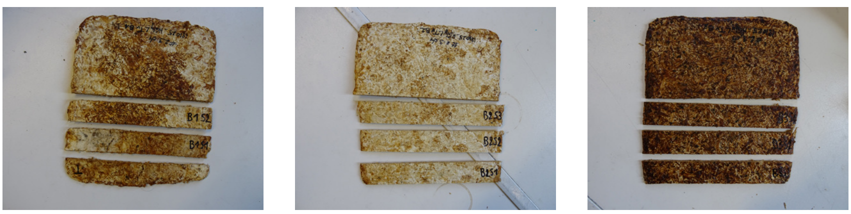
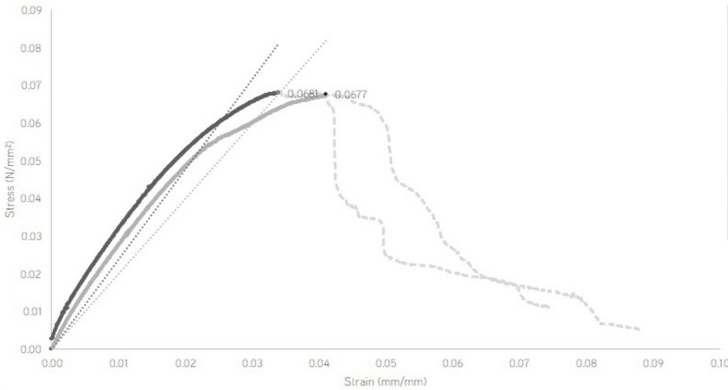


Figure 54
Tests B1 (a); B2 (b) and B3 (c)

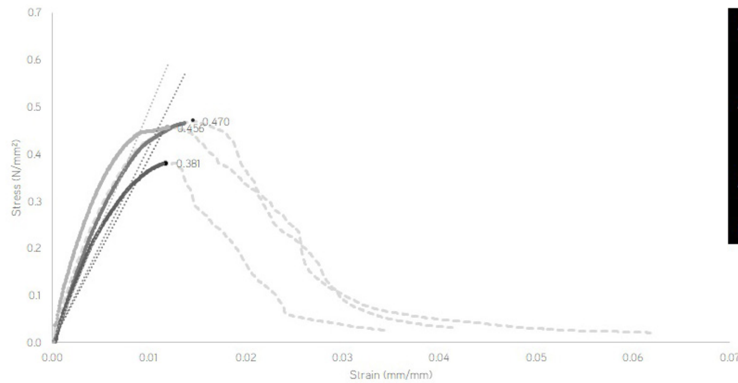
Sample measurements

DRY SAMPLE	dry weight (0.01g)
B1S1	5.7
B1S2	6.3
B2S1	4.3
B2S2	4.7
B2S3	5.2
B3S1	6.2
B3S2	5.7
B3S3	5.2

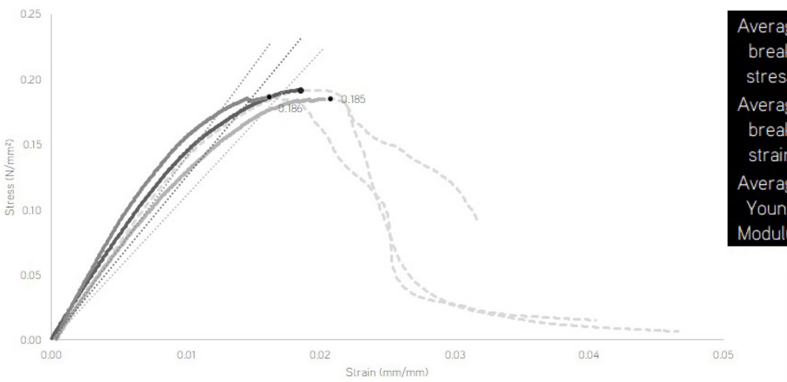
Tensile tests



Average break stress	67.90	+/- 0.20	kPa
Average break strain	0.04	+/- 0.00	mm/mm
Average Young Modulus	2.11	+/- 0.10	Mpa



Average break stress	362.67	+/- 38.66	Kpa
Average break strain	0.01	+/- 0.00	mm/mm
Average Young Modulus	48.59	+/- 6.47	Mpa



Average break stress	187.76	+/- 2.87	KPa
Average break strain	0.02	+/- 0.00	mm/mm
Average Young Modulus	14.32	+/- 1.72	Mpa

Comments on tensile tests

The three samples are illustrated above. The results were all very consistent and thus quite reliable. The break was never sudden but always quite gradual, as the dotted lines illustrate in the graphs. In terms of tensile strength, these samples do not reach the strength of the BC samples. It has to be noted that the tables provide measurement data for mycelium samples in KPa for the strength and MPa for the stiffness. In comparison with eachother, samples B2 have a considerably higher strength and stiffness. This already illustrates that a good balance of heat pressing and glycerol soaking has to be found, since samples B1 had less of those, and samples B3 more.v

Other samples

This list is composed of tests for which the research was performed, but for which the tests did not make it to production due to diverse reasons.

ECA dip-soak

An important issue with BC is **its lack of water resistance.** A study (Ayadi et al. 2013) reveals a simple method to make cellulose sheets (not specifically BC) hydrophobic using an ECA **(ethyl cyanoacrylate)** monomer solution dip-coating. Polymerization takes place wherein each individual fibre is coated forming a PECA layer while the cellulose fibres morphology remains unaltered. Furthermore, the mechanical properties of the cellulose are improved as well, influenced by the RH (relative humidity) conditioning before and after the coating. Although, this improvement could be because of very thin cellulose sheets used, where the newly formed coating dominates the mechanical performance, as mentioned in the study. The PECA layer formed by hydrogen bonding actually would reduce inter-chain interaction between cellulose fibres. The best performance is reached at a **solution of 3.5 wt% of ECA in toluene**. The hydrophobic and mechanical resistance reaches a plateau at this concentration, pointing out that it corresponds with a total covering of cellulose fibres at the surface.

Due to the high cost of ECA, this technique was not used nor further investigated

The following experiments did not make it to production because of not enough BC material being available.

Kakishibu / Tannic acid

This tanning agent would enhance watertightness and improve crosslinking.

