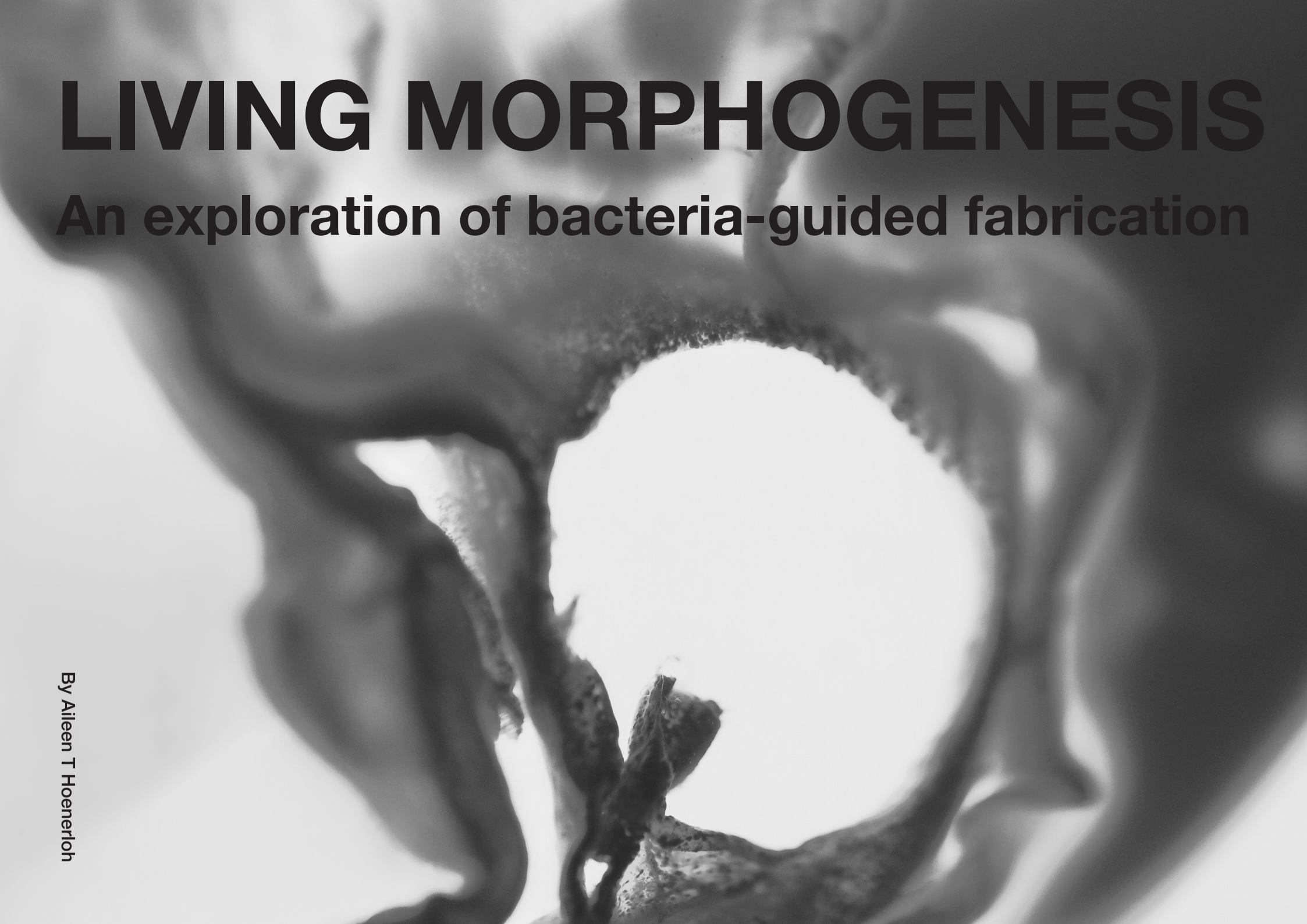
LIVING MORPHOGENESIS

An exploration of bacteria-guided fabrication



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ABSTRACT

This thesis contributes to biofabrication design practices by developing a co-designing process with living materials to bridge the gap between design ambition and material biofabrication. An interdisciplinary collaborative approach is proposed by redefining material exploration at the intersection of design and microbiology based on the example of bacteria-produced cellulose. Bacterial Cellulose (BC) naturally grows into flat sheets at the air-nutrient interface on the surface of a liquid culture. While BC is fast gaining recognition across scientific domains due to its versatile properties – including high durability, water-holding capacity, and lightweight nature – its fabrication has traditionally been limited to post-growth moulding of the flat material sheets. Applying conventional fabrication techniques, however, overlooks the material's inherent self-forming capabilities.

For this reason, the methodology developed in this thesis seeks to understand BC growth behaviours through a cross-disciplinary lens with the aim of facilitating the exploration of BC's intrinsic morphological behaviour. Carefully designed environments implement aeration and customised scaffolds as design tool to enable the growth of three-dimensional BC. The described iterative experimentation not only identifies key design parameters to guide the BC's growth into novel shapes, but also reveals the emergence of complexity and spatial variability within its materiality. Additionally, the thesis investigates novel preservation methods in order to document and capture these complex and rapidly evolving BC formations.

The findings introduce a novel organism-guided and designer-led approach to BC fabrication, enabling the creation of uniquely complex three-dimensional structures. This marks a significant advancement in Biodesign research, demonstrating the importance of exploratory methods in understanding and utilising living materials for new forms of fabrication.

ACKNOWLEDGEMENTS

I would like to start by thanking all of my supervisors for their support and guidance over the past five years as I navigated the challenges of pursuing a PhD. Thank you, Martyn, for your continued encouragement to stay curious while developing experiments and for teaching me how to be a Biodesigner. If it wasn't for our meeting during my Master's, I likely would have never found my passion for the field. Thank you to Ben for guiding me through the process of the PhD and for helping find a balance of the creative and the systematic research. And thank you, Thora, for supporting me as a supervisor in the lab with my experiments to see the bigger picture, and for being there for me as a friend through the highs and lows of this rollercoaster.

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PROLOGUE

My academic journey began in architecture, where I developed an interest in fabrication methods, both manual and digital. Working in the wood workshop and exploring digital fabrication processes gave me an appreciation for the relationship between material and form. During my postgraduate studies at Cardiff University, I was introduced to parametric design strategies. During this time my understand of how digital models could be translated into physical objects through various fabrication methods deepened. However, I also became increasingly aware of the material waste generated by these processes, such as discarded acrylic from laser cutting or excess filament from failed 3D prints. This awareness led me to explore more sustainable alternatives, eventually focusing on natural and reusable materials compatible with digital fabrication. My master's thesis centred around clay 3D printing, where I designed an interactive system that allowed users to modify printed geometries in real time.

It was around this time that I met Martyn Dade-Robertson, who introduced me to the newly forming the Hub for Biotechnology in the Built Environment (HBBE) at Newcastle University. The HBBE's vision of integrating biology with design aligned with my growing interest in sustainable fabrication, and I applied for a PhD position in biodigital fabrication. While the PhD research was intended to explore large-scale biomaterial printing using robotic systems, delays in setting up the HBBE workshop during the COVID-19 lockdown shifted my focus toward smaller-scale fabrication found within living systems. Without a formal background in microbiology and limited by the facilities found in my family's home, I turned to the Do-It-Yourself (DIY) biology community, where open-access knowledge and hands-on experimentation provided an entry point into working with living materials.

The HBBE offered a unique interdisciplinary environment with access to specialised facilities and experts able to give insights into a large spectrum of fields, ranging from material sciences, microbiology, design, fabrication, and more. While the COVID-19 lockdowns initially delayed access to these resources, they later played a crucial role in my research, allowing me to explore living materials from different angles. Throughout my research, I worked alongside technicians, fellow PhD students, postdoc fellows, and professors, who contributed their expertise through

formal feedback as well as many informal daily conversations in the lunchroom. Both the direction of my experiments and the development of my methodologies for working with living biomaterials were influenced by these conversations.

The influence of the DIY community is also reflected in my approach to writing. Unlike other theses in the Biodesign field, the main body of this work follows the conventions of scientific publishing by avoiding the first-person perspective, maintaining the objectivity expected in STEM disciplines. However, the language has been deliberately shaped to be accessible beyond academic circles. Where possible, scientific terminology is minimized in favour of clear, direct explanations to ensure that the research can be understood by a wider audience, including those in DIY and maker communities. Given that much of my own learning was enabled by publicly available knowledge, this is a way of contributing back, making the science behind Living Morphogenesis as open and approachable as possible.

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Bacterial cellulose grown with submerged aeration disc with distinct elongated growth features

CHAPTER 1

INTRODUCTION

Chapter Summary

This opening chapter provides the framework for the research presented in this thesis by situating it within the rapidly evolving field of Biodesign. It first distinguishes between the nuanced branches that define the field before specifying the position of this work. The chapter defines the core research focus and the specific research question and aims that guide the exploratory and experimental research approach. A structured roadmap to the thesis is provided in the form of concise summaries of each chapter before a brief statement acknowledges collaborative work and publications of results presented.

1.1. Branches of Biodesign

The term ‘Biodesign’ represents an interdisciplinary field that has emerged at the intersection of biology, design, and technology. The origin of Biodesign can be imagined as far back as the first domestication of animals and plants by humans. However, in contemporary contexts, it is more often associated with the cutting-edge research and design of biotechnological innovation and sustainability efforts. While the biomedical field has used the term for many decades to describe processes such as modified cell cloning, in a more recent development the term has found its way into the vocabulary of designers and architects who aim to harness the potential of biological systems for their creative practices (Kindregan, 1972). Despite its rapid growth, or arguably because of, the full scope of the field has yet to be explicitly defined. Multiple attempts to categorise Biodesign work have been made in the past decades with coinciding themes. The exhibition “En Vie – Alive” showcased artworks divided into five categorised based on the level of interaction between the living system and the design process (Collet, 2013). Each group identified the role of nature differently: nature as model, nature as co-worker, creation of a synthetic nature, nature and technology hybrids, and provocative futuristic ideas. While the first two groups incorporated biology in its existing form, the third and fourth group more heavily relied on manipulating the biological process through synthetic biology to expand its functionality. Building on Collet’s identified branches of Biodesign, ‘Growing Design’ was introduced by Camere & Karana (2017) to emphasise the role of the living organism as part of any Biodesign fabrication process.

Myers took an overarching approach to outlining the core building blocks of Biodesign in the form of three foundational methodologies: Design with Biology, Design for Biology, and Design by Biology (Myers, 2018). Design with biology refers to the utilisation of a living organism or natural process as part of the design process, such as growing furniture from fungi. In contrast, Design for Biology does not involve the integration of living process but rather focuses on creating tools, including lab equipment, and finding solutions to advance biological research. In between the two previous methodologies sits ‘Design by Biology’ which observes biological systems to inspire the development solutions for specific design problems. Myers’ breakdown of Biodesign encompasses both Collet’s and Camere & Karana’s defined branches, while leaving space for

further developments.

In particular the concepts of nature as model and nature as co-worker have sparked interest in research related to challenges in the built environment which can be linked to the many parallels between nature's building methods and architectural construction processes. However, while Biodesign has begun to inspire architectural practices, its potential is far from fully realised.

1.1.1. Biology and Architecture

The concepts of bio-utilisation and bioinspired design have arguably been used in the built environment since the first human shelters were built in caves. But Biodesign philosophies in architecture were particularly fuelled by the sustainability movement at the end of the 20th century, aiming to use fewer non-renewable resources and optimising aspects of the built environment ranging from individual building materials to space management (Collet, 2021). This movement emerged from a growing awareness of the environmental impact of traditional building practices and the need to develop sustainable alternatives. Engineers and architects began to look at nature to learn from biological systems. These systems in nature have been self-optimising through natural selection for billions of years and offer new perspectives on efficient utilisation of resources as well as resilience and adaptability to environmental factors (Pawlyn, 2016). A key aspect of nature that is relevant to the human design strategies is the concept of morphology, which, in a strict biological sense, means "the study of form and structure" (Ritterbush, 1968, p.7). It refers to the way in which nature assembles, builds, and forms organisms both internally and externally. This is of particular interest because "morphogenetic mechanisms are more goal-seeking than blueprint-following" (Davies & Levin, 2023, p.47), meaning that an organism, such as a tree, has an inherent drive to optimise and will adapt its form in direct response to the environmental factors surrounding it.

In Biodesign, different methods exist to implement knowledge of natural form and optimisation into human design. A well-known method is Biomimicry, which is defined as "a practice that learns from and mimics the strategies found in nature to solve human design challenges" (Biomimicry Institute, 2024). It was first coined by Otto Schmitt in the 1950s under the term 'Biomimetics' (Bhushan, 2009) and later popularized as 'Biomimicry' by Janine Benyus in 1997, with the understanding that it is "design through analogy to biology" (Kennedy, 2017, p.51). It falls into Myers' category of 'Design by Biology' and does not utilise living functions of biology. Instead, it learns from its structural, logical, or aesthetic principles to solve a specific design problem through modern technological tools (Benyus, 2002). Early examples of innovation through biomimetic design include Leonardo da Vinci's flying machines (Ridley, 2019) and Georges de Mestral's de-

velopment of VELCRO (Stephens, 2007), which were inspired by winged animals and the form of burrs respectively. Today, Biomimicry is being implemented to solve large-scale and small-scale problems across all fields of science and design, including textiles design, biomedical science, agriculture and the built environment (Zhong et al., 2017; Zhang, 2012; Othmani et al., 2021; Sheikh & Asghar, 2019).

Computational tools are often utilised during the biomimicry design process to 3D model prototypes because large sets of diverse data need to be brought together. A particularly suitable tool is parametricism, coined by Patrik Schumacher, combined with simulation tools and artificial intelligence (Kadar & Kadar, 2020). Instead of manually creating multiple 3-dimensional design prototypes, parametric tools can be employed to "specify relationships among various parameters on [the] design model" (Jabi, 2013, p.9), which are then translated into changes of the prototype through mathematic algorithms. The biomimicry approach draws parallels between these computational algorithms and evolutionary processes in nature (Pawlyn, 2016), and the parameters can be provided by the design brief or identified through site research and continuously redefined through iteration and versioning (Jabi, 2013).

Another less-established method for architectural Biodesign distances itself from the use of computational tools and mathematical algorithms to mimic nature. Instead, it aligns with Myers' category of 'Design with Biology' and aims to harness the responsive, adaptive, or self-assembling capabilities of different living organisms, also referred to as living systems, in the form of new biomaterials (Armstrong, 2011). A living material can be created by many organisms, including fungi, bacteria, algae, and yeasts. It can be a single or combined culture, but it is always a made of multiple individual entities of the organism (Whitesides & Grzybowski, 2002). It is this inherent complexity within living materials that creates emergent behaviours, characteristics, and properties that can be utilised for new sustainable building practices. The concept of emergence refers to the process by which complex structures, patterns or behaviours occur as a result of interactions between smaller and simpler individual components. This concept is rooted in the theory of emergent systems, where the collective behaviour of individual components leads to new properties and functionalities that are not directly evident from the individual parts alone or intentionally executed, in contrast to hierarchical systems (Steels, 2019). Parametric design can

be implemented to simulate aspect of this emergence as part of a biomimetic approach (Kadar & Kadar, 2020). However, working on design strategies that integrate the living material directly allows the designer to harness the full potential of its emergent functionality, or even enhance it (Ramirez-Figueroa, 2018). This method is referred to as biological fabrication. The concept of biological fabrication, or biofabrication, in architecture can be described as “the emerging and often radical approach to design that draws on biological tenets and even incorporates the use of living materials into structures, objects and tools” (Myers, 2018, p.8). It is here where the research of this thesis positions itself.

1.1.2. The scope of Biological Fabrication

The term 'Biological Fabrication' is a relatively new branch within the field of Biodesign and is used to describe research with biomaterials, such as fungi, bacteria, yeasts, and algae, across multiple disciplines without a clear definition. This has led to terminological inconsistencies which is a recognised issue within biologically informed disciplines (Kapsali, 2022). To accurately identify the specific domain of Biodesign to which this research adds, the term's various contextual interpretations in literature are examined and compared with conventional definitions found in dictionaries with a focus on the active integration of behaviours and characteristics of living organisms.

As of today, no entries for the term 'biological fabrication' exist in dictionaries. Instead, individual entries for each of the two words are offered. The following four online dictionaries were chosen to synthesise a conventional definition of the term based on entries of 'biological' and 'fabrication: *Oxford English Dictionary* [1], *Cambridge Dictionary* [2], *Merriam-Webster Dictionary* [3], and *Collins English Dictionary* [4]. The following definitions for the adjective 'biological' can be found:

Oxford English Dictionary

1. "Of or relating to biology or the phenomena of living organisms"
2. "Involving or consisting of living organisms; derived from living organisms"

Cambridge Dictionary

"Connected with the natural process of living things"

Merriam-Webster Dictionary

1. "Of or relating to biology or to life and living processes"
2. "Used in or produced by applied biology"

Collins English Dictionary

1. "Biological is used to describe processes and states that occur in the bodies and cells of living things
2. "Biological is used to describe activities concerned with the study of living things"

The following definitions for the noun ‘fabrication’ can be found:

Oxford English Dictionary

1. “The action or process of fabricating; construct, fashioning, manufacture; also, a particular branch of manufacture” (fabrication)
2. “The process of fabricating in the manufacture of finished products”

Cambridge Dictionary

“To produce a product, especially in an industrial process”

Industry [*noun*]

“the companies and activities involved in the process of producing goods for sale, especially in a factory or special area”

Merriam-Webster Dictionary

“To construct, manufacture; specifically, to construct from diverse and usually standardised parts”

“Something made from raw material by hand or by machinery”

“to make or form by combining or arranging parts or elements”

Collins English Dictionary

1. “a fabricating or being fabricated; construction; manufacture

fabricate [*verb*]

“If something is fabricated from different materials or substances, it is made out of those materials or substances”

Based on these definitions, 'biological' is an adjective and refers to biology, which is the science of life and living organisms. It represents phenomena, processes, and states inherent to the life functions and behaviours of living organisms. The term includes both the application of these living processes and the study of it.

The term 'fabrication' can be summarised as the skilled, and often industrial, creation or construction of (physical) products. It involves both the constructing and manufacturing of items from either raw or standardised materials. It does not define the scale of the fabricated item or its components and specifies that both the use of manual and digital tools are viable.

Combining both summaries the following definition of 'biological fabrication' emerges:

> *"The process of constructing or manufacturing products, structures, or systems that involve or are derived from living organisms. It encompasses the use of knowledge of biology to create products, often through industrial processes, with the intrinsic characteristics and functions of living biological organisms. It merges the application of biological processes with the production of goods to leverage biological systems for commercial outputs."*

The derived definition clearly identifies the importance of the biological system to the process of fabricating a product. However, no indication is made to whether the biological system is in a living or inert state or to what extent the biological system is manipulated. A similar broadness of the term is found in literature. Based on the level of human manipulation of the biological system, four different definitions of the term can be derived from literature. The human manipulation consists of two areas, the internal and the external modification. The former refers to the genetic modification of the living organism on a cellular level while the latter manipulates the organism's behaviour through manipulation of environmental factors only.

The first identified understanding of 'biological fabrication' refers to a fabrication process without human intervention, neither internally or externally. The justification for using the term 'fabrication' when referring to natural biological processes is made through a comparison between the saliva used by edible-nest swiftlets and additive manufacturing processes (Jessel *et al*., 2019).

In agreement that nature can fabricate, Keating states “biological cells are both factories and product: a designer and fabricator” (Keating, 2016, p.28). To further clarify the influence of human interference, Keating (2015 & 2016) distinguish between ‘natural biological fabrication’ and ‘digital biological fabrication’. The latter involves human-controlled computational tools to aim towards achieving parallelisation in the production process found in nature but with digital controls and materials (Keating, 2015). Similarly, Greanya (2015) differentiates between ‘biological fabrication’ as a natural process and ‘biofabrication’ as a human-driven process which can incorporate internal and external artificial influences, such as synthetic biology, computer simulations, and digital fabrication processes.

The second identified understanding of the term is the most commonly used amongst designers and leans on the previously mentioned definition of ‘digital biological fabrication’ (Keating & Oxman, 2013), while excluding internal manipulation of the organism. It broadly describes a fabrication process in which the instinctive behaviour of a living organism, in particular growth, is accompanied by intentional and artificial interference of humans. Oxman *et al*. (2013) describe ‘biological fabrication’ as the use of self-fabricating biological organisms which are integrated into a digital fabrication process, giving the example of silk worms producing a material skin around an artificially fabricated pavilion frame. In agreement, Wang et al. (2005) describe ‘biological fabrication’ as a controlled fabrication process in which external influences, predominantly the creation of artificial environments, are implemented to change targeted natural behaviours of organism. Further defining the relationship of human and biology, Collet describes the process as “co-working with living systems” (Collet, 2017, p.1). The human’s role is to guide the fabrication process of the organism and this guidance is developed based on the characteristics of the organism, implying a deep-rooted understanding of its behaviours and capabilities.

The third understanding of ‘biological fabrication’ more heavily focuses on the interference of the organism’s fabrication behaviour internally and can be found most often in publications of chemical engineering and synthetic biology. This aligns with Greanya’s (2015) definition of ‘biofabrication’ which is also used with the same connotation by Costa (2019). In contrast to the previous understanding, the fabrication process is not limited to the macro scale but can occur on a nano, micro, and macro scale alike (Qin *et al*., 2008; Natalio *et al*., 2017). Both internal and

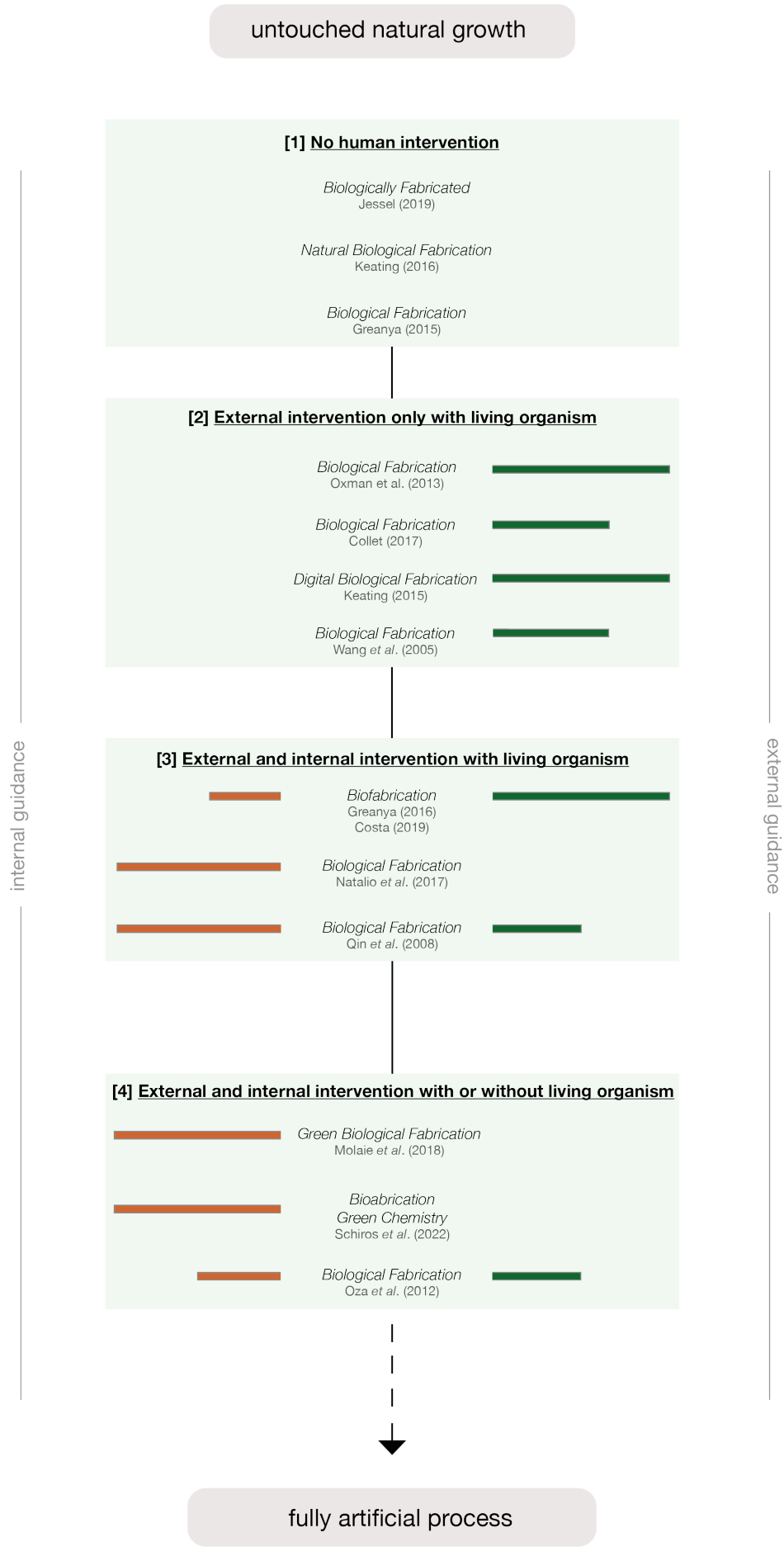

Figure 1.1: *Sequential order of identified definitions of the term 'biological fabrication'*

external interference by humans is required, or heavily relied on, to facilitate this type of 'biological fabrication', which is interchangeably referred to as 'biofabrication' (Costa, 2019) and linked to a frequent use of custom-build bioreactors. In particular the development of Engineered Living Materials (ELM) finds alignment in this interpretation (Natalio, 2019).

The fourth, and last, identified understanding moves away from the concept of utilising an active and living biological process and establishes the connection to biology through the use of materials made by nature. Also referred to as 'green biological fabrication' (Molaie *et al*., 2018) or 'green chemistry' (Schiros *et al.*, 2022), this interpretation is mainly found in chemistry research which aims to reduce the use of harmful chemicals by creating "bio-inorganic materials […] synthesized by living systems" (Oza *et al.*, 2012, p.505). While the preparation of the material involves living organisms and their metabolisms, it is not the living aspect which is of importance to the process. This understanding is most closely comparable to the use of timber in construction or PC for the fabrication of paper-based materials.

The four interpretations of the term can be sequentially aligned on a spectrum, ranging from least to most interference by humans (Figure 1.1). Within each of the four groupings, different degrees of internal and external influence are identified. However, the complexity of 'biological fabrication' is not only linked to the level of interference by humans. It also concerns the question "where is the information" (Dade-Robertson, 2021, p.78), referring to the conceptual location of process-guiding parameters. Dade-Robertson proposes a triangular visualisation in which he refers to in silico, in vivo, and in vitro as three corner domains of information and acknowledges the interconnection between them (Dade-Robertson, 2021). While Dade-Robertson uses the diagram to analyse individual biological fabrication processes, mapping the area of each identified contextual definition of the term in literature allows to precisely position the approach to biological fabrication of this thesis (Figure 1.2).

Living Morphogenesis positions itself within the second contextual understanding in which the organism is alive. Its natural metabolic functions are utilised exclusively through external manipulation, such as a constrained growth environment. The information of the morphological development of the material is shared between the artificially created physical environment and the responsiveness, or agency, of the living organism. Attributing agency to living systems and organ-

isms goes further than acknowledging responsiveness based on environmental influences and recognises a degree of autonomous behaviour (Okasha, 2024). This creates a continuous feedback cycle between the two domains and informs the iterative development of experimentation. Computational tools are utilised to facilitate the design and fabrication of the artificial environment but do not assume the role of predicting the organism's response behaviours to it.

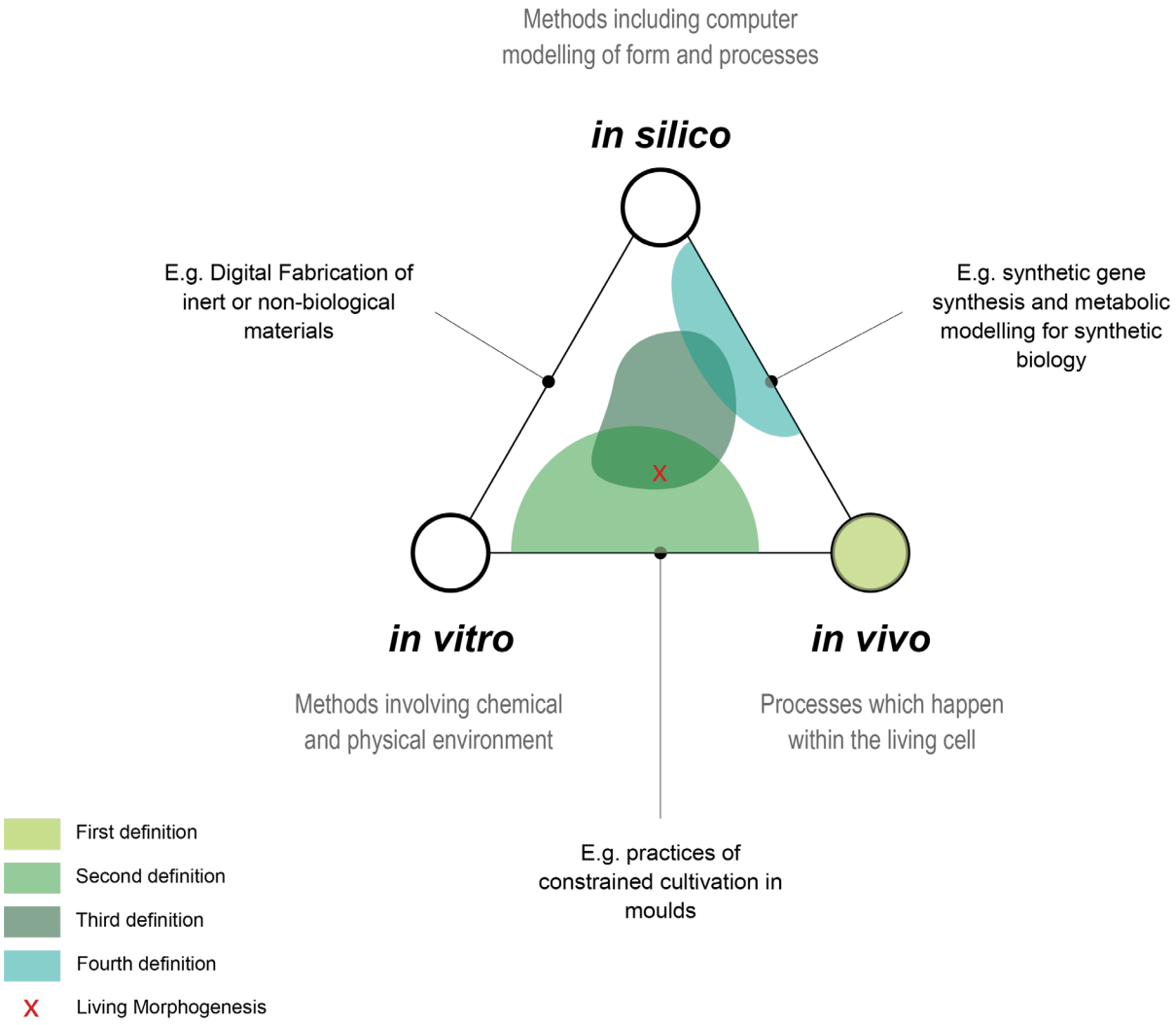

Figure 1.2: *Mapping of identified contextual interpretations into diagram of 'Domains of Information'. Diagram adapted from Dade-Robertson (2021)*

1.2. Research Focus

A part of the current movement towards sustainability in the built environment, driven by climate change, is the development of novel biotechnologies that introduce living systems into the material fabrication process. Biomaterials of particular interest for architectural application are those produced as part of the organism's natural metabolism, including mycelium, bacterial cellulose, and cementation through microbially induced calcite precipitation (Heinrich *et al.,* 2019). The research in this thesis focuses on bacterial cellulose (BC), a biomaterial produced by microbes that shares many similarities with plant-derived cellulose (PC) (see Chapter 2.1.1), which is already widely used across various industries. BC is a biomaterial that is accessible for exploration outside of laboratory settings due to limited requirements for sterility or specialised equipment. This also opens up the potential for explorations on a larger scale, which are the most relevant for future applications in architectural settings. To date, commercial applications of BC are limited, and fabrication processes heavily rely on those developed for PC or on the post-processing of the naturally flat BC sheets through moulding or drying techniques. This, however, fails to acknowledge any inherent capabilities of the living material to self-morph or to be responsive to its environment.

The primary aim is to investigate the potential of fabricating 3-dimensional BC and to establish a design methodology that facilitates the development of design parameters to guide the emerging material form.

The research brings together the scientific microbiological knowledge of BC cultivation and different perspectives of designing with unpredictable emergent complexity. The work is divided into two parts with each investigating a research question looking at a different aspect of the development of a novel fabrication method.

Question 1: *How can the growth environment of BC be manipulated to alter the natural fabrication behaviour of the organism to fabricate the material into complex 3-dimensional forms?*

The most commonly practiced method for growing BC is through a static culture using liquid containing nutrients and bacteria cultures (see Chapter 2.2). Through this method the material forms on the liquid surface into flat sheets, where the organism has equal access to nutrients and oxygen. The manipulation of this interface offers an interesting starting point to encourage novel responses of the organism.

> **Question 2:** *To what extent can the implementation of a synthetic growth environment guide and control the characteristics and emerging morphology of the BC?*

Living systems inherit complex internal mechanisms to respond to the exposure of different stimuli., which imparts them with many advantages compared to traditional non-living materials. Biomaterials can react to their environment and adapt their morphology, materiality, growth rate, and more. Due to the complexity of reactivity occurring on the nano- and microscale within the organism, designing with and for these living materials on a macro scale entails an unknown degree of unpredictability. Understanding what this unpredictability means for the design process and how it responds to the implementation of design parameters is key to the advancement of these living materials.

The research methodology developed for this thesis is intentionally flexible and unrestricted to allow the possibility of inventing new phenomena that cannot be hypothesised. The absence of predetermined objectives that constrain the direction of research allows for flexible open-ended exploration, which can cultivate an environment in which novel insights can naturally emerge. This approach is particularly suited for fundamental research in the field of biotechnologies, where the nature of living systems still has a lot left to be explored.

Based on the exploration-guided nature of the methodology, a single initial objective was identified as point of origin for the research:

> **Objective 1:** To develop a comprehensive understanding of existing fabrication methods for bacterial cellulose with focus on their manipulation of the organism's growth environment.

As the experimental research advanced, a progressive identification of objectives emerged from the findings and insights. This evolving focus ensured that research remained relevant and responsive to the material's emerging potential and led to the discovery of unique properties that may have otherwise been overlooked. The following four objectives emerged:

Objective 2: To develop a systematic research approach of exploring and analysing the behavioural response of the organism to physical intervention in its growth environment.

Objective 3: To identify different methods of providing scaffolding for bacterial cellulose growth that are biocompatible with the organism and facilitate new morphological expression.

Objective 4: To analyse the morphological development of BC in the presence of aeration scaffolding towards behavioural patterns in its growth in order to identify design parameters.

Objective 5: To develop methods of documenting different aspects of the morphological expression and novel material characteristics of and within the BC forms.

The research of this thesis presents the investigation and development of a fabrication method for BC through an iterative design methodology, which expands the organism's capabilities from growing 2-dimensional shapes into 3-dimensional forms. The transdisciplinary approach bridges understanding of microbiological mechanisms with critical and innovative design thinking to establish a co-designing process with a living system. Lastly, it contributes to the growing body of knowledge for the development of novel biotechnologies in the built environment.

1.3. Thesis Structure

This thesis is structured into nine chapters of which Chapter 5, 6, and 7 form the core of the experimental and physical output of this research. The discussion of results is divided into two categories – biological concepts and design implications – to acknowledge the interdisciplinary nature of this research.

Chapter 2 provides background on bacterial cellulose and state of the art with a focus on fabrication methods and applications. It starts with a distinguishment between different cellulose types before expanding on the metabolic process within the bacterial cell responsible for the fabrication of BC fibres. Existing and potential future uses of BC in the food and fashion industry as well as in the medical and material sciences are summarised, offering the contextual background to position this research. Lastly, the broad spectrum of industrial fabrication processes for BC and individual bioreactor setups is briefly summarised and categorised based on the potential of producing 3-dimensional BC masses.

Chapter 3 comprises of the derivation of the methodological approach to the explorative experimentation in Chapter 5 to 7 and discusses the value of design practice as research method in the field of biodesign. The first part of the chapter illustrates the dependency on hypotheses of the two fundamental modes of research, confirmatory and discovery, following an in-depth analysis of the role of exploration with posteriori hypotheses in interdisciplinary research. The second part introduces the methodology of Design Research before elaborating on the branch Research through Design and its relevance within the field of biodesign research.

Chapter 4 outlines the base protocol to fabricate BC, which is implemented and adapted for the experiments in Chapters 5 and 6. Each of the three core components of the experimental setup, including the nutrient source, inoculation process, and growth vessels, are first explained before different options for each, which can be selectively combined into bioreactors, are presented. The second part introduces the process of growing BC in a static culture and the subsequent step of harvesting the biomaterial from the liquid.

Chapter 5 is the first of three chapters presenting the lab-based research of this thesis and focuses on the exploration of three different materials to function as scaffolds for shape-defining BC growth. The first two scaffold materials, textiles and mycelium, are tested in the context of the parallel development of the BioKnit prototype, which is a collaborative effort to combine the two living materials of mycelium and BC into a human-scale prototype through the use of knitted scaffolding. The third scaffold type, aeration, investigates the potential of guiding the BC growth through interfering with the metabolic process of the bacteria and providing targeted extensions of the air-liquid interface.

Chapter 6 builds on the results of chapter 5 and continues a more in-depth investigation of aeration as a shape-guiding scaffold for fabricating 3-dimensional BC shapes with minimal intervention. The first part of the chapter focuses on identifying behavioural patterns of the BC growth in the presence of aeration scaffolds and how changes of the core components of the experimental setup, summarised in chapter 4, influence those behaviours. This includes the volumetric upscaling of the bioreactor setup. The second part further scrutinises the shape, materiality, and positioning of the scaffold, introducing custom made aeration devices. In addition, the subsequent effect of the scaffold design on the predictability of the emerging BC shape is observed and analysed.

Chapter 7 presents and analyses the development of two preservation approaches, hydrated and freeze-dryed, to capture the essence of the BC shapes fabricated through aeration scaffolding in the previous chapter. Each approach enables the documentation of different characteristics of the BC, including the shape emergence, distribution of material maturity, interior structure, and volumetric expansion.

The **Chapters 5, 6, and 7** include a brief discussion of the potential biological principles behind the observed growth behaviours of the BC in the corresponding chapter and of potential limitations in the methodological approaches. Additionally, a summary of identified starting points for future investigations based on the results of each chapter is given.

Chapter 8 dicusses the broader implications of biodesign research with living materials in respect to their limitations of predictability in responsive behaviours. The methodological approach developed in Chapter 3 is analysed towards its application for the experimentation in Chapters 5 to 7 and the derivation of design parameters for guided BC fabrication. This leads into the argumentation that digitality exists within living organisms and how the term Biodigital Fabrication may be more suitable to define the developed BC fabrication method. Finally, the chapter concludes the research by summarising the results of the research and highlighting the original contributions to the wider field of biodesign with living materials. In addition to identifying existing limitations, it offers speculative future perspectives for the work.

1.4. Collaborations and Publications

The unique setup of the HBBE created what can be called an environment for informal collaboration in which discussions about work progress were regular occurrences during lunch breaks and social gatherings. While these conversations undoubtably influenced the direction of the experiments presented in this thesis, they are not considered as collaborative work. However, those experiments in Chapters 5 and 6 that were driven by active collaborations are marked clearly and the names of the collaborators as well as their contribution are listed. Any tasks not clearly assigned to a collaborator, e.g. experimental design, experiment setup, or data analysis, were contributed by the author. In addition to regular exchanges with fellow researchers and formal collaborations, a key facilitator for the work presented in this thesis was Oliver Perry. As the technician of the HBBE workshop, he offered invaluable support in the fabrication of custom tools, e.g. the Sliceatron 3000 (see Chapter 7.2.1), and the consideration of health and safety aspects of the work. Table 1.1 breaks down experiments including formal collaborations.

In addition, parts of chapters have already been published in journals or conference proceedings:

The results of Exp. D and E in Chapter 5.2 were published under the title “Multi-Organism Composites: Combined Growth Potential of Mycelium and Bacterial Cellulose” in 2021 in the journal *Biomimetics* (Hoenerloh *et al.*, 2022).

The partial results of Exp. A to C in Chapter 5.1 and Exp. D to E in Chapter 5.2 are published as conference proceedings. “Bioknit Building: Strategies for Living Textile Architecture” at the *International Conference of Construction, Energy, Environment & Sustainability* (Scott *et al*., 2021) and “Knitted Cultivation: Textiling a Multi-Kingdom Bio Architecture” at the *Fifth International Conference on Structures and Architecture* (Scott *et al*., 2022).

The developed methodological approach of explorative experimentation in Chapter 3, the complete results of Exp. I in Chapter 6.1 as well as partial results of Exp. K, P, and Q in Chapter 6.2, together with the preservation methods developed in Chapter 7 were published as “Living Morphogenesis: Bacteria-Driven Form Exploration through Aeration Scaffolding” in the peer-reviewed conference proceedings of *ACADIA: Habits of the Anthropocene* (Hoenerloh *et al*., 2023).

Table 1.1: *Breakdown of collaborators and respective contributions*

Experiment	Collaborator	Contribution
B - Fabric Tensioning	Jane Scott	Fabrication of six knitted material samples with various knitting patterns used as scaffold for the BC.
C - Yarn Differences	Jane Scott	Fabrication of knitted material samples made from five different yarns used to test BC adhesion.
D – Mycelium Compatibility	Dilan Ozkan	Joint development of experimental plan and data analysis together with the author; fabrication of mycelium blocks.
E – Mycelium Pocket	Dilan Ozkan	Provision of inoculated mycelium substrate; joint development of experimental plan and data analysis with author.
	Jane Scott	Fabrication of knitted material sample in the form of an open pocket
F - BioKnit	Jane Scott; Ben Bridgens; Elise Elsacker; Dilan Ozkan; Romy Kaiser; Armand Agraviador	Joint development of concept, experimental plan, experiment execution, and analysis of data of the main frame made with knitted fabric and mycelium.
	Oliver Perry	Joint design and fabrication of the large-scale moulds to grow BC sheets inside of.
Chapter 7.1. – Freeze Drying	Joshua Loh	Offering knowledge about material preparation for freeze-drying; handling of the freeze-drying machine.
Chapter 7.2.2. – Computer Tomography	Saimir Luli	Offering knowledge about CT imaging of soft tissue; access to and operation of the CT machine.

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Uneven surface of statically grown bacterial cellulose

CHAPTER 2

BACTERIAL CELLULOSE CONTEXT

Chapter Summary

In this chapter, a literature review on bacterial cellulose (BC) is presented. It introduces the living biomaterial with a focus on design-led fabrication, application, and exploration while acknowledging and integrating the underlying microbiological processes.

The first section briefly introduces the existing types of cellulose before defining which specific type is being used for the research of this thesis. To position the bacterially produced material within the broader landscape of cellulose, distinct similarities and differences are summarised. Following this, the biological process dictating the synthesis of cellulose fibres within the bacterial cell is elaborated on with a focus on identifying potential parameters to influence the formation of bacterial cellulose.

The subsequent section summarises existing fabrication techniques and processes to cultivate bacterial cellulose as a biomaterial. Different types of established bioreactors used in scientific research are introduced before the focus shifts towards design-led and experimental fabrication approaches. The focus of the following comparison of the presented fabrication methods lies on the morphological development of the BC throughout its growth process to identify potential opportunities for interventions.

Moving on from fabrication processes, the third section addresses existing and potential future applications of BC across a wide spectrum of fields, ranging from commercially available products to highly speculative design ideas. The versatility of BC is highlighted together with a shifting mindset of exploring the use of BC as a holistic material to include its living characteristics and behaviours.

The final section synthesises the presented literature to identify an opportunity within BC research with the potential of advancing the understanding of the biomaterial, and consequently, opening up opportunities for future applications. This identified gap serves as the starting point for the research in the following chapters of this thesis.

2.1. Definition of Cellulose

2.1.1. Different Cellulose Types

Cellulose is the most abundant polymer on earth and can be found in every genus of plants, which classes it as a renewable and sustainable material (Heinze *et al*., 2018). On the molecular level, cellulose is a polysaccharide and hence a form of sugar. Cellulose containing materials have been utilised by humans to produce an array of different products for millennia and include those products made from wood and other plants. One of the earliest uses of cellulose in a refined form was for paper making. Beginning with the development of papyrus around 3000 B.C. in Ancient Egypt, developing into the first paper made from plant pulp in Southeast Asia in the 7th century (Wiedemann & Bayer, 1983; Maniaci, 2006). Since then, continuous advances have been made in the isolation process of cellulose to achieve a pure polymer that can be further processed and modified into new materials, ranging from textiles to building materials and food additives (Klemm *et al*., 2005).

Depending on how the cellulose is synthesised, different terminologies are used to distinguish its origin. The most commonly used and available form of cellulose is plant cellulose (PC) which is derived, as the name suggests, from plant material. In plants, the cellulose's function is the protection of the cell's interior and strengthening of the cell walls while not fully removing the eukaryote's ability of adjusting its shape as needed throughout its life cycle. For this, the PC has developed additional functional properties such as good tensile strength and a degree of water permeability (Sakurai, 1991). PC was first discovered in 1838 by the French chemist Payen as a resistant and fibrous residue of plant tissue after extensive chemical treatments. In the following year, the term 'cellulose' was introduced to describe this material (Payen, 1838, cited in Klemm *et al*., 2005). Apart from plants, other organisms and microbes can produce cellulose, which is then referred to as microbial cellulose (MC). This umbrella term is used to summarise cellulose which is produced by microbes such as bacteria, fungi, and algae (Sietsma *et al*., 1969; Northcote *et al*., 1958). The constitution and abundance of the cellulose varies among microbial species. While only selected fungi and algae incorporate this polymer into their cell walls where it functions sim-

ilarly to PC, the bacterial genus *Acetobacter* is the most efficient producer of MC. To distinguish the cellulose synthesised by bacteria from MC, it is more specifically referred to as BC.

The pure form of BC does not include additional polymers, such as lignin, pectin, or hemicellulose, which can be present in plant cell walls (Sakurai, 1991). This higher purity of BC, compared to PC and other forms of MC, implies a less energy-intensive process to harvest the cellulose for further applications and enables a bottom-up approach for biofabrication. In contrast to PC, however, the BC still contains living bacteria when harvested as a sheet and requires steps to deactivate the microorganism and filter out any residue of the culture medium to achieve a pure material. A summary of the differences in physical properties of BC and PC can be found in Table 1. While the chemical formula for both BC and PC is the same ($C_6H_{10}O_5$), the up to 100 times thinner fibres of BC result in a much higher porosity and water holding capacity due to the tighter 3-dimensional network they can form. This makes BC the favoured cellulose types for applications in the medical field, such as wound healing (see Chapter 2.3) (Naomi *et al*., 2020).

Table 2.1: *Comparison of properties for bacterial and plant-based cellulose. Reproduced from Wang et al. (2019.)*

Properties	BC	PC	References
Tensile strength (MPa)	20 – 300	25 – 200	Feng et al., 2015; Gibson, 2012
Young's modulus (MPa)	Sheet: 20,000 Single fibre:130,000	2.5 – 0.170	Lynd, Weimer, van Zyl, & Pretorius, 2002; Nishi et al., 1990
Water holding capacity (%)	> 95	25 – 35	Rebelo et al., 2018; Islam, Taous, & Joong, 2012; Boulos, Greenfield, & Wills, 2000; Goto & Yokoe, 1996
Size of fibers (nm)	20-100	micrometer scale	Monika, Justyna, & Artur, 2011; Genet et al., 2005
Crystallinity (%)	74 – 96	40 – 85	Park, Baker, Himmel, Parilla, & Johnson, 2010
Relative hydrophilicity (%)	40 – 50	20 – 30	Bishop, 2007
Purity (%)	> 99	< 80	Klemm et al., 2005
Degree of polymerization	14000 – 16000	300 – 10000	Tahara et al., 1997
Porosity (%)	> 85	< 75	Elham & Amir, 2013
Total surface area (m^2/g)	> 150	< 10	Islam et al., 2012; Alexander, Ibon, & Jürgen, 2002

2.1.2. Bacterial Cellulose Production

The first discovery of BC in a scientific setting was made in 1886 by A. J. Brown who reported the formation of a thick gelatinous film in a culture inoculated with Bacterium *aceti*, today known as *Komagataeibacter xylinus* (Yamada *et al*., 2012). Since then, other cellulose-producing bacterial strains have been identified (Wang *et al.*, 2019). The cellulosic material is the product of a metabolic process during which the aerobic bacteria oxidise a carbon source to generate energy (Ashjaran *et al*., 2013). Aerobic bacteria require access to oxygen to survive and grow. The mechanisms of the oxidative fermentation by the bacteria, which results in the production of BC, can be summarised into six steps (Figure 2.1):

1. The process begins with the uptake of glucose, or another carbon source, by the bacteria. The simple sugar molecule functions as energy source for the organism.
2. Inside the bacterial cell, the glucose is metabolised into energy through a series of chemical reactions. As part of this, the glucose is converted into individual glucose-6-phosphate molecules. Carbon dioxide is a by-product of this process due to the oxidative nature of the cellular respiration.

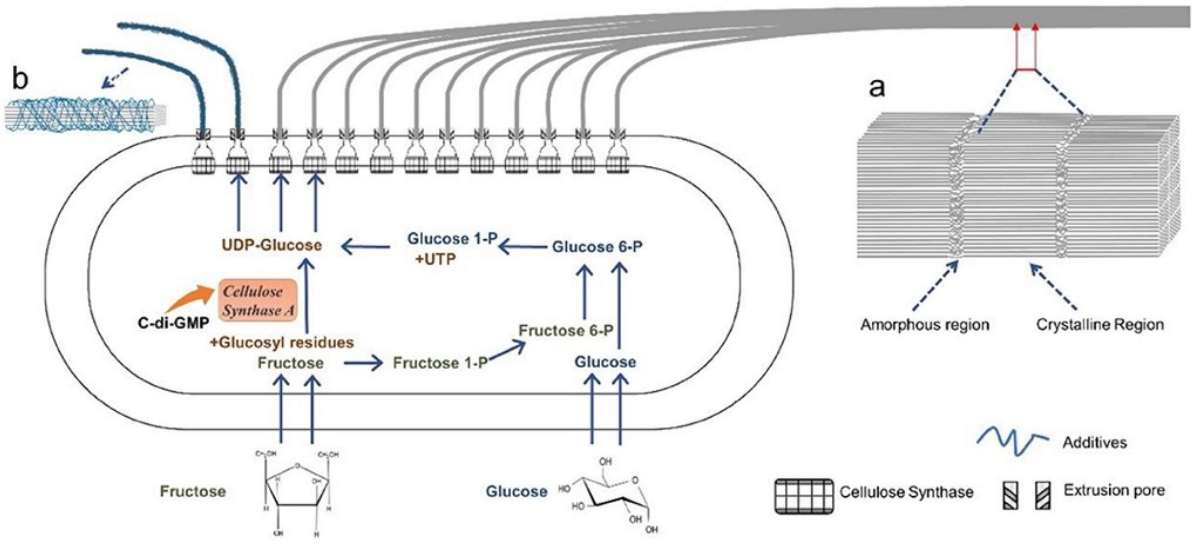

Figure 2.1: *Schematic illustration of the intracellular biosynthesis of cellulose molecules and the extracellular assembly of cellulose molecules into nanofibers by a bacterium. A ribbon-like nanofiber is produced in standard fermentation (a), and loosely-gathered nanofibers are harvested in the presence of additives (b). Reproduced from Zhong, 2020.*

3. Before all glucose-6-phosphate molecules are metabolised into energy, the bacterium *K. xylinus* has the ability to convert some into longer glucose molecule chains. These polymerised glucose molecules form the initial building blocks for cellulose.
4. The polymerised molecules are transported to the outer membrane of the cell before being extruded out. Outside of the bacterial cell, the building blocks assemble into long, parallel chains, called fibrils (Figure 2.2).
5. With growing length, the fibrils begin to intertwine through hydrogen bonding between the individual glucose molecules, resulting in one thicker and longer cellulose fibre. At this stage, the formation of a 3-dimensional network of these fibres begins and the growth of BC can become visible to the naked eye.
6. Over time, more glucose is converted into fibrils and the cellulose fibre network continues to expand and grow in thickness, until it has formed into a strong gelatinous material. If the culture medium is agitated continuously during the fermentation process, the individual fibres accumulate into smaller individual masses instead forming a mat on the surface (see Chapter 2.2.1).

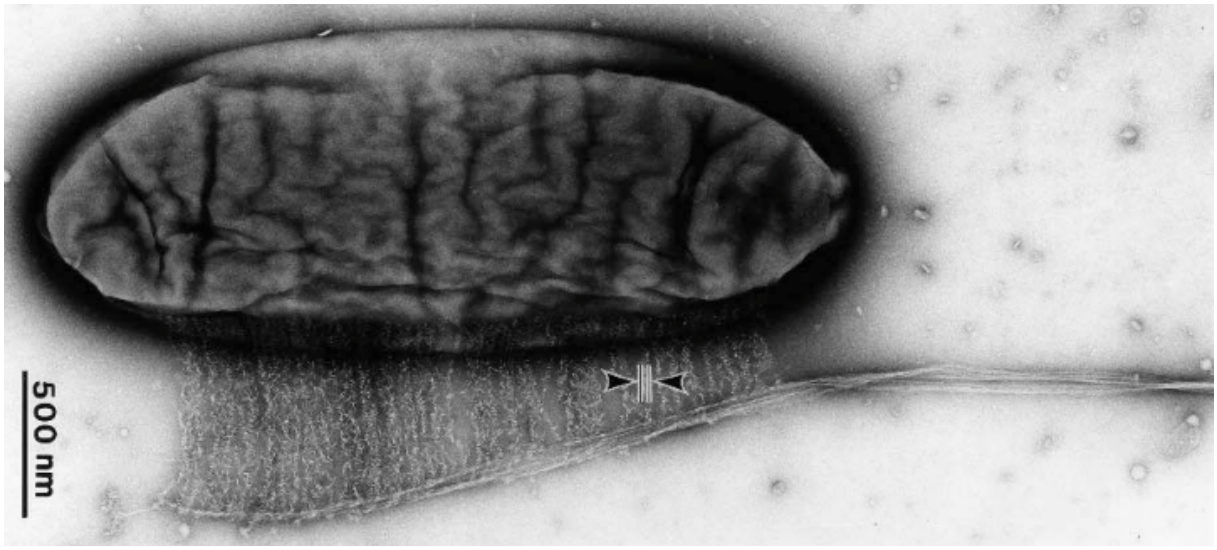

Figure 2.2: *Structure produced during incubation for 5h at 4 °C by the cell which had been incubated for 3 min at 28 °C (negatively stained). The cell was stored for 1.5h at 4 °C before the experiment. Initially the ribbon assembly was produced and thereafter the coarse band-like assembly. The width of the strand between the arrowheads is 24 nm. Reproduced from Hirai, Tsuji & Horii (2002)*

During the fermentation process, in particular during the oxidation of glucose, gluconic acid is produced and released into the medium, which lowers the overall pH of the culture. This change in environment slowly decreases the fermentation activity of the bacteria while simultaneously turning the growth environment more hostile towards other organisms and less receptive to contaminants (Singhania *et al*., 2022). To date, it is not fully understood why these types of aerobic bacteria synthesise BC as part of their metabolism. However, two established theories exist:

1. The first theory categorises the BC as an aid for a continuous metabolic process of the bacteria. The cellulose fibrils connect into a porous network so that the bacteria and fermentation gas, carbon dioxide, become entangled in it. The presence of the gas lifts the sheet to the surface of the nutrient medium where the bacteria have optimal access to oxygen (Ruka *et al*., 2012; Schramm & Hestrin, 1954).
2. The second theory categorises the BC as a form of protection which hinders harmful UV rays from damaging the dormant underlying bacteria. The BC also forms a physical barrier for other environmental conditions and contaminants (Scott Williams & Cannon, 1989). This also includes chemical protection of the organism (Florea *et al.*, 2016).

