BACTERIAL COMPOSITES



MSc. Thesis, Eduard Georges Groutars

	This project is based on the notion of designing with bacteria, the new possibilities that this would bring and the consequential role of the designer. It takes place at the Bionanoscience department of the TU Delft where the designer, Eduard Groutars, is collaborating with researcher Kui Yu, who is developing a novel composite material made by bacteria.
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PREFACE

This project started from a fascination with the natural world and the dynamics of materials that are alive. When compared to the materials that we as humans produce, which are usually static, the materials made by mother nature seem to be much more intelligent, efficient and elegant. Constantly evolving, adapting and growing, it is my personal belief that if we could implement such qualities in the products and building that we produce, a lot of todays ecological problems would be history.

It is because of this fascination and ambition that I started to take an interest in the field of biodesign, where designers, artists and engineers are collaborating with living organisms to develop new materials and product ideas. Many projects within this emerging field propose a new harmony between biology and human technology that not only offers a wide array of possibilities but also forces you to think about the role that a designer can fulfill in this new context. One thing that is for sure is that Bio-design is an interdisciplinairy field, in which designer will need to learn how to collaborate with biologists and vice-versa.

I therefore traded the model-making workshop for a microbiological laboratory. Here I consider myself to be very lucky and I am gratefull to Elvin for providing me with the opportunities, connections and inspiration. I would also like to thank Marie-Eve for welcoming me into her research-group and giving me with a lot of freedom and possibility to learn. Lastly, I would like to thank Kui Yu for sharing his secret recipes and teaching me how to grow a material with big potentials using the worlds smallest organisms.

EXECUTIVE SUMMARY

This project started with analyzing the different roles and potentials that bacteria have for the growth of new materials with ecological benefits. Here questions arise about the role that a designer can fulfill in the development of such novel materials. What new skillset does he/she need to obtain? How will a collaboration with biologists take place?

In order to get a better understanding of the potentials of materials grown by bacteria and the subsequent role of a designer, a Material Driven Design (Karana et al., 2015) project was performed in collaboration with scientists from the Aubin-Tam research group, part of the Bionanoscience department of the Delft University of Technology. The starting point of the design project was a composite material consisting of three ingredients that are grown by three separate species of bacteria.

In order to gain an understanding of how this material was grown and produced, the designer performed a plethora of experiments investigating; the growth of the organisms and the amount of material they produced; how the ratio between the three ingredients influenced the resulting material; how the way in which the material was processed resulted in its final form and properties. This led to the understanding that this material is highly programmable in its form and properties such as its flexibility, strength and surface roughness.

With this in mind, user studies were performed in which it was found that the versatility of the material was considered interesting and intriguing by participants. They wonder what it is and how it is made, finding it hard to believe that bacteria grew such a material. This led to a material concept in which the designer proposes to play with these varying properties of the material, resulting in contrasting material experiences and highlighting the material its ability to appear as something that is both natural and man-made at the same time. This was done by exploring various processesing potentials of the material and analysing how different parameters of these processes influence the resulting material its properties. In doing so, the designer provided a framework by which future designers can program and explore this bio-based material that shows a lot of different potentials.

TERMINOLOGY

Material Driven Design	A design methodology that takes a Material Proposal as the starting point of a design process, hereby facilitating the design for novel material experiences and the discovery of unique potentials for this material.
Biofabrication	Utilizing the metabolism of living organisms in order to produce materials.
Material Proposal	The starting point of the design process, a bacterially grown composite, consisting of three ingredients; Cellulose, Calcite and P.G.A.
G.H. Bacteria	The Gluconacetobater Hansenii, bacterial species used in this project to grow Cellulose.
S.P. Bacteria	The Sporosarcina Pasteurii, bacterial species used in this project to grow Calcite.
B.L. Bacteria	The Bacillus Licheniformis, bacterial species used in this project to grow P.G.A
Cellulose	A common polysacharide or biopolymer that, for example, forms the constructive element in plant cells.
Calcite	A common mineral and polymorph of calciumcarbonate. It for example constitutes the shells of marine organisms.
P.G.A.	Polyglutamic acid, a polymer with a glue-like consistency.
Medium	The liquid in which the bacteria are grown, containing their nutrient and building blocks they need to produce the materials.
S.E.M.	Scanning Electron Microscopy, a beam of electrons is used to scan the surface of a material, providing the ability to zoom in to about 500.000 times.
Micro-Structure	In this project refers to the way in which the ingredients are layered, the homogenity and density of these layers.

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Figure 1: The raw materials that make up a bicycle as part of the Materialism artwork by Studio Drift, 2019

1.INTRODUCTION

This chapter will first sketch the context in which this graduation project is taking place. A context in which growing materials using living organisms such as bacteria is defined as promising and ecologically advantageous method. Within this context, the scope of this project is defined as exploring the design potentials of such novel materials, grown by bacteria and investigating the role that a designer can play in this development. Furthermore, the scope in which the project is performed, relevant research questions and the overall structure of this report will be explained.

1.1 PROJECT CONTEXT

- MaterialismOn the left (fig. 1) is an overview of the total amount of raw material that is
required to produce a single bycicle. This was done as part of an artwork by Studio
Drift where they question the role that various materials play in our modern lives.
They also propose that many of these materials, their origin and environmental
cost often go unnoticed by consumers (Studio Drift, 2019).
 - Problems Whether or not this holds true is a complicated question to answer. However, it can be stated that many of the materials, used by mankind, amount to a plethora of ecological problems in the world today. Take for example the plastic we use in our day to day lives, a substantial amount of which ends up in our waters, harming all ecosystems (Jambeck & Law, 2015). Another consideration is the amount of energy , often in the form of heat, that is invested into a material. An example here is cement, making up concrete and most of our buildings, the production of which requires temperatures of 1450°C, amounting to 8% of the total CO² emissions globally (Andrew, 2018)

Demand Many of these negative ecological developments call for the use of more sustainable materials and means of producing them. Materials that require less energy or precious resources to produce. Materials that do not involve harmfull substances during their production. Materials that are more easily recycled by both man and nature. Here designers can play a key role in the deployment and promotion of such sustainable alternatives through the selection of materials they use (Ashby, 2012). In addition, designers can aid in the development of more sustainable materials by offering a new understanding of their potentials. (Barati & Karana, 2019)

A Look at With the above in mind, interesting opportunities present themselves in the field of Biofabrication, where materials are produced through the growth of living organisms and cells (Mironov et al, 2009). Living organisms, through evolution, have become very adept at dealing with a scarcity of resources and growing materials from a limited selection of components. (Darwin, 1859, Wegst et al., 2015). With this in mind, Biofabrication shows the potential to produce materials far more efficiently than traditional means. Requiring less energy to be invested energy in their production (Jones et al., 2017), no precious resources to be extracted from the earth's crust (Holt et al., 2012), whilst resulting in materials that are often biodegradable due to their biological origin.

Introduction

Biological Humans have been collaborating with living organisms in the production of food, Technology medicine and clothes for thousands of years. (Arnold, 2005). Take for example the constructuion of the living root bridges in Meghalaya, India (fig. 2). First described in 1844, some of these living structures, made from the roots of local trees, are said to be over 500 years old (Lewin, 2012). Biofabrication is in this sense not new.

NovelYet, there is an increasing interest in crossovers between biology and humanDevelopmentstechnology and the opportunities that these bring. This can be in part, attributed
to the growing need for sustainable solutions as mentioned on the previous page.
A second factor is the increase in knowledge and possibilities concerning biology.
Biologist are more and more adept at understanding and altering an organism its
inner workings down to the molecular level (Ran et al, 2013, Collins et al., 2003).

Growing Design This has led to the emergence of the field of Growing Design, which entails growing materials from living organisms to achieve unique material functions, expressions, and sustainable solutions for product design (Camere & Karana, 2017). Such new collaborations with living organisms lead to designers becoming more involved in early stages of material development (Rognoli et al., 2015) and the need for designers to adopt new sensibilities in order to engage complex interdisciplinary problems (Camere & Karana, 2018).

> In this novel field, a large selection of projects and materials, involving living organisms in their production and use, were analyzed, (see the benchmarking analysis in appendix A). These projects were classified based on the type of organims they incorporate. Here, three prominent categories are; Fungi, Algea and Bacteria.

Fungi-basedFungi can be used to grow a mycelium network, comparable to the roots of a plantMaterials(Jones et al. 2017). This mycelium network can be grown in a pure form or in
combination with an organic material such as wood-chips or yute. The result is a
lightweight and isolating material. It can be grown into a mold and, once finished
and sterilized, be used for a wide vareity of applications (Appendix A)such as the
construction of a pavillion at the Dutch Design week (fig 3)

Algea-basedMicro-algea are single celled plants, often found in aqateous environments andMaterialsthey produce approximately half of the oxygen on this planet (Chapman, 2010).They do this through photosynthesis, combining CO2 and sunlight into sugarand O2. A number of projects (fig. 4 and Appendix A) make use of this ability bycovering man-made materials in a layer of living algea which consequentially willabsord carbondioxide and other harmfull materials out of our environment.



Figure 2: A living bridge constructed out of fig tree roots, Meghalaya, India.



Figure 3: A pavilion with the facade panels grown using Mycelium, Pascal Leboucq and Krown Design.



Figure 4: H.O.R.T.U.S. XL by ecoLogic Studio, a large 3D printed sculpture inhabited by living micro-algea.

Introduction

Bacteria; Bacteria are the oldest, smallest (fig. 5) and most simple form of life on this planet.
 Life at the Despite, or because of their simplicity, they are the most abundant form of life on earth, versatile in their metabolism and present in virtually every habitat (Campbell et al., 2017). They are key to every ecosystem and hereby make all other forms of life on this planet possible, including Humans (Campbell et al., 2017).

Bacteria in Due to both their simplicity and versatility, bacteria are considered to be very usefull and promising in combination with human technology. They offer a wide range of possibilities when it comes to the fabrication of novel materials (Nussbaumer et al, 2017; Venil et al., 2013; Iguchi et al., 2000) and the potential to provide new, living functionalities to the man-made environment. (Smith et al. 2020; Lehner et al. 2017; Virginie & Jonkers, 2011) To illustrate this, a small selection of projects involving bacteria is presented, see also the Appendix B for more relevant projects.

Growing Biocouture as developed by Suzanne Lee in 2004 (Fig. 6, Appendix A) is a project
Clothes in which bacteria are used to grow cellulose. This cellulose is grown into sheets of material which are processed like regular fabric, producing clothes. These clothes are biobased, biodegradable and require little more than sugar to produce (Iguchi et al., 2000). In addition, it has been shown that using food waste could be a potential input for growing this material. (Li et al., 2016).

Living A new type of conrete with living bacteria mixed into into it (Fig. 7, Appendix A)
 Concrete Once casted, the bacteria stay dormant for up to 50 years. When the concrete cracks, the bacteria become active and start to produce calcite, filling up the cracks, essentially giving the concrete self healing abilities (Virginie & Jonkers, 2011).

Future Base on these and many more projects, (Appendix A), potential uses for bacteria and other living organisms in the production and use of products can be identified. Firstly, there is the potential to grow functional and biodegradable materials using little resources or invested energy using the metabolism of the organism. (Nussbaumer et al, 2017; Venil et al., 2013; Iguchi et al., 2000). Here one can think of upcycling waste streams into materials that can be worn and used. Also, a new category of living materials can be envisioned, enriched with living organisms that can photosynthesize, heal themselves, and sense and clean the environment. (Smith et al. 2020; Lehner et al. 2017; Virginie & Jonkers, 2011).

The role of the
designer?As mentioned, these new possibilities bring about questions as to how this
changes the role of the designer. Evident here is that designer will need to adopt
an interdisciplinairy mindset (Myers, 2012)

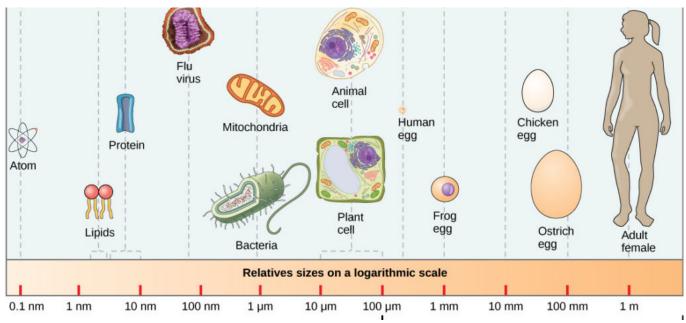


Figure 5: A bacterial cell and its relation to the sizes of other organisms.



Figure 6: A jacket (left) and shoe (right) as part of the Biocouture project developed by Suzanne Lee.



Figure 7: A sample of the living concrete (left) and footage of the bacteria filling up the crack (right). (Jonkers, 2011)

1.2 PROJECT SCOPE

Through a collaboration with scientists at the Aubin-Tam research group, the designer will focus on the design and development of a composite material grown by bacteria. In doing so, attention will be paid to the role that a designer plays in this collaboration with scientists (and bacteria) and the potentials that this material has for future applications.

New Roles for Based on the previously described context of this project (1.1 Project Context), opportunities for the development of novel materials grown by bacteria are identified within the field of Growing Design. As exciting as these opportunities are, questions remain as to how a designer is supposed to adopt such a new way of working in collaboration with living bacteria. What knowledge and skills does a designer need to obtain in order to do so? What new design sensibilities (Camere & Karana, 2018). This project aims to answer such questions by means of a practical example, real world collaboration. By learning from and reflection on this experience, the designer will aim to provide foothold for the next designer that wishes to engage in this new field.

Collaboration For a designer to be able to work with bacteria, it is already clear that an with Scientists intedisciplinairy collaboration is key. (Camere & Karana, 2018; Myers, 2012). To be more specific, a designer does not have the proper training or knowledge to work micro-organisms. Therefore, a collaboration with biologists is considered to be very valuable, providing the designer with the neccessary tools to be able to work in a microbiological laboratory. At the same time, designers, coming from a different background, can provide new insights to the potentials of such innovations (Barati & Karana, 2019) that would otherwise stay confined to the lab environment. In this way, designers could also incite biologists to look beyond the border of their scientific discipline. Where the role of a designers stops and that of a biologist begins and what these two disciplines can learn from each other will be investigated through the collaboration with the Aubin-Tam group.

BacterialWithin the Aubin-Tam research group, a bacterially grown composite is currentlyCompositebeing developed by the PhD candidate, Kui Yu. This material is grown with three
separate bacterial species, shows very promising mechanical properties and will
be further explained in Chapter 2. Here the designer has been given the unique
opportunity to collaborate on the development of this innovative material and
learn how to work with bacteria in a high-tech lab environment. It will therefore
be this collaboration and specific material proposal that will be the focus of a
Material Driven Design project.

Material Driven This project will be performed conform the Material Driven Design (MDD)
method (Karana, 2015). In this method, a material proposal (Chapter 2) is taken as a starting point for the design process which consists of four main steps:
1) Understanding the material at hand, done through tinkering or experimenting with the material to get a feel for the process and create a vareity of physical samples. Performing a technical characterization to determine the material its physical and mechanical properties. Performing and experiential characterization to determine the material.
2) This will lead to the formulation of a Material Experience Vision (M.E.V.) which will express an envisioned interaction unique to the material and its characteristics.
3) This M.E.V. will then be validated by the investigation and manifestation of

3) This M.E.V. will then be validated by the investigation and manifestation of experiential patterns in line with the M.E.V.

4) Based on this a material concept will be developed that expresses a certain future potential, unique to this material.

Note that material proposal in this case is considered to be underdeveloped, meaning that there is still a lot of variation in its properties due to the fact that it is part of an ongoing research. Because of this, a large portion of the efforts made by the designer will be focussed on 1) Understanding the material. Especially step 3) Manifesting experiential patterns, has been largely left outside of the scope.

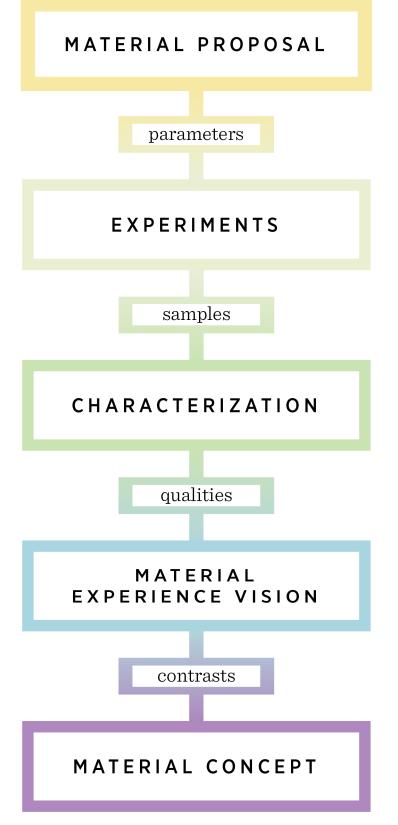
Explore and Communicate Potentials

Through this Material Driven Design project, the focus will not neccesarily be
 on coming up with new (product) applications for this material. The aim will be
 more on the uncovering new material potentials in terms of form, functionality,
 experience and affordances (Barati & Karana, 2019). The focus will thus be on
 developing and communicating these potentials to future designers

1.3 RESEARCH QUESTIONS

Potentials of Bacteria in Design	 What current examples are there of designers collaborating with bacteria? What roles do bacteria fulfill in these examples? How the the designers in these cases collaborate with these bacteria? Based on these current developments, what future innovations, involving bacteria, can be envisioned?
The Role of the Designer	 How can a designer work with bacteria in a laboratory environment? What new tools and limitations present themselves, working in a laboratory? What does the designer need to learn about biology and other disciplines? To what extent does a designer need to collaborate with a microbiologist and what can both parties learn from each other? To what extent should a designer follow the lab-protocols,e.g., should the designer play the role of a scientist or that of a creative and are these roles conflicting? To what extent can the designer control the growth of a living organisms? How does a lack of this control influence the outcome of the process?
The Material Proposal	 What characterizes the proposed material? What parameters can be altered in the growth and production of the material? Which of these parameters are relevant for experimenting with this material? How do these influence the resulting material? What parameters are interesting but outside of the scope of this project?
Technical Characteristics	 What are the unique technical characteristics of the material? What characteristics can be varied in this material?
Experienciential Characteristics	 What are the unique technical characteristics of the material? What characteristics can be varied in this material?
Potentials of the Material	 What are the unique potentials of this material? What are the main limitations of this material? Can these limitations be overcome in the near future and how?

1.4 REPORT STRUCTURE



In chapter 2: The material proposal will be defined according to its ingredients, micro-structure and the protocols required for its growth and production. From this, parameters relevant for changing the material its properties are defined.

In Chapter 3: With these parameters in mind, numerous experiments were performed which resulted in material samples with different properties.

In Chapter 4 & 5: These samples are analyzed for both their technical and experiential properties. Based on this characterization, the material its unique technical and experiential qualities are defined.

Chapter 6: Based on these qualities, unique to the material, a Material Experience Vision is defined, aimed at positioning the material and providing a goal to strive towards b.m.o. the material concept proposal.

Chapter 7: With the envisioned interaction in mind, three material concepts are proposed based on contrasting properties that the material can attain.

Figure 9: An overview of the structure of this report



2.THE MATERIAL PROPOSAL

This chapter will define the material proposal, the starting point of the Material Driven Design project. This material is being developed by Kui Yu, a PhD candidate at the Aubin-Tam research group. The material its three ingredients and how they interplay to form the resulting composite will be explained. Then, the process by which it is grown and produced will be explained. This will lead to an overview of the parameters relevant for the production of this material, which will serve as a basis for the experiments that will be done with the material.

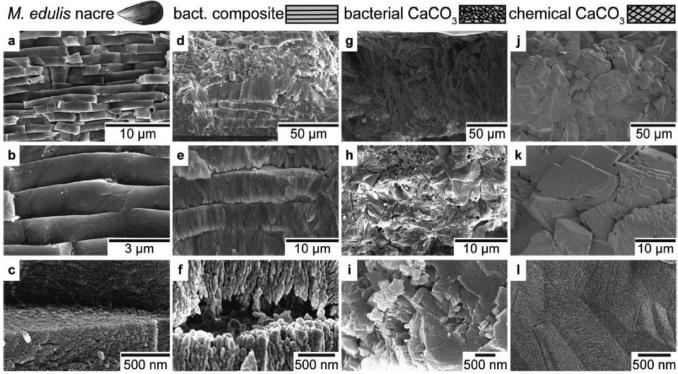


Figure 10: S.E.M. pictures of; a,b,c; M. edulis nacre; d,e,f; Bacterial composite comprised of bacterially produced calcite and Polyglutamic acid; g,h,i; Bacterially produced calcite; j,k,l; chemical calcite; Spiesz et al, 2019

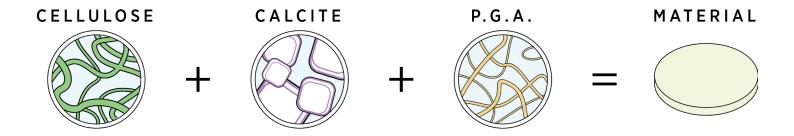
2.1 AUBIN-TAM RESEARCH GROUP

Biological Composites

The material proposal is part of the research taking place at the Aubin-Tam group. Here scientists are developing biocomposites inspired by the hierarchical nanostructures that can be found in nature. An example of this is a bacterially produced composite material, inspired by the layered structure that can be found in the nacre produced by mollusks (Fig. 10) (Spiesz et al., 2019).

Cellulose Within this research group, Kui Yu, a PhD candidate, is exploring various ways of biofabricating composite materials and analyzing their microstructure and P.G.A.
 resulting properties. Among his developments is a biofabricated composite that is composite of; bacterial cellulose, forming flexible organic fibers; bacterial calcite, forming a stiff inorganic matrix; and Polyglutamic acid, a biopolymer, acting as a glue, strengthening the calcite crystals.

The resulting material shows promising mechanical properties and is selected as the material proposal, the starting point for this design project. The characteristics of these ingredients, how they are grown and processed into the resulting composite will be explained on the following pages.



2.2 THE INGREDIENTS

- The MaterialThe material is a composite consisting of three ingredients; Cellulose fibers,
Calcite crystals and Polyglutamic Acid polymers (P.G.A.). All of which are either
grown by or with bacteria. Combined, these ingredients result in a material that
shows properties of all three of these ingredients.
 - Cellulose is the most abundant biopolymer on this planet, most of it being produced by plants. (Klemm, 2005) Common examples of this are paper, wood and cotton. In this case the cellulose is produced by Gluconacetobacter Hansenii bacteria. As viewed in on the right (fig 11), bacterial cellulose is much finer, with fibers a hundred times smaller in diameter than plant cellulose. (Aramwit, 2016). These cellulose fibers are characterized by their flexibility whils being strong along their length (Iguchi, 2000).
 - Calcite Calcite is a common mineral that is for example produced by sea shells and coral reefs (Tanaka, 1959). In this project, Calcite is produced with the aid of Sporosarcina Pasteurii bacteria. In the presence of these bacteria, calcite forms small crystals (fig 12) which are, in comparrison to cellulose, very hard, stiff and brittle (CES Edupack, 2019).
 - P.G.A. Polyglutamic Acid is a polymer, consisting of chains of glutamic acid monomers, produced by bacteria (Bajaj, 2011). It occurs in the fermented Japanese food Natto and has found its way to a number of skincare and beauty products, see the image (fig 13) on the right. In this project, P.G.A. is produced with the help of Bacillus Licheniformis. These polymers influence the way in which the calcite crystalizes, rendering them more ductile and tough (Spiesz 2019).

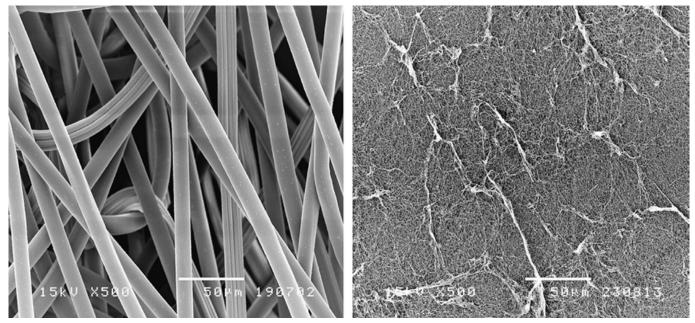


Figure 11; Plant cellulose fibers (left) and bacterial cellulose fibers (right). (Aramwit, 2016)



Figure 12; S.E.M. image of calcite crystals (left) (Spilde, 2009), White cliffs consisting of limestone (right)



Figure 13; Left, Natto, fermented soy beans containing P.G.A. Right, skincare product containing P.G.A.

2.3 MICRO-STRUCTURE

NaturalNatural materials such as bone or nacre derive their strength and toughnessMicro-Structuresfrom combining organic and inorganic ingredients into hierarchical structures
spanning across different length scales. (Spiesz et al., 2019; Wegst et al. 2015).
Here the organic and inorganic ingredients often represent soft and hard
materials respectively, the combination of which grants such materials their
unique combination of strength and toughness (Wegst et al. 2015).

- Micro-Structure In the case of this material proposal, the cellulose and P.G.A. are organic of the Material materials or polymers, which are relatively soft (CES Edupack, 2019). Especially when compared to their inorganic counterpart, the calcite crystals, which are relatively hard (CES Edupack, 2019). Like the examples of the natural world, it is also with this material the case that it derives its strength and toughness from its micro-structure. The way in which the ingredients are layered up and intertwined with each other on a microscopic scale is therefore defining for the material its ultimate strength and toughness. Here the whole is indeed greater than the sum of its parts.
 - Layering Like the nacre inspired composite (Spiesz et al., 2019), the aim of Kui Yu is to create a material with a similair, hierarchical structure inspired by the materials found in nature. If we look at the microstructure of the material (fig. 14) we can see that it consists of layers. These layers are the result of the filtration process (explained further in this chapter) and promote the material its final strength and toughness.
- Cellulose fibers &When zooming in (fig. 15), we also observe that the calcite crystals are wrappedCalcite Crystalsin cellulose fibers, thus providing this alternating hard-soft contrast. Note herethat if the calcite crystals grow too big, the will disrupt the layered structure andhomogenity of the material, therefore, decreasing its mechanical performance.
 - P.G.A. Polymers Zooming in further (fig. 16), we can see that the surface of the calcite crystals is different from those grown without the presence of P.G.A. (Chapter 4). On these images, the P.G.A. polymers themselves are indistinguishable, they are much smaller than cellulose fibers. But we can assume that they are interwoven into the calcite crystals, hereby improving their toughness (Spiesz et al., 2019).

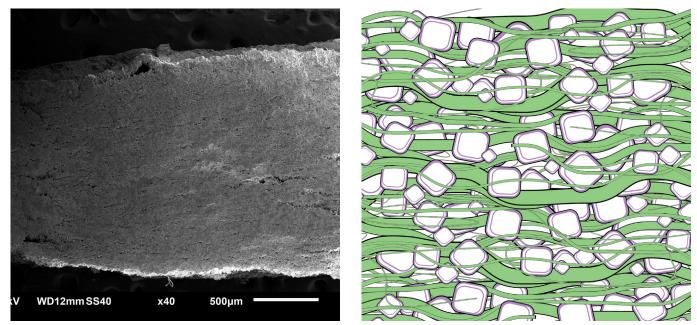


Figure 14; Left, S.E.M. image of the layered structure, represented in a diagram on the right.

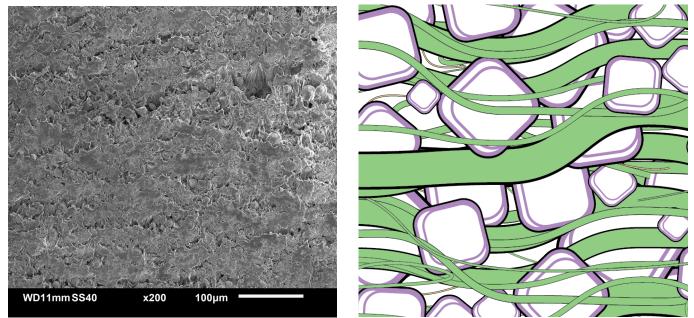


Figure 15; Left; S.E.M. image, right; diagram of the alternating cellulose (green) and calcite (purple) structure.