Heat press on plasticized BC (EGCC)

Both techniques were exerted, a combination of both could have been interesting, as both heat and cross linking agent EGCC could improve cross-linking even more.

Heat press on wet double layer as a means to combine BC

Heat press on wet BC and fibres as a means to combine into a composite

8.1. Water absorbance

This relates to '**RQ 8** *What watertightness treatments are there and do they have an influence on strength and appearance?'*

For applications in biomedical circumstances, bacterial cellulose is often praised for its high water absorbance capacity, but of course for architectural applications the opposite property is desirable. This aspect was not the focal point of the research, but to extend the explorative aims of the investigations, a basic test on water absorbency of all made samples was performed. The aim is to have a rough insight on the **magnitude of the absorbency** and a **means of comparison between the different post-processings** which were carried out. Some of the post-processings did have specific water-repellent aims, such as the beeswax and coconut oil coatings (experiments G, H, I). The other ones did not, but it is still relevant to clarify whether differences are present.

The test was performed by placing two small samples of approximately 25mmx50mm of each test in a water-filled plate. The original sample weight (0h), the weight of the samples after 12h and the weight after 48h were measured and the weigh increase was calculated. The results are shown in Table 18.

Table 18

Values of absorbed water in weight after 12h and 48h.

sample	0h (0.01g)	12h (0.01g)	48h (0.01g)	Increase from 0h to 12h	Average increase	Increase from 0h to 48h	Average increase	Increase from 12h to 48h	Average increase
B'	5	10	11	100%	83%	120%	102%	10%	10%
B''	6	10	11	67%		83%		10%	
C'	6	11	13	83%	102%	117%	108%	18%	5%
C''	5	11	10	120%		100%		-9%	
D'	8	17	17	113%	81%	113%	71%	0%	-7%
D''	10	15	13	50%		30%		-13%	
E'	12	17	16	42%	38%	33%	42%	-6%	3%
E''	12	16	18	33%		50%		13%	
F'	15	22	26	47%	34%	73%	56%	18%	16%
F''	18	22	25	22%		39%		14%	
G'	7	11	12	57%	**54%**	71%	**69%**	9%	**10%**
G''	6	9	10	50%		67%		11%	
H'	29	35	37	21%	**25%**	28%	**28%**	6%	**3%**
H''	28	36	36	29%		29%		0%	
I'	9	13	14	44%	**47%**	56%	**58%**	8%	**7%**
I''	10	15	16	50%		60%		7%	
J'	6	13	12	117%	101%	100%	71%	-8%	-15%
J''	7	13	10	86%		43%		-23%	
K'	7	17	17	143%	107%	143%	114%	0%	4%
K''	7	12	13	71%		86%		8%	
L'	23	80	86	248%	228%	274%	248%	8%	6%
L''	22	68	71	209%		223%		4%	
Q'	13	16	18	23%	24%	38%	38%	13%	11%
Q''	8	10	11	25%		38%		10%	
R'	11	15	18	36%	32%	64%	53%	20%	16%
R''	7	9	10	29%		43%		11%	
S'	27	50	52	85%	86%	93%	96%	4%	5%
S''	23	43	46	87%		100%		7%	
T'	11	28	30	155%	131%	173%	141%	7%	4%
T''	12	25	25	108%		108%		0%	

When placing the samples in the water, a clear difference was already visible between samples G, H and I and the other ones. While all samples immediately let themselves submerge in the water, these samples were clearly water repellent to a certain extent. When looking at the results, this is less clearly distinct. Unfortunately not any of the samples is completely water repellent (0% increase in weight). The **best result** is found for **sample H (beeswax coating)**. With an increase of 'only' 28% after 48h. Also samples E (EGCC soaking), F (glycerol soaking), I (coconut oil and beeswax), Q (heat press-dried), R (dried and heat pressed) have values below 60%. Most other samples show values coming closer to 100%. One sample scores really bad: the SCOBY tests (L) absorbed almost 2.5 times its own weight after 48h.

It can be concluded that some **crosslinking agents** such as EGCC and glycerol inhibit new water absorbance and that the same happens with **heat pressed samples**. Furthermore the use of beeswax as a water-repellent coating is a lot more effective than coconut oil, although coconut oil made it possible for the beeswax to be spread more evenly.

This discussion also relates to the following original research question:

RQ 8 *What watertightness treatments are there and do they have an influence on strength and appearance?*

The influence on strength and appearance is handled further on.

Table 19
Overview of all stress, strain, stiffness and density results

Label	Sample	Category	Average break stress (Mpa)	Standard deviation (Mpa)	Average break strain (mm/mm)	Standard deviation (mm/mm)	Average Young Modulus (Gpa)	Standard deviation (Gpa)	Density (kg/m^2)
A	Undried, wet	Reference	0.68	*0.42*	0.09	*0.03*	0.01	*0.01*	
B	Only air dried	Reference	31.75	*9.19*	0.04	*0.01*	0.45	*0.06*	144.49
L	SCOBY	Reference	16.09	*0.84*	0.03	*0.00*	0.71	*0.06*	50.70
C	Alkalic cleaning, air dried	Reference	16.71	*6.34*	0.07	*0.04*	0.18	*0.10*	59.31
K	Alkalic cleaning, air dried	Reference	47.50	*19.06*	0.06	*0.02*	0.62	*0.48*	97.06
D	Ethylene glycol	Soaking	91.89	*29.03*	0.08	*0.01*	0.89	*0.29*	192.17
E	EGCC	Soaking	85.08	*13.49*	0.12	*0.01*	0.67	*0.10*	240.42
F	Glycerol	Soaking	57.32	*13.30*	0.13	*0.02*	0.37	*0.04*	276.57
G	Coconut oil	Soaking	24.62	*6.98*	0.12	*0.04*	0.22	*0.11*	102.52
H	Beeswax	Soaking	10.85	*6.43*	0.11	*0.05*	0.11	*0.05*	129.27
I	Coconut oil and Beeswax	Soaking	14.20	*3.87*	0.08	*0.04*	0.29	*0.28*	89.66
J	Citric acid solution	Soaking	17.00	*12.60*	0.07	*0.01*	0.24	*0.20*	76.58
M	Sewn wet and dried	Seams	13.44	*2.00*	0.07	*0.02*	0.22	*0.09*	
N	Dried and sewn	Seams	11.90	*1.85*	0.04	*0.00*	0.32	*0.04*	
O	Seam by drying	Seams	8.97	*1.21*	0.06	*0.00*	0.17	*0.03*	
Q	Drying by heat press	Heat press	82.67	*25.41*	0.01	*0.01*	11.58	*2.81*	171.25
R	Dried and heat pressed	Heat press	19.39	*5.29*	0.04	*0.02*	0.63	*0.38*	144.49
S	Double layer by drying	Seams	15.91	*5.83*	0.06	*0.02*	0.16	*0.13*	95.20
T	Mixed	Mixed	21.06	*16.28*	0.01	*0.00*	1.02	*0.81*	110.30
U	Mixed, spread on fibers	Composite	9.96	*2.03*	0.01	*0.01*	0.78	*0.14*	118.25
V	Composite fibres	Reference	23.33	*2.70*	0.03	*0.00*	0.60	*0.06*	
W	Grown comp, air dried	Composite	6.84	*1.06*	0.02	*0.00*	0.28	*0.06*	93.33
X	Grown comp, heat press	Composite	8.94	*1.41*	0.01	*0.00*	0.52	*0.11*	98.91
Y	Composite by drying	Composite	18.28	*1.12*	0.05	*0.00*	0.32	*0.03*	151.62
MYC	Plasticised Mycelium	Mycelium	0.07	*0.00*	0.04	*0.00*	0.00	*0.00*	
			0.36	*0.04*	0.01	*0.00*	0.05	*0.01*	
			0.19	*0.00*	0.02	*0.00*	0.01	*0.00*	