Figure 2.3: *Bacteria growth curve for bacterial cellulose with four distinct phases. Redrawn from Sushil (2018)*

The rate of production of BC in static culture conditions (see Chapter 2.2.1) correlates with the four distinct phases of bacteria growth as summarised in the general bacteria growth curve (Figure 2.3). The fastest BC growth can be observed during the second phase during which cell division occurs rapidly due to high availability of nutrients and oxygen at the surface of the medium. Once an equilibrium between living and dead cells has established within the culture and the availability of nutrients decreases, the growth speed plateaus. Eventually, the equilibrium breaks, and the dead cells overtake the living ones in numbers. While the remaining living cells continue to metabolise available glucose, the speed of growth significantly decreases (Sushil, 2018).

Another factor influencing the growth speed, in addition to the living cell count, is the thickness of the BC matrix on the surface of the static nutrient medium (Figure 2.4) (Dudman, 1960). Initially, the bacteria throughout the full volume of the medium actively metabolise the glucose utilising the dissolved oxygen. Once this has been depleted, only the bacteria on the upwards facing side of the BC continue to metabolise new fibres while the others go dormant. This aerobic zone with access to environmental oxygen has a thickness of only 800–1000 µm. In this zone, the most influential factor for the continued metabolism is the access to a carbon source, which needs to be transported via diffusion through the lower anaerobic layers of the BC (Hornung *et*

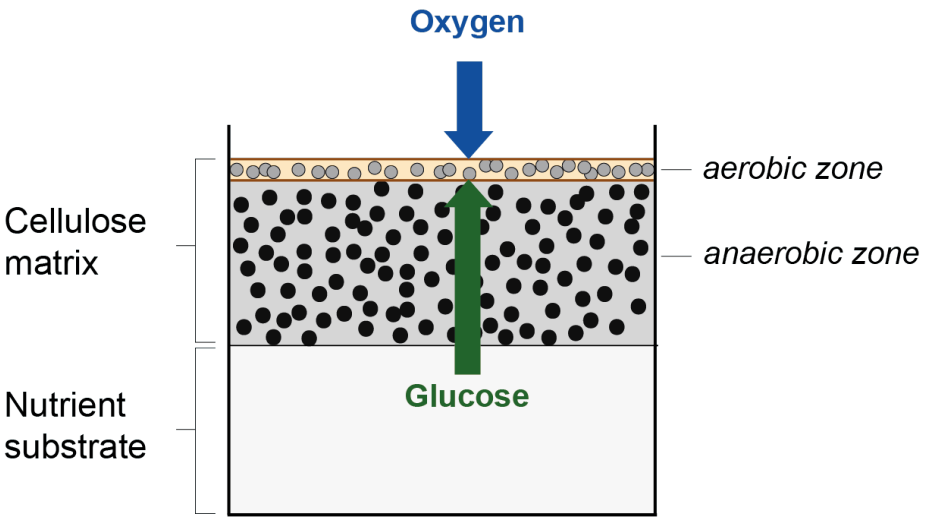

Figure 2.4: *Cross section of static BC culture showing aerobic and anaerobic layer within growing BC sheet. Adapted from Hornung et al. (2006)*

al., 2006). With increasing thickness of the BC layer, less and less carbon molecules reach the aerobic layers, which eventually results in halted BC production.

The BC synthesis process described above refers to a culture that is grown with one single bacteria strain, also called a pure culture. Another culture method for BC is to use a symbiotic culture of bacteria and yeasts (SCOBY), such as the ones commercially used for brewing kombucha. In these SCOBY, multiple bacteria strains and yeasts are combined and their metabolic processes create an interdependence. This co-culturing improves the environment for the BC synthesise while also decreasing the receptivity for contamination faster (Villarreal-Soto *et al*., 2018; Kaewkod *et al*., 2019). During the first stage, the yeasts show increased activity and multiply using the dissolved oxygen in the medium while releasing carbon dioxide. Once the dissolved oxygen concentration declines, the fermentation process begins and the yeasts change to metabolise the glucose into alcohol, more specifically ethanol (Maicas, 2020). While the yeast growth declines, the bacterial growth increases. The nutrient medium now contains two sources

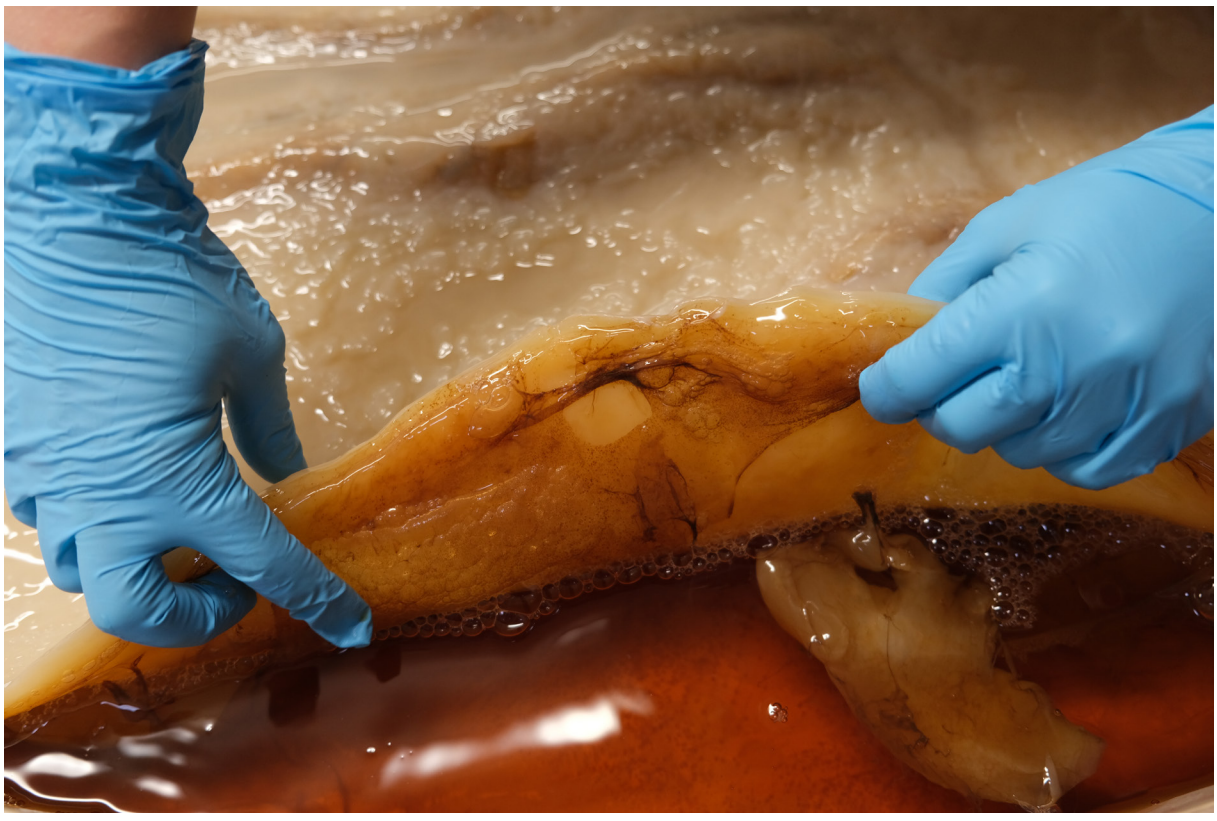

Figure 2.5: *Brown yeast cluster underneath a BC sheet grown from SCOBY culture*

of carbon which the bacteria can metabolise, glucose and ethanol, with the latter enhancing the metabolism of glucose into cellulose (Ryngajłło *et al*., 2019). Similarly to the culturing of a single strain, the formation of BC on the surface limits the availability of oxygen for the organisms within the medium. This environmental condition increases the fermentation behaviour of the yeasts and ultimately increases the production of ethanol for the bacteria to feed on, creating a co-dependent cycle. In addition to the formation of a gelatinous BC sheet on the surface, in a SCOBY culture the dead yeast cells form clusters and strings which attach to the underside of the BC and are brown in colour (Figure 2.5).

Based on the metabolic process, the key factors influencing the BC synthesis and the growth speed of the BC are the availability of oxygen and of a carbon source, usually glucose or ethanol. Both of these factors can be adjusted or manipulated as part of the cultivation setup, such as in bioreactors which are designed to enhance or influence the BC fabrication (see Chapter 2.2.2). Oxygen and glucose, therefore, also show potential as indirect communication link to the bacteria to influence their metabolism and the following BC growth behaviours.

2.2. Bacterial Cellulose Fabrication Processes

With a steadily growing interest in the use of BC in various commercial sectors and fields of research (see Chapter 2.3), different fabrication methods have been developed over time. Depending on the requirements for the BC, such as its shape, physical properties or the overall yield, the fabrication setup can be modified and manipulated as needed. The BC material can be cultured in either fully static nutrient medium or in an agitated condition, with each giving different results of material properties. While there are limited possibilities of modifying a static culture, the agitated state can be induced through a variety of methods. This has led to the development of an array of different bioreactors, all ensuring that the bacteria's requirements for access to oxygen and glucose are fulfilled. These bioreactors allow the optimisation of particular BC characteristics while also defining the required level of human input and energy usage. The BC produced through an agitated process often functions as raw bulk material which is intended to be further filtered and processed into its final form. In addition to established bioreactors, less conventional methods of fabricating BC materials have been explored, predominantly in a creative design setting. It is these design-based fabrication approaches which often intend of utilising the BC as a fully established material in its pure grown state and, therefore, also develop towards influencing the growth shape of the BC.

2.2.1. Static and Agitated Culturing

The traditional and still most commonly utilised culture method is the static cultivation due to the high BC yield that can be achieved and the low-maintenance setup. Depending on the inoculation method and bacterial culture used (see Chapter 4.1), this process does not always require sterile preparation and can be used in Do-it-Yourself projects as well as larger-scale commercial productions (see Chapter 2.3). As the name suggests, this fabrication method utilises an inoculated nutrient medium kept in a static state to facilitate the formation and maturing of BC on the surface (Figure 2.6, left). The advantages of this method lie in the simplicity of the setup as well as the low amount of energy required during the growth stage. Limitations of this culture method are longer culturing times and the required space. Because the fermentation is an aerobic process, the surface area of the growth medium dictates the size of the BC sheet that can be harvested at the end of the growth phase (Figure 2.6, right). Without any additional enrichment of the growth medium with dissolved oxygen, the thickness of the BC growing at the surface is limited by the ability of the oxygen to penetrate the cellulose matrix and reach the bacteria (see Chapter 2.1.2). The cultivated BC pellicle, or sheet, can be utilised in its raw form or blended into a paste for further processing.

Figure 2.6: *(left) Static culturing method of BC with SCOBY; (right) static culture with customised shape during growth and after harvesting*

The agitated, or shaking, culturing method aims to solve a shortcoming of the static culture by supplying the liquid medium with oxygen through continuous movement during the fermentation process. This counteracts the depletion of the dissolved oxygen in the nutrient medium. The term refers to any BC culture which is kept in continuous motion during the growth stage, either inside an orbital shaker incubator (Figure 2.7) or any form of customised bioreactor (see Chapter 2.2.2). Due to the movement of the liquid culture, tall growth vessels, such as beakers and flasks, are chosen instead of shallow wide containers. The BC produced through this method appears in the liquid medium in a variety of shapes, including small spherical shapes, pellets, and irregular masses (Krystynowicz *et al.*, 2002; Schramm & Hestrin, 1954; Dudman, 1960). The size of each pellicle is influenced by the bacterial concentration in the medium, the bacterial strain, the rotation or agitation speed, as well as the culture time and presence of additives. The size of the spheres and pellicles in one single culture can vary and increased growth time can result in individual small masses joining together into one large mass. Aside from the shape of the pellicle, the characteristics of BC from agitated culture also differ to those from static cultivation. A key observation made during of the BC produced via agitated culturing is a higher occurrence of mutant cell, caused by the applied shear stress, unable to produce the BC fibrils (Schramm & Hestrin, 1954; Nguyen *et*

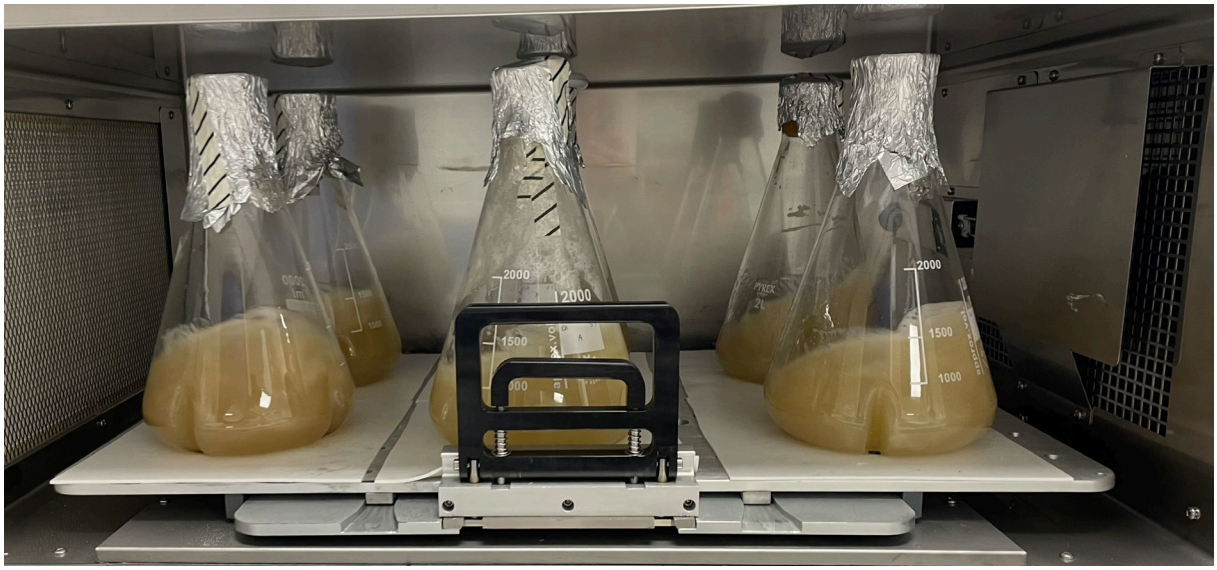

Figure 2.7: *Agitated culturing of BC inside shaking incubators that continuously move beakers to keep the inoculated medium in motion.*

al., 2008; Valla & Kjosbakken, 1982). This can result in a comparably lower yield than from a static culture of the same inoculum concentration and BC with lower degree of polymerisation, a lower crystallinity index, as well as inferior mechanical properties (Watanabe *et al*., 1998; Wang *et al*., 2019). To increase the BC productivity in agitated cultures, the balance between oxygen supply and volumetric agitation power needs to be fine-tuned to the individual culturing setup (Kouda *et al.*, 1998), as well as the compatibility with different bacterial strains and nutrient mediums (Krystynowicz *et al*., 2002).

2.2.2. Bioreactors for BC Yield

When considering a large-scale cultivation or fermentation of BC, designing an automated system which allows adjustments to growth factors, such as oxygen availability, agitation, or nutrients supply, offers a financially feasible solution. Such systems are referred to as bioreactors and are designed with the aim to provide the most suitable environment for a specific biological or biochemical reaction to happen. Oftentimes, bioreactors are designed to automate a manual process to meet the demands of industrial environments, but they can also describe a low-cost setup at the early experimentation stage (Mandenius, 2016). To create the desired biosphere, the environment for the bacteria culture or organism to synthesize, engineering expertise as well as a good understanding of the biological process is needed.

The design of a bioreactor is tailored to meet the specific needs of the organism and can vary widely in the complexity of the setup. The dimensions of the bioreactor also vary depending on the intended use, ranging from small scale systems measuring in mm^3 all the way to industrial and plant scale of up to 500 m^3. The operation mode of a bioreactor can be continuous, batch or batch-fed, usually depending on how the nutrients are supplied, how the cells form within the reactor, and how the final material is harvested. All three operation modes can be implemented alone or as a hybrid model in a bioreactor developed for the cultivation of BC. The most used types of BC bioreactors have been summarised and grouped into three fabrication approaches to compare their BC yield, with a particular focus on the possibility of cultivating non-planar and 3-dimensional material.

Batch Feeding

A batch feeding bioreactor utilises the basic principle of a static batch culturing method with the addition of regular supplementing with fresh nutrient medium or oxygen. An example of a batch fed process is the aerosol bioreactor from Hornung *et al.* (2007) (Figure 2.8, left). Their aerosol bioreactor sprays the BC pellicle growing on the surface of a growth vessel filled with inoculated liquid medium with fresh nutrients in regular intervals after an initial static phase of 5 days during which the pellicle establishes. The nutrient aerosol is being created through ultrasound

waves in a separate tank and directed to a specially designed distribution hood that is placed above the growth vessel. Eight channels connect the aerosol tank to the hood and, by constantly changing the input channel, turbulence inside the hood ensure even mixing and distribution of the nutrients onto the pellicle underneath. Once the aerosol was introduced at continuous 5-minute intervals, a growth of 2mm/day was recorded in the thickness of the pellicle. This linear growth was recorded for over 40 days until contamination occurred. Compared to traditional static cultures, which reach their maximum growth potential after around 20-30 days, the method of batch feeding increases the productive growth stage significantly. While the spraying interval can be adjusted to form one coherent BC pellicle, pausing the aerosol flow for a few hours can lead to the formation of distinct layers which can be separated during harvested (Hornung *et al.*, 2007). Even though this bioreactor achieves a higher yield of BC per m^2 and time than the traditional static culture, it does not answer the issue of space requirements and increases the need for sterility and maintenance throughout the BC fabrication.

A less commercially oriented and hybrid-mode batch feeding bioreactor was developed by artist Stefan Schwabe who explored the idea of a continuous growth which is harvested by slowly rolling up the BC from the surface (Figure 2.8, right) (Schwabe, 2012). Instead of supplying fresh

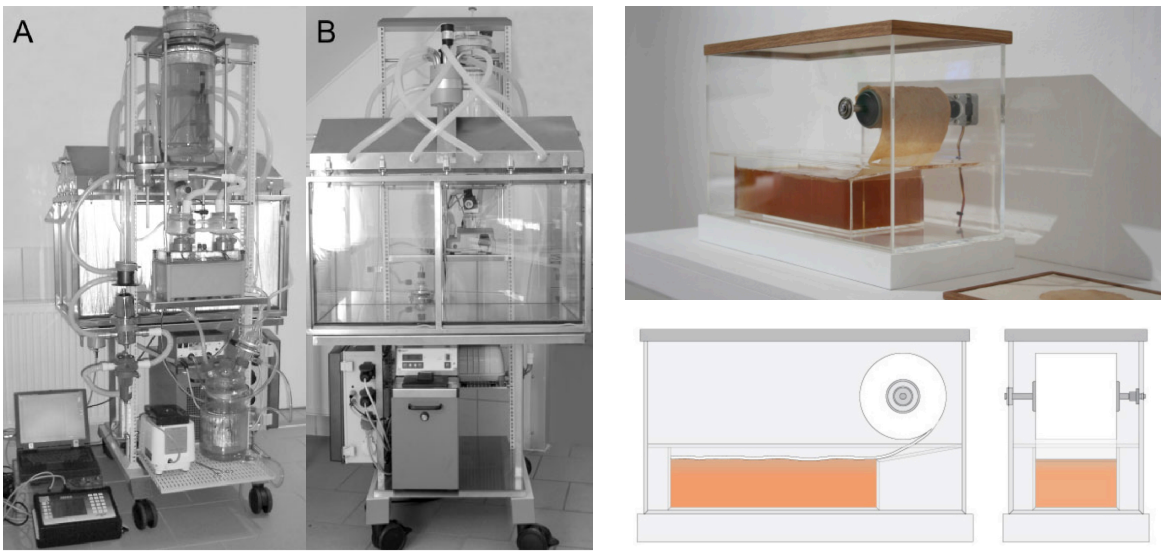

Figure 2.8: *(left) "The aerosol bioreactor (left hand: back; right hand: front)". Reproduced from Hornung et al. (2007); (right) Experimental continuous fabrication setup for growing toilet paper from bacterial cellulose by Stefan Schwabe. Reproduced from Schwabe (2012).*

nutrients from the top, this bioreactor implements the notion of continuously re-exposing the existing nutrient medium by slowly pulling the growing BC to the side and out of the growth vessel. This cultivation method, in theory, can grow one long and continuous BC sheet until all nutrients of the liquid medium is depleted. The possibilities of prolonging the growth cycle by replenishing the existing medium with new nutrients or refilling the growth vessel both exist. However, the speed at which the BC is cultivated remains unchanged to a traditional static culture and, similarly to the aerosol bioreactor, the setup requires additional space and maintenance due to the mechanical components.

Taking the concept of batch feeding a step further is the Engineered Living Manufacturing System (ELF) that incorporates a secondary drip-feeding mechanism which allows the creation of a functionally graded material (Figure 2.9) (Dade-Robertson *et al.*, 2023). ELF is a modifiable bioreactor setup which allows the fabrication of functionally graded BC through the inclusion of synthetic biology practices. In contrast to the interval-timed aerosol bioreactor, the batch feeding mechanism is implemented in the form of a sensor which triggers an increase of the liquid nutrient level as needed once the topmost layer of BC has matured. Before beginning the growing process, a digital 3D model of the desired final material is created. Through horizontal slicing of the model, the exact timing and location of the deposition of an additive or chemical agent onto

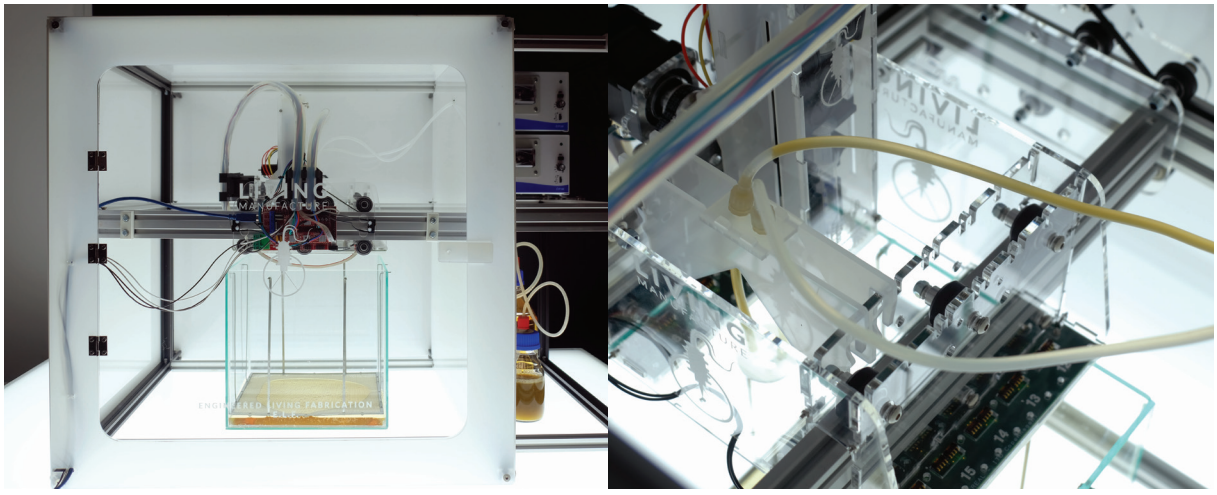

Figure 2.9: *Engineered Living Manufacture (ELF) fabrication system utilising batch feeding mechanisms to grow layered BC and integrate aadditives. Reproduced from Dade-Robertson et al. (2023)*

the growing BC pellicle is calculated. The ELF is an example of an experimental bioreactor which builds on existing culturing methods but aims to produce a final material product that does not require further post-processing before its application.

Two main batch feeding strategies can be identified from the examples. The first strategy relies on adding new nutrients to the existing culture to the top layer of the growing BC, either as aerosol or in liquid form. The second strategy targets the availability of oxygen instead and continuously increases the air-liquid interface. The main focus of batch feeding BC is increasing the yield and, in the case of the ELF, adding desirable properties to the material. But even through adding mechanical and computational elements, due to the underlying principle of static cultivation, the dimensions of the BC are limited by the growth vessel and a customisation thereof would require a time-consuming rebuild of the bioreactor.

Submerged Fermentation

Rotating Disc Bioreactors (RDB) are batch reactors which utilise mechanical movement to increase the yield of the BC and sits at the intersection of static and agitated culturing. Multiple discs are attached to a rod in the centre and half-submerged into the inoculated culture medium, which is filled into a large container on the bottom of the bioreactor. The rod with the arrayed discs

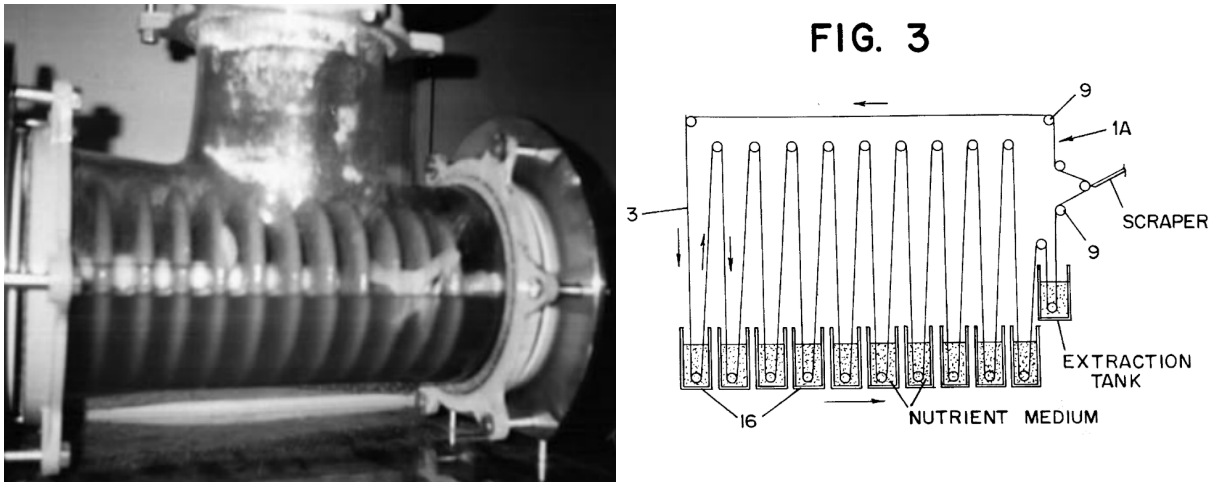

Figure 2.10: *(left) Biosynthesis of BC in the RDF. [...] Cultivation in RDF. Reproduced from Krystynowicz et al. (2002); (right) Conveyor belt batch feeding bioreactor as shown in Patent. Reproduced from Bungay & Serafica (2000)*

is continuously rotated during the fermentation process and the BC matures through a dip-coating process (Figure 2.10, left). The layer of inoculated medium which attaches to the discs, and later to the growing volume of BC, is exposed to oxygen and metabolises BC fast due to the very thin spreading of the liquid. This section of the discs is then re-submerged as part of the continuous rotation and the process is repeated (Serafica, 1997). A similar concept was patented in 2000 and proposed a batch feeding bioreactor with a continuously running conveyor belt which runs submerged in inoculated medium and exposed to environmental oxygen to increase the surface area for the BC to develop on (Figure 2.10, right). In the concept drawings, a scraper can be lowered onto the moving belt to scrape off and harvest the developed BC (Bungay & Serafica, 2000). Beneficial attributes of this fabrication method include the even distribution of the BC across the discs or belt which supports a homogenous maturing of the BC and an increased yield compared to traditional static culturing (Krystynowicz *et al*., 2002). Another advantage of this method is that the culture medium can be enriched with new nutrients, or additives, at any time without disturbing the growing BC. However, even though the yield of BC can increase through this method, it is at the cost of the mechanical properties similarly to the BC of agitated cultures due to the present shear force (Campano *et al.*, 2016).

The plastic composite support (PCS) biofilm reactor is another version of a submerged

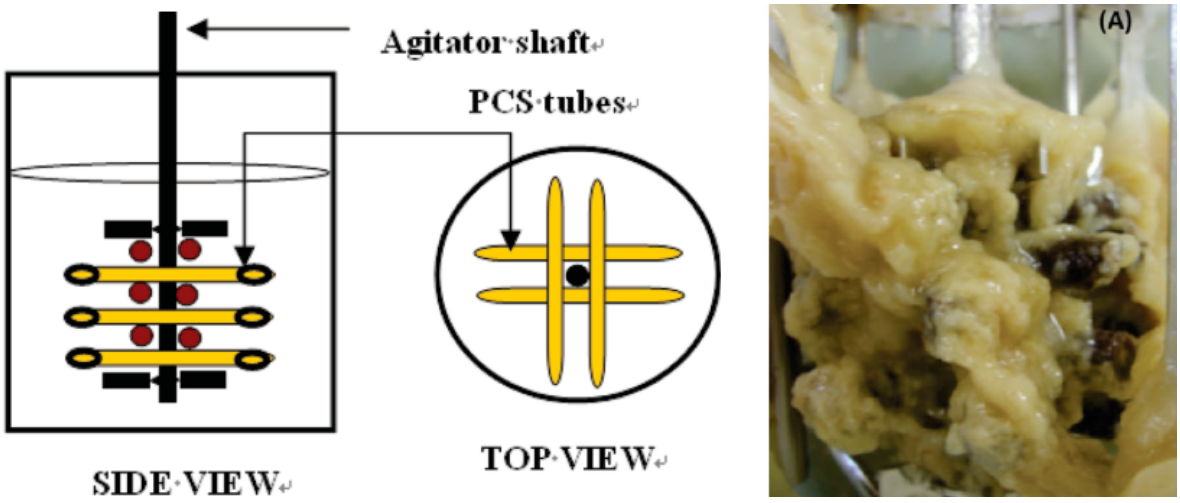

Figure 2.11: *(left) Schematic of PCS biofilm reactor to cultivate BC; (right) BC grown on PCS biofilm reactor after 120 hours. Reproduced from Cheng et al. (2011)*

fermentation bioreactor utilising a rotating motion that can be used to cultivate BC. It utilises a stirring motion of a support scaffold which is fully suspended into inoculated culture medium. The setup resembles the rotating element of the RDB, however, it is fully submerged and rotates along the vertical axis instead of the horizontal one (Figure 2.11, left) (Cheng *et al*., 2009, 2011). Similarly to the RDB, the tubes of the PCS reactor provide a surface for the BC to attach to and begin to layer up into a 3-dimensional mass. The difference between the two methods is the way in which oxygen is supplied. The stirring motion of the PCS reactor increases the dissolved oxygen within the inoculated nutrient medium to enhance the cell metabolism within, while the RDB only increases the oxygen availability for a small volume of medium at a time.

The BC produced via submerged fermentation can achieve more irregular and larger 3-dimensional volumes (Figure 2.11, right) compared to static fermentation. The geometry of the scaffold design and the dimensions of the bioreactor are the most influential shape-defining parameters. This indicates that an interior scaffold on which the BC matures can provide a level of control over the morphological development throughout the growth phase. The submerged state of the scaffold also offers a solution to break away from the otherwise shape-dictating planar air-liquid interface.

Bubble Column

A well-established method for fermentation in a submerged state is the bubble column reactor (BCR), which can be operated in batch, batch-fed, and continuous mode. In addition to aerobic processes, these types of reactors are also suitable for the controlled reaction of oxidation, chlorination, polymerisation, hydrogenation and more. Apart from the fuel industry, these types of reactors are also used for large-scale fermentation processes and wastewater treatment (Kantarci *et al*., 2005). The BCR is designed to increase the oxygen concentration is a large body of liquid and maintain the oxygen level for a specified duration, allowing for the desired reaction to occur at a faster speed. The standard setup of a BCR for BC consists of a large tank, often metal or glass, and an aeration device or sparger attached to the bottom of the tank which is filled with inoculated nutrient medium (Figure 2.12). And while the setup seems simplistic, the underlying principles of multiphase fluid dynamics influence the functionality of the BCR significantly, espe-

cially in chemical engineering applications (Rzehak *et al*., 2017). Design parameters for the setup of the BCR include the column dimensions, the volume distribution within the column, the bubble characteristics, and the internal design of the column, such as the placement and shape of the sparger (Degaleesan *et al*., 2001). An example of an experimental development of the BCR for BC cultivation was developed as part of the Bacto-Basic Project during the International Genetically Engineered Machine (iGem) Competition (SoundBio iGem, 2019). In addition to the standard components of the BCR, the developed prototype includes additional features, such as probes to measure the pH, temperature and dissolved oxygen, a venting system to release gas build-up at the top. An exterior water bath was also added to maintain ideal growth temperatures. Measuring the changes within the culture medium throughout the BC growth phase enables a more precise analysis and understanding of the cell behaviour (Parvulescu *et al*., 2021) and can suggest improvements of the setup in further iteration.

A variation of the BCR that is more specifically designed for aerobic reactions, which also integrates additional probes and design parameters into the built, is the airlift reactor. Instead of

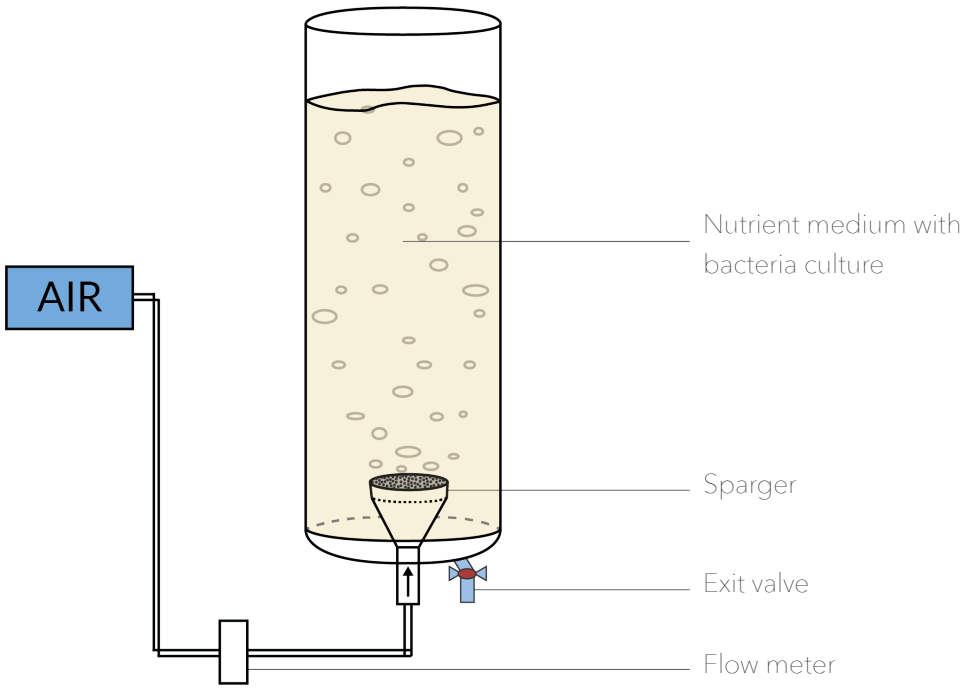

Figure 2.12: *Schematic drawing of a BCR. Adapted from Arjomandzadegan et al. (2014)*

evenly aerating the whole volume of medium, a two-zone system of inner and outer area is introduced to increase and control the liquid circulation with an internal loop (Sharma *et al*., 2015). A tube of smaller diameter than the column is inserted into the centre and the aeration from the sparger is directed only into this area (Figure 2.13). This creates an upwards stream in the centre of the reactor and a slower downward movement in the outer zone which improves the clustering opportunities of BC cells. For the cultivation of BC in particular, various shapes and sizes of the column design have been tested, including spherical vessels (Choi *et al*., 2009; Song *et al*., 2009) and arrayed inner tubes (Zuo *et al*., 2006). The main benefits of this BCR adaptation are improved material properties due to reduced shear stress with higher yield and comparably lower energy-consumption to stirred cultivation (Kouda *et al*., 1998; Campano *et al*., 2016; Shoda & Sugano, 2005).

The BCR and its variations develop and upscale the agitated cultivation method to industrial scales while maintaining a low-maintenance setup without moving parts. In contrast to previously mentioned batch feeding and submerged fermentation methods, the BC cultivated in BCRs is not

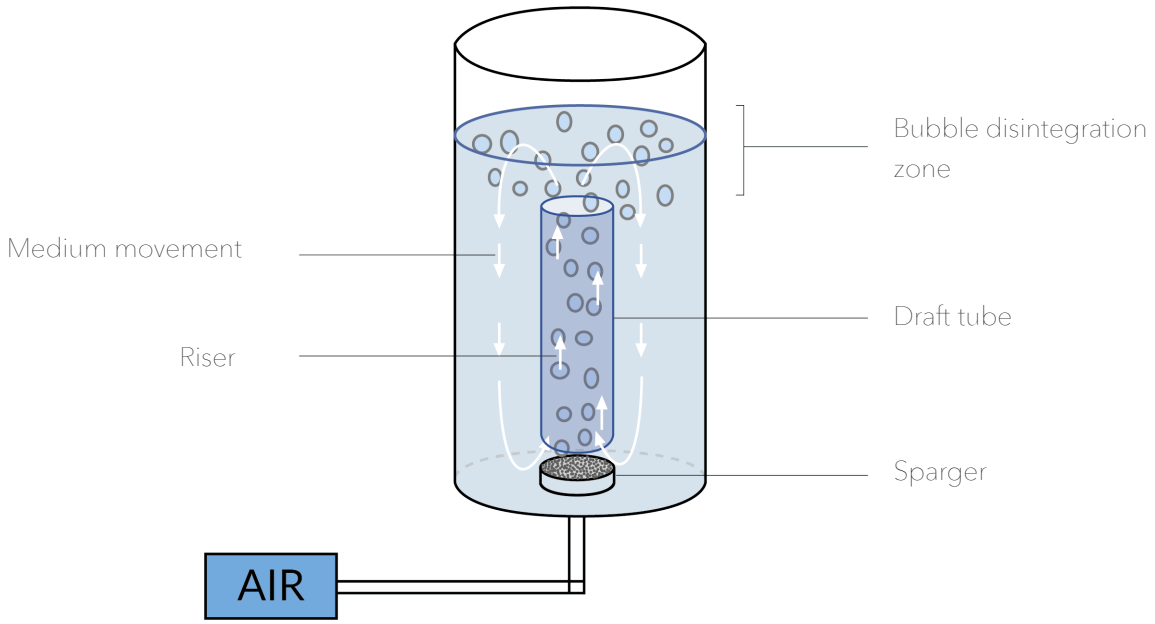

Figure 2.13: *Schematic drawing of an air-lift reactor. Adapted from Sharma et al. (2015).*

attached to any kind of scaffolding but creates free-floating pellicles and masses (Shoda & Sugano, 2005), as described in Chapter 2.2.1. While this process initiates a more organic form emergence, it is heavily dictated by parameters such as airflow direction, strength, and vessel design. An investigation into the potential of BCRs for controlled BC shape cultivation has likely not been considered due to their main focus being on increasing the yield for further material processing.

When comparing the three types of mechanical bioreactors, different approaches can be identified for the establishment of ideal culture conditions in which oxygen and nutrients are available to the bacteria. Figure 2.14 summarises the diversity of BC shapes which can be achieved through the different approaches, showing a morphological diversity not possible to achieve through a static culture method. This indicates that the addition of a moving element to the fabrication setup holds the potential of manipulating the shape development of the BC during growth.

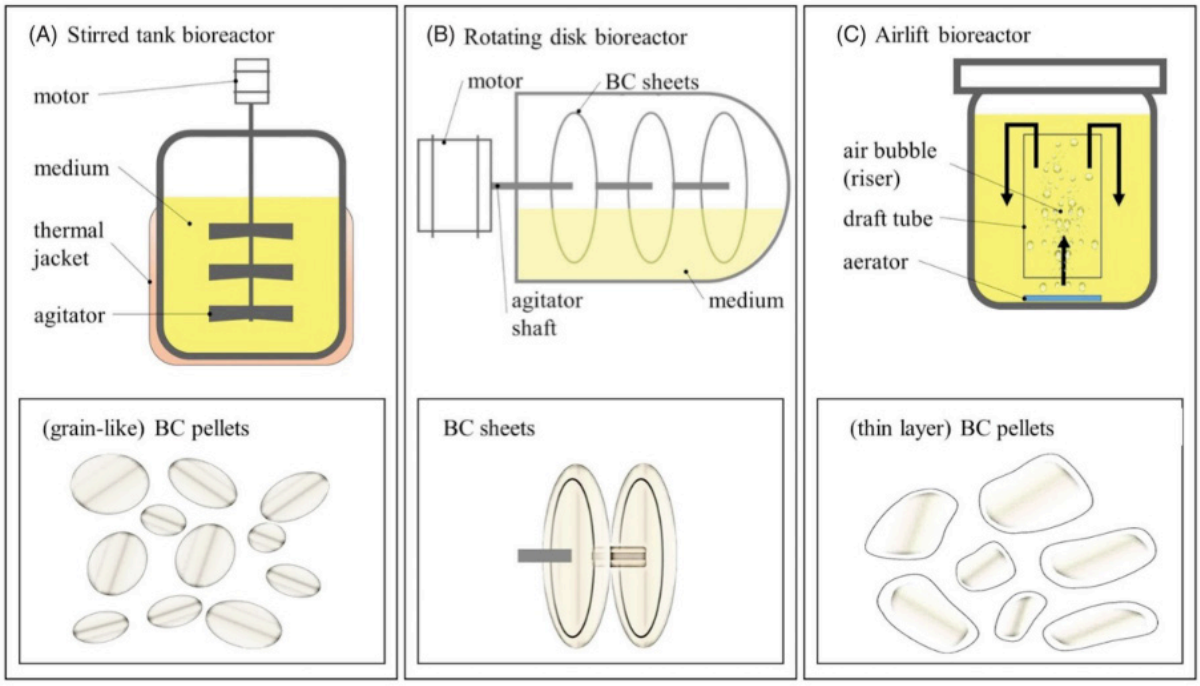

Figure 2.14: *Bioreactor designs (top figure) and shape of BC cultivated from fermentation using the specific bioreactor (bottom figure). (A) Stirred tank bioreactors and grain-like BC pellets with size 5-3mm, (B) rotating disc bioreactors and BC sheets, (C) airlift bioreactors and thin layer BC pellets. Reproduced from Blanco Parte, F. et al. (2020)*

2.2.3. Fabrication Methods for Growing BC ‘to shape’

Unlike the previously introduced methods of fabricating BC in bioreactors, of which all but one focus on increasing the yield of BC mass, only a few selected methods and prototypes exist for shape-oriented BC fabrication. In contrast to the yield-focused fabrication methods, the overall produced mass of BC is of secondary interest in the shape-oriented processes. Instead, these processes are developed with the aim to fabricate BC in the shape required for its intended use to cut out any post-processing steps of moudling, demoulding or shaping. The method of growing BC ‘to shape’ allows the scientist or designer to maintain all of the material’s properties without sacrifices due to breaking up the natural fibre matrix. The scale at which these shapes are grown ranges from small millimetre objects to clothing items and is predominantly limited by the manual nature of these process (Roussel *et al.* 2023). Both, single strain and SCOBY, cultures have been used for these methods.

While the same two principles of manipulating the growth process are adapted for these processes, the majority of example utilise the controlled exposure to oxygen. A process of guiding BC growth into hollow spheres was introduced by Laromaine *et al*. (2018). To create the sphere,

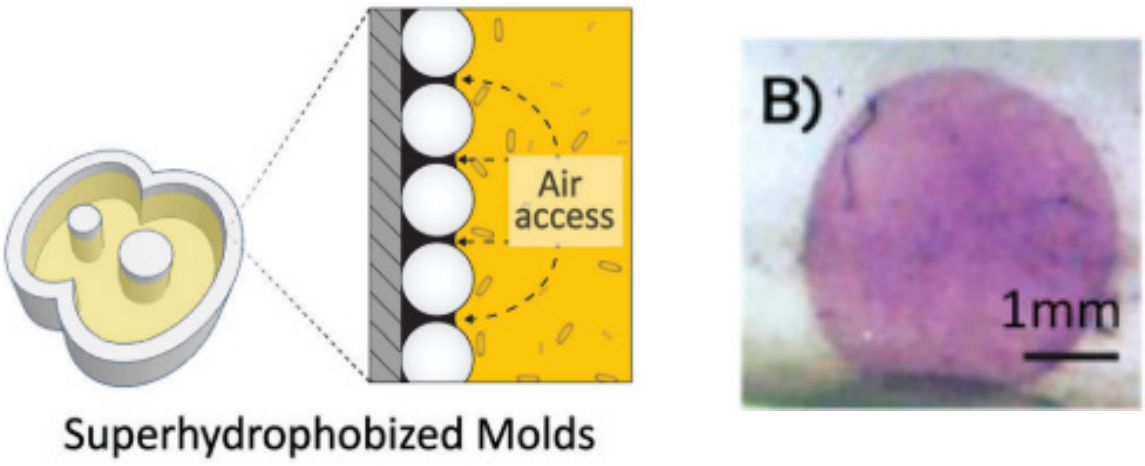

Figure 2.15: *(left) Schematics of the air–water interface stabilization into liquid marbles or superhydrophobized molds. Reproduced from Greca et al. 2018; (right) Digital image of the BC sphere stained with crystal violet to facilitate its visualization. Reproduced from Laromaine et al. (2018).*

a small drop of no more than 8 µL of growth medium inoculated with a single strain of bacteria is placed on a super-hydrophobic surface. Due to the hydrophobicity, the surface tension of the droplet of liquid retains an almost spherical shape while it sits on the surface and is incubated (Figure 2.15, right). The BC growth appears during static growth on the extended air-liquid interface which, in this case, is the outside of the drop. After three days, a thin layer of BC forms which shows a high level of permeability of gas and water (Laromaine *et al.*, 2018). The same concept was applied by Greca *et al*. (2018) to create larger scale and customised hollow BC shells. Instead of a flat super-hydrophobic surface, silicone moulds were coated with a specially developed spray that creates a thin hydrophobic layer between the mould and the nutrient liquid, allowing oxygen to enter to trigger the fibre synthesis of the bacteria in these areas (Figure 2.15, left). However, the BC cultivated through this method only retains its 3-dimensional shape while filled with a liquid or other substance, such as air (Zolotovzky, 2017).

Another method based on the principle of an increased exposed surface area was developed by Ruehs *et al*. in the form of 3-dimensional BC foam (2020). A culture medium inoculated with a single strain is foamed with the help of a stabiliser, xanthan gum, and a foaming agent, cremodan. After an initial incubation time of 24 hours, the network of synthesized cellulose fibres is strong enough to stop the foam from collapsing. The viscosity of the medium plays a vital role

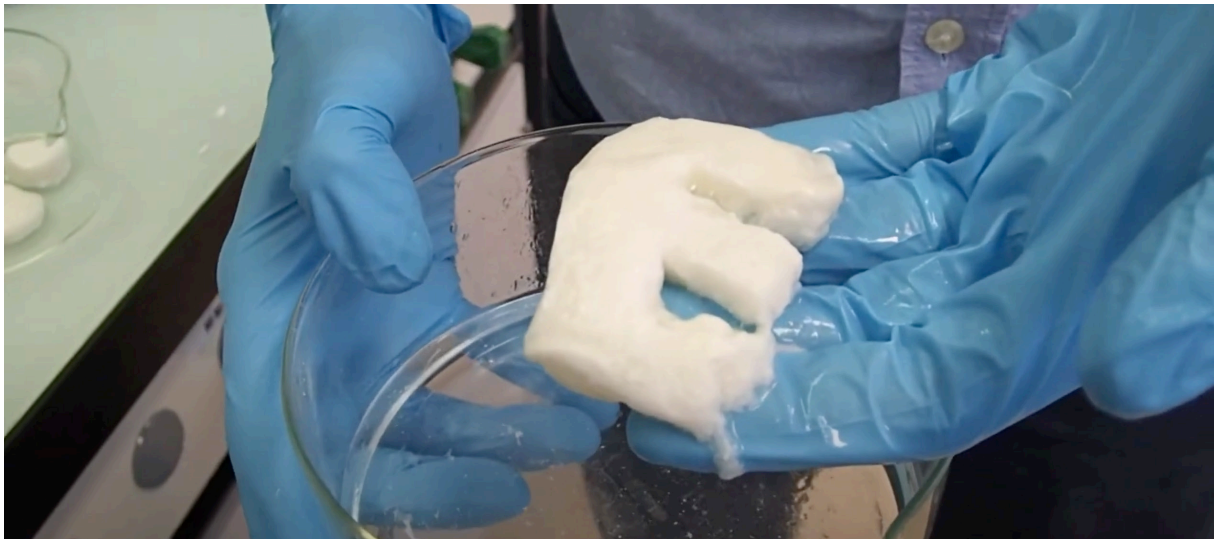

Figure 2.16: *BC foam created in mould shaped as the letter E.Image is a screenshot taken from ETH News for Industry (2019)*

in the process, allowing the bacteria enough movement to spread through the volume evenly, while making the foam structurally stable enough to bridge the time until the BC has grown stable enough. The foam also significantly increases the surface area of the medium exposed to oxygen which speeds up the BC growth process. The inoculated foam creates a malleable matter which can be moulded into any desired shape. Because the foam takes on the shape of the container or mould it is in, an upscaling potential for larger scale 3D shapes exists (Rühs *et al.,* 2020). While this method allows for BC to grow throughout the full volume of the shape, the properties of the material change significantly due to the porosity of the foam template and the additives.

The two previous methods require highly controlled and sterile environments to ensure a precise cultivation of the BC and are therefore limited in their upscaling capabilities by the dimensions of laboratory equipment and the feasibility of the cultivation process. A process developed for the BC cultivation from SCOBY was explored by Chan, Shin & Jiang (2018) to grow the BC into the shape of stencils for clothing items, such as a vest top (Figure 2.17). Based on the static culturing method, inside a wide and shallow container, a negative mould for the stencil is produced in foam and placed on the air-liquid interface. This effectively blocks the access to oxygen in specific areas, resulting in a BC surface pellicle growing in the desired shape. A drawback of this method is that not the full surface area of the large vessel is utilised, meaning that the full

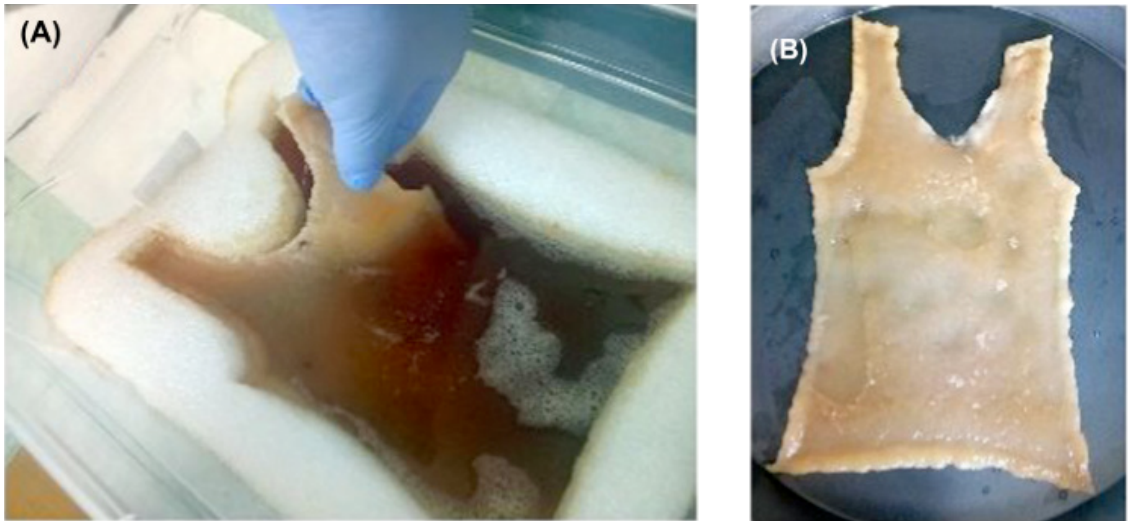

Figure 2.17: *Static culture with polystyrene blocker in order to obtain tailor-shaped BC (Mass Transfer Laboratory, University Politehnica of Bucharest): (A) experimental setup; (B) tailor-shaped BC wet membrane. Bucharest. Reproduced from Parvulescu et al. (2021).*

growth potential of the BC is stunted compared to a traditional static culture.