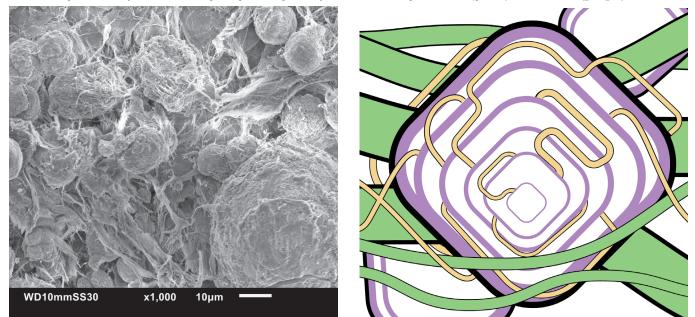
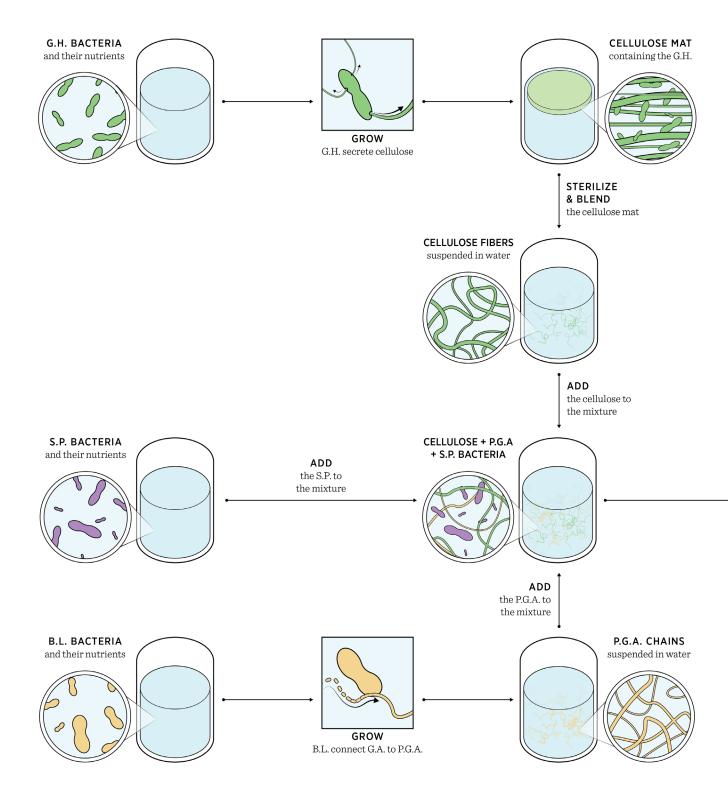


Figure 16: S.E.M. (left) and diagram (right) of the calcite crystals permeated by P.G.A. polymers (orange).



2.3 PROCESS

Overview

Shown above is an overview of the process of growing and processing the material as proposed by Kui Yu.

- It starts with the growth of cellulose by the G.H. bacteria (in green)
- Secondly, the growth of P.G.A. using the B.L. bacteria (in orange).
- These are then combined and calcified by the S.P. bacteria (in purple).
- The resulting mixture is then filtrated to render a solid material.

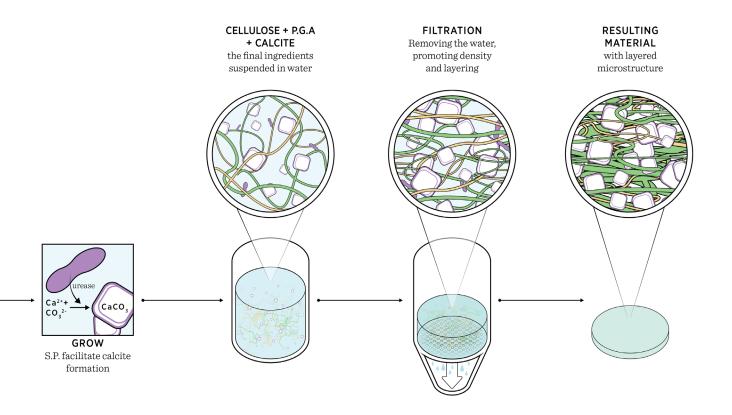


Figure 17: An overview of the process steps required to produce the material.

These process steps will be explained in more detail on the following pages. Please note that the specific protocols, such as the required ingredients, for making this material are still classified and can therefore not be shared, yet.

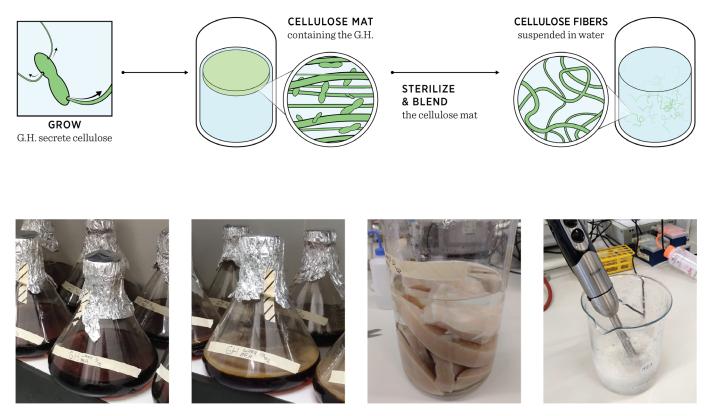


Figure 18: Overview of the cellulose growth by the Gluconacetobacter Hansenii.

Growing Cellulose As shown above (fig. 18), the cellulose is grown by the Gluconacetobacter Hansenii (G.H.) bacteria. These bacteria are placed, together with the medium they need, in a sterile glass flask. The flask is then closed with aluminium foil to avoid contaminations whilst still giving the bacteria acces to the oxygen. It is then placed in a 30 $^{\circ}$ C environment where the bacteria are left to grow for up to 6 weeks.

The bacteria will then start to secrete cellulose fibers from numerous glands along their cell wall (Villareal-Soto et al. 2018). By doing this they form a biofilm at the air-water interface, consisting of numerous layers of cellulose fibers. The bacteria reside in this biofilm, protected. Depending on the strain of bacteria, the container and access to oxygen, this biofilm can grow up to a milimeter in thickness per day.

When the biofilm is sufficiently thick it is harvested. This is done by boiling it, sterilizing the bacteria, and then rinsing the cellulose mat repeatedly with water. This rinsing will get rid of most of the dead bacteria, after which the cellulose mat will turn white. After this, the mat cut into pieces and blended using a blender to render a suspension of very fine fibers with an increased total surface area. This increase in surface area is essential to growing the calcite crystals in between the cellulose fibers.

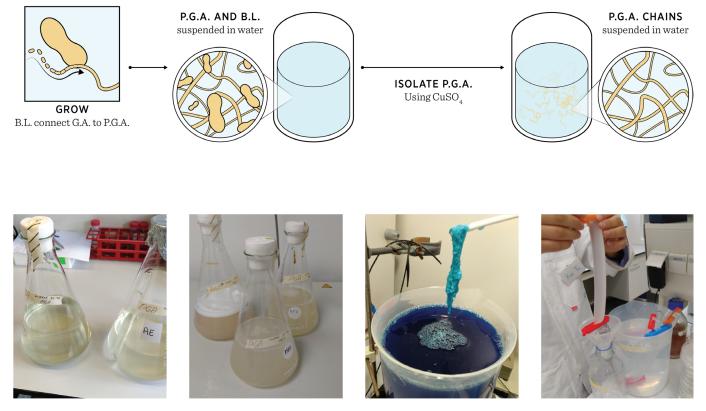


Figure 19: Overview of the growth of P.G.A. by the Bacillus Licheniformis and following purification.

Growing P.G.A. This is the most time intensive and difficult material to produce. The process as described (fig. 19), is for the sake of clarity, simplified.

The Bacillus Licheniformis (B.L.) bacteria and the nutrients they need are grown in a liquid medium at 28 °C. In the course of 48 hours they will start to connect Glutamic Acid (G.A.) monomers, present in the liquid, herby forming long polymer chains of Polyglutamic Acid (P.G.A.). These P.G.A. polymers are anionic, which means they posses negatively charged areas along their length, which will facilitate the calcite formation (described on the next page).

The resulting liquid is then sterilized by boiling. After this, the P.G.A. polymers have to be isolated from the liquid, this is done by adding $CuSO_4$. The dissolved $Cu2^+$ ions bind to the negatively charged P.G.A., causing it to precipitate at the bottom of the container.

The precipitated P.G.A. (blue in the above figure) is then redissolved into a P.V.A. (Polyvinyl Alcohol) solution which can consequently be dialyzed to get rid of the Copper ions. The result is a liquid containting purely P.G.A. polymers which can be used further in the process.

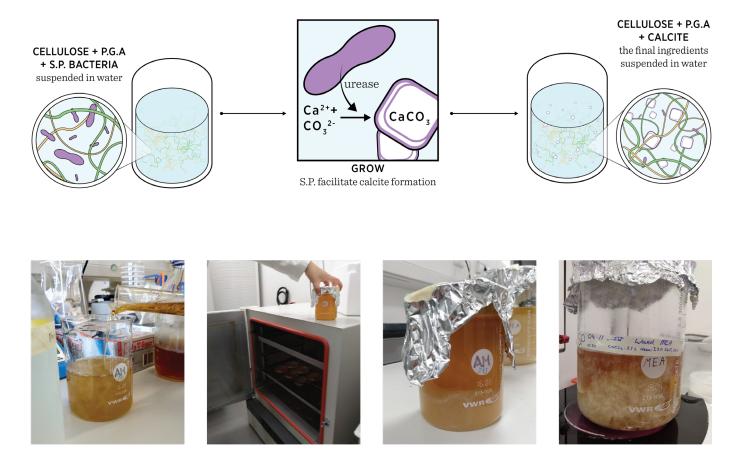


Figure 20: Overview of the calcification procedure by the Sporosarcina Pasteurii.

CalcificationIn the image above, the calcification procedure is visualized (fig. 20). TheprocedureCellulose and P.G.A., grown in the previous steps, are combined, suspended in aliquid. To this liquid, the Sporosarcina Pasteurii (S.P.) bacteria, their nutrients,Urea, and ion sources are added. These ion sources will provide the Ca²⁺ and CO₃²⁻ions required to form CaCO₂ or calcite.

The mixture is grown for 24 hours at 28° C. During this time, the S.P. bacteria will grow into large numbers and start secreting Urease. This is an enzyme that will break down the Urea, present in the liquid into Ammonia and Carbonate. This causes the solution to become alkaline, (P.H. > 9.0). Due to this rise in P.H. value, the formed carbonate and calcium ions present will form $CaCO_3$. This is a solid state, the ions thus go from being dissolved into a liquid to a solid, crystalline form.

During this phase transition, from a dissolved ion to a solid crystal, the nucleation sites, where these crystals will start their growth, are of importance for their eventual structure (Anbu et al., 2016). The P.G.A.polymers, having negatively charged areas, provide excellent nucleation sites for the calcite crystals, this is suspected to be one of the reasons that the P.G.A. affects the calcite crystal structure. (Spiesz et al, 2019)

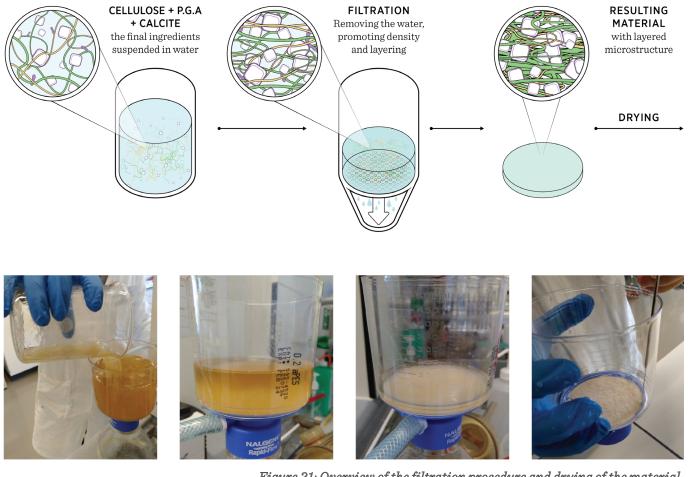


Figure 21: Overview of the filtration procedure and drying of the material.

Filtration and After the calcification procedure we are thus left with the material its main ingredients; Cellulose, Calcite and P.G.A., suspended in a liquid. Here, the filtration procedure is required to go from this liquid suspension to a solid material. The liquid is therefore poured into a filter cup which is connected to a pump. This pump will generate a pressure difference, causing the water to be pulled through the filter whilst the solid ingredient stay behind.

During this process, the ingredients become more densely packed together and will start to layer themselves horizontally in a self-assembling process as described by Kui Yu. It is therefore this filtration process that gives the resulting material its density, layered microstructure and consequential mechanical properties.

Even though the material is no longer in a liquid phase after the filtration, it still contains a lot of water. This wet solid is therefore still very soft and pliable, it is only after drying that it will become a stiff material with its final properties and dimensions.

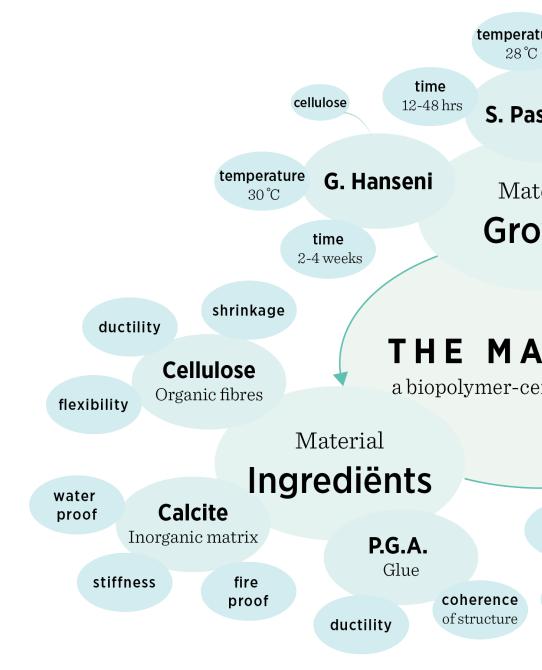
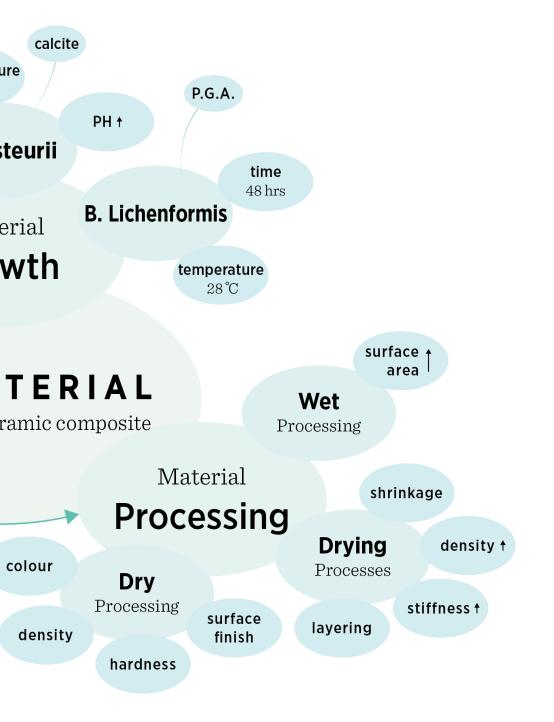


Figure 22; Material taxonomy, an overview of the parameters, relevant for the production of the material in question

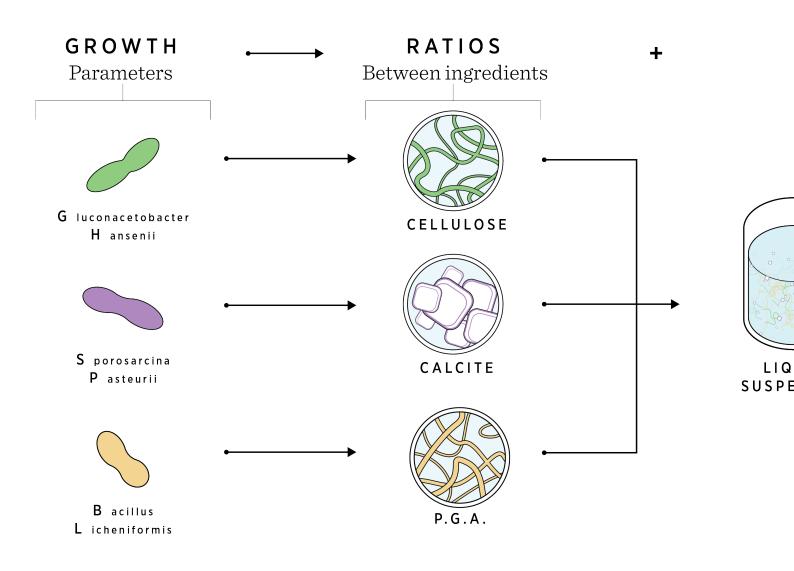
2.4 MATERIAL PARAMETERS

Material	Based on the process of growing and producing the material as defined by Kui
Taxonomy	Yu, a material taxonomy (fig. 22) is generated to provide an overview of the
	parameters relevant in this process (Karana et al., 2015). Based on this overview,
	we can distinguish three main categories along which these parameters can be
	divided.

Growth The generation of the material starts with the growth of the bacteria. This growth can be influenced by a number of parameters such as the bacteria their medium, growth time and temperature.



Ingredients	This growth consequentially results in an amount of and ratio between the material its ingredients. With each ingredient having different characteristics, this ratio between ingredients heavily influences the material its properties.
Processing	With the ingredients starting off being suspended in a liquid, the way in which this liquid suspension is processed into a solid material is also defining for the material its final form and properties.
	Note that this is the process as defined by Kui Yu, in the next section, the process as a whole will be altered and varied to evaluate its respective outcome.



3. EXPERIMENTS

This chapter will provide an overview of the different experiments performed in order to gain an understanding of the material and its components. Initially, the experiments were focussed on understanding and controlling the biological processes required to grow the different ingredients. Secondly, experiments were aimed at controlling the production of these ingredients and the ratio's between them. In a later stage, the experiments were aimed at exploring various ways of processing these ingredients and shaping the resulting material.

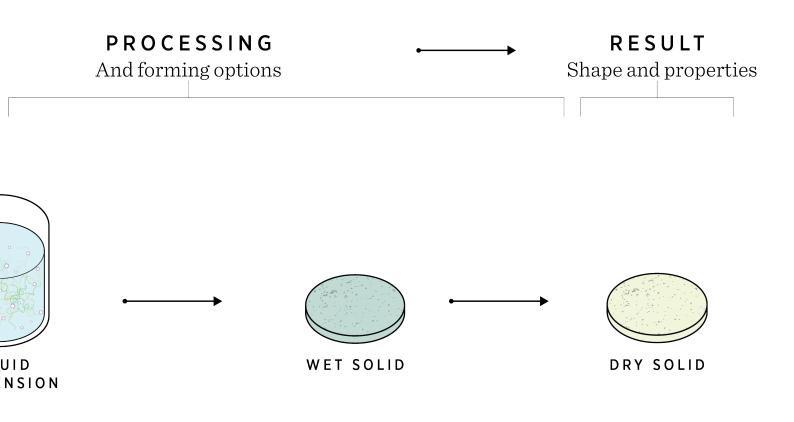


Figure 23; Overview of the different phases of material growth and production.

3.1 APPROACH TO EXPERIMENTS

The process of creating this material as defined in the previous chapter is divided into three parts which will serve to structure the results of the experiments as shown in the image above (fig. 23). Also see Appendix B for an extensive lab journal, documenting all of the experiments.

Growth Parameters	Initially, the experiments were aimed at gaining an understanding of the biological processes and how these can be steered.
Ingredient Ratios	Secondly, the experiments were aimed at varying the ratio's between the ingredients and evaluating how this affected the material.
Forming Processes	Lastly, the experiments were aimed at exploring the different options for processing the material from a liquid into a solid, hereby also defining its shape.

3.2 GROWTH PARAMETERS

In order to get a better understanding of how the different bacteria grow this material, what influences their growth and how this can be optimized; experiments were where the protocolsfor growing these bacteria were altered in order to see how the bacteria respond.

This was done for the cellulose growth by the G.H. bacteria and the calcite growth by the S.P. bacteria. The protocol for growing P.G.A. using the B.L. bacteria is much more complex and therefore left out of the scope of experiments.

Calcification Different ways of growing bacterial calcite were tried out, this included;

- Calcifying materials other than cellulose and repeating calcification cycles on the same material (fig. 24).
- Varying the amount of nutrients that the bacteria got.
- Varying the amount of bacteria and their activity.
- Varying the amount of ions (building blocks) present in the liquid.
- Varying the growth time (fig. 25)

Here, the above variation yielded different amounts of calcite as shown on the right (fig. 25) . Here important parameters, having the most effect on the yield of calcite, were: The amount of bacteria and their activity, The amount of ions present in the liquid and the growth time. These three parameters and their effect on the calcite yield will be discussed in Chapter 4.

Cellulose growth The growth of cellulose was found to be a bottleneck in the production of this material. This mainly because it takes up to 6 weeks to grow and the amount of cellulose is limited to the surface area of the container. Here the experiments were therefore aimed at optimizing the cellulose production. (fig. 26) Parameters that were of a notable effect in these experiments were: The access to oxygen, amount of nutrients and type of container. These parameters will also be discussed in Chapter 4.

> Overall On overall these experiments provided insight into the growth of these bacteria and how to steer it. In addition, they helped with familiarizing with the laboratory protocols and gaining the confidence to make more radical changes in the growth and production of the material.



Figure 24: Different materials combined with Bacterial Calcite, from left; Sand, Chitosan, Unblended cellulose mat



Figure 25: Varying calcite yields as a result from varying growth times, from left; 0.1 g/18hrs, 1.1 g/25 hrs & 2.2 g/42 hrs



figure 26: Different ways of growing bacterial cellulose, from left; in a glass flask and in a plastic tray



figure 27: Overview of an experimental set-up (left) and the isolation of P.G.A. using CuSO4 (blue) on the right.

3.3 INGREDIENT RATIOS

The previous experiments granted insights into what influences the bacteria their growth and how this results in a certain amount of material. With these insights, the composition or ratio between these ingredients can be controlled up to a certain extent.

With this in mind, a range of material samples was created in which the starting amount of cellulose and P.G.A. was known and the calcification process was steered according to the previously defined parameters (See also Chapter 4:Technical characterization). This led to a range of objects with a known composition of ingredients as shown (fig. 28). Comparing these samples with varying ingredient ratios among each other, differences between their properties can already be defined. Note that these will be quantified further in chapter 4.

- Dimensions The sample made out of pure cellulose remained very thin due to the cellulose itself not taking up much volume. By increasing the amount of calcite, the samples become much thicker.
 - Shrinkage With an increasing amount of Calcite, the samples seem to shrink more when drying. Here the sample containing purely cellulose did not shrink and the sample containing a ratio of 33% cellulose and 67% calcite shrunk to about 60% of its original diameter when drying. Interestingly, the samples with an even higher calcite content showed less signs of shrinkage. Also the addition of P.G.A. seems to reduce the amount of schrinkage.
 - Warpage Related to this shrinkage is the fact that the samples warp when they dry. Here it became evident that, the more calcite that was added to the composition, the thicker the material, the less warpage.
- MechanicalWith an increasing amount of calcite, the samples become much more stiff. ByPropertiesincreasing the amount of P.G.A., the samples seem to become more flexible and
ductile. These properties will be further quantified in the next chapter.
 - SurfaceBy adding more calcite, the roughness of the samples increases, it also becomes& Colourmore stone-like to the touch. By increasing the amount of P.G.A. in the samples,
become more smooth and plastic-like to the touch. Samples with a higher calcite
content also seem to become more brown-ish of colour.



figure 28: Samples with varying ingredient ratios, not that the percentages are an approximation.

3.4 PROCESSING AND FORMING

- Wet to Dry After growing the bacteria and attaining a certain composition of ingredients, the material is still suspended in a liquid. How it is then processed from this liquid suspension into a dry solid is defining for the material its final shape, micro-structure and properties. Here we distinguish between different phases of the material process which are also shown below (fig. 29);
- LiquidAs mentioned before, the bacteria and materials are grown in a liquid medium,Suspensionthe result of the growing procees is thus that the solid materials are suspended
-drifting- in a liquid.
- Wet Sludge This is an intermediate phase in which the material is still considered to be liquid but the excess of water has been decanted -poured off- and thus the concentration of solid matter in the liquid suspension is much higher.
 - Wet Solid In this phase, the material has become solid but still has a high moisture content. It is therefore still very soft and maleable. Note that the exact point of transition between a wet sludge, (liquid containing solid matter) and a wet solid (containing liquid matter) is hard to pinpoint exactly.
 - Dry Solid This is the final phase in which the moistere content of the material has largely evaporated and the material has become rigid and stiff. Note that this is not considered to be the material its final form since it can be re-wetted again.
 - Forming On the right (fig. 30) is an overview of the different manners in which the material
 Options can be processed from a liquid to a solid. Note that these options are not exclusive
 but the ones that have been experimented with (see also Appendix B). Some of
 these options will be discussed on the following pages.

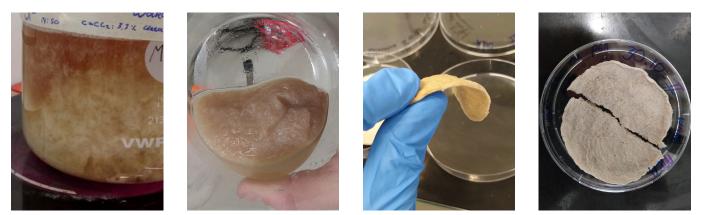


figure 29: Overview of the different phases; from left; Liquid suspension; Wet sludge; Wet solid and a Dry solid

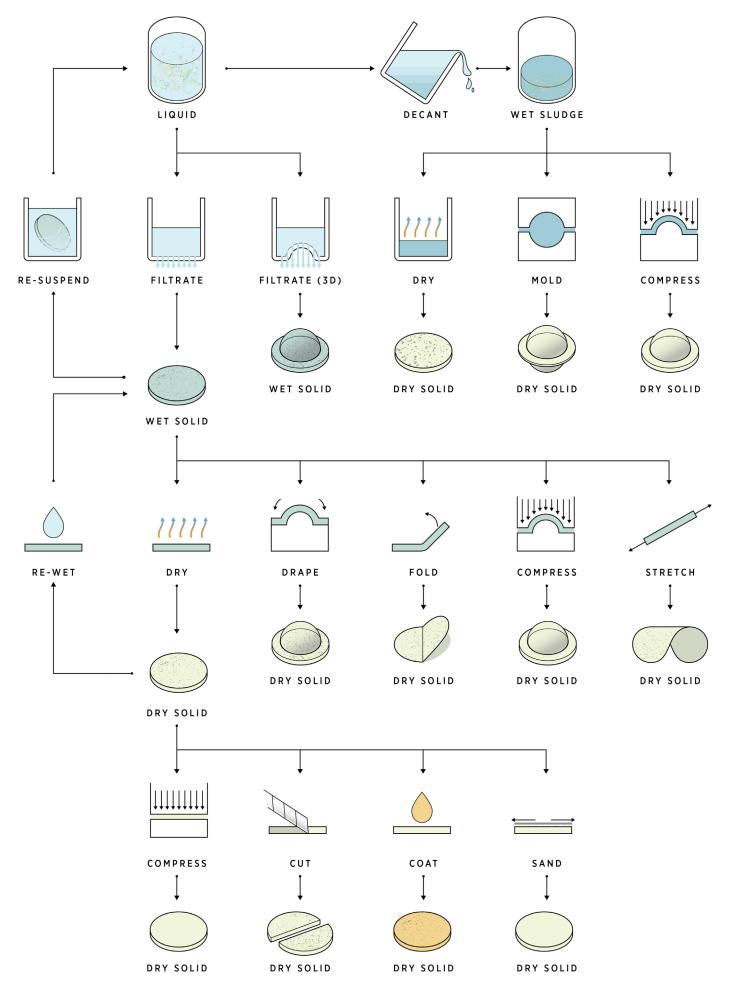
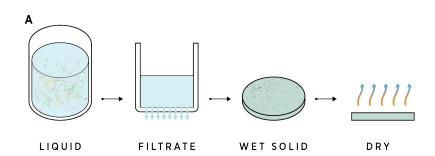


figure 30: Overview of the different forming processes and consequential phase transitions.

	In order to explore different options of processing and forming the material,
	variations on the existing protocols were made.
A: Filtration	 Conform protocols, the liquid can filtrated and the resulting wet solid dried. Filtration procedure can take up to 8 hours; Material size is limited to the diameter of the filter. Filtration does seem to promote a layered microstructure. Material shrinks upon drying. Drying time, dependant on humidity and temperature, influences properties.
B: Decanting & Evaporation	 An alternative to the filtration procedure. The excess of water is decanted and the remaining wet sludge left to dry. Evaporation can take a long time, in the example it was 12 hours. No size limitations due to a maximum filter diameter. Result has poor density, feels spongy, very little tear resistance Expected is that, by skipping the filtration, the micro-structural did not become layered.
C: Folding	 Keeping to the filtration procedure, the wet solid can be folded into a desired shape which it will hold upon drying. The material becomes stiffer along the fold due to its geometry. The minimum bending radius is defined by the material its thickness. The material will still warp and shrink upon drying, proving it difficult to control the shape.
D: Draping	 In a similair manner, the wet solid was draped over an existing shape, copying this shape once dried. Again, shrinkage occurs, making it impossible to fully controll or replicate a shape.
E: Re-Wetting	 A dry solid can be re-wetted to become a soft and malleable wet material again. It can then be folded or draped into a novel shape and left to dry. Upon drying a second time, the material hardly shrinks. This can be very usefull to controll the final shape.
	 The dried material can also be completely re-suspended in water, upon which it can be filtered and processed again. The recycled material (re-suspended and re-processed) appears to be more white of colour. Apart from the colour there is no noticable difference between the original

material and the one that has been recycled





RESULT



RESULT



RESULT



RESULT



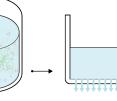




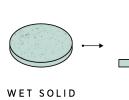
LIQUID

в

С



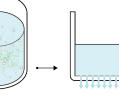
DECANT

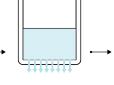


WET SLUDGE

LIQUID







FILTRATE

FILTRATE

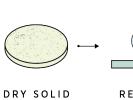


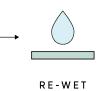
LIQUID

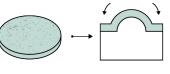
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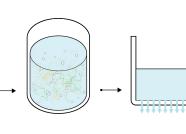








WET SOLID



WET SOLID

RE-SUSPEND

LIQUID

DRAPE

DRAPE

FOLD

DRY

Experiments

	experiments were performed where the material was either compressed or
	stretched into shape, providing more controll over the final dimensions.
A: Stretching	 After filtration, the wet solid was clamped on a mold, preventing it from shrinking during drying. The material, wanting to shrink but not able to, became tightly strung. This resulted in a well defined, double-curved surface, allowing for reproducibility. Interesting is how this tension introduced into the material will influence its mechanical performance.
B: Partial Stretching	 Instead of stretching the sheet of wet material across its entire circumference, it can also be stretched parially, along specific lines. This resulted in parts of the material becoming tensed up around a defined geometry. While other parts of the material are allowed to shrink and warp, attaining an undefined geometry. The material sample seemed to attain a stiffness along the spokes.
C: Compression (Wet)	 The wet solid can also be compressed into a shape and left to dry afterwards. While wet, the material can be easily formed, allowing for freedom of form in compression. The material is, when wet, however, not able to handle a lot of compressive stress and will rupture. After compression, the material will shrink less but still warp during drying.
D: Compression (Dry)	 The material can also be left to dry and compressed when it is completely dry. The material is able to handle significantly more pressure when it is dry. Being dried and already stiff, this method allows for less freedom of form than compressing it while wet. The material does seem to become very hard, smooth and defined.