8.2. Results

After an extensive overview of all different tests which were carried out, the results are compared and assessed. The results are grouped all together, or in specific smaller groups where comparison is more relevant, such as specific seam comparison or composite comparison. In the end the most interesting experiments will be highlighted. The graphs do not include stiffness data, to keep the graphics clear. When relevant, the link to the stiffness will be made.

Reference sample results

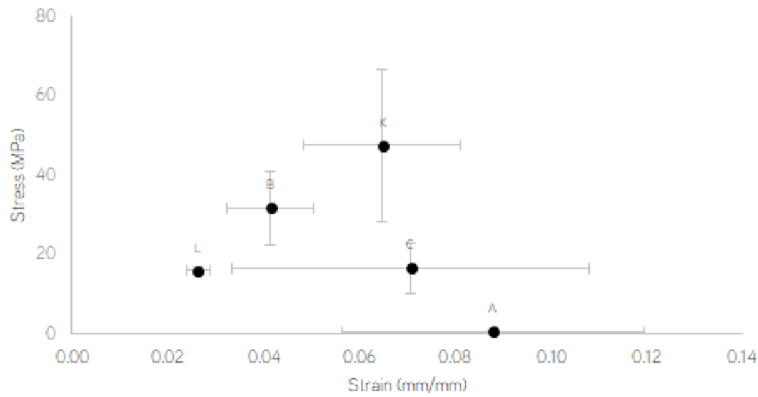


Figure 55
Overview of reference sample results

As a base of materials to start from, some reference samples were defined. These represent 'original' states of the material such as freshly harvested wet BC (A) of DIY-grown SCOBY (L). Samples K and C represent alkalic cleaning, which was the base for all future tests.

It can be seen that the undried material (A), although feeling very strong and though, did not show any strrength. It has to be said that the samples were not freshly harvested, but kept in humid conditions (first water, then ethanol) for a week until tested. This could have affected the strength of the samples.

The alkalic cleaning performed on all samples (C and K), which is commonly performed in BC-literature and renders the sheet completely clean and pleasingly white, does not improve the strength of the material, in comparison to a non-treated material (B). It does decrease the stiffness by increasing strain at break. The DIY-grown SCOBY (L) has a little smaller strength that could be attributed to non-homogeneous growing, but the difference is little.

Post processing results

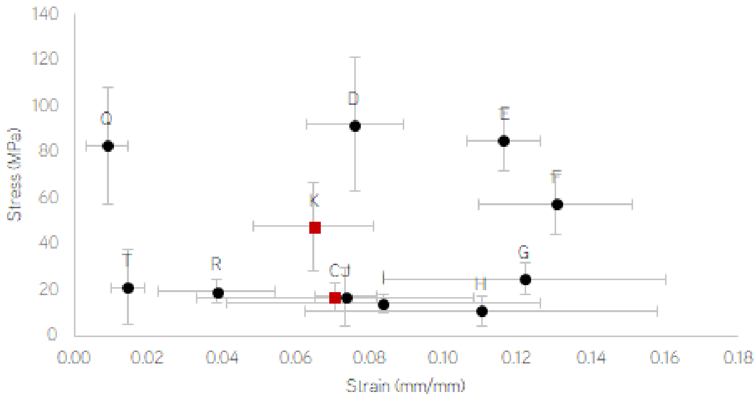


Figure 56
Overview of stress-strain failure averages of post processing samples.

Different ways to alter the plain BC were explored. Multiple soaking experiments were conducted with the aim to induce **cross linking or plasticise** the material (D to J). The influence of a **heat press** (Q and R) and of **mixing BC** (T) was explored as well. In Figure X also reference samples K and C are shown in red.

In comparison with those reference samples some samples did not improve break stress but did gain higher stiffness such as the mixed BC (T) and the heat pressing of dried BC (R). The mixed BC was not produced very well, the spreading out was not homogeneous and contained holes. It was visible from the tests that failure occured in those holes. By applying a better production protocol, failure stress could be improved as well.

Some samples only lost stiffness, becoming more ductile. This is the case for sample I (coconut oil and beeswax) and even more for samples H (only beeswax) and G (only coconut oil). This is a very interesting observation. These coatings were applied first and foremost to improve water repellency, but it was already a hypothesis that they could improve ductility of the samples. As the reference samples (especially B, air dried) feel very brittle and fragile, applying these coatings makes them easily malleable, as is now also proven in the results by increasing failure strain. Samples F (glycerol soaking) also considerably improved malleability, but also improved strength.