A manual adaptation of the dip-coating process, which is found in large-scale bioreactors of submerged fermentation, enables the growth of 3-dimensional BC on scaffolds of varying complexities. During the fabrication process, the scaffolding object is briefly submerged in inoculated medium and placed in an incubator until the new BC layer has established (Figure 2.18) (Rühs *et al.*, 2020). This process is repeated until the desired thickness of BC is achieved. The adhesion of the first layer of medium can be influenced through manipulation of the scaffold's surface texture and roughness, either through the choice of material or the addition of coatings, such as polydopamine. The surface roughness presents itself as a design parameter to guide the growth of a material with gradient characteristics based on varying material thicknesses. Compared to PCS biofilm reactors the BC grown via this method resembles the shape of the internal scaffolding

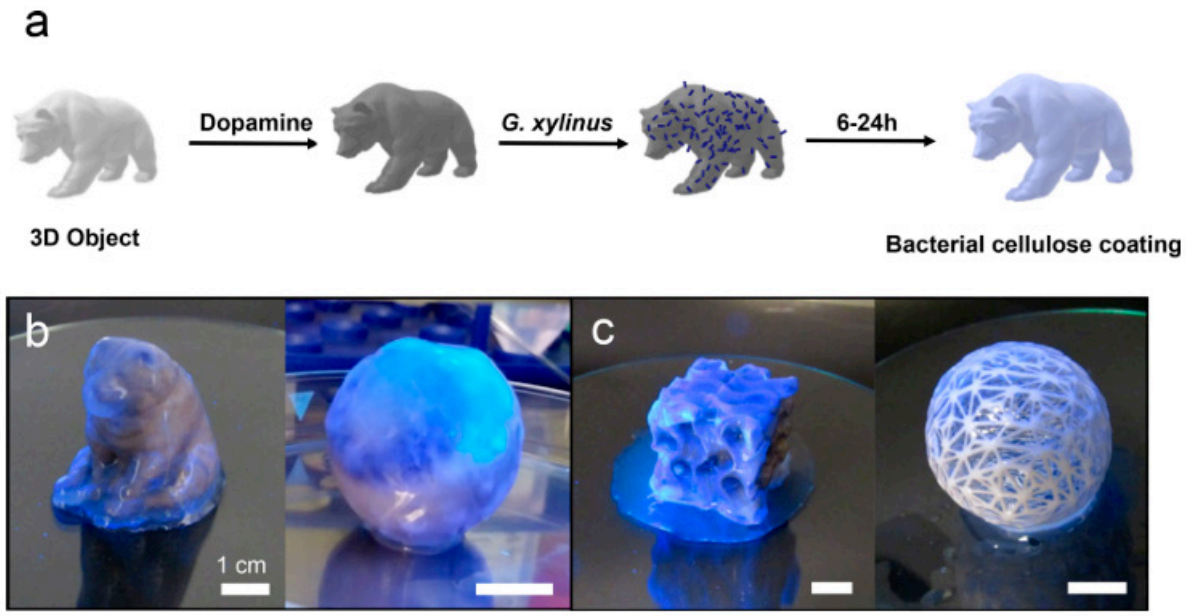

Figure 2.18: *Coating of 3D objects with bacterial cellulose via the surface adhesion of Gluconacetobacter xylinus. (a) Schematics showing the preparation of a 3D object for bacterial cellulose coating via polydopamine deposition. Polydopamine is deposited by immersion in a dopamine solution at pH 8.5.26 Treated objects are then covered with bacteria by dip-coating in a concentrated solution of G. xylinus. After removing unattached bacteria, the bacteria-coated objects are immersed in bacterial growth media to form bacterial cellulose around the objects. (b) Bacterial cellulose coatings formed on 3D nylon objects. (c) Bacterial cellulose grown on nylon objects with complex-shaped architectures. (b) and (c) are UV light images of BC-coated objects dyed with Fluorescent Brightener 28. Scale bars: 1 cm. Reproduced from Rühs et al. (2020).*

more closely. Due to the BC's immediate reliance on the internal scaffold for its shape definition, it can be argued that this method does not create 3-dimensional BC shapes but rather composite materials.

The BC shapes achieved through these cultivation methods range in scale and complexity, each with its own benefits and drawbacks. Because these methods are often developed to solve one particular issue, they are not universally applicable, easily automated, or commercialised. However, the creative approaches to working with the bacteria and its BC synthesis to achieve shape-based results showcase the potential and advantages of the living material.

2.3. Application and Uses of Bacterial Cellulose

The knowledge about BC and ways of utilising it as a material have developed and transformed drastically, in particular during the last century. From a living culture which was utilised to create a fermented beverage and a dessert, it now also holds great potential for applications in the medical field. Advanced explorations in biomedical research have identified promising attributes of BC for its application in the fast-evolving area of tissue engineering. Material research focused on other potential uses are predominantly found in the broad field of Biodesign (see Chapter 1.1), including but not limited to Engineered Living Materials (ELM), fashion design and product packaging. However, the creative and explorative approach to implementing BC as new material is continuously expanding across all areas of life.

Food Industry

The oldest documented consumption of BC dates back to the 18th century. During that time a dessert food called Nata de Piña, which translates to 'cream of pineapple', was discovered in Southeast Asia. It is believed to be an accidental discovery made when a cloth made of pineapple leaves was forgotten in acidic pineapple juice and a gelatinous layer began to form on the liquid surface after a couple of days (Vergara *et al.,* 1999). Nata de Piña became a popular dessert due to its sweet taste, but its availability was limited to the seasonal harvest of pineapple. In 1949, a chemist working for the coconut industry experimented with producing the gelatinous sheet from a different naturally sweet liquid which led to the development of Nata de Coco, 'cream of coconut' (Vergara *et al*., 1999). The food gained international interest in the 1970's which resulted in the upscaling of the fabrication method and the expansion of the production from the Philippines to locations worldwide. Today, Nata de Coco can still be found as a dessert on its own or as chewy addition to beverages.

An even earlier use of the BC-producing fermentation is that of Kombucha tea. Instead of consuming the BC, however, it was the fermented liquid which was of interest due to its believed health benefits. The definite origin of the fermented beverage is still debated amongst historians with many different legends around it. The oldest possible origin, however, dates back to the Tsin

Dynasty around 220 B.C. in northeast Asia, historically referred to as Manchuria (Jayabalan *et al*., 2014), where the tea was traded under the name Mo-Gu. Through the expansion of trade routes, first to Japan and later to Europe via Russia, the beverage gained popularity amongst consumers and scientific research into its health benefits began. The development of the name Kombucha has two possible explanations, with one being the misapplication of the Japanese word 'kombu', meaning kelp and the Chinese word for tea, 'cha' ('kombucha', 2022). The other explanation goes back to a physician Kombu who imported the beverage to Japan in 414 A.D. to treat the reigning Emperor Inkyo (Dufresne & Farnworth, 2000). Nowadays, the term kombucha is used to refer both to the fermented drink as well as the fermentation process with SCOBY.

In most countries, BC is now an approved food-safe additive that can be incorporated into food items either in its raw material form or processed into a thickening and suspension agent (CPKelco, 2024). Depending on its intended use, the BC is cultivated either in a static or agitated setup from a SCOBY or individually selected bacteria strains. And while the human digestive system lacks the enzyme cellulase to break down the cellulose fibres, it is considered an essential component of a balanced diet (Zhong, 2020).

Medical Sciences

The application of BC and its derivatives in the medical and biomedical field is vast and complex due to the intrinsic versatility of the material's nanofibre matrices. Components of plant-derived cellulose have been utilised in the field for centuries (Sindhu *et al*., 2014) to make wound dressings, aid in drug delivery, and to treat renal failure (Hoenich, 2006). BC presents as promising alternative and replacement for PC due to the similarities of these two cellulose types. BC offers additional benefits due to its high purity, comparably easy manipulation through synthetic biology, high biocompatibility, and the speed at which it can be grown. Additionally to the high fabrication speed, BC cultivation offers the possibility of a customised growing-to-shape process which presents as invaluable time-saving feature, especially for application which utilise the material in its hydrated and sheet-like state.

Many review papers of the application potential of BC and the current states of development have been published in the last ten years which provide an extensive overview of the state of the

art (Torres *et al*., 2012; Jankau *et al.*, 2022; Petersen & Gatenholm, 2011). Noteworthy examples of application include medical implants (Sepúlveda *et al.*, 2016) and complex scaffolding for tissue engineering processes. In these instances, the BC is combined with other substances, such as gelatine or collagen, to form composite materials. Preliminary data also suggests that BC can be fabricated to possess various mechanical properties, which could make it suitable for implants in high stress functions, such as heart valves (Mohammadi *et al*., 2019; Millon & Wan, 2006) and the creation of artificial blood vessels with small diameters (Klemm *et al*., 2001). Implants for the cartilage-based parts of ears and noses have also been explored using various fabrication methods (Backdahl *et al*., 2014; Nimeskern *et al*., 2013). Through the high biocompatibility and porous cellular structure of BC, the integration of natural blood flow is possible and allows for an easier attachment and integration into wound sites compared to other implant materials (Kołaczkowska *et al.*, 2019). The same properties make BC a suitable scaffolding material for an array of tissue cultivation, including human skin and bone tissue (Pang *et al*., 2020). The most advanced research of BC for medical application is in the area of wound healing, particularly for burn wounds where the material development has reached the level of multiple clinical trials (Zmejkoski *et al.*, 2018; Portela et al., 2019; Czaja *et al.,* 2007) and the availability of a selected few BC-based wound dressings (Farah, 1990; Frankel *et al.*, 2004). The good water-holding capacity of BC plays a key role in providing optimal moist conditions for healing while also enabling a slow-release process of antibacterial components (Gupta *et al*., 2016; Junker *et al*., 2013). BC for wound dressing is one of few applications for which the material is grown in static condition and utilised in its original sheet form due to its ability of moulding precisely onto different body parts (Sindhu *et al.*, 2014).

While the research of BC in the biomedical field is an essential contributor to understanding its versatile potential, the requirements for sterility, reproducibility, and material purity limit the work to single strain BC cultures from highly controlled lab environments. The published research data from the field is written with a focus on its molecular or cellular structure, making it less accessible to design-based researchers and those who aim to use BC in its raw form with a holistic view on the material's capabilities and its responsive growth behaviours. The knowledge gained from the rigorous research relating to ideal growth conditions and biological synthesis mechanisms, however, provide a strong basis to develop research into shape-oriented growth processes.

Cosmetics, Fashion, and other fields

The advances in BC cultivation and its versatile materiality have sparked interest in its implementation across many other commercial and scientific fields. In cosmetics, BC is being researched as alternative material for petroleum-based components and as a substitute for PC, which currently has many different applications, such as thickening agent, emulsion stabiliser, and texture modifier (Savary *et al*., 2015). A particularly well-researched application of BC is beauty face masks. Similarly to its compatibility for wound dressings, BC as face mask material can increase hydration of the skin and release topical cosmetic pharmaceutical drugs, including antioxidants and vitamins (Nowak *et al.*, 2021). For this, the BC can either be statically grown and cut to shape (Amnuaikit *et al.*, 2011) or processed into a gel. To add specific nutrients, the porous BC material can then be soaked in a bath until the desired concentration of nutrients has been absorbed. Comprehensive reviews of studies around the application of BC in cosmetics were published by Bianchet *et al*. (2020) and Oliveira *et al.* (2022) but will not be discussed further.

An area in which BC is explored as holistic material and as an alternative to leather is the fashion industry. The BC in its raw sheet form is a nonwoven bio-fabric (Parvulescu *et al*., 2021) and its bio-compostable properties show potential to combat the environmental impact of fast-fashion (Niinimäki *et al*., 2020). The statically grown BC sheets, either in hydrated or dried state, can be handled similarly to traditional fabric which is cut to shape using stencils and subsequently sewn together to create an item of clothing. Suzanne Lee (2011) implemented this method to create a fashion line of jackets and coined the term ‘biocouture’ to describe the intersection of biology and high-end fashion. Since then, more designers and companies have explored the potential of BC as fabric material (ONExONE, 2020; Material District, 2019). This has led to the development of various post-processing methods to change the texture, colour, and weathering behaviour of the BC. Taking a different approach to the use of BC in fashion is the start-up company Nanollose who developed a process to spin BC into fibres which can then be woven or knitted into clothing, following a similar process to the one of viscose produced from PC (Nanollose Ltd., 2024). In this instance, BC is utilised as alternative source material for an existing commercial process of fibre spinning. Taking it one step further is the research by Morrow et al. (2023) which discovered a method of growing BC into fibres that can be knitted without further

need of spinning and processing.

More individualised applications of BC in other fields include the development of diaphragms for speakers (AIAIAI, 2023), as food packaging material (Cohen *et al*., 2019), and as conductive material for electronic circuits (Gama *et al*., 2016). Moving away from applications of BC which require sterile conditions, extensive chemical manipulation, and post-processing to extract specific molecular components of the material, allows the emergence of a new bio-materiality in an array of fields and from different research communities (Collet, 2020). And it is within this same movement that the potential of BC as material for the built environment can be explored.

2.4. A Gap in Bacterial Cellulose Research

As the summary of BC utilisation in Chapter 2.3 shows, the biomaterial has been used for decades across different disciplines and has been a part of human daily life in various forms. One area in which BC has not made significant advances in yet, however, is the built environment and building material sciences. The applications of BC presented in Chapter 2.3, whether commercially established or in development, range from utilising only specific molecular components of BC to large material sheets in their naturally flat and unprocessed form. BC is predominantly treated as a bulk material which can be processed similarly to PC to achieve any desired form and shape. This, however, can fail to utilise the full potential of this growing and living material. In some instances, the post-growth shaping of the BC as an additional process is necessary, such as for the implementation of BC in the biomedical field. Here, the final material is required to conform with health and safety regulations. For the fabrication of composite materials, the blending and subsequent mixing of BC with additional components also naturally requires a final step of reshaping the mass. The application of BC in design-based fields, however, can benefit from the ability of growing the material to shape and minimising or fully eliminating the need for a multi-step material fabrication process.

The different methods of fabrication, summarised in Chapter 2.2, show an array of material shapes which can be achieved, ranging from the traditionally grown flat sheet to irregular masses expanding into all three dimensions. The methods of supplying the bacteria with nutrients and oxygen have a direct impact on the morphological development of the BC. Irregular and novel shapes of 3-dimensional BC masses can be achieved during submerged fabrications that integrate an element of mechanical agitation, such as stirring. Further, the addition of a scaffold for the BC to attach to shows an increased upscaling ability of the material. Current state of the art, however, utilises the integrated scaffold as the shape-defining element of the final material state and approaches the fabrication with a desired growth outcome in terms of BC yield and materiality.

A potentially limiting factor in the advanced application of BC is its unknown potential of self-forming and receptiveness to guided form-finding during the active growth stage, even

though first attempts have been made using a combination of physical manipulation and synthetic biology (Zolotovsky, 2017). Through an experimental approach of working with the BC, which allows the designer to develop an understanding of its growth behaviours in relation to various environmental triggers, a fabrication method for controlled and predicted growth may be identified. Instead of focusing on achieving specific material characteristics or BC yield, the intention of the fabrication method lies in the analysis of the organism's receptiveness to external stimuli and the morphological emergence of the BC. Identifying a set of design parameters for growing complex 3-dimensional BC forms can aid the discovery of the material's full potential in advanced future applications.

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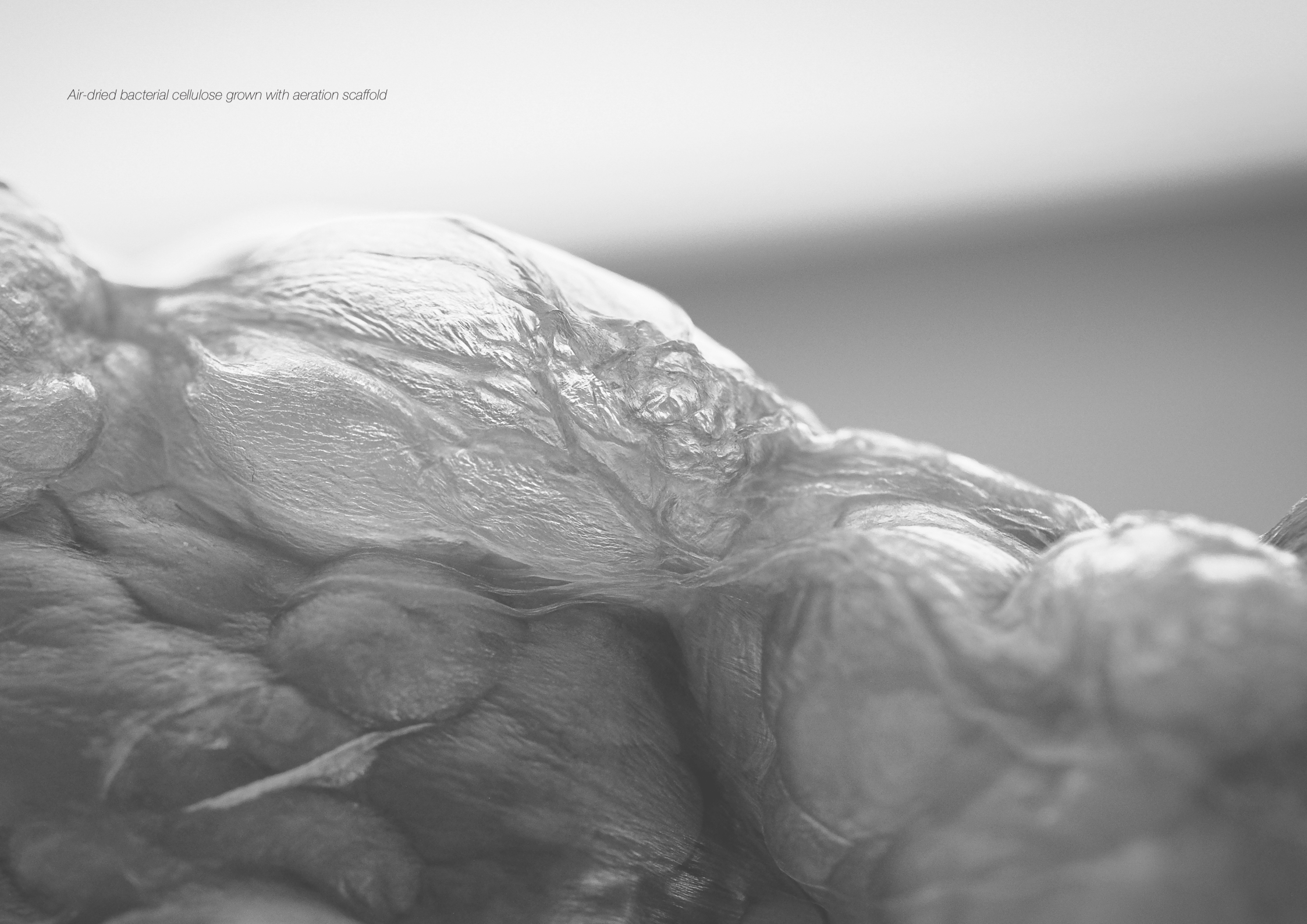

Air-dried bacterial cellulose grown with aeration scaffold

CHAPTER 3

EXPLORATIVE RESEARCH METHODS

Chapter Summary

This chapter provides an overview of different research methods and methodologies, and their connections to interdisciplinary design research, with the aim to establish the context and motivation for the methodological approach developed for this thesis. Thematically, the chapter is split into three sub-sections, progressing from the broadest conventional views on scientific research to more specific design-related methodologies and, finally, the detailed methodological approach for the research of this thesis.

The first section compares the two overarching approaches to research, confirmatory and exploratory, and explores their interdependency. Their connections to a priori and posteriori hypotheses is summarised and the effect each of these two types of hypotheses have on the research development and expectations of the researcher. The origin of the exploratory research approach is summarised as well as the methods employed to undertake an exploratory research project.

In the following section, the role of design and designers in research is explored. The three branches of Design Research (DR) – Research into Design (RiD), Research for Design (RfD), and Research through Design (RtD) – are briefly introduced before analysing the methodological approach of RtD in more detail. The research field of Human-Computer Interfaces (HCI) is introduced as example of applied RtD. Possible adaptations of the methods identified in HCI to make them applicable to the field of bio-design and for working with a living non-human organism are discussed, with a focus on the four areas of prototyping, feedback loops, multidisciplinarity, and process-oriented research.

The final section describes the developed approach for the research presented in this thesis, which is built around exploratory research and DR. Through the use of RtD principles, a mixed-method approach is employed that combines methods from the disciplines of architectural design, material science, and microbiology.

3.1. Confirmatory and Exploratory Research

Scientific research is the systematic and methodical pursuit of knowledge through the formulation, testing, and validation of hypotheses. It is driven by the researcher's curiosity in a particular field of interest and follows the rigorous application of the scientific method to acquire objective knowledge (Wilson, 1952). Broadly, the "scientific method is […] the persistent application of logic as the common feature of all reasoned knowledge" (Cohen, 1936, p.192), and this logic can be split into two categories: general principles and specialised techniques (Gauch, 2003). While the latter is specific to each individual field and continuously developed to fit the scope of the research project, the general principles are expected to be learned and applied by every researcher across disciplines to ensure an objective perspective, high productivity, and innovation (Gauch, 2012).

While the origin of the scientific method cannot be precisely defined, a key figure in its development was Francis Bacon in the early 17th century, who laid the groundwork for what is now known as the confirmatory research approach within the scientific method. Bacon introduced his 'Plan of Work' (lat. *Distributio operis*) divided into six stages, following the bibles testament of the creation of paradise in six days (Dawkins, 2016). In Novum Organum, the second stage, Bacon discusses the discovery and development of new scientific methodology to identify the 'real' truth through inductive methodologies, which offered an alternative to the predominantly deductive methods at the time (Quinton *et al*., 2024). The fourth stage, 'The Ladder of the Intellect' describes the application of this newly developed methodology. The three-step process begins with a proposed truth that is tested through newly developed experiments, followed by a revision of the truth and a further developed experiment. According to Bacon, the iterative and design-based process of analysis, experimentation, evaluation, and redevelopment allows the human to reach more fundamental truths based on observation rather than abstract reasoning (Bacon, 1620[2000]; Buchanan, 2001; Quinton *et al*., 2024).

The most common application of the scientific method is through the use of *a priori* hypotheses, as proposed by Bacon, which are formulated prior to starting the research, based on results from previous research. In this case, the research is used as a tool to test an existing or

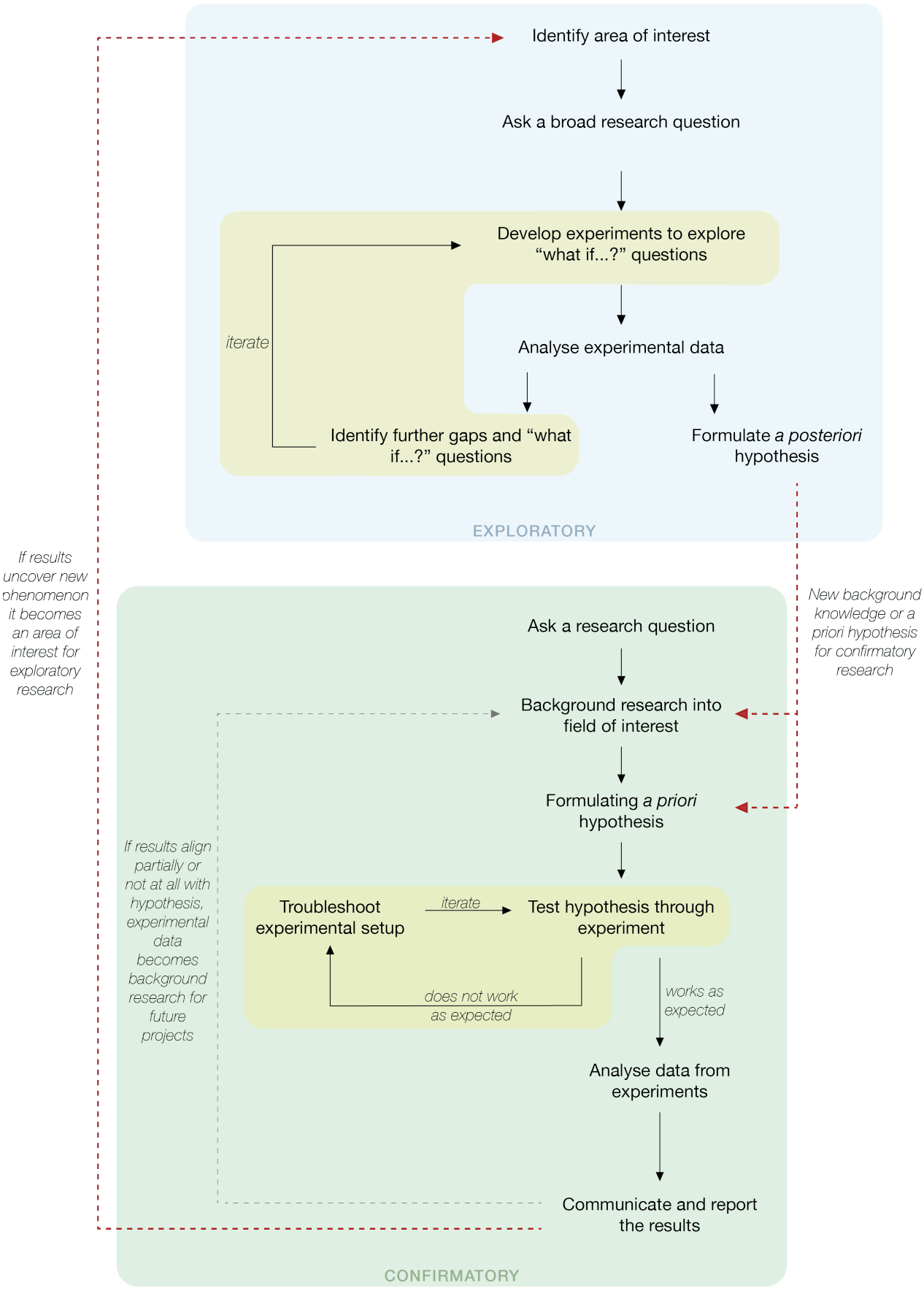

Figure 3.1: *Step-by-step process of exploratory research (top) and confirmatory research (bottom) with indicated interdependency between the two research approaches. Iteration loops within each research approach highlighted in yellow.*

anticipated understanding of a specific phenomenon (Popper, 1963) and is referred to as confirmatory research. While this modern scientific method is not a fixed sequence of steps, it can be summarised through a flow chart with two internal iteration loops (Figure 3.1). The process begins by phrasing a research question about an area of interest, followed by an extensive review of existing literature, data, and results of previous experiments in that area. Based on the acquired foundational understanding, a hypothesis is formulated about a specific phenomenon. The following step of testing the hypothesis through experimentation is iterated until the results of the experiment produce the expected type data. This data is then analysed before the results are reported. Communicating the experimental methods and the results effectively is a key requirement for any empirical science as it ensures that the research is reproducible by other researchers, meaning that the credibility of the results can be confirmed (Schmidt, 2009; Hansson, 2021). If the results align only partially or not at all with the hypothesis, this data becomes part of the background research for future projects and can lead to new research questions or hypotheses to test. It can also uncover fundamentally new gaps in knowledge. Because the hypothesis is based on existing theories it is essential in guiding the direction and execution of the research, making this a theory-led research approach.

Within research through *a priori* hypotheses, multiple approaches to applying the scientific method exist. Two particularly influential voices were Karl Popper and John R. Platt who both identified a potential of confirmation bias if a researcher focuses solely on confirming one predicted outcome. While Popper proposed to aim to refute the hypothesis rather than to confirm it (see Chapter 3.1.1), Platt favoured the use of multiple, parallel hypotheses. He proposed a new systematic method for scientific research and coined the concept ‘strong inference’ (Platt, 1964), which can be summarised into three steps. First, the researcher develops alternative hypotheses thematically surrounding the main one. Second, one or multiple experiments are developed to refute as many of these alternative hypotheses as possible. In the third step, these experiments are executed, and refuted theories are disregarded. The researcher then returns to step 1 and repeats the process, formulating further hypotheses to potentially refute. Instead of testing the main hypothesis directly, knowledge surrounding the specific theory is gained and narrowed down until researched assumptions can be made to deem the project successful. Through this looped but

linear process of investigation, Platt claims quick advancements can be made in a scientific field due to the exclusion of distracting irrelevant theories and the deepening understanding of the topic that the researcher earns, which leads to more definitive conclusions (Platt, 1964).

Another application of the scientific method employs research as a tool to understand unexplained or previously undiscovered phenomena and is referred to as explorative research or discovery research, due to its nature of discovering new theories progressively (Brink & Wood, 1989). Instead of formulating hypotheses prior to starting the experiment, this approach focuses on broadly exploring an area of interest first and then developing hypotheses based on experimental observations. These inductively generated hypotheses are referred to as *a posteriori* and are predominantly used when researchers aim to make advances into unexplored fields or phenomena to lay the foundation for new knowledge (Franklin, 2005). In contrast to theory-led research with *a priori* hypotheses, this approach is often chosen when existing literature does not provide sufficient, or any, answers and practice-led research through exploration is required to establish foundational knowledge in the field (Franklin, 2005). Research built around *a posteriori* hypotheses aims to employ a holistic viewpoint to their research, providing an overarching understanding that considers all scales and levels of the work (Brink & Wood, 1989). Similarly to confirmatory research, exploratory research begins with the identification of an area of interest and the formulation of a research question. In the following step, experiments are developed and conducted to explore a multitude of "what if…" questions surrounding the main research interest. Based on the analysis of the acquired data, new gaps in the research field are identified and further branches of exploration are iterated on. Simultaneously, the researcher can formulate *a posteriori* hypotheses to create theories of how the data can be explained. In contrast to confirmatory research, which relies heavily on quantitative data collected through experiments (Platt, 1964), exploratory research data can be both experimental or observational (Jaeger & Halliday, 1998).

The broad viewpoint which exploratory research takes on the research question allows the researcher the "simultaneous measure of many features of an experimental system" (Franklin, 2005, p.888) by creating new branches of investigation. It is this all-observing mindset which philosopher Francis Bacon idealised in his interpretation of explorative research. He stated that scientists shall not form theories about their subject of interest prior to experimentation but to

explore “without premature reflection or any great subtlety” (Bacon, 1620[2000]). This idealised approach to begin without any previous knowledge, however, would produce a dataset so large that it could only, if at all, be processed with the help of supercomputers today. It is, therefore, neither a realistic nor an economic approach for most research projects. Instead, the researcher can take advantage of existing background theories, knowledge, and understanding to broadly frame the phenomena being explored, creating a research focus while not narrowing it down to one specific hypothesis (Franklin, 2005). The repeated process of generating new and increasingly intricate hypotheses regarding a phenomenon within this focus eventually achieves a deeper understanding of the phenomenon and its surrounding conditions (Reiter, 2017). It is this growing number of untested hypotheses generated by explorative research which allows a starting point for confirmatory research and creates a dependency of the two approaches (Figure 3.1) (Jaeger & Halliday, 1998). Broadening the field of interest from a singular hypothesis and asking open-ended questions of ‘what if…?’ exposes specific areas of interest to investigate further in theory-led *a priori* research. Vice versa, datasets from confirmatory research can narrow down and uncover completely unknown phenomena which initiate background exploration (Franklin, 2005).

3.1.1. Exploration as Research Method

Exploratory research is described as inductive process which continuously introduces new ideas and theories to the pool of knowledge. It is a way of "making sense" (Reiter, 2017, p.144) rather than striving for a specific truth. By injecting the research process with new pathways to explore, the researcher avoids the risk of fixating on a single theory and pursuing it to the point of diminishing data returns. This process has been described by the German philosopher Hans-Georg Gadamer through the hermeneutic circle (Figure 3.2), which was originally introduced by Martin Heidegger in 1927 as a concept of a process to understand and interpret text more holistically (Gadamer, 2004 [1994]). The hermeneutic circle attempts to capture the complex relationship between the text, its interpretation, and the interpreter's existing beliefs, knowledge and expectations. The researcher figuratively enters the circle of exploration after acquiring broad background knowledge and preliminary explanations of the phenomenon of interested. They then collect data of any type, including historical, contextual, or even biographical info about the researcher who discovered the above mentioned background knowledge to "compare details to context" (Gadamer, 2004[1994], p.145). The latter is particularly relevant in theological research, where the

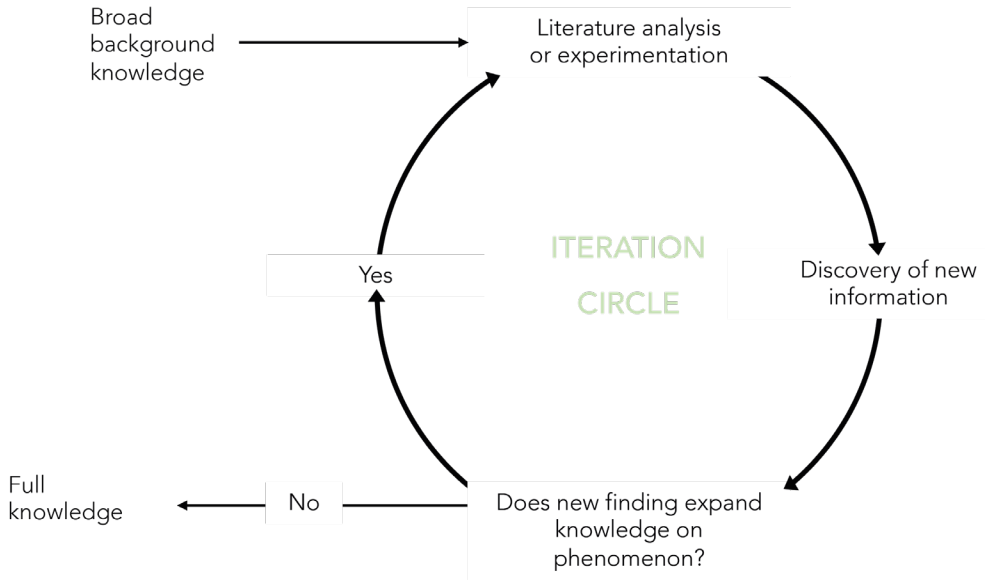

Figure 3.2: *Concept of the hermeneutic circle for knowledge generation after Heidegger.*

hermeneutic circle is a key methodology to interpret scripture. The growing collection of data subsequently uncovers new areas and aspects of the phenomenon which results in a more in-depth understanding of it. As new fields of interest open up during the data collection, the researcher continues to ask new questions and collect data to answer them (Gadamer, 2004). The circle will only come to a close once all new data purely adds to the already discovered information rather than introducing new areas of interests (Reiter, 2017).

Another strategy to explain the design of explorative research is the implementation of the bottom-up approach which generates new theories to expand on the research topic (Sabatier, 1986). The iterative nature ensures that newly gained knowledge is incorporated to further steer the direction the research, ensuring continued relevance of the research. The underlying process is a development from data and observations to noticeable patterns, and ultimately to posteriori hypotheses. This approach allows the researcher to explore multiple directions of interest before narrowing down the investigation based on observed connections between observations. Instead of the converging top-down approach of confirmatory research, the diverging bottom-up approach visualises into a pyramidic shape with four steps (Figure 3.3, right). The smallest part of the pyramid defines the base level of understanding and assumptions about the research topic,

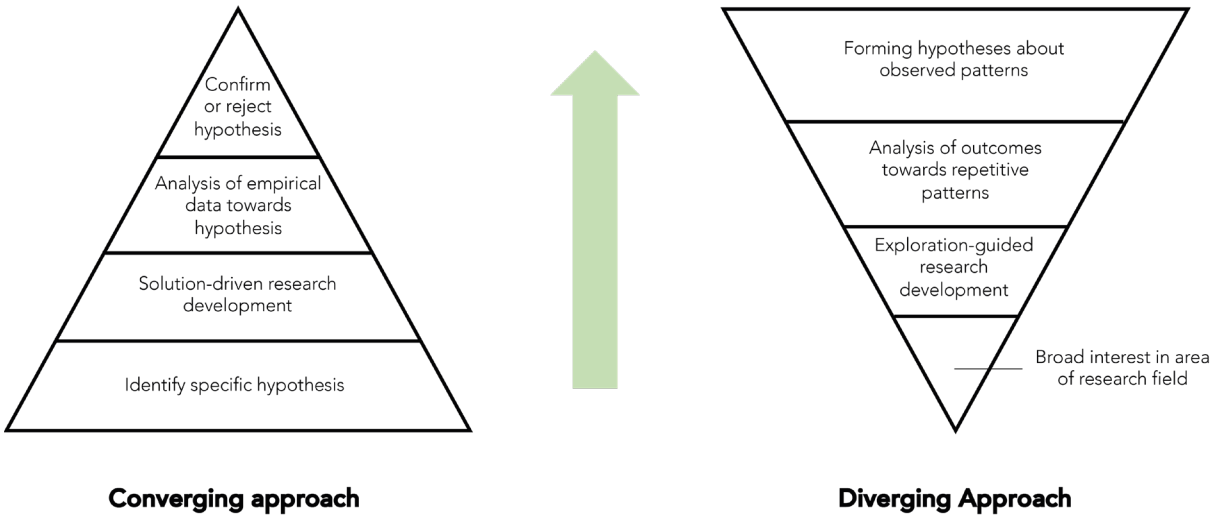

Figure 3.3: *Comparison of a bootom-up converging (left) and top-down diverging (right) research approach*

which defines the start point for the investigation. The active research then begins with the initial exploration and collection of data within the defined research scope, before moving towards an analysis of repetitive behaviours or characteristics. In the final step the researcher draws tentative conclusions from the analysis and formulates hypotheses to explain elements of the research topic (Sabatier, 1986).

Before beginning the active stage of the research, the researcher must choose how narrow or broad they wish to extend the scope of exploration by defining the background knowledge and theories. This will highly depend on the phenomena being investigated and the nature of it, as well as the media utilised to collect the data (Brink & Wood, 1989). For the scope of the research to remain adaptable throughout it requires continuous iterative reconsideration. This ensures that the researcher continues to be receptive to unexpected data recoveries and explanations (Bacon, 1620[2000]) but simultaneously allows them to identify a starting point for more detailed investigation (Franklin, 2005). This is also where the importance of the background knowledge lies. Before arguing with or against an opinion during the research process the researcher is required to form their own judgement first, as “criticism never starts from nothing” (Popper, 1963 p.238). Defined as background knowledge, in this instance, is every reliable un-refuted theory surrounding the field of interest which the researcher is aware of but doesn’t question during the critical investigation (Reiter, 2017; Popper, 1963). An additional benefit of incorporating background knowledge is the potential to identify an endpoint of the research and close the hermeneutic circle, which is near impossible when not considering existing theories (Reiter, 2017).

Philosopher Sir Karl R. Popper (1963) overarchingly theorised about confirmatory approaches throughout his lifetime. However, by pointing out the shortcomings of the approach he makes an argument for the importance of exploratory research data and its credibility within the science community. At the core of Popper’s understanding is that scientific knowledge grows by repeatedly overthrowing scientific theories to be replaced by better ones, with the definition of ‘better’ being that the theory has not yet been proven to be wrong but is testable by scientific means. This understanding has been widely accepted as core mentality within scientific research today (‘Criterion of falsifiability’, 2024). He emphasizes, however, that this deductive process of eliminating possible truths will not ever result in reaching the final, unimpeachable truth the researcher may

be striving for. It is not possible for the researcher to have certainty that an un-refuted theory will not be proven wrong once a different experiment setup is tested or newer technologies are developed. Additionally, the confirmatory research is more likely to develop into a dead-end because of the selectivity of the linear theory testing (Popper, 1963) (Figure 3.1). This is where explorative research differs through unpredictability in its development which can result in the collection of diverse data sets. For this, however, constant flexibility of the researcher is required to be able to fluidly adapt the methods and procedures employed (Brink & Wood, 1989). Contrary to confirmatory research, in which experiments are often considered as failed when the collected data discredits the hypothesis, the exploratory approach is most successful if the researcher adopts a 'no failure possible' mindset and identifies all gathered data as a success in the advancement of the investigation. Explorative research outcomes leave room for future alternative hypotheses by acknowledging that all explanations and evidence are either partial or incomplete (Reiter, 2017). Furthermore, due to the continuous development of new technologies and scientific methods, refutation of a theory, or parts of it, have to be anticipated. It should not be seen as a sign of failure because the researcher gained a deeper understanding nonetheless and can develop further research from it (Popper, 1963). And as Bacon fittingly states "[t]ruth emerges more readily from error than confusion" (Book II. XX, 1620[2000], p.130).

3.1.2. Exploratory Research and Interdisciplinarity

Exploratory research requires the researcher to look beyond existing theories and experimental methods in order to uncover new phenomena and to establish explanations for them (see Chapter 3.1.1). A possible approach to this is the combining of experimental methods and theories from multiple research fields through collaboration which can be summarised into three types: multidisciplinary, interdisciplinary, and transdisciplinary. While these terms are often used interchangeably, they each define a different approach to collaborative work. Choi & Pak (2006) identified the following definitions:

> **Multidisciplinarity:** "Draws on knowledge from different disciplines but stays within the boundaries of those fields" (NSERC, 2004 cited in Choi & Pak, 2006, p.353)
>
> **Interdisciplinarity:** "The ability to analyse, synthesize and harmonize links between disciplines into a coordinated and coherent whole" (CIHR, 2005 cited in Choi & Pak, 2006, p.354)
>
> **Transdisciplinarity:** "[Provides] holistic schemes that subordinate disciplines, looking at the dynamics of whole systems" (Choi & Pak, 2006, p.355)

To further distinguish between the last two terms, transdisciplinarity has been defined as "[a] specific form of interdisciplinarity in which boundaries between and beyond disciplines are transcended and knowledge and perspectives from different scientific disciplines as well as non-scientific sources are integrated" (Vrije University Amsterdam, 2005 cited in Choi & Pak, 2006, p.354). This suggests that 'interdisciplinary' can be used as an umbrella term to refer to collaborative work with two or more scientific and non-scientific fields in which methods and knowledge are integrated into one to address complex research questions and real-world problems. It brings together researchers and research techniques from different fields to work towards

a common goal, utilising the unique perspectives and expertise of each field to achieve a more comprehensive understanding or solution (Aboelela *et al*., 2007).

Each field contributes its own set of methods for data collection and data analysis which requires a flexible, and often unique, approach to the specific problem being investigated. To purposefully combine the fundamentally different research methods from multiple fields it is necessary for the researchers to gain a holistic view of the problem investigated and the research fields involved. In the medical field, holistic refers to "perspectives that do not reduce human experience to a single dimension of descriptors" (Aboelela *et al*., 2007, p.330). In Biodesign, it more directly refers to a multi-scale perspective that does not conceptualise living organisms as mere technology but rather considers their inherent complexity and capabilities in the larger context of design problems (Crawford, 2022). Because successful interdisciplinary work is a learned skill, Nancarrow *et al*. (2013) propose a set of principles. While these principles were proposed for teamwork within the healthcare sector, many of them can be generalised and transferred to individual interdisciplinary research projects. These include the importance of identifying framing objectives, the development of a common language, and the adoption of a flexible approach which benefits from continuous re-evaluation and adaptation. Edwards *et al.* (2000) agree that, due to the uncertain nature of interdisciplinary work compared to working within the boundaries of only one field, flexibility is a key element of the research approach.

In exploratory research, flexibility is a key requirement for multiple reasons. Not only does a flexible and open-minded mindset of the researcher allow for the fluid redirecting of the research based on findings, but it also ensures that the research itself can address a wide array of different research questions. Another link between exploratory research and interdisciplinary work is that the process of merging methods from multiple disciplines inevitably creates new perspectives, tools, and mindsets (Choi & Pak, 2006). This also develops a new way of problem-solving by the researchers as they combine their abilities of relating to the problem from through background experiences and enhance their "ability to rearrange those experiences in new relations to meet the new situation" (Billings, 1934, p.260). As a result, new and customised tools, whether theoretical or experimental, are developed to approach complex and new research questions. This synergy indicates that interdisciplinarity is a desirable attribute for exploratory research.

When approaching explorative research within the relatively new field of Biodesign, interdisciplinarity is inherent to the process through its merging of biological and creative design methodologies. The development of new tools for Biodesign research is essential because existing tools for designing and planning will not suffice to fully understand the extend of new biotechnologies. By relying on processes already known and used for non-living systems, the biodesigner risks "collaps[ing] the multidimensionality inherent in the organism" (Ramirez-Figueroa, 2018, p.4). Biomimicry (see Chapter 1.1.1) is an example for this top-down approach in Biodesign in which a variety of biological processes are analysed towards their beneficial properties for the built environment but eventually translated into already existing material systems using tools. The uniqueness of the biological components, and arguably its relevance for living biomaterials, are lost along the process and the approach of biomimicry is reduced to a design for aesthetic rather than functionality (Armstrong, 2011). Therefore, employing an explorative approach to understanding living systems is essential to create a holistic view which acknowledges their partly or fully unexplored multidimensionality and complexity.

3.2. Design in Scientific Research

Design is a creative practice that involves the imaginative process of conceptualising and crafting visual, functional and aesthetic solutions to address specific problems. It has always played a critical role in scientific research, whether as a tool for the development of experimental apparatus, the generation of visualisations to aid in data presentation and interpretation, or the theoretical development of research studies themselves. In the last century, there has been a growing interest in the use of design as a research method in its own right, which had led to the emergence of a new research methodology called Design Research (DR). Research through Design (RtD) is a branch within DR that is of particular importance for the research approach in this thesis and will be introduced in this sub-section.

3.2.1. Origin of Research through Design

Research through Design as a research approach is part of a larger, more comprehensive methodology of Design Research (DR), which can be traced back centuries to Francis Bacon and, arguably, Galileo Galilei (Buchanan, 2001; Llasera, 1986). While neither of these philosophers used the direct terminology of DR, their publications describe a way of working which is linked to design-based practices of acquisition of knowledge. In Francis Bacon's lifework Instauratio magna, translated from Latin to 'Great Instauration', first published in 1620, he described his dream of recreating paradise on earth but with the important difference of human beings having reached full 'Enlightenment' (Zagorin, 1998) (see Chapter 3.1). The enlightenment through the advancement of knowledge would enable mankind to create 'artificial things' based on observations made from nature which are not inferior to the natural, but rather multiplications thereof (Llasera, 1986). Bacon's approach to knowledge is considered to be design-based, with 'design' referring to the systematic, empirical, and iterative framework that provides structure for the research process.

Following Bacon, Galileo Galilei published 'Dialogues Concerning Two New Sciences' in 1638 and laid the foundations for modern physics (De Angelis, 2021). On the first page, Galileo acknowledged the creative design ability within humans that allows them to create instruments and machines (Buchanan, 2001). No further mention of design can be found in Galileo's publication, which mirrors the scientific development of the time. The importance in Western culture shifted away from creative design, which could not be explained through set rules, and towards new learnings of theoretical liberal arts, also called neoteric. During this 'Battle of the Books', design remained confined to fine arts and literacy as part of the paleoteric (meaning 'old') learning, as it was not considered to lead towards truth (Levine, 1991; Buchanan, 2001). While it was considered a learned skill, artisans were understood to only acquire practical knowledge and no means to explain the reasoning behind it.

In the following two centuries, the practice of design was predominantly present at academies and art schools, which focused on teaching the process of creating and developing creative thinking. The next notable development towards Design Research as its own independent methodology was made in the 1920s by Bauhaus student Wilhelm Wagenfeld. The industrial designer

aimed to improve the functionality of everyday objects, such as lamps and typewriters, by studying their ergonomics and developing new prototypes. His goal-oriented iterative design process involved creating and refining prototypes, exemplified by his award-winning Wagenfeld Lamp, marking a significant step towards modern DR methodologies (Büchner, 2019; Woodham, 2004).

Within the broader field of Design Studies, Herbert Simon was a prominent voice for Design Science who "frame[d] design as a logical search for satisfactory criteria that fulfill a specific goal" (Huppatz, 2015, p.34) and claimed that "[e]veryone designs who devises courses of action aimed at changing existing situations into preferred ones" (Simon, 1988, p.67). He argued that design should be less about intuitive and informal decision making, but instead follow a problem-solving-oriented framework that strives for rigorous inference. This view agrees with Wagenfeld's approach to industrial design for optimised functionality. Simon offered a technical interpretation of design and emphasised that design can and should be an equally accepted research methodology to traditional science disciplines, defined as those that study how natural phenomena occur. (Simon, 1996).

An example of these Design Science principles translated into a practical framework is 'A Pattern Language' by Christopher Alexander (Alexander *et al*., 1977). He proposed a design language consisting of 253 patterns of which each pattern summarised one recurring problem within architecture practice and a proposed solution. Alexander claimed that through the intentional combining of patterns, which is learned through active engagement with design and community, the unique and complex design problems of constructing buildings and spaces can be solved. While the design guidelines are still met with support by many architects and urban designers, they have found a larger following in the Computer Sciences, particularly in participatory fields such as Human-Computer Interactions (see Chapter 3.2.2) (Dearden *et al*., 2002).

A direct critique of Simon's proposal of design as systematic process removed from one's individual creative thinking was voiced by Donald Schön through 'The Reflective Practitioner'. Schön introduced the two concepts of 'reflection-in-action' and 'reflection-on-action' (Schön, 1983[2016]), advocating for design practice guided by the individual's advancing personal skill and competence. The first concept refers to the ability of adapting to a situation as it happens through continuous internal deliberation, and the latter refers to the process of analysing a situa-

tion after it had occurred (Figure 3.4). The concepts are interconnected in the way they generate new knowledge for the researcher or individual, allowing quicker reaction in action and widening the perspective for post analysis. Schön argued that because design practice is a form of "reflective conversation with the situation" (Schön, 1983[2016], p.v), the addition of reflective thinking and real-time reflection to the design process can improve the designers' work beyond the capabilities of a rigid goal-oriented framework. Due to the uniqueness and inherent complexity of design situations, they cannot be standardised or considered to be repeatable scenarios. Linked to this, Schön emphasised the importance of experimentation and exploration, whether it is in the professional practice of architecture, engineering, medicine, or teaching (Schön, 1983[2016]).

The most important publication made towards the establishment of DR as an acknowledged and validated research methodology was made by Christopher Frayling in the early 1990s. Frayling challenged the public image of scientists and artists often depicted in overdrawn stereotypes by the media and argued that, if analysed more closely, the rigorous scientists rely more on intuitive design work than portrayed and vice versa. While scientific researchers claim to work

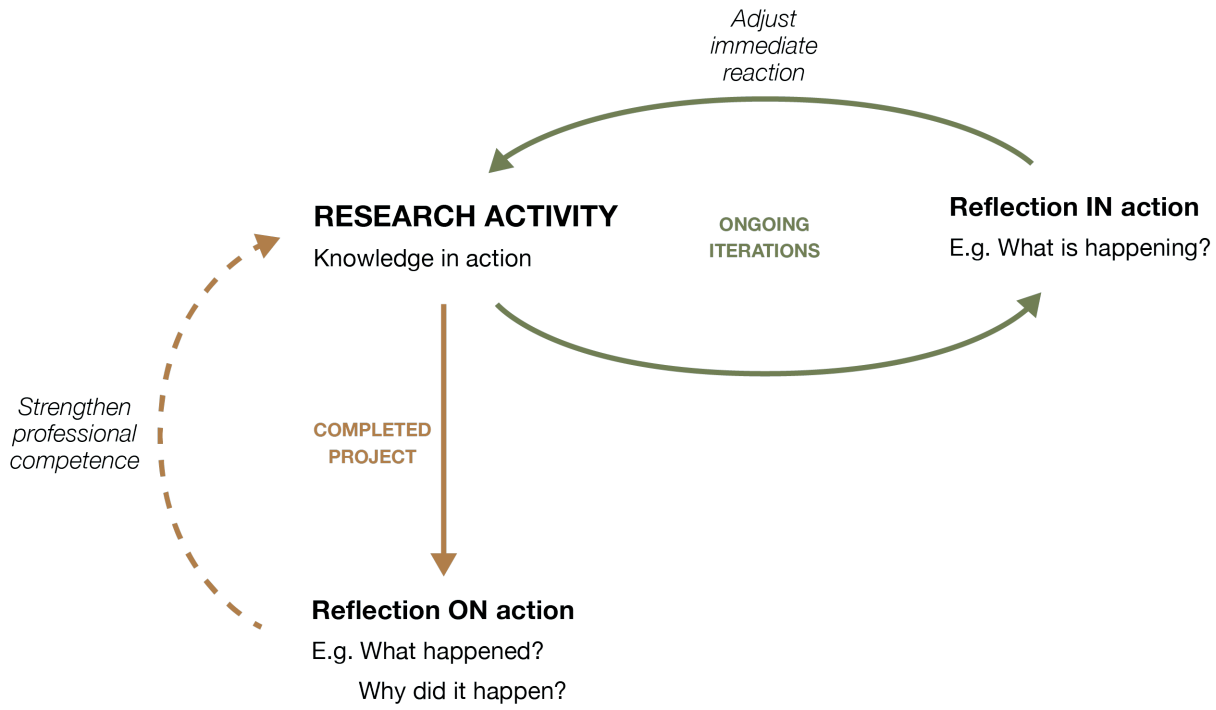

Figure 3.4: *Diagram of workflow following Schön's reflective practice with reflection-in-action during research activities and relfection-on-action following a completed research project*

explicitly through repeatable protocols following strict validated rules, they do not acknowledge or discuss their subjectivity influencing their decision-making. The same subjectivity is found in the 'cognitive idiom' of art which lead Frayling to conclude that some art is also research. He divided DR into three sub-categories (Frayling, 1993) (Figure 3.5):

1. **Research into art and design (RiD)** – done by historians or anthropologists to investigate certain aspects of existing art or design processes, using existing models, rules, and procedures
2. **Research for art and design (RfD)** – describing the process of gathering reference material and information before the creative practice to inform the design of the artefacts created afterwards
3. **Research through art and design (RtD)** – the process of conducting research and creating the artefact in the same step

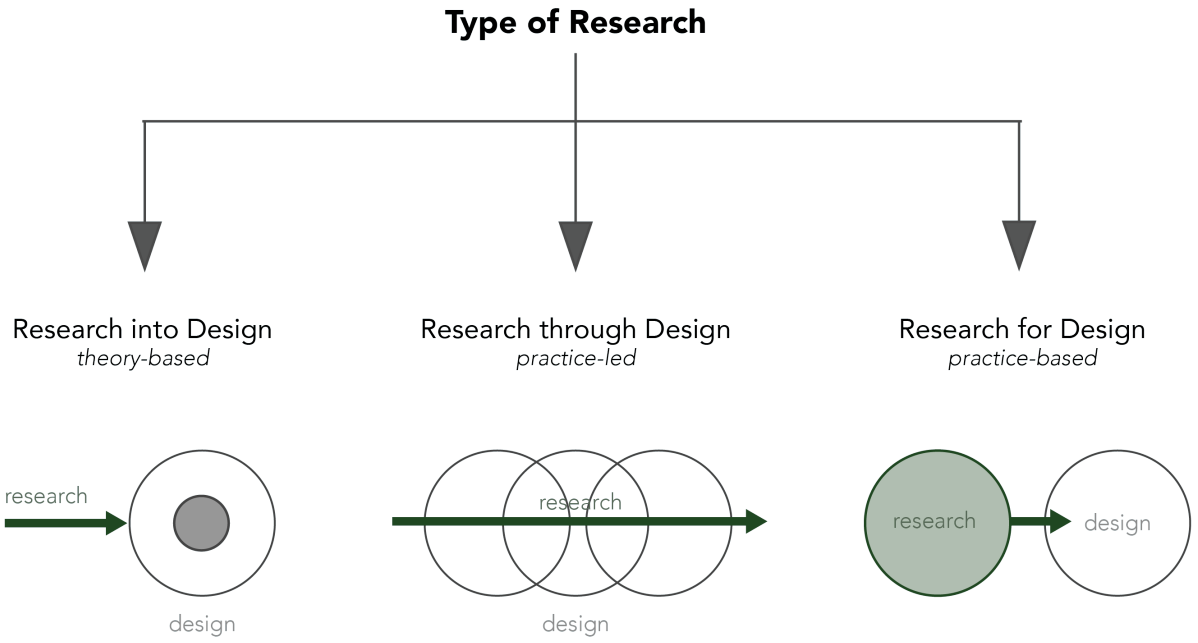

Figure 3.5: *Frayling's division of Design Research into three categories of Research into Design, Research through Design, and Research for Design. Diagram based on Pontis (2010) and Manolakelli (2023)*

RiD and RfD both employ systematic methods to extract data and information either from an existing artefact or from a situational environment in order to produce an artefact for it (Frayling, 1993). RiD is a theory-based research which utilises existing knowledge and artefacts to learn more about design without practicing design work. Schön's proposed framework for reflective practise can be seen as example for the theoretical nature of outputs from RiD. Contrasting, RfD is a practice-based approach in which the practical design process is a tool for the optimisation of a design solution. Wagenfeld's work on functionality optimisation through prototyping embodies the essence of this approach. RtD differs from the previous two approaches in that it utilises the act of designing and crafting as tool to generate knowledge through a practice-led orientation. RtD produces a diverse range of outputs that can range from physical prototypes to theoretical and conceptual frameworks because the focus predominantly lies on the exploration of the process and not on a specific design goal. Similarly to the principles of the scientific method (see Chapter 3.1), in order for any output of DR to be considered credible contributions, a rigorous documentation of the step-by-step development of the design process is required, in addition to the communication of the results and the presentation of the physical output, if there are any. However, the diversity of possible output types poses a challenge for a standardised method of reporting results and can be considered a part of the research itself (Borgdorff, 2010).