In order to counteract the shrinkage occuring in the material, forming

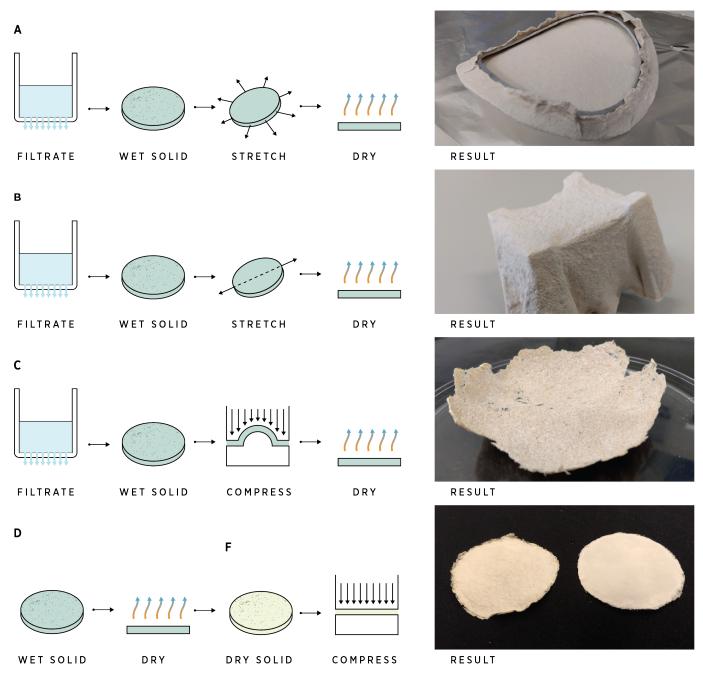


Figure 32 : Overview of different processing experiments.

3.5 INSIGHTS FROM EXPERIMENTS

Performing the vareity of experiments granted insight and understanding into how the material grown, produced and eventually be manipulated.

Relevant Parameters

evantBased on the experiments, a number of parameters, relevant for the growth and
production of the material are defined. These parameters influence the resulting
material in different ways as is depicted in the figure on the left (fig. 33). Here,
again the distinction has been made between the growth of the material by
bacteria and how different parameters of this growth influence the material
its composition of ingredients. This ratio of ingredients will consequentially
influence the final material its properties. Equally as important to the final
properties of the material and also its resulting shape, is the way in which it is
processed from a liquid suspension to a solid material. Note that these parameters
also influence each other and that there are also parameters left outside of
the scope of this analysis. Nevertheless, how the parameters depicted (fig. 33)
influence the resulting material will be further investigated and quantified in the
technical characterization, Chapter 4.

Designer or In addition, it is now possible to reflect on the way that the designer approached Scientist the experiments and how this lead to an understanding of the material. Here it is viewed that in the beginning of the process, more effort should have been made to follow the protocols as described by the scientist. Here, following protocols and controlling the experiments is deemed as crucial to come to an initial understanding of the complicated biological and chemical processes involved. Of course it is interesting to take a more intuitive approach and allow for unexpected things to happen but this should be done after an initial understanding has been manifested through controlled experimentation

> Nevertheless, through the experiments performed which, at times, might have been too exploratory in nature, an understanding of the process was manifested and interesting parameters for further characterization defined.

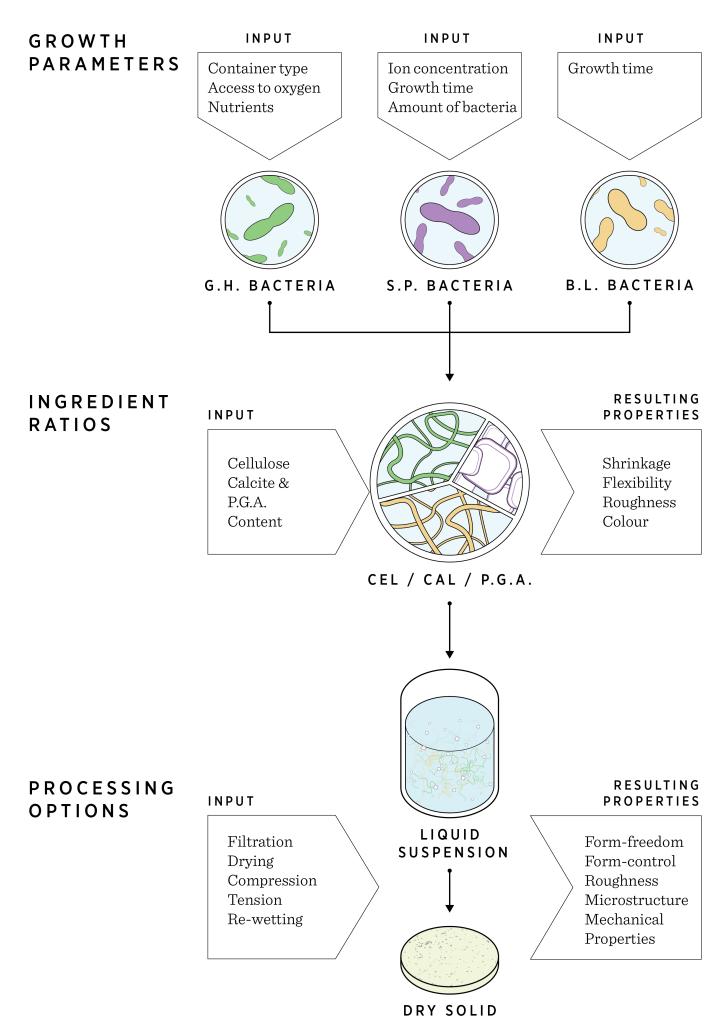


Figure 33 : Different parameters relevant in each phase of the material growth and production **45**



4.TECHNICAL CHARACTERIZATION

This chapter will discuss the various technical parameters that are involved in the development and forming of this composite material. It builds on the results obtained from the experiments and is thus structured in the same manner as the previous chapter. First the parameters relevant to the growth of the material will be discussed, then the ratios between the ingredients and the way in which they are processed. The aim is to quantify how the above influence the properties of the resulting material. Based on this, the material its unique technical qualities will be formulated as a conclusion to this chapter.

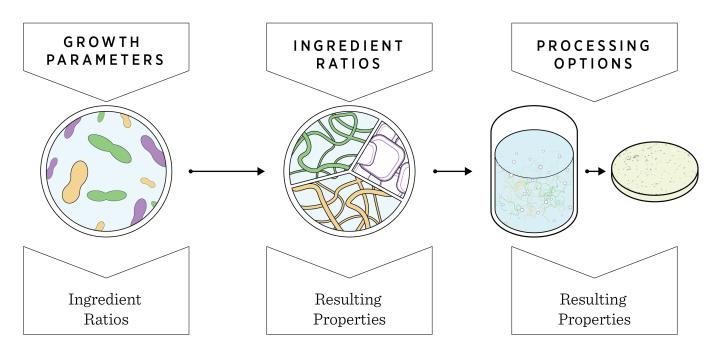


Figure 34: Overview of the focus of the T.C., divided across the three phases of the material production.

4.1 AIMS OF T.C.

Growth	The first part of this chapter will investigate how;
Parameters	 The type of container, amount of nutrients and access to oxygen influence the amount of cellulose produced by the G.H. bacteria The growth time, ion concentration and amount of bacteria influence the total amount of calcite produced by the S.P. bacteria
Ingredient Ratios	The second part wil investigate how the ratio between ingredients influences the microstructure, density and flexural performance of the material.
Processing Options	The third part wil investigate how different ways of compressing the material will influence its micro-structure, density and flexural performance.
	Lastly, this chapter will conclude with comparing the material its mechanical performance to other materials and will summize with defining the technical qualities of this material.

4.2 GROWTH PARAMETERS

During the experiments performed with growing the material, a number of parameters relevant to this growth were defined. These parameters consequently influence the amount of a specific ingredient that is produced.

CelluloseIn Chapter 3, parameters relevant to the growth of bacterial cellulose wereGrowthdefined as follows;

- The type of container; the material it is made of and its surface area.
- The type of bacteria used.
- The medium; e.g. the amount of glucose.
- Access to oxygen.

On the right (fig. 35) is an overview of different experimental set-ups in which the above parameters were varied. The growth rate was obtained by measuring the increase in thickness of the cellulose mat over the course of weeks. This thickness multiplied by the total surface area of the air-water interface, inherent to the container, gives the relevant cellulose growth rate in cubic centimeters. Here it was found that;