Then some tests did considerably increase the strength of the BC. Samples Q (heat press on wet BC), D (Ethylene glycol soaking) and E (EGCC soaking) all improved failure stress, presumably by increasing cross-linking by means of their cross-linking agent soakings or heat treatments. In terms of strain, sample D remained comparable to the references. For sample E, failure strain increased as well. Very impressive is the heat pressed sample Q which not only improved strength considerably (82.67 MPa failure strength) but also gained high stiffnes. In general, all tried samples have stiffnesses (largely) under 1GPa. Sample Q reaches 11.58 GPa due to the heat treatment. This discussion also relates to the following original research questions:

RQ 4 *What post-processing techniques can improve the strength of bacterial cellulose?*

RQ 5 *What are the mechanical strengths of these post-processing techniques?*

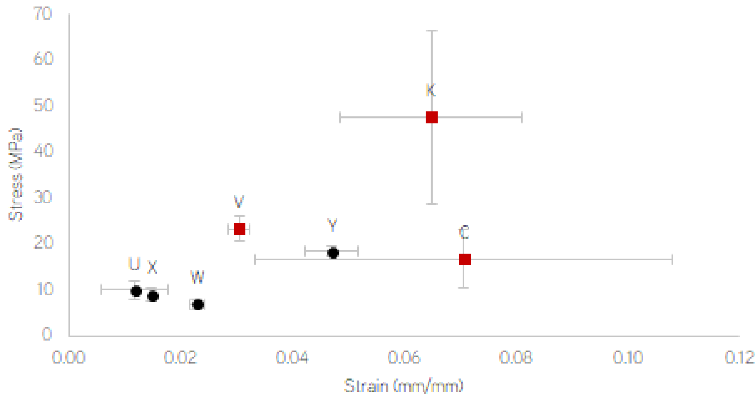


Figure 57
Overview of stress-strain failure averages of composites.

The search for a strong biomaterial membrane led to developing composites. Natural fibres such as flax combined with a BC matrix could generate a material that has higher mechanical strengths compared to plain BC. In comparison with the fibres, a plain material with transverse stiffness would be developed. Unfortunately, the results for these tests are not fulfilling this hypothesis. Sample K and C reflect the plain BC reference material, data point V represents the plain fibres. All tried composites have lower or equal strength values, although stiffness in general has increased.

Sample Y has a double layer of BC, making the resulted data even worse. Test U (mixed BC spread on fibres) has the highest stiffness, which seems logic since the plain mixed BC (sample T) also has a higher stiffness. Although the difference with samples X and W (in-situ grown composites) is minimal. It can be concluded that the added value of the composites at this stage is only an improved stiffness.

While these results are not pleasing, there are some reasons that can explain itproviding working points to improve. It was already very clear when testing that the **adherence** between BC and fibres is not satisfactory at all. As mentioned before, a protocol should be developed that completely embeds the fibres in the BC. For example, the mixed BC could be pressed from both sides into the fibres. The in-situ grown composites, which showed very promising when harvested, also only resulted in a deposited layer of BC on the fibres. Embedding the fibres in-situ in the BC is certainly something that needs attention.

Another problem was that the strains at break of the BC and the fibres proved very different, explaining the double failure curves that were shown. This induced, although providing a more stiff materials, that the materials did not work together as composites. In the production protocol of the in-situ grown samples (W and X), it was already tried to tension the fibres while the BC grew. The results show that more pretensioning is needed in order to align the failure strains of fibres and matrix.

This discussion also relates to the following original research question:

RQ 9 *How can composites be created and what is their strength, water-tightness and appearance?*

Seam results

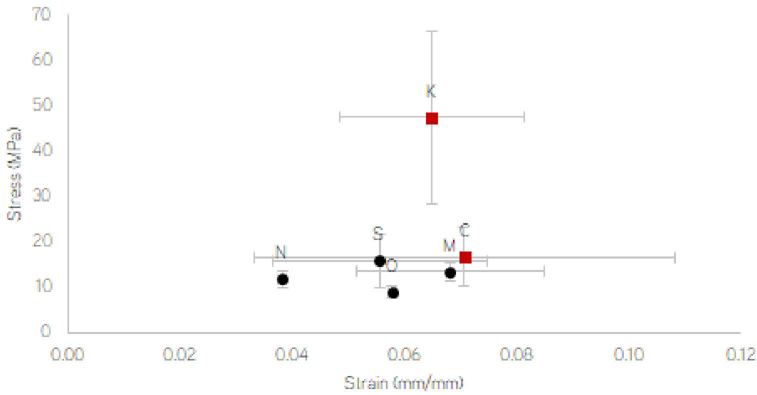


Figure 58
Overview of stress-strain failure averages of seam tests

The seam tests illustrate an exploration on connection possibilities in an architectural application of BC membranes, as for which a concept is drawn on page 72 and 73. The aim is to create connections that are stronger than the plain material, but also remain in the same realm of techniques as the BC, i.e. with natural materials or without any interfering at all. As explained before, it was found in the explorative research that wet BC combines by itself when drying. The strength of those connections is tested (sample O). Also a sewn connection (N) and a self-combined and sewn connection (M) are tried. The sewn connection (N) failed in its connection by the threads. The other samples did not fail in the connection but in the plain material. This already illustrates well that the self-combined connections which were discovered in the explorations, are stronger than the connections, although it has to be noted that the connections were double, as elaborated before.

The data shown in Figure 58 is not so informative for all samples, as the failure strengths of samples M and O represent the failure of plain BC, which is thus the same material as reference samples K and C. The sewn connection N does show its failure stress.

Sample S is the same as the plain BC material, just like reference samples K and C, only that a double layer of BC was combined. This is the same build-up as the connection part of O. The strength of this double layer of BC is not much higher than the reference samples, which is not what was expected. A double strength was expected.