The essence of the RtD approach proposes that design can be used as tool that enables discovery, which contrasts the traditional understanding of design as a tool of invention. To further define the scope of RtD, a definition based on dictionary entries for each individual component can be compiled. The Oxford English Dictionary defines the three words as followed ('research', 2010; 'through', 2017 & 'design', 2012):

Research, *noun*

1. The act of searching carefully for or pursuing a specified thing or person; an instance of this. Frequently with after, for, of.

2. Systematic investigation or inquiry aimed at contributing to knowledge of a theory, topic, etc., by careful consideration, observation, or study of a subject. In later use also: original critical or scientific investigation carried out under the auspices of an academic or other institution. Occasionally with of; now frequently with into, on.

through, *preposition*

III. Expressing agency, means, or cause.

a. By means of; by the intermediate agency of; with the aid of; via. In early use also: † by the primary agency or action of (obsolete).

b. In accordance with, according to or conforming with.

Design, *noun*

1. A plan or scheme conceived in the mind and intended for subsequent execution; the preliminary conception of an idea that is to be carried into effect by action; a project.

2. The art of drawing or sketching; (hence) the process, practice, or art of devising, planning, or constructing something (as a work of art, structure, device, etc.) according to aesthetic or functional criteria; (also) this as a subject of study or examination.

Combining the three highlighted definitions of the dictionary, RtD can be summarised as ‘the systematic investigation aimed at the contribution to knowledge of a theory by careful consideration, observation, or study of a subject with the aid of a process, practice, or art of devising, planning or constructing something’. In simpler terms, it describes the search for new knowledge through the artistic process of creating an output, which can be physical or theoretical. Prototyping is a key element of this methodological approach and represents both the process and the artefact of the research.

Prototyping is a key element of the RtD methodology and represents an embodied design practice in which both the experimental process and the artefact of the research are equally important. This makes it particularly suitable for interdisciplinary projects where the merging of field-specific methods is integral to the theoretical and experimental research process. A practice-led methodology can foster an in-depth learning by the researcher of the involved disciplines through the hands-on learning approach. Additionally, RtD not only facilitates the discovery of new phenomena but also enables the development of foundational knowledge in a new emerging field, such as Biodesign.

3.2.2. Working with Research through Design

When working within DR, researchers often choose to borrow theories and methods from various fields after evaluating their suitability for the specific problem or brief they are addressing. Social science methods are more commonly used for RiD and RfD due to their interpretative nature (Koskinen *et al*., 2011). However, explorative and experimental methods are more appropriate for RtD, allowing researchers to fully immerse themselves in the context of the situation and gain a holistic viewpoint (Gaver, 2012). This initial step of identifying the appropriate combination of methods from various disciplines requires the designer to have extensive knowledge of interdisciplinary methodologies, marking the first scientific analysis of RtD. This approach ensures that designers can produce academically rigorous new knowledge while retaining the ability to experiment and employ creative practice as part of the research (Veselá, 2021).

Human-Computer Interaction (HCI), also known as Human-Computer Interfacing, is a branch of Computer Science that evolved from the field of Human Factors in Computing, focusing on the evaluation of computational systems at the interface with humans (Karray *et al*., 2008; Bannon, 1995). Although dominated by cognitive science and psychology, the field has recently incorporated the practice of design through the implementation of the RtD methodology, in particular to develop new interfaces and improve users' experience with technology. The suitability of RtD for the field lies in its "reflective practice of reframing the underlying situation and goal of the project" as well as the "shift to investigating the future as a way of understanding the world that should be brought into being" (Siek *et al*., 2014 p.178). A significant publication illustrating this was William Gave's work in the early 2000s on an interactive coffee table designed to stimulate user curiosity (Gaver *et al*., 2004; Siek *et al.*, 2014).

The field of HCI emerged in the 1980s when computers became more readily available for everyday households for personal computing, such as video gaming and word processing (Vijay, 2022). This necessitated more effective and intuitive communication with the computer to increase its accessibility. HCI developed in response as the design component which connects user, machine, and system (Karray *et al*., 2008). HCI research focuses on the interaction between users and machines, emphasising both human behaviour and machine response. The tool for this

communication and interaction is the interface consisting of buttons, touchscreens, and motion detection (Vijay, 2022). HCI employs methods from multiple disciplines, including computer science, psychology, and behavioural sciences. The study of information systems is also essential, allowing comprehensive analysis of hardware, software, database, network, and the people who feed into the development of the interactive system (Zimmerman *et al*., 2004)

HCI combines experimental psychology of a living, thinking, and acting user with aspects of creative design processes, utilising psychological theories about human behaviour to inform the iterative design practice (Figure 3.6). Each design iteration revolves around a prototype interface, a physical or conceptual one, starting from low fidelity to high fidelity until achieving the desired result and resolution (Siek *et al*., 2014). This process, building on Maher, Poon & Boulanger' concept of co-evolution of problem and solution, acknowledges that the problem being investigated cannot be fully understood until hypothetical solutions are thought through and tested periodically (Maher et al., 1996; Cross, 2013). The process of evaluating the potential impact of specific solutions allows the researcher to identify wider or more specific aspects of the problem environment that have not previously been seen and develop a new iteration of higher fidelity. This leads to HCI

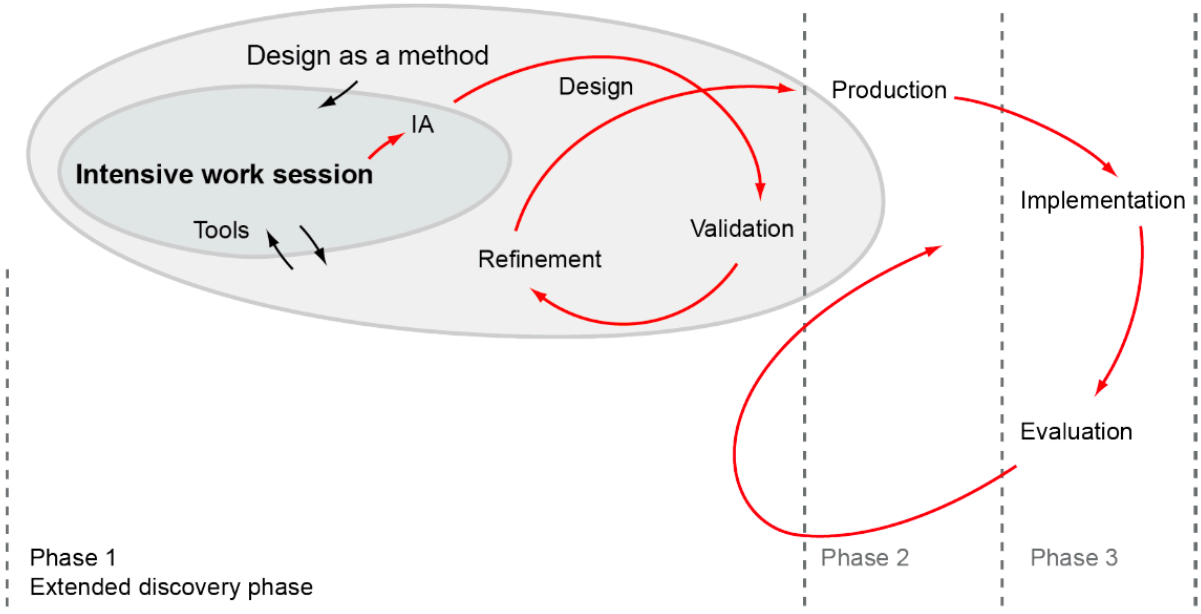

Figure 3.6: *Integrated design process for HCI. Reproduced from Zahedi, Guite & de Paoli (2011).*

being describes as a process of integration and decomposition at once (Zimmerman *et al.*, 2004).

The research processes and outputs of HCI can reach great complexity through the combination of interdisciplinary approaches and the element of (un-)predictability in behaviour of the user. The complexity is also directly linked to the problem for which the interface is being developed and the initial information provided to the designer to solve it. This influences the way the designer approaches the 'problem framing' stage which Schön describes as "the process in which, interactively, we name the things to which we will attend and frame the context in which we will attend to them" (1991 p.40). Meaning, the designer creates the environment in which they want to formulate the solution by identifying outlining questions. When approaching the problem, the first step is to identify what level of functionality and usability the solution needs to offer. The functionality defines the level of interaction the user will have with the interface of the product, such as turning it on an off, the usability describes how intuitive and appealing the interface is for the user (Te'eni *et al*., 2006). Finding a balance of functionality and usability is a key challenge of the design and research process. When identifying the desired user activity with the interface, it can be divided into three levels: (1) physical, (2) cognitive, and (3) affective. The latter has been a more recent development and describes the way in which the interface is turned into a pleasurable experience by evoking emotions within the user and increases the desire of continued use. The other two levels refer to the mechanical process of interaction, such as pressing buttons, and the way the user comprehends the functionality of the system respectively (Karray *et al*., 2008). Current technologies support the designer to choose from three types of interactions, also called modalities, to create input when developing the system, which can be (1) visual based, (2) audio based, or (3) sensor based. By combining aspects of all three, resulting in a multimodal interaction, the system can become a fully immersive experience for the user but also implement multiple levels of failsafe for events in which one singular interaction cannot be clearly interpreted by the computer (Jaimes & Sebe, 2007).

Measuring the effectiveness of an HCI prototype, which is arguably directly linked to the success of the RtD process, is often seen as subjective due to the element of personal experience from the user. This makes it difficult to compare systems and evaluate whether a prototype is an improvement to the previous version or not. In the 1960s, design researcher Bruce Archer

proposed brief guidelines to generate objective knowledge in design through systematic research methods (1968). While Archer's approach concerns itself with the overarching field of RD (Frankel & Racine, 2010), it has led to the proposal of multiple ideas to evaluate HCI prototypes through methodical analysis. One proposed method is to evaluate the balance of functionality and usability achieved within the system (Karray *et al*., 2008; Nielson, 1993). The question the researcher then tries to answer is: has the system reached the desired complexity and have all interaction levels been incorporated without overcomplicating the handling of it for the user? While this approach begins to integrate variables which can be evaluated using surveys, they still heavily rely on the user's personal and subjective experience. A more elaborate proposal was made by Professor William Gaver (2012) who suggests evaluating the overall research process involved in the HCI, rather than solely focusing on the physical prototype or end-product. Gaver divides the output of an RtD process into four categories: (1) Inspiration, (2) Persuasion, (3) Provocation, and (4) Contribution. Questions asked for each category are:

(1) Does it encourage the designer/researcher to look at the problem from a different perspective?

(2) Does the outcome convince third parties of the value of the new development?

(3) Does it challenge current believes and assumptions?

(4) Does it offer new theory, knowledge, and/or practice protocols?

The success and effectiveness of the HCI then depends on how well it performs in each category. In agreement, Cross argues "successful design behaviour is not based on extensive problem analysis, but on adequate 'problem scoping' […] and prioritising criteria" (2013, p.91), suggesting that a holistic view of the development process is more indicative of success than a focused analysis of the user's experience.

In order to holistically analyse the research process, however, systematic documentation of thoughts, ideas, and decisions is necessary from those involved. Therefore, in addition to being a crucial element in validating design-based research within the wider scientific community (see Chapter 3.1), the documentation process is also necessary for the objective evaluation of the

research outcomes. This leads to the question why, within the field of RD, there is still an overarching lack of the aforementioned communication through documentation. One explanation is that there is no simple step-by-step framework which designers can refer to when approaching the reporting of DR results (Dorst, 2013). Due to the diverse educational background designers have, academic language used in publications discussing frameworks are not naturally accessible. Another explanation for the lack of systematic documentation is the extremely complex process of creativity which is embedded in the research process. The Oxford English Dictionary defines creativity as "[t]he faculty of being creative" and the "ability or power to create" ('creativity', 2010). It is the skill in design-based research which is individual to each designer and researcher based on life experience, education, thought patterns, and more. While it is not possible to comprehensively document the extent of individual creativity, Dorst & Cross argue that there is one specific event in creative design projects which shapes the implemented creativity and influences the process (2001). This event is often referred to as 'creative leap' and is further defined as "a mental creative moment in which one discovers and illustrates a new design idea, or restructures and develops an old one" (Alhusban, 2012 p.iv). It is the same event which Schön refers to when describing the successful 'problem framing', the moment in which the designer gains full insight over the extent of the problem and a solution to solve it holistically crystalises (Schön, 1991).

An influential factor for the development of the design process is the input information that is available to the designer at the beginning of the process. Depending on the type and quantity of information, the direction of the 'creative leap' can be influenced (Dorst & Cross, 2001; Boden, 1990). Additionally, the information can shape the creativity of the designer by acting as subconscious boundaries to work within and lead to 'personal' creativity rather than 'historical' creativity. The difference between the two is that the latter represents those ideas that are unique worldwide and have not been thought of before, while the first describes ideas new and unique to the individual designer but not in the context of wider society (Boden, 1990). The less information is provided at the beginning of the process, the more likely is the occurrence of 'historical' creativity (Dorst & Cross, 2001) which signifies the contribution of new knowledge within the scientific community. Because the defining moment of insight is only identifiable retrospectively, it must be preserved through rigorous observation and documentation of the whole design process. The

documentation can be divided into four parts, namely (1) the thought process involved in making decisions, (2) the problem's context, (3) the final product and (4) its intended impact, and a reflection on the actors who facilitated the design process (Siek *et al.*, 2014; Dorst, 2013). Breaking down the creative process into these elements of significance supplies the designer with a template to structure transcripts and makes the research more accessible on an interdisciplinary level. When considering the actors involved in the research process, in RtD in particular, there are multiple be considered. Apart from the researchers and designers, the test users' backgrounds and feedback also play a vital role. In the early 2000s, the idea of co-designing and co-creating was introduced to the field of DR and draws parallels to the concept of co-evolution of problem and solution. It emphasises the constant feedback between those who design and those who are designed for, as well as the intentions of the investors (Koskinen, 2011).

In summary, rather than relying on one specific research method, RtD is a methodology that brings together and combines methods from various fields into one interdisciplinary research endeavour. This approach allows design researchers to implement their creative skills while working within the boundaries of scientific knowledge generation. In the field of HCI, creative design is utilised as a tool to establish successful communication between living and non-living participants. To achieve this, prototypes of interfaces are produced iteratively based on feedback loops from test users and through extensive problem framing by the researchers, enabling a holistic approach to problem-solving. To meet scientific standards, the systematic documentation is a key component of DR and RtD but is oftentimes neglected by researchers. Breaking down the complexity of the design process into elements of significance allows the researcher to identify the critical moment shaping the creative design development retrospectively and the "storage of design knowledge in the recording system of scientific literature" (Baytas, 2022, para.43).

3.2.3. Adaptation to Biodesign

The modern understanding of the term ‘Bidoesign’ is an interdisciplinary field of scientific and artistic work that encompasses cutting-edge research of biotechnological innovation (see Chapter 1.1). In a broader and generalised sense, the term is used to describe the “designing with, as, or for living matter” (Myers, 2018). And while Biodesign is still predominantly used to describe processes of designing ‘for’ biology, such as the development of medical devices or appliances, design historian William Myers argues that “biodesign refers specifically to the incorporation of living organisms or ecosystems as essential components” (Myers, 2018 p.8). With his interpretation, Myers pushes the new design practice further into interdisciplinary territory, proposing the integration of fields of such as biology, material science, and ecology into the creative design flow. It is here where the need for efficient communication between humans and microorganisms emerges and parallels to the emergence of HCI as a research field (see Chapter 3.2.2) can be drawn to Biodesign. In particular its method of enabling an interaction between humans and another reactive system is of relevance. Instead of building a communication link with non-living computational systems, Biodesign aims to establish communication of two living actors speaking fundamentally different languages but also utilises design as a tool to do so. The need for this communication has slowly become apparent through research into sustainability in the built environment, in which harnessing natural processes of living systems is showcasing potential to replace fossil-based materials (Goidea *et al*., 2022). And while the beneficial effects of certain microbes on the human immune health are increasingly discovered, there is a lack of understanding how their presence can be integrated into everyday architecture (Shin *et al*., 2015; Thoemmes *et al.*, 2022; Beckett, 2021). Many aspects of HCI design research draw on RtD principles and can be directly and indirectly translated to fit the scope of Biodesign with living microorganism. A key difference that needs to be considered, however, is that Biodesign aims to design with emergence (see Chapter 1.1.1) and recognises the possibility of yet unknown complexity. In contrast, computational systems in HCI function within boundaries of capabilities and are significantly more predictable than living systems (Hartson, 1998).

Four core methodological principles for Biodesign were established using the HCI framework. They also form the skeleton for the developed research approach of this thesis:

[1] Prototyping

The iterative development of prototypes plays an integral role in RtD and in HCI research. It is through these prototypes that the researcher determines how well the interface facilitates the desired communication. Additionally, it tests the feasibility of translating the hypothetical design concept into a physical product by exploring the required expenditure of work (Hartson, 1998). Translated to Biodesign, prototyping allows the researcher to test new material compositions and construction techniques in a computer simulation, as mock-up installation, or as prototype in a real-life setting to analyse the impact of the natural environment on its function. It also permits a softer transition from working exclusively with biological principles actioned by non-living parts to the integration of living biological components through a proof-of-concept stage and subdivision into different aspects of the design. One example of this is the project Pulp Faction which developed a "3d printed material [assembled] through microbial biotransformation" (Goidea, 2020) using mycelium to connect modular material components. The iterative prototyping process began with a focus on individual aspects, such as substrate composition, fungal species, and printing parameters, and gradually grew in scope by bringing multiple aspects together. The employed RtD methodology with a focus on making through material practice facilitated the fabrication of a final physical prototype 'Protomycokion' at architectural scale (Goidea *et al*., 2022).

[2] Feedback Loops

A vital part of the development process of an HCI systems is the incorporation of user feedback. This user response has significant influence on the design direction of the system allowing the researcher to identify flaws as well as evaluating the overall impact. This feedback can be acquired either in a direct or an indirect form, depending on the needs of the research. While direct feedback is communicated verbally or in written form using a common language, indirect feedback is collected in the form of behavioural observations, such as body language and brain wave functions. The latter requires the researcher to analyse and interpret the data based on

those aspects identified as relevant to the HCI system (Tan & Nijholt, 2010). When adopting the principle of feedback to Biodesign practice it can be split into two general approaches. First, feedback from colleagues, engineers, biologists, and other scientists on the impact and integration of a prototype into an environment, which is a familiar process for architects and designers working collaboratively. And second, feedback taken directly from the living microorganism throughout the development of the prototype and during its implementation. It is the latter approach which acknowledges the autonomy of the microorganism but simultaneously poses as the more challenging one due to the limited availability of resources to support the data analysis. Without a common language, the researcher is restricted to collecting indirect feedback data from the microorganism. Examples of this are observations made of the growth behaviour, such as growth speed or species competition, as well as the survival rate and spatial spreading. Projects which showcase this engagement with a living microorganism are 'Guided Growth' (Zolotovsky, 2017), 'Bio-Material Probes' (Ramirez-Figueroa, 2018), and Ozkan et al.'s work on digital fabrication of fungi (2021). In 'Bio-Material Probes' Ramirez-Figueroa aims to identify strong and usable traits of various living systems by embracing the unknown and allowing the organisms to show their own agency (2018). And while each probe proposes a method to work in a design context in the future, the development of each is fully guided by the living organism and avoids steering the development towards a specific outcome.

[3] Interdisciplinarity

The RtD methodology is collaborative by definition and in the field of HCI this is manifested in the combination of methods from computer sciences, psychology, product design, and more. In Biodesign it is predominantly the methods and workflows of biological research, material science, and design which are brought together. This list, however, is not exhaustive and the researcher can incorporate other fields such as engineering and additional life sciences depending on the scope and goal of the project (Myers, 2018). From this stems another level of collaboration in Biodesign which accounts for the bringing together of living and non-living actors which are often forgotten about, such as the environmental characteristics of humidity and temperature (Keune, 2021). Because of the design element in HCI, a main challenge of the field is the rigor-

ous documentation of the research which is to the standards of systematic sciences such as engineering and computer science in which researchers have set ways of following and writing process protocols. The same challenge can be found in Biodesign which combines creative design practice with the systematic methods of biology. In order to validate results and contribute new knowledge in life science research, each experiment needs to be executed in multiples with a minimum of triplicates to account for variability within the biological system. Depending on the audience the Biodesigner is aiming at, adopting the method of multiples and protocol writing for each prototype ensures a higher chance of acceptance within the science community. Presenting precise instructions on how to replicate the prototyping distances the research output from subjective and expressive art, while simultaneously making it more accessible to researchers from varying disciplines. A good example of this is the comprehensive work 'Mycelium Matters' by Elise Elsacker (2021) in which she explores the potential of mycelium as building material component through a range of fabrication methods. For each physical prototype group, Elsacker documents the design decisions and workflow of lab-based practices in one protocol which can be understood by biologists, engineers, and designers alike. The before mentioned 'Pulp Faction' project also showcases a complex methodology combining methods of biological lab work with material science testing protocols and computational design processes from engineering and architecture (Goidea *et al*., 2022).

[4] Process-oriented

The last core principle of RtD implemented into Biodesign is the equal focus on the final output as well as the research and design process to achieve it. In HCI, the process of receiving the user's feedback is essential for the researcher to gain a holistic view of the problem or communication that the system is being designed for. Each step of the iterative process produces a prototype which has the potential to fulfil all requirements. Because these research projects oftentimes employ a bottom-up approach within a set of boundaries provided by stakeholders without a specific end-product in mind, it is essential for the researcher to be able to interpret the feedback the users offer to steer the design development. An empathetic approach to the communication with the user permits a deeper understanding of their needs and a more fine-tuned reaction in

the design development. This is particularly true in Biodesign where the researcher has limited frameworks available to deepen the dialogue with the microorganism. It is the "socially engaged nature in artistic research" (Veselá, 2021 p.2) which is brought into the biological approach and challenges the researcher to rethink methods of communication and relating to the non-human actor. The term 'naturecultural' was first introduced by Donna Haraway to describe the inseparability of culture and nature in ecological relationships (Haraway, 2003; Malone & Ovenden, 2016) and further developed into the field of Biodesign through the understanding that "engaging with living organisms is intrinsically connected with different levels of care" (Keune, 2021 p.14). This means that each iteration of the design process can stand for a further development of the sense of care and relating with the non-human organism (Puig de la Bellacasa, 2017). Each of the previously introduced projects, 'Guided Growth' (Zolotovsky, 2017), 'Mycelium Matters' (Elsacker, 2021), and 'Bio-Material Probes' (Ramirez-Figueroa, 2018) showcase this development of a relationship between researcher and microorganism, with Ramirez-Figueroa's work clearly defining the framework for this approach.

Working with a living non-human actor requires a rethinking of the communication tools available and highlights the importance of an empathetic approach towards the collaboration with the microorganisms. It is this approach of 'Nature as a co-worker' (Collet, 2017) which begins to acknowledge the organism's own agency and values its feedback for the iterative development of prototypes (Crawford, 2022). This resonates with fundamental theory of evolution in which "feedback and adaptation are [the] core principles of biological design that allow plants and animals to evolve in response to changing conditions" (Andréen & Goidea, 2022 p.481).

Ultimately, explorative research in Biodesign allows the researcher to account for the uniqueness and autonomy of the living organism by employing a divergent bottom-up approach and keeping an open mind to discover unexpected results. Nature and biology are indefinitely complex, and the behaviour of the living cannot be predicted, especially if its potential is not known to begin with. To create new materials for the built environment which push the sector towards sustainable development "a new kind of biology is needed" (p.72 Armstrong, 2011).

3.3. Summary of Research Approach

The aim of this thesis is to investigate the potential of fabricating 3-dimensional BC and to identify design parameters to guide the emerging material form (see Chapter 1.2). This positions the work in the broader context of the emerging branch of Biodesign in which the researcher develops a greater understanding of the behaviours of a living non-human organism with a focus on morphology. The scale goes beyond that of petri dishes and material samples that can only be observed with magnification under the microscope.

The aim is not to pursue the confirmation of one specific theory explaining the behaviour of the living organism, but rather ask open-ended questions about the challenges and possibilities of the co-working design process. By employing the bottom-up approach to the development of the experimentation, the end-product of this work is defined by an organism-guided process interpreted by the researcher towards the aspect of morphology and shape-finding, and reflecting on all actors involved in the process (Sabatier, 1986). This entails the embracing of a non-linear development during which new branches of interest are discovered continuously through unexpected findings. Areas of interest were chosen based on a literature review (see Chapter 2) and used as boundaries that frame the research development in order to confine the scope of this work to the time-constraints of a PhD conducted during the COVID-19 pandemic. These boundaries were redeveloped and narrowed down iteratively throughout to direct the research towards the overall aim of developing a biofabrication process for 3-dimensional bacterial cellulose. The work of this thesis clearly positions itself within the exploratory research approach and presents experimental findings that broaden the inspiration for more in-depth investigations in the future.

The overarching methodology followed in this work lies within the field of DR and was chosen to follow the scientific framework during a creative design process of the research development. Within the field of DR, specifically the methodology and methods of the branch RtD were used to develop the Biodesign approach of this research. The disciplines of microbiology, architectural design, digital fabrication, and material science were brought together, and their methods combined into one interdisciplinary approach based on the research framework developed for the field of HCI (see Chapter 3.2.3). The key adaptation of the HCI framework to Biodesign was

the transition to a non-human living organism as co-actor in the researcher with the practice of design chosen as tool to facilitate a dialogue without common language. At the core of this dialogue is an empathetic approach to develop a set of interpretation guidelines that improves our understanding of the needs of the microorganisms in order to support their autonomous behaviours. Acknowledging the organism's autonomous behaviours allows moving away from a purely anthropocentric perspective of design in which the microorganism is exclusively seen as harvestable material process (Keune, 2021) and towards an active collaboration between human and non-human life.

Following the HCI framework, the design of the communication is approached in multiple steps to reach the desired complexity. First, the vital needs of the microorganism are identified to ensure the survival throughout the experimentation. Second, the favourable conditions, which enhance the microorganism's behaviour but are not essential, are determined. From these, design variables are developed, and the desired degree of human intervention is explored. During the last stage, the limits of the microorganism's autonomy are tested through increasingly complex human manipulation. Inspired by Gaver's proposed method of objective analysis (2012), a set of

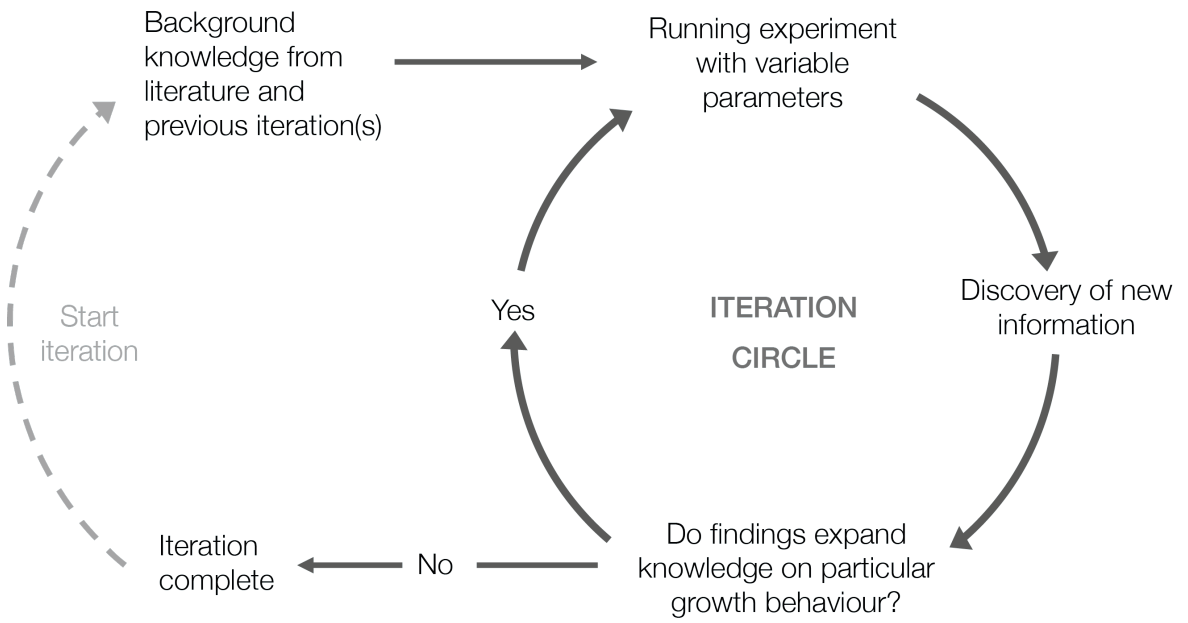

Figure 3.7: *Experimental iteration circle developed based on the principles of the hermeneutic circle.*

four questions was developed as a basis to assess each iteration of experimentation with a focus on understanding the microorganism's participation:

1. Does the microorganism have all vital needs met and how far does the organism flourish past the survival point?
2. Does the microorganism react to the applied triggers and how do the reactions manifest morphologically?
3. How does the behaviour of the microorganism differ to the previous iteration and what changed in experimental setup can this be accounted to?
4. Does the microorganism show potential for further development of this design strategy through unexpected behaviour?

While the main focus of the research lies on the organism-guided morphological development through creative design exploration, selected methods from scientific research were adopted to achieve a base level of credibility of the results (see Chapter 3.1). The general principles are followed through the detailed description of the experimental setups and the documentation of growth behaviours of the BC in regular intervals. All information needed to repeat each experimental setup is provided. The specialised techniques of microbiology include the use of replicates, biological and technical, for each experiment to develop confidence that observed results are real phenomena. The aim of biological replicates is to test variation within one biological organism and technical replicates test variation in the protocol or measuring equipment (Bell, 2016). Biological and technical replicates were implemented together when fundamental behaviours of the BC growth were explored to acquire reliable background knowledge for further exploration (see Exp. C in Chapter 5.1, Exp. D in Chapter 5.2 & Exp. I in Chapter 6.1). Following experiments, testing adjustments of individual parameters of the experimental setup were conducted with biological replicates where plausible. However, due to the dimensions and size of each experimental setup, together with the longer running time of each experiment, replicates were not always identical in every aspect, but rather testing the same principles of intervention (e.g. Exp. P in Chapter 6.2).

The decision whether technical replicates were necessary to hypothesise about the organism's behavioural response to a particular environmental stimuli were made based on an iteration circle following the concept of the hermeneutic circle (Figure 3.7) (see Chapter 3.1.1). Another factor influencing the decision to not test identical replicates for every iteration was the overall nature of this work to explore rather than to confirm. This leads to the acknowledgement that the micro-organism's behaviour to customised interventions is a unique expression of its autonomy rather than a replicable process.

Summarised, the work of this thesis employs a predominantly design-based, exploratory experimental methodology that is developed from the RtD within DR. The research methods of HCI are taking as guidance for the development of a new Biodesign approach that facilitates the establishment of a co-working relationship with a living microorganism. While certain elements of scientific data validation are implemented, a level of uniqueness and unpredictability is acknowledged as part of the creative design research process. The impact of this work on the wider Biodesign community lies within the developed methodological research approach as well as in the final physical outputs from the experimentation with a living system and its morphogenetic behaviours.

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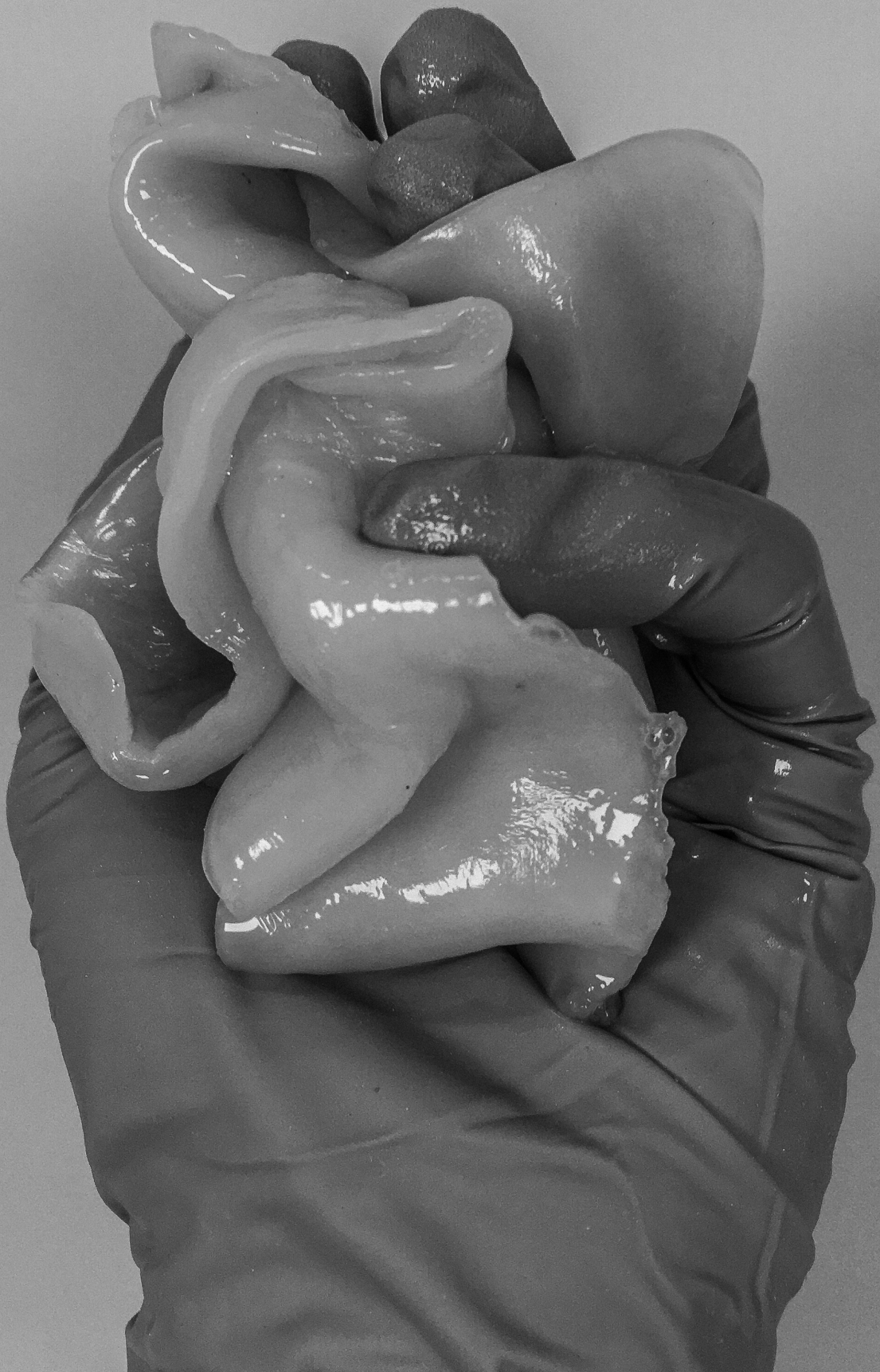


Demonstration of the flexibility of a sterilizable grown bacterial cellulose sheet

CHAPTER 4

CULTIVATION OF BACTERIAL CELLULOSE

Chapter Summary

In this chapter the lab-based methodology for cultivating bacterial cellulose in a laboratory environment is described. The experimentation in this thesis followed an explorative research approach which involved the iterative development of experiments (see Chapter 3.3). Therefore, the lab methods needed to accommodate the variability of growth settings and adapt to evolving requirements of the designer's intervention. While the specific parameters of each growth setting varied, a set of essential requirements are needed for the BC production to occur (see Chapter 2.1). For the bacteria culture used in this research, those basic growth conditions are access to nutrients and oxygen simultaneously. In addition, a growth vessel is required to host the nutrient medium and the bacteria culture. Therefore, three categories of parameters emerged of which each experimental setup utilised at least one option:

1. Nutrient source – the type of liquid medium used to feed the bacteria culture (see Chapter 4.1.1)
2. Inoculation type – the method used to add the bacteria culture to the nutrient medium (see Chapter 4.1.2)
3. Growth vessel – the shape and dimension of the vessel which holds the nutrient medium (see Chapter 4.1.3)

In the following subsections each category is summarised, and a symbol is assigned to each option of the category. These symbols are used in Chapter 5, 6, and 7 to represent the setup of each experimental growth setting in a comparable fashion.

Additionally, the traditional method of growing BC sheets from kombucha SCOBY is explained and tested. The key observations of the bacteria's behaviour during growth in addition to existing literature (see Chapter 2) formed the background knowledge from which the first iteration of experimental cultivation was developed in the subsequent chapters.

4.1. Growing Bacterial Cellulose

Two different methods of cultivating BC exist, with the first utilising a single bacteria strain, also called pure culture, and the second utilising a symbiotic co-culture of bacteria and yeasts, also called SCOBY (see Chapter 2.1.2). A pure culture, in which one single strain of bacteria is cultivated, is most often used when BC of high purity and sterility is required. This is the case for the majority of medical applications and biomedical research, such as burn wound dressings or implant coatings, where biocompatibility is required (Portela *et al*., 2019; Rühs *et al*., 2020). In the Do-It-Yourself community and creative Biodesign, the kombucha SCOBY is predominantly chosen as it only requires regular household items as food source and can be grown in a non-sterile environment. The main use of the kombucha SCOBY, however, is in the food industry where it is used to create a fermented tea beverage.

The experiments described in this thesis utilise a kombucha SCOBY to grow the BC which was chosen for two particular reasons. Firstly, the work of this thesis begun in early 2020 when Covid-19 lockdown restrictions were in place and all experimentation needed to be accommodated in a regular household kitchen. The level of sterility and sophistication of equipment needed for work with a pure culture exceeded the available tools. An essential machine for sterility is the autoclave in which the bottles and the medium are heated to 121 °C at 15 psi for 15 minutes before cooling back down to room temperature. This process sterilises and decontaminates any tools, bottles, and substrates. Secondly, while using a pure culture may seem more appropriate for academic research at first glance, there is also a benefit in working with a more resilient and commercially available culture. By increasing the accessibility of this research for others to continue or interpret for their own projects, the audience widens past academic and industrial scientists.

4.1.1. Nutrient Sources

Depending on which culture type, pure or SCOBY, is used to grow the BC different nutrient media are prepared to feed the bacteria. For kombucha cultures, a tea-based medium is most widely used, in particular in the food industry (see Chapter 2.3). For pure bacteria cultures, Hestrin-Schramm (HS) is a commonly used media which requires the mixing of multiple chemicals and nutrient powders, followed by sterilisation through heat. Both media types have been used in the experiments of this thesis and the preparation is as followed:

Tea-based Medium

The symbol used to indicate tea-based medium was used in an experiment is:

Tea-medium is used in the food industry to produce a consumable beverage from SCOBY and many variations of this medium's recipe exist depending on the desired taste. The recipe used for the experiments in this thesis was developed specifically to enhance the cultivation of the BC, rather than for the production of a beverage (Kretzer, n.d.). A traditional kombucha medium used to create the drink does not include apple cider vinegar as it would alter the taste. For home experimentation, the added vinegar helps reduce the risk of contamination by lowering the pH of the medium and making it less inviting for other bacteria and fungi. The pH lowers from around 5 to 3.4 - 3.7 once the vinegar has been added. This value is lower than the identified ideal pH for culturing BC from pure culture, which is a pH of 4.5 (Aswini *et al*., 2020). The following ingredients and quantities are needed to prepare the tea-based medium:

Table 4.1: *Recipe for tea-based growth mediumbased from Kretzer, n.d.*

Ingredient	Quantity (per Litre)	Function
Granulated sugar	80 grams	Provide sucrose that is split into glucose and fructose, which the bacteria can metabolise further (see Chapter 2.1)
Apple Cider Vinegar	70 ml	Reduce pH of the medium to decrease risk of contamination
Water	930 ml	Dissolve the granulated sugar and enable movement of the bacteria
Black Tea bag	2-3 grams	Provides nitrogen which is essential for growth, maintenance, and metabolic activity of the bacteria

To prepare the medium, 500ml of the water are brought to boiling point and transferred to a sterilised container, such as an autoclaved 2000ml beaker or a plastic box cleaned with ethanol (Figure 4.1). The tea bag is added to the hot water and left to steep for 15 minutes. After removing the tea bag, the sugar and cider vinegar are added and stirred until fully dissolved. Lastly, the remaining cold water is added to the container and stirred again. Once the medium has a homogenous light orange colour, it can be set aside to cool below 30° Celsius. The medium can either be stored in clean bottles for up to a week or used immediately once it has cooled down to temperature.

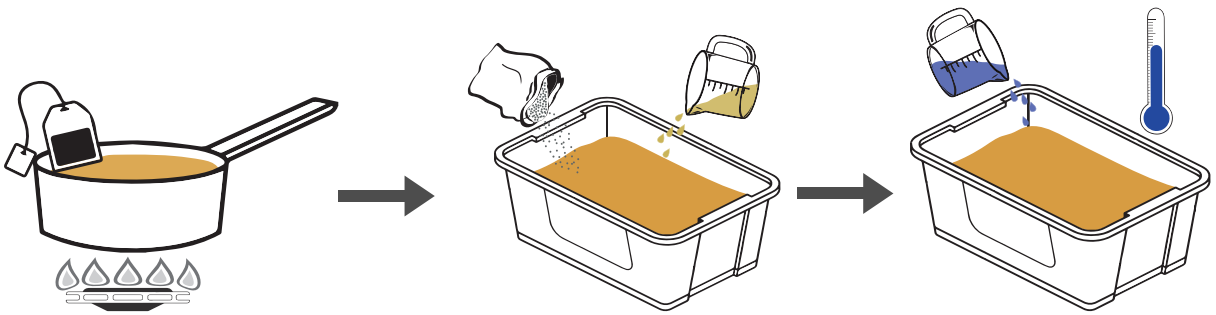


Figure 4.1: *Schematic showing the three steps of preparing tea-based medium: Boiling water to prepare black tea, adding granulated sugar and apple cider vinegar, and finally cooling the medium down to room temperature byt adding cold water*

Different types of tea can be used to prepare this media and depending on whether it is black, green, or white tea, the BC will be darker or lighter in colour. The colour of the BC pellicle can also be influenced through additives, such as food colouring, or varying sources of sugar. Fruit sugars with natural colours have proven to be a potent dye. Especially bright coloured berries and beets are successful in visibly changing the pellicle's colour as it grows (Bloch, 2019).

Hestrin-Schramm Medium

The symbol used for Hestrin-Schramm medium is:

Hestrin-Schramm is a specialised nutrient solution which is one of the preferred medium for the cultivation of pure culture BC. HS media contains a high concentration of glucose and yeast extract, which makes it highly receptive to contamination by various bacteria and fungi. To avoid contamination, this medium must be prepared, handled, and used in a sterile environment. The preparation of one litre of HS media requires the following ingredients (Hestrin & Schramm, 1954):

Table 4.2: *Recipe for Hestrin-Schramm medium and the function of each ingredient (Aswini et al., 2020 & Son et al., 2001)*

Ingredient	Quantity (per Litre)	Function
Glucose	20 grams	For the bacteria to metabolise for energy and BC production (see Chapter 2.1)
Yeast Extract	5 grams	Vitamins and nitrogen for cell growth. Buffering agent for stable pH
Citric Acid	2,7 grams	Buffering agent to maintain pH
Peptone	5 grams	Provides nitrogen which is essential for growth, maintenance, and metabolic activity of the bacteria
Deionised water	1000 ml	Dissolve all ingredients and allow movement for the bacteria
Hydrochloric acid (HCl)	as needed	Lower the pH level
Sodium hydroxige (NaOH)	as needed	Increase the pH level

The preparation of HS media involves multiple steps of mixing, sterilising, and combining (Figure 4.2). To begin the preparation of 1 litre of medium, two bottles are needed. 500 ml of deionised water are filled into a 1000 ml Duran bottle, then the glucose is added. With the help of an electro-magnetic (EM) stirrer, the glucose is fully dissolved in the water. In a separate Duran bottle 250ml of deionised water are added and placed on the EM stirrer. Once a vortex has been formed in the water, the dry nutrients (peptone, yeast extract, sodium diphosphate, and citric acid) are slowly added until fully dissolved. With the vortex still active, the pH of the medium is adjusted to the desired level. The bottle is then removed from the EM stirrer and the remaining 250ml of water are added. Both bottles are closed with a lid and placed in the autoclave for sterilisation. In the autoclave, the bottles and the content will be heated to 121 °C at 15 psi for 15 minutes before cooling back down to room temperature. In a last step, the 500ml of nutrient solution is added to the glucose in a sterile environment, either under a Bunsen burner flame or inside a laminar flow cabinet.

When HS is used for pure culture BC cultivation, the pH is adjusted to 6.0 with either HCl (to increase acidity) or NaOH (to increase alkalinity). To increase the medium's resistance to contamination, the pH of HS used for this thesis was lowered to the same value of 3.5 that the tea-based medium was measured at. As it was the same kombucha culture used with the tea-based and the HS medium, it was known that a lower pH would not affect the BC growth negatively.

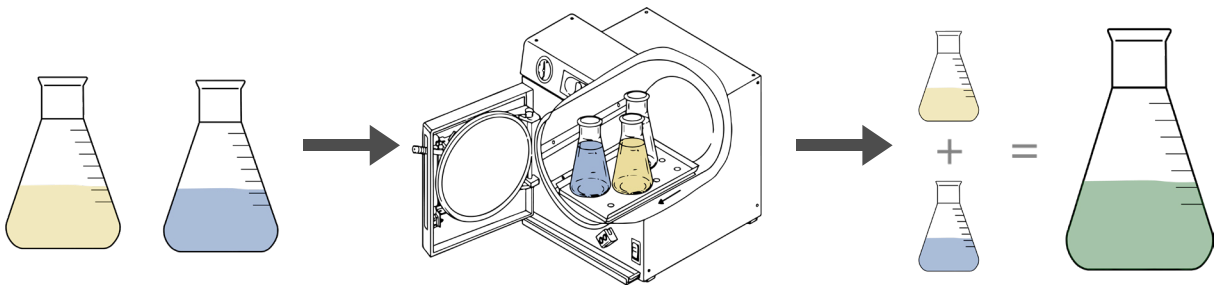


Figure 4.2: *Schematic showing the three steps of preparing HS medium: Preparing the nutrient solution and glucose solution in seperate bottles, autoclaving both solutions, and finally combining both solutions into a larger bottle*

4.1.2. Inoculation Methods

The inoculation of media describes the process in which a living organism, such as bacteria, viruses or fungi are added to a substrate or body of nutrients for multiplication and growth. In the case of BC, this can be done through one of two methods. The first one is full SCOBY inoculation and the second is inoculation with liquid. Each has their own benefits and drawbacks which required a thorough analysis of the desired outcome of an experiment prior to choosing a method.

SCOBY Inoculation

The symbol used to indicate SCOBY inoculation is:

The traditional way to inoculate media using kombucha cultures used in the food industry is the SCOBY inoculation. The term SCOBY can be used interchangeable with freshly grown BC and refers to the 3-dimensional cellulose fibre matrix which hosts living bacteria and yeasts that form a symbiotic culture. In this thesis, the term SCOBY is used in the context of the inoculation method and BC in the context of the produced material. To inoculate fresh medium using a SCOBY, it is simply placed inside the liquid medium and left to ferment. The living organisms inside the SCOBY will feed off the nutrients in the fresh media and replicate to grow in numbers. As part

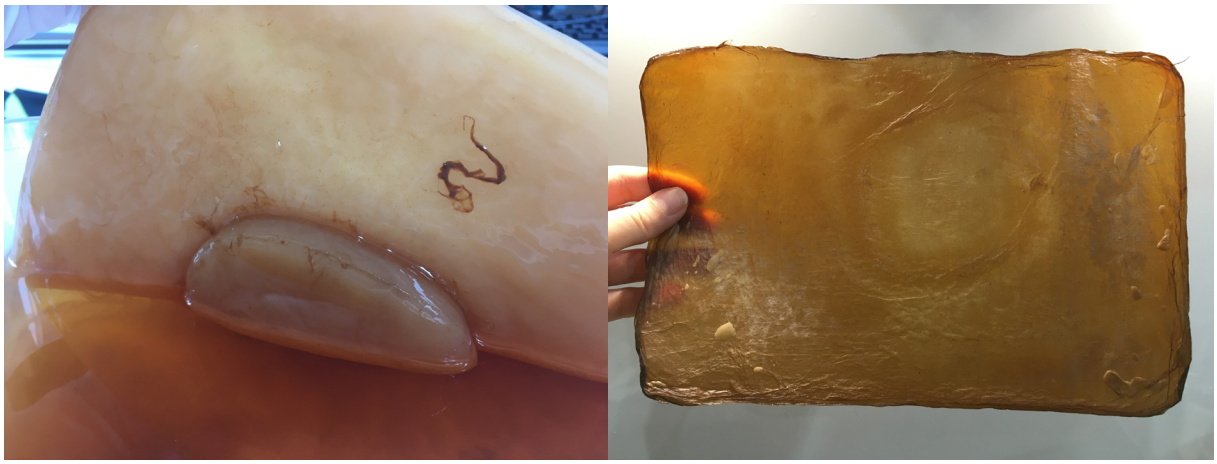


Figure 4.3: *(Left) SCOBY attached to newly grown pellicle; (right) dried BC with visible material change where the SCOBY was attached*

of this metabolic process, a new layer of BC will grow on the surface which also hosts yeast and bacteria cells. This newly formed SCOBY can then also be used to inoculate fresh media.

The main benefit of the SCOBY inoculation is the simplicity of it as it does not require any more handling after being placed inside the medium. While SCOBYs do not come in a standardised size, they are on average 150 to 200 mm in diameter. If a SCOBY is bought online for home brewing, it is likely in a round shape due to the vessel it was cultured in and recommended to inoculate anywhere between 1000 ml to 3000 ml (Happy Kombucha, n.d.). One SCOBY can be used to inoculate multiple consecutive batches of fresh medium.

The main drawback of this method is the potential uneven distribution of bacteria inside the medium. While the new bacteria and yeast multiply and freely move within medium, the SCOBY holds the highest concentration of them per square centimetre. This can result in thicker BC growth in the area where the SCOBY attached to the growing pellicle. In other cases, the SCOBY floats to the surface and hinders the growth of new BC in that area. But in either case, detaching the old SCOBY from the newly grown BC can leave a visibly mark and create a single BC sheet with varying properties (Figure 4.3).

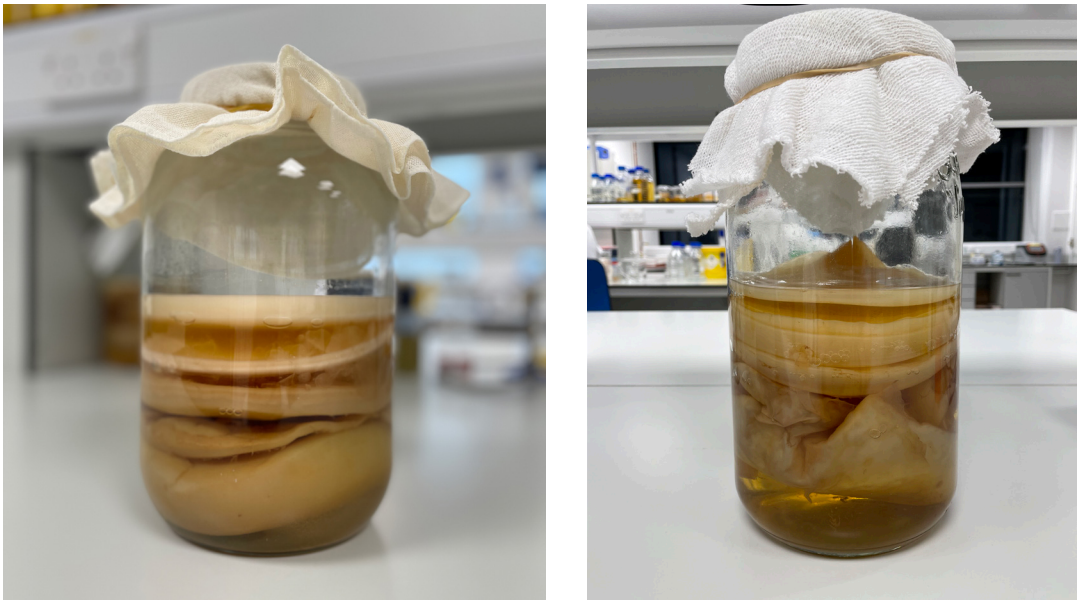


Figure 4.4: *SCOBY hotelmade with (left) HS medium and (right) tea-based medium.*

SCOBY Hotels

A SCOBY hotel is the term used for a large glass vessel which holds multiple SCOBY at a time and keeps them alive by frequently replacing the medium every 3 months. In kombucha homebrewing, the SCOBY hotel is used to store spare SCOBY, which occur as the inoculation SCOBY dublicates during a fermentation cycle. Both the initial and the new SCOBY are viable for further inoculation. In the experiments described in Chapters 5 & 6, the newly formed SCOBY is referred to as BC and harvested as material. The spare SCOBY sit in fresh medium inside a hotel which, over time, also ferments and attains a high concentration of bacteria and yeast. This potent liquid can be used in addition to SCOBY inoculation to speed up the fermentation or used by itself in the form of liquid inoculation (Villarreal-Soto *et al*., 2018).