- The type of material that the container is made out of makes the biggest difference. Bacteria do not seem to grow well in a container made of PVC.
- The Kombucha culture, a mixture of cellulose producing micro-organisms (Villareal-Soto et al., 2018), seemed to grow faster, however, these cultures proved less predictable and were eventually contaminated.
- Increasing the amount of glucose (by roughly 20%) rendered more cellulose.
- The acces to oxygen was not measured, however, the PVC tray was closed of and the steel tray was not, so this could explain the difference in growth rate Note that there are still a lot of other factors affecting the growth speed of cellulose, big differences in growth rates between the same set-ups have been observed. Therefore this data is merely meant as a handlebar.

```
CelluloseNote that the cellulose, after it has been grown, still contains about 99% water. ACompositionthick cellulose mat will therefore become very thin after it has been dried (fig. 36).In Appendix C3 09-12 it was measured that the cellulose, when dried, retains<br/>about: 1,2% of its original weight and 2,0% of its original volume. This means that<br/>the growth rate of the steel tray, which was the fastest, amounts to about 1,31<br/>grams of bacterial cellulose per day.
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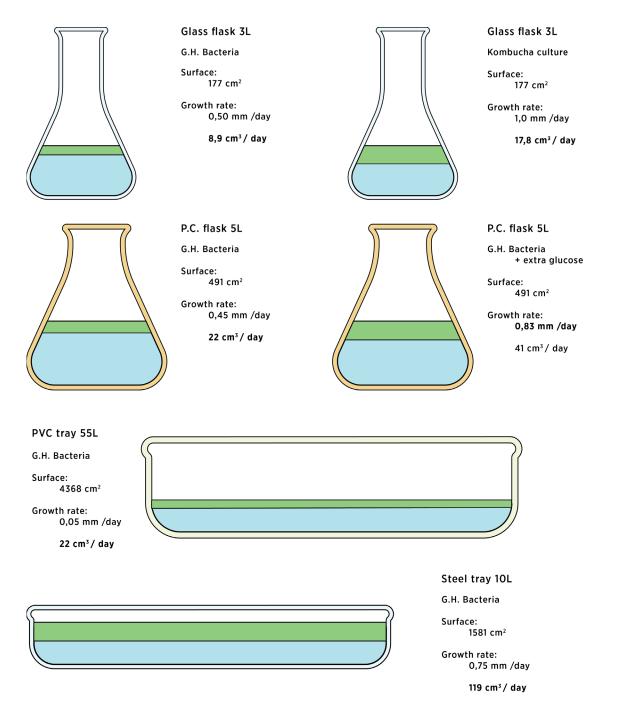


Figure 35: Overview of different Cellulose growth setups and their cellulose yield.

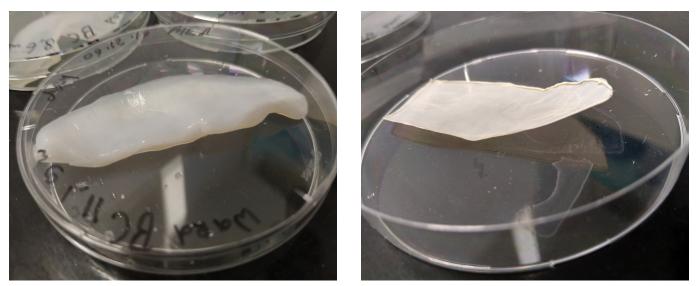


Figure 36: A piece of cellulose when it is wet (left) and the same piece when dried (right).

Test: 08-10

Two solutions with the varying calciumchloride content and the same growth time.





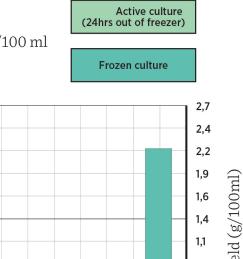
Growth time: Starting amount CaCl2 * 2H2O: Theoretical CaCO3 yield: Actual CaCO3 yield: Efficiency:



 $48\,\mathrm{hrs}$ 4,0g/100ml 2,7g/100ml 2,5g/100ml 93%

Test: 09-10

Multiple solutions with the same Calciumchloride content 4,0 g/100 ml Varying the growth time.



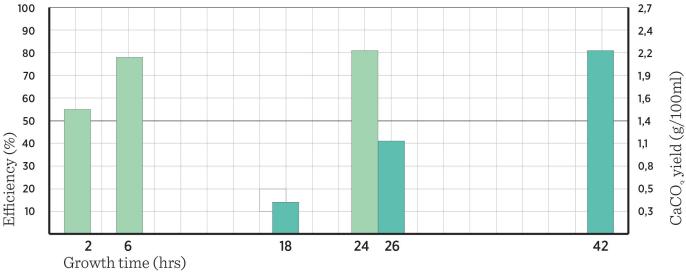


Figure 37: Overview of the results testing the Calcite formation rate

CalciteThe amount of calcite that is produced by the S.P. bacteria is dependent of theFormationfollowing parameters;

- Concentration of Calcium (dissolved CaCl2) ions.
- Growth time
- The amount of S.P. bacteria present and their health/activity

lon Concentrations The Sporosarcina Pasteurii (S.P.) bacteria facilitate the formation of calcite crystals by secreting Urease. This is summarized in the simplified chemical reaction shown below.

$$CH_4N_2O + 2H_2O + CaCl_2 \xrightarrow{\text{urease}} CaCO_3 + 2NH_4^+ + 2Cl_2$$

Based on this reaction; the amount of Calcite $(CaCO_3)$ that can theoretically be formed is determined by the amount of Calciumchloride $(CaCl_2)$ that is added to the medium. If it is assumed that the bacteria convert 100% of the CaCl₂ into $CaCO_3$ then adding 1,00 gram of $CaCl_2$ would render 0,68 gram of $CaCO_3$. This was confirmed in the experiment performed on 08-10 (fig. 37, Appendix B), where the amount of calcite formed was directly related to the concentration of ions present in the medium. Here the bacteria converted 93% of the Calcium Ions into Calcite.

Growth Time The second parameter, the time the reaction takes, also influences the amount of calcite rendered. This was validated in the experiment performed on 09-10 (fig. 37), Appendix B). Here an increase in time rendered an increase in conversion rate and thus the CaCO₃ that was yielded.

Amount and
Activity of
BacteriaThe same experiment also showed that parameter 3, the amount and health of
the S.P. bacteria, heavily influences the CaCO3 yield. Here the bacteria that were
cultivated at 28 degrees for 24 hours were much more numerous and active than
those that just came out of the -80 degrees freezer. This resulted in a much faster
conversion of Calcium Ions into Calcite.

P.G.A. With these parameters in mind, the amount of calcite created by the S.P. bacteria
 Formation and can therefore be regulated. Concerning the growth of the P.G.A., for this the
 defined protocols were followed resulting in an amount of material that is
 somewhat known. The ratio between the different ingredients can therefore be controlled by regulating the amount of cellulose and calcite.



figure 38: The material samples used for the 3P bending test

4.3 INGREDIENT RATIOS

Samples Like mentioned earlier, the ratios between the different ingredients -Cellulose, Calcite and P.G.A.- heavily influence the resulting material and its technical characteristics. In order to examine this, five samples with varying ingredient ratios were produced (fig. 38). This was done by varying the calcite content while keeping the other factors constant. These were consequently measured for their density (Hildebrand H300S), and tested for mechanical performance by means of a three point bending setup. In addition, other samples have been examined using a Scanning Electron Microscope (JEOL JSM 6010 LA), to view how their microstructure changes in relation to the ingredient ratios.

Sample name	Calcite content	Cellulose content	P.G.A. content	Density (g/cm ³)
1/2/0	33%	66%	0%	1,374
1/1/0	50%	50%	0%	1,302
2/1/0	66%	33%	0%	1,180
1/1/1	33%	33%	33%	1,352
1/1/2	25%	25%	50%	1,387

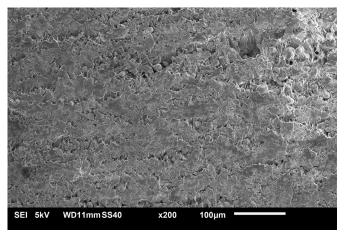


figure 39: 50% Calcite, 50% Cellulose

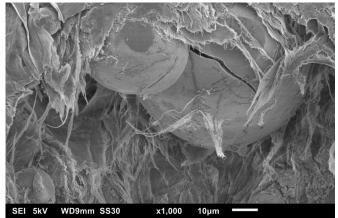


figure 41: 50% Calcite 50% Cellulose

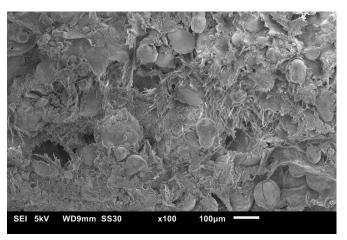


figure 40: 80% Calcite, 20% Cellulose

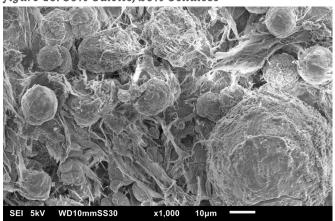


figure 42: 33% Calcite 33% Cellulose 33% P.G.A.

Micro-Structure In the images shown above, the micro-structure of three samples with varying ingredient ratios are displayed (fig. 39-42). Comparing figure 39 withfigure 40, it is evident that the increase in calcite content results in the calcite crystals getting noticeably bigger in diameter. With this increasing diameter, the calcite crystals start to disrupt the homogenity and layering of the material its microstructure as observed in figure 40. It is assumed here that this disruption will decrease the material its mechanical performance. So not only will the material properties change due to the higher calcite content and the inherent properties of calcite (stiff and brittle). The effect that these calcite crystals have on the homogenety and layering also plays a role.

Comparing fig. 41 with fig. 42, it is observed that the addition of P.G.A. results in the calcite crystals becoming differently structured. Here the P.G.A. polymers are intertwined with the calcite crystal and consequently increase their ductility (Spiesz et al., 2019).

Mechanical Properties	In order to test how these ingredient ratios influence the material its mechanical properties, a three point bending test was performed using a Zwick-Roell Z010 test press. This to measure the material its response to flexural stress in relation
	 to the ingredient ratios. During this test, a number of unexpected occurences distorted the test and its results. The samples warped significantly during drying, due to this they were not completely flat resulting in inhomogenities in the results. At a deflection of 6-8 mm, the sides of the tool came into contact with the samples (fig. 43), this caused a dramatic increase in the force required to deflect the sample and a corresponding bump in the resulting graph (fig. 44) At a deflection of 10-12 mm, the sides of the sample started to slide
	 downwards (fig. 43). This resulted in a decrease of the required force, shown as a plateau in the graph (fig. 44) Due to the above factors, the actual point of failure in the material could unfortunately not be defined. It was therefore impossible to define the strength and toughness of the material samples. What could be calculated were the yield point, and stiffness of the material samples in relation to their ingredient ratios. Visit Appendix C for more information on these calculations.
Calcite Ratio	 Comparing different calcite ratios (fig. 44); an increase in calcite content leads to: Intitially a higher stiffness (E_{flex} of sample 1/1/0) but followed by a drop in stiffness (sample 2/1/0). This drop could be attributed disruptions in the microstructure by calcite crystals grown too large (previous page) A decrease in the amount of deformation the material can take before yielding (strain at yield). A lower density which could be attributed to the amount of air inclusions in the material increasing.
P.G.A. Ratio	 Comparing different P.G.A. ratios (fig. 45); an increase in P.G.A. content leads to: An increase stifness (E_{flex}) An increase in the amount of deformation that the material can take before yielding (strain at yield). An increase in density of the material.
	From these findings, it is extrapollated that; Calcite promotes stiffness in the material but only up to a certain extent, too much calcite will result in the micro- structure of the material becoming disrupted, decreasing stiffness. P.G.A. promotes flexibility in the material, a higher P.G.A. content also increases

the amount of elastic deformation that the material can handle.

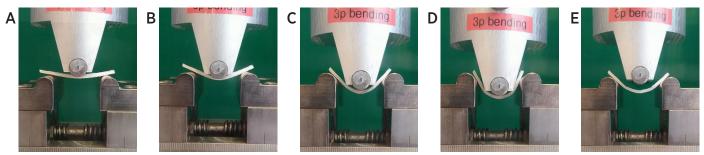
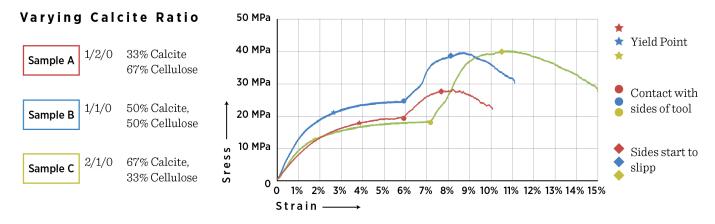
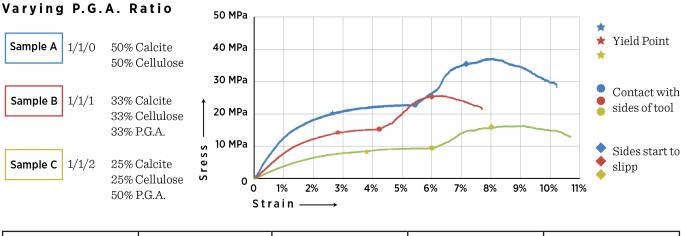


Figure 43; Images of the test setup with A: Initial deflection, B: Sides of tool in contact with sample, C & D: Sides of sample sliding downward and E: tool retraction revealing the residual deformation.



Ratio (Ca/Ce/P.G.A.)	Density (g/cm ³)	Strain at yield (mm/100mm or %)	Stress at yield (MPa or N/mm ²)	E _{flex} (Mpa or N/mm²)
1/2/0	1,37	3,9	17	1091
1/1/0	1,30	2,6	17	1438
2/1/0	1,18	1,8	10	1007

Figure 44; Results of the comparrison betwee n samples with different calcite ratios.



Ratio	Density	Strain at yield	Stress at yield	E _{flex}
(Ca/Ce/P.G.A.)	(g/cm^3)	(mm/100mm or %)	(MPa or N/mm ²)	(Mpa or N/mm ²)
1/1/0	1,30	2,6	17	1438
1/1/1	1,35	2,8	14	927
1/1/2	1,39	3,8	11	643

$Figure \ 45; Results \ of \ the \ comparisson \ between \ samples \ with \ different \ P.G.A. \ ratios$

4.4 FORMING PROCESSES

In order to understand whether or not and how the forming processes (as described in Chapter 3.4) influence the material its mechanical properties, material samples with varying ways of production were prepared. Here, the material its response to compression and how this influences its performance was investigated. Evident from the previous chapter, there are more ways of processing the material with consequential changes in its properties. However, compression was selected as an interesting case due to the material its apparant ability to withstand compressive forces.

- Samples Three samples were prepared with the same ingredient ratios and filtration procedure as shown in the figure on the right (fig. 46).
 - Sample 1 was prepared and dried in a regular fashion.
 - Sample 2 was filtrated and compressed with a 100 kg when it was still wet.

• Sample 3 was dried and compressed with 16 000 kg when completely dried. These samples where then sanded for flatness, cut into three pieces, measured for their density, examined b.m.o. S.E.M. and subjected to a three point bend test.

Compressive Behaviour of the Samples

Even though the samples were not compressed in triplicate or in a controlled set-up, it is still possible to analyse the behaviour of Samples B and C under compressive stress. Given the dimensions before and after compression (fig. 46)

- B shows a strain of 20% along its diameter and 50% along its thickness
- C shows a strain of 4% along its diameter and 13% along its thickness
- Sample C withstood a compressive stress of 83 MPa without showing any signs of failure. Unfilled concrete fails around 30 MPa (CES Edupack, 2019)
- Comparing the strain along the thickness, Sample C displays a stiffness that is a 1000 times greater than that of Sample B.

Of course, further testing is required to validate the above statements but they indicate an interesting relationship and highlight the difference between the material when it is wet and when it is dry.

Micro- On the right are pictures of two material samples, obtained via S.E.M.. Note that
 sample A (as described in figure 46) was unfortunately not viewed under the
 S.E.M. ((JEOL JSM 6010 LA). Comparing the microstructure of samples B (fig.
 47) and C (fig. 48), a difference between them can be observed. Where sample
 C seems to have retained a regular and somewhat layered micro-structure, the
 structure of sample B seems to have become somewhat dislodged. This makes
 sense when comparing the strain both samples underwent during compression.



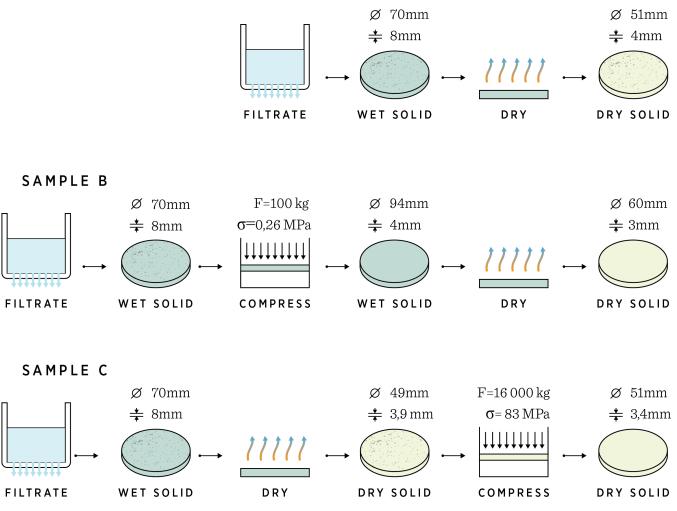
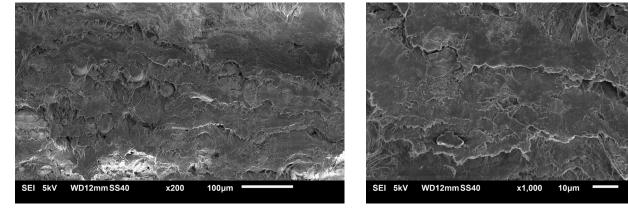


Figure 46; Phases of producing samples A, B and C with corresponding dimensions and the compressive force/stress



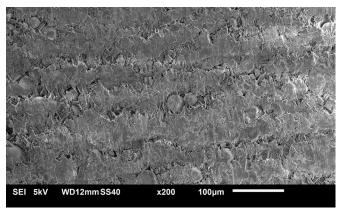


Figure 47; S.E.M. images of the microstructure of sample B

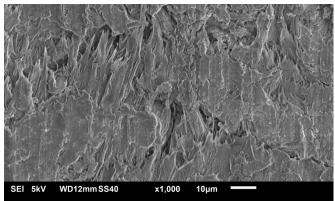


Figure 48; S.E.M. images of the microstructure of sample C

- 3P Bending The three samples described on the previous page were subjected to a three point bending test using the Zwick Roell Z010 test press. Based on this test, the material its flexural performance such as stiffness, strength and toughness in relation to the way in which it was processed can be determined. The results of this test are displayed on the left (fig. 49). See also Appendix C for a more detailed description of the results of this test.
 - **Sample B** Comparing the results of Sample A with Sample B (fig. 49 & 50), it is concluded that compressing the material when it is still wet, results in;
 - A decrease in density; this makes sense given that the volume of sample B turned out to be slightly bigger (fig. 46).
 - A slight increase in stiffness (E_{Flex}).
 - A decrease in the amount of elastic deformation and stress (Strain and Stress@ yield) that it can take.
 - A decrease in the total amount of deformation and stress (Strain and Stress@ failure) that it can take.

It is therefore concluded that the properties of the material deteriorate when it is compressed whilst wet.

Sample C Comparing the results of Sample A with Sample C (fig. 49 & 50), it is concluded that compressing the material when it is wet, results in;

- An increase in density.
- An big increase in stiffness (E_{Flex}).
- A decrease in the amount of elastic deformation (Strain@yield) that it can take.
- An increase in the amount of stress (Stress@yield) that it can take.
- A decrease in ductility (Strain@failure).
- An increase in strenght (Stress@failure).
- A decrease in its toughness

Based on this it is concluded that the compression lead to a change in the material its properties, not neccesarily for the better or worse.

Comparing samples B and C; a trade-off exists between compressing the material when it is wet, allowing for a lot of form freedom but deteriorating its properties and compressing it when it is dry, bring less form freedom but improving its stiffness and strength.

ToughnessThe value for the toughness was obtained measuring the area under the curve up
untill the point of failure (See also Appendix C). Note that little sources exist on
the flexural toughness of materials to compare and validate this value. In existing
literature, Flexural Toughness was described as an indicative value. (Wang et al.,
2013) It is also in this research considered a mean to compare between samples.

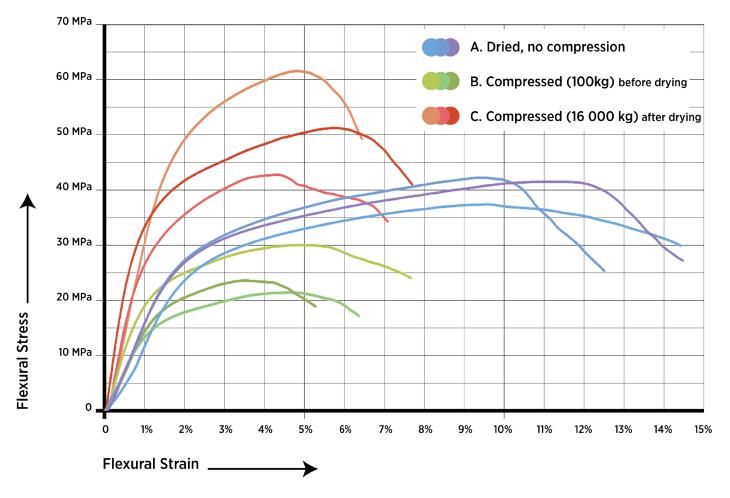


figure 49; Stress strain curve of sample 1/1/0_B and point of interest located along it

Sample	Density (g/cm ³)	E _{Flex} (GPA)	Strain @ Yield (%)	Stress @ Yield (MPa)	Strain @ Failure (%)	Stress @ Failure	Flexural Toughness (MJ/m ³)
А	1,34	1,57	3,4	31,5	10,2	40,1	5,8
В	1,23	1,85	2,1	21,5	4,3	24,7	1,7
С	1,54	4,04	2,8	46,0	4,9	51,5	4,1

figure 50; Table showing the different values measured in the 3P bend test.

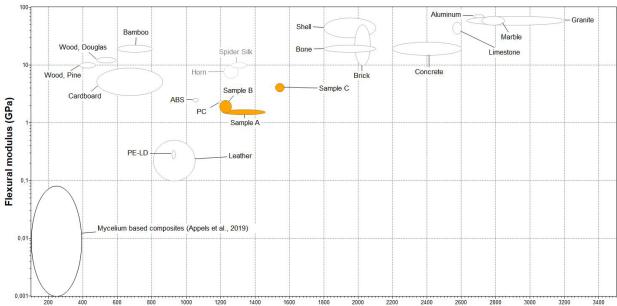
4.5 COMPARRISON

Based on the mechanical tests performed, it is possible to compare the different samples of this material and their flexural performance to existing materials. Here the Cambridge Engineering Selector (CES Edupack, 2019) database was used to compare the data obtained from the 3P bending test (4.4) with that of existing materials. This was done for the material its measured density, flexural stiffness, flexural strength and compressive strength.

- Flexural Stiffness In the table on the top left (fig. 51) we can see a comparrison between the density (X-axis) and flexural stiffness (flexural modulus, Y-axis) of different materials. Here we can see that the measured samples A, B and C are performing relatively poor when compared to various types of wood that are stiffer yet lighter. When compared to another biofabricated material, like mycelium based composites (Appels et al, 2019), the material samples perform very wel in terms of flexural stiffness and strenght (fig. 51 & 52).
- Flexural StrengthIn the table on the left (fig. 52) we can see a comparison between the density
(X-axis) and flexural strength (flexural modulus, Y-axis) of different materials.
Here the material samples are performing very well when compared to conrete,
brick and different types of stone, being stronger and more lightweight. Again,
when compared to wood, the material appears less strong and lightweight.
Interestingly, the samples do apear to fall in between the wood and stone
category., highlighting that they are part cellulose part mineral.

Like mentioned on the previous page, little sources exist about the flexural toughness of materials and also CES Edupack does not support this comparisson. This is important to note since, based on the comparissons of the stiffness and strentght (fig. 51 & 52) one could assume that sample C steadily outperforms Sample A which is, when we are looking at toughness and ductility, not the case.

Compressive Note that the compressive strength of the material was not properly tested.
 Strength However, since sample C was subjected to 83 MPa of compressive force whilst not failing, the compressive strength of the material appears to be one of its main strenghts. Therefore a comparrison was made (fig. 53), asuming that the compressive strength of the material is somewhere between 83 and 93 MPa whilst knowing that it could very well be higher. Here sample C outperforms concrete and even most types of wood whilst being remarkably lightweight when compared to the various types of stone.



00 600 800 1000 1200 1400 1600 1800 2000 2200 2400 2600 2600 3000 3200 3400 Density (ka/m^3) Figure 51: The Flexural stiffness of samples A,B and C compared to various other materials.

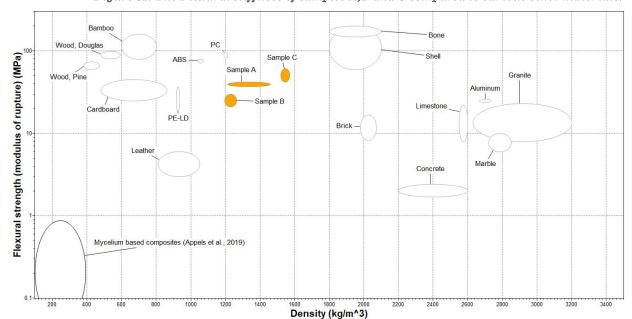
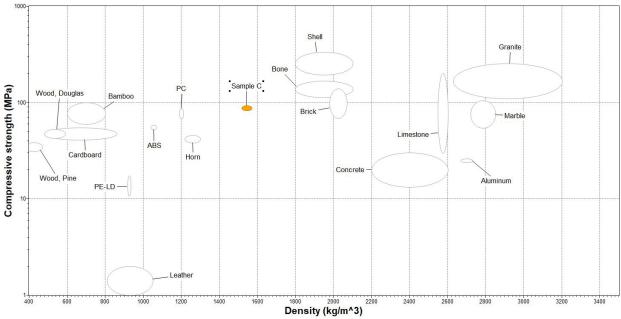


Figure 52: The Flexural strength of samples A, B and C compared to various other materials.



 $Figure \ 53: \ The \ Compressive \ strength \ of \ samples \ A, B \ and \ C \ compared \ to \ various \ other \ materials.$

4.6 T.C. CONCLUSION

Based on the technical characterisation conclusions can be drawn on the material its mechanical and physical properties in relation to its ingredients and the way in which it is processed. Based on this, technical qualities, unique to the material can be defined.

- GrowthThe organisms involved in growing this material are understood well enoughParametersto influence their growth and the formation of the material. They are also
understood well enought to realize that that there are a lot of parameters at play
which can, within the scope of this project, not be fully controlled. Heterogenity in
the material, its inredient ratios and distribution is therefore still present.
- Ratios of The ratios between the ingredients of the material are defining for the resulting ingredients material, it's microstructure and mechanical properties. Depending on these ratios, the material can range from flexible to stiff and ductile to brittle. In adittion, the material its surface, colour and the way that it shrinks during drying change with the ratio between the ingredients (REF). Efforts have been made to identify an optimal ratio of ingredients but this requires further testing. This supposed optimal ratio is of course also dependent on the mechanical performance that a design requires and can therefore be varied intentionally.
- Processing The way in which the material is processed from a liquid suspension into a dry solid is also defining for its resulting density, microstructure, surface roughness and inherent mechanical properties. In addition, these processes determine the form of the material and how well this form can be controlled. Inhibiting this control over form are the shrinkage and warpage that occur when the material goes from a wet to a dry phase. This transition is also reversible by re-wetting the material, meaning that it shows a lot of potential for being recycled.
- Mechanical In general the mechanical properties of the material are considered to be very good, especially for a biofabricated material. It combines a low density with a high compressive and flexural strength. Here it is also assumed that the methods of preparing the material and the resulting ratios were not optimal yet and thus the material can do better.
- OtherOther comparisons can be made based on the fact that this material requiresPropertiesno heat to produce and is relatively fireproof. Due to its absorbance, it also shows
potential for combining it with various coatings .

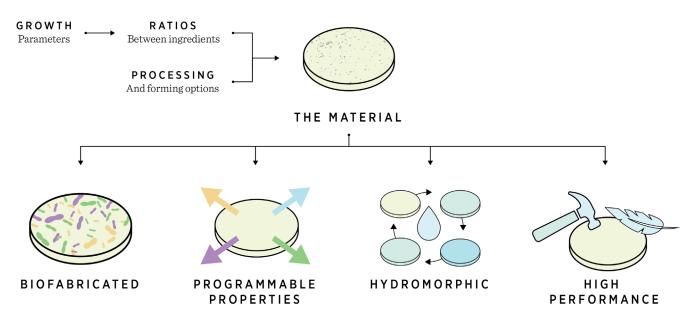


Figure 54: The Compressive strength of samples A, B and C compared to various other materials.

Based on the technical characterization, four unique technical qualities of the material are defined as shown in the figure above (fig. 54).

- **Biofabricated** The material its ingredients are grown by bacteria with subsequent variance in its properties due to the unpredictability of these biological processes.
- ProgrammableThe material its stucture, mechanical characteristics and appearance canPropertiesbe varied according to the ratio of its ingredients and the way in which it is
processed.
- HydromorphicThe material its water content is defining for it stiffness, ductility and formability.Upon drying it will shrink and warp. It is not waterproof and can therefore be
reshaped and recycled by adding water.

High Performance The material has a very low density in relation to its compressive and flexural strength and is considering the fact that it is grown by bacteria; high performance.

FurtherFor the further development and characterization of this material, it isTestingrecommended to;

- Further test its performance under compressive loads.
- Determine the optimal ratio of ingredients and verify this ratio b.m.o. a thermographic analysis
- Investigate the water absorption in relation to different ratios, densities and in combination with crosslinked alginate.



5. EXPERIENTIAL CHARACTERIZATION

This chapter entails the Experiential Characterization (E.C.) of the material, aimed at finding out how people perceive this material, what they associate it with, how it makes them feel and what it makes them do. Two separate tests have been performe, based on the insights that these rendered, experiential qualities of the material will be formulated.

5.1 GOALS OF THE E.C.

Based on the Technical Characterisation we can conclude that many of the material properties can still be altered. This results in a lot of variation in the manifestation of the material samples. In this sense, the material is regarded as being underdefined, with a lot of variation in how it looks, feels and smells.

Initial tests With this in mind, the initial Experiential Characterization (E.C.) was aimed at exploring these varying properties and how people responded to them. The aim of the tests was, therefore, exploratory in nature; not aimed at investigating a specific phenomenon or answering a defined research question. The goal was to get a broad impression of how people react to the different manifestations of the material.

Tests with
materialThe second series of experiential tests was performed with material designers,