This discussion also relates to the following original research question:

RQ 7 *How can seams be created and what is their strength?*

Strength and water absorbency

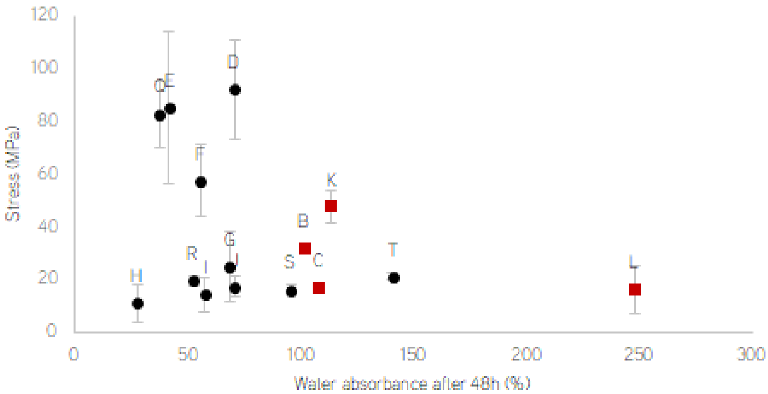


Figure 59
Stress versus water absorbency rates

Among other parameters, strength and water absorbency are important for architectural membrane materials. In this graph both are plotted in order to quickly identify possible materials, by searching for high strength and low water absorbency rates. Samples Q, D and E already came out as the most promising results in terms of strength, especially sample Q for its high stiffness. Also in this graph, they all have lower water absorbency rates compared to the reference materials that did not have post-processing (B, C, K, L, pictured in red squares). The lowest water absorbency rate is found for sample H which was coated with beewax. Unfortunately it did not improve strength. A combination of a heat pressed sample, coated with beeswax is a possible future track to achieve optimal performances. Although it has to be noted that the water repellency needs further development in order to achieve full watertightness of this originally water absorbing material.

Mycelium composites

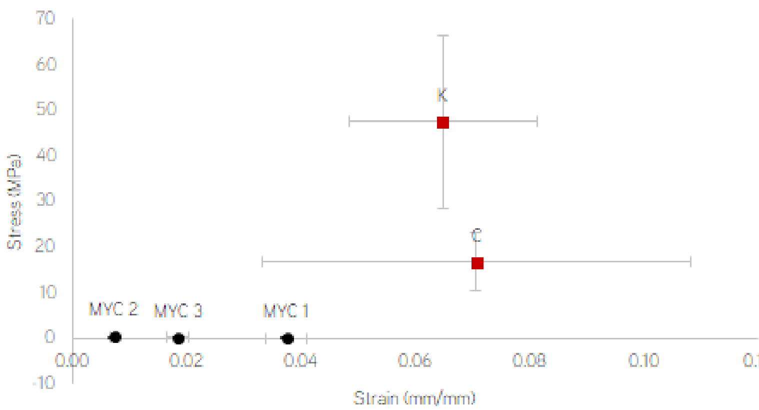


Figure 60
Overview of stress strain failure averages of mycelium composites

A small range of plasticised mycelium composites, provided by Elise Elsacker, were also tested. The three tests have different glycerol soaking times in order to plasticise the normally solid mycelium. The results are not satisfying, as the failure stress is very low in comparison to the BC reference samples. A clear difference is visible although between the three samples in terms of strain, as the sample with the lowest heat press temperature and least soaking reached the highest strain. It can be concluded that for this application, where tensile strength is crucial, the mycelium samples are not adequate.

Comparison with literature

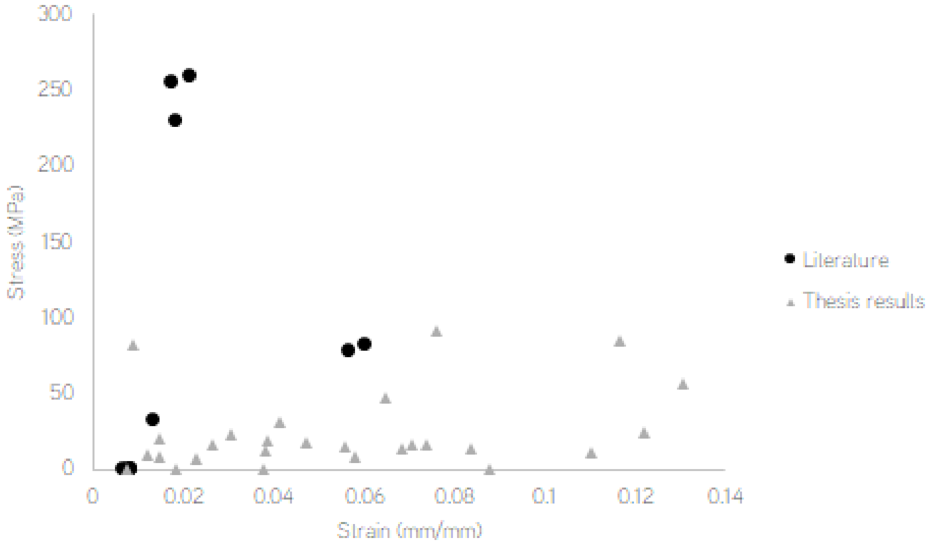


Figure 61
Comparison of own data with literature

In the state of the art, an overview of already defined strengths of BC in literature is given. This data is now supplemented with the own gathered data in order to place all explorations in this thesis into perpective. In Figure X both are combined. As was already mentioned in the state of the art, one early research (Yamanaka et al, 1989) mentioned exceptionally high results in comparison with other literature. The results from the thesis are in the same range as the other literature pictures here. In general quite low stresses were achieved. Some samples do achieve higher stresses, coming close to 100MPa. These are the aforementioned succesful samples Q, D and E. In general, quite high strains are also noted.