Two separate SCOBY hotels were created for the research described in this thesis: one using tea-based medium and one using HS medium (Figure 4.4). For the tea-based medium 5 new SCOBY from 'Happy Kombucha' were placed in a 3000 ml jar sterilised with 70% ethanol. Fresh medium was added until the SCOBY were covered by roughly 50mm of liquid. To allow for oxygen exchange, the jar was covered with a thick muslin cloth and elastic band. New medium was added whenever evaporation had caused the top SCOBY to be exposed or a new SCOBY or roughly 15mm thickness had formed at the surface.

To create the HS hotel, 5 new SCOBY from the same manufacturer were taken and rinsed with deionised water to clean off any residue medium. They were placed in a sterilised 3000ml jar and covered with 50mm of fresh HS medium, similar to the tea-based hotel. This was done inside the laminar flow hood to minimise contamination. The hotel was fed with new medium every 2-3 weeks, encouraging the growth of new HS SCOBY. Once 5 HS SCOBY had grown, they were removed and used to start a new hotel using the above method. This extra step was implemented to reduce the traces of tea-based medium in the final SCOBY hotel. The feeding and medium exchange frequency was then matched to the one of the tea-based hotel.

Liquid Inoculation

The symbol indicating the use of liquid inoculum is: 

To inoculate fresh medium with liquid, the medium of a well-established SCOBY hotel is taken and added to the nutrient solution. Each SCOBY hotel is left to mature for at least 6 weeks to achieve a high concentration of bacteria and yeasts. The medium is then stirred for a few seconds to help the even distribution of the living organisms from the liquid inoculum. Depending on whether the fresh medium of the experiment is tea-based or HS, the according SCOBY hotel was chosen to extract the liquid inoculum from.

The main drawback of this inoculation method can be a reduced speed of BC growth due to the lower concentration of bacteria and yeast added. It also requires more preparation by creating and maintaining a SCOBY hotel. As a benefit, however, the BC sheets grown using this method were significantly more even in thickness and did not have a visible mark from a detached SCOBY.

As the concentration of fresh medium to liquid inoculum plays a role in the speed of the BC growth, the best concentration was determined through a set of experiments. For both the tea-based and the HS medium, five concentrations were tested. Each medium was tested in triplicates to improve reliability of the result. Every 24hrs the thickness of the BC and any visible changes were noted and photographed. After 14 days, the concentration with the most growth and best evenness in appearance was determined.

For the setup of the experiment, the ratios of 1-to-1, 1-to-5, 1-to-10, 1-to-50, and 1-to-100 of inoculum to medium were tested. For each ratio, a falcon tube with an overall content of 10ml was placed inside an incubator at 28° Celsius to ferment. The cap of the falcon tube was only loosely screwed on and opened fully inside the laminar flow every 2 days for oxygen exchange. The ratios in millilitres were as followed:

Table 4.3: *Summary of liquid inoculum to medium ratios tested to identify the ideal ratio for further experimentation*

Ratio	Liquid Inoculum	Fresh medium
1-to-1	5 ml	5 ml
1-to-5	2 ml	8 ml
1-to-10	1 ml	9 ml
1-to-50	0.2 ml	9.8 ml
1-to-100	0.1 ml	9.9 ml

A difference in growth speed was observed between the two media. While the tea-based samples grew quicker the higher the ratio was, with the 1-to-1 ratio showing the quickest speed, the HS samples showed the fastest growth in the 1-to-50 and 1-to-100 samples, closely followed by the 1-to-20 samples. The 1-to-1 and 1-to-5 started off with fast growth until day 6, from where the growth plateaued. Another difference in growth behaviour was the formation of fermentation gases in the tea-based samples. These gases formed into a bubble and began pushing the thin BC growth at the surface upwards, resulting in an angled position of the BC. In areas in which the gas pushed the BC away from the liquid medium, the growth stopped fully. The result of this was a few pellicles with uneven thicknesses in the tea-based samples. The samples of lower ratios (1-to-50 and 1-to-100) were most affected by this (Figure 4.5).

While liquid inoculation is common practice for BC grown from pure culture, it is less often used with kombucha SCOBY. However, the results of this experiment justified the use of this inoculation technique for both media types as they produced thick and mostly evenly grown BC pellicle in similar, or arguably better, quality than from SCOBY inoculation due to a very even layer structure.

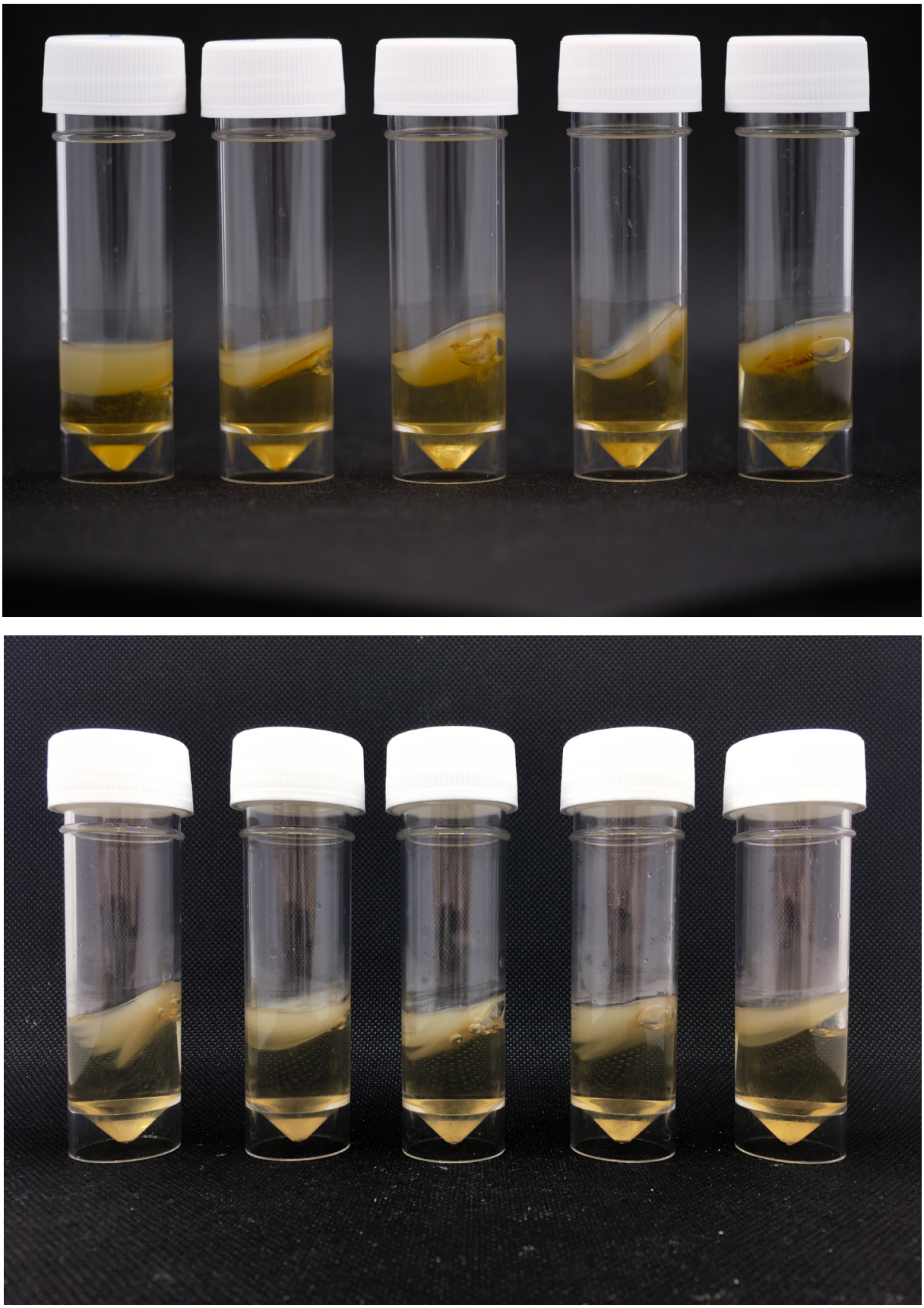


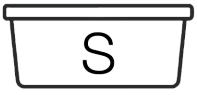
Figure 4.5: *Comparison of concentration test with (top) tea-based medium and (bottom) HS medium after 30 days showing visible differences in uneven BC orientation, gas bubble formation, and thickness of BC. Ratios from high to low (left to right).*

4.1.3. Growth Vessels

Due to the large variety of experimental setups, the growth vessels varied in size and materiality to accommodate each experiment. Growth containers were either made of hard plastic or glass.

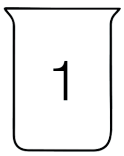
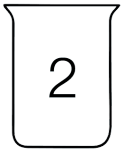
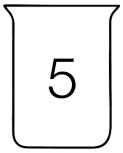
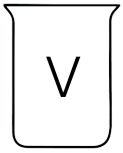
Two different types of plastic vessels were used for the experiments. The first type was a clear store-bought organisation box with standard, loose-fitting lid in the sizes 150 x 250 x120 mm, 190 x 330 x 110mm, and 260 x 360 x 140mm. These boxes will be referred to as S, M, and L box. Additionally, a 600 x 400x 70 mm shallow transportation box without lid was used. This will be referred to as XL size. To sterilise these containers, 70% ethanol and/or 1% Virkon solutionwere used.

Table 4.4: *Summary of plastic growth vessels*

Size S	Size M	Size L	Size XL
150 x 250 x 120 mm	190 x 330 x 110 mm	260 x 360 x 140 mm	600 x 400 x 70 mm
			

The glass vessels were either lab beakers of the brand Duran and Fisher or tall home décor vases. The beakers ranged in size from 1000 ml tall form, 2000 ml tall form, and 5000 ml squat form. The vases were round with a 120 mm diameter, 400mm height, and a volume of 4500 ml.

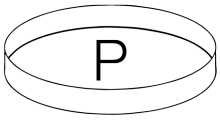
Table 4.5: *Summary of glass vessels used*

Tall Form 1000 ml	**Tall Form** 2000 ml	**Squat Form** 5000 ml	**Vase** 4500 ml
d = 81 mm, h = 180 mm	d = 101 mm, h = 255 mm	d = 200 mm, h = 250 mm	d = 120 mm, h = 400 mm
			

All glass vessels were autoclavable and sterilised before being used for an experiment setup.

The growth container was chosen depending on the requirements and desired outcomes of each experiment. The glass vessels offered greater medium depth with less surface area exposed to air, while the plastic containers offered less medium depth with a larger surface area exposed to air.

Additionally, a series of small samples was grown in petri dishes. The symbol used for these experiments is:

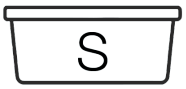


4.2. Static Kombucha Growth

As the name suggests, the fermentation of BC in a static culture does not involve any kind of movement, neither of the medium or the developing pellicle. For this cultivation method, liquid medium is inoculated with the desired bacteria and left to ferment in an open container (see Chapter 2.2.1). The process of static BC cultivation was tested to gain foundational knowledge on the organism's growth behaviour and to develop an understanding of smells, colours, and textures that occur during BC growth. These observations then formed part of the background knowledge that informed the development of experimental setups in Chapter 5 to 7 (see Chapter 3.4).

For the experiment, one SCOBY culture from 'Brew Your Bucha' was purchased and used repeatedly. This experiment was conducted in a home kitchen due to a lockdown restrictions. Surface areas and equipment used were sterilised with 99% isopropanol, also called rubbing alcohol, and single-use gloves were worn while handling the SCOBY. Once experimentations were moved to the laboratory, 70% ethanol was used to sterilise equipment and work surfaces instead.

4.2.1. Experiment Setup

Nutrient Media	Inoculation	Vessel	Duration (days)
			28

For the experiment, one SCOBY culture from 'Brew Your Bucha' was purchased and used repeatedly, together with 3000ml of medium following the tea-based recipe (Table 1). To allow for continuous air supply inside the box but decrease the risk of air-borne contamination, a rectangle (50 x 120mm) was cut out of the lid and covered with cotton fabric (Figure 4.6). In later experiments conducted in the lab, the lid was fully replaced by a large cotton fabric spanning over the whole opening of the container. The most common way for BC to grow is as a biofilm on top of a static liquid at ideal conditions of 27-30° Celsius. To replicate these conditions, the growth container with medium and SCOBY was placed on a warm shelf and left to ferment without being moved. The lid was carefully lifted every 24 hours to document the growth of the BC on the surface. The transparent walls of the growth container allowed observation of the growth in thickness without having to lift the pellicle (Figure 4.7).

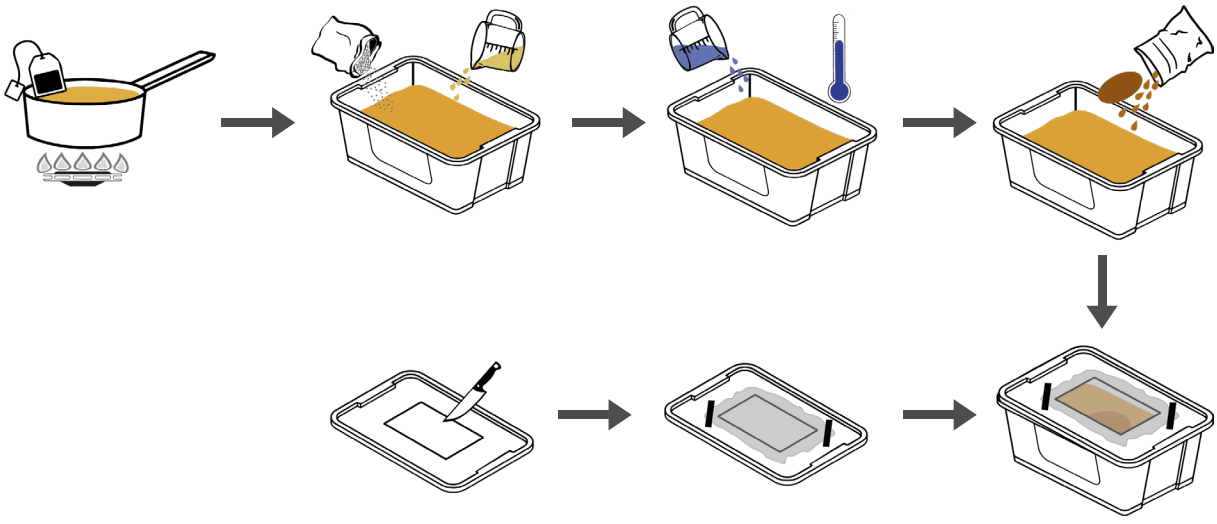
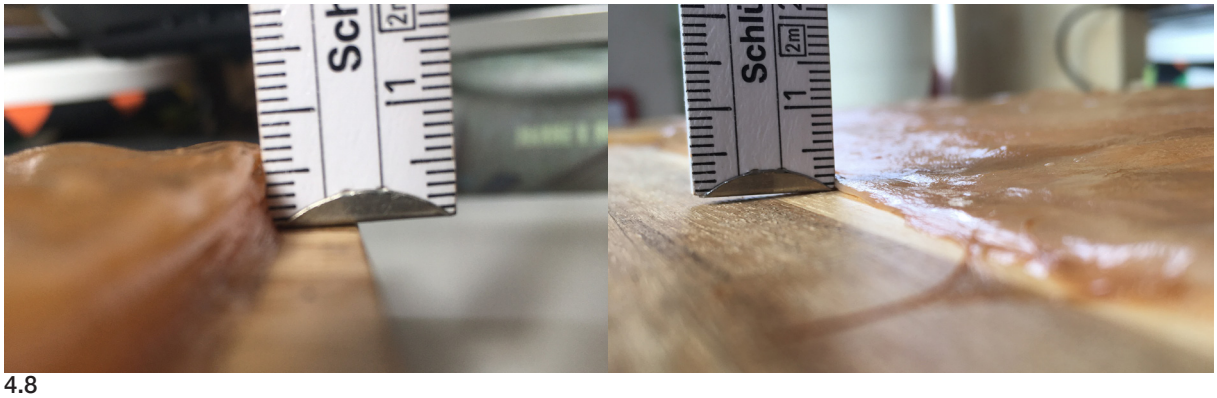


Figure 4.6: *Process of growing bacterial cellulose with SCOBY inoculation in tea-based medium preparing the lid of the plastic growth vessel with an aeration opening covered by cloth.*

After the growth plateaued and showed no more change for 7 days, the experiment was ended and the pellicle removed from the liquid. This process will be referred to as ‘harvest’. To dry the harvested BC the pellicle was placed on a wooden board and left in a well-ventilated area. After 2 days, the pellicle was turned over to achieve an even drying on both sides. Once the pellicle was visibly dried, it was peeled off the wooden board and stored in an airtight container after documentation. The dried BC changed from a thick opaque material to a paper-thin and translucent material (Figure 4.8).

4.7



4.8

Figure 4.7: *(left) Top view of static culture after 3 days with SCOBY in the centre of the sevvel and a yeast cluster on the left side; (right) side-view of the feathery yeast cluster.*

Figure 4.8: *(left) BC thickness immediately after the harvest; (right) BC thickness after 48 hours of drying on a wooden board*

4.2.2. Harvest Process

Harvesting the pellicle refers to the removal of the newly grown BC from the growth medium after it has been cultivated for the desired amount of time. The goal of the harvesting process is the removal of excess medium and bacteria from the BC, and the concomitant interruption of fermentation. In the first step, the BC pellicle is removed from the nutrient medium and rinsed under cold water until it runs clear in colour (Figure 4.9). If the BC was grown using full SCOBY inoculation, in a second step the mother SCOBY is carefully detached by pulling it away from the new growth. Lastly, the BC pellicle is then washed with antibacterial dish soap by carefully lathering it on both sides. After rinsing the soap off, the BC is padded dry using paper towels.

In the case of a highly contaminated experiment in the lab, all living organisms need to be killed off before harvesting the BC. Contamination includes the growth of other organisms, such as mould, algae, fungi and more. Spores of these organisms can be found in the air and exposure of growth medium to air can cause an unwanted inoculation of it with these environmental organisms. To kill off any contamination, the growth vessel and its content were either or Virkon powder was added to the nutrient medium to create a 1% solution. Once the Virkon solution had changed colour from pink to transparent, the BC could be safely removed from the vessel.

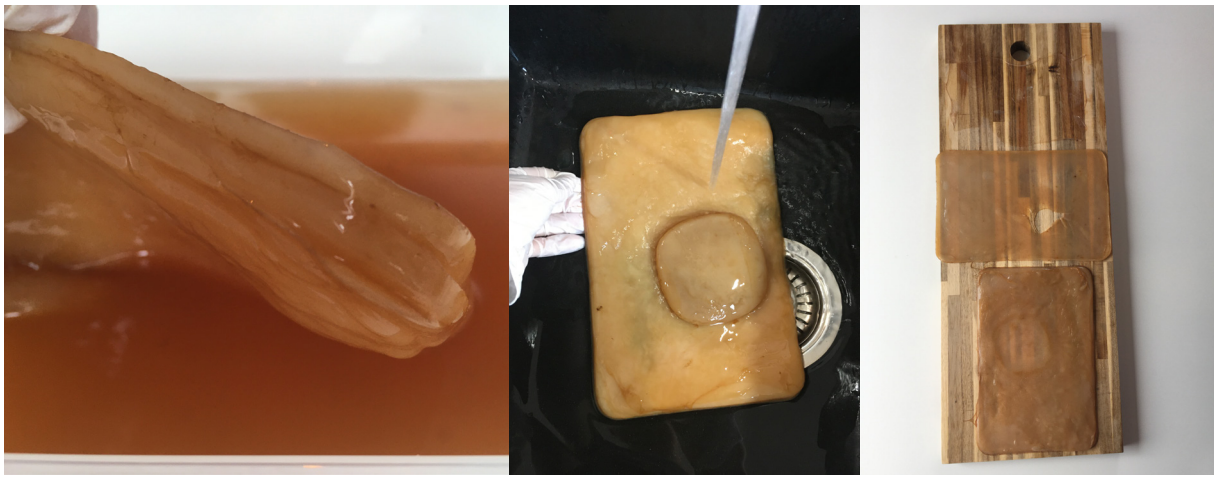


Figure 4.9: *(left) BC during harvest showing multiple merged layers; (middle) washing of BC with cold tap water; (right) placing of BC on wooden board for air drying*

4.2.3. Observations

After repeating this experiment of growing BC statically, multiple times, the following of observations about the growth behaviour of the BC could be made. In a static condition, the biofilm of BC begins to be visible after 48 hours of fermentation (Figure 4.7) and grows steadily for up to 15 days covering the full surface area of the medium. If the growth box is moved during fermentation and a layer of medium forms above the new pellicle, a new detached layer of BC will begin to grow on top of it (Figure 4.9, left). After the peak of 15 days, the growth speed decreases and eventually halts after around 30 days. After 30 days, no more increase in BC thickness was observed. The fermentation intensifies the smell of vinegar noticeably during the first week of growth and creates gas bubbles underneath the growing pellicle which can alter the pellicle shape.

Based on these observations, basic principles to work with Kombucha BC had been determined to begin the explorative experimentation towards 3-dimensional growth in Chapters 5 to 7:

1. The growth vessel, as well as the shape and size of the air-liquid interface of the medium inside the vessel, need to be chosen depending on the desired outcome of the experiment because the newly grown BC takes on the shape of the medium surface created by the horizontal section of the growth vessel.
2. The availability of nutrients in the medium influences the overall growth of pellicle colour, thickness, production of gases, and speed of the culture.
3. BC can be grown in a non-sterile environment and certain contaminations, such as small areas of white mould, can be removed without affecting the growth process.
4. The BC can be grown at room temperature (21° Celsius) but the growth speed decreases when the temperature drops below 25° Celsius.
5. Fermentation gases build up underneath the growing pellicle and can collect in bubbles, pushing the pellicle away from the nutrients in the media. This stops the growth in that area of the BC because the bacteria can no longer access the nutrients. The bubbles can be pushed out to the sides of the pellicle to avoid this from happening.

4.3. Autoclaved tea-based medium

To test whether the Kombucha medium could be made more resilient against contamination, a version of autoclaved tea-based medium was compared with non-autoclaved medium. A heat treatment is not common practice for this medium type due to the nature of work for which it is used, including homebrew kitchens and large-scale static growth.

To account for possible crystallisation of the sugar during the autoclaving process, the medium was prepared in two separate bottles, following the principles of HS medium preparation (see Chapter 4.1.1). Bottle A was prepared with 500ml of filtered water and 100% of the sugar (Table 4.1). For bottle B, 430ml of filtered water was brought to boiling point and then left to steep- with 1 tea bag for 15 minutes. After removing the tea bag, 200ml of cider vinegar were added. The electromagnetic stirrer was used to evenly combine the components. Both bottles were then autoclaved. The colour of the glucose water in bottle A changed from clear to translucent yellow, while the content of bottle B only appeared slightly darker than before. However, once the solutions had cooled down to room temperature, the colour of bottle B had changed into an opaque yellow-brown colour. To finish the preparation of the autoclaved medium, 500 ml of the glucose solution were added to the second bottle under a flame. For comparison, a fresh bottle of kombucha medium was prepared using the original recipe, not autoclaving the contents.

The experiment setup included two 1000ml tall form beakers, one with 200ml of autoclaved medium, one with 200ml of fresh medium. The beakers were autoclaved prior to setup, and the beaker with the autoclaved medium was prepared in sterile conditions under a flame. As liquid inoculum, 100ml of liquid was added from the kombucha SCOBY hotel. The top of the beaker was covered with a double layered cotton cloth secured by a rubber band and the culture left to ferment for 30 days.

After 30 days, a significant difference in the thickness of the new pellicle growth could be observed (Figure 4.10). The non-autoclaved medium beaker showed a 10mm thick pellicle with even thickness and a mostly matt and even surface. The pellicle in the autoclaved kombucha beaker varied from 4-6mm and the surface had a shiny appearance. The colour of the autoclaved medium had changed back to a translucent orange-yellow colour with dark brown residue sitting

at the bottom of the beaker. The colour was visually identical to the non-autoclaved medium, apart from the residue at the bottom. The colour of the two pellicles was identical, an opaque cream yellow. No fermentation bubbles were visible in the non-autoclaved medium beaker, while two medium sized (8-15mm) bubbles were visible underneath the autoclaved medium pellicle. Both pellicles had thin layers of growth hanging underneath. Neither of the pellicles showed signs of contamination.

The results of this test show that no benefits in BC growth can be achieved through autoclaving the medium. Neither of the setups were contaminated during growth duration, suggesting that the low pH of the non-autoclaved medium is sufficiently resistant against contamination for the purposes of this thesis.

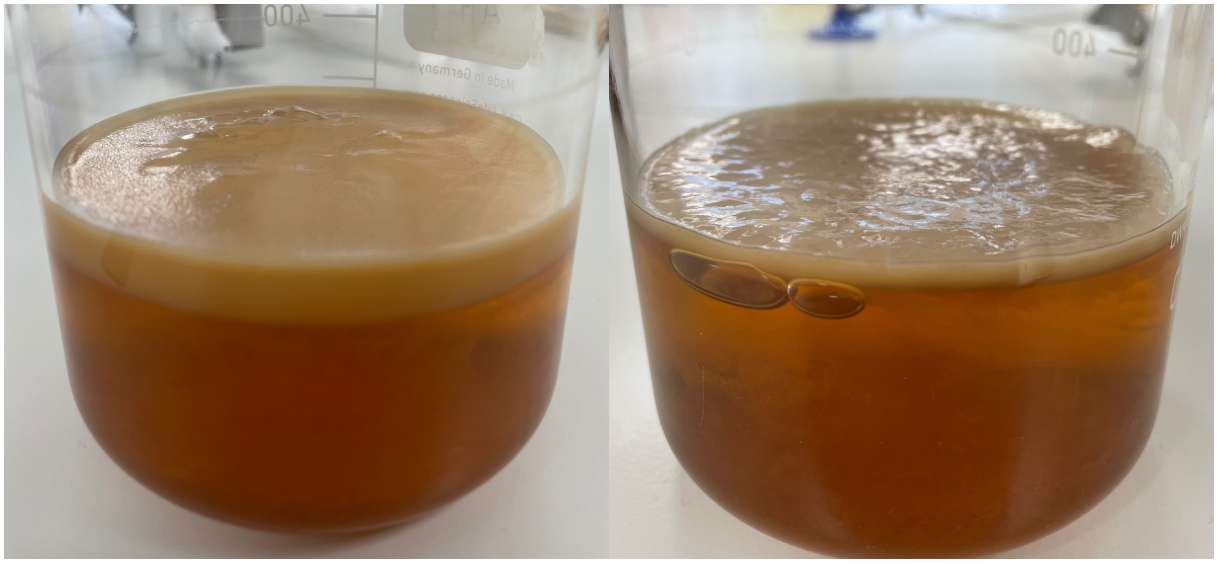


Figure 4.10: *(left) BC thickness in non-autoclaved tea-based medium and (right) BC thickness in autoclaved tea-based medium after 30 days of growth*

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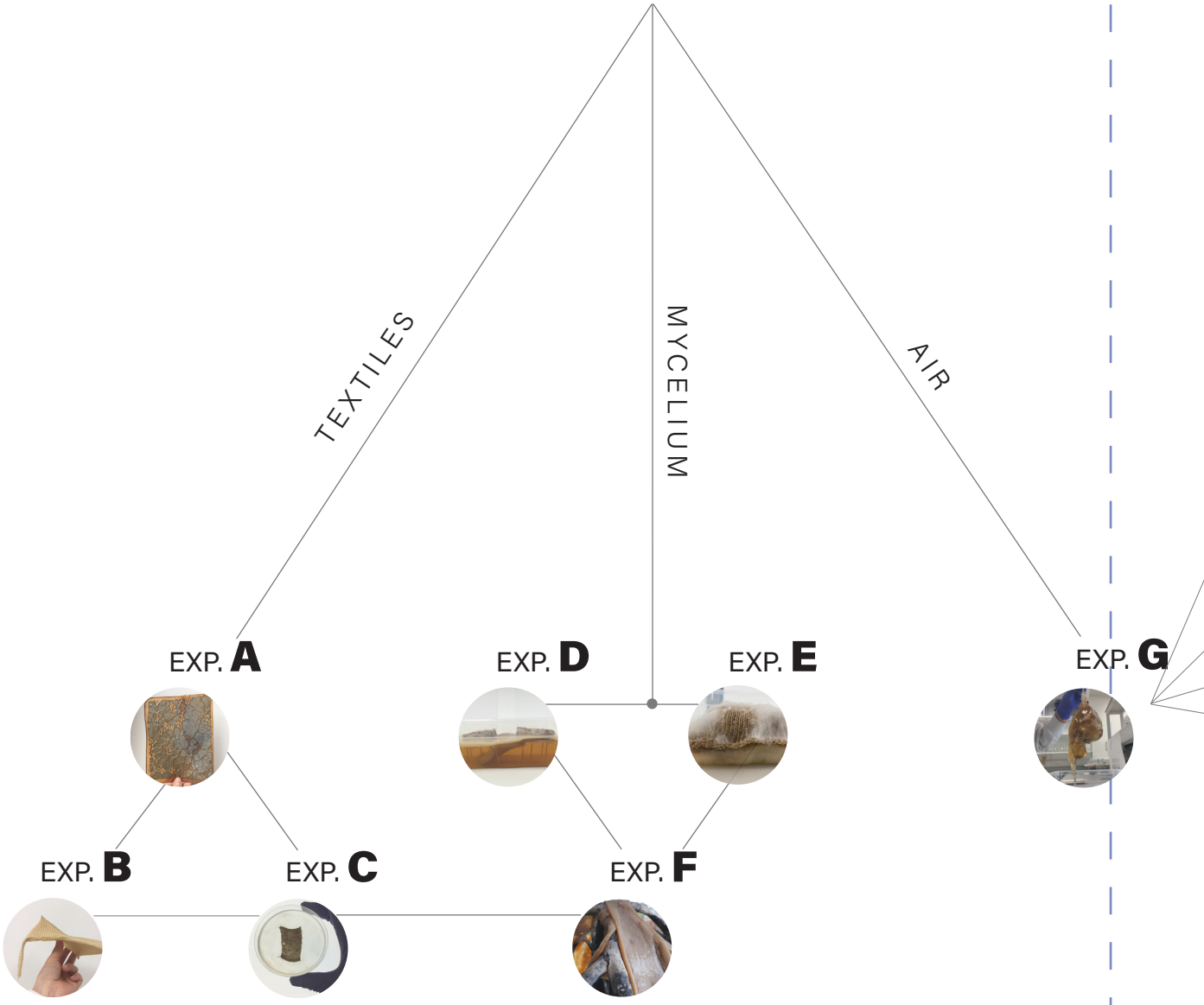
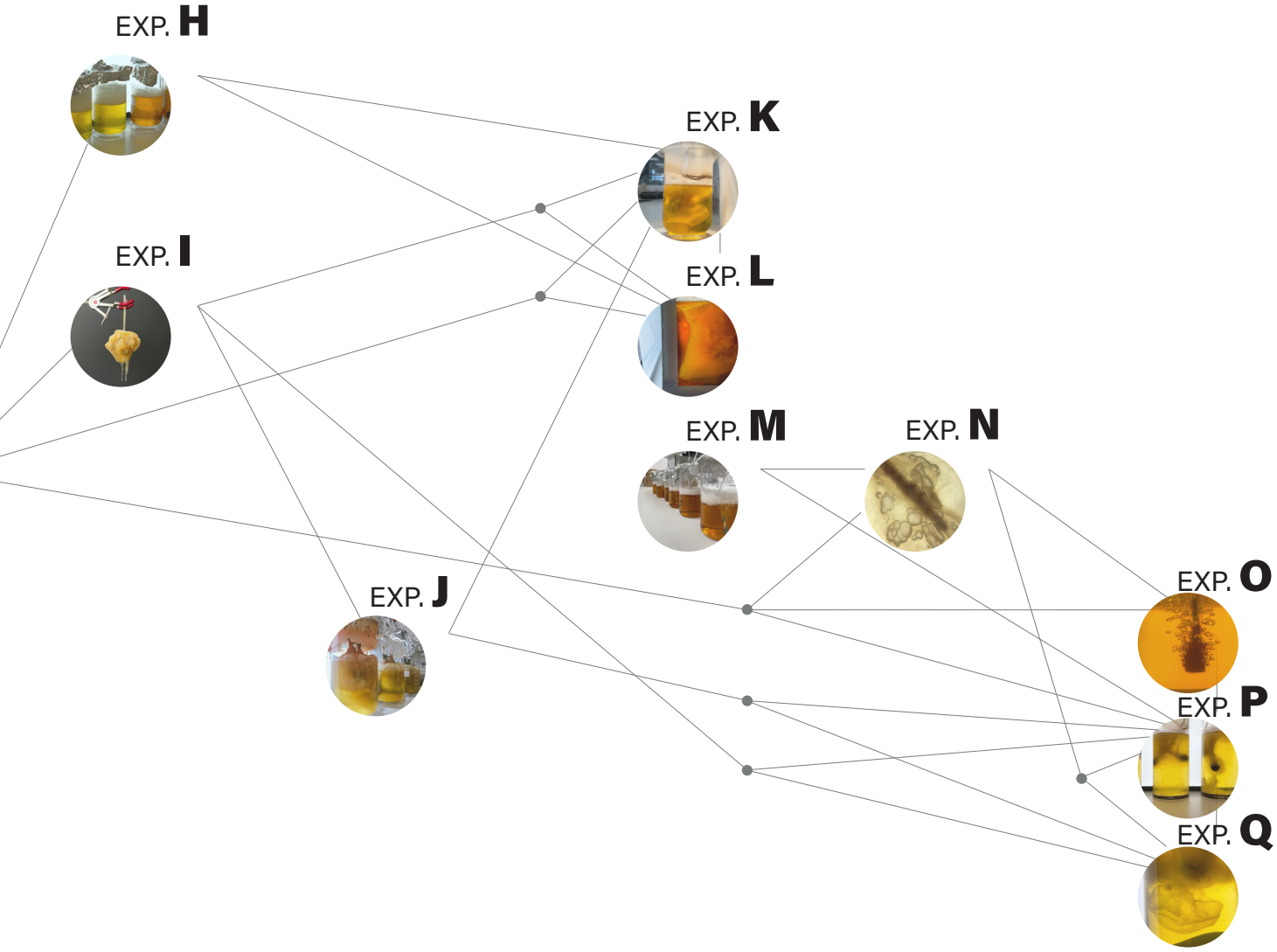
Scaffolding Material Tests



A | Fabric Integration
B | Fabric Tensioning
C | Yarn Differences
D | Mycelium Compatibility
E | Mycelium Pocket
F | BioKnit Prototype

Aeration Scaffolding



G | Effect of Aeration
H | Comparison of Medium Recipes
I | Identifying Growth Stages
J | Combination of Air Stones
K | Upscaling in 5L Beaker
L | Upscaling in Fish Tank
M | Alternative Straw Materials
N | 3D Printed Air Stones
O | Comparison ABS and PLA
P | Material Combinations
Q | Customised 3D Printed Scaffolds

A small mushroom growing from a mycelium composite tile on the surface of a bacterial cellulose culture

CHAPTER 5

SCAFFOLDING MATERIALS

Chapter Summary

For the first experimental phase in this research, a variety of non-living and living materials were explored as scaffold to aid the formation of 3-dimensional BC objects by creating composite materials. The experiments were designed to first, test the compatibility of the additional material with the living bacteria, and second, explore methods to shape or guide the development of the BC into complex forms. While a variety of BC composites of 3-dimensional form have been reported or published already (see Chapter 3.2.), the here presented experiments aim to minimise the need for post-processing the BC after it has finished the growth stage and focus on providing the living organism with an environment that encourages a new growth behaviour.

This part of the experimentation was limited to testing three materials as composite components which include textiles, mycelium, and aeration devices. These materials were chosen due to their relevance of ongoing prototyping work within the Living Construction theme of the Hub for Biotechnology in the Built Environment (HBBE) (Chapter 5.1.1. and 5.1.2.) and based on the environmental conditions required for the metabolic process of the bacteria (see Chapter 3.1.1.) to produce BC (Chapter 5.1.3).

Each material tested can be categorised into internal and external scaffolding, depending on how the composite with the BC is formed and at which stage the shaping of the BC is occurring. Internal scaffolding is integrated into the growing BC to either influence the shape post-growth (Chapter 5.1.1.) or to guide the assembly of BC fibres during the active growth phase (5.1.3.). The external scaffolding is already in the desired 3-dimensional form and can provide a frame for the bacteria to deposit the cellulose fibres on or function as a positive mould for the BC to dry onto (Chapter 5.1.2.).

5.1. Textiles as Scaffold

Textiles have played an important role for the humans for millennia, with the oldest known fibres dating back 34,000 years (Lavoie, 2009). With their use ranging from garments to tools, such as rope and baskets, fibres can be found in nearly every aspect of our modern life. In architectural application and research, fabrics have gained increasing interest as a flexible formwork for complex and organic concrete structures (Veenendaal *et al*., 2011). In recent years, textiles have started to assume yet another identity in research as versatile biomaterial either as host or component of Engineered Living Materials (Lee *et al*., 2020). Notable examples range from the use of fibres as host for microorganisms and their metabolisms on a microscopic level (Sherry *et al*., 2023; Beyer *et al*., 2019) to large-scale prototypes utilising complex fabric structures as scaffolding and shape-defining components (Scott *et al.*, 2021).

Bacterial Cellulose and textiles have been explored as composite materials with a focus on improving mechanical properties of the BC (Damsin, 2019; Puello Acosta, 2018; Frajova, 2021), implementing BC as environmental protection coating on historic silk fabrics (Wu *et al*., 2012), and testing the ability of advanced wound dressings (Meftahi *et al*., 2010). The application of BC as a fully functional textile has also been investigated in material science and the grassroots research community in the form of a vegan leather alternative. In fashion, notable examples include the 'BioCouture' project (Lee, 2011; Lee *et al*., 2020), compostable sneakers prototypes (ONExONE, 2020), 'Nullarbor' garments (nanollose, 2023), and handbags (Material District, 2019). These projects either use the BC as a sheet-like material to cut out stencil components or use the BC to produce fibres that can be spun into yarns and knitted into fabrics. While the first approach includes the drying of the BC over a positive mould to retain a 3-dimensional product, the second approach utilises the inherent ability of textiles to stretch and bend from a flat into a complex 3-dimensional form. However, the potential of combining the malleable characteristics of a traditional fabric with the shape retention abilities of dried BC has not been explored in explored in great detail.

The first set of experiments in this chapter focuses on the use of different fabrics, varying in sample size, yarn material, knit pattern, and stitch size as potential non-living component to guide

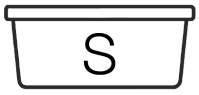
the growth of BC into desired forms. Exp. A, B, and C help develop a deeper understanding of the growth behaviour of BC when introducing foreign objects into the growth culture during the active growth stage and how this can affect the drying process. The following questions guided the start of the exploration of BC and fabric composite materials:

1. Do the bacteria survive in the presence of foreign objects of varying materiality?
2. How does the BC grow with the foreign objects? Does it integrate into its microscopic fibre structure or does it grow surrounding the whole knitted piece of fabric?
3. What are the limiting factors for the growth of the BC onto the integrated fabric scaffolds?

Additionally to the methods of growing BC introduced in Chapter 4, the textiles were prepared prior to their addition to the inoculated medium to reduce the contamination risk and remove any residue of chemicals and dirt. All fabrics were either machine washed at 30 degrees or hand-washed at cold temperatures, depending on the yarn type, using non-bio laundry detergent. The fabrics were fully air-dried and then stored in a closed plastic container until use.

A | Fabric Integration

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			16/68

5.1

Figure 5.1: *Hand-knitted fabric next to the growth container size S*

Experiment Method:

This first experiment explored the potential of integrating different types of fabrics into the BC with the aim to use the fabric as a scaffold to encourage the growth into all three dimensions through the matrix of the knit pattern.

The types of fabrics chosen were a home-made hand-knit (270 x 130mm) using the Garter stitch (Figure 5.1) and a blue shop-bought cotton lace (Figure 5.2). The knitted fabric was made using 'Knitting essentials' acrylic yarn in the colour 'super chunky oatmeal' and 8mm needles. The handknitted fabric was chosen due to its visible 3-dimensionality within the stitch pattern which was a result of the choice of yarn and needle size. The result was a fabric with an open structure and potential for the

BC to grow within. The lace fabric was chosen due to the varying density within the pattern. A flower pattern showed a finely knitted fabric embedded within a loosely connected irregular grid of strings. Within this grid, holes of varying sizes (15 x 8mm to 25 x 15mm) were created, which were of particular interest to understand the BC's potential to enclose foreign materials. Each experiment used as growth vessel size S with approximately 2.5 litre of tea-based medium that was inoculated with 1 full SCOBY of approximately 120g.

Two different methods for the integration of the fabric were tested in this experiment:

1. The handknitted piece was suspended onto the surface of the liquid medium and held in place through strings attached in each corner. Prior to placing the knit, it was submerged into fresh medium and kneaded by hand until fully saturated with liquid. The SCOBY was positioned to float under the knit in the middle of the growth container and added to the nutrient medium before the knit was added. To decrease contamination, the growth vessel was covered using a cotton cloth. The setup was then placed near

5.2

5.3

Figure 5.2: *Store-bought blue cotton lace*
Figure 5.3: *Placing store-bought lace fabric on top of a 7-day old BC pellicle, one piece saturated with medium and the other piece dry*

5.4

5.5

Figure 5.4: *Irregular growth of BC on hand-knitted fabric*

Figure 5.5: *Area indicating where the SCOBY was pushing against the fabric and speeding up the BC growth (Day 6)*

a radiator to achieve a stable growth environment at around 20° Celsius. This setup was left in a static position for 16 days before the BC composite was harvested.

2. The cotton lace was added to the growth setup 7 days after the nutrient medium was inoculated with bacteria. At this stage, a 2mm layer of BC had formed at the air-liquid interface, which allowed the lace to stay on the surface without needing additional fixations. The lace was divided into two parts and placed on the pre-grown BC with a small gap in between (Figure 5.3). The right piece was fully saturated with nutrient medium in a similar fashion to the hand-knitted fabric. The left piece was placed on the BC without prior soaking in medium. This setup was left to grow for an extended period of 68 days*.

Observations Summary – Handknit:

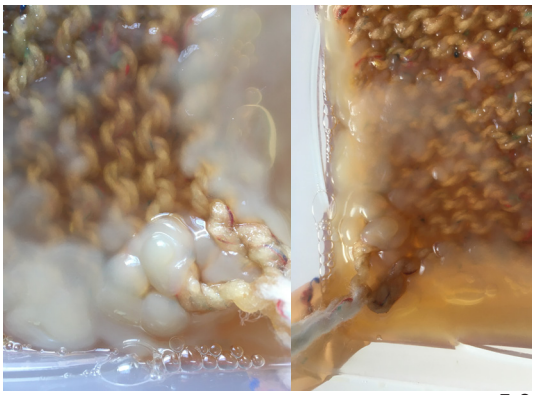
During the growth period, the BC on the handknit was observed to be maturing irregularly, with the side was closest to the radiator growing faster. To even out the growth, the setup was rotated by 90 degrees every 48 hours. On day

9, a 1-3mm thick transparent to opaque pellicle had grown to cover the whole surface of the knit with an irregular surface texture (Figure 5.4). The most mature area of the BC had formed directly above the mother SCOBY, which floated in the centre of the growth vessel underneath the knit (Figure 5.5). At this stage, the BC had also started to grow over the exposed corners, climbing on the knit for up to 6mm above the liquid surface (Figure 5.6).

After a further 7 days of growth the BC composite was harvested. At this stage, the knit was fully covered by a 3-5mm thick white-opaque BC layer with a blister-like surface texture (Figure 5.7). Each of the blisters was 1-2mm in thickness and appeared to be made of fully mature BC rather than filled with liquid. While there is no obvious correlation between the location of the blisters in the central part of the knit, Figure 5.8 shows preferred growth in the grooves created by the yarn in the corners.

The underside of the knit shows that the BC only grew on the top surface which had exposure to oxygen. Where the corners lifted off the medium, the BC grew around and encased the fabric and grew through stretched loops of the knit. A further climbing of the BC upwards on the corners was not observed.

Once the BC composite was fully air-



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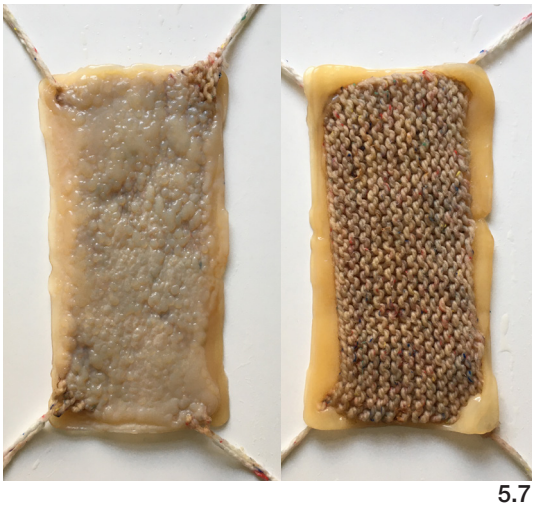

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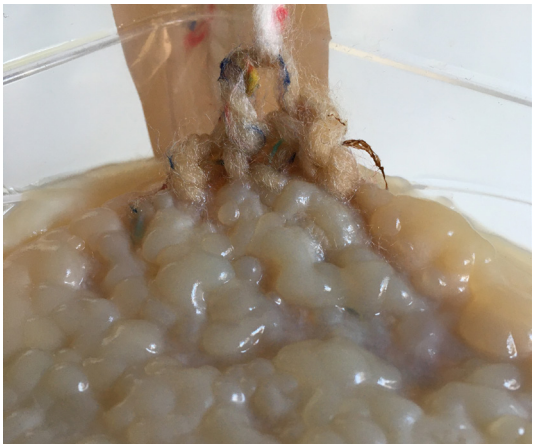
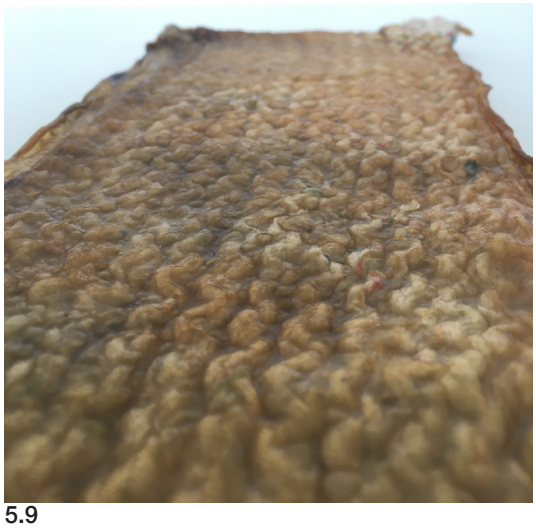


5.8

Figure 5.6: *Growth progress of BC after 9 days showing attachment around exposed fabric corners*
Figure 5.7: *BC growth of on suspended knit fabric after 16 days*
Figure 5.8: *Thicker BC growth visible in the grooves of the knit in the corners on day 16*



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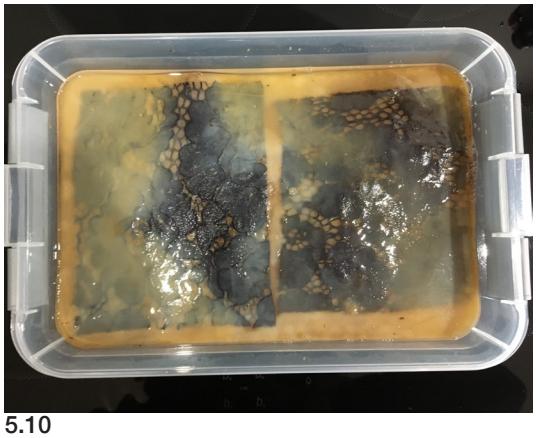


5.10

Figure 5.9: *Dried BC on handknitted fabric only faintly showing uneven growth*

Figure 5.10: *BC growth on the two patches of lace showing an increased maturity on the outside of the vessel*

Figure 5.11 (right): *Dried cellulose with enclosed lace, top showing dry lace and bottom showing hydrated lace*

dried, the blisters-like surface was less apparent (Figure 5.9), and the BC provided stability for the knit to stay in a flat position with minimal bending. The BC shrunk onto the knit and, depending on the thickness of the BC, the profile of the knit pattern was more or less pronounced.

Observations Summary – Lace:

After 68 days of growth, different levels of maturity were visible in the BC grown over the pieces of lace. However, the unevenness spread over the whole surface of the growth vessel with no direct link to the saturated or unsaturated lace (Figure 5.10). The least growth was observed in the centre of the growth vessel, where the two pieces of lace met, and was fully transparent. The profile of the lace was still visible through the BC film. Around the edges of the vessel, the BC was more mature and began to change into an opaque appearance. Overall, the thickness of BC was thinner than the previous knit experiment. This could be contributed to a number of reasons. First, contamination of the culture with fruit flies started in the first 7 days and continued until the harvest. Second, the culture was placed in a different location in the house with less consistent temperatures which can influence the growth speed. Lastly, the materiality of the lace could influence the

bacterial activity.

Before drying, the BC was cut in half to separate both pieces of lace. The section view showed that the piece of hydrated lace was more effectively encased by the BC than the dry piece. In particular areas of larger gaps in the lace showed bridging of the BC between the bottom and the top layer. Once fully air-dried, the BC shrunk onto the lace in both cases and exposed the 3-dimensionality of the surface pattern (Figure 5.11). Areas of more mature BC growth, along the edges of the growth vessel, showed a less distinct patterning. Additionally, only the top layer of BC, which grew after placing the lace, exposed the pattern, while the bottom layer of BC dried predominantly flat.

Developments for Next Iteration:

This set of experiment showed that BC can grow alongside and through different types of fabrics. The bacteria showed no preference between the synthetic and the natural yarn type in regard to the attachment to the fabric. However, the coarseness of the knitting pattern influenced the ability of the BC to grow in between the fabric's seams.

The following questions arose:

1. The BC attaches to the fabric when it is positioned at the air-liquid interface. However, the growth is flat and does not take on 3-dimensional shapes of the fabric's knit pattern. How does the material behave when the BC-fabric composite is stretched and shaped after the harvesting and before drying?

   → *Experiment B (Chapter 5.1.)*

2. Coarse knitting patterns allow the BC to grow through the loops of yarn and encapsulate the fabric by bridging a top and bottom layer of growth. What is the smallest size of loop that the BC can grow through?

   → *Experiment C (Chapter 5.1.)*

3. The BC showed the potential to grow alongside artificial and organic fibres with no clear indication for a preferred materiality. Can a preference be detected for different types of artificial and organics yarns?

   → *Experiment C (Chapter 5.1.)*

* *The prolonged growth time was a result of personal circumstances not allowing the harvest of the BC sooner and not planned*

B | Fabric Tensioning

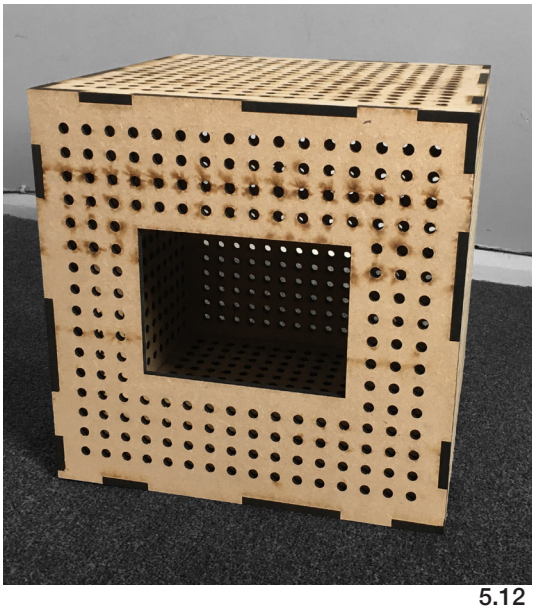
Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			21/28

Collaborators: Jane Scott (Fabrication of six knitted material samples with various knitting patterns used as scaffold for the BC)

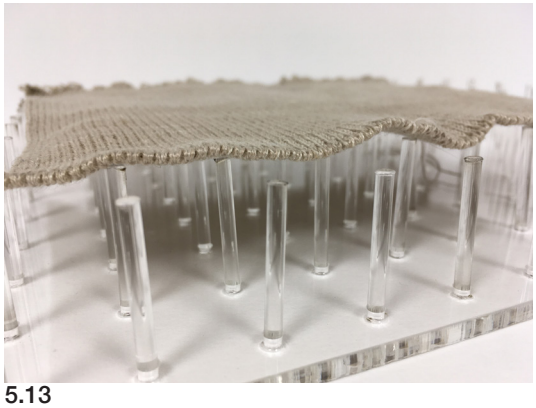
Experiment Method:

This set of experiments explored the potential of shaping a BC-fabric composite into 3-dimensional shapes during the drying stage. Shaping BC into form after the active growth phase has been documented by a number of researchers (3.2.3. Fashion Industry and Material Sciences) but focuses predominantly on draping the flat BC sheet over positive moulds to dry onto. In contrast, the composite material in this experiment is suspended into air and pulled into various shapes using string and a customised drying box (Figure 5.12). The sides of the box were constructed with a tight pattern of holes to attach the string onto and allow for adaptive decision making during the suspension stage to optimise the position for each composite sam-



5.12

Figure 5.12: *Tensioning box made of MDF board with a pattern of holes to attach the cellulose to and one opening on the side*



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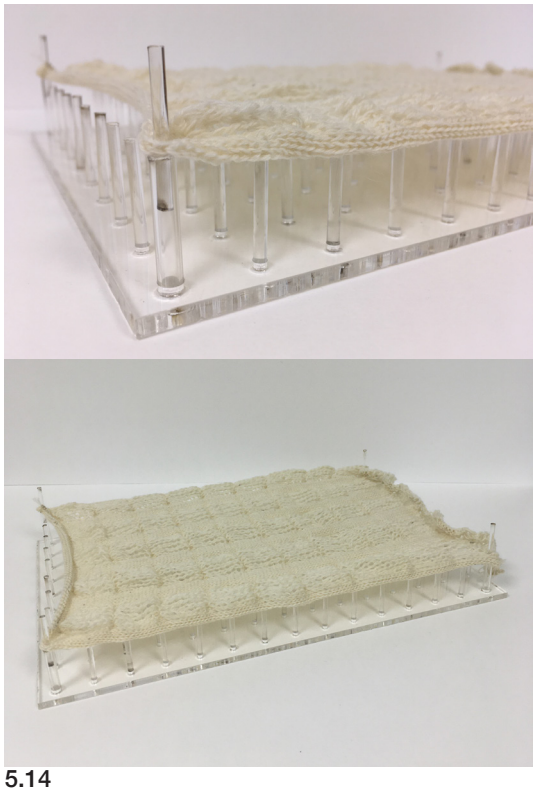


5.14

Figure 5.13: *First iteration of fabric scaffold*
Figure 5.14: *Fixation of fabric in the corner of the scaffold*

ple (400 x 400 x 400mm).