who were already familiar with the Material Driven Design method (Karana,
2015) and therefore biased. The goal of this test was to generate ideas and input
for the design process.

5.2 INITIAL TESTS

Test setup

Performed with 6 participants, IDE students. 4 participants were unaware of my graduation topic, 2 were aware that I am working with bacteria but not what the material was. Each participant was presented a variety of samples and asked to explore them whilst thinking aloud, this was video recorded. Afterwards they were interviewed about what they thought the material was, what they associated it with and how they felt about it. The Experiential Characterization Map (REF) was used as guide for these tests but followed loosely.

Results Performative level: the most prevalent actions were rubbing or tracing -exploring texture- and bending -exploring flexibility.

Sensorial level: most samples were classified as; moderatly hard, matte, warm, fibred and light weight. Notably, the contrasts between samples in terms of roughness, elasticity and regularity were adressed.

Emotional level: Surprise was expressed regarding the flexibility of the samples and pleasure induced by the vareity in textures.

Interpretive level: Interpretations varied between different samples and their faces. Notable contrasts between; agressive and calm, natural and professional, sober and frivoulous. Also, the samples were interpreted as being made from a recycled material, carboard and gypsum.

Insights The varying material properties arouse interest and bring about different material experiences.

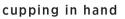
The material is being interpreted as ordinary and of a relatively low-grade -recycled cardboard- in both its mechanical properties and origin.

PERFORMATIVE

scratching



"I like both the translucent and the textured one"





"It does not seem to rub off"

ticking

carefull bending



"I don't dare to bend it too much"

tracing



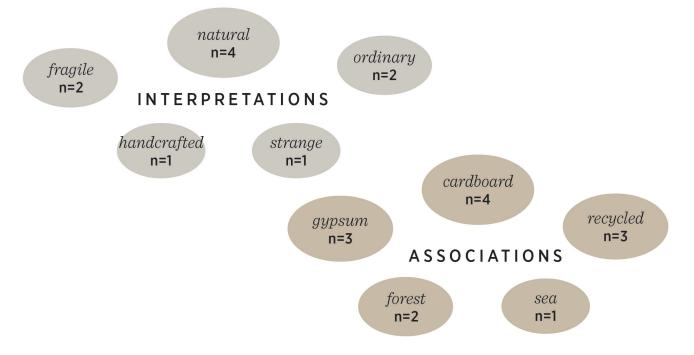
"Textured but still smooth, like some beauty product"



"It sounds like gypsum"



"Like a mushroom, something you would find in a forest"



 $Figure \ 55: visual \ representation \ of \ the \ results \ obtained \ in \ the \ first \ test$

5.3 TESTS WITH MATERIAL DESIGNERS

- Test setupPerformed with 3 participants who are aware of the bacterial origin of the
material and have expertise with the Material Driven Design method (Karana,
2015). They were presented 3 samples of the material, varying in shape and
texture, and asked to explore these whilst thinking aloud, this was video recorded.
After this they were asked to fill in the Experiential Characterization Map and
reflected on this afterwards.
 - **Results** The results of this test are visualized in the illustration to the right (fig. 56).
- PerformativePrevalent actions were pushing and bending -testing the material its strength-
and tracing and rubbing -exploring texture-. Other notable actions (n=1, not
shown) were; Holding against the light, tearing and smelling the samples
 - SensorialThe participants were unanimous in that the material is; rough, matte,levellightweight, irregularly textured and fibred. The participants did note that the
samples varied concerning; roughness, hardness, strength and transparency.
 - AffectiveRecurring pleasant emotions were surprise, curiosity and fascination. This due to
participants knowing that the material is grown by bacteria but don't know how.
Unpleasant emotions were reluctance, doubt and insecurity, in addition,
all participants expressed that the material looked fragile and they were therefore
very carefull in handling it.
- InterpretiveAll participants interpreted the material as natural, a consideration here is thatlevelthey already had a clue about the materials 'natural' origin.

Two participants mentioned a duality due to contrasting interpretations, for example, the material was interpreted to be both ordinary and strange.

Insights The material appears more fragile than it is, causing people to be overly carefull with it.

The material arouses curiousity, people knowing what it is wonder how bacteria could have made such a thing.

The varying textures and surfaces bring about pleasure.

Performative level



pushing _{n=2}



bending _{n=2}

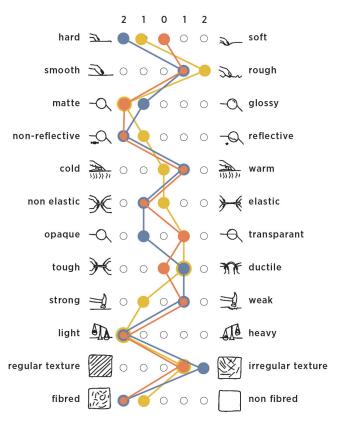


rubbing n=3

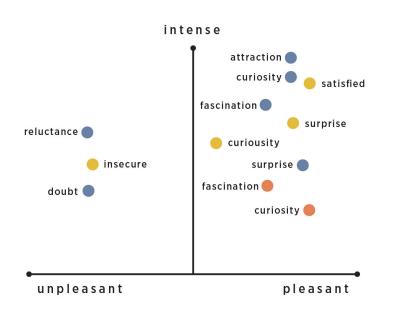


tracing _{n=2}

Sensorial level



Affective level



Interpretive level

Natural _{n=3}

Due to fibers and irregularity



Cosy _{n=2}

Looks soft and is warm to the touch





Dual n=2

Multiple contrasting interpretations both apply

5.4 E.C. CONCLUSION

Because of the variability in shapes, appearances and properties that the material allows for, the experiential characterization was also performed with a wide variety of material samples. This was aimed at exploring the different experiences participants had in relation to this vareity and led to valuable insights. However, it is also this vareity, introduced in the material, that inhibits an in-depth experiential characterization of it. This combined with the small sample size adds up to the fact that these tests were very exploratory in nature. In order to gain a proper understanding of the material its experiential characteristics, the appearance of the material must be more defined and tested in a more controlled setup with more participants. Yet, for the sake of continuity in the design process, conclusions will be drawn based on the small tests that were performed. Here, recurring patterns of interpretation and experience have been identified which are used to define the material is unique experiential qualities.

- Performative On the performative level, all participants were observed to be overly carefull with the material samples. Slightly probing and bending it but not daring to go too far. Also they often traced the different surfaces, exploring the variety of textures that the samples had.
 - Sensorial Recurring sensorial properties assigned to the material were: fibred, irregular and lightweight. However, many participants also noted the sensorial properties to vary between samples and surfaces. Here contrasts between; rough / smooth, opaque / translucent and glossy / matte, were expressed. The contrast between paper- and stone-like was also mentioned often.
 - Interpretive These contrasting properties also recurred in the interpretations that people assigned to the material, it being both ordinary and strange, futuristic and nostalgic at the same time. It was often interpreted to be natural due to it's fibredness.
 - Affective Curiousity and fascination were expressed due to the material its contrasting sensorial properties. This was experienced to be pleasurable. Less pleasant were the emotions of doubt and insecurity, due to the material appearing as fragile and the participants fear of breaking it.

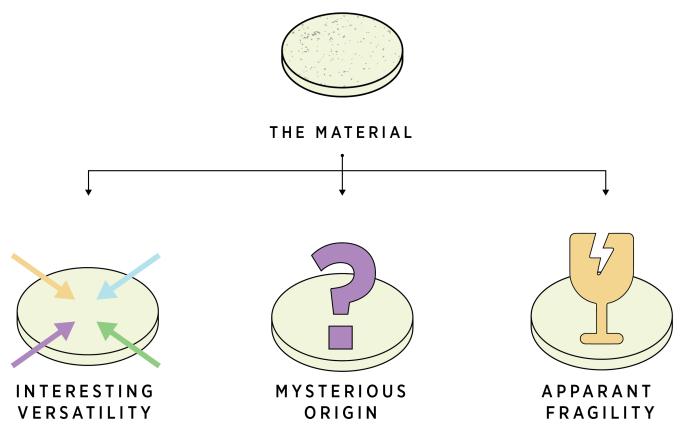


Figure 57: The material its experiential qualities.

	Based on the characterization, unique experiential qualities of the material are defined which are also shown above (fig. 57).
Interesting Versatility	The varying sensorial qualities of the material cause people to investigate its different surfaces which in experienced as pleasing.
Mysterious origin	The material its conflicting properties also arouse questions about what it is. It is interpreted to be natural but participants wonder how it was made.
Apparant fragility	The material is percieved as fragile and delicate. Participants are overly carefull in handling it and experience insecurity in doing so.
Further Testing	Like mentioned, to gain a more in depth and valid understanding of how people experience this material, further testing is required. Most of all, this would require a more controlled set-up in which the properties of the material are kept constant. In addition, this test would require a bigger sample size in which a critical comparrison is made between the participants that have prior knowledge about the bacterial origing of the material and those that dont.

6.MATERIAL EXPERIENCE VISION

This chapter will provide a synthesis of the previously found unique qualities of the material, both in the technical and experiential sense. The relationships between these different qualities will, combined with a benchmarking analysis of similair materials, lead to the identification of a plausible domain for this material, defined in the form of a Material Experience Vision.

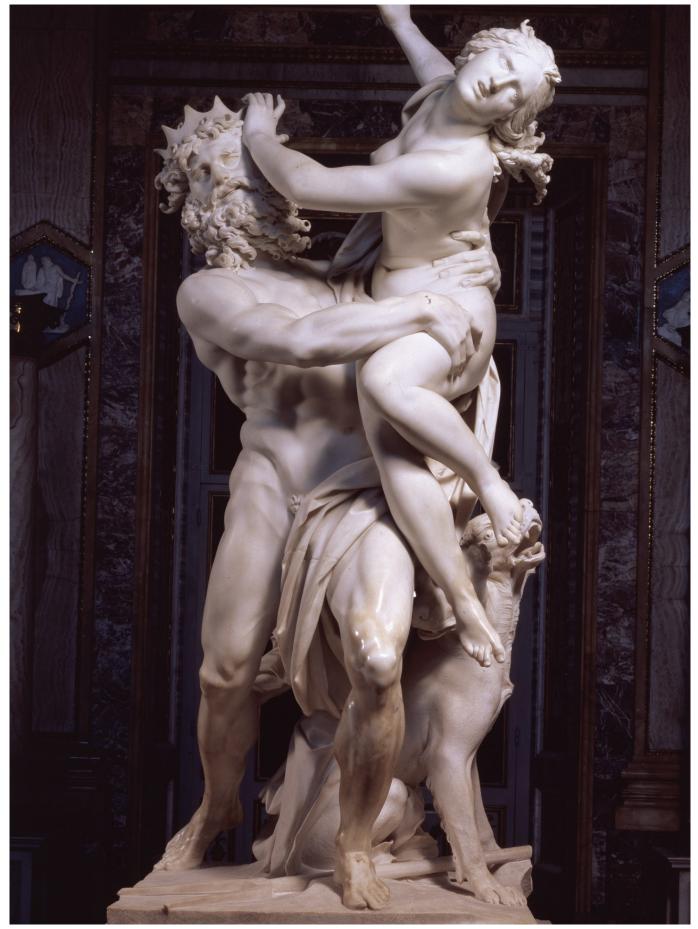


Figure 58: Ratto di Proserpina, Gian Lorenzo Bernini, 1622, Carrara marble

6.1 UNIQUE QUALITIES

Technical:	Based on the findings of technical characterization in Chapter 4, the material its unique technical qualities are as follows;
Biofabricated	The material its ingredients are grown by bacteria with subsequent variance in its properties due to the unpredictability of these biological processes.
Programmable Properties	The material its stucture, mechanical characteristics and appearance can be varied according to the ratio of its ingredients and the way in which it is processed.
Hydromorphic	Hydromorphic: The material its water content is defining for it stiffness, ductility and formability. Upon drying it will shrink and warp. It is not waterproof and can therefore be reshaped and recycled by adding water.
High Performance	High performance: The material has a very low density in relation to its compressive and flexural strength and is considering the fact that it is grown by bacteria; high performance.
Experiential:	Based on the findings of the experiential characterization in Chapter 5, the material its unique experiential qualities are as follows;
Interesting Versatility	The varying sensorial qualities of the material cause people to investigate its different surfaces which in experienced as pleasing.
Mysterious Origin	The material its conflicting properties also arouse questions about what it is. It is interpreted to be natural but participants wonder how it was made.
Apparant Fragility	The material is percieved as fragile and delicate. Participants are overly carefull in handling it and experience insecurity in doing so.

Benchmarking In order to understand related materials, what characterizes them and how they
 Analysis: compare to the material in question, a benchmarking analysis was performed
 (Appendix A). From this, qualities that define similair materials and possible gaps for the application of this material can be formulated:

- Unique Based on the analyzed projects that involve either bacterial cellulose or calcite, it is concluded that the material in question is unique in that it encompasses qualities from both of these categories.
- GrownReviewing examples of biofabricated materials, it is concluded that suchAestheticmaterials often posess certain look and feel, inheritent to the fact that they are
grown. Designers tend to embrace this 'grown aesthetic' and its resultant
imperfections since they tell an 'honest' story about the material its origin. This
brings rise to the notion that a biofabricated material could also be dishonest.

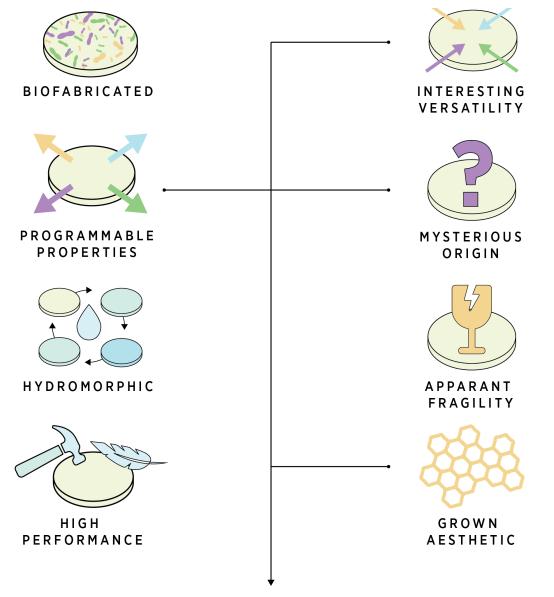




Figure 60: Ratto di Proserpina, Gian Lorenzo Bernini, 1622, Carrara marble

6.2 PROGRAMMABLE PARADOX

In one of his many masterpieces (fig. 60), Gian Lorenzo Bernini, when he was only 23 years old, managed to manipulate hard marble to look as soft and pliable as a womans thigh. This soft and pliable expression of a hard and brittle material offers a paradox, one that makes you wonder at the material its properties and the way it was made. The Material Experience Vision is thus defined as;

Program and emphasize paradoxal qualities in the material, inciting people to investigate, uncover and wonder.

Here, the paradox that is of interest is that between something that is either natural or man-made. The goal of the concept, following this interaction vision, will therefore be to play with peoples perception about what is natural and what is man-made about this material.

PROGRAMMABLE PARADOX

natural vs MAN-MADE

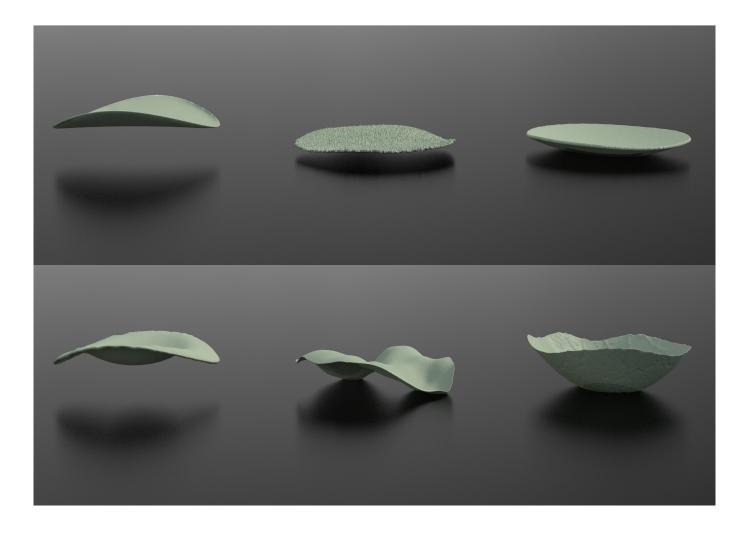


Figure 61: The M.E.V., the meanings distilled from it and the related material qualities.

6.3 EXPERIENCE PATTERNS

In order to link the material experience vision to formal properties of the material and concept to be, an effort was made to manifest experience patterns in line with the material driven design method (Karana et al., 2015). Here two meanings; Natural and Man-made, aimed at eliciting the envisioned paradox, were distilled from the material experience vision. Based on these two, seemingly paradoxical meanings, experiential patterns can be defined, aimed at providing insights into the interrelationships between the experience vision and formal qualities of the material. (Giaccardi & Karana, 2015; Karana, 2009). Note that ideally, such experiential patterns are defined through a study that involves an adequate sample size of participants. In this case, however, this step is performed based on the insights of the designer, aimed at providing clarity in the design process.

The meanings distilled from the experience vision and formal material qualities that are attributed to these meanings are visualised in the figure above (fig. 61). These material qualities will consequentially be used as handlebars in generating the material concept.



7. MATERIAL CONCEPTS

Based on the Material Experience Vision, three material concepts are proposed that emanate the material its contrasting qualities. This is done by illustrating potentials unique to the material, defined as affordances or forming processes that the material allows for. These forming processes consequentially influence the material its shape and properties, which is explored by means of a parametric design model. Based on this exploration, a relationship between the way in which the material is grown and processed and its envisioned experience is communicated.

7.1 GOAL OF THE CONCEPTS

The goal of the three material concepts proposed in this chapter is to communicate the material its ability to attain contrasting properties such as flexible and stiff, smooth and rough, and having either a controlled or an uncontrolled shape . These contrasting properties will lead to different interpretations of the material being either natural or man made, bringing about the envisioned material experience.

AffordancesIn line with the Materials Potentials framework (Barati & Karana, 2019), threeas Materialproposed material concepts are based on potentials that are unique to thePotentialsmaterial. These potentials are defined as affordances that the material possesses.The term affordances is traditionally interpreted as; possibilities for actions that
are offered to an animal by its environment (Gibson, 1979; Heft, 2001). However,
in the case of conceptualizing the potential of a material, the term affordance can
also enable the description of a material in terms of its process-ability (Gaver,
1996). So, as defined in chapter 3, the material affords to be processed in different
ways, these different ways of processing consequently bring about vastly different
properties in the material. It can therefore be stated that affordances, as a
material potential subsequentlially influence the material its forming, functional
and experiential potential.

Exploring these
 Potentials
 The three concepts will therefore communicate the material its ability to
 be processed in three distinct ways. Here, a number of parameters (see also
 Chapters 3 and 4) are relevant in these processes and defining for the resulting
 material its form and properties. The relationship between these parameters and
 the resulting material have been further explored through a parametric design
 model (Grashopper 3D, 2019; Rhinoceros 3D, 2019; Appendix E). This in order to
 simulate how the material can behave and bring about different experiences.

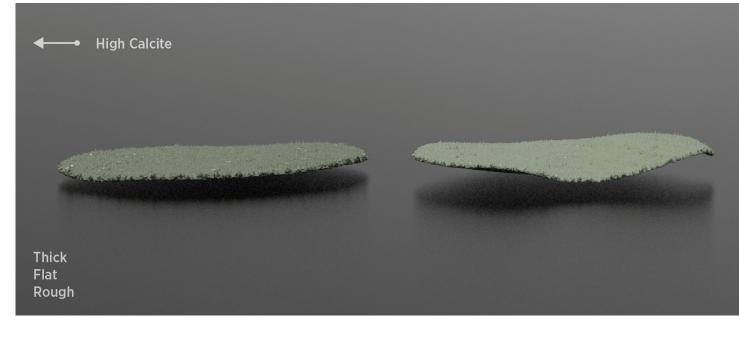
7.2 CONCEPT: DRYING

This concept is aimed at communicating the different behaviours that the material displays upon drying and what causes it to do so.

ParametersAs defined in chapters 3 and 4, the material, when dried, will shrink, warp andand Propertiesbecome rigid. Defining for this behaviour, is the ratio between the material its
ingredients (fig. 62). Here, a higher calcite content will result in a thicker material
that is of a darker colour and a much rougher texture. Due to this increased
thickness, the material will become less warped and shrink more (fig. 63). If the
amount of cellulose is increased, this will result in a thinner material that is more
smooth and prone to becoming warped upon drying (fig. 63).

Parametric With this in mind, a parametric model was created to simulate this transition
 Exploration from a thick and rough material to a thin and warped material (fig. 64, Appendix E). This model was made with the cellulose content as the main parameter that influence the material its thickness, roughness, colour and warping.

PotentialWith these variations, the material can be made to look very natural due toApplicationsits properties changing between a rough and fibred texture and a warped and
uncontrolled form. Note that this lack of control over form does provide challenge
in translating it to an application. However, an interesting aspect to mention here
is the increase in translucency, revealing the material its inner structure, as the
thickness decreases.



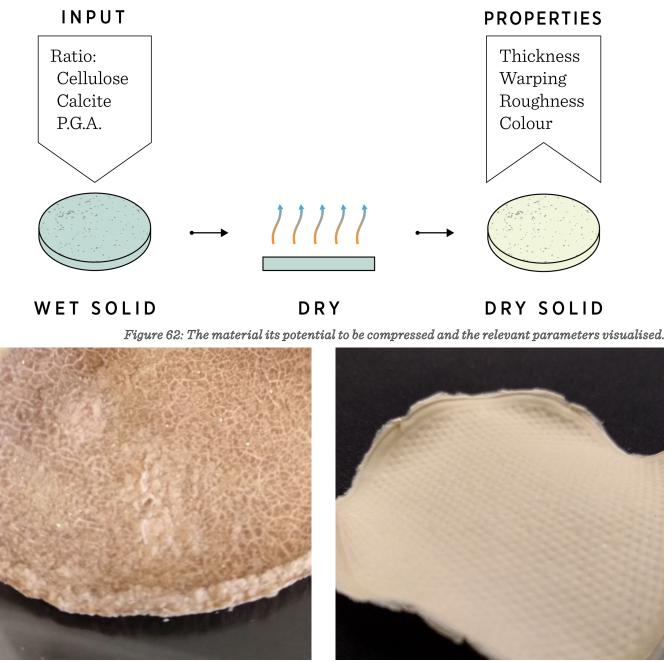
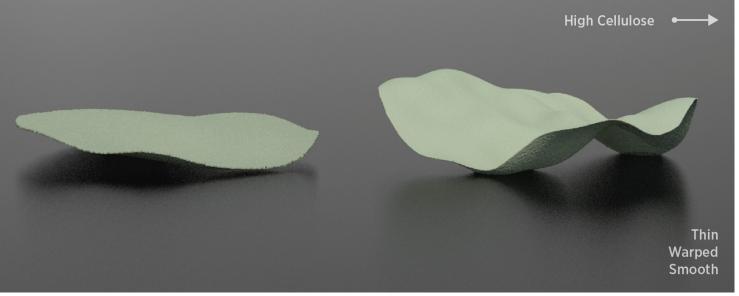


Figure 63: Material samples with a high calcite (left) and cellulose (right) content.



 $Figure \ 64: A \ parametric \ simulation \ of \ different \ drying \ behaviours \ of \ the \ material.$

7.3 CONCEPT: COMPRESSION

This concept is aimed at communicating the behaviour and changes in properties that the material displays upon being compressed.

Parameters	As defined in chapter 4, the material behaves very differently under compression
and Properties	in relation to its wettness or moisture content (fig. 65). Compressing the material
	when it is wet and pliable allows for a lot of form-freedom but the material is
	not able to handle a lot of pressure, it will also become weaker through this
	compression and will still shrink upon drying, inhibiting controll over the form
	(fig. 66). On the other hand, compressing the material when it is dry allows for a
	lot more pressure to be applied, strengthening the material but, since it is already
	dry and stiff, does not allow for a lot of form-freedom (fig.66.)
Parametric	These relationships have been simulated through a parametric model in which
Exploration	the moisture content and the amount of pressure were varied, influencing the
	resulting smoothness, form-freedom and -controll (fig. 67, Appendix E).
Potential Applications	The method of compression shows potential for creating a very hard and smooth material that could be considered as a replacement for tradtional ceramics. Especially considering the fact that it requires no heat but pressure to produce and is a lot less brittle than ceramic materials, allowing the final shape to be much thinner. However, there exists a tradeoff between form-freedom and controll,
	therefore a optimum between the moisture content and the amount of pressure that is applied has te be defined.

High Pressure Low Moisture

Thick Controlled Smooth



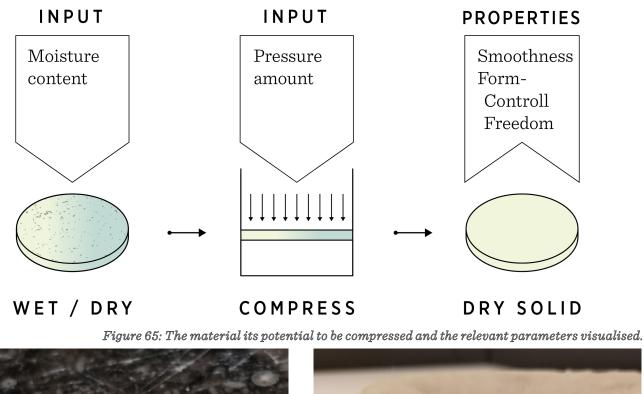




Figure 66: Low moisture content/ high pressure (left); high moisture content/low pressure (right).

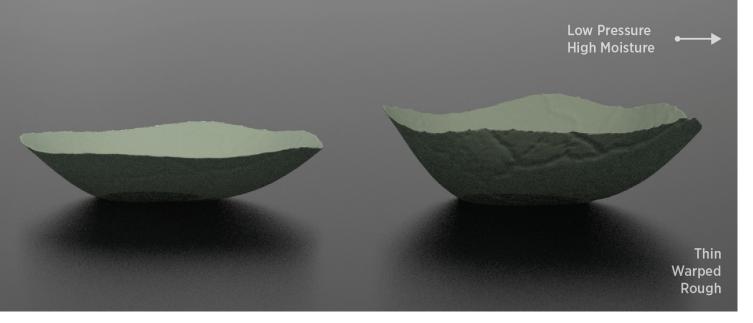


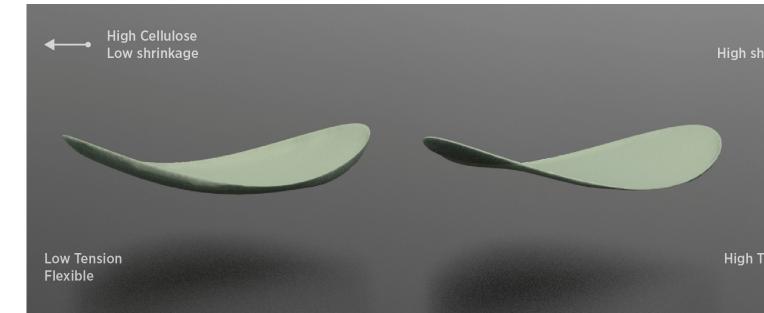
Figure 67: A parametric simulation of different compressive behaviours of the material. 83

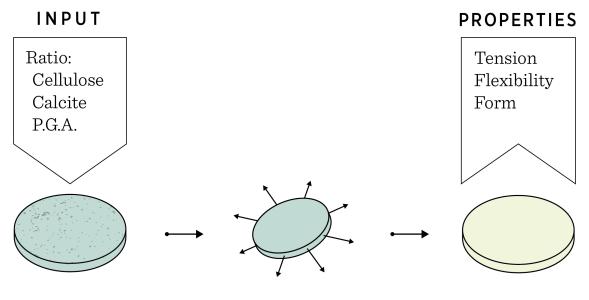
7.4 CONCEPT: TENSION

This concept is aimed at communicating the behaviour and changes in properties that the material displays upon being stretched while drying.

Parameters Here an important factor is the amount of shrinkage that the material undergoes and Properties upon drying. This shrinkage factor has been identified and linked to the thickness and ratio between the ingredients (Chapter 3) but is as of yet not fully understood. Yet, here its is assumed that increasing the amount of calcite will initially lead to a reduction in shrinkage and secondly to a decrease in shrinkage (see also chapter 3, p. 36). This shrinkage, when stretching the material, will result in tension building up in the material (fig. 68). The ratio between the ingredients will therefore influence the amount of tension introduced in the material and its inherent shape and flexibility.

- ParametricThese relationships were simulated in a parametric design model (fig. 70,ExplorationAppendix E). Here a decrease in cellulose content will result, initially lead to the
material becoming more tense, and secondly, becoming more stiff.
- PotentialBy stretching the material onto a shape and hereby controlling the amount andApplicationsway in which it shrinks upon drying shows potential for making reproducible
shapes. Also by controlling the thickness the degree of translucency can be set,
allowing for the creation of stone-like yet paperthin lamp shades. Varying the
degree of flexibility in the material can also allow for the creation of a chair-
seating in which the amount of comfort can be varied.





WET SOLID

STRETCH

DRY SOLID

Figure 68: The material its potential to be stretched and the relevant parameters visualised.



Figure 69: A stretched sample with a low tension (left) and high tension (right).

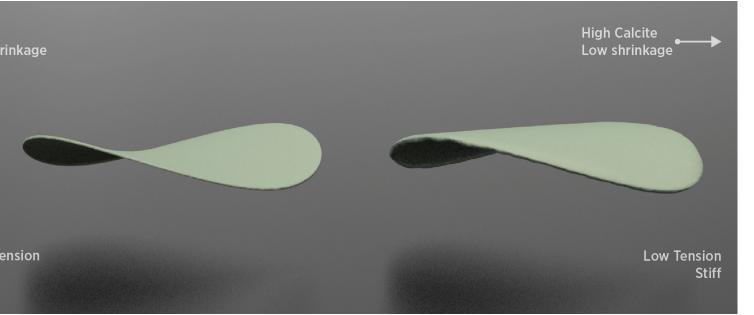


Figure 70: The material its potential to be stretched and the relevant parameters visualised.

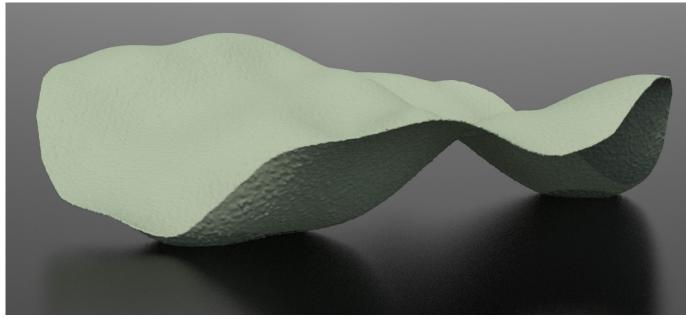


Figure 71: Simulation of a material that has warped upon drying

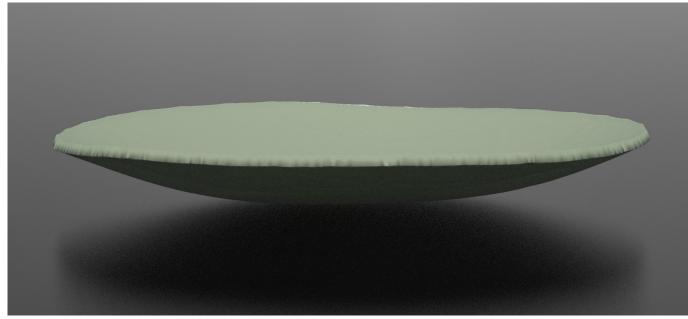
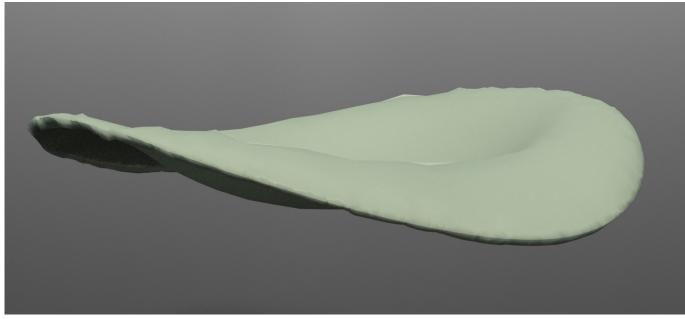
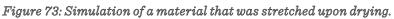


Figure 72: Simulation of a material that was compressed.





7.5 CONCEPT DISCUSSION

Given the analysis of the material its properties and the vast amount of parameters that are relevant for producing it, the goal of the concepts was to provide insight into the behaviour of this material along a selection of these parameters. In doing so, it was illustrated that this material has the potential to be grown and processed into different shapes with contrasting properties and inherent experiences.

ParametricHere the use of a parametric design tool (Grashopper 3D, 2019; Rhinoceros 3D,Design Tool2019) was found to be helpfull in simulating and exploring these said parameters.
This in part because it allowed for more control over the outcomes as opposed
to doing experiments in which a lot of unexpected things can happen. Yet, it is
precisely because of these unexpected things, that such a digital tool can never
replace the physical making process. The 'unexpected', is part of the material
and organisms their behaviour and can not be overlooked. Nevertheless,
the parametric design tool has been deemed usefull in communicating the
found interrelationships between parameters and properties. Given a proper
understanding and quantification of such interrelationships, it can be a valuable
tool in the kit of a designer.

MaterialThe three material concepts, presented as potentials for processing the material,Potentialseach provide interesting contrasts between form, functionality and experience
of the material. For example, the final manifestation of the material can vary
between a rough and a smooth surface, a controlled and uncontrolled geometry
or a material that is either flexible or stiff. With this in mind, a range of objects
can be created that are all grown by bacteria yet appear to be entirely different in
their origin and expression. This allows for the envisioned experience of creating
a material with paradoxal properties, inciting people to wonder what it is and how
it is made. In addition, this material has the unique potential to challenge people
their preconceptions of what a biofabricated materials can become.