Comparison with common membrane materials

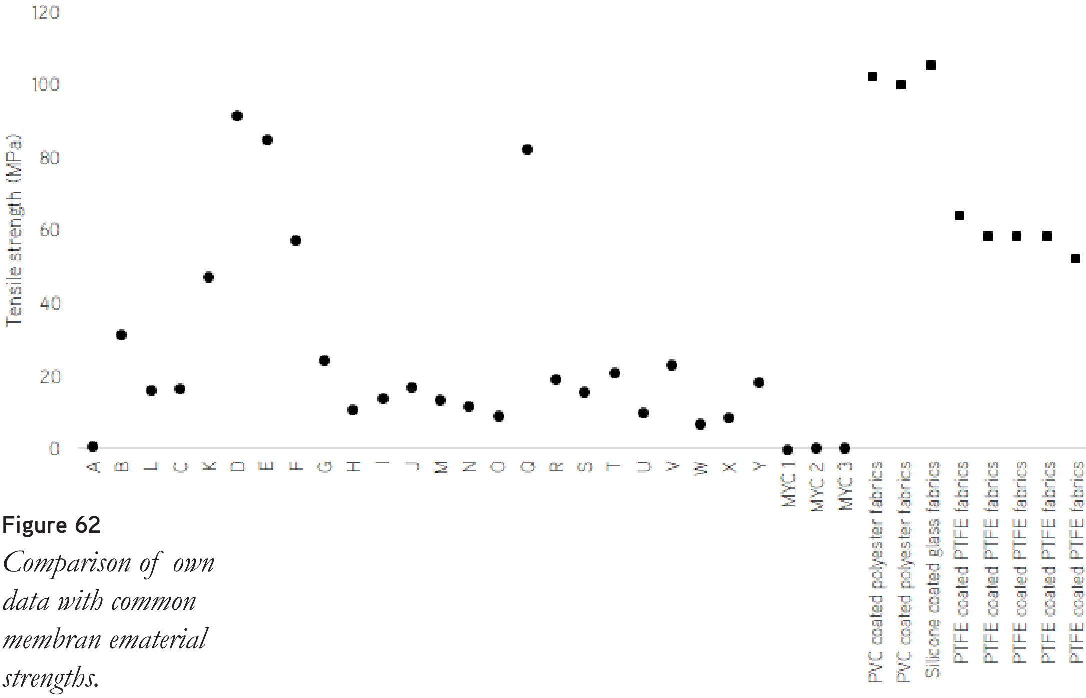


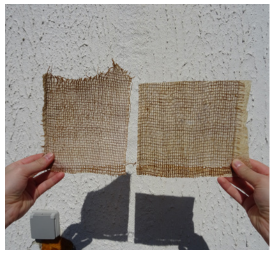
Figure 62

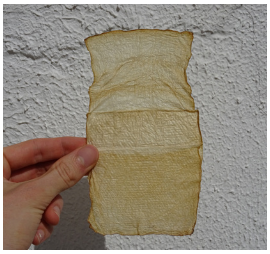
Comparison of own data with common membran ematerial strengths.

Here an overview of common membrane material data allows to conclude wether BC can, or could ever be used as a totally new interpretation of this temporary architecture, making it disposable in a nature-friendly way, among other positive properties. On the right side are pictured the oil-based membrane material strengths. In this overview strain and stiffness are not considered, as they were not found for the common membrane materials.

The comparison proves quite satisfactory. While the lowest of all gathered results of the experiments do not achieve the strength of common membrane materials, the best results (samples D, E, Q), do. The strength of glycerol soaked (F) and even the alkalic cleaned reference material (K) approaches that of the PTFE fabrics, the failure stresses of the Ethylene glycol and EGCC-soaked (D and E) as well as the heat pressed (Q) even approach the tensile strengths of PVC-coated polyester fabrics.












9 Conclusions

9.1. Is bacterial cellulose a valuable replacement of common membrane materials?

This thesis constituted of a wide exploration of possibilities concerning bacterial cellulose in different shapes. Composites, soakings, coatings, seams, heat pressed and mixed samples, they were all part of a broad characterisation of possibilities with the material. All of these have been described mainly in terms of strength, but also in terms of water absorbance and appearance. After a comparison with literature and common membranes, it is now possible to make a first statement on whether bacterial cellulose's realm could be extended from a microbiologist and avant-garde designer material, to an ecological answer to fossil fuel-based tensile membrane materials.

Looking at the **strength** comparison with common membrane materials, the answer is definitely positive. The strengths of common materials are reached within this research, and some literature even finds higher strengths for bacterial cellulose. Furthermore, as this was only an exploration, there are still more possibilities to be examined. The found results also have a broad margin of optimisation to be carried out.

The other aspect which was looked at is **water repellency.** Unfortunately, this aspect still requires a decent solution. Some coatings such as the beeswax or processings such as the heat pressing already decrease the water absorbency rates, but a totally water repellent material was not reached.

In terms of **appearance**, some samples had a specific pleasing aesthetic, such as the plasticized samples with ethylene glycol, EFCC and glycerol. These were rendered pleasingly white transparant. Most astonishing is the attractive pattern found on the heat pressed sample, figured in detail on page 2.

If a best result in general of this whole research has to be pointed out, the **heat pressed sample Q** definitively wins. In terms of strength, this material gained the highest stresses with an impressively high stiffness, in comparison with the other experiments. Furthermore the appearance and texture are very (most) pleasing and the water absorbency rates where the lowest of all samples, although this is still a working point.

9.2. Exploiting bio-based characteristics for membrane set-up

Next to the research done which is summarised hereabove, a highlight of this thesis is the development of a connection concept between sheets. On pages 72 and 73 the concept is explained more in detail. The results of the seam connection tensile tests were not completely satisfying. For example it does not seem logic that a double layer of BC does not create a double strength. Therefore more research in this topic of connections still has to be conducted.

9.3. Studies to be done

Although the research had the aim to not only focus on tensile testing and be a bit more horizontally spread, only a few aspects of the material were covered. Before being able to definitively approve bacterial cellulose as a structural architectural membrane, the following research is still needed.

First of all a further characterisation with more precise data of the **tensile tests** is needed. As only three or four specimens were used for each test, more are needed to gain decisive information. A further optimisation of this strength is also needed.

The **water repellency**, as already mentioned before, still needs a solution.

Also the quest for strong **connections** needs more attention.

Not covered at all, are an assessment of **durability** in outside conditions. This is of course related to water repellency. Once that a good water repellent technique for BC is found, the material has to be subjected to prolonged outside conditions.

If technically bacterial cellulose is completely characterised and evaluated as being a possible replacement or complement to tensile membrane structures, one aspect still has to be defined. A **lifecycle assessment (LCA)** can make or break the development of this material, since its research is based on the assumption that it could be an ecological, bio-degradable, non-resource intensive alternative to fossil-fuel based membranes.

9.4. Cost and upscaling

A drawback BC is facing now is a relatively **high production cost**, a statement made in biomedical and cosmetic contexts. Some research investigates techniques to cut this cost down, for example by increasing the yield by optimising the culture media composition (Ramana, Tomar, and Singh 2000)) or by using waste as a carbon source. Now mostly glucose is used, but fruit juices from discarded fruit, sugar cane molasses, agricultural waste and brewery waste could all provide a cheap and environmentally friendly alternative to glucose. They additionally **close a gap towards a circular economy**, which brings us to an ideal solution by using local waste to 'grow' local building materials. For example, a study showed that a Hestrin-Schramm (HS) medium with added sugarcane molasses proved better for BC production than glucose. (Jozala et al. 2015, Islam et al. 2017).