Additionally, a scaffold was designed to hold the fabric in a flat plane on the surface of the air-liquid interface of the medium (Figure 5.13). Exp. A identified one of the main challenges of growing the BC-fabric composite to be the placement of the fabric on the air-liquid interface for an even integration. Being able to place the fabric directly on the surface through the scaffold allowed for a more accurate comparison of the different fabric types being tested in this experiment. The scaffold consisted of two parts: a bottom plate with a 15x15 mm regular grid pattern of holes and acrylic rods cut to 30mm length. The rods were slotted into the holes of the plate in the formation needed to for each sample. Fabrics without stretch were placed loosely on the rods, while those with stretch were attached to the corners (Figure 5.14). This scaffold was placed at the bottom of the growth vessel and the tea-based medium was poured into it until it reached the top of the rods, leaving the fabric partially submerged. The SCOBY was placed next to the scaffold in the medium, not sitting on the rods.

In total, fabrics with six different knitting patterns with varying degrees of stretch were chosen. The yarn used for the knits was raw wool and linen, and each sample was knitted

using a different knit structure (Figure 5.15):

Fabric 1: 1x1 Rib [raw wool]

Fabric 2: 1x1 Rib [linen]

Fabric 3: Knife Pleat [linen]

Fabric 4: Rib with Float [linen]

Fabric 5: Tuck Rib [raw wool]

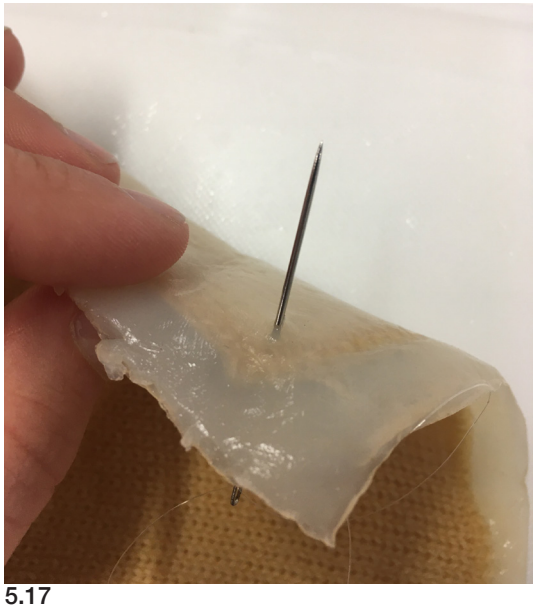
Fabric 6: Drop Stitch [raw wool]

After placing the fabric on the scaffold, the setup was left to grow for 21 days at room temperature before the composite material was harvested and prepared for drying. Once tensioned inside the box, the composite material was left to air-dry for a minimum of 7 days. To release the dried material, the strings were cut loose, and the sample carefully removed through the opening on the side of the box.

The fabrics were tested consecutively, in no particular order, instead of in parallel to allow for adjustments and improvements of the setup between each experiment.

Figure 5.15: *Six different fabrics tested, each made with a different knit pattern*

5.16



5.17

Figure 5.16: *Thin and transparent BC on fabric 5 after a couple of days of growth showing a decrease in liquid levels*

Figure 5.17: *Process of attaching string to the fabric-BC composite for tensioning inside the box*

Observations Summary:

The custom scaffold enabled the fabric to stay at a specific height within the growth vessel for the duration of the growth phase. In particular, fabrics without stretch or creasing within the pattern were evenly submerged into the medium. However, the liquid level of the medium decreased due to evaporation and conversion into BC and resulted in the fabric beginning to droop/sag in between the acrylic rods it was lying on (Figure 5.16). This uneven landscape of the fabric resulted in irregular BC growth, with a stronger appearance in the areas further submerged due to the sagging.

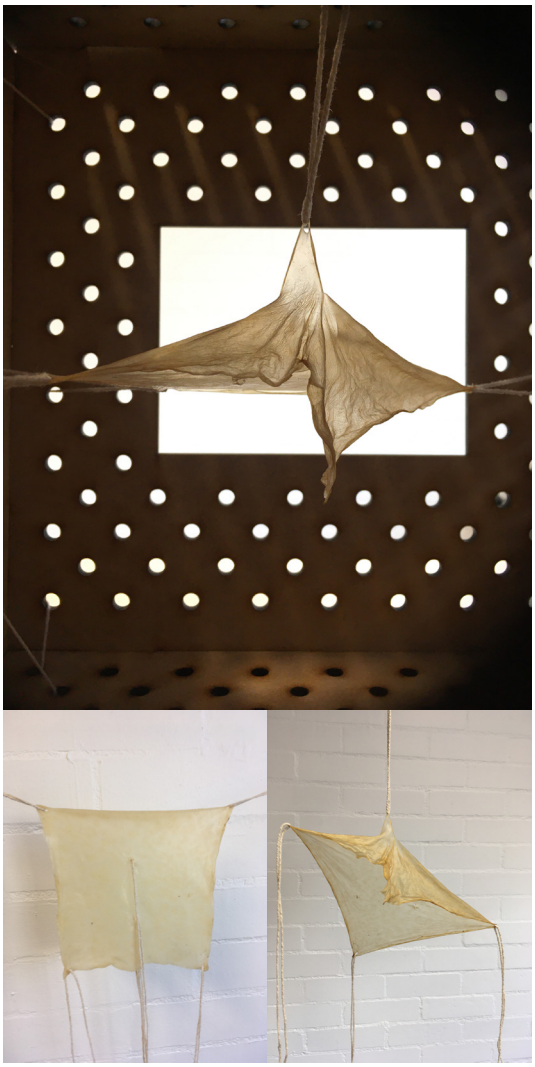
To counteract the decrease of medium in the vessel, a syringe was used to inject more liquid underneath the growing pellicle on the air-liquid interface. To not disturb the growth on the fabric, the medium was added in the corner of the vessel in which the SCOBY was placed, opposite the scaffold. This method allowed a precise levelling of the medium until the fabric was half-submerged. Depending on the temperature inside the lab, the medium needed to be topped up every 2-4 days throughout the 21 days of growth.

It was not possible to fix fabric 6 or fabric 3 onto the scaffold in a way which allowed it to be flat and evenly submerged into the liquid.

This was due to the high level of stretch in the knit pattern which resulted in a curling of the material. After 10 days of irregular growth, it was decided to stop the experiments and exclude the fabrics. Similarly, the BC on fabric 4 grew predominantly detached from the fabric due to the loose strings within the pattern sagging below the air-liquid interface. After 7 days, the experiment was stopped, and the fabric excluded from further development.

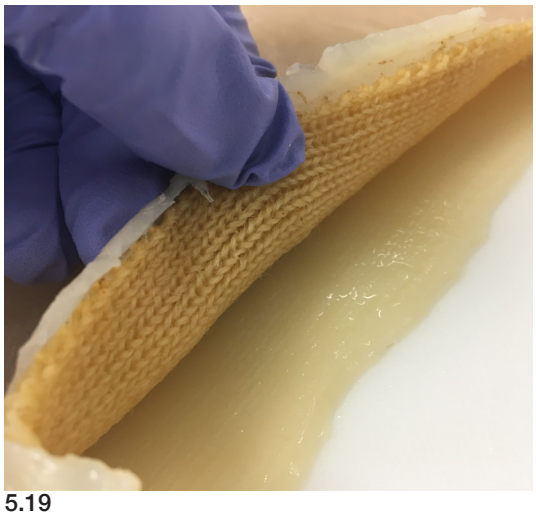
On fabric 2 and 5, even growth of BC was achieved using the syringe method of refilling the medium regularly. After 21 days of growth, the fabrics were harvested and prepared for the tension drying. During this stage it was observed that, even when evenly submerging half of the fabric, the BC only grew on the side exposed to air and did not weave itself into the fabric. Leading from this, the composite material with fabric 1 was created using the same process of the lace integration (Exp. A). The scaffold and SCOBY were placed in the growth vessel with medium seven days before the fabric was placed on the scaffold. At this stage, the BC had grown to a thickness of 3mm and matured to an opaque colour. Before placing the fabric, it was saturated with fresh nutrient medium, and the setup left to grow for 21 days.

To prepare the harvested fabric-BC



5.18

Figure 5.18: *Detached cellulose sheet before (left) and after (right) tension drying and inside the tensioning box (top)*



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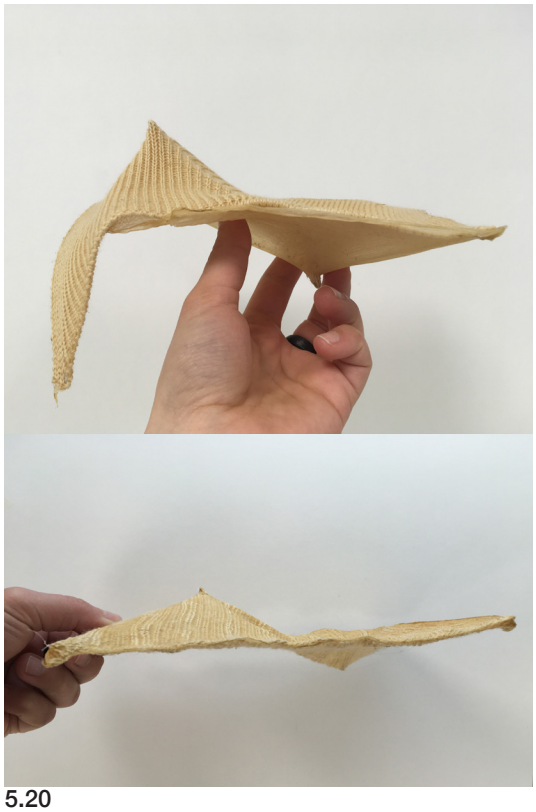


5.20

Figure 5.19: *BC encasing fabric 1, but not showing any attachment to the fabric*

Figure 5.20: *Dried BC and knit composites with fabric 1 and 5*

composites for tension drying, as much liquid as possible was removed from the fabric by placing it fabric side down onto paper towels and applying gentle pressure. Once there was no further liquid dripping from the composite, strings of yarn were attached to the piece using a sewing leather needle. The needle was used to pierce through both layers of material and the yarn attached through multiple looping and knotting (Figure 5.17). The loops were spread over an area of 2-3 mm to avoid the yarn ripping out of the fabric during tensioning.

During the handling of the composites, the weak attachment of BC to fabric was noted. A shearing motion quickly resulted in a partial or full detachment of the BC layer. In the case of fabric 2, the BC fully detached and was tensioned inside the box without reattaching the fabric (Figure 5.18). Through this, a comparison of tension dried BC to tension dried composite was made possible and aided in understanding how much strength the dried BC contributes to the fabric.

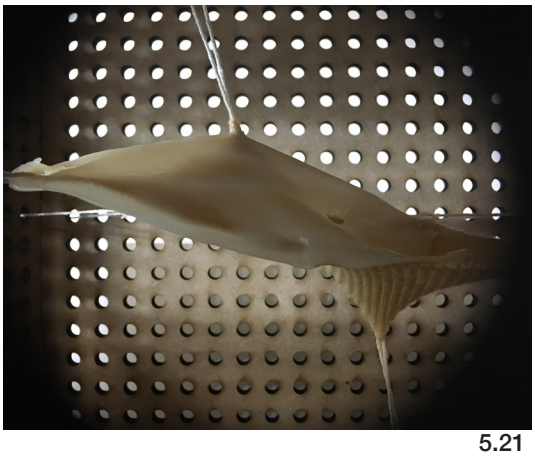
During the harvest of fabric 1, it was noted that the BC had fully encased the piece fabric. Similarly to the lace experiment, the bottom and top layer of BC had merged along the outside edge of the fabric, comparable to shrink-wrapped plastics. However, while cutting the

composite into a rectangle before attaching the strings, the BC layers split and exposed the fabric within (Figure 5.19). The splitting also revealed that there was no attachment of the bottom layer to the fabric, while the attachment of the top layer could withstand light pulling apart from the fabric. Before tensioning the composite to dry, the thinner bottom layer of BC was removed.

Compared to the BC growth in Exp. A, the thickness and maturity of the BC grown on these fabrics is similar to lace composites rather than the knitted fabric. The overall thickness ranged between 3-5mm and visually the BC grew more evenly opaque.

Stretching the composites inside the drying box was possible and resulted in dried materials which were able to hold the shape they had dried in (Figure 5.20). In particular fabrics 1 and 5 showed good shape retention even while being held upright.

Due to varying stretch capacities of the two composite components, applying increased tension on any single point could result in the BC detaching from the fabric. Additionally, the perforation of the BC through the needle prior to attaching the yarn proved to create a weak point prone to further ripping under tension (Figure 5.21).



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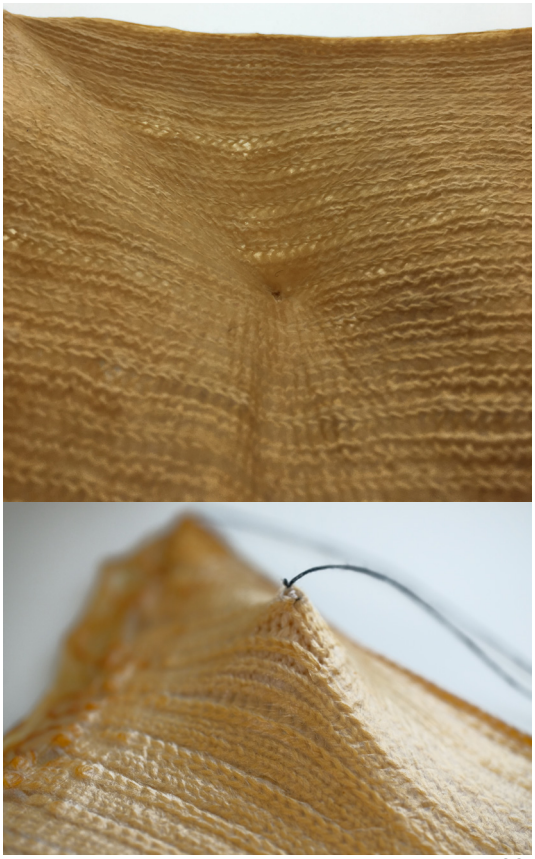


5.22

Figure 5.21: *Fabric 1 tensioned inside the drying box with visible detachment of the BC as well as holes in the BC through rupturing*

Figure 5.22: *Composite holding peak shape and showing BC dried into the stretched fabric texture*

While the BC and fabric separated on a centred downward pull and dried individually, pulling upwards on a point in the centre caused the two materials to be pressed together. This resulted in the BC drying onto the fabric with stronger attachment and into the expanded individual 'pores' of the fabric (Figure 5.22). These areas of stronger attachment, geometrically expressed as a peak, also showed stronger shape retention when placed on a flat surface compared to other parts of the composite.

Developments for Next Iteration:

The prototype of the fabric scaffold aided in the even placement of the material and a more even BC growth. However, the distance between the rods caused a sagging of the fabric which was enhanced by decreasing liquid levels. Additionally, the scaffold did not solve the issue of only one-sided BC growth on the fabric. The following questions arose:

1. Is a further development of the fabric scaffold possible which incorporates a mechanism to feed more medium into the vessel without disturbing growth at the surface and eliminates or reduces the potential of sagging of the fabric?

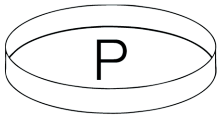
   → *Future Works (Chapter 5.5.)*

2. How does flipping the BC-fabric composite at the halfway point of the growth phase affect and influence the way the BC grows onto the fabric? Is it possible to achieve one continuous BC sheet encasing the fabrics instead of two individual sheets that are 'naturally laminated' at the edges?

   → *Experiment C (Chapter 6.1.)*

C | Yarn Differences

Bioreactor Summary:

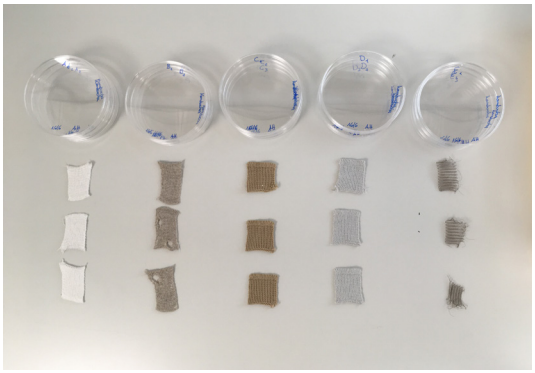
Nutrient Media	Inoculation	Vessel	Duration (days)
			30/48

Collaborators: Jane Scott (Fabrication of knitted material samples made from five different yarns used to test BC adhesion)

Experiment Method:

This experiment explored an alternative way of growing a BC-fabric composite material with the focus on achieving a stronger attachment and interweaving of the BC with the fabric. The aim was to identify the most suitable yarn to create a BC-fabric composite material for the 'BioKnit' prototype, which was developed within the Living Construction group of the HBBE. The 'BioKnit' combined the biomaterials mycelium and BC with a knitted scaffold to create a self-supporting structure (Scott *et al.*, 2021).

Following the results of Exp. A and B, five different types of yarn were tested to identify whether a difference in attachment strength of the BC can be achieved (Figure 5.23). The yarns were chosen after they proved to be compatible



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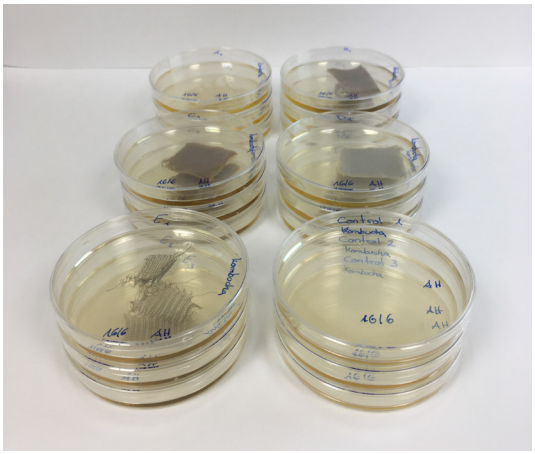


5.24

Figure 5.23: *Five yarn types lined up and size comparison to petri dishes used in the experiment*
Figure 5.24: *Six sets of fabric samples and control in triplicates after 24 hours of initial growth and following placement of fabrics.*

with the other biomaterial, mycelium, in parallel testing for the 'BioKnit' (see Chapter 5.3).

Fabric 1: Soft combed cotton (2/20 Nm)

Fabric 2: Linen (2/28 Nm)

Fabric 3: Merino worsted lambswool (2/30 Nm)

Fabric 4: Acrylic (2/35 Nm)

Fabric 5: Monofilament (1/50 Nm)

The measurement Nm stands for Numeric measure and is a unit of measure that describes the fineness or thickness of yarn. With 1/50 Nm indicating that 50 meters of yarn weigh 1 gram.

To be able to fit the fabric into a petri dish, each yarn was machine knitted into a small sample of 30 x 30 mm using identical setting (stitch length @25, take down (=tension of yarn) @28-64, comb @150, rib). Following the scientific standards of further 'BioKnit' experimentation, each yarn sample was tested in triplicates.

Alternative to growing the BC as a floating element on the surface of a body of liquid, it is also possible to grow BC by layering thin coats of medium on top of an initial layer of inoculated medium in intervals (Hsieh *et al.*, 2016). To test whether a layered growth of BC improves the integration of fabric, this method was adopted for the experiment and adjusted for the use with a kombucha culture. A control set without any fabric was added to track the unhindered growth of BC through this layering method.

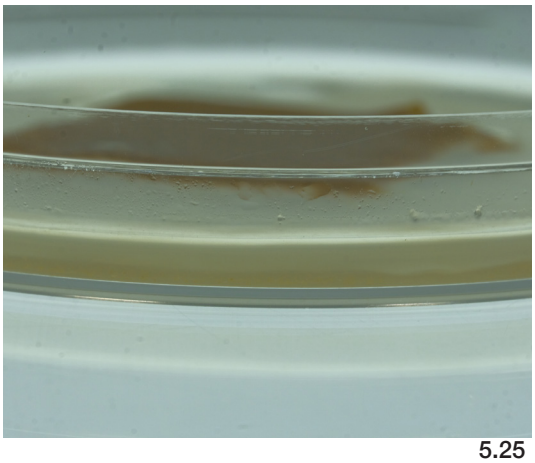
An initial layer of 15ml fresh medium was inoculated with 1ml of SCOBY hotel liquid inoculum inside the petri dish and placed in the incubator at 28 degrees Celsius. After 24 hours, each fabric sample was saturated in fresh medium and placed in the centre of the petri dish (Figure 5.24). After another 24 hours in the incubator, regular feedings of the BC with 1.5ml of medium were done every 3 days. After 14 days of initial growth, the feedings were increased in interval to once a day, and the amount of liquid reduced to 1ml. Before every feeding, the growth of each sample was documented and photographed.

After 30 days of BC growth, set 1 of samples was harvested and air-dried. Set 2 was continued to be fed for an additional 18 days without disturbance to the BC. For set 3, the growing BC-knit composite was flipped upside down inside the petri dish and continue to be feed from the other side for 18 days. This additional step was added to test for increased BC growth on both sides of the fabric without the detachment seen in Exp. B.

Observations Summary:

Within the initial 14 days of growth, the BC was growing in thickness correlating to the amount of medium it was fed, while not maturing in colour. Instead of developing an opaque white or orange hue, the BC remained translucent with scattered brown grains, suspected to be particles of the black tea leaves used to prepare the medium. The gel-like appearance of the BC resembled the appearance of premature BC appearing in the liquid surface of a static culture after 48 hours of growth. Even after increasing the feeding intervals, the BC did not mature in colour, but continued to increase in thickness (Figure 5.25 & 5.26). This indicates a lack of nutrients or oxygen which hinders the bacteria in producing enough fibrils to create fully formed matrices (Klemm *et al.*, 2001).

Apart from the monofilament sample, which did not lay flat due to the more rigid characteristics of the yarn (Figure 5.28), all other samples were fully covered by the BC after 25 days. At this stage, the BC followed the curvature of the fabrics' corners, which showed similarity to the BC attachment in Exp. B (Figure 5.27). Through the transparent bottom side of the petri dish, it was visible that the BC had not grown underneath the fabric (Figure 5.31). During the harvest of set 1, it was discovered that



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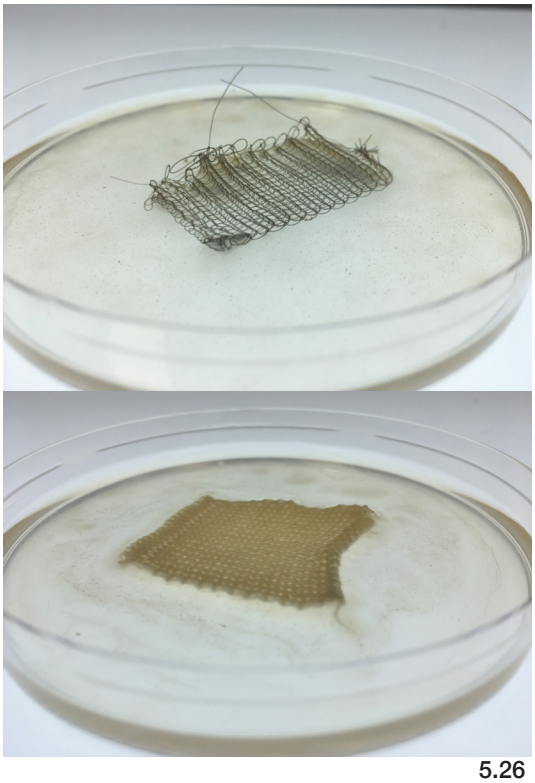

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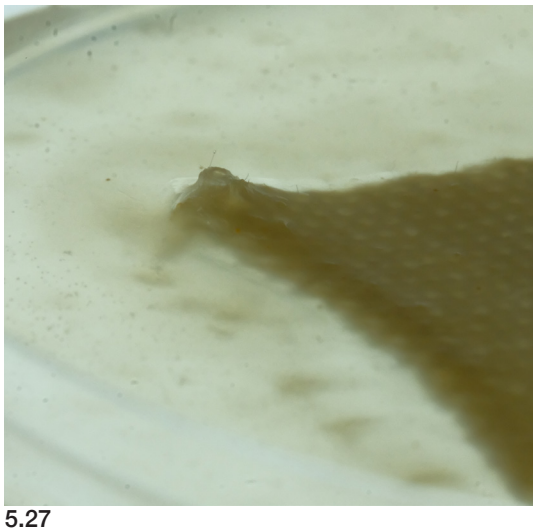
Figure 5.25: *Thickness of BC visible through the side of the petri dish*

Figure 5.26: *BC growth after 10 days*



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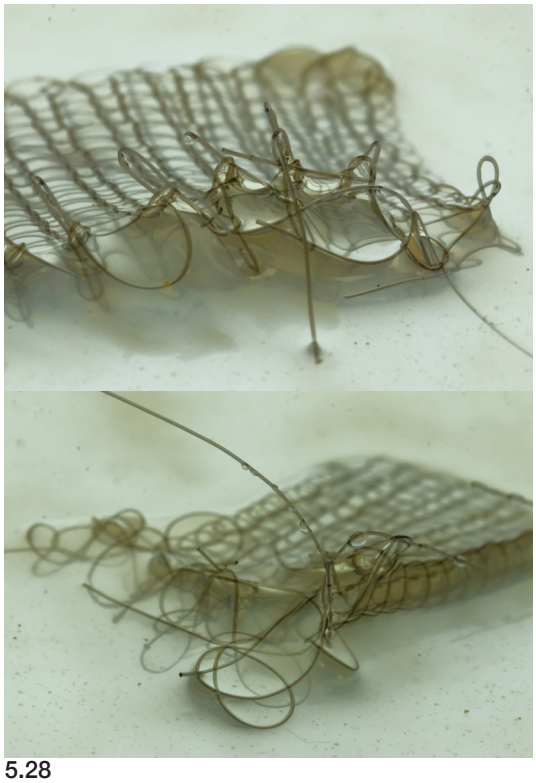


5.28

Figure 5.27: *BC growth after 25 days fully covering the fabric and bending around the corners of the fabric*

Figure 5.28: *Medium that stuck in between loops of the fabric through surface tension grew BC film*

the BC had grown into the surface texture of the fabrics while not penetrating the fabric's structure completely. The transparency of the pellicle in the hydrated state made it difficult to see the full extent of the BC growth. Once air-dried, however, the BC became more visible through an orange discolouration and the paper-like surface texture.

Before removing the BC composites from the petri dish, they measured between 2.7 and 3.5 mm in thickness and were floating on a 1mm layer of medium. As soon as the composite was lifted, a considerable amount of liquid dripped from it back into the petri dish. The translucent BC was not able to hold most of the liquid it had grown on and acted similarly to a sponge; it was possible to squeeze liquid out and leave indents in areas where pressure was applied. The BC composites had an average thickness of 1.5mm after they had been handled during harvest and were placed on top a wooden board to dry. The texture of the BC was softer compared to a statically grown pellicle and did not show the same stability and tensile strength while manually applying force.

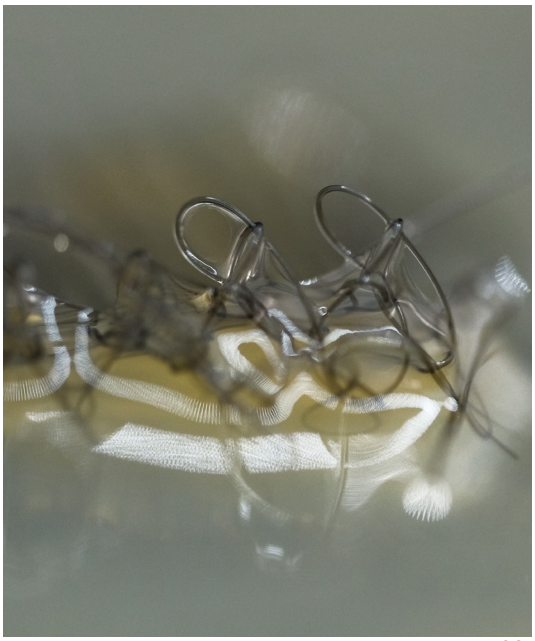
An observation made through close-up photography was that the medium, which spread through the larger loops of the monofilament fibre fabric, also started to grow a thin BC

film, spanning the distance in a vertical position (Figure 5.29). Through surface tension of the medium inside the loops of filament, the fibres offered enough attachment for the medium. This was particularly visible in the dried BC-knit composite (Figure 5.30). The same occurred with drops of medium which attached to the fibres of the fabric patch that stuck out of the otherwise flat sample.

No difference in BC maturity was observed between set 1 (30 days) and set 2 (48 days) with varying growth periods. The BC in set 2, however, did grow a further 1mm in thickness within the 18 days of additional growth.

After the turning of the BC in set 3, new growth established on the newly upward facing surface of the fabric and fully enclosed it. No division or separation between the initial and new growth was visible in the cross-section of the BC. During harvest, the BC did not detach from the fabric which suggests a stronger attachment to the fabric and in between BC of different growth stages.

Overall, no yarn type hindered the growth of the BC. However, different ways and strengths of attachment were observed. The open structure of the monofilament allowed the BC to grow fully through the fabric patch without needing to flip the growing composite half-



5.29

5.30

Figure 5.29: *BC forming a layer in between loops of the monofilament samples*

Figure 5.30: *Dried BC-composite with monofilament fabric*

way. This yarn type showed the most coherent and strong attachment with the BC as composite material. However, the BC grown on tighter knit structures of the other yarn types resulted in a more evenly flat grown layer.

Developments for Next Iteration:

It was concluded that all yarn types can be considered as suitable material for the further development of the BioKnit prototype.

While no further experimentation with yarn types was conducted, ideas for Future Works were developed:

1. Can the observed growth of BC on liquid held on yarn loops due to surface tension be developed into a fabrication method for 3-dimensional hollow BC shapes?

   → *Future Works (Chapter 5.5)*

2. Is it possible to replace the monofilament yarn with a dissolvable material which, after the BC growth period through dip-coating, leaves only the BC shape behind? Could this yarn nurture the bacteria and yeasts through slow-release nutrients?

   → *Future Works (Chapter 5.5)*

Figure 5.31 (right): *(top) Harvested and dried control BC with no added fabric; (bottom) Close-up of underside of dried BC, harvested after 33 days without flipping*

5.2. Mycelium as Scaffold

In parallel to the group of experiments exploring textiles as scaffolding material, the compatibility of bacterial cellulose with the living biomaterial of mycelium was investigated to support the development of the HBBE BioKnit prototype. Mycelium is the root network of fungi and can be grown into composite-materials with notable structural properties (Elsacker, 2021). Previously, BC and mycelium had been researched as composite material with the BC acting as nutrient source for the mycelium to feed on either as part of a paste recipe (Elsacker *et al*., 2021) or as the sole nutrient source (Poncelete, 2018; Euale, 2022). However, both biomaterials exhibit complimentary characteristics desirable for an alternative building material, including fire retardance and heat insulation from the mycelium, and high tensile strength paired with high water holding capacity and water-resistance of the BC. For this reason, a composite material joining the two biomaterials with equal priority is of interest.

Mycelium presents itself as a possible scaffolding material for BC due to the ability of growing it at a small and large scale, ranging from simple blocks to complex 3-dimensional forms (Nguyen *et al*., 2022; Dessi-Olive, Oliyan& Buntrock, 20). In contrast to the previous set of experiments with textiles, the mycelium is utilised as a scaffold for the BC to ‘climb’ onto while growing, forming a skin around the already established 3-dimensional form. Whether the BC can grow further into the structure of the mycelium scaffold is also investigated.

The mycelium used in the following experiments was grown from a composite paste made of various materials, including sawdust, wheat flour, and coffee grounds, which creates a strong and robust final material (Ozkan et al., 2021). Another method to grow mycelium, which requires sterile lab conditions, produces a leather-like mycelium film on the surface of a nutrient medium. In the case of the latter, a material made of 100% pure mycelium is grown. The reason for choosing a mycelium composite over pure mycelium was the intended implementation of it in the large-scale BioKnit prototype which required a certain level of compression strength that was not achieved with the mycelium leather. The BioKnit prototype was designed to proof the concept of a self-supporting structure grown from two living biomaterials, mycelium and BC, using a knitted structure as scaffold to define the shape of the structure and act as soft mould for the mycelium

to grow inside of. To identify the potential application and benefits of BC as part of this prototype, two sets of experiments were designed to test the compatibility of the two materials.

The aim of the first set of experiments was to test the ability of BC to grow in the presence of mycelium which is at different stages of its life cycle (Exp. D). The second set explored the growth potential of mycelium in the presence of BC and the subsequent drying of both materials (Exp. E). Based on the results of each compatibility test, the application of the BC within the BioKnit prototype is decided on, tested, and executed (Exp. F).

D | Mycelium Compatibility

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			21

Collaborators: Dilan Ozkan (Joint development of experimental plan and data analysis together with the author; fabrication of mycelium blocks)

5.32

Figure 5.32: *Close-up of air dried (top) and living mycelium (bottom) tiles*

Experiment Method:

The aim of this set of experiments was to test the possibility of growing BC in the presence of mycelium at different stages of being alive. Mycelium tiles were produced from wheat straw, wood shavings, coffee grounds, and oyster mushroom spawn (sourced from GroCycle, UK) at a ratio of 1:1:1:1. The dry components were mixed together and sterilised for 15 minutes at 121 degrees Celsius before the spawn was added and thoroughly mixed in. Once sterilised, the mixture was handled in sterile conditions. The inoculated mixture was added into sterilised boxes of 100x100x30mm with airtight lids and kept in a dark location at ambient temperature for three weeks. The first sample was first air-dried for 8 days and then heated in the

oven for 2 hours at 60° Celsius. This ensured a fulyl dried sample. The second sample was air-dried for 2 weeks at ambient temperature. The third sample was used directly after being taken out of the sealed container while still in its active growth phase. The samples are referred to as oven-dried, air-dried, and living respectively (Figure 5.32).

To grow the BC, the mycelium tiles were placed inside the growth vessel which was filled with 2500ml of tea-based medium. Following the method developed for the integration of fabrics in Exp. B, the mycelium tiles were positioned half-submerged into the medium and held in place either by two glass jars (Figure 5.33, bottom) or through wooden toothpicks attached in each corner (Figure 5.33, top). The SCOBY used to inoculate the medium was placed inside the box in the centre and between the two mycelium tiles without touching the tiles or the sides of the growth vessel (Figure 5.34).

These setups were left to grow for 21 days, and daily observations were noted and photographed. Where a substantial amount of gas pockets was produced underneath the growing BC sheet, the development was noted and then the gas was removed by gently pushing them to the edges of the growth vessel using a gloved hand. To harvest the composite

5.33

Figure 5.33: *Two methods to hold the mycelium tile in place at the air-liquid interface*

materials, they were gently lifted off the medium and rinsed with water only before air-drying on a wooden board for 7 days. A control experiment was set up using identical method but without the placement of mycelium tiles (Figure 5.35).

The detailed experimental setup, methods, and results are published by Hoenerloh, Ozkan & Scott (2022).

Observations Summary:

It was possible to grow BC around each of the three iterations of mycelium tile but with varying degrees of maturity and thickness. All samples grew a BC sheet at the air-liquid interface as well as on the submerged underside of the mycelium tiles. However, during the harvesting process, the connection between these two areas was identified as a weak point in the BC and ripped partially or fully in two of the three samples.

Each sample produced a noticeable amount of fermentation gasses underneath the growing BC sheet on the air-liquid interface which hindered uniform BC growth, in particular in the case of the oven-dried sample.

Oven-dried

The growth progress was measured on day 14, it measured 12mm at the edged to 2mm in the centre above the SCOBY. The largely ir-

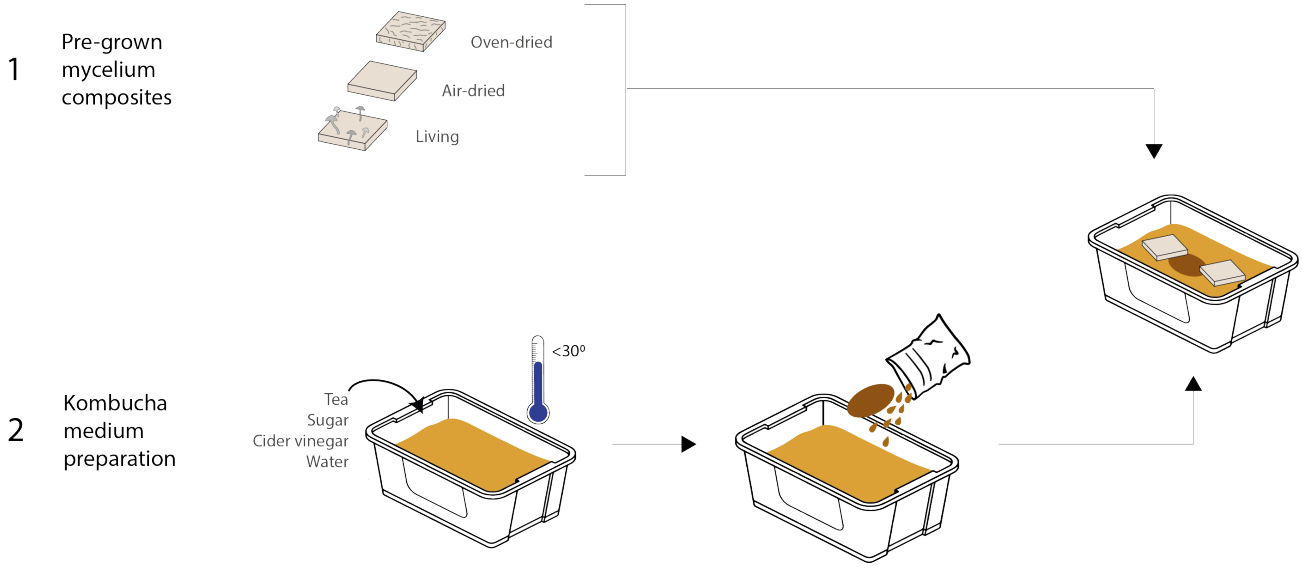


Figure 5.34: *Experiment setup to grow BC around established mycelium tiles split into two processes of 1] preparing mycelium tiles at different staged of life and 2] preparing the BC culture and growth vessel (Hoenerloh, Ozkan & Scott 2022)*

regular thickness can be led back to one large pocket of fermentation gases which had formed over the SCOBY in the centre of the vessel and in between the mycelium tiles. New BC growth was lifted off and no longer in contact with the medium, which halted the growth progress. The BC had grown into a convex shape around the fermentation gasses (Figure 5.36) and attempts to remove the gas were unsuccessful in flattening the BC. During harvest, the thin 2mm area of the BC ripped and partially detached from the mycelium tiles.

While placing the mycelium tiles inside the growth vessel, the hydrophobicity and light weight of the oven-dried samples resulted in the tiles floating on the surface even with glass jars placed as weights. However, after 3 days of growth, a thin layer of BC locked the tiles into position, and they were no longer able to move around the vessel. Overall, no inhibition of BC growth was noted due to the presence of the mycelium apart from the excessive fermentation gas production. Once dried, the sample showed integration into the mycelium tile through exposed wood shavings of the paste material in the previously submerged area of the tile (Figure 5.37).

5.35

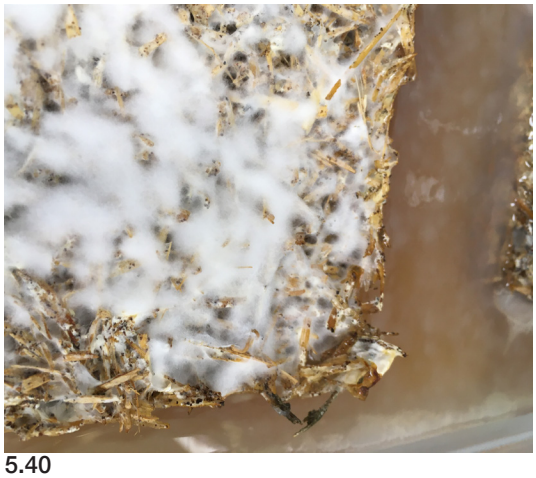
5.36

5.37

Figure 5.35: *Setup with control (left), air dried (middle), and oven dried mycelium (right)*
Figure 5.36: *Oven-dried mycelium sample with convex BC growth above the SCOBY*
Figure 5.37: *Dried BC on oven-dried mycelium*

5.38

5.39



5.40

Figure 5.38: *Increased BC growth around fruiting body and primordia of mycelium tile*

Figure 5.39: *BC grown on submerged side of mycelium tile still attached after BC grown on air-liquid interface detached during harvest*

Figure 5.40: *Continued growth on living mycelium tile during the first 6 days of the BC growth*

Air-dried

Steady growth of BC was observed with the air-dried mycelium sample. On day 6, the BC measured 2-3mm throughout and on day 14, the thickness had increased to 10-13mm. In areas adjacent to dried fruiting bodies and the preceding primordia formation on the mycelium tiles, the BC grew thicker and irregular in shape (Figure 5.38).

While the BC grew more evenly compared to the oven-dried sample, the attachment to the submerged part of the mycelium tile was weaker. During the harvesting process, the BC grown on the air-liquid interface ripped and fully detached from the mycelium tiles, leaving behind a thinner layer of BC grown on the submerged half of the mycelium (Figure 5.39). Once the BC had fully dried, it had shrunken onto the tile, creating a skin-like layer with strong attachment.

Living

For the first 6 days, the BC grew steadily to an even thickness of 2-3mm, and fermentation gases collected in small bubbles underneath the pellicle. The mycelium also continued to grow on the exposed top surface and formed white spots, in particular underneath the glass jar weight (Figure 5.40). By day 14, the BC had

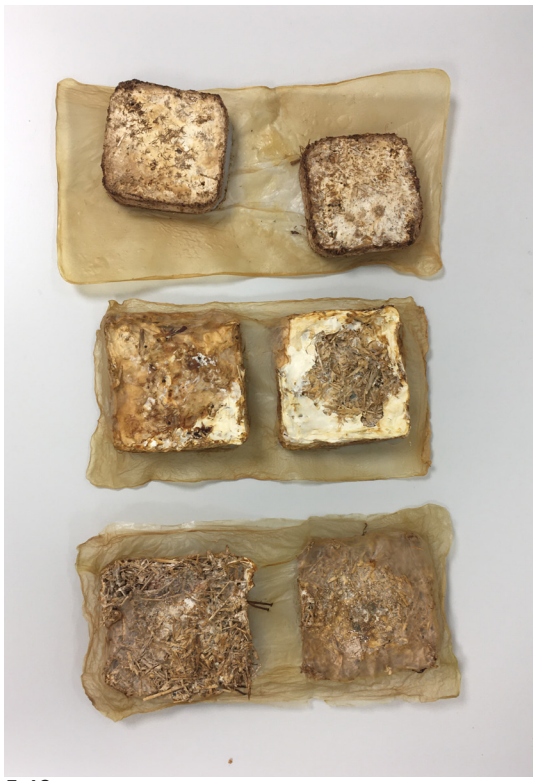
grown to a thickness of 3-5mm, and the collection of gas bubbles underneath had increased to a foam-like appearance. Due to the small size and large quantity of the bubbles, it was not possible to remove them from under the growing BC and is suspected to be the reason for the reduced growth.

BC growth was observed not just along the mycelium tile and fruiting bodies (Figure 5.41) but also into the inside of it, which was possible due to the lack of hydrophobic skin on the outside of the tile. The paste materials, such as the wood chips, were still exposed and able to soak up the medium. While this resulted in a more intertwined connection between the BC and mycelium, it also resulted in green mould growing on the tile which could be caused by the prolonged wet state of the mycelium, presence of new bacteria, or one of the ingredients of the medium. During the harvesting process, the mycelium tiles began to crumble and fall apart during the rinsing stage and the BC skin grown around the previously submerged side of the tile did not provide any form stability. Due to the mould contamination, the composite was oven-dried at 100 degrees Celsius for 1 hour after harvest.

To summarise, the strongest BC-mycelium composite was achieved with living myceli-

5.41

Figure 5.41: *BC growing around a mushroom/fruiting body on the side of the living mycelium tile*



5.42

Figure 5.42: *Dried BC-mycelium composites with (top to bottom) oven-dried, air-dried, and living mycelium*

um tiles, followed by oven-dried mycelium and, lastly, air-dried mycelium (Figure 5.42). However, the most matured BC was grown on the oven-dried sample, followed by the air-dried, and lastly the living mycelium sample.

Developments for Next Iteration:

Following the predominantly successful results of this experiment, no further experiments were conducted to test the viability of growing BC in the presence of mycelium and this method was considered a viable option for the BioKnit prototype. The following questions and areas of interest arose:

1. Is it possible to reverse the growth order and cultivate mycelium in the presence of freshly grown BC?

   → *Experiment E (Chapter 5.2.)*

2. What is the impact of the mycelium on the nutrient supply for the bacteria and how does it influence the growth of BC on fruiting bodies and submerged parts? Is it possible to grow mycelium in a desired 3-dimensional shape to, once fully dried, function as a scaffold for the BC to grow on in a fully submerged state?

   → *Future Works (Chapter 5.5.)*

E | Mycelium Pocket

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			14 + 12

Collaborators: Dilan Ozkan (Provision of inoculated mycelium substrate; joint development of experimental plan and data analysis with author), Jane Scott (Fabrication of knitted material sample in the form of an open pocket)

Experiment Method:

The aim of this experiment was to test a reversed growth order of Exp. D and test the viability of growing mycelium on established BC. Because the mycelium substrate is a loose mixture which only solidifies during the mycelium cultivation, a form of scaffolding or containment was required. Due to the experimentation being part of the BioKnit investigation, knitted fabrics were chosen to contain the mycelium substrate. A pocket of the dimensions of 110 x 150mm was knitted (linen, circular plain, 8gg Dubied (referring to the type of knitting machine) with one side left open to be filled and sewn shut at a later stage.

During the first growth phase, the knitted pocket was placed on the fabric scaffold (Exp.

5.43

Figure 5.43: *Positioning of knitted pocket in kombucha culture*

B). with the bottom side lying flat. The top half of the pocket was pulled upwards and attached to the top corners of the growth vessel using string (Figure 5.43). The separation of layers was added to avoid the BC 'biowelding' them together during growth. During the 14 days of BC growth, the same method of adding medium throughout, as in experiment B, was adopted.

During the harvest, the BC-knit composite was washed with antibacterial dish soap and thoroughly rinsed to remove all residue of bacteria, medium, and soap from the fabric.

Simultaneously to the BC growth, mycelium substrate of the composition strawbale, wood shavings, and coffee grounds (ratio 1:1:1) was mixed, autoclaved, and pre-inoculated with oyster mushroom spawn (ratio 3:1) for 5-10 days. The process of pre-inoculation reduces the risk of contamination during the initial stage of mycelium growth and is the preferred method for large-scale application of the biomaterial. This method was also chosen for the assembly of the BioKnit prototype (Kaiser et al., 2023).

Before the pre-inoculated mycelium was filled into the knitted pocket, the substrate was mixed thoroughly, and large pieces of mycelium growth were broken up. Once filled, the pocket was sewn shut using standard yarn and the BC was folded multiple times, creating a total of 9 layers, on the bottom side of the pocket (Figure 5.45). One side of the pocket was left without BC cover to allow oxygen flow to the mycelium

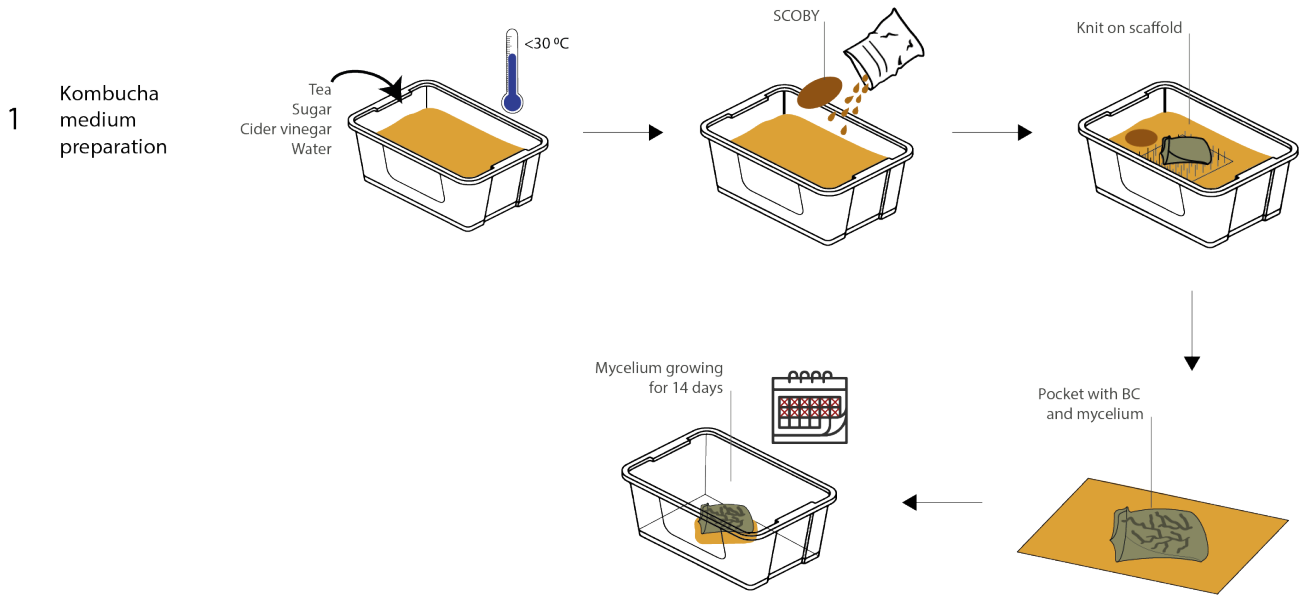


Figure 5.44: *Experiment setup of growing BC around a knitted pocket using a scaffold and subsequent growing of mycelium inside the pocket (Hoenerloh, Ozkan & Scott 2022)*

substrate.

The pocket was placed, BC side facing down, inside a plastic box with lid and left to grow for 12 days in a dark room at 28 degrees Celsius (Figure 5.44).

The mycelium growth was documented after 1, 7, and 12 days from the placement of the pocket inside the plastic box.

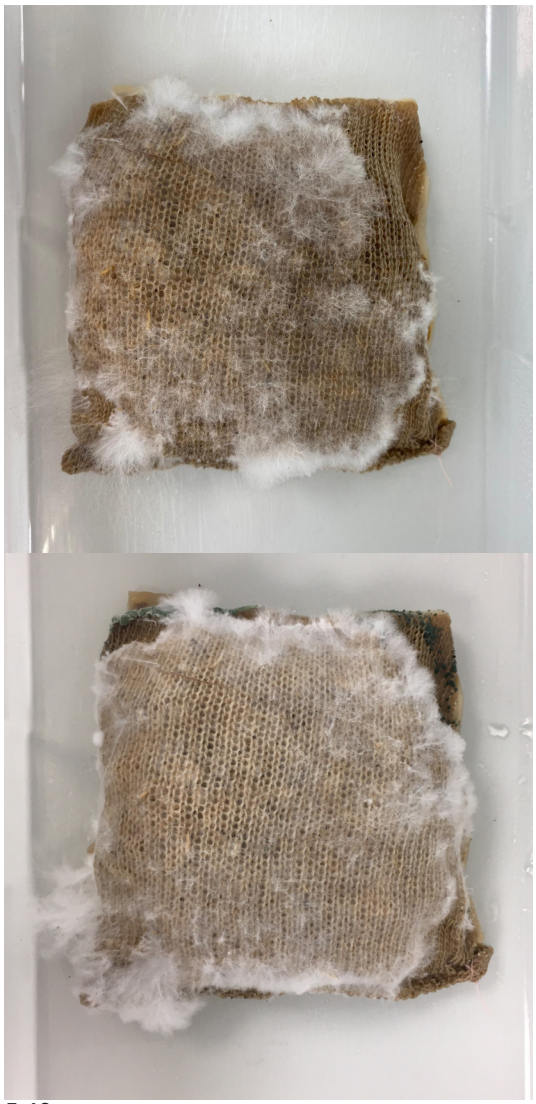
Observations Summary:

The BC grew successfully onto only one side of the pocket without joining the two sides together. However, due to the high absorbency of the yarn used, the top layer of the pocket absorbed some of the medium which led to a darker discolouration. The BC measured at 5mm during the harvest, and the thinner thickness can be explained with the shortened growth time of only 14 days. The BC was intentionally harvested early to maintain a highly flexible and foldable material for the second phase of the experiment.

The mycelium growth inside the soft scaffolding of the pocket was uneven and progressed slower than anticipated. The most prominent white growth occurred near the edges of the pocket (Figure 5.46) where the still hydrated BC provided higher levels of moisture for the BC. After 7 days, the mycelium began to

5.45

Figure 5.45: *Composite of BC and mycelium with knit as scaffold*



5.46

Figure 5.46: *Mycelium growth after 7 (top) and 14 (bottom) days*

grow away from the pocket and along the sides of the plastic box and growth on the top of the pocket did not progress further. Parallel experimentation with mycelium and various yarn types showed lower growth coverage on linen compared to wool-based yarns (Scott et al., 2022). This can explain the overall limited visible mycelium growth on the pocket.