Of the three presented concepts, the potential of compression is deemed as the most interesting to pursue further. This because it makes it possible to turn something that is grown into a hard, smooth, almost ceramic like material. A ceramic-like material that requires no heat to produce and could potentially be a lot tougher than traditional ceramics. It also provides an interesting paradox between the natural origin of the material and its final shape and properties that are smooth and defined.



8.CLOSING

This chapter will discuss the potentials that bacteria can have in the development of new materials and the role that designers can play in this development. It will also discuss the potential that the material in question has and how it can be further developed in the near future.

9.1 GENERAL DISCUSSION

The Potentials of Bacteria

Through the analysis of existing projects, it was found that collaborations between designers and bacteria provide a lot of opportunity for the growth of novel materials that can be ecologically beneficial. This because such grown materials require less energy and precious resources to produce whilst often being bio-degradable. In addition, bacteria show potential for developing a new type of smart materials, inhabited by living bacteria that could provide the material sensing, cleaning and self healing capabilities.

The Role of the
DesignerFor designers to engage in this new field where materials and products are grown,
there are some conditions. In working with living organisms, a designer has to
to adopt an interdisciplinairy approach and collaborate with biologists in order
to understand the biochemical processes involved. Because of the complexity
of these processes, it is deemed important that the designer initially sticks to
following the protocols set by the biologists and tries to controll the experiments
as much as possible. This to come to a neccesary understanding of the complex
way in which the material is grown and produced.

However, it is also viewed that the designer should not try to become a biologist him/herself. Once a proper understanding of the relevant processes is reached, she/he can also think outside the box and adopt a more intuitive approach towards experimenting with the material. This in order to allow for unexpected occurences to happen that can lead to new perspectives on the potentials of the material. In addition, not trying to controll every aspect of the experiments and making assumptions about the underlying processes can allow the designer to make faster progress in realising prototypes. In doing so it is viewed that the designer can provide an added value to the development of such materials by exploring new and unorthodox ways of experimenting with them.

Potentials of the Material

The material that was the subject of this thesis is considered to be very unique in its combination of ingredients and the fact that these ingredients are grown by bacteria. It displays outstanding mechanical performance and is, given the fact that it is grown by bacteria, also considered to be unique in this aspect. In addition, it offers a lot of versatility in its final form and properties, resulting from the way in which it is grown and processed. This allowed for the creation of objects with a lot of variation between them in terms of their; form, surface roughness, colour and stiffness. Also, the level of control that the designer had over the final form of the material varied with each method of processing the material. It is therefore stated that this material is very versatile in its form, function and experience potential.

Yet it is also this versatility which proved to be a challenge in designing with the material. Due to the amount of parameters involved in the making process, this project has been more about understanding the material and identifying its potentials than it was about carrying them out. In doing so, the added value of this design project is considered to lie in the communication of all these parameters and processes at play in the growth and creation of this bacterial composite. This communication was done by proposing a parametric design model in which the interrelationships between the material its parameters and properties are simulated.

Viability In terms of the viability of this material finding its way to real-world applications in the near future, a couple of questions remain. The first one being about scalability. During this project it was found to be extremely challenging to scale up the growth of bacteria, requiring hundreds of liter to, for example, be able to produce a single chair. This is indeed a challenge relevant for many biofabricated materials and should be adressed in collaboration with biotechnologists that posses expertise on large-scale fermentation. Here, the amount of nutrients and energy required to grow bacteria on such a scale should also be critically compared to the resources invested in more traditional materials. Another aspect to be considered is the fact that the material is in its current form not water proof which inhibits a lot of applications. On the other hand, this also means that the material that for example allows it to be coated with other biopolymers such as alginate, presumably rendering it waterproof.

All of these challenges and opportunities considered, the fact that this material is still part of a research in progress speaks to its futre potentials and that of materials grown by bacteria in general.

9.2 CONCLUSION

This project has shown that bacteria can be used to grow a material that has excellent mechanical properties and also displays a versatile array of form, functional and experience potentials. Due to this versatility and the amount of parameters involved in producing this material, it was also a challenge for designer to properly understand the processes involved in making the material. Therefore, a large part of the efforts were spend on attaining an overview of the different parameters at play and their interrelationships with the final properties of the material. This lead to the development of a parametric design tool aimed at communicating these interrelationships, a parametric design tool that, if properly grounded, can provide to be usefull for future designers willing to engage with this new way of working. Through the simulated material potentials and the experiments that underly it, the material has shown the potential to exhibit contrasting expressions that challenge the conception of what is a natural and what is man-made.

9.3 RECCOMENDATIONS

For further development of this material by future designers who wish to explore its potentials, its is recommended to;

- Initially, stick to the protocols described by biologists in order to come to and understanding of the material.
- Eventually, allow for a more intuitive approach to the experiments in order to generate new perspectives on the potentials of the material.
- Investigate the amount that the material shrinks in relation to its ingredient composition and thickness.
- Test the material its behaviour under compressive loads.
- Research the phenomenah described in Chapter 7 to strengthen the argumentation behind the parametric model.
- Look into options or material combinations that could render the material waterproof, e.g. combining it with crosslinked alginate.
- Collaborate with bio-technologists in order to scale up the growth of the bacteria and realise larger prototypes.
- Collaborate with experts in the field of industrial ecology and Circulair Design to properly asses the scenario of producing this material on an industrial scale.

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BACTERIAL COMPOSITES

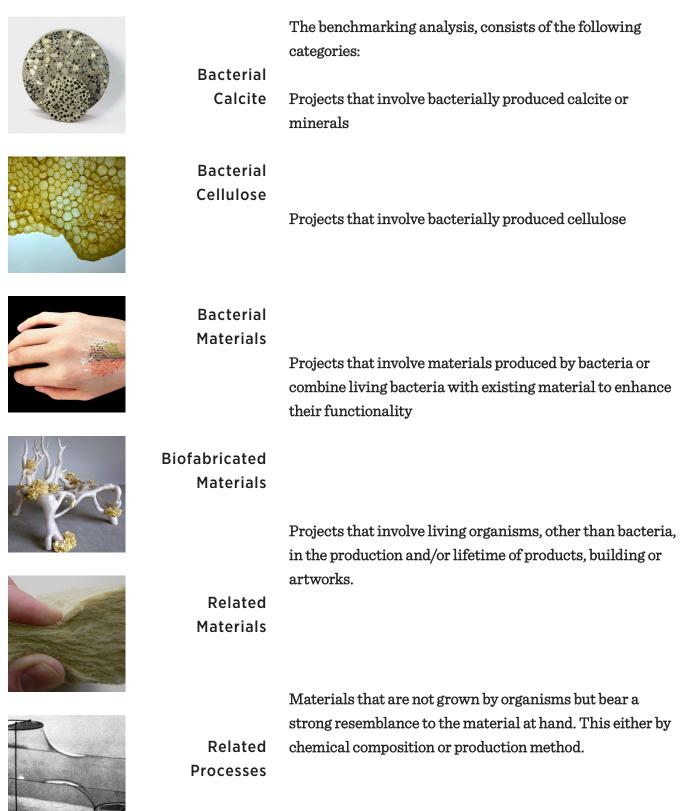
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A. BENCHMARKING ANALYSIS



A.1 BACTERIAL CALCITE

Name & Year Kev Words Manufacturer Composition Process

Living Concrete, 2011 Alive, Self-healing, In production Henk Jonkers et al, TU Delft Concrete, Bacillus Alkalinitrillicus, Nutrients Bacterial endospores embedded in the concrete become active when the concrete cracks. The bacteria will start producing calcite, filling up the crack. Alive, large scale, self healing.

Applications Purpose

Technical

Experiential

Concrete, hard, rough, strong





Name & Year Key Words Manufacturer Composition Process

Technical Experiential Applications Purpose

Living Building Materials (LBM), 2020 Alive, Regenerative, Responsive, Research Will Srubar et al. CU Boulder Sand-hydrogel scaffold, Cyanobacteria Sand-hydrogel scaffold supports the bacterium, increasing the humidity and temp. activates the bacterium causing biomineralisation of CaCO3, providing toughness and strength to the matrix.

Alive, self healing, self replicating.

Name & Year Key Words Manufacturer Composition Process

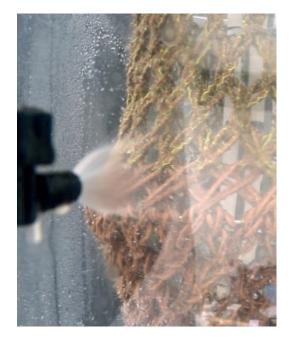
Technical

Purpose

Experiential

Applications

Calcified Collum, 2019 Grown, Structural, Prototype Bastian Beyer, Daniel Suarez Fibers (Jute/PE), S. Pasteurii, Calcite Soft fibrous materials are CnC knitted into a collum and calcified in 8 steps for 3 days by bacteria to produce a rigid structure. Static (dead), light-weight, stiff Rough, Rigid, alien, Novel building materials Self assembling and greenly produced building materials



A.1 BACTERIAL CALCITE

Name & Year	Dune, 2009
Key Words	Grown, Environmental, Conceptual
Manufacturer	Magnus Larsson
Composition	Sand, S. Pasteurii, Nutrients
Process	Bacteria and their nutrients can be
	added to sand where they will produce
	calcite, glueing sand particles together,
	effectively producing limestone.
Technical	Large scale, resource efficient,
Experiential	Architectural, large scale, humane
Applications	habitat
Purpose	Forming limestone structures in





Name & Year Key Words Manufacturer Composition Process

Technical

Experiential Applications Purpose Biobrick, 2014 Grown, Structural, In production Biomason, USA Sand, S. Pasteurii, Nutriens Bricks are manufactured by adding S.P. Bacteria to sand. Here they will produce calcite, glueing sand particles together, effectively producing limestone.

(Allegedly) Similair performance to regular bricks, Energy efficient. Brick, hard, rough, strong Building materials

A.2 BACTERIAL CELLULOSE

Name Key words Manufacturer Composition Process

Technical

Purpose

Experiential

Applications

Xilynium Cones, 2013 Grown, Formed, Prototype Jannis Huelssen Acetobacter Xylinium, Bacterial cellulose Cellulose is grown by the A.X. bacteria, upon drying, the material its shrinkage is utilised by wrapping it onto a reprodicible shape. Absorbant, Biodegradable, reproducible, Leather like, fragile, natural



A.2 BACTERIAL CELLULOSE



Name & Year Key Words Manufacturer Composition Process

Technical Experiential Applications Purpose Biocouture, 2004 Grown, Fashion, Prototype Suzanne Lee Acetobacter, Bacterial Cellulose Acetobacter grows cellulose in thick mats, these are then dried and treated (with mineral oil?) to be then processed like regular (cotton or leather) fabrics. Absorbant, light weight, biodegradable Leather like, translucent, alien Clothes and fabrics

Making the fashion industry more

Name & Year	SS-GR1 Loudspeakers, 1991
Key Words	Grown, High performance, Product
Manufacturer	Sony
Composition	Hi-fidelity loudspeakers with the
	tweeter membrane made out of
Process	Bacterial Cellulose
	Bacterial cellulose grown by (most
	likely) the Acetobacter and pressed
Technical	into the shape of the tweeter.
	Bacterial cellulose was selected for its
	accoustic abilities, low-density and
Applications	high toughness.
	High performance loudspeakers,





Name & Year Key Words Manufacturer Composition Process

Technical Experiential Applications Purpose Microbial Cellulose, 2016 Grown, Recycled, Formed, Research Urban Morphogenesis Lab, Bartlett Acetobacter, B. Cellulose, Organic waste

Experiments with different types of food waste as input for cellulose growth by bacteria. In addition, experiments with forming B.C. utilising its shrinkage. Biodegradable, waste=food, formed Translucent, leather like, large Utilizing food waste in urban

A.2 BACTERIAL CELLULOSE

Name & Year Kev Words Manufacturer Composition Process

Wound Dressing, 2020 (2004) Grown, Medical, Biocompatible, Research Sulaeva et al, BOKU university, Vienna Bacterial Cellulose and Alginate Bacterial cellulose impregnated with alginate provides a moist, biocompatible yet antibacterial environment for healing complex wounds.

Technical Experiential **Applications**

Protection, moisture retention, nonsticking.





A.3 BACTERIAL MATERIALS

Name & Year Kev Words Manufacturer Composition Process

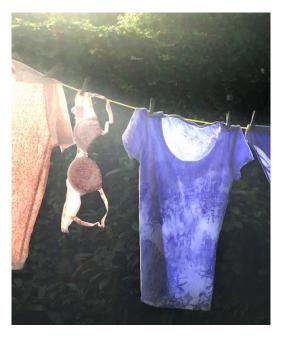
Qmonos, 2015 G.M.O., Biofabricated Proteins, In Production Spiber inc., Japan G. modified bacteria, Spider silk protein

TechnicaL

Experiential **Applications**

Bacteria can be genetically modified to produce around 600 different types of proteines. How the company 'spins' the fibers from these proteins is a mystery High performance, flexible, versatile, resource efficient. Soft, smooth,

Name & Year	Bacterial Pigments, 2012
Key Words	Large scale, Pigments, In Production
Manufacturer	Pili inc, France
Composition	Various pigments produced by bacteria
Process	Bacteria are fed sugar and produce
	pigments via numerous enzymatic
	pathways. Unclear whether these are
Technical	wildtype bacteria or not.
	Renewable, non-polluting, versatile,
Experiential	UV resistant (?)
Applications	Natural, soft-spoken, intricate,
	Fashion but dyes and pigments in



A.4 BACTERIAL PROJECTS



Name & Year Key Words Manufacturer Composition Process

Technical Experiential Applications Purpose

Living Language, 2015 Alive, Bacteria, growing forms, Conceptual Ori Elisar Paenibacillus Vortex, Agar plates Living ink is used to visualize the evolution of an alphabet. The P. Vortex bacteria spread out and act as a single hive mind in search for food (similair to the slime mold). Alive, intelligent, natural forms Intricate, alien

Name & Year Key Words Manufacturer Composition Process

Biologic, 2015 Alive (?), Bacteria, Responsive, Prototype Tangible Media Group, MIT Latex with a B. Subtilus/E.Coli biofilm Bacterial cells are printed onto a latex sheet. In response to humidity and temperature, these cell will swell or shrink (hygromorphic). Technical The change in cell size results in the sheet bending. Changes in colour are Experiential also achieved.

Applications Responsive, Nano-actuator, Macro-





Name **Key Words** Manufacturer Composition Process

Technical Experiential Applications Purpose

Living Tattoo, 2017 Alive, Bacteria, Sensorial, Conceptual Liu et al, MIT Living bacteria, GMO's (?), hydrogel Hydrogel layers containing lving bacteria are 3D printed in tiny layers. These bacteria change their colour based on the presence of certain chemicals, Alive, Sensorial, unclear how immediate Futuristic **Biological sensors**

A.4 BACTERIAL PROJECTS

Name Hybrid Living Materials (Vespers III), Kev Words 2019 Manufacturer Alive, E. Coli, 3D printed, conceptual Composition Mediated Matter group, MIT Process E. Coli, hyrdrogel, chemical signals Bacteria producing various pigments and enzymes are applied (unclear how) to an 3D printed object. Chemical signals, deposited using polyjet AM Technical will activate the bacteria at controlled

Technical Experiential Applications

locations. Alive, dynamic, reproducible

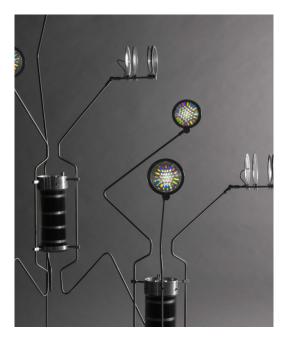




Name Key Words Manufacturer Composition Process

Technical Experiential Applications Purpose Ambio, 2014 Alive, bioluminescent bacteria, prototype Teresa van Dongen Glass tube, photobacterium, medium Bioluminescent bacteria are isolated from an octopus. These bacteria are cultivated in high concentration to provide an atmospheric bioluminescent light. Alive, atmospheric lighting, no electricity Delicate, charming, futuristic

Electric life, 2019
Alive, electro-active, bacteria,
prototype
Teresa van Dongen
Container, Micro-organisms, nutrients
Electro active micro-organisms
secrete electrons in their metabolism.
These electrons are used to power
LED's
Unclear what the capacity of this
biological battery is.
Futuristic



A.5 GROWN MATERIALS



Name & Year Key Words Manufacturer Composition Process

Technical

Experiential

Applications

Living Root Bridges of Megalaya, ±1500

Plants, Alive, Old, Infrastructure Khasi & Jainta tribes Roots of the Ficus Elastica (fig tree) Young pliable roots are guided through the air over a river. In the course of many years, more roots are added which grow to thick sizes, providing functional bridges, some of these bridges are more than 500 years. over bricks. Energy efficient.

Name Key Words Manufacturer Composition Process

Technical

Purpose

Experiential

Applications

Interwoven, 2016
Plants, Grown, Roots, Prototype
Diana Scherer
Grass roots
Grass is grown on top of a mold,
containing the soil and nutrients that
the plant needs. The roots grow into
the mold forming patterns . The grass
is then removed .
Grown, lightweight, transparant,
fragile
Natural, Delicate, Fascinating





Name & Year Key Words Manufacturer Composition Process

Algea Lab, 2017 Algea, Grown, Bioplastic, Prototype Studio Klarenbeek Dros with Atelier Luma

Algea derived biopolymer

Algea are cultivated, harvested, dried and processed into a paste which is 3D printed without the use of heat. After printing the material dries to form a hard solid.

Grown, CO2 negative, water-resistant Textured, organic yet manufactured Packaging but also for long term use

Technical Experiential Applications

Purpose

A.5 GROWN MATERIALS

Name & Year Key Words Manufacturer Composition Process

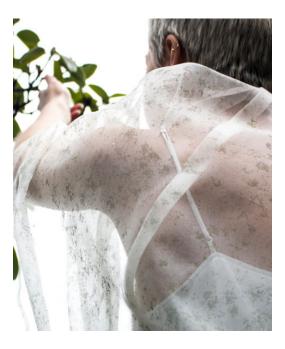
Technical

Purpose

Experiential

Applications

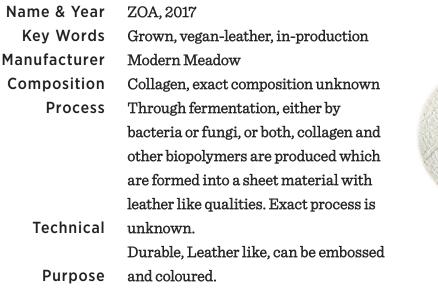
Biogarmentry, 2018
Alive, Algea, Photosynthesis,
Prototype
Roya Aghighi
Textile, Micro-Algea
Living algea are grown onto a fabric
which can be worn by a person. The
algea require moisture and sunlight to
grow and perform photosynthesis.
Alive, Photosynthesis, Color-changing
Transformative, requires care
Living textiles that clean the air





Name & Year Key Words Manufacturer Composition Process

Technical Experiential Applications Purpose H.O.R.T.U.S. XL, 2019 Alive, Algea, 3D-printed, Prototype ecoLogic Studio 3D printed structure, hydrogel, Micro-Algea A large scale 3D printed structure contains hydrogel in its openings. This hydrogel allows for algea to live, grow and perform photosynthesis. Large scale, A.M., Alive, Photosynthesis Futuristic yet natural, requires care Buildings, living facades





A.5 GROWN MATERIALS



Name & Year Key Words Manufacturer Composition Process

Living Bricks, 2019 Alive, Mycelium, Biowelding, Prototype The Living Mycelium and Sawdust Fungi (mycelium) are grown together with sawdust into buckets. The mycelium blocks are left alive and stacked upon which the still alive fungi weld the blocks together to form an arch. This project was showcased at the Centre Pompidou in 2019 but

Name & Year Key Words Manufacturer Composition Process The Growing Pavilion, 2019 Grown, Mycelium, Large scale Pascal Leboucq, Krown Design Mycelium panels placed on timber frame

The mycelium is grown into large molds, then baked and placed on the facade of the pavilion.

Noticably, this was not a perfect

ambitious scale of the project.

process with contaminations and

still alive parts visible, inherent to the

Technical Experiential Applications





Name & Year Key Words Manufacturer Composition Process

Technical Experiential Purpose Mycelium Chair, 2013 Mycelium, grown, 3D printed, Prototype Eric Klarenbeek 3D printed bioplastic inhabited by mycelium A shell of bioplastic is 3D printed and inhabited by mycelium. The fungus fills up the shell, strengthening it and also blooming, in the form of mushrooms on the outside. 3D printed, grown, strong (?) Futuristic yet natural looking

A.6 RELATED MATERIALS

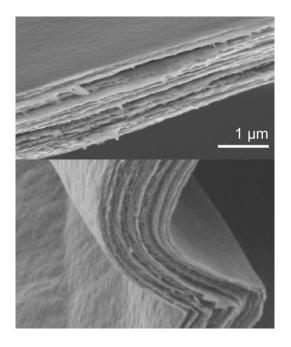
Name & Year Key Words Manufacturer Composition Process

Technical

Applications

Mineral Wool, 1840 Mineral Based, Versatile, in Production Rockwool Molten Stone Stone, variable minerals, are mined and molten at around 1400 C. The rock is then spun in a process similair to that of making cotton candy, to form a wool made up out of thin fibers. Isolation, Bio-compatible, Lightweight





Name & Year Key Words Manufacturer Composition Process

Technical Applications Ceramic Paper, 2018 Flexible and conductive ceramics, research Max Planck Institute Vanadium Pentoxide Thin layers of minerals are deposited on top of each other to produce a thin film. Hard but flexible, electrically conductive

Future batteries, sensors and artificial muscle actuators

Name & Year Key Words Manufacturer Composition Process D-Shape, 2006 Calcite, 3D printed, Large Scale Enrico Dini Magnesium- and Calcium-Carbonate, Sand A 3D printing process where a chemical binder agent is jetted by

Technical Purpose Sand A 3D printing process where a chemical binder agent is jetted by a nozzle onto a layer of sand. It is chemically similair to the calcification performed by the S. Pasteurii bacteria.

E Large Scale, construction Develop a new building method.



A.6 RELATED MATERIALS



Name & Year Key Words Manufacturer Composition Process

> Technical Experiential Purpose

Bio Iridescent Sequin, 2019 Nano-Cellulose, Iridescent, Prototype Elissa Brunato Cellulose Unclear, Likely the cellulose is refined to a nano scale, suspended in water and pipetted into a mold were it will self-assemble into layers upon evaporation. Iridescent, Biodegradable Delicate, Shimmering, Fascinating A more sustainable fashion industry

Name & Year Key Words Manufacturer Composition

Nanocellulose Fiberboard, 2015 Cellulose, Fermented (?), Recycled YungTin Ling Cellulose from plants such as flax with nanocellulose used as a binder agent. Process Apparantly the nanocelluse is produced by bacteria (Dezeen.com), yet it is unclear how this is done. The mixture of fibres and nanocellulose is Technical compressed into shape. Experiential Like MDF, probably not waterproof Applications Interesting organic looking patterns





Name & Year Key Words Manufacturer Composition Process

Technical

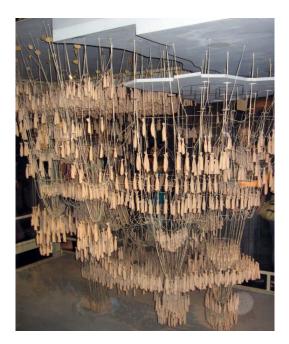
Experiential Applications Recurf, 2016 Recycled clothes, PLA, HVA, TU Delft Recycled clothes and bioplastic Discarded clothes are shredded, combined with PLA and heat-pressed into shape, forming a hardened composite material. Recycled, lightweigth, accoustically dampening Warm, fibred, narrative about its former life Products, accoustic panels, outdoor?

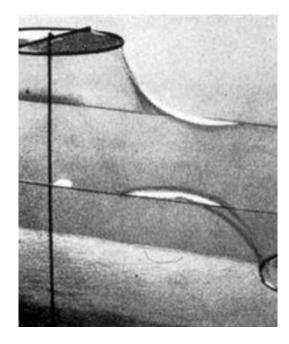
A.7 RELATED PROCESSES

Name & Year Key Words Manufacturer Composition Process Sagrada Famillia, 1882 Funiculair Structure Antoni Gaudi Ropes and Weights In order to model the Sagrada Familia, Gaudi used ropes and weights to model arches. By doing this he optimised the structure and its load bearing capacite. Using tensile forces to model a structure that could deal with compressive forces.

Relevance

Going from tension to compresion,





Name & Year Key Words Manufacturer Composition Process

Relevance

Soap film maquette, 1952 Tensile membrane structures Frei Otto Soap and metal wires By studying how a soap bubble would form under different boundary constraints, Otto was able to model tensile structure before computers were used to do so. To form a material under tension, defining boundary constraints, also to

limit shrinkage.

Name & Year Key Words Manufacturer Composition Process In Tension, 2019 3D print, Cellulose, tensile structures Megan Mcglynn PLA, Nanofibrillated cellulose A sheet is stretched over a print bed, heated PLA is printed over this sheet. When the tension is released a 3D structure is formed.

Relevance

Forming a material in unexpected ways through tension, going from a 2D to a 3D object.



RELATED PROCESSES A.7



Name & Year Key Words Manufacturer Composition Process

Terramia, 2020 Thin walled structure, Stiffening, Drones, Mud & Digital Lab Canvas and mud A canvas tent is set up and drones are used to spray a stiffening agent, made primarily from mud, onto the structure, the canvas becomes rigid to provide an lightweigth building Relevance material. The transition from a flexible to a stiff