Related to the cost, is also the question of **upscaling**. Can bacterial cellulose grow out of the lab? A personal thought on this is the following. Although the material for this thesis was grown in the lab, a lot of DIY-actors and designers just grow it in their kitchen or garage, by using a SCOBY culture. This was also investigated in this research, although the SCOBY samples had a lower strength and very high water absorption rates. But since it is already possible to grow out of extremely sterile conditions, already one large difficulty for upscaling is scratched. The biggest issue then becomes the need for space, time and nutrients. As mentioned above, nutrients could be found in local waste streams, which would be an ideal solution. When more possibilities of this material will be understood, a more precise statement on the upscaling can be formulated.

9.5. Critical reflection on the approach

The unusual approach, which is explained in detail in Chapter 4, was an important part for the production of this thesis. Besides from consulting available academic literature, interaction with an online DIY community was used as a tool to bridge the gap to (micro)biology from an architectural engineering perspective. Also was it used as an inspiration, since the DIY context has all kinds of experiments and designers working with novel techniques, in a way that is very accessible.

Some research questions were defined in order to assess how this particular way of working was experienced.

- *How did academic literature and open knowledge platforms relate? What were the uses of each in the light of this research?*

Both were actively used, but for different aims. Academic literature was consulted to have an overview of what is already performed for BC research. Since the information (even from long ago) is still easily available, a very consistent overview of what was already studied could be defined. Also the methodologies were of use, since academic literature consistently mentions information on how the material is produced. Although the information is available, it is not always easiliy understandeable for an architectural engineer.

- *How was the balance between both?*

In the beginning the focus lay more on the DIY forums, when I was not yet acquainted with the material. Further to the end the focus shifted more to academic literature, since it became more easily understandeable as more knowledge was gathered.

- *What was the added value of including DIY knowledge and forums?*

The gap to difficult academic literature was bridged by the open knowledge platforms, where a lower treshold exists. Gathering information on these sites allowed to understand the functioning of the material, but also see it in a different context. For example, an academic research never focuses on home-grown DIY SCOBY, while the lack of needs of sterile environments is truly important for a possible upscaling.

- *What were downsides of including DIY knowledge?*

The downside was that it is all very explorative and not always reliable. Also, since a forum is time-based and about discussing, 'old' knowledge gets lost on the way. A search function can dig into that database, but does not always bring everything back, and the authors maybe are not available anymore. Since in an academic paper everything should be consitently described, the need for interaction is smaller.

- *To what extent was there a real ongoing discussion with back and forth knowledge sharing during the research in the DIY forum?*

A couple of times, the ongoing work of my research and experiments were shared on the forum. This was done with questions for advice when facing problems, or simply to share my findings. I was pleasantly surprised by the interaction these posts generated, which helped me to for example try out other things, or simply to solve problems I encountered.

10.1. Data overview of literature and common tensile materials

Overview tensile strength literature — Source and Author	Year	context of research	Methodology		Tensile strength (Mpa)	deviation (%)	Young modulus (Gpa)	deviation (%)	Elongation (%)	deviation (%)	Density (g/cm^3)	deviation (%)	Yield (g/L)	deviation (%)
Nanofiber-Based Hybrids for Nanocomposites	*2017*	Create green hybrids based on biodegradable constituents.	Plain pellicle		79.1	13.6	2.49	9.6	5.6	17.8				
Qiu and Netravali			Hybrid BC - MFC (50%)		84.1	14.7	2.83	6.9	6	22.3				
The structure and mechanical properties of sheets prepared from bacterial cellulose	*1989*	Research on mechanical properties of BC	Plain air drie	20°C	256		16.9		1.7					
			Hot pressed	150°C 49 Mpa	231		18.0		1.8					
Yamanaka et al.			Hot pressed	150°C 49 Mpa	260		16.9		2.1					
			Mixed BC		85		4.9				0.99			
Effect of different additives on bacterial cellulose production by Acetobacter xylinum	*2009*	Material property of agitated BC cultures with differen	Plain pellicle		34	0.88	2.7	1.12	1.3	38.46			1.3	23.08
			0% CMC		2.3	13.04	0.3	10.00	0.8	62.50			1.5	0.00
Cheng, Catchmark and Demirci			0.2% CMC		2.4	12.50	0.3	9.68	0.7	71.43			4.8	0.00
			0.5% CMC		2.4	12.50	0.3	9.68	0.8	62.50			6.5	0.00
			0.8% CMC		2.3	13.04	0.3	10.00	0.8	62.50			8.1	0.00
			1.0% CMC	highest yield	2.5	12.00	0.3	9.68	0.6	83.33			8.4	0.00
Physical properties of bacterial cellulose	*2006*	How alterations of the culture medium can not only increase yield, but also improve mechanical strength, here with lignosulfonate			Tensile Load(N)									
			HS 10245		*10*		0.33						4.4	
Keshk			HSL 10245		*18.28*		0.61						7.2	
			HS 13772		*15.51*		0.51						8.7	
		Different strains (number)	HSL 13772		*18.69*		0.62						11.4	
		HS (without lignosulfonate)	HS 13773		*15.03*		0.50						10.1	
		HSL (with lignosulfonate)	HSL 13773		*19.23*		0.64						16.2	
			HS 13693		*13.81*		0.46						7.9	
			HSL 13693		*21.53*		0.71						16.3	