Throughout the mycelium growth stage, the BC stayed fully hydrated due to a lack of air circulation. After 12 days, clear signs of contamination were visible on the mycelium and the BC, predominantly at the edges of the pocket where both biomaterials met. To avoid the spread of the contamination, the experiment was ended, and the composite material dried in an industrial oven for 8 hours at 50 degrees Celsius.

Once dried, the pocket had fully hardened to hold its shape and the BC had shrunk onto the fabric, exposing the ribbed pattern of the knit (Figure 5.47). The oven-drying resulted in a darker and more brittle BC, while the mycelium was only visible in the form of a patchy white skin on the top surface of the pocket. The previously fuzzy appearance of the mycelium disappeared during the drying.

Comparing the growth behaviour of this experiment with Experiment D, the mycelium growth was observed to be more inhibited by

the of the BC than vice versa. This suggests the growth order of this experiment is less suitable for the development of the BioKnit prototype.

Developments for Next Iteration:

This experiment concluded the exploration of growing mycelium and BC simultaneously and in the presence of each other for the BioKnit prototype. The following ideas and conclusions resulted from this experiment:

1. While it was possible to grow the mycelium in the presence of living and hydrated BC, it did increase the contamination risk due to the high level of humidity and the inability to autoclave the setup. A similar contamination problem occurred in experiment D (Chapter 5.1.2.). Can the contamination risk be reduced by combining the materials only once they have finished their growth phase to dry together?

   → *Experiment F (Chapter 5.2.)*

2. How can the properties of the yarn used to knit the fabric scaffold be used to improve the process of growing BC on only one side of a knitted pocket?

   → *Future Works (Chapter 5.5.)*

5.47

Figure 5.47: *Mycelium-BC pocket after 8 hours of oven-drying*

F | BioKnit

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
		 XL & Customised mould	21 / 28

Collaborators: Jane Scott, Ben Bridgens, Elise Elsacker, Romy Kaiser, Dilan Ozkan, and Armand Agraviador (Joint development of concept, experimental plan, experiment execution, and analysis of data of the main frame made with knitted fabric and mycelium); Oliver Perry (Joint design and fabrication of the large- scale moulds to grow BC sheets inside of)

5.48

Figure 5.48: *Large cellulose sheets placed on mycelium and knit composites*

Experiment Method:

The final experiment with mycelium and BC was split into two phases. After facing contamination in both previous experiments, the decision was made to grow the two materials separately and combine them into a composite for the drying phase. A preliminary study was conducted on a smaller scale to test the methods and identify potential issues before beginning the fabrication of the large-scale panels of the BioKnit

The mycelium was grown and shaped through soft scaffolding of knit and the BC was cultivated in a SCOBY-inoculated static culture. The growth vessels were each filled with 6000ml of tea-based medium and inoculated with 1 SCOBY, divided into quarters. Once the

mycelium had grown to the desired stiffness, the samples were pre-dried for 3 days to reduce the moisture content. In the following step, the BC was harvested as flat sheet and draped over the mycelium-knit with care taken to gently press the flexible BC into crevasses and corners of the shape (Figure 5.48). The samples were then left to air-dry at 28 degrees Celsius for 10 days.

The same method of separate growth was implemented in the fabrication of the BioKnit panels (Figure 5.49). However, the growth vessel was upscaled (2000 x 470mm) and customised in shape to fit the arched design of the BioKnit. Each wooden growth vessel was lined with plastic foil and sealed using silicone before being filled with 33 litres of tea-based medium. Each vessel was inoculated with 5 full SCOBY and roughly 500ml of liquid inoculum. The vessels were covered with plastic foil to limit airborne contamination and opened intermittently for air circulation inside.

After 28 days of growth, the BC was harvested and placed on the dried mycelium-knit structure. To secure the BC in place it was either sewn onto the knit or sewn together, where looped around a mycelium column. The prolonged growth period was chosen due to fluctuating temperature within the building that the panels were grown in.

Observations Summary:

During the preliminary study, the BC grew to an even thickness of 3mm over the three-week growth phase. During the first 7 days, no growth was visible, which can be explained with the comparably lower ratio of inoculum to medi-

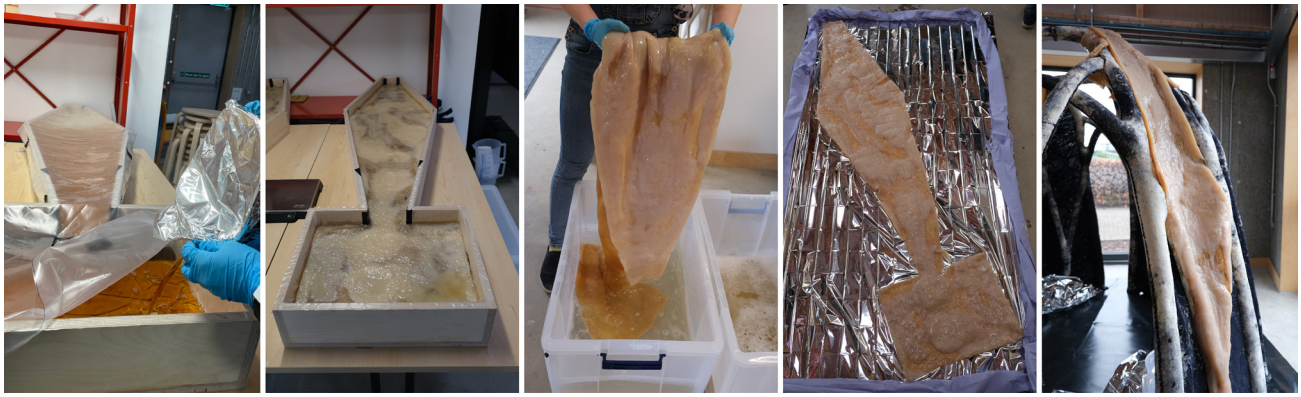
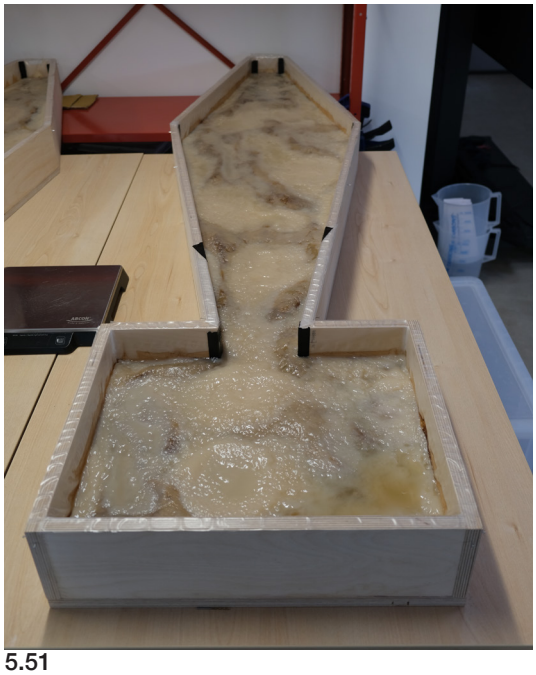


Figure 5.49: *Five steps of growing the large BC panels to be assembled with the BioKnit prototype*

5.50



5.51

Figure 5.50: *Preliminary study showing white speckles on top of the BC (top) and close attachment to the composite (bottom)*

Figure 5.51: *Irregular BC growth visible in the BioKnit panels after 28 days of growth. Opaque areas indicating inoculation SCOBY underneath*

um compared to previous experiments.

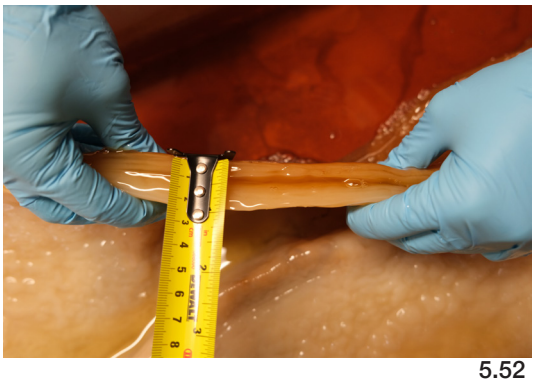
After placing the BC on the mycelium-knit shape, the BC adopted the overall shape of the composite and dried onto it, forming a leather-like skin. Sharp inward edges of the composite were rounded up by the dried BC. White speckles, similar in appearance to paint splatters, were observed across the BC after it was fully dried (Figure 5.50). This indicates a reaction of the mycelium to the BC and a potentially continued growth

The BC for the BioKnit panels grew irregular in thickness, ranging from 2mm to 24mm after 28 days. An increased amount of fermentation gases was observed during the growth of the BC panels. While regular attempts were made to remove the gas bubbles to the sides of the mould, convex areas began to form within the BC which stopped the local growth progression. The thickest areas of BC grew directly above the inoculation SCOBY (Figure 5.51) and had a rich opaque-orange colour. The BC above gas bubbles only grew into immature and transparent-orange colour.

Due to the size and weight two people were required to handle the BC panels during harvest and to place them on top of the mycelium-knit BioKnit frame (Figure 5.52 & 5.53). Similarly to the preliminary study, the BC adapt-

ed to the curvature of the frame and rounded off hard edges. Where the BC spanned over a hollow space in the frame, it began to sag under its own weight. In these areas, the BC had to be sewn onto the frame to avoid a full detachment.

Once fully dried, the BC formed a thick leather-like skin over the frame and showed a similar level of attachment to the preliminary study and Exp. E. The colour of the BC changed to a translucent dark orange which functioned similarly to windowpanes (Figure 5.54), in particular where the BC spanned a hollow space of the scaffold.



5.52

Developments for Next Iteration:

This experiment concluded the development of the 'BioKnit' prototype, and no further experiments were conducted to text the growth potential of BC and mycelium. The completed 'BioKnit' prototype was exhibited in the OME_, the experimental house of the HBBE on the campus of Newcastle University.

Figure 5.52: *BC grown in customised moulds for BioKnit panels. Growth after 28 days*

Figure 5.53 (next page, left): *Draping the BC panel over the mycelium-knit frame and fixating it through sewing*

Figure 5.54 (next page, right): *BC panel draped over frame and spanning a hollow space with thin knit lining. View from the inside of the BioKnit frame*

5.3. Air as Scaffold

The idea for the last tested scaffolding principle was developed based on the biological and environmental requirements which need to be present for the acetic and aerobic bacteria within the SCOBY to produce the BC biofilm. As elaborated in Chapter 3, the bacteria require simultaneous access to oxygen and a nutrient medium, which provides nitrogen and carbon, to be able to metabolise the BC fibrils. The possibility of using each of these requirements as design parameter were considered before deciding on exploring the potential of a scaffold made of air. Due to the nutrients being fully dissolved within a larger volume of liquid, a directed and controlled accumulation of them within said volume would not be possible. While adding nutrients through a microfluidic system (Derme et al., 2016; Gatenholm *et al*., 2014) or continuous drip feeding of nutrients into a nutrient-deprived liquid would momentarily result in a space of higher nutrient concentration, the physical phenomenon of diffusion would quickly result in the nutrients evenly distributing. However, placing a permanent or semi-permanent scaffold within the medium which evenly sparges air at a continuous flow rate ensures a controlled and continuous increased presence of oxygen in specified areas.

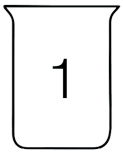
Apart from the design of the bubble column bioreactors (see Chapter 3.3.2.), this concept of an artificial air-liquid interface submerged into an inoculated medium had been explored in various studies. In the pursue of artificial blood vessels, BC cultivated from a single strain was grown as a biocompatible coating in a thin layer around a hollow and perforated silicone tube connected to an oxygen source (Bodin *et al*. 2007). In a more general exploration of growth-based fabrication methods for BC, an additional oxygen flow directed at a submerged non-living component was implemented to guide the BC to grow into a skin fully encasing the component (Derme, Miterberger, and Di Tanna 2016). Another explorative approach to artificial air-liquid interfaces within a BC growth culture investigated the effects of commercially available fish tank aeration stones suspended into a medium towards the BC yield and pellicle formation (Puello Acosta 2018). While these studies invert the idea of a traditional mould and explore the potential of an internally form-guiding scaffold, the free morphological development of the BC is discouraged either through short growth stages or adaptations of growth conditions within the medium. The

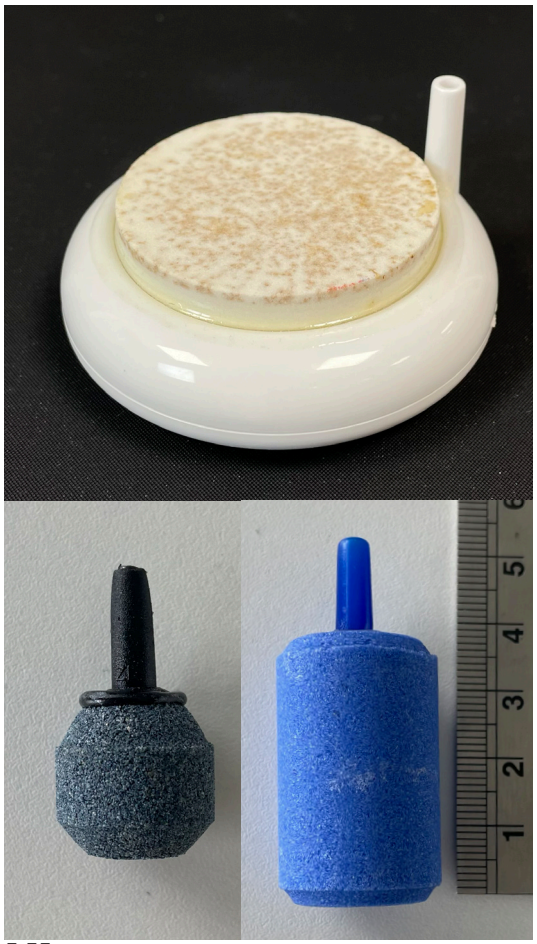
approach of controlling the liquid air interface, however, holds potential to enable a more complex relationship between the bacteria and their environment, enabling emergent and generative forms.

In this initial experiment with aeration scaffolds, the influence of two types of air stream created by commercially available aeration devices within an inoculated growth medium are observed and analysed toward their potential for further exploration.

G | Effect of Aeration

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			21



5.55

Figure 5.55: *Aeration disc (top) and air stone (bottom) used for the experiment*

Experiment Method:

This set of experiments aims to explore the potential of growing BC in a submerged state around and internal aeration device. For this, two different types of commercially and readily available aeration devices developed for fish tanks were chosen (Figure 5.55):

[1] an aeration disc by HOMSFOU with a solid plastic base and a smaller insert of porous stone at the top (50mm diameter, 50mm height).

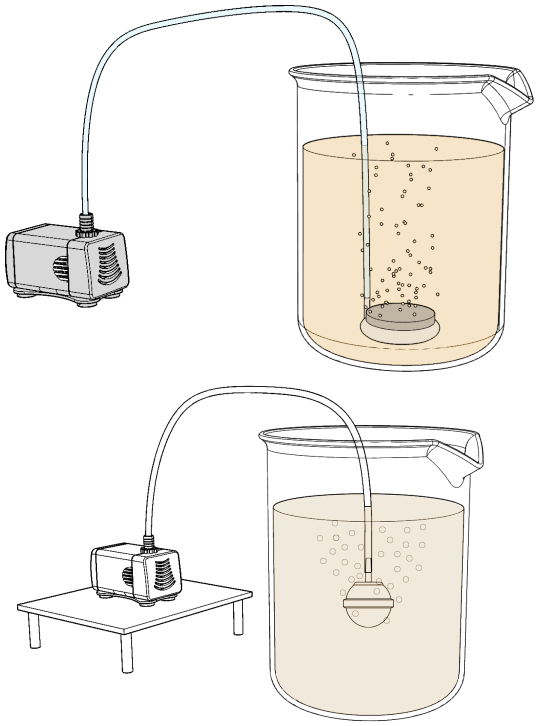
[2] air stones by Pawfly Aquarium, made completely from porous material either spherical (17mm diameter) or cylindrical (23mm diameter, 40mm height) in shape.

Both devices are intended to create a column of air bubbles going from the bottom of the tank to the top to increase the oxygen availa-

bility in the water. On the aeration discs, the attachment for the air tube is on the side. The air stones attach to the air tube in the centre top of the stone (Figure 5.56). To fix the aeration discs into place, they were glued to the bottom of the growth vessel with silicone glue 48 hours before the start of the experiment. The air stones were suspended from the top of the vessel halfway into the growth medium. While the air stones were free to move around the growth vessel during the experiment, their height was fixed using wires and tape at the top of the beaker.

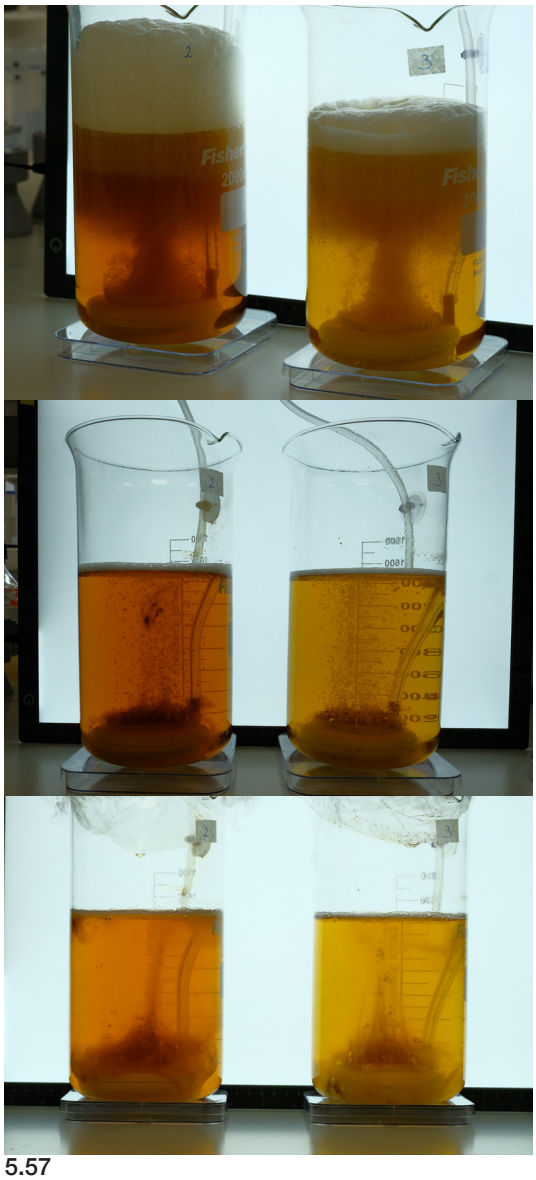
To reduce the contamination risk, the vessels were sterilised using 70% ethanol and the aeration devices were set to run in water for 15 minutes to remove any particles left in the porous surfaces. Each growth vessel was filled with a total of 1100ml liquid, consisting of 1000ml tea-based medium and 100ml liquid inoculum.

The air tubes attached to the aeration devices were connected to an air pump and which provided airflow at constant pressure for 21 days (2W, 0.012 MPA pressure, max. output 1000cc/min). The growth progress of the BC and the air-drying after harvest were documented via photography and by noting done unexpected changes and behaviours of the BC.



5.56

Figure 5.56: *Diagram of setup with air pump, aeration device, and beaker with inoculated medium. The aeration device is either attached to the bottom or suspended halway into the medium*



5.57

Figure 5.57: *Experiment using aeration discs on the day of setting up, after 3, and 5 days (top to bottom). Showing decreasing airflow from the aeration discs with progressing growth duration*

Observations Summary:

BC growth was observed with both types of aeration devices with a clear distinction in the morphological development of the BC over the 21 days. Immediately after starting the air pumps, a layer of foam began to build up the surface on both setups. While the foam produced from the aeration discs was made of fine foam bubbles which stabilised into a 50mm tall foam layer, the air stones produced uneven and larger foam bubbles which did not create a stable foam layer due to consistent collapsing. The foam build-up from the aeration discs plateaued after 2 hours and decreased after 12 hours, until no longer visible after 24 hours (Figure 5.57). From this point, the growth of a surface pellicle could be observed. The uneven foam from the air stones occurred until the end of the experiment and its continuous disturbance of the medium surface prevented the formation of surface BC pellicle entirely (Figure 5.58, bottom).

Within 2 hours, the first brown particles were visible on the porous side of the aeration disc and their appearance resembled a small dandelion seed with a fine hair-like structure. This correlates with the growth mechanism of kombucha, in which the yeast growth faster in the presence of high oxygen concentration (Chapter 2.1.2). After 24 hours, both types of

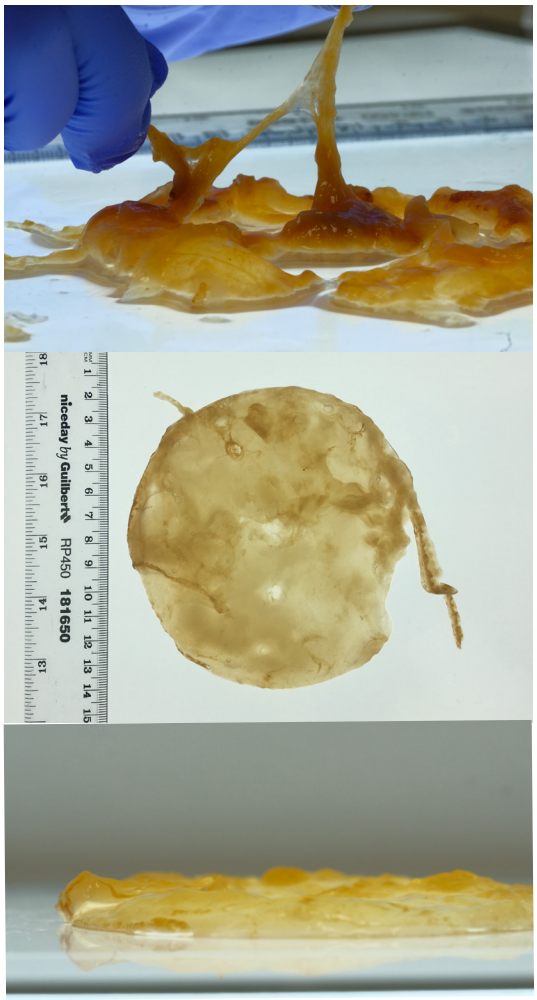
aeration device were fully covered in a layer of biofilm with a fuzzy appearance, containing both brown and white particles. This biofilm continued to grow in both setups until the end of the experiment. On day 8, the biofilm around the aeration discs began to partially lift off the porous surface and, in some cases, the biofilm folded in on itself. However, the biofilm remained attached to at least one side of the discs in every setup. In contrast, the biofilm forming around the air stones began to pull the two aeration devices together and creating one larger cluster of growth (Figure 5.58, bottom).

During the first 3 days of growth, air bubble columns were visible from both aeration devices. However, they decreased in strength due to the growing biofilm on top of the porous surfaces. Simultaneously, a gel-like and near transparent growth of underdeveloped BC occurred inside the medium which began to either trap air bubbles into reservoirs or force the air coming from the aeration devices to carve pathways to the surface (Figure 5.59). In these “bubble pathways” individual air bubbles stacked up on top of each other and slowly move through the gel-like BC. In the case of the aeration discs, the pathways always started from the aeration device but ended either on the underside of the surface pellicle of midway through the medium,

5.58

5.59

Figure 5.58: *Setup of growth vessel with air stones on day 0 (top) and after 21 days of growth (bottom)*
Figure 5.59: *Visible vertical bubble pathways and lifting off of the BC from the aeration disc*



5.60

Figure 5.60: *Bubble pathways connecting the biofilm from the centre of the aeration discs and the ring from the perimeter*

which was confirmed during the harvest (Figure 5.60). The pathways from the air stones always ended midway. Each pathway was active for only a few days before the bubbles within would either stop moving or no new air could escape from the aeration device due to blockage from the growing biofilm. The availability of static oxygen from trapped air bubbles or the slow-moving bubbles pathways enabled the BC began to develop further, forming an opaque wall around these elements of air and increasing their visibility.

Once the BC growth was harvested from the medium, it immediately began to lose volume through a continuous dripping of liquid (Figure 61, top). The shape of the BC immediately after removal from the liquid was plump and comparable to fully inflated balloon. Within the first 10 minutes, the BC growth began to shrink and eventually shrivel. After 7 days of air drying, the volume of the BC had reduced to the extent that the internal aeration devices were visible (Figure 5.61, bottom). Apart from acting as skin around the aeration stones, large air pockets that had been trapped within the gel-like BC were also encased by the BC skin.

While imaging the freshly harvested bubble pathways from the aeration disc setup under the microscope, two distinct elements were

visible: a translucent gel and brown cell clusters within the gel (Figure 5.62). These are assumed to be immature BC and yeast cells respectively. However, no tubular shape or differences in material density were visible.

Developments for Next Iteration:

The results of this experimentation with air as scaffold showed promising growth of BC into unique, unexpected, and complex 3-dimensional shapes. This resulted in an array of questions and ideas for further exploration emerging:

1. How can the shape of the BC grown underwater be preserved so that it can be documented and analysed?

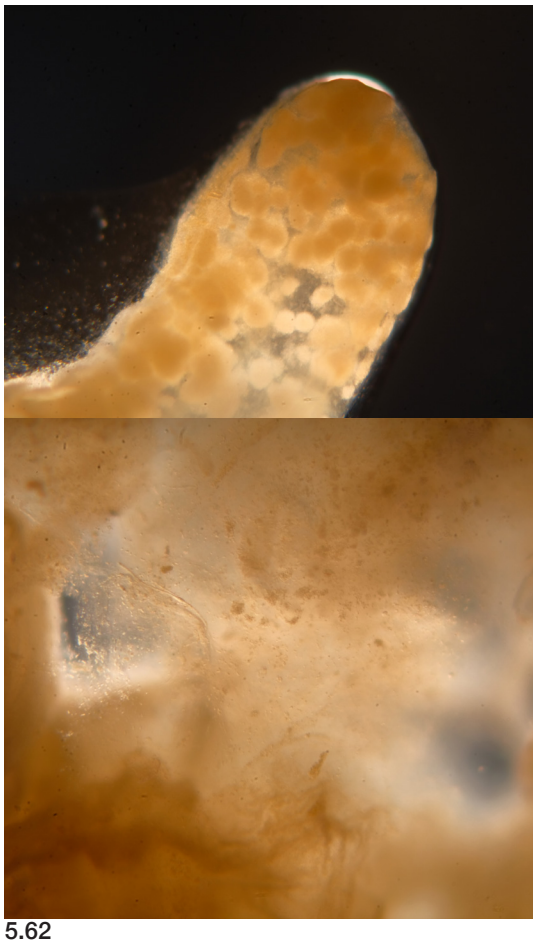
   → *Chapter 7*

2. Is it possible to adjust the medium in a way that reduces the appearance of brown yeast within the BC and also slows down the formation of fuzzy biofilm that blocks the porous surface of the aeration device?

   → *Experiment H (Chapter 6.1.)*

5.61

Figure 5.61: *BC growth from air stones setup directly after harvest (top) and after 7 days of air drying (bottom)*



5.62

Figure 5.62: *End of bubble pathway under the microscope showing a combination of translucent gel and brown cell clusters (top) and similar colouration in the ring formation (bottom)*
Figure 5.63 (right): *Close-up of large air bubble developing between the aeration discs and the biofilm*

3. Is it possible to identify repetitive growth behaviours and distinctive growth elements of the BC? To what extent can these behaviours be categorised?

   → *Experiment I (Chapter 6.1.)*

4. What is the limit of upscaling for this type of aeration scaffold? How does the vessel size effect the development of the shape?

   → *Experiment K & L (Chapter 6.1.)*

5. Is it possible to steer the development of the 3-dimensional shape by customising the aeration scaffold?

   → *Experiment N, P & Q (Chapter 6.2.)*

6. What is the form-giving potential of the foam which is created from the inoculated medium during the first hours of the experiment? Can it be used as scaffold?

   → *Future Works (Chapter 5.5.)*

5.4. Discussion

The observed behaviours of the BC growth in response to fabric, mycelium and air in its growth environment made it possible to identify the most suitable composite material to further explore the aim to develop 3-dimensional BC through growth (see Chapter 1.2). The focus of the discussion lies on the compatibility and growth response of the BC with each tested material and the potential for further development of a composite based on the materials characteristics.

The collected preliminary qualitative data has informed the starting point for further in-depth experimentation of BC form generation and their development (see Chapter 6). It is also the first implementation of the developed methodology of the adapted hermeneutic circle from chapter 3.3. This data offers three potential starting points for stage 2 of the experimental work and poses as ‘background data’ for the next iteration circle.

A limiting factor of the presented results are that they represent visual observations of the BC growth and do not provide understanding of the micro-structure of the growth. By the time BC is visible to the naked eye, the synthesis of BC fibres on a nano and micro scale has occured for multiple days already (see Chapter 2.1.2). For the context of this thesis, these visual observations are sufficient to inform the development of further experiments because the aim is to implement design strategies on a macro-scale.

5.4.1. Integrating Textiles

The results of Exp.A to C indicate that fabrics can be integrated into the setup of a static culture method of BC to fabricate a composite material with a low risk of contamination. Through demonstrating a consistent pattern of growth across fabrics of varying size, materiality, and stitch or knit pattern, the BC showed a sufficient level of biological compatibility with the fabric components to create composite materials.

The most notable characteristic of the fabricated fabric composites is the one-sided growth of the BC on the upwards facing side of the fabric. This behaviour of growing only towards the oxygen-rich environment aligns with the mechanisms of BC synthesis described in Chapter 2.1. Placing the fabric component on the air-liquid interface results in the liquid and bacteria moving upwards through the fibres of the yarn via capillary action (Sherry *et al*., 2023; Adler & Walsh, 1984). This creates a new air-liquid interface on the top surface of the, now hydrated, fabric where the BC begins to grow. The speed at which the inoculated medium moves through the fabric is increased if the yarns of the fabric are already hydrated due to decreased surface tension (Adler & Walsh, 1984), which also results in a faster onset of visible BC growth. However, prior hydrating of the fabric component only affects the growth timeline and not the overall growth potential of the BC in the setting. An example for implemented differentiated capillary action is sportwear which utilised the moisture transfer within specific areas of the garment for thermal regulation and sweat wicking. In the context of creating BC composite materials, this concept offers the potential of exploring spatially directed growth by varying absorption capabilities of the artificial air-liquid interface created by the fabric. This inverts the idea of restricting the air-liquid interface through the addition of foam blockers on the surface (see Chapter 2.3.2) while simultaneously advancing the fabrication into a composite material.

The artificial air-liquid interface formed by the hydrated fabric is not limited to creating a horizontal plane but can expand into a vertical orientation. Depending on the absorption capacity of the fabric, BC growth can be achieved above the surface level of the medium, as seen in the corners of the handknitted fabric in Exp. A. However, the materiality as well as the fibre orientation play a significant role in the absorption capability and therefore the ability to transport nutrients

to these locations (Sherry *et al.*, 2023). Furthermore, a targeted in-depth investigation of this phenomenon is required to determine the extent to which the BC can grow outside of the liquid culture as the observed growth of 6mm in Exp. A was not significant in comparison to the overall BC growth.

Achieving BC growth on both sides of the fabric component required additional steps of manual intervention during the growth process. The first method requires pre-growing a layer of surface BC in the static culture before placing the fabric component on top of it. Similarly to placing the fabric directly in the liquid, capillary action moves liquid and nutrients from the BC through the fabric, where the growth continues and re-joins with growth around the outside of the fabric sample. This creates a pocket-like growth of the BC with the fabric component inside. However, the BC only exhibits attachment to the fabric on the grown post-placement. Another method, which achieves BC attachment to both sides of the fabric, requires a flipping of the BC and fabric composite after an initial growth stage following the setup of Exp. B. Rotating the BC upside down exposes the dormant bacteria in the anaerobic zone of the submerged side of the pellicle and transforms it into the aerobic zone, where BC synthesis can resume (see Chapter 2.1.2). To enhance the integration of the fabric and strengthen the connection between the first BC growth and the second, the characteristics of the fabric component can be adjusted to include gaps, such as in lace (Exp. A), or drop stitches in knitted fabrics. This leads to one continuous, instead of layered, BC growth and encapsulates the fabric more tightly due to the multiple BC connections within the fabric and not solely around it. However, the flipping process requires high accuracy to not enable a layer of growth medium to pool on the newly exposed surface as this would function as a barrier and result in a fully detached second BC layer.

During the drying process, the BC decreases in volume due to the high-percentage water loss and shrinks onto the fabric, which remains the same in volume and dimensions. The irregular drying of the two components improved the attachment at the cost of flexibility as the volume shrinkage of the BC tightens the fibre matrices (Clasen *et al.*, 2006). The handling of the hydrated composites, however, exposed the weak connection between the BC and the fabric and resulted in detachment of the two components during the stretching into position in Exp. B. As previously discussed, the BC does not grow through the fabric but rather on the top surface

of it and an attachment in a hydrated state only exists on a superficial surface-to-surface level. When the composite is then stretched, the different degrees of elasticity result in a fast detachment between the two components. While each tested fabric had a directional stretch based on the knit pattern, the BC has only limited non-directional stretch due to the way the fibre matrix is assembled (Damsin, 2019; Wood *et al*., 2023). The dried composite materials resulted in a more visually form-stable composition than each component on its own and they displayed a degree of shape memory when dried in a specified shape (Exp. B). This type of shape memory of the BC has been utilised in a variety of art projects and is one of the conventional ways of post-processing BC into 3-dimensional form (Ozkan *et al.*, 2023). Results of Exp. B show that it is possible to create 3-dimensional shapes of the BC-fabric composite materials through the post-processing step of stretching and tensioning. However, these shapes are limited to those made from manifold surfaces, ignoring possible emergent shape development by the organism.

The focus of the textile experiments was testing the compatibility of the BC with the fabric and analysing whether the presence of the non-living component would hinder, or enhance, the growth potential of the BC. Overall, the BC was able to grow in the presence of all tested yarn materials and fabric sample sizes. Observed differences in BC maturity and thickness were not considered significant if the control culture without fabric addition showed the same growth pattern. The data in the form of photographic documentation and visual observation does not support a nuanced analysis on a micro or nano level to identify metabolic changes in the BC synthesis. Furthermore, the circumstances of the COVID pandemic during the early experimentation of this thesis resulted in the use of multiple different SCOBY suppliers and the need to grow of BC in multiple geographical locations, depending on the lockdown requirements at the time. Nonetheless, the results add to the growing understanding that BC from SCOBY is compatible with non-living components to create composite materials and, more specifically, offer a first insight into the effects of materiality, fabrication pattern, and growth order on the material attachment.

5.4.2. Bacterial Cellulose-Mycelium Composites

The results of Exp. D to F suggest that growing BC and mycelium composites is feasible, but the order of primary and secondary organism plays an important role in the successful growth of both living organisms and minimising of unintended external contamination.

The presence of mycelium, whether in a dormant or inactivated state, influenced the growth of the BC when the mycelium was set primary and the BC secondary organism. This growth order resulted in an increased fermentation process during the BC cultivation, made visible through the accelerated accumulation of aeration gas underneath the BC pellicle on the liquid surface. This barrier of CO_2 in between the growing BC and the nutrient medium hinders the continued maturation either completely or slows it down significantly (see Chapter 4.1.2). A potentially influential factor for this increased yeast activity within the culture is the difference in pH between the mycelium, which is usually between 5.5 - 6.5, and the tea medium at around 3.5. A SCOBY culture can grow at a pH ranging from 3.5 to 5 whereas activity is slowed in more acidic environment (Hwang et al., 1999). A higher pH could therefore speed up fermentation as a natural reaction by the culture. Another explanation could be the addition of new enzymes and compounds from the mycelium into the BC culture which break down organic matter or trigger enhanced reactions by the yeasts. To identify the exact reason behind the increased fermentation, however, more in-depth experiments and analyses of the behaviour are needed utilising biological and technical triplicate samples as well as experiments to isolate the reaction.

Another notable difference in BC growth behaviour was a faster maturing material in the presence of mycelium fruiting bodies and pinheads (see Figure 5.38). In contrast to the body of the mycelium tile, which is made as a composite with woodchips and other organic material (see Chapter 5.2), the pinheads and fruiting bodies do not contain any additives. Pure mycelium contains high levels of nitrogen absored from the growth substrate (Litchfield et al., 1963), which is a key nutrient for the BC synthesis, and its increased localised presence may be fuelling the fabrication process in those areas. The assumption that the BC can access certain beneficial nutrients from the mycelium is supported by the observations made during Exp.D, where a thin layer of BC growth occurred on the submerged underside of the mycelium tiles. This is in contrast to the BC

behaviour in Exp. A to C, where no BC growth was recorded underneath the much thinner fabric samples. Another explanation is that the oxygen, which is stored within the mycelium as part of its cell respiration (Darby et al., 1950), can be partly accessed by the yeasts and bacteria to continue the BC synthesis. In that case, the mycelium would, similarly to the hydrated fabric at the liquid surface, act as an artificial air-liquid interface. However, it needs to be taken into consideration that the BC grown on the submerged mycelium was not fully matured and not comparable in thickness to the BC grown on the medium surface. The collection of additional data during the growth via sensors, such as dissolved oxygen, CO_2 and pH, would allow a more in-depth analysis of the changes in environment that influence the growth behaviour.

The reversed growth order of BC as primary and mycelium as secondary organism resulted in significantly more visible contamination of both organisms (Exp. E). Apart from different pH levels, the environmental growth conditions for both organisms are similar, requiring around 27 degrees Celsius, high levels of humidity, and little to no light. If the organisms are grown in subsequent order, the primary organism remains in a growth-encouraging environment even after its harvest and dry out. While in a hydrated state, and without removing all residue of the nutrient medium, the sugar within the BC offers a breeding ground for a variety of other organisms, including mould spores. In order to achieve reliable results of the combined growth potential of BC and mycelium the experimental setup needs to be iterated to include ventilation around the composite while the mycelium grows, as well as including a thorough cleanse of the BC to remove all residue of organic matter within it. Considering the results of parallel testing of mycelium and fabric, the material of the yarn for the scaffold of the composite should also be reconsidered to improve the compatibility with mycelium (Scott *et al*., 2022).

The best results for BC and mycelium composites without any form of contamination were achieved when both organisms were grown in separate environments and only joined together during the drying stage. The most natural and unhindered growth as well as good attachment between components was considered a success of the composite material. Similarly to the BC's behaviour on fabric, the flat sheet reduced in volume and shrunk onto the mycelium shape it was draped over until it formed a hardened and partly transparent skin. In the case of the BioKnit panels, gravity held the large panels in place during the drying and the additional pressure onto

the mycelium resulted in a strong attachment once dried. A particularly tight attachment was also observed in areas where the mycelium had created a while film on top of the fabric scaffold. These same areas had an increased presence of white speckles which can be interpreted as signs of reactivated mycelium growth, triggered by the localised increase of humidity. The lignocellulose of BC has been identified as a nutrient source for the mycelium to feed on and various ways of utilising it as feedstock have been explored (Elsacker, 2021). In particular, inoculating sterilised BC sheets with a liquid mycelium culture have shown to achieve a leather-like composite material with close resemblance in colour to the speckles observed in Exp. F (Euale, 2022). The reactivation of the mycelium through the absorption of liquid from the BC, which simultaneously supports the drying of the latter, suggests an interdependency of the two living materials. While the further exploration of this was outside the scope of this thesis and did not match the design agenda or timeline of the BioKnit prototype, it offers a starting point for future investigations into the interrelationship of BC and mycelium.

The final prototype of the BioKnit shows that mycelium and BC can be combined into a composite of pavilion scale, while the assembly process simultaneously highlights the challenges of upscaling the BC fabrication, in particular due to the weight of the hydrated BC. In the context of this thesis, however, the BC is not fabricated into 3-dimensional shapes but moulded onto mycelium shapes post-growth. While the interdependency of the two living materials shows potential for utilising mycelium as submerged 3-dimensional scaffold for BC growth, it would not explore emergent form due to the pre-defined shape of the mycelium.

5.4.3. Air Scaffolding

The results of Exp. G suggest that internal air scaffolding is capable of influencing the growth behaviour of BC into complex 3-dimensional forms which cannot be achieved through other fabrication processes and offer a first insight into possible design parameters to guide the form development.

The first way in which the air bubbles influences the BC growth was the foam formation on the surface of the medium. The foam of the aeration discs was made of significantly more stable and smaller bubbles than the foam from the hanging air stones, which consisted of bubbles varying in size that did not build up in height. The size of the foam bubbles can be linked back to the pore size of the aeration devices which influence the way the air coming from the pump is sparged. The material of the aeration discs was made from visibly smaller particles than the air stones, which showed a variety of particle sizes (Figure 5.64). A visible difference in the air bubble density can be seen in the photo documentation (Figure 5.57 &5.58). Food foams exhibit similar characteristics and aim for equal small-sized bubbles to achieve maximum stability (Müller-Fischer & Windhab, 2005). In addition to influencing the stability, the size of the air bubbles also influences the dissolved oxygen content in the medium due to the speed at which the bubbles move through the liquid (Navisa *et al*., 2014). The higher oxygen concentration could explain the

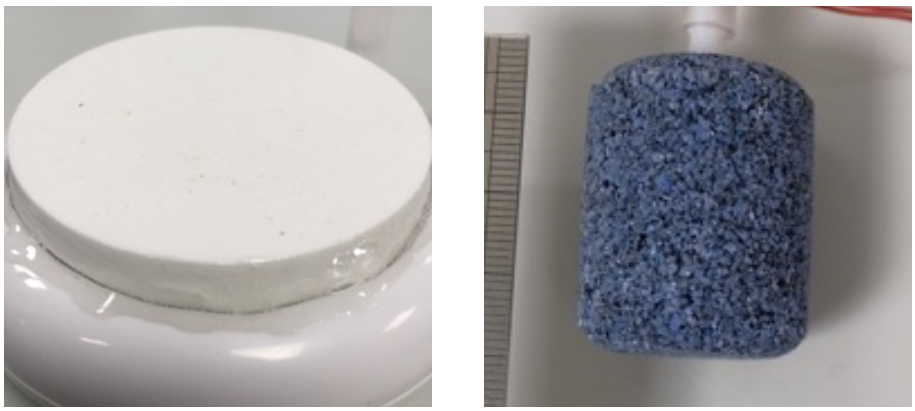


Figure 5.64: *Visual difference in pore size of aeration disc (left) and air stone (right)*

sped-up yeasts production, visible through the long brown strings and fuzzy biofilm skin, on the aeration discs compared to the air stones, which aligns with the SCOBY fermentation process (see Chapter 2.1.3). Lastly, the foam plays an active role in the growth of a surface BC sheet, or the lack thereof, due to the agitation it causes on the liquid surface. Similarly to agitated cultures (see Chapter 2.3.1), the BC cannot form into a sheet on the surface as long as the surface is in motion, and instead forms into smaller individual floating masses within the medium. This can be seen in the aeration discs samples which, after the initially strong foam formation, began to show a growing surface BC pellicle once the airflow from the discs had reduced.

However, not only the pore size of the aeration scaffold influences the formation of a surface pellicle. The speed at which the expanding biofilm of yeast and BC covers and blocks the pores is equally important and is determined by multiple factors and parameters of the growth environment. The BC synthesis in a static culture has previously been compared to the cell-wall production of green plants (Schramm *et al*., 1957). However, the stages of the observed BC growth in both aerated samples could more accurately be compared to the development of biofilms in aquatic environments. The categorisation of BC as a fibrous component for biofilms supports this comparison (Gilmour *et al*., 2023). Drawing these parallels allows a more in-depth understanding of how the BC forms in response to the aeration scaffolds and offers possible explanations for the varying levels of material maturity within each grown shape. First, a distinguishment between attached and dispersed biofilms can be made. The dispersed biomass begins with planktonic, or free-floating, cellular microorganisms which continuously absorb nutrients in their direct vicinity and multiply into microcolonies. In wastewater treatment facilities, these dispersed biofilms develop into a sludge (Sehar & Naz, 2016) at the bottom of the tank, whereas the dispersed biofilm in the aerated samples grew into a gel-like immature BC which expanded throughout the full volume of the growth vessel. The gel-like materiality likely materialises as the “cells which are introduced into the fresh medium become attached around the surface of air bubbles existing in the agitated liquid [and the] cells start to reproduce and synthesize cellulose ribbons” (Czaja *et al*., 2004, p.406). This thicker consistency of the translucent biofilm directly influenced the ability for air to pass through, resulting in the development of vertical tunnel-like pathways from the aeration surfaces. Applying the same principle as above, the localised and slower moving air bubbles

increase the oxygen availability for the bacteria and accelerate the BC maturation along the walls of the pathways.

In addition to dispersed biomass, an attached biofilm occurred directly on the aeration scaffolds. The development of attached biofilms can be divided into multiple distinct stages (Alotaibi & Bukhari, 2021; Karaguler *et al*., 2017). During the first stage of biofilm development, planktonic cells move through the liquid volume based on the circulation within it. In the context of Exp. G, the airflow from the scaffolds dictates these movements (Sumida *et al*., 2013). When these cells come into contact with a surface, they begin to form a conditioning layer directly on top, which forms the basis for continued biofilm growth and the starting point of pores within the material becoming blocked. The successful, but reversible, attachment is influenced by multiple factors, including the surface roughness and localised fluid movement (Sehar & Naz, 2016), and can take from minutes to hours. The larger surface area of the porous surfaces on the aeration scaffolds, compared to the plastic and silicone components, offers favourable conditions for the conditioning layer to form and can explain the initial visible growth isolated in those areas. In a SCOBY culture, the yeasts are the first within the symbiotic culture to multiply and form biofilms (see Chapter 2.1.3) and have a brown, feather-like appearance. Various yeast species, including *Candida* which is present in almost all kombucha SCOBY (Harrison & Curtin, 2021), are able to adhere to abiotic surfaces (Alonso *et al*., 2023), which include those of aeration scaffolds. In combination with the observed fuzzy appearance of the initial growth in the experiment, this leads to the assumption that the yeasts function as conditioning layer for the subsequent attachment of cellulose-producing bacteria, adding to the symbiotic behaviours of the SCOBY in this unconventional setting.

The attachment of the biofilm to the surface is considered irreversible once the bacteria produce extracellular polymeric substances, such as cellulosic fibres (Vu *et al.*, 2009), and show exponential cell multiplication. In the aeration experiments, an increase of visible biofilm growth could be observed within a few days of starting the airflow and the fast biofilm growth on the porous surfaces meant a visible reduction in airflow as the biomass began to block the individual pores. This can be interpreted as indicator for this next stage of biofilm formation. The last growth stage is the maturing during which the biofilm takes on a more defined 3-dimensional shape (Alotaibi & Bukhari, 2021). During the emergence of the BC shape, the position of the aeration

scaffold may change as it accommodates the growing biofilm (Figure 5.58).

The shaping of the biofilm is continuously influenced by multiple factors of the growth setup. The ring-shaped BC growth around the aeration discs (Figure 5.60) indicates that the smooth plastic surface of base was not suitable for irreversible attachment of the microorganisms in contrast to the porous top surface. In addition, the principle of higher dissolved oxygen content in areas of slow-moving bubbles combined with the localised infusion of air via the scaffolds offers an explanation for the varying degrees of maturity, judged based on the opaqueness of the BC, observed within each harvested BC shape. More mature BC was observed near the air outlets while a even decrease in maturity occurred the further the BC was from the scaffold. In both aeration systems, however, mature BC growth was predominantly observed in the liquid volume above the air outlets of the scaffold. This indicates that the oxygen distribution within the liquid volume varies depending on the scaffold position and shape, comparable to the different zones created within airlift bioreactors (see Chapter 2.3.2). The possibility of creating differentiated oxygenated areas within the growth vessel based on scaffold shape and position was identified as potential design parameter for the continued experimentation.

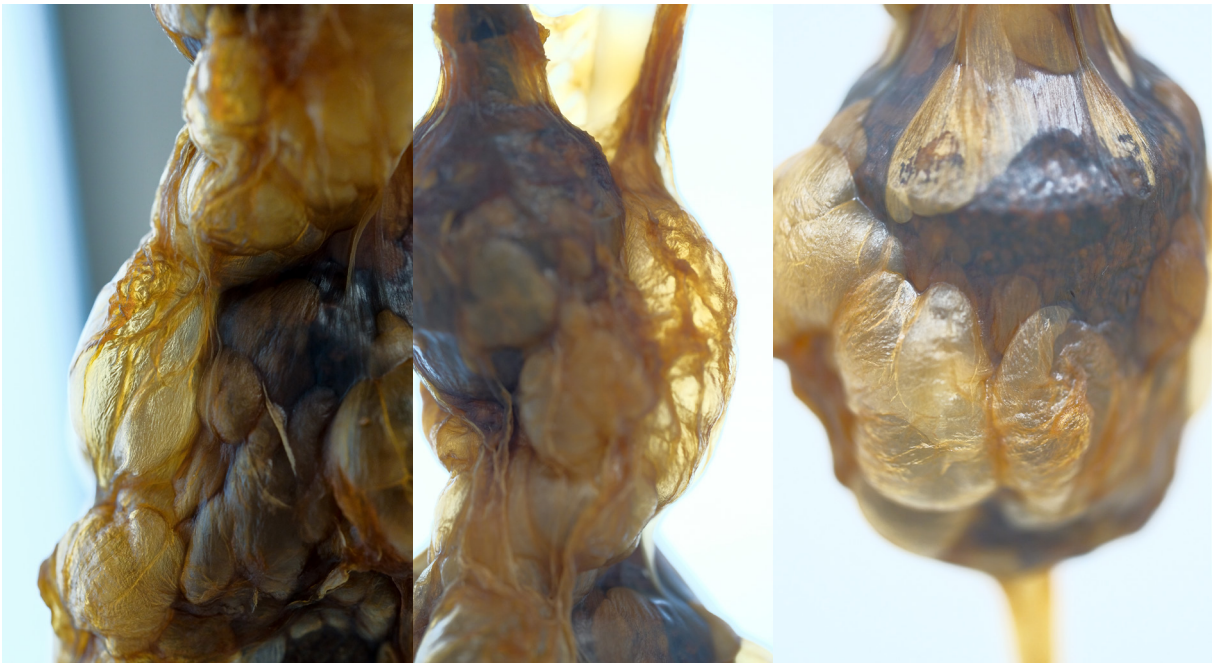


Figure 5.65: *Encapsulated air reservoirs inside the air-dried BC*

The main challenge identified during Exp. G is the preservation and documentation of the shapes that are grown via this method. The 3-dimensional BC shapes exhibited the same drying behaviour as flat sheets of BC and reduced in volume significantly as the water either drips out of or evaporates from the material. In addition, the immaturity of the BC, particularly in the outer parts of the shape, accelerated the water loss even further. The same was observed during Exp. C during the harvest of the immature batch-fed BC samples. Without further intervention, the BC dried onto the scaffold, forming a skin around the joined air stones. The structural changes of the BC fibre network induced by the air-drying process reduced its gas permeability (Clasen et al., 2006) and led to the encapsulation of the air-reservoirs within (Figure 5.65). However, the original position of these air reservoirs within the full 3-dimensional shape could not be recovered or recorded.

Out of the three tested composite types, the aeration scaffolding achieved the most unique shape development of the BC without the need of post-growth processing or shape-defining measures in the form of moulds. The results of both types of aeration scaffold within the inoculated medium showed great potential for further exploration of design parameters to guide the shape development of the BC, with the additional challenge of developing methods to preserve and document these shapes (see Chapter 7).

5.5. Future Explorations into BC Composites

The results of BC and fabric composite grown utilising the customised scaffold showed potential for further development of the experimental setup. The scaffold successfully positioned the fabric at the desired height The fluctuating liquid levels inside the growth vessel resulted in a partial sagging of the fabric on top of the rods which could be resolved by adding a thin metal mesh in between the fabric and the scaffold. This would leave the positioning of the fabric unaffected by the liquid levels. Additionally, an inlet for a silicone tube could be installed in the lower half of the growth vessel to allow topping up the liquid via syringe as needed without disturbing the BC growth on the surface (Figure 5.65). This process could also be automated with sensors and timed feeding pumps.

Further potential for iteration exists in the use of monofilament fabrics as dip-feeding scaffold to grow BC onto. Based on the observations in Exp. C, the loops within knitted monofilament can allow inoculated medium to span across through surface tension, allowing for a thin layer of BC to develop. Combining this with the dip-coating growth method (Rühs *et a*l., 2020) and the principle of rotating disc reactors (see Chapter 2.2.2) (Krystynowicz *et al*., 2002), it holds the

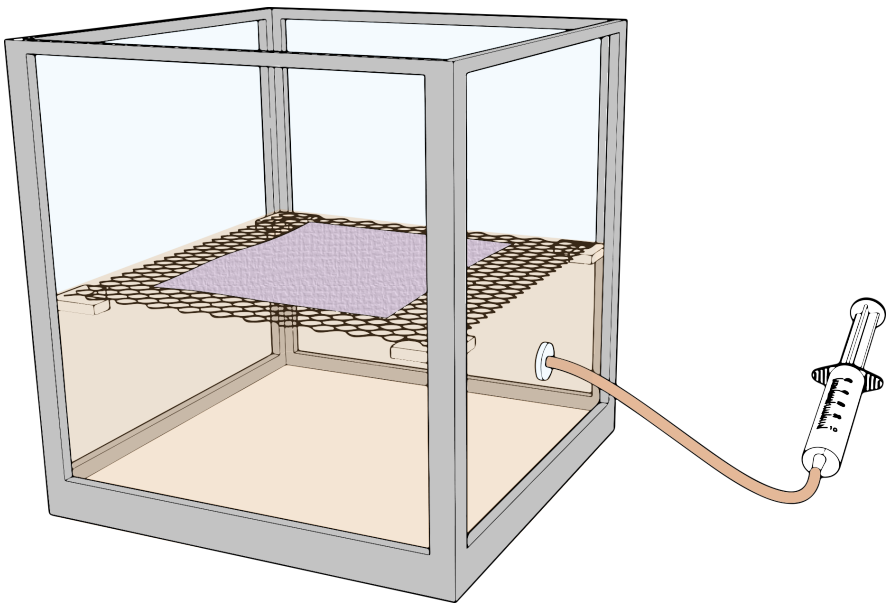


Figure 5.65: *Concept for second iteration of fabric scaffold, developed into a growth vessel with permanent mesh scaffold for fabric placement and tubing for manual topping up of growth medium*

potential of creating larger-scale BC shapes made from fragmented into individual parts within a wire-like scaffold. Thinking further, a customised dissolvable yarn could be explored that provides structural support and nutrients to the growing BC until the shape has matured sufficiently. This method would require a certain level of shape-guidance through the assembly of the scaffold but equally maintains a degree of shape emergence through changeable dipping patterns.