material.

Name & Year Key Words Manufacturer Composition Process

Relevance

Gravity Stool, 2012 Magnetism, Epoxy Jolan van der Wiel Epoxy and magnetic particles Magnetic particles are mixed into epoxy, with the use of a large magnet the expoxy is then formed into a unique shape and left to harden. The contrast between the defined top half of the chair and the very chaotic parts in between.





Name & Year Key Words Manufacturer Composition Process

Relevance

Liquid Marble Hard, fluid Matthieu Lehanneur Marble A large slab of marble is CnC'd into its rough shape and then polished by hand.. poor interns A modern version of Bernini's sculpture, in which too, solid marble is made to look like something pliable.

B. LAB JOURNAL

Goals of experiments

B1. Oktober Exploring the calcification process by cultivating S.P. bacteria.
 Experimenting with varying parameters in the calcification process.
 Trying out different materials in combination with bacterial calcite

B2. NovemberExploring ways of filtrating and forming the resulting material.Experiment with varying ingredient ratios of the material.

- B3. December Characterizing the bacterial cellulose and its growth parameters.Define the ingredient ratios and produce samples accordingly.
 - B4. Januari Scale up the growth and filtration process.
 Try out with different ways of forming the material in its wet phase.
 Experiment with recycling and reshaping the material.
 - **B5. Februari** Define the cellulose growth rates and important cellulose growth parameters. Experiment with forming options including compression.
 - **B6. March** Experiment with forming the material through tension, partial tension, freely, partial compression and full compression.

B1. 03-10 Calcite formation

Questions_

How can the S.P. bacteria be grown? How much calcite will they produce? Do the ingredients influence the calcite yield? How long wil it take? Can calcite be combined with other materials?

Set-up

- Sporosarcina Pasteurii bacteria (1 freezer stock tube)

- 200 ml of SP_C medium, 300 ml of demi water

- Various concentrations (0,2%, 0,5%, 1,0%, 2,0% and 4,0%) of CaCl2 and Urea divided across 5x 100ml

- Other ingredients to be calcified like sand and chitosan

- Every 100 ml was divided into;

- 50 ml, pure, to check the calcite yield

- $2 \mathrm{x}\, 25 \mathrm{ml}$, one with sand and one with chitosan

- These were set to grow at $28\,C$ for $24\,hours$

$Results_{-}$

After 1 night of growth all samples had a distinctive ammonia smell, indicative that the bacteria wereactive

The 50 ml tubes had increasing amounts of residue on the bottom, later tested to be CaCO3 (with HCL). The calcite was brown of colour, likely to be caused by organic matter.

Only the fourth, 2,0% variant of the petri dishes filled with sand showed some 'glueing' of the sand particles though it was far away from sandstone.

Conclusion_

Still not the most impressive yield of Calcite, perhaps the small volumes in the small tubes is not a good idea, next test will be of a bigger volume. Chitosan, according to Kui, dissolves in acid, precipitates in alkaline environments, it therefore



Test setup



Bacterial calcite formed in tubes



Calcified Sand

B1. 08-10 Calcite formation II

Revisit the previous experiment; this time with larger containers

Questions_

- How do varying ingredients produce different results?
- Do the bacteria need extra nutrients?
- Can materials like glass and tissues be calcified?

Set-up

- Three different beakers (500 ml) inoculated with SP

- Beaker 1: 200ml SPC medium, 300ml Demi water, 10 g urea, 10 g CaCl2, SP freezer stock

- Beaker2: 200ml SPC medium, 300ml Demi water,
- 10 g urea, 20 g CaCl2, SP freezer stock
- Beaker3: 300ml SPC medium, 200ml Demi water,
- 10 g urea, 10 g CaCl2, SP freezer stock
- $3x\,25ml\,was$ pipeted from each beaker into a petri
- $dish\ containing\ sand,\ tissue\ paper\ and\ glass\ beads$

- These were set to grow at 28 C for 24 hours

$Results_{-}$

After two nights of growth, beaker 1, 2 and 3 produced 5.4, 10,8 and 5.7 grams of calcite respectively.

The tissues, sand and glass pearls were left to calcify for two nights.

The sand became a lot more solidified than in the previous experiments.

The tissues became quite stiff

The glass beads have some calcite-ish scale on them but not

Conclusion_

The starting amount of CaCl2 directly influences the calcite yield and can probably be even higher. The increase in nutrients (beaker 3) did not provide a significantly higher calcite yield.

The bacteria produce more calcite if left for longer



Beakers with calcite formed



Calcified tissue



Calcified glass beads



Calcified Sand

B1. 09-10 Calcite Growth Time

Questions_

How does the growth time relate to the amount of CaCl2 that is converted into CaCO3 (calcite)?

Set-up

Inoculate 4 beakers with the same ingredients; 200 ml SPC medium, 300 ml demi water; 10 g urea, 20 g CaCl2, SP freezer stock
Let these beakers grow at 28 C for 18, 25, 42 and 63 hours
Filtrate the water out, let the residue dry and

- Filtrate the water out, let the residue dry and weigh the remaining calcite

Results_

After 18 hours, beaker I rendered 0.2 grams of calcite. this means that 15% of CaCl2 got converted to CaCO3

25 hours, beaker II rendered 1,1 gram, 41% conversion 48 hours, beaker III, 2,2 gram, 81% 62 hours, beaker IV, 3,0 gram, 93 %

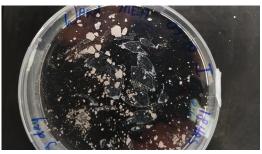
The residue was tested with hydrocholic acid (HCL), this resulted in bubbles forming (CO2) indicating that it was indeed calcite.

Comparing beakers 1, 2 and 3, the calcite seems to become darker, more brown, with longer periods of growth. Beaker 4 was harvested in a different manner -with more rinsing- and is therefore difficult to compare.

Conclusion_

The bacteria apparently need some time to start up, in the first 18 hours not much is happening yet.

A conversion of a 100% seems practically impossible.



Calcite remaining from beaker 1



Calcite remaining from beaker 2



Calcite remaining from beaker 3



 $Calcite\ remaining\ from\ beaker\ 4$

B1. 10-10 Calcifying Tissues

Questions_

Can stacks of paper tissues be calcified? What if we repeat this calcification process by adding new medium and bacteria every 24 hours? Can the resulting composite be rewetted to be reshaped?

Set-up

- Stacks of 12 paper tissues were placed in petri dishes.

- To each petridish, 75 ml of calcification medium (30ml SP_C, 45ml demi water, 2% Urea, 4% CaCl) and bacteria were added.

- These were left to grow for 24 hours, after this, the calcification procedure was repeated for two times.

- One of the samples was compressed, to squeeze water out.

- The resulting composite was examined for stiffness, after this they were rewetted to see if they could be reshaped.

Results_

The results became reasonably stiff but far from petrified.

The formed calcite does not seem to fully penetrate the stack of tissues, leaving voids in between.

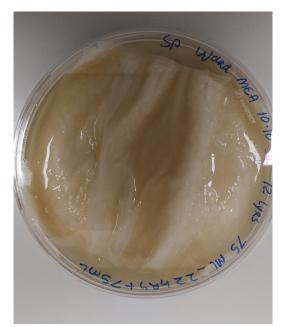
The samples smell quite badly due to the prolonged growth time.

Rewetting the samples resulted in them becoming very pliable, they became stiff again after drying.

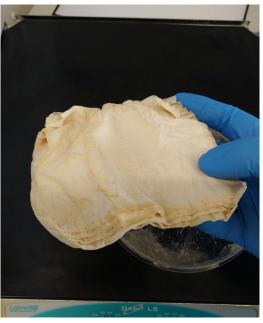
Conclusion_

Either more calcification steps or higher concentrations of ions and bacteria are needed to really petrify these structures.

The cellulose/calcite ratio is still quite high, promoting flexibility but decreasing stiffness Squeezing out the water can be effective as opposed to filtration



Growing the bacteria on the tissues



Calcified tissues



Reshaped tissues

B1. 28-10 Calcite Cellulose and P.G.A.

Having the bacterial Cellulose and Polyglutamic acid ready; the first experiment creating a bacterial composite

Questions_

How does the amount of calcite influence the resulting composite? What other factors are relevant in creating this material?

Set-up

- Three beakers were inoculated with; Beaker I and II; 500 ml, 30 gram cellulose 10 grams of CaCl2 Beaker III; 500 ml, 30 gram cellulose, 20 grams of CaCl2

- Beaker I and III were left to grow for 24 hours, II for 36

- The resulting suspension was filtered and dried in the 37 degree incubator

Results_

- Sample I, resulting from beaker I, was a bit flexible but still seemed to have a high calcite content, it was also fairly rough

- Sample II, was very brittle and broke rather quickly

- Sample III, gained a very interesting texture, warped a lot during drying, became a lot darker in colour.

Conclusion_

Calcite/cellulose ratio makes a big difference in resulting stiffness and appearance of the samples Filtration takes a long time.

Interesting textures appeared, also resulting from the filtration process, the upper part being rough and the bottom part smooth with hints of the filter texture.

The wet-> dry transition, also evident with the



Sample 1



Sample 2



B1. 29-10 Active Culture Calcification

Questions_

What if active (unfrozen) bacteria are used for the calcification process?

Set-up

- SP bacteria are taken out of the freezer 24 hours before the start of the calcification process.

- They are grown in SP growth medium at 28 C.

- Then added to the calcification medium, similar to the experiment of 09-10

- Like the experiment of 09-10, three identical beakers were inoculated and left to grow for varying times

Results_

After one night, the incubated growth medium became a lot less transparant, indicating the growth of bacteria.

After only two hours, the beakers showed signs of calcification taking place (ammonia smell and suspended material showing).

Beaker I, 2 hours, taken out and filtered, rendering 1.5 gram of calcite, a 54% conversion of CaCl2 to CaCO3

Beaker II, 6 hours, 2,1 gram, 78% Beaker III, 24 hours, 2,2 gram, 81%

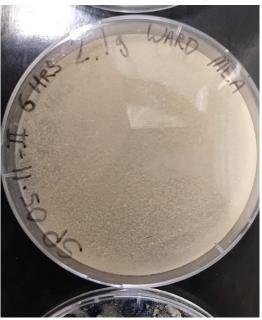
Conclusion_

Active or unfrozen bacteria can calcify incredibly fast. 54% conversion at just two hours. In comparrison frozen bacteria converted 15% in 22 hours!

Again, a colour change in the resulting material can be observed.



Results of beaker 1



Results of beaker 2



Results of beaker 3

B2. 04-11 Bypassing Filtration

Questions_

Would alternatives processes to filtration of the material result in the same material? If not, how will the results differ?

Set-up

Grow the SP bacteria in one beaker containing;
500 ml, 120 g cellulose, 0.5 g PGA, 10 g CaCl2
After growth, divide the resulting suspension in
3 parts

- I, let all the water evaporate

- II, filter using sieve, let the remaining water evaporate

- III, filter using sieve, compress the remaining water out

Results_

I, a very loose, spongy and brittle material, crumbles upon touching

II, after filtering using sieve, a wet sludge remained, this was draped over a 3D shape and left to dry. The dried sample retained the shape but it did shrunk and became very thin. It did result in a difference in texture on the top and bot side.

III, after compressing the water out, it became significantly more hard and stiff that sample II

Conclusion_

Filtration process can be bypassed with variable results.

Letting all the water evaporate out results in an inferiour material.

The wet sludge, remaining after using the sieve, can be molded but will shrink a lot.

Compression promotes hardness and stiffness and is usefull for getting rid of a large part of the water content in the material.



Sample 1



The wet sludge



Sample II



Sample II



Sample III 117

B2. 06-11 Growing Large(r) Quanties

Questions_

How can the S.P. bacteria be grown in a larger batch? Will the calcification process still be as effective?

Set-up

- Fill a large beaker with; 1500 ml, 300 g cellulose, 50 g CaCl2, SP freezer stock

- Allow it to grow and calcify for 72 hours

- Divide it over 3 smaller beakers and process these in different ways.

Results_

After growth, the material had a very white and clean appearance, this could be because of a high calcite content with relatively little biological components.

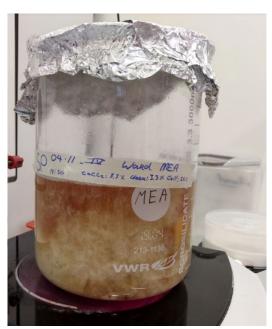
Two parts were filtrated and folded into a new shape when wet. These held their new shape very well.

One part was decanted (getting rid of surplus water) and left to dry in a 3D mold. The resulting dried material did not hold the shape of the mold very well due to shrinkage.

Conclusion_

It is counterintuitive that these samples turned out so white after such a long growth time. One explanation for this could be the high amount of inorganic compounds (calcite) in relation to the amount of bacteria and the medium they had at their disposal. Thus, keeping the inorganic concentrations high and the concentration of organisms low will result in a white appearance.

The molding of the wet sludge was inneffective, folding the wet solid did prove to be effective.



The large beaker



 $One \ of \ the \ samples \ that \ was \ folded$



One of the samples that was molded

B2. 07-11: Varying Ratios

Questions_

How can different material samples with different ingredient ratios be processed?

Set-up

Inoculate three beakers with SP bacteria;
Beaker I: 500ml, 60 g cellulose (coarsely blended), 10 g CaCl2
Beaker II: 500 ml, 30 g cellulose (finely blended), 10 g CaCl2
Beaker III: 500 ml, 30 g cellulose, 20 g CaCl2
Let these grow for 24 hours, filtrate the water out

- Test various post-processes in the machine workshop

Results_

The resulting samples again had a fairly white colour.

All samples had a very high calcite content, being very stiff, brittle and rough.

Sample I with the coarsely blended cellulose had an interesting resulting texture but also appeared very brittle and, due to its inhomogenity, tore apart fairly easily.

Sample III, with the highest calcite content, has a topside that appears like an extraterrestrial landscape, very pleasing to the touch.

Conclusion_

The high calcite contents of these samples result in in thick samples with a high surface roughness. They are also of a high stiffness and presumably very brittle. It is expected that the optimum ratio of ingredients lies in a much lower calcite content.



Sample I with coarsely blended cellulose



Sample III with a very high calcite amount



Detail of sample III texture

B2. 08-11 Workshop Experiments

Questions_

What other post processes can be relevant in forming this material? What processes can be relevant in treating this material?

Set-up

Take the previously made material samples to the machine workshop at the IDE faculty, TU Delft.
Experiment with various ways of; compressing, cutting, re-wetting and treating the material.

$Results_{-}$

The samples that were compressed seemed to hold up, the material appears able to handle compressive forces.

One sample was tested for fire-proofness, it held up, did not catch fire and isolated very well.

Re-wetting and compressing the material seems to work very well in forming ing, the resulting sample holding it's shape very well and drying fast.

The material is very absorbant and therefore becam quite dirty. This can also be used to treat the material in various ways.



Fire test







Sample pressed in a vice



Roller pressed sample

B3. 09-12 Bacterial Cellulose Characterization

Questions_

What is the density of the wet bacterial cellulose? What is the density of the dry bacterial cellulose? What is the water content of wet bacterial cellulose?

Set-up

- Grow the bacterial cellulose in the course of 4 weeks.

- Cut of 5 pieces, measure their volume in water and weigh them separately.

- Let them dry at 37 C in the incubator for 24 hours

- Weigh and measure their volume again.

Results_

The 6 pieces measured as follows:

Wet

Sample #	Weight (g)	Volume (cm^3)	Density (g/cm^3)
1	8,5	10	0,85
2	6,0	6,0	1,00
3	4,0	4,5	0,89
4	6,7	7,0	0,96
5	5,6	6,0	0,93

Dry

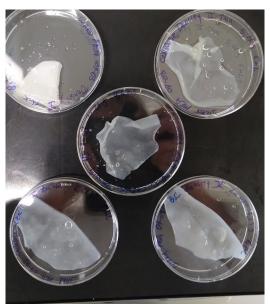
Sample #	Weight (g)	Volume (cm^3)	Density (g/cm^3)
1	0,10	0,19	0,53
2	0,07	0,14	0,50
3	0,05	0,09	0,56
4	0,08	0,14	0,57
5	0,07	0,12	0,58

Conclusion_

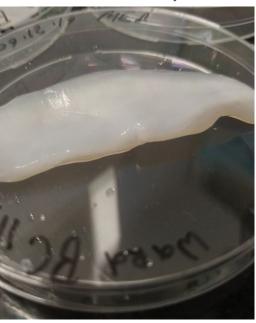
Bacterial cellulose has a very high water content.

After drying it contains about:

- 1,2% of its wet weight
- 2,0% of its wet volume
- 55% of its wet density



Pieces of wet cellulose



Detail of wet cellulose



Dried cellulose

B3. 10-12 Defining Ingredient Ratios

A series of samples was created with the ratio of ingredients (Calcite, Cellulose and PGA) controlled as much as possible.

Questions_

Can a series of samples with controlled ratios of ingredients be made?

To what extent can these ratios be controlled? How will properties like stiffness, microstructure and surface roughness vary among these samples?

Set-up

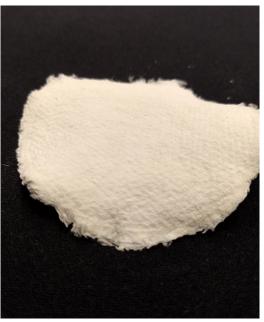
- 10 samples were created with the following ingredients;

#	Ce/Ca/P.G.A.	Cellulose	CaCl2	P.G.A.
Ι	1/0/0	60 g	0 g	0 g
II	1/1/0	60 g	1,25 g	0 g
III	1/2/0	60 g	2,50 g	0 g
IV	1/4/0	60 g	5,0 g	0 g
V	1/8/0	60 g	10,0 g	0 g
VI	2/2/1	60 g	1,25 g	0,5 g
VII	2/4/1	60 g	2,50 g	0,5 g
VIII	2/8/1	60 g	5,0 g	0,5 g
IX	1/1/1	60 g	1,25 g	1,0 g
Х	1/2/1	60 g	2,50 g	1,0 g

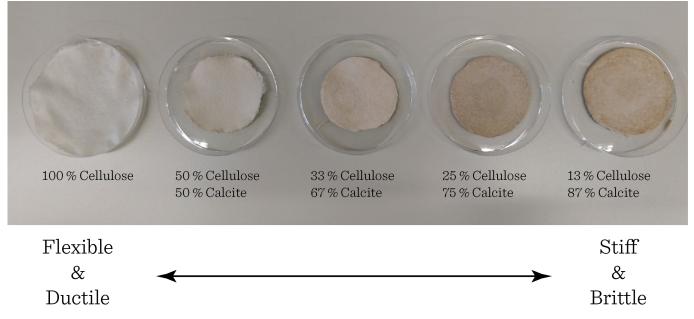
Here, the added cellulose has a wet weight of 60 grams, based on the results of the 09-12 experiment, this will render about 0,8 gram of dry cellulose.



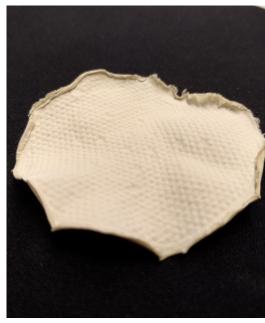
100% Cellulose



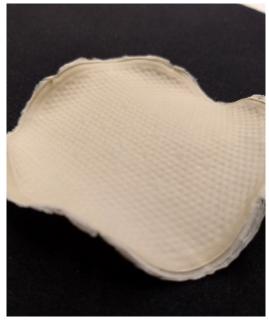
33% Cellulose 67% Calcite



Samples I to V with varying Cellulose/Calcite ratios



50% Cellulose 50% Calcite



33% Cellulose 33% Calcite 33% P.G.A.

Results_

The samples noticeably change with the varying ratios;

Becoming more stiff with higher clacite contents.
Showing the most shrinkage at 1/2/0, with higher calcite .the amount of shrinkage seems to decrease.
Becoming darker in colour with higher calcite content.

- Becoming rougher in surface with higher calcite content.

- Becoming more flexible with higher P.G.A. content

- Becoming more plasticky to the touch with higher P.G.A.

Conclusion_

Experimenting with controlling these ratios gives essential insights into the properties of the resulting material and something that should have been done earlier in the process.

Still, the ratios of the ingredients are not fully controlled due to parameters of the process like growth which can not be fully controlled.

The optimum Cellulose Calcite ratio in terms of mechanical performance seems to be somewhere

22 % Cellulose 67 % Calcite 11 % P.G.A. 29 % Cellulose 57 % Calcite 14 % P.G.A. 20 % Cellulos 60 % Cellulos 60 % Calcite 20 % P.G.A.

Samples VI to X with varying Cellulose/Calcite/P.G.A. ratios

B4. 06-01 Scaling Up the Filtration

Questions_

- Can an alternative, bigger, ceramic filter be used?

Set-up

Start with 300 grams of wet cellulose and 10 grams of CaCl2, add SP bacteria, grow overnight.
Filtrate the resulting suspension using a ceramic filter that measures 220mm in diameter, placed on a glass flask connected to a pump.

- Filter paper, (Macherey-Nagel[™] MN 710) is cut to size and placed inside the ceramic filter.

$Results_{-}$

The filter paper failed during the first attempt, resulting in the material being sucked through and ending up in the glass flask, the second time, a double layer of filter paper was used.

The filtration went very fast, in a matter of minutes, as opposed to the hours that the bottletop filters take.

The resulting wet sheet was easy to sever from the filter paper but quite thin and fragile. Also, the distribution of the material was not homogeneous, resulting in very thin, weak spots. This heterogenity in material distribution can be countered by properly stirring the suspension, pouring it in the filter and lastly, turning on the pump.

The resulting wet sheet has a clearly defined pattern on the bottom, caused by the filter porer.

Conclusion_

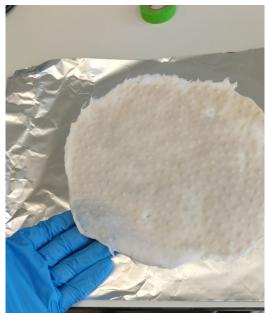
Effective in producing samples of a larger size, which are very usefull for forming experiments.



Test setup



Material, inhomogeneously distributed, inside the filter



Resulting wet sheet with the underside up

B4. 07-01 Draping the Material

Questions_

Can the material be formed by draping it over a 3D shape when it is wet?

Set-up

- Use the sheet produced on 06-01
- Drape it over a 3D shape
- Dry it overnight

Results_

When the material was still wet, it seemed to take on a promising shape.

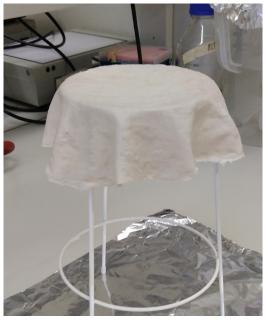
When it dried, it shrunk a lot, losing a lot of definition.

The material did appear to 'shrink around' one edge of the shape, which did become very clearly defined.

Conclusion_

The shrinkage plays a big role in the forming of the material and needs to be taken into accound.

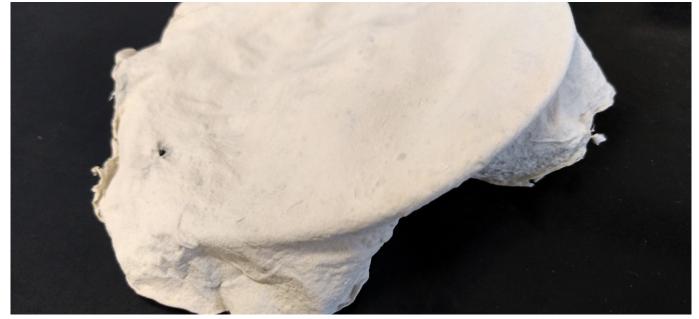
This same shrinkage can also be used to define shapes in the material.



Wet material



Dried material



 $Clearly \ defined \ edge \ in \ the \ shrunk \ material$

B4. 08-01 Draping the material II

Questions_

Can the shrinkage around an edge, evident from the previous experiment, be controlled?

Set-up

Start with 300 grams of wet cellulose and 10 grams of CaCl2, add SP bacteria, grow overnight.
Filtrate the resulting suspension using the 220mm ceramic filter.

- Place it over a 3D mold

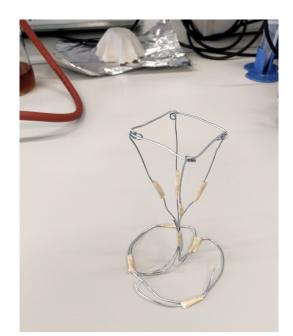
$Results_{-}$

The sample again shrunk a lot during drying. In doing so, it did follow the contour of the mold slightly but not as clearly defined as was aimed beforehand.

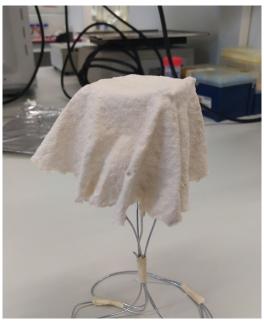
The edges did take on a lot of folds.

Conclusion_

The material seems to have 'creeped' over the edge whilst shrinking. In a follow up experiment one should try to keep it in place at certain points.



The mold



The dried shape



The dried shape

B4. 08-01 Draping the material III

Questions_

Can the material be kept in place, to have it shrink around defined edges?

Set-up

Start with 300 grams of wet cellulose and 10 grams of CaCl2, add SP bacteria, grow overnight.
Filtrate the resulting suspension using the 220mm ceramic filter.

- Place it over a 3D mold and keep it in place using custom made clamps.

$Results_{-}$

The thin metal wire was not strong enough to keep the material from shrinking, the whole mold was bent.

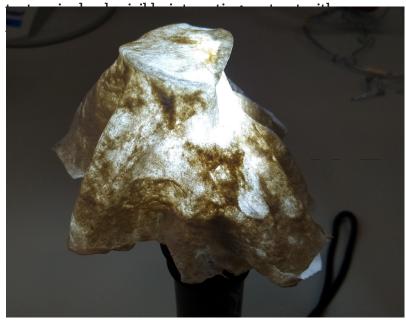
The top side did take on an interesting tensile shape.

Conclusion_

Metal wire is not an adequate material to make drying molds out of.

The flowing folds of the material that still have a very stone-like texture provide for an interesting aesthetic.

When illuminated from behind, the inner cellulose



Illuminated from within



The wet material



Dried material



Dried material

B4. 09-01 Recycling the Material

Questions_

- Can the material, when dried, be resuspended in water and filtrated again to produce a new solid?

Set-up

- Use the samples made on 07-01

- Place them in distilled water and wait for them to lose their structure ergo, become resuspend fibres in liquid.

- If this does not work, use the blender
- Re-filtrate the material.
- Drape it over a new shape

Results_

After being placed in water, the material, allthough becoming very soft, did retain its structure, even after 24 hours of submerging.

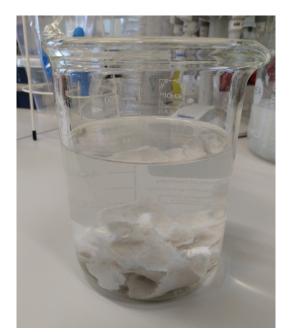
After these 24 hours, the blender was used to resuspend the fibers in water. One test piece was not blended but kept in water for 72 hours, after which it still held its shape.

The new suspension was refiltered and shaped, again it shrunk a lot when drying.

Conclusion_

The material appears to retain its mechanical properties after being recycled, this has to be properly tested though.

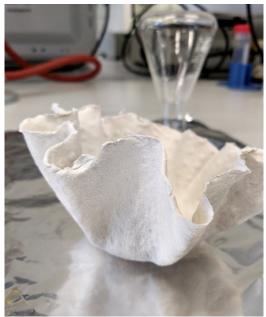
The shrinkage again occurs, probably because the cellulose fibers have become fully swollen -saturated with water- due to the resuspension. The material seems to have become more white. This makes sense because your effectively rinsing it with water during recycling.



After 24 hours of submergence



The recycled material, draped over a rounded shape



The dried shape

B4. 13-01 Tensile Forming the Material

Questions_

- Can the materials shrinkage be prevented by clamping the material to a mold?

Set-up