Overview tensile strength common fabrics — Source and Author	Year	context of research	Methodology		Tensile strength (kN/m) warp	Tensile strength (kN/m) weft	Elongation (%) warp	Elongation (%) weft	Density (g/cm^3)	Thickness (mm)	Weight (g/m^2)	Tensile strength (Mpa) warp	Tensile strength (Mpa) weft
European Design Guide for Tensile Surface Structures	*2004*												
Blum, Bögner, and Némoz		**PVC coated polyester fabrics**	Type 3	(French design	115	102					1200		
		example 610 g	*610 gsm*		*48*	*42*				*0.47*	*610*	*102*	*89*
		example 900 g	*heavy duty 900 gsm*		*80*	*80*				*0.80*	*900*	*100*	*100*
		PTFE coated glass fabrics	G5		*124*	100					1200		
		Silicone coated glass fabrics			107	105					1100		
		example Fiberflon	*SIL - 076W*		*80*	*80*				*0.76*	*1100*	*105*	*105*
		PTFE coated PTFE fabrics			84	80					830		
		ETFE foil 0.050					450	500	1.75	0.050	87.5	64	56
		ETFE foil 0.080					500	600	1.75	0.080	140.0	58	54
		ETFE foil 0.100					550	600	1.75	0.100	175.0	58	57
		ETFE foil 0.150					600	650	1.75	0.150	262.5	58	57
		ETFE foil 0.200					600	600	1.75	0.200	350.0	52	52

10.2. Norm interpretation

A first comparative material that has a set of norms available is **leather**. Unfortunately, tensile strength norms were not found. Methodologies in literature are based **on tensile strength norms of thin plastic sheets**, for example by *ASTM D-882-02* in the research about hybrid composites (Qiu and Netravali 2017). A similar ISO norm is available, *ISO 527-3: tensile properties of plastics: test conditions for films and sheets.*

This norm handles foil and sheet material plastics with a thickness below 1mm. It could be a comparable material, although the norm already states *'not normally suitable for determining tensile properties of cellular materials and plastics reinforced by textile fibres'*. This would exclude composite tests. Since no other reference exists, the conditions are still taken as a starting point and adapted following the needs. In order to keep consistency all tests over different compositions of BC are following the same methodology.

Following norm ISO 527-3, test specimen type 2 as shown in Figure 17 is used, which is preferred. The strips are 25mm wide, also corresponding with the used grip width. The norm prescribes 100mm starting length between clamps. Due to the limited length of the specimens and to ensure that the specimens can be decently clamped, this length was reduced to 60mm.

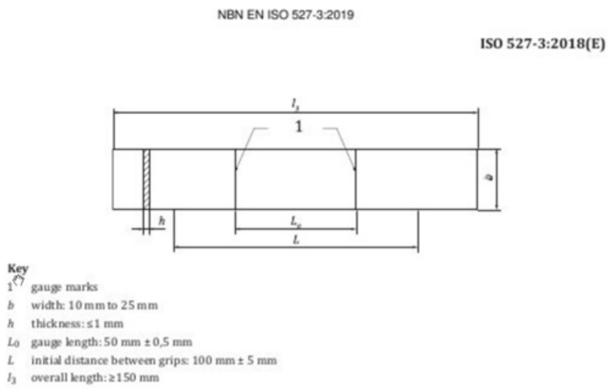


Figure 63
specimen type 2 design, an excerpt from ISO 527-3:2018

Some comments relating to the norm:

- *Anisotropy* is not relevant, as BC should have constant properties in different directions.
- *Cutting:* The samples were not clean-cut punched as prescribed but cut with scissors.
- *Number of specimens:* A minimum number of 5 samples is prescribed. Furthermore, following the homogeneous but irregular nature of the BC samples, an even higher number would be desirable. However, the aim of this research is to gain quick and explorative knowledge of a broad set of possibilities with BC. Due to a limited amount of materials available and the aim to test a multitude of different samples, a lower number of 4 samples per test is defined. In a further perspective, a higher number of samples will be required in order to gain more precise measurements.
- *Conditioning:* 23+-2°, RH 50+-10%, min. 16h before is prescribed. These guidelines were not followed, the samples were stored at room temperature before testing.

10.3. Data processing

The obtained load-displacement curve is converted to a more useful stress-strain curve by means of the following formula

$$\sigma = F/A \text{ [MPa]}$$

$$\varepsilon = \Delta L/L_0$$

where:[22]

F = exerted force [N]

A = original cross-section of the specimen [mm^2]

ΔL = displacement of the testing bench [mm]

Lo = original length of the test piece [mm]

10.4. Shrinkage upon drying

	At harvesting		Dried		Thickness difference		Weight difference		Moisture content	Total average drying transitions		
	weight	avg thickness	weight	avg thickness								
	g	0.01mm	g	0.01mm	mm	%	g	%				
Sheet 1	196.6	2.4	4.2	0.19	-2.4	-93%	-192.4	-98%	98%	Thickness change avg		Deviation
Sheet 2	196.5	2.6	4.0	0.19	-2.4	-92%	-192.5	-98%	98%	-92%	+/-	1.03%
Sheet 3	124.7	1.7	2.3	0.11	-1.5	-93%	-122.4	-98%	98%	Weight change		Deviation
Sheet 4	18.0	0.7	0.5	0.06	-0.6	-91%	-17.5	-97%	97%	-98%	+/-	0.38%

Figure 64
First sheets: weight and thickness measurements with shrinkage rates

10.5. SCOBY production protocol

The culture liquid, with proportions stated in Figure 32:

- Boil the water and infuse the tea for 15min
- Dilute the sugar
- Leave to boil for 20-30min in order to approximately sterilise the liquid.
- Let the liquid cool down below 30°C. If not, the SCOBY bacteria will be killed.

Growth:

- Clean a plastic container and pour in the culture liquid and the starter liquid and/or SCOBY.
- Cover the container and place in a warm dark space around 25°C for a couple of days to weeks, depending on the desired thickness and growing rate.

Harvesting:

- At any moment the film which appeared on the surface of the liquid can be retrieved.
- Rinse and wash with soap in order to kill the bacteria.
- The SCOBY (if unwashed) or culture liquid can be used again as a source of bacteria. Nutrient will have to be added to ensure a good culture growth.

Drying:

- Place on a (massive) wooden plank and flip regularly until dry.

Ingredients to produce your fabric from a Scoby

Preparation for	Water	Tea	Sugar	Cider vinegar	Starter*	Scoby
~ 265 cl	200 cl	3 gr ≈ 2 teabags	200 gr	20 cl	45 cl	1 Scoby
~ 1055 cl	800 cl	12 gr ≈ 8 teabags	800 gr	80 cl	175 cl	1 Scoby
~ 1320 cl	1 000 cl	15 gr ≈ 10 teabags	1 000 gr	100 cl	220 cl	1 Scoby
liquid of culture	You have a smaller or a bigger container? Adapt the recipe, it's proportional!					

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