Start with 300 grams of wet cellulose and 10 grams of CaCl2, add SP bacteria, grow overnight.
Filtrate the suspension using the 220mm ceramic filter.

- Place the resulting, wet, material over an alluminium mold.

- Clamp the material down using wooden pegs

Results_

After one night of drying, the material held the shaped that it was clamped in. In doing so, it tensed up, the internal stress, resulting from the material wanting to shrink whilst being held in place resulted in a tight surface that you could use as a drumhead.

The texture left by the filter pores on the underside of the sheet dissapeared due to the shrinkage.

The sides of the material had to be cut off to be able to remove it from the alluminium clamping mold. After this removal, the material, not being supported by the alluminium, felt very thin and flimsy.

Because of this thinnes, it does provide very beatifull translucent capabilities, where the organic cellulose texture can be observed.

Conclusion_

An effective way of making clearly defined, reproducible shapes out of the material. If this tension, introduced in the material, does the mechanical properties any good remains to be seen.



Clamping setup



Dried result



Dried result



The inner structure illuminated

B4. 14-01 3D Filtration of the Material

Questions_

- Can the material filtered over a 3D shape, directly forming it in its wet-dry transition?

Set-up

- Produce a suspension containing 500 grams of wet cellulose and 20 grams of CaCl2 as starting ingredients

- Filtrate this suspension using a 130mm ceramic filter.

- Place a small, dome shaped sieve in the middle of this filter, surround it with alluminium foil and cover the whole with filtration paper.

$Results_{-}$

The filtration took very long due to a lot of blockage by the paper, aluminium foil and material heaping up at choke points.

After 8 hours, the procedure was aborted because it did not seem to be able to provide a solid material as a result.

Also, the material accumulated, as a wet sludge, at the sides of the filter but not where it was intended to accumulate.

Conclusion_

When tried without the alluminium foil, the material would not accumulate around the intended 3D shape but would rather slide past and underneath it.

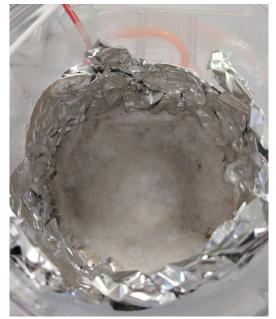
3D filtration still seems like a viable option but a different setup is needed.



Test setup



2nd test setup



Resulting -still very wet- sludge

B4. 14-01 Re-wetting the Material

Questions_

- Can an already dried piece of the material be made wet and reshaped?

- How fast will the material lose its rigidity when becoming wet?

- How will it hold its shape when drying for the second time?

Set-up

- Use one of the samples made on 07-01
- Wetten the sample using distilled water
- Flatten the sample when wet
- Clamp the wet and flat material into a new shape.

$Results_{-}$

When adding water, the material initially seemed to hold its shape fairly well for about 30 seconds. When applying a small force though, it quickly yielded, being far less stiff that when it was dry. Note that it did require some force to flatten the material, it did not become completely flat either. Here parts that experienced a lot of deformation in the previous forming proces, would not return to normal.

After clamping and drying, it did hold its new shape fairly well.

One interesting aspect is that this time, it hardly shrunk when drying.

Conclusion_

If the material is deformed too much during the first time drying, it will not return to normal. In a similair fashion, it will not shrink after it has already shrunk the first time drying. This can be usefull.



The intitial shape



Pushing the wet material downwards



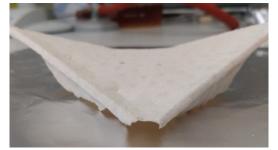
Flattened sheet



Marks of the initial deformation



Re-clamping



B4. 15-01 Bacterial Cellulose Growth

Questions_

- How fast does the cellulose mat form?

- What factors influence the formation of the cellulose mat?

- What method yields the highest amount of cellulose per day/week?

Set-up

- Various ways of growing Bacterial Cellulose have been deployed throughout the last 4 months.

- Parameters that were varied;

- Container size
- Container material
- Oxygen supply
- Organisms used

Carbon source (type of sugar)
For each 'batch' of cellulose grown, the parameters were documented and the growth monitored.

The thickness of the resulting cellulose mat was measured in relation to the time it took to form.
The surface area of the cellulose mat was measured.

$Results_{-}$

Glass flask, 3L, G.H. bacteria Yielded a cellulose mat of 177 cm2 that grew 0.5 mm in thickness per day

PVC tray, 55L, G.H. bacteria Yielded a cellulose mat of 4368 cm2 that grew 0.05 mm in thickness per day.

Stainless steel tray, 10L, G.H. bacteria Yielded a cellulose mat of 1581 cm2 that grew 0.75 mm in thickness per day.

PC flask, 5L, G.H. bacteria Yielded a cellulose mat of 314 cm2 that grew XXX mm in thickness per day.



G.H. growth in a glass flask

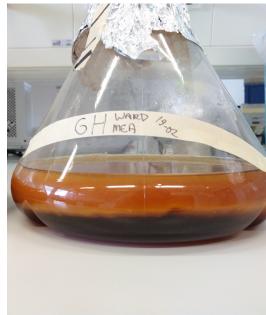


G.H. growth in a PVC tray





Kombucha culture showing heterogentity



Bacterial calcite formed in tubes



G.H. bacteria in P.C. flasks with extra sugar

Glass flask, 3L, Kombucha culture Yielded a cellulose mat of 177 cm2 that grew XXX mm per day

P.C. flask, 5L, Kombucha culture Yielded a cellulose mat of 314 cm2 that grew 0.82 mm per day

P.C. flask, 5L, Kombucha culture, Extra sugar Yielded a cellulose mat of 314 cm2 that grew 0.82 mm per day

Conclusion_

The container size directly relates to the potential surface area of the cellulose mat, thus, a shallow yet broad container should be ideal for optimizing the cellulose yield.

The container material is important, the PVC container for example, provides a large surface area but inhibits the growth rate. This is probably due to the PVC containing compounds that are toxic to bacteria. Glass, P.C. and stainless steel do provide for favourable growth conditions.

The kombucha culture, comprised of a multitude of organisms living in symbiosis (REF) seemed promising at first but became less potent during the second and third batch that was grown with it. Probably due to the culture becoming weaker. The cellulose yielded by the kombucha culture also appears far less homogeneous than that grown by the G.H., pure strain bacteria.

Providing additional sugar to the medium also seemed to speed up the cellulose growth.

It is noted that the parameters above were found to be influential but further testing is needed to prove this.

B4. 28-01 Forming Material under Partial Tension

Questions_

Can the material be formed with part under tension and other part left to shrink and warp freely?

Set-up

- Produce a sheet of material containing 500 grams of wet cellulose and 20 grams of CaCl2 using the big filter

- Place this over a custom rig, clamp the material to the 4 vertical pillars.

$Results_{-}$

After drying it became evident that the clamps were not strong enough to keep the material its designated parts in place. Part of the rig had also come loose.

Still, the 4 'legs' were clearly defined in the material, these parts also feel very stiff along their length.

The overal compressive strength of this sample appears to be very good, especially along the length of the parts that were in tension.

Conclusion_

This could be a workable method, given that the rig is strong enough to keep the material from shrinking in the designated places.





Wet material



Dried material



B5. 18-02 Forming Material over a sheet

In line with the idea to make a table out of the material, a test was performed, folding the material over a sheet of PMMA

Questions_

Will the resulting connection between the material and the PMMA be solid?How will the shrinkage of the material influence the outcome?

Set-up

- Produce a sheet of material containing 500 grams of wet cellulose and 20 grams of CaCl2 using the big filter

- Clamp this to a sheet
- Calmp the underside (facing away from the sheet)

$Results_$

The shrinkage of the material resulted in an uneven underside, making the table instable. The connection between the material and sheet was very solid.

Weird blisters appeared on the surface of the material, first time I have seen those.

Conclusion_

The underside of the table needs to be rigged, kept



Test setup



Bacterial calcite formed in tubes



B5. 22-02 Compression Tests

Questions_

By how much force can the material be compressed in its dried state? By how much force can the material be compressed in its wet state? Can the material be pressed into a 3D, double curved shape? How will the material dry and warp after being compressed?

Set-up

-Grow two large beakers overnight with the following ingredients;

 $3500\,ml, 500\,g\,cellulose, 20\,g\,CaCl2$

- Filtrate these using the 130 mm filter.

Let one of the samples dry and compress it with 37 tonnes (around 25 MPa) on the hydraulic press.
Compress the other sample while it is still wet into a 3D shape with 3 tonnes on the hand press.

Results_

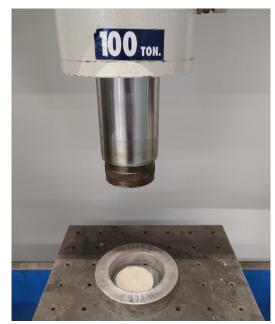
The dried sample showed signs of local failure but in general withstood the pressure quite well. It was, however, not completely dry yet and warped after the compression in the final stage of drying.

The sample increased in diamter but decreased in height.

The mold in which the wet sample was compressed failed at around 1,5 tonnes. The material itself also showed signs of failure. The second sample did seem to become a lot more desne due to the compression in its wet phase.

Conclusion_

The moisture conten makes all the difference for the end result. Presumably, the material can handle even higher pressure when it is completely dried.



Before and after



33% Cellulose 67% Calcite, 24 000 kg dry



33% Cellulose 67% Calcite, 1-2 000 kg wet

B6. 10-03 Tensile Forming

Questions_

Can a sample, formed by tensile stress (like the samples made on 13-01) be made as thin as possible?

Set-up

- Grow a beaker overnight containing: 1000 ml, 150 g cellulose, 10 g CaCl2
 Filtrate the resulting suspension using the large (270mm) ceramic filter.
- Place the resulting sheet on a double curved clamping mold with woodn pegs to preven it from shrinking

$Results_{-}$

After filtration, the resulting sheet proved to be very thin and fragile. I did manage to place it on the mold but after drying it proved to be too thin. A large tear propagated through the material.

Interestingly, the material also became more concave in respect to the aluminium mold. Here the material, being very thin, presumably shrank even more.

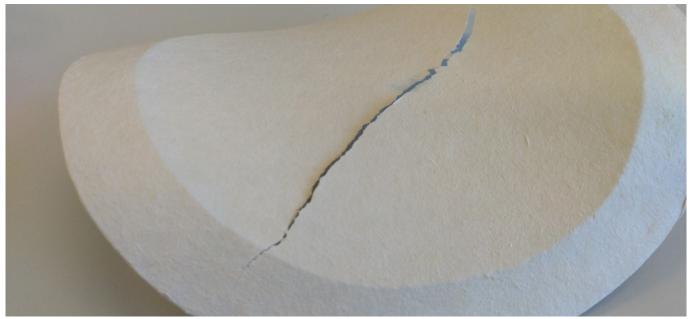
Due to the high calcite content, it did have a very peculiar feel to it.



Test setup



Bacterial calcite formed in tubes



C.TECHNICAL CHARACTERIZATION

This Appendix contains the data obtained from two three point bending tests.

C.1 VARYING INGREDIENT RATIOS, 3P TEST



Samples The samples, shown above, were made in similair manner with the ratio between the ingredients being varied. Each Sample was cut into 3 pieces which were consequently measured and tested for their flexural performance.

Their density was measured using a Hildebrand H300S Electronic Densimeter.

Sample name	Calcite content	Cellulose content	P.G.A. content	Density (g/cm ³)
1/2/0	33%	66%	0%	1,374
1/1/0	50%	50%	0%	1,302
1/2/0	33%	66%	0%	1,180
1/1/1	33%	33%	33%	1,352
1/1/2	25%	25%	50%	1,387

These samples were then tested for their flexural performance using a Roell Zwick Z010 Test press with the resulting graphs shown on the right.



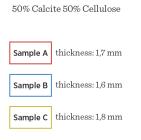
33% Calcite 67% Cellulose

50 MPa

40 MPa







2/1/0

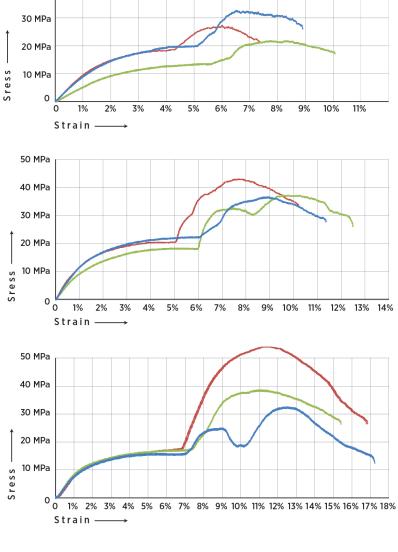
1/1/1

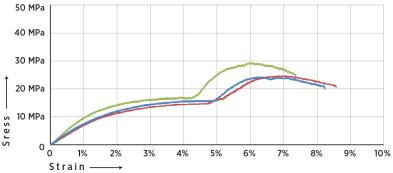
 Sample A
 thickness: 2,4 mm

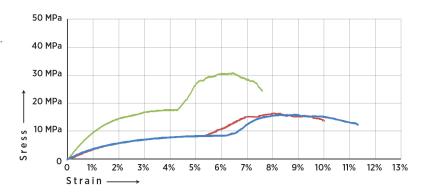
 Sample B
 thickness: 2,4 mm

 Sample C
 thickness: 2,2 mm

67% Calcite 33% Cellulose

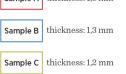




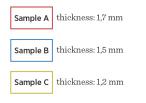


33% Calcite 33% Cellulose 33% P.G.A.

 Sample A
 thickness: 1,3 mm



1/1/1 25% Calcite 25% Cellulose 25% P.G.A.



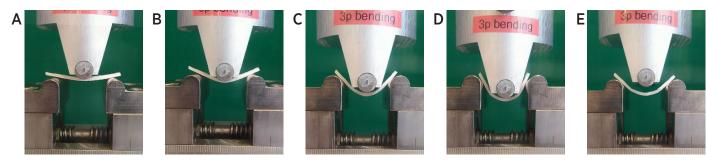
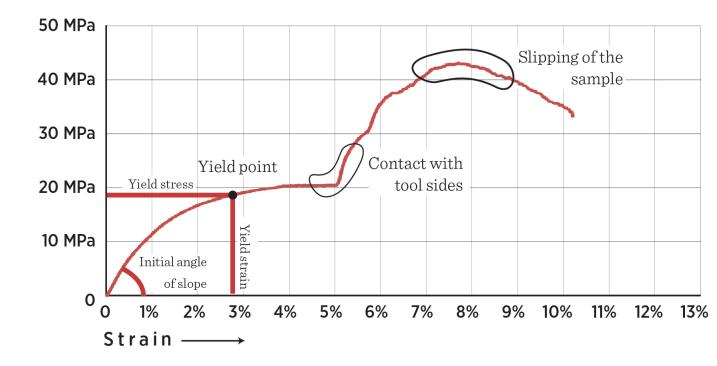


figure #; Images of the test setup with A: Initial deflection, B: Sides of tool in contact with sample, C & D: Sides of sample sliding downward and E: tool retraction revealing the residual deformation.



Interpreting the Results

Shown on the left are stress/strain curves of all the tested samples. Allthough there is a lot of variance between these curves, an exemplar curve, shown above, will be used to discuss some points of interest, identifyable in all results.

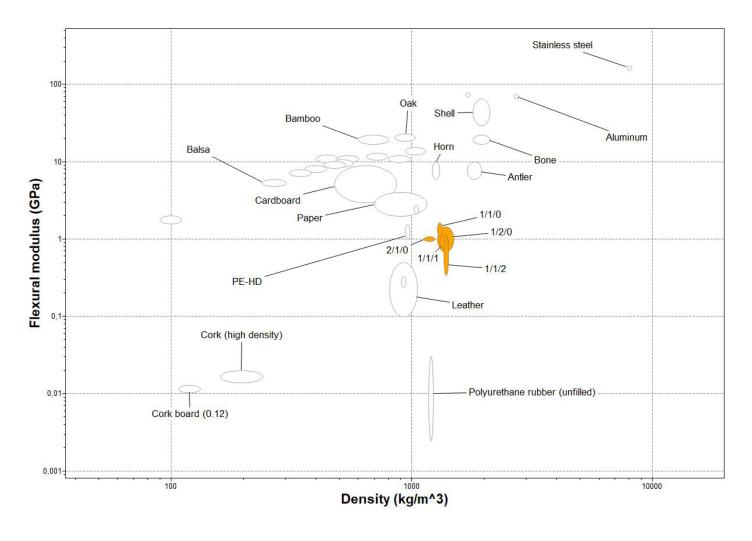
Due to the sample coming in contact with the side of the tool as mentioned on the previous page, all curves show a nod about halfway. Here the measured force, required to deform the sample, did increase but this should not be interpreted as and increase in maximum stress. Also, due to the slipping of the sample, all of the curves decline near the end. The point of failure is however, not evident from these results.

We can therefore only interpret the first part of the curve. Given the measured residual deformation, the total elastic deformation can be calculated and used to identify a yield point, where the material goes from elastic to plastic deformation. Given the initial curve of the slope, the flexural elastic modulus of the material can be calculated.

The Results Based on these calculations, the density, yield point and flexural modulus of the three samples are defined as follows.

Ratio (Ca/Ce/P.G.A.)	Density (g/cm ³)	Strain at yield (mm/100mm or %)	Stress at yield (MPa or N/mm²)	E _{flex} (Mpa or N/mm ²)
1/2/0	1,37	3,9	17	1091
1/1/0	1,30	2,6	17	1438
2/1/0	1,18	1,8	10	1007
1/1/1	1,35	2,8	14	927
1/1/2	1,39	3,8	11	643

Using the Cambridge engineer Selector, we can then compare the performance of this material to other materials.



C.2 VARYING PROCESSES, 3P TEST



Three samples were prepared in triplicate with the same ingredient ratios, growth and filtration procedure.

Sample A Sample A was prepared and dried in a regular fashion.

Dimensions before drying (diameter x thickness)	Dimensions after drying (diameter x thickness)	Density
70 mm x 8mm	51 mm x 4mm	1,34 g/cm ³

Sample B Sample B was filtrated and compressed with a 100 kg when it was still wet.

Dimensions before	Compressive force	Compressive stress	Dimensions after	Dimensions after drying	Density
compressing			Compression		
70 mm x 8mm	100 kg	0,26 MPa	94mm x 4mm	60mm x 3mm	1,23 g/cm ³

Sample C Sample C was dried and compressed with 16 000 kg when completely dried.

Dimensions	Dimensions after drying	Compressive force	Compressive stress	Dimensions after	Density
drying		10100	511055	compression	
70 mm x 8mm	49mm x 4mm	16 000 kg	83MPa	51mm x 3mm	1,54 g/cm ³

The samples were then sanded to ensure flatness and cut into three pieces to be subjected to a 3P bending test using the Zwick Roell Z010 test press.

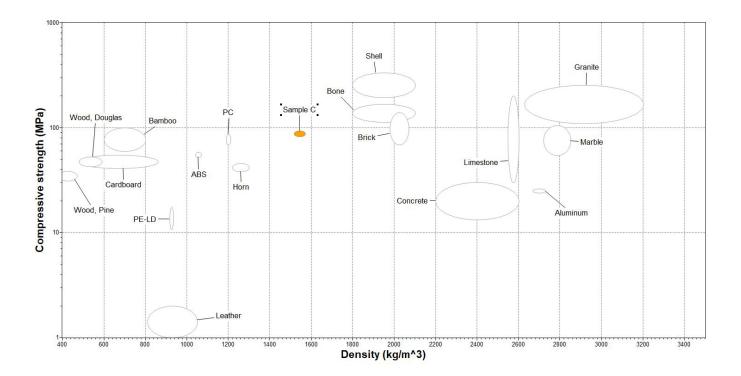
Their density was measured using a Hildebrand H300S Electronic Densimeter.

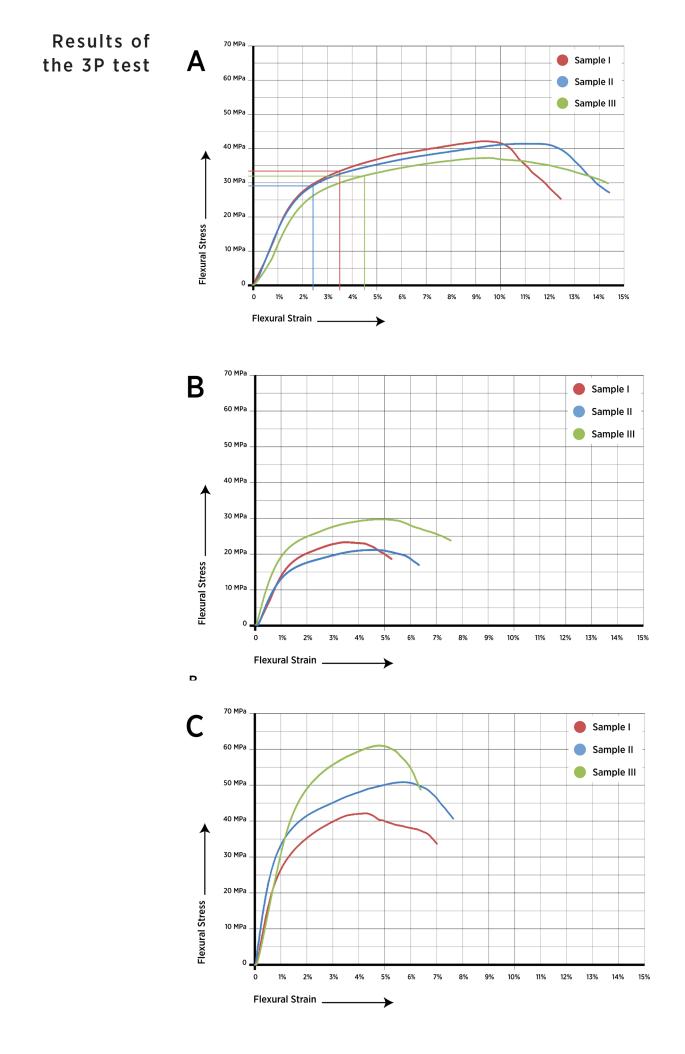
Compressive Strenght of Sample C

There was no accurate way of measuring the amount of compressive force that sample C underwent, the indicator dial of the hydraulic press was hereby used as a reference. This indicator gave the amount of pressure that was distributed over the surface of the tool. Given the diameter of this tool (100mm) the consequential force, applied to the sample, could be calculated. In addition to the above incongruency, there was only one sample compressed in

this setup and thus the obtained date can not be validated.

What we do know is that the sample underwent a huge amount of compressive stress (approximately 83 MPa) and that it did not fail. This knowledge can be used to compare its compressive behaviour to other materials. In the graphe shown below, the material sample is compared to the data of other materials, obtained through the Cambridge Engineering Selector. Here the compressive strenght of





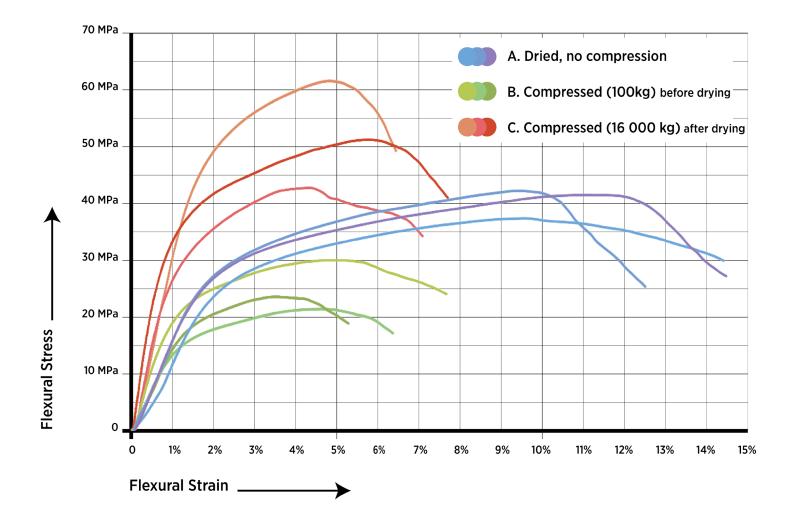
Technical Characterization

	Density (g/cm³)	Residual deformation (mm)	Flexural (E) modulus (GPa)	Flexural Strain @yield (%)	Flexural Stress @yield (MPa)	Flexural Strain @Failure (%)	Flexural Stress @Failure (MPa)	Flexural Toughness (MJ/m³)
Sample I	1,457	3,5	1,613	3,49	33,5	9,49	41,95	5,7
Sample II	1,213	4,6	1,682	2,30	29,0	11,39	41,24	6,6
Sample III	1,363	3,6	1,405	4,53	32,0	9,59	37,14	5,2
Average	1,34		1,567	3,4	31,5	10,2	40,1	5,8

В

_	Density (g/cm³)	Residual deformation (mm)	Flexural (E) modulus (GPa)	Flexural Strain @yield (%)	Flexural Stress @yield (MPa)	Flexural Strain @Failure (%)	Flexural Stress @Failure (MPa)	Flexural Toughness (MJ/m³)
Sample I	1,247	3,2	1,510	2,13	21,3	3,54	23,29	1,2
Sample II	1,261	3,3	1,617	1,92	17,8	4,58	21,21	1,5
Sample III	1,195	3,7	2,467	2,16	25,4	4,79	29,72	2,3
Average	1,23		1,86	2,07	21,5	4,3	24,7	1,7

C	Density (g/cm³)	Residual deformation (mm)	Flexural (E) modulus (GPa)	Flexural Strain @yield (%)	Flexural Stress @yield (MPa)	Flexural Strain @Failure (%)	Flexural Stress @Failure (%)	Flexural Toughness (MJ/m³)
Sample I	1,519	2,6	3,787	2,91	39,0	4,288	42,40	2,9
Sample II	1,528	3,4	4,830	2,25	42,4	5,702	50,95	4,9
Sample III	1,569	2,3	3,493	3,32	56,7	4,830	61,11	4,5
Average	1,54		4,04	2,83	46,0	4,94	51,5	4,1



	Density (g/cm³)	Flexural Strain @Yield (%)	Stress @Yield (MPa)	Flexural Strain @Failure (%)	Flexural Stress @Failure (MPa)	Flexural (E-)modulus (GPa)	Flexural Toughness (MJ/m³)	
sample A	1,34	3,4	31,5	10,2	40,1	1,57	5,8	
sample B	1,23	2,1	21,5	4,30	24,7	1,85	1,7	
sample C	1,54	2,8	46,0	4,94	51,5	4,04	4,1	

Flexural Properties

Tougnhess

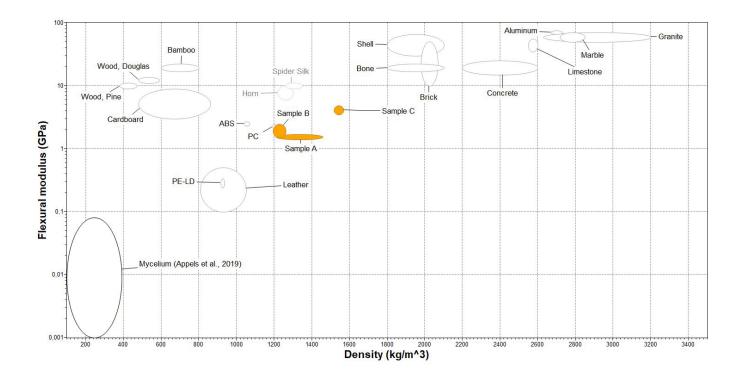
The yield point was defined by measuring the residual deformation and defining the elastic deformation.

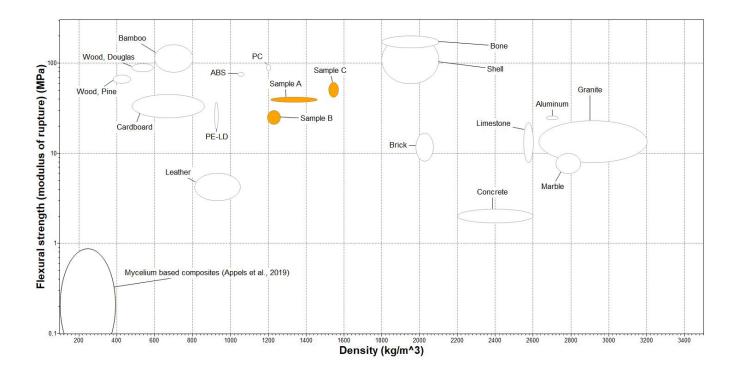
• The point of failure was defined as the top of the curves.

• The flexural modulus by measuring the the slope of the initial curve

• The flexural toughness was defined by measuring the area under the curve using OriginLab graphing software. This data was then controlled via a calculation by hand. There is unfortunately little data available to controll or compare the obtained values.

The samples their flexural modulus and flexural strength were then compared using CES Edupack software. Unfortunately this database does not support the comparrisson of ultimate flexural strain or flexural toughness.





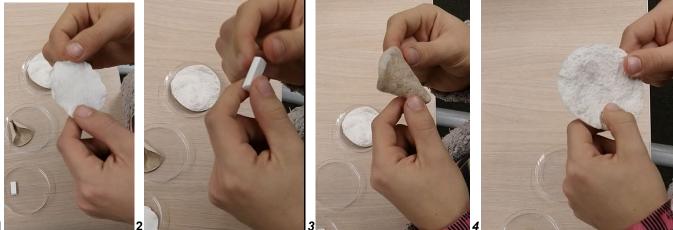
C.EXPERIENTIAL CHARACTERIZATION

This appendix contains the data obtained during two experiential tests performed on;

C.1 INITIAL TESTS

P1_06-11_Florien

Did not know anything about the material or my graduation

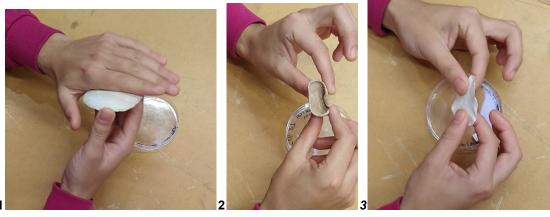


- 1 1
- Feels like chalk and paper
- Looks 'sustainable
- 2
- This looks like a crayon but could also be something plastic-ish
- 3
- This reminds of a mushroom or a leaf
- But it could also be some sort of an ecological cup or something
- It rubs of a little
- 4
- And this looks a bit like paper-mache or chalk or something
- And it also looks like a mushroom

Participant interpreted three samples as **recycled**, **natural**, **ordinary** She tried bending most of the samples

P2_06-11_Max

Participant was aware of the bacterial nature of the material



1 1

Participant was stroking the material

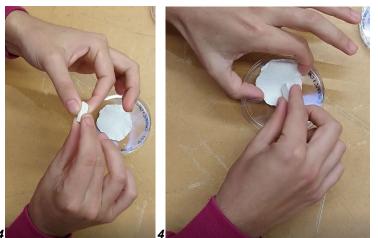
- This feels like gypsum with some sort of fibers on the edges
- It also comes of when I rub it
- It smells a bit like cheese
- One side feels soft while the other one is much rougher, something to scrub my hands with
- I feel like breaking it, just to see what the inner structure is made of

Participant interpreted the top side of this sample as nostalgic

2

Participant was bending the material

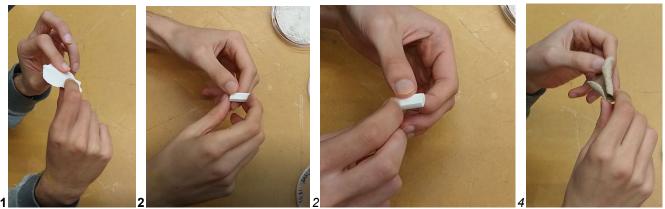
- This one looks like a potato chip
- It is more bendable, kind of like cardboard
- It smells less like cheese, a bit like cheese and cardboard
- It feels like something natural, something you can find in a forest
- 3
- This looks like gypsum but feels more like paper, like flexible gypsum
- Does not rub off



4

- This one,... ewl, gives me jingles, kind of like touching a piece of chalk, it is smooth but also produces vibrations
- Can you also use it like a piece of chalk, -tests it- oh no, it does not rub of
- It is very smooth but when it gets a little wet it becomes sort of rough-ish, or like stone, I don't know, cant place it. Participant interpreted this sample as **manufactured and calm**
 - Participant noted the contrast between this sample and the others which had 'natural fibres' in them

P3_06-11_Emiel



1

It feels quite fragile, I don't dare to bend it too much

- Participant notices the difference in texture, top and bottom
- It feels like cardboard that has gotten wet

Participant interpreted this material as **ordinary**, **manufactured**, **calm and sober 2**

And is this the same material? Is it part of the experiment? It sort of looks like it.

Participants scratches the material with his finger

Hmm, I wasn't expecting it to be this hard

Participant interpreted this material as **ordinary**, **manufactured**, **calm and sober 3**

Feels like 'kroepoek' but a bit more dense

It smells like sesame paste/peanut butter

It does give off a bit when you rub it.

Feels more brittle, I think because it is more dry

Participant interpreted this material as natural, masculine and agressive,



5

Participant was ticking on the material with his nail

process of drying. , being flat innitially)

This one appears harder and more brittle, and it makes the same noise as gypsum Participant interpreted this material as **natural, strange, handcrafted and frivolous.** The bottom side was interpreted as **futuristic** and the top side **nostalgic.**

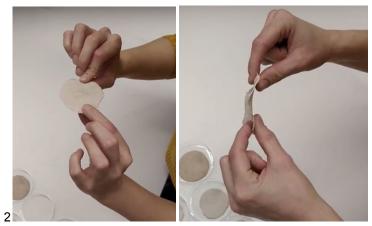
- This sample felt as if it came from the sea
 - He especially noted the contrast between the top and bottom side, with the top side appearing to have formed by something alive (participant knew I am working with bacteria) and the bottom side being formed through the

P4_07-11_Joy

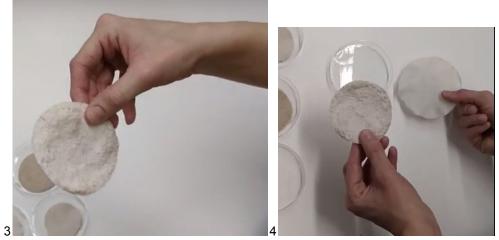


Hmmm, this one has the flexibility of a brick

It feels rather nice though



This one is very thin, it feels like it has some bend to it but it also feels very fragile

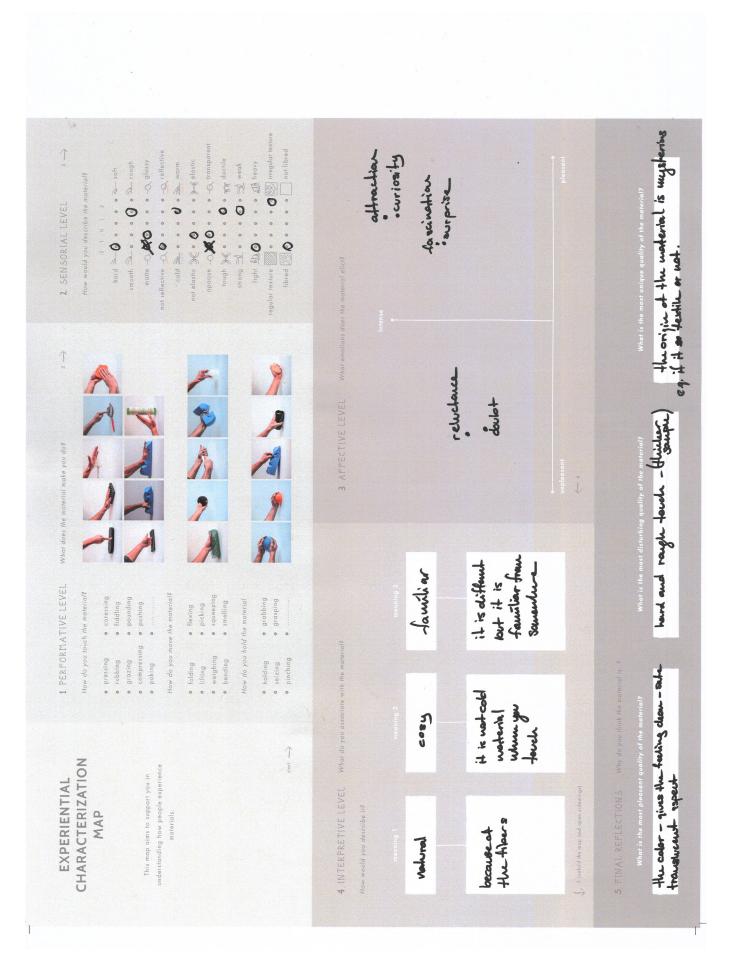


With this one I really like the texture, it has an organic feel to it where this one (points to other sample) feels more like a flat pancake

P5/6 N/A

Unfortunately the raw data of participant 5 and 6 got lost when cleaning up my drive.

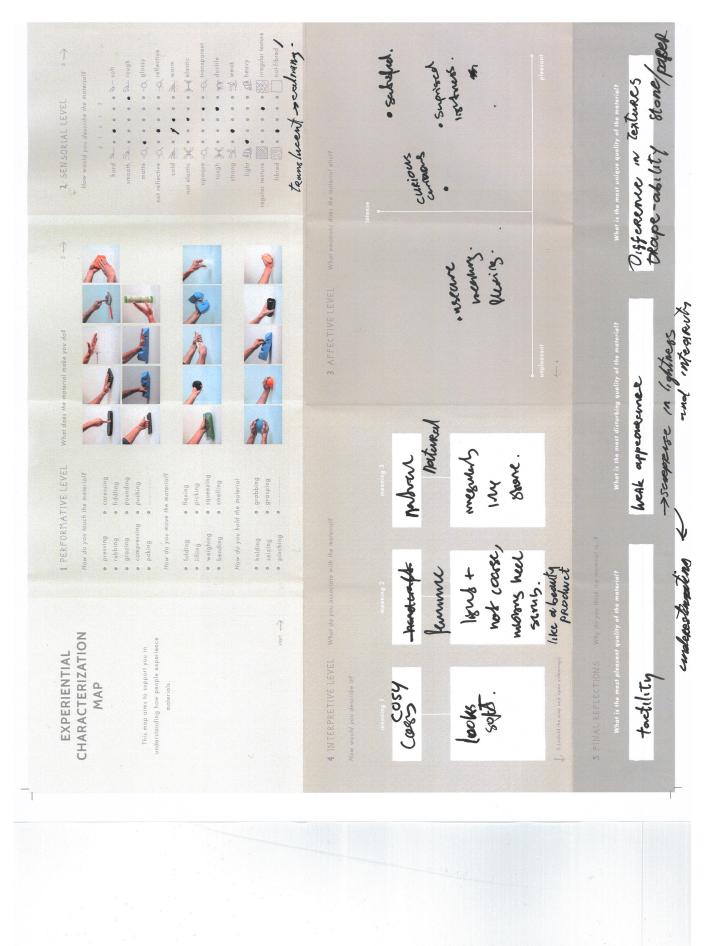
C.2 TESTS WITH MATERIAL DESIGNERS



 SENSORIAL LEVEL 3→ SENSORIAL LEVEL 3→ How would you describe the material? 1 0 1 2 <li 0="" 1="" 2<="" li=""> 1 0 1 2	cold > 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0	0 *	ngni ⊑(A • • • • • • • • • • • • • • • • • • •		What emotions does the material elicit ²							What is the most unique quality of the material?	
	*		2		TIVE LEVEL What emotions of	3						srial? What is	
re LEVEL tings)		calm	aloof	vulgar	sober	nostalgic	feminine	strange	not sexy	professional	manufactured		
INTERPRETIVE LEVEL (set of meanings)	OR	aggressive .	cosy .	elegant .	frivolous	futuristic .	masculine .	ordinary .	toy-like	natural .	hand-crafted .		

1

C.2 TESTS WITH MATERIAL DESIGNERS



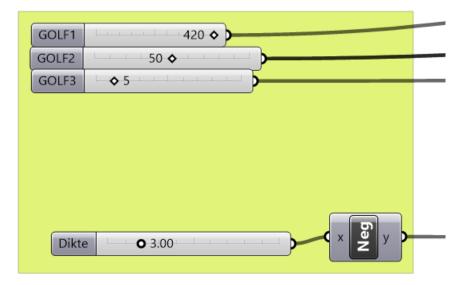
Experiential Characterization

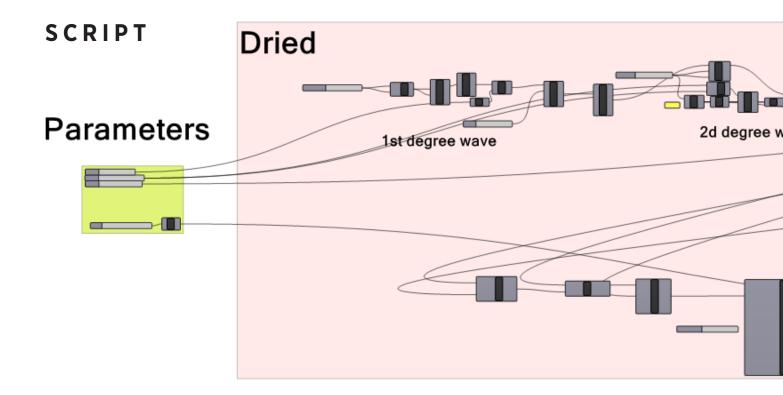
E. CONCEPT EXPLORATION

This part contains the Grasshopper scripts that were developed in collaboration with Leandre Sassi to simulate the material its behaviour along different

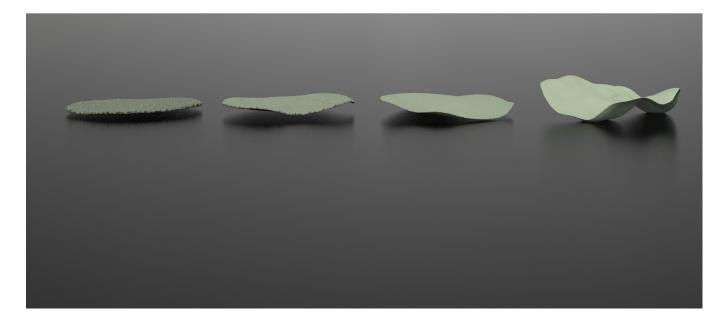
E.1 DRYING

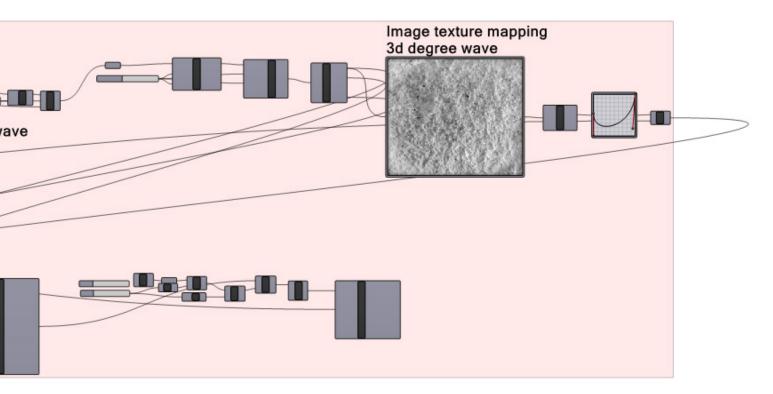
PARAMETERS





RENDERS

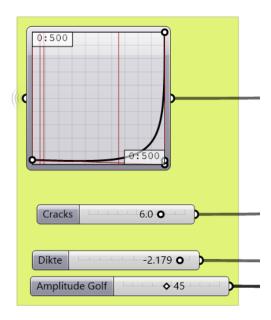




Concept Exploration

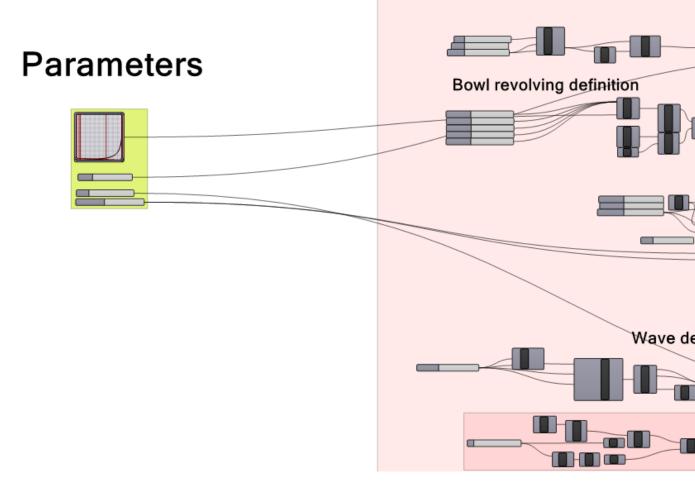
E.2 COMPRESSION

PARAMETERS



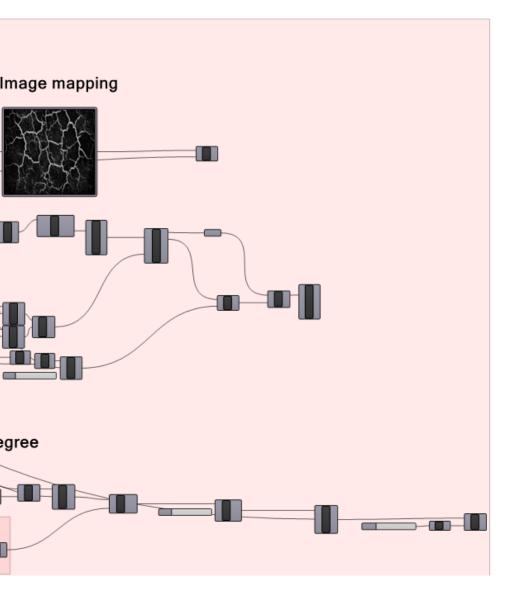
SCRIPT

Compression



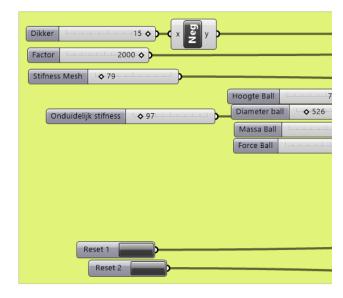
RENDERS

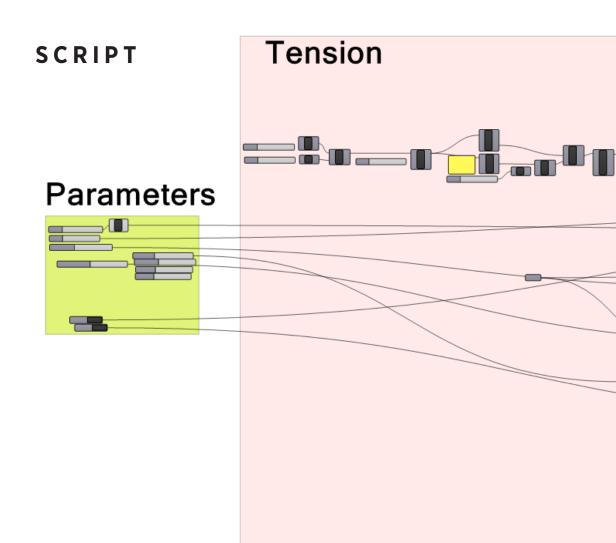




E.3 TENSION

PARAMETERS





RENDERS