Briefly discussed in Chapter 5.4.2., observations of BC growth on the submerged side of the mycelium tile in Exp. D signifies potential for utilising mycelium as positive mould for BC to grow onto. Further investigations into the compatibility of the mycelium with the BC would be required to identify the source of nutrients or oxygen within the mycelium in order to enhance this presence for more mature BC growth. Additionally, new mycelium substrate recipe could be explored to include nutrients for the BC during the second growth phase. In the context of emergent shapes, the mycelium would need to be grown in a pre-defined mould. The development of pinheads and fruiting bodies, which showed the strongest influence on BC growth, can only be partially controlled (Ozkan *et al*., 2022).

The partially successful growth of BC and mycelium through the use of fabric scaffolding in Exp. E generated an interest in the further optimisation of the experimental setup. The type of yarn used for the pocket scaffold influences both the mycelium growth as well as the attachment of the BC, and a customisation of materiality and knit pattern could improve the compatibility with both. Drawing from the results of Exp. C, a monofilament fabric would improve the BC attachment and simultaneously decrease the unwanted residue of medium on the other side of the pocket. This method, however, does not explore the potential of BC shape emergence but instead aims to explore the possibility of a composite material with two living organisms.

The foam created during the first hours and days of Exp.G sparked interest as potential building block for 3-dimensional BC due to its stability. The principle of BC foam shapes has shown promising results with the use of 3-dimensional negative moulds (Rühs *et al,* 2018). To provide a less shape-restricting environment for the foam to develop, a small-looped 3-dimensional wire mesh, similar to the previously described dip-coating mesh of monofilament, could be provided for the foam to grow within. However, previous research utilised a pure single strain culture to cultivate the BC and the compatibility with SCOBY would also need to be tested.

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A network of air reservoirs developed in bacterial cellulose grown with an aeration scaffold

CHAPTER 6

AERATION SCAFFOLDING

Chapter Summary

The focus of this chapter is the further exploration of aeration scaffolds as a potential method of guiding and influencing the growing BC form based on the principle of internal scaffold which provides the required environmental conditions for the metabolic process of BC growth. The results of Exp. G (Chapter 5.1.3.) showed promising results in evoking a submerged BC growth away from the air-liquid interface which formed in a more concentrated mass than BC produced through agitated cultures (see Chapter 3.3.1). In addition, the observations of novel growth behaviours throughout the active growth phase of the BC led to the identification of design parameters that can be explored through customisation of the aeration scaffold and the setup of the bioreactor.

The first group of experiments (Chapter 6.1.) set out to further explore the behaviour of complex 3-dimensional BC growth in a controlled synthetic environment within a customised bioreactor with the aim of identifying patterns in the growth behaviour and linking them to specific parameters of the experimental setup. The systematic approach utilised to identify the influence of various parameters of a bubble column reactor (Kantarci *et al*., 2005) was used as a starting point for the further development of the bioreactor setup. Characteristics such as the column's size, bubble characteristic, and sparger design, were translated into equivalent components of the developed bioreactor, such as the growth vessel's dimensions, bubble stream appearance, as well as the positioning of the submerged aeration scaffold. In the following group of experiments (Chapter 6.2) additional characteristics, such as materiality and design of the aeration scaffold, were explored after identifying their potential importance in the form development of the BC. Furthermore, the experiments of Chapter 6.2 began to explore the potential of predicting the 3-dimensional BC form based on the design of the aeration scaffold. While existing research into bubble column reactors and others aided in developing the first sets of experiments, it was the iterative development explained through the adapted hermeneutic circle (see Chapter 3.3) which led the direction of exploration.

Throughout the first sets of aeration experiments, additional steps for the lab method of cultivating BC (see Chapter 4) were developed to accommodate the changing environmental condi-

tions and desired experimental outputs. These include the design and fabrication of a customised lid for increased sterility and precise positioning of the aeration scaffold (Exp. K, Chapter 6.1), an added UV sterilisation step for components not suitable to be autoclaved (Exp. H, Chapter 6.1.), and adjusted growth times based on each individual case.

Each bioreactor was based on a 'skeleton' setup consisting of the main components needed to achieve a controlled aeration through a submerged scaffold (Figure 6.1). The base setup included:

1. A growth vessel made of glass, preferably with a volume extending in the vertical rather than the horizontal direction to achieve depth;
2. An air pump with either fixed or adjustable air flow rate and pressure;
3. An aeration scaffold, either a store-bought aeration device or a custom fabricated one;
4. A silicone tube connecting the air pump and the aeration scaffold;
5. A firm lid covering the opening of the growth vessel.

Throughout the experimentation, a set of active and passive design parameters were developed to guide the shape formation of the BC. The passive design parameters are implemented before starting the BC growth and modify the setup of the bioreactor to individual requirements of the particular experiment. The active design parameters give the human designer the option to directly manipulate growth conditions for the BC during its growth stage. For all aeration experiments, liquid inoculum was chosen as inoculation method due to the more even distribution of bacteria within the medium and the possibility of the SCOBY blocking parts of the aeration scaffold due to a lack of space within growth vessel.

6.1. Commercial Aeration Tools

For the first half of the aeration experiments, commercially available components were used as aeration scaffold. These included the same spherical and cylindrical air stones introduced in Exp.G in varying sizes (Exp. H-K), as well as a porous rubber hose intended for the use of plant irrigation (Exp. L). The experiments of this subsection focus on gaining a deeper understanding of the effect of a submerged aeration on the growth of BC with the aim of identifying direct and indirect, active and passive, design parameters which can be used to guide the form-finding process of the BC.

The order in which the experiments are presented is structured to first explore reoccurring growth behaviours in a repeated bioreactor setup to identify patterns, before beginning to vary elements of the initial bioreactor setup, such as growth vessel dimensions and arrangement of aeration devices, to test their potential as design parameter. By experimenting with multiple combinations and arrangements of aeration devices (Exp. J & K), additional insights into the bridging capabilities of the BC were gained to expand on the previous observations with individual air stones and develop customised aeration scaffolds in the following subsection 6.2.

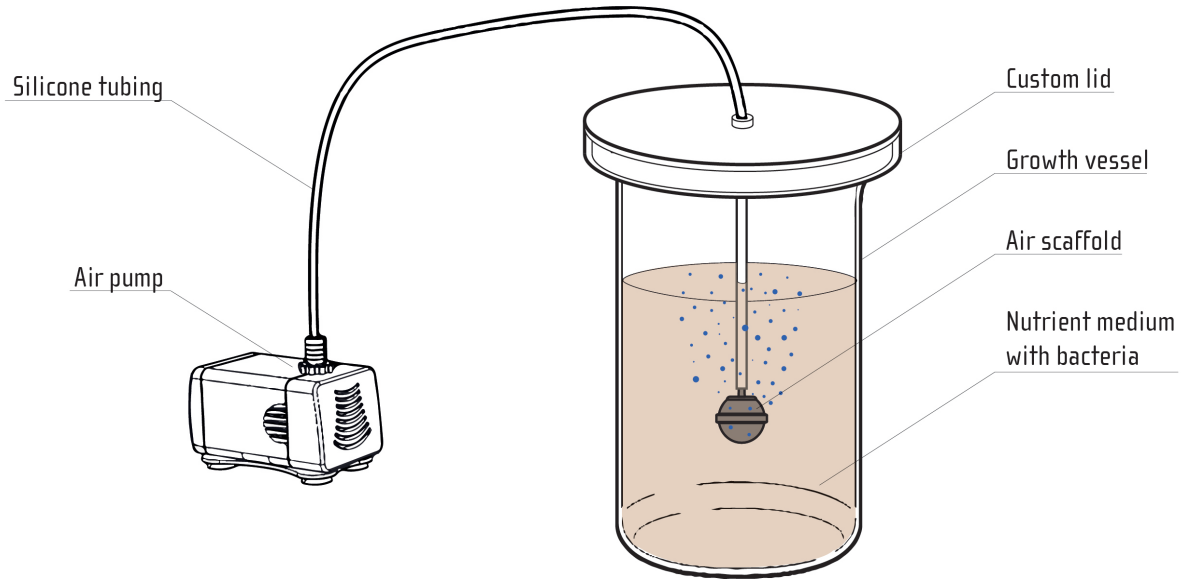
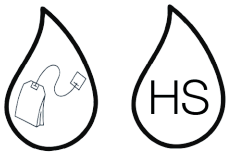
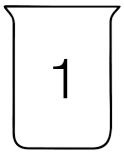


Figure 6.1: *Core components of the bioreactor developed for the aeration experimentation*

H | Comparison of Medium Recipes

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			18

6.2

Figure 6.2: *Media comparison setup with one spherical air stone per growth vessel*

Experiment Method:

The aim of this experiment was to identify potential differences in BC growth based on the nutrient medium to feed the bacteria and yeasts culture of the SCOBY. Additionally, a specific interest was placed on the formation of brown yeast in the early stages of the growth due to their formation resulting in a progressing blockage of the aeration surfaces in the first aeration experiment (Exp. G). For this experiment, a new SCOBY hotel was set up using HS medium with a lowered pH (see Chapter 4.1.2.).

Each medium was tested in a duplicate setup with an individual air stone (17mm diameter) suspended into 500ml medium inoculated with 50ml of liquid inoculum from the corresponding SCOBY hotel. The air tubes were

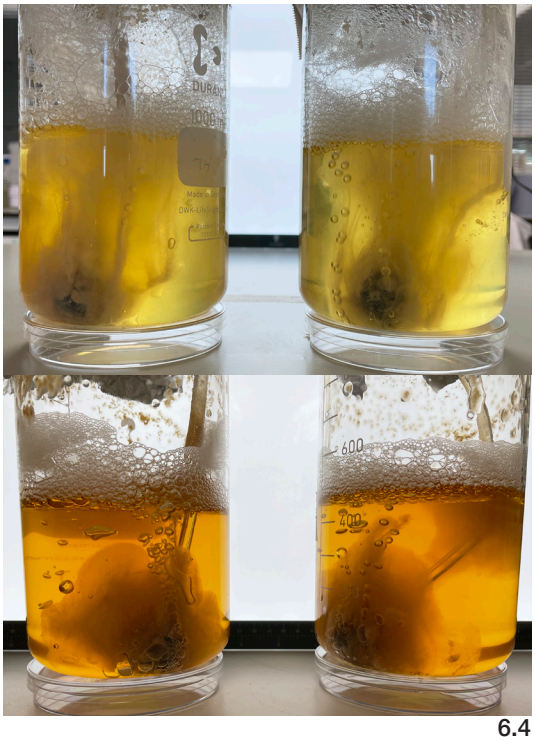
not fixed into one particular position and the air stones were positioned at the bottom of the growth vessel (Figure 6.2). Due to the HS medium being more prone to contamination, the experiment was prepared inside the laminar flow hood after the growth vessels were autoclaved. The air stones were also sterilised under UV light after running in water for 15 min prior and air drying. Aluminium foil was used to cover the growth vessels before they were moved to the lab bench and connected to the air pumps. A set of dual air pumps (4.0 L/min., 0.016Mpa, 4.0W) with adjustable flow rate were used with one pump for each medium type.

Observations Summary:

It was possible to grow BC with both media types, however, notable differences in the development and maturing of the biofilm were observed.

Within the first 10 minutes of the aeration starting, a visible white and stringy biofilm began to form only on the air stones of the HS samples. Their appearance resembled that of a string of feathers and occurred in direct proximity to the origins of air bubble streams from the air stone. Simultaneously, a layer of foam began to build up on both the tea-based and HS samples, with the HS foam reaching a taller height

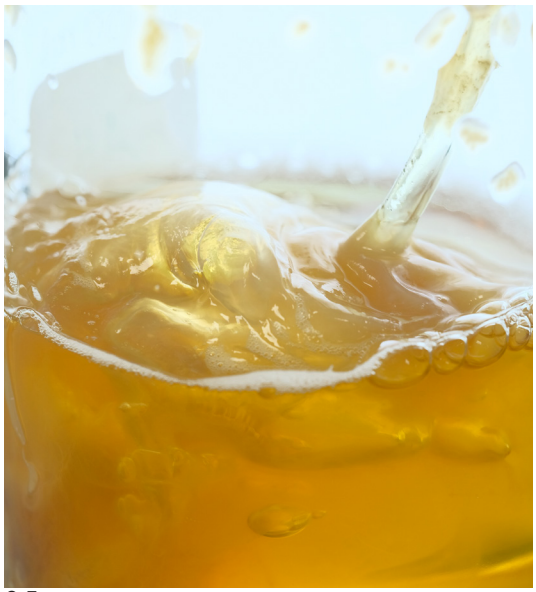
6.3



6.4

Figure 6.3: *Comparison of HS (top) and tea-based medium (bottom) after 72 hours of growth*

Figure 6.4 : *Growth after 7 days in HS (top) and tea-based medium (bottom)*



6.5

6.6

Figure 6.5: *Reduction in liquid levels exposing parts of the previously submerged BC growth in tea-based sample*

Figure 6.6: *White crust on surface pellicle and bright yellow discolouration or submerged air reservoirs on HS sample on day 17*

up to the top of the vessel.

After 72 hours, the medium of the tea-based samples had turned cloudy and the growth around the air stones was only visible with an additional light source behind the vessels (Figure 6.3). The transparency of the HS medium remained unchanged throughout the experiment. At this stage, a clear difference in the development of the biofilm was observed. The biofilm of the tea-based samples grew in two separate places, the air stone and at the surface around the air tubing, while the HS samples formed one continuous biofilm attached to the whole length of the silicone air tube leading out of the vessel. This growth trend continued for several days (Figure 6.4). The overall larger biofilm growth within the HS medium resulted in a faster decrease of air flow from the air stone and an earlier formation of bubble pathways to the surface. Throughout the experiment, the biofilm shapes of the tea-based samples were visible with defined edges and distinctly darker in colour, while the HS biofilms were more difficult to distinguish due to being close in colour to the medium with undefined, feathered out edges.

By day 14, the BC growth inside the HS medium had darked in colour and developed into a more defined shape which encased the whole air stone and the part of the silicone tube

that was submerged in the medium. The tea-based samples continued to grow two separate biofilms. In both cases the liquid levels had reduced to less than 400ml and no visible airflow was left in either sample. The reduced liquid levels meant that parts of the previously submerged BC growth were now exposed to the air and continued to mature similarly to a static culture (Figure 6.5). At this stage of the experiment, both HS samples showed first signs of contamination in the form of a white crust and bright yellow discolouration (Figure 6.6). On day 18, the contamination had spread across the exposed surface as well as part of the submerged growth of BC and prompted an early termination of the experiment.

The position of the air stone within the vessel was near identical in all samples, pressed against the glass, and resulted in each biofilm growing predominantly towards the inside of the growth vessel. The observed differences in BC growth for each medium type are summarised in Table 6.1.

Table 6.1: *Comparison of observed growth characteristics in tea-based and HS medium*

Tea-based Medium	Hestrin-Schramm (HS) Medium
Less stable foam during the first 24 hours of the experiment	Immediate formation of white strings after turning on pumps
Yeast began to form within 24 hours on lower half of air stone	Growth forming around whole aeration setup (air stone and air tube)
Growth formation beginning only around air stone but with defined outline	Growth is white/translucent with undefined edges
No contamination throughout growth phase	Two types of contamination appeared from day 14
Formation of static air pockets inside the growth	Predominantly formation of bubble pathways in the beginning, then air reservoirs

Developments for Next Iteration:

Based on the observations of this experiment, it is possible to grow BC using the aeration scaffold method from both medium types. No difference in the maturity of the BC were observed, however, the colour of the BC is lighter when grown in HS medium. The following conclusions were drawn based on the observations to improve the setup of experiments using aeration scaffolding.

1. Because the HS medium offers a beneficial breeding ground for a large variety of organisms, the sterility of the experimental setup needs to be improved. A customised lid design is necessary to allow for air flow to the medium while also restricting the entry of contaminants into the medium.

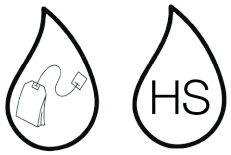
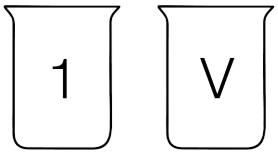
   → *Experiment K (Chapter 6.1.)*

2. HS medium is only suitable for smaller scale experiments due to the increased risk of contamination and the need for a sterile environment. Experiments utilising large-scale or particularly tall vessels should only be setup with tea-based medium.

   → *Experiment L (Chapter 6.1.)*

I | Single Air Stone

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			14 - 21

Experiment Method:

The aim of this set of experiments was to identify growth patterns and reoccurring growth behaviours during the cultivation of BC on submerged aeration devices.

In total, this experimental setup was run 14 times in varying combinations of growth vessel and medium type and over the duration of multiple months. The two growth vessels used were a 1000 ml tall form glass beaker filled with 500ml medium (Figure 6.7) and a cylindrical vase filled with 2300ml medium. Both tea-based and HS medium were used and inoculated with liquid from the corresponding SCOBY hotel at a ratio of 1:10 inoculum to medium. All setups were prepared inside the laminar flow with autoclaved and UV sterilised growth vessels and

6.7

Figure 6.7: *Triplicate setup of HS medium samples with the new lid design prior to starting the aeration*

aeration devices.

For the first set of experiments, aluminium foil and plastic foils were utilised to covered to top of the growth vessels as protection from contamination. In parallel to this experimentation running, a customised lid was developed as result of continued contamination issues (Exp. J). This lid was used for later setups of this experiment (Figure 6.7).

A selection of the BC growths from this set of experiments was further analysed during the development of shape preservation methods (see Chapter 7)

Observations Summary:

Through the repeated execution of this experimental setup, it was possible to identify a set of growth behaviours that can be separated into three different stages (Figure 6.8). These stages can be summarised into the initial stage of biofilm formation, the active development of shape, and the maturing of the BC.

The observational data cannot predict the exact duration of each stage as there are many additional factors influencing the BC growth, such as the room temperature, age of the SCOBY hotel for the liquid inoculum, and porosity of the individual air stones. However, all three stages occur during every experimental setup that is not terminated early due to contamination or other reasons.

Stage 1: Biofilm Formation

During the first stage, strong aeration is coming from the air stone due to unobstructed porous surfaces and creates a layer of foam on the surface of the medium. The foam either holds its shape and height or is in a continuous loop of building up and collapsing every couple of minutes

The first signs of biofilm growth appear directly on the porous surface of the air stone within 24 hours of starting the aeration and consists of two elements. The first element is a stringy white growth varying in length between a couple of millimetres to centimetres (Figure 6.9). One end is attached to the air stone while the other end moves freely with the air stream. The second element is a white translucent film encasing the whole air stone. The thickness of this film is only a few millimetres at this stage but begins to impact the way in which the air escaped from the air stone. While the overall aeration remains strong, the air begins to carve specific ways through the biofilm.

Towards the end of the first growth stage, air pockets begin to form on the underside of the air stone. This area generally has the least

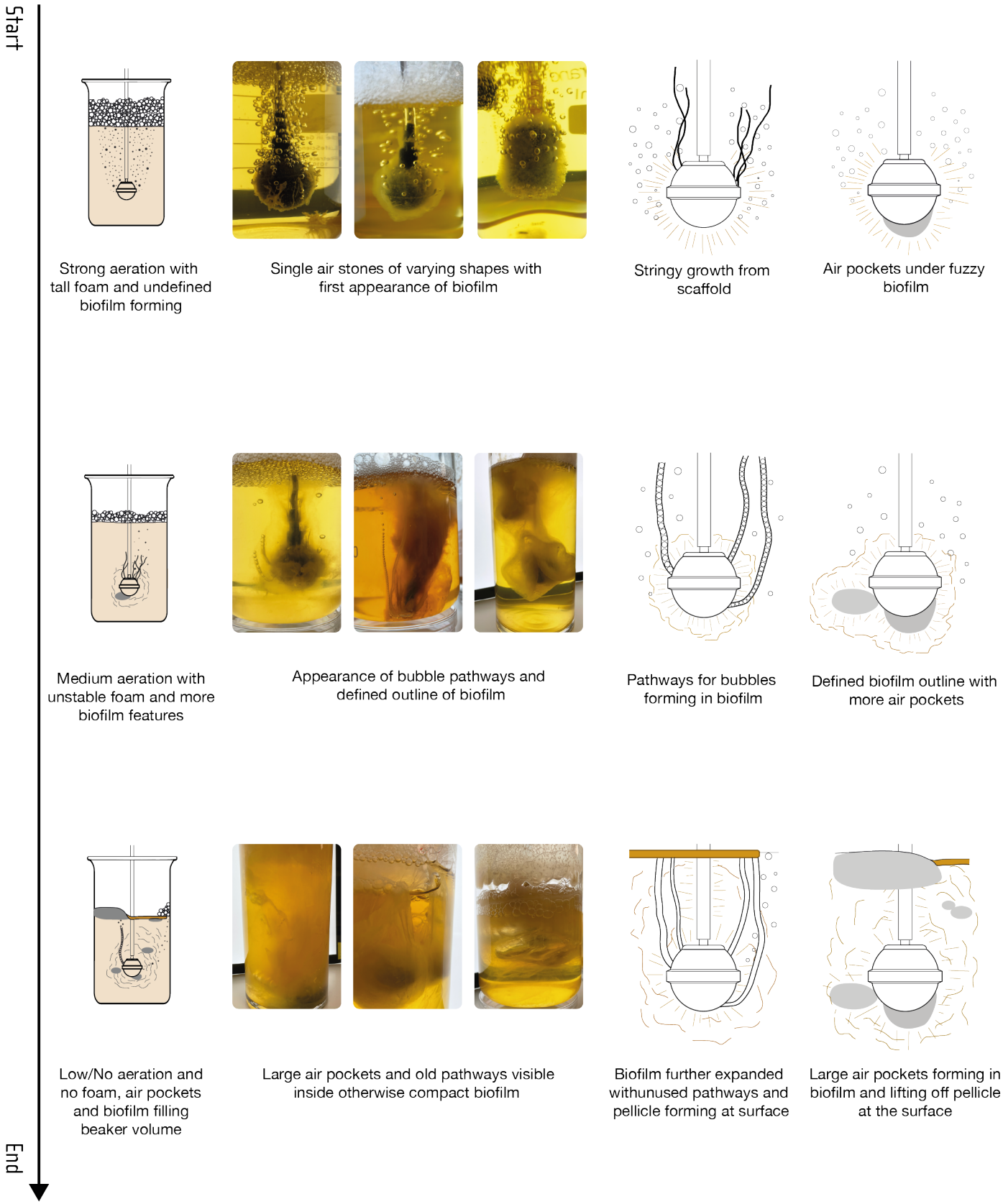
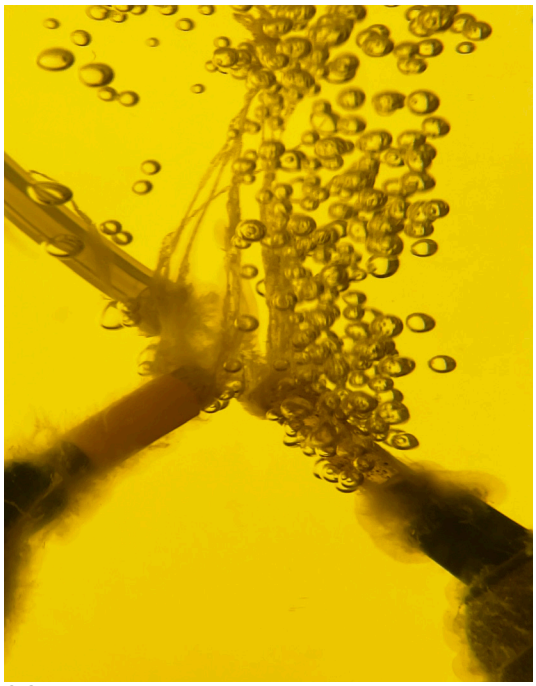
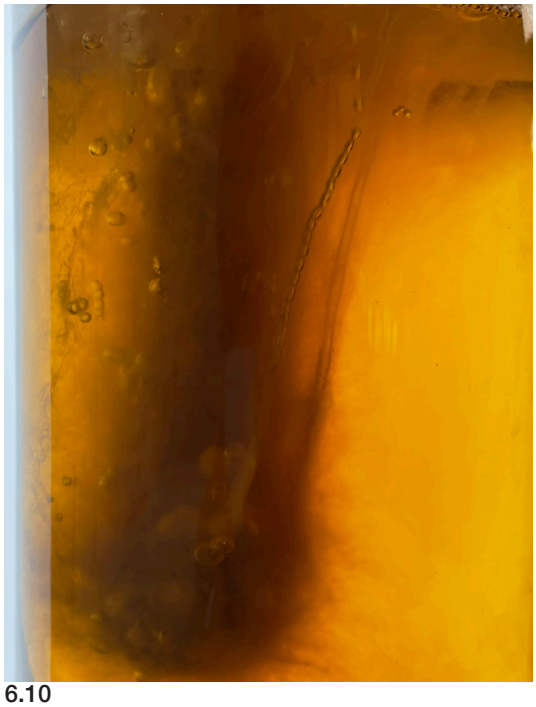


Figure 6.8: *Identified distinct growth stages of BC grown with a submerged aeration scaffold (Hoenerloh et al., 2023)*



6.9



6.10

Figure 6.9: *Two biofilm features of Stage 1 including a stringy growth and a fuzzy biofilm around the aeration scaffold*

Figure 6.10: *Active bubble pathways with small air bubbles lined up and moving towards the liquid surface*

amount of air flow due to the unequal densities of air and water resulting in only upwards air streams. As the air pocket begins to develop, the biofilm is lifted off the air stone and continues to mature in colour to an opaque white appearance.

Stage 2: Shape Development

The second stage of growth begins once the aeration has visibly decreased through blockage of the porous surface and the biofilm on the air stone has begun to grow away from the air stone itself with more defined outlines. At this stage, bubble pathways are created by the more concentrated air stream coming from the air stone as they carve their way through the biofilm (Figure 6.10). Additionally to the visible feathery biofilm, the presence of a gel-like growth within the medium can be identified through the location of bubble pathways and larger static air pockets within it. The gel is made up of loosely connected BC fibrils which have formed within the medium due to the increased availability of oxygen (Klemm *et al*., 2001).

More air pockets begin to form on and around the air stones within the biofilm and gel-like BC (Figure 6.11). The air pockets, also called air reservoirs, can be ‘active’ or ‘passive’. ‘Passive’ describing a state in which air is static

within the pocket and does not move in or out. 'Active' describing a state in which air continuously collects within the pocket until it is released in one large burst. In both cases does the increased availability of oxygen result in maturing of the BC around the pocket.

Stage 3: Maturing

The last stage begins when the aeration activity from the air stone has either fully stopped or reduced to a level of minimal air movement, such as one active air reservoir or bubble pathway. The lack of disturbance on the surface of the medium results in a BC pellicle growing in static or near static conditions. Minimal foam formation is possible but will only occur in one concentrated area of the liquid surface.

The BC previously noticeable with gel-like consistency has continued to mature and is no longer transparent but either translucent or fully opaque. All elements of growth previously observed begin to be pulled together by the maturing gel-like BC and a more compacted shape of BC emerges. Depending on the depths of the air stone within the medium and the remaining aeration, the surface BC pellicle and the submerged growth join together. If large air reservoirs have formed near the surface, they can lift off the surface pellicle and prevent further ma-

6.11

Figure 6.11: *Example of air reservoirs in varying sizes and the outlines of previously existing bubble pathways*

turing, similarly to fermentation gases in static cultures (see Chapter 2). The longer the experiment is left to run in this growth stage, the more time does the surface pellicle have to mature in thickness and stiffness which influences the shape of the BC growth once removed from the medium (Figure 6.12).

Developments for Next Iteration:

The results of this set of experiments made it possible to propose a categorisation of the growth behaviour of BC grown submerged around an aeration scaffold. This led to the emerging of the following questions:

1. How does it affect the growth if multiple aeration elements are inside the growth vessel? Can patterns be observed in the BC behaviour depending on how the aeration scaffolds are arranged?

   → *Experiment J (Chapter 6.1.)*

2. How does the space available and quantity of medium influence the growth behaviour? Will growth continue for longer if there is more medium available?

   → *Experiments K & L (Chapter 6.1.)*

3. How can these shapes be preserved in order to document their 3-dimensionality and the interior structure built by the bacteria?

   → *Chapter 7*

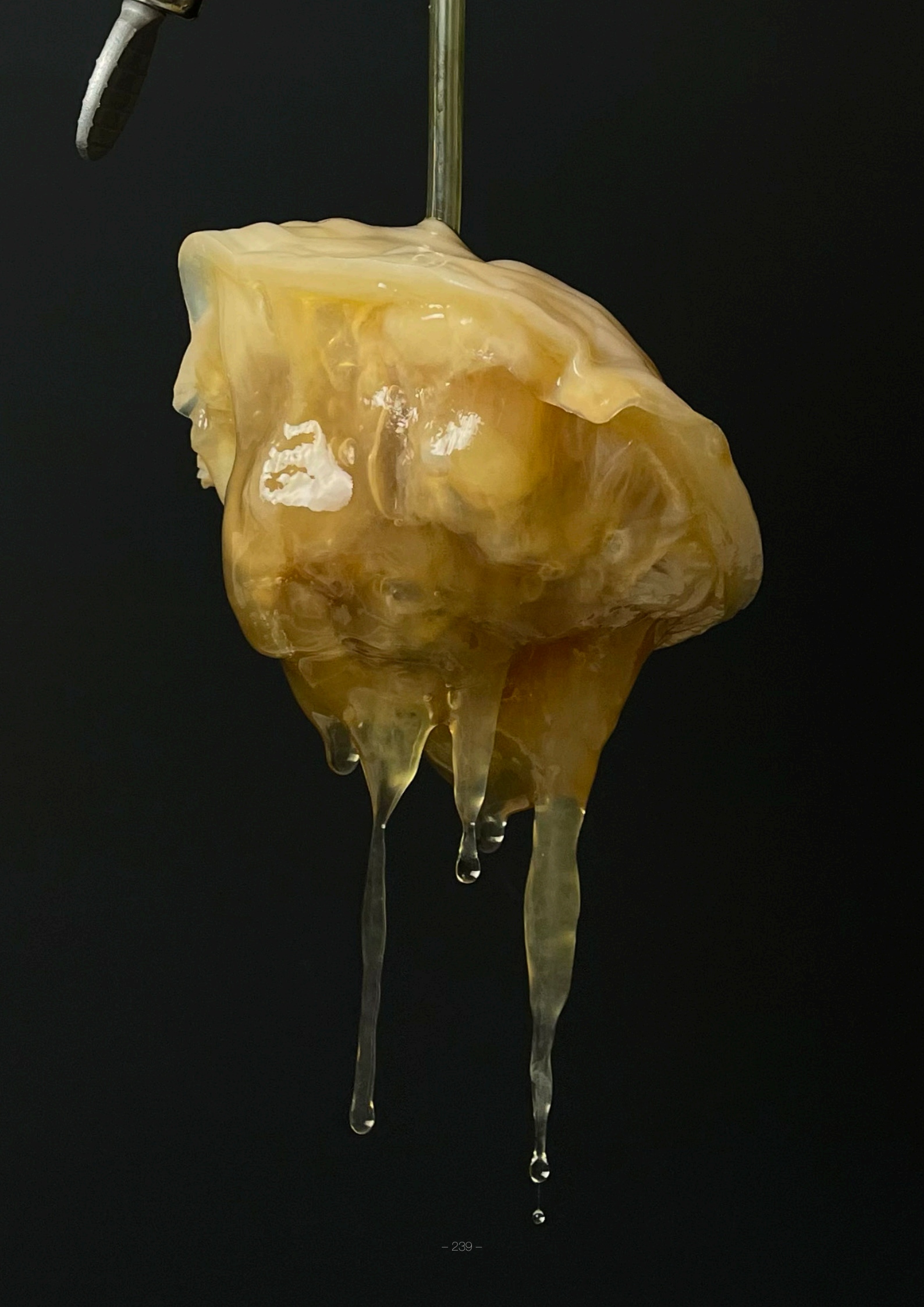
4. Can the adjustment of air flow via the pump's regulator be used as active design parameter to steer the growth and either prolong or shorten the time during which the air flow occurs on the scaffold?

   → *Experiments K (Chapter 6.1.), P & Q (Chapter 6.2.)*

5. Is it possible to enrich the growth medium with additives that encourage the formation of a more form-stable biofilm that can retain liquid more easily?

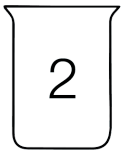
   → *Future Works (Chapter 6.4)*

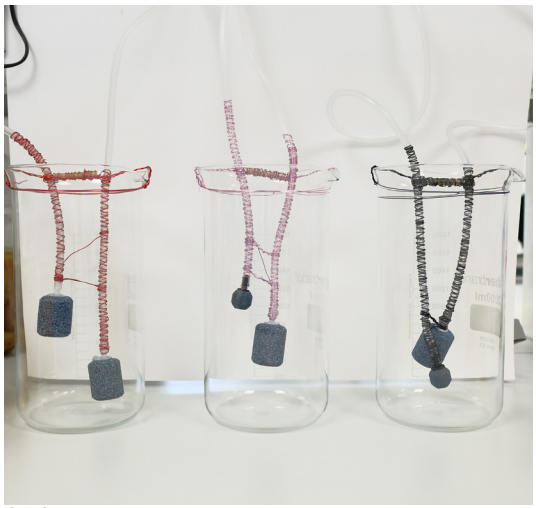
Figure 6.12 (right): *Close-up of freshly harvested BC growth from a tea-based culture grown in a tall vase with visible influence of the surface pellicle on the overall shape*



J | Combination of Air Stones

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			14



6.13

Figure 6.13: *Setup with two air stones per beaker prior to UV sterilisation*

Experiment Method:

The aim of this experiment was to test how the shape of the aeration scaffold influences the shape of the BC growing around it. For this, three types of air stone combinations were created from spherical (17mm diameter) and cylindrical (23mm diameter, 40mm height) air stones. To connect the air stones, aluminium wires were used in three different colours to differentiate them (Figure 6.13). The following three air scaffold combinations were created:

(1) Two cylindrical air stones joined with 30mm height difference

(2) One cylindrical and one spherical air stone joined with 10mm height difference

(3) One cylindrical and one spherical air stone joined at the same height

Additionally to joining the two air stones together, the wiring was also wrapped around the silicone tubing to increase their rigidity and to minimise their movement during the growth stage. This was done to avoid a twisting of the air tubing that could lead to reduced air flow, as was observed in Exp. H (Chapter 5.1.3.).

All parts of the setup were autoclaved or UV sterilised and assembled on the workbench under a Bunsen flame due to the dimensions of the setup. The growth vessels were filled with 1500ml of HS medium and inoculated from the HS SCOBY hotel at a ratio of 1:20 inoculum to medium before the air scaffolds were suspended into the lower third of th e vessel's volume (Figure 6.13). The same metal wire was used to fix the aeration scaffolds to the rim of the vessel and lock them into position. Each set of air stones was connected to a dual air pump which meant they were supplied with the same airflow throughout the experiment.

After the growth vessels were set up with aeration scaffold and connected to the pumps, they were covered with plastic foil and removed from under the Bunsen flame. To start the growth phase of the experiment, the air pumps were all turned on simultaneously.

6.14

Figure 6.14: *Foam formation immediately after turning on of the pumps (top) and after 48 hours (bottom)*

6.15

Figure 6.15: *An 'active' air reservoir underneath the surface pellicle in setup (2)*

As a result of contamination occurring early in the experiment, the growth time was cut short and 1% Virkon powder was added to the growth vessels to kill off all contamination and BC growth.

Observations Summary:

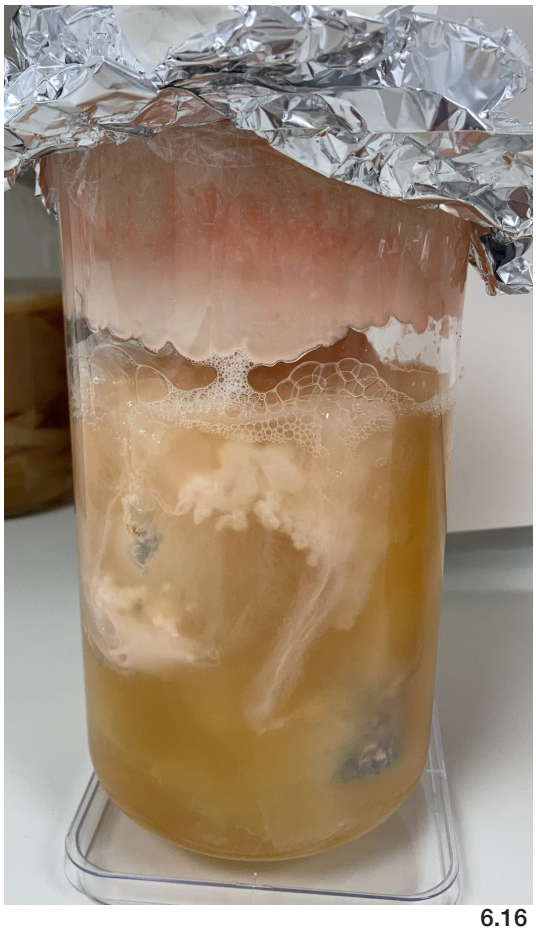
All three aeration scaffold setups grew unique BC forms during this experiment even though contamination occurred early on and the growth time was shortened to 14 days. The final forms shared a similar overall volume but each had developed a different network of air reservoirs that were partially visible from the outside.

Immediately after starting the pumps, foam began to build up on all three beakers (Figure 6.14, top) and leaked through the lids until they, eventually, had to be removed. In the case of setup (1) and (2) the plastic foil was reapplied after 6 hours when the foam height had decreased (Figure 6.14, bottom). The foam in setup (3) only began to settle down after 48 hours, at which point the plastic foil was reapplied. Despite the strong foam formation and leakage, which resulted in a decrease in medium, the BC growth during the first 7 days showed the same morphological development as observed during Exp. I. A white fuzzy biofilm grew on the air stones and also on the wires and air tubing

connecting the air stones, encasing the whole aeration scaffold. With progressing time, the biofilm grew in size and matured to a less translucent colour while simultaneously reducing the airflow. At this stage, a difference in the shape development of BC was visible depending on how the air stones were arranged, with the BC around the cylindrical stones yielding a thicker biofilm layer than the spherical ones. All setups also showed varying sizes of air reservoirs and bubble pathways (Figure 6.15).

A new observation was that BC began to grow in a thin layer in the top third of the growth vessel which was previously covered by foam. This BC was irregular in thickness and fully separate from the BC growing around the aeration scaffold. On day 8, setup (1) began showing signs of contamination through pink discolouration on the BC growing on the exposed vessel walls. Contamination was also visible inside the submerged BC growth in the form of thick white strings (Figure 6.16). In an attempt to avoid contaminating the other two setup, all vessels were sealed airtight with Parafilm sealing film and aluminium foil. The following day (day 9), setup (2) also showed signs of contamination in the form of a yellow-white crust on the exposed part of the BC on the liquid surface (Figure 6.17).

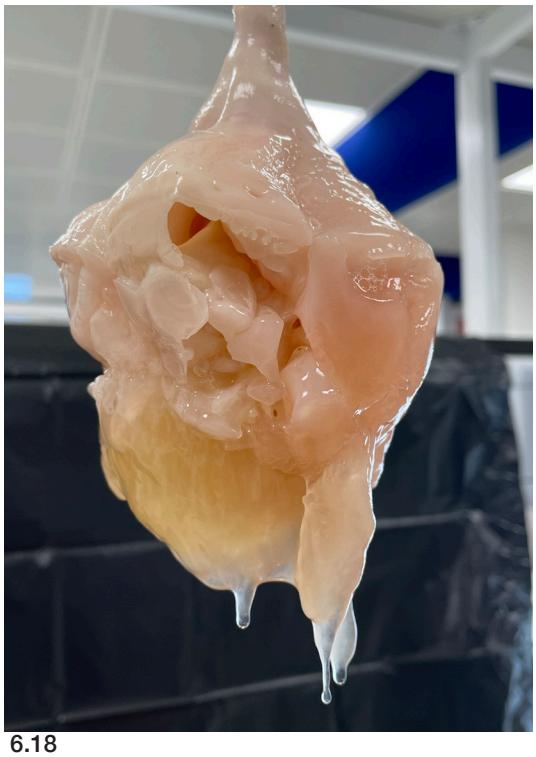
After 14 days, all three setups showed



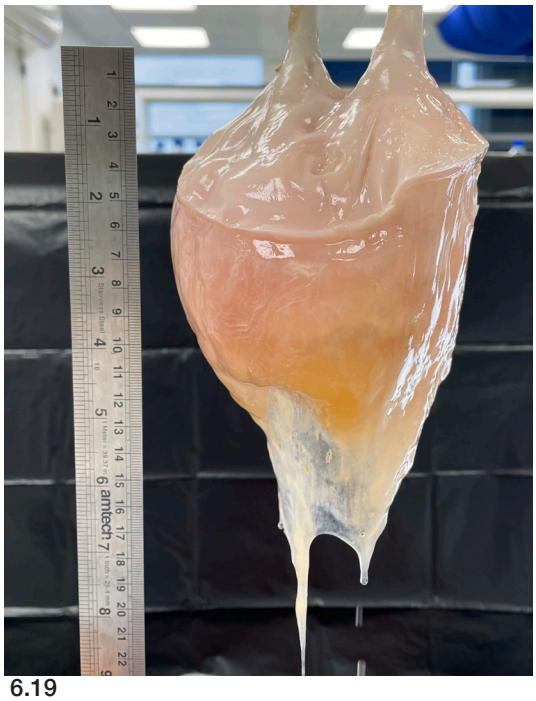
6.16

6.17

Figure 6.16: *Contamination of setup (1) after 9 days of growth*
Figure 6.17: *Contamination of setup (2) after 9 days of growth*



6.18



6.19

Figure 6.18: *Shape of BC grown from setup (2) with visible carved out areas from air reservoirs*
Figure 6.19: *Shape of BC grown from setup (3)*

signs of strong contamination and the decision to end the experiment early was made. Additionally to the previously described contamination in setup (1) and (2), mould began to grow on the exposed BC parts of all setups due to the lack of airflow. Setup (3) was the only one not showing signs of contamination on the submerged BC. 14 days after adding the Virkon to the growth vessels, the BC was harvested and the shapes documented (Figure 6.18 & 6.19).

Developments for Next Iteration:

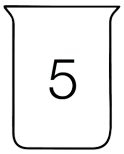
Due to the strong level of contamination that occurred during the experiment, the growth behaviour on the aeration scaffolds was only observed and documented during the first two stages of growth determined in Exp. I. The importance of sterility was made apparent and prompted the development of the bioreactor:

1. Similarly to the conclusions of Experiment H (Chapter 5.2.1.), a customised lid is needed for HS aeration experiments. It should allow the exchange of oxygen while not allowing contaminants to enter the growth vessel. Additionally, the lid should be able to fix the silicone tubing into position.

→ *Experiment K (Chapter 6.1.)*

K | Upscaling in 5L Beaker

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			20

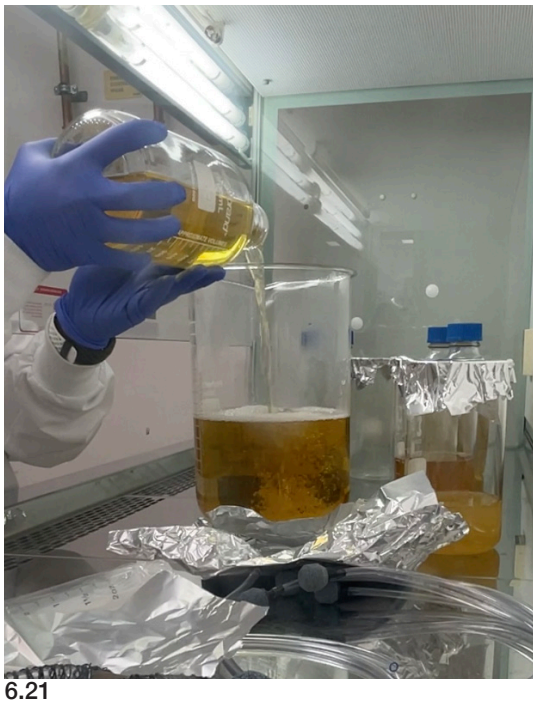
Experiment Method:

The aim of this experiment was to test whether a larger growth vessel and an increased availability of nutrient medium will influence the shape development and overall shape of the BC. To have comparable results, the aeration scaffold no. (2) from Exp. J was replicated and suspended into a 5,000ml growth vessel. The only difference in aeration scaffold was the horizontal distance between the two air stones, which was increased to 60mm. To straighten the silicone tubing, it was soaked in boiling water for 5 minutes before being stretched into a straight position for cooling. This step improved the predictability of how the aeration scaffold will position itself inside the growth vessel.

Following contamination issues in previ-

6.20

Figure 6.20: *Customised lid for improves sterility*



6.21

Figure 6.21: *Setting up the experiment inside the laminar flow hood for a sterile environment*

ous experiments, a customised lid was developed which allowed the exchange of oxygen without letting dust particles enter (Figure 6.20). The design of the lid was influenced by the one of petri dishes.

To set up the experiment, the aeration scaffold was cleaned by running it inside water for 15 minutes before UV sterilising it for 1 hour. The lid was also UV sterilised while the growth vessel was autoclaved. Then, 3,000ml HS medium were added to the vessel and inoculated with 300ml inoculum from the HS SCOBY hotel. The silicone tubes of the aeration scaffold were threaded through the bottom of the lid before being placed on top of the growth vessel together (Figure 6.21). The air pump was attached to the tubes and the full setup transported to the lab bench. One dual air pump with individually adjustable airflows was used for this experiment (2.5L/min per outlet, 4.4W). The strength of the air pressure was set to the lowest setting for the first two days and then incrementally increased until it reached 100% on day 8.

To record the growth of the BC, a timelapse was setup using an iPhone SE and a timed desk lamp directed at the growth vessel. Photos for the timelapse were taken at 6h intervals (Figure 6.22). After the harvest, the BC shape was shock-frozen, 3D scanned, and

freeze-dried as part of the shape preservation exploration (Chapter 7).

Observations Summary:

An overall faster growth and BC shape development was observed in this experimental setup compared to previous aeration experiments. This resulted in a BC growth of compact and defined shape with increased maturity of the internal structure of air reservoirs. The incremental increase of air pressure for the first week resulted in a slower blockage of the air stones and created a larger number of bubble pathways and air reservoirs than previously observed. After each increase of air pressure, a newly forming pathway of the air could be observed.

The air pump was started at the lowest air flow setting for both outlets and the foam only reached a maximum height of 30mm during the initial starting of the air (Figure 6.23). Dark speckles formed on top of the foam within a few minutes of aeration and began to stick to the side of the vessel. They did not grow as contamination and are assumed to be either leftover dust from inside the air stones or a chemical reaction from the HS medium to the material of the air stones. Within 24 hours, a thick white fuzzy biofilm had formed around the entirety of

6.22

Figure 6.22: *Timelapse setup of the growth with iPhone stand*

6.23

Figure 6.23: *Large 5L growth vessel with aeration scaffold directly after turning on the air pumps*

the aeration scaffold. There were also free-floating elements of biofilm distributed throughout the medium, turning the clear liquid translucent. After 72 hours, major BC growth in the form of a white and fuzzy biofilm had formed floating just underneath the surface and attached to the larger air stone of the aeration scaffold, reducing the airstream. A flat sheet of translucent biofilm had also formed right at the bottom of the growth vessel, unattached to the growth around the aeration scaffold. The transparency of the medium had further declined. The air pressure from the pump was increased by 50% which unblocked parts of the air stone covered in biofilm and resulted in additional airstreams.

On day 5, the flat sheet of biofilm had lifted off the bottom and partly attached to the main growth around the aeration scaffold. The appearance of the film resembled a jellyfish (Figure 6.24). At this stage, the medium had returned to a transparent state, making the extent of the white opaque BC growth clearly visible. Another notable change in the BC growth was the development of multiple active air reservoirs of which some were interconnected. An audible 'pop' occurred when bursts of air were released from the top-most air reservoir.

The network of connected 'active' air reservoirs continued to grow, building a net-

work with multiple ways for the air to escape from the air stones and giving the BC growth a defined shape. A general pulsating of the BC was noticeable when air moved through the air reservoirs. From day 8, no further foam was produced and the BC pellicle growing on the surface started to mature faster. The skin of the air reservoirs in the submerged BC matured to a white colour, making them more easily detectable within the overall growth and showing the extent of the cave-like system they had formed (Figure 6.26). The noise of air releasing from the air reservoirs changed to a higher pitch.

On day 10, first signs of mould contamination appeared on an area of exposed BC grown along the exposed walls of the vessel. On day 11, the contamination had progressed and the BC on the exposed vessel walls was removed under the laminar flow hood. The network of air reservoirs had developed into a defined 3-dimensional shape (Figure 6.26). On Day 17, first signs of contamination occurred on the surface pellicle. By day 20, the contamination has spread into the air reservoirs (Figure 6.25) and the experiment was ended. After the shape preservation process (Figure 6.28), the BC growth showed clear influence of the cylindrical shape of the growth vessel, in particular through the rounded surface pellicle (Figure 6.27). Com-

6.24

6.25

Figure 6.24: *Growth after 5 days showing a jelly-fish-like biofilm lifting off the bottom of the growth vessel*

Figure 6.25: *Signs of contamination inside the BC*

parable form behaviour was observed in Exp. J with scaffold no.2.

Developments for Next Iteration:

The results of this experiment showed that the BC will keep expanding from the initial biofilm formed around the aeration scaffold until it reaches the walls of the growth vessel, which influences the final shape. The following ideas for iterations were developed:

1. The larger volume of the tank allowed the BC to expand further and create a much larger scale object than in the previous smaller vessels. What is the maximum that the BC will bridge into a full solid item? Can the shape of the vessel also influence the growth shape?

   → *Experiment L (Chapter 6.1.)*

2. Is it possible to reuse the aeration scaffold to feed in supplements into the existing growth before the harvest? Could this be additional nutrients or a solution which aids in the shape preservation/solidification of the shape?

   → *Future Works (Chapter 6.4)*

6.27

Figure 6.26 (left): *Close-up of the elaborate systems of connected air reservoirs after 11 days of growth*

Figure 6.27: *Final shape of the BC fully frozen with discolouration of the BC from contamination and visible opening of the air reservoirs on the side*

Figure 6.28 (next page): *Harvesting the BC by shock-freezing to preserve the shape (see Chapter 7)*


NITROGEN

L | Upscaling in Fish Tank

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
		Fish Tank 600 x 400 x 400mm	26

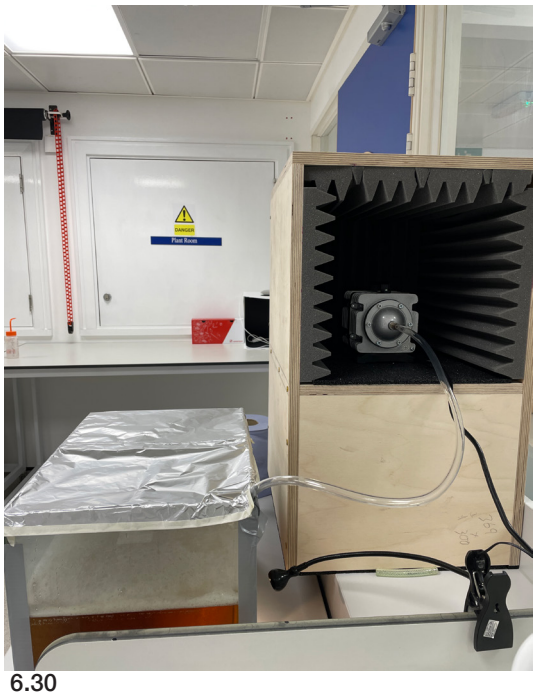
Experiment Method:

The aim of this experiment was to test whether there is a limit for how large the BC can be grown into a solid shape using the developed aeration bioreactor setup.

Instead of combining multiple smaller-scale aeration devices into one large scaffold, a porous rubber hose used for garden irrigation systems was utilised to upscale. The porosity of the hose was created through the manufacturing process of compressing small chunks off recycled rubber into shape, fusing them together without fully sealing gaps with glue. When used for gardening, the hose is connected to a water outlet with up to 0.7 bar and laid flat near plants. Due to the porosity of the hose, the water slowly leaks in single droplets with a constant flow. For

6.29

Figure 6.29: *Aeration scaffold made from porous garden hose and shaped into a spiral inside the empty growth vessel (top) and during aeration testing in water (bottom)*



6.30

Figure 6.30: *Final setup with air compressor inside custom build noise cancelling box and desk lamp directed at the aeration scaffold from the outside*

the aeration experiment, the assumption was made that the porosity of the hose will also let air escape in small bubbles. This was tested in water prior to setting up with medium (Figure 6.29).

In order to achieve a continuously strong airflow from the hose, a stronger air compressor was chosen. Due to the increased vibration of the air compressor on the workbench, which could result in disturbance of the growth medium and consequently influence the BC growth, an insulation box was designed and fabricated (Figure 6.30). While the added vibration sparked interest as a potential design parameter, it did not fit the scope of this experiment.

To shape the irrigation hose into the desired aeration scaffold shape of a spiral (Figure 29, top), a 4mm aluminium wire was inserted into the hose at the full length of 105cm. One end of the spiral was closed off with a corresponding plug and the other end was attached to a 12mm silicone tube via adaptor. To counteract the lightweight nature of the hose, a 1,120g piece of marble was attached to the bottom of the spiral via cable ties.

To setup the bioreactor, the interior of the growth vessel was sterilised with 70% ethanol and the aeration scaffold was left to run in water for 15 minutes in a separate vessel. UV was

not used to sterilise the hose due to the potential of damaging the rubber material. After filling the growth vessel with 19,000ml of tea-based medium, it was inoculated with 700ml liquid inoculum from the corresponding SCOBY hotel. Additionally to the lower ratio of inoculum to medium, this larger bioreactor had to be moved into a separate room of the lab in which the average temperature was consistently lower than 20 degrees Celsius, which was acknowledged as potential factor slowing down the BC growth. The top of the growth vessel was covered with aluminium foil to minimise contamination. To document the growth of the BC, a desk lamp was directed at the aeration scaffold from the outside of the fish tank in addition to the light table.

Observations Summary:

Overall, the BC grew on the spiral aeration scaffold but not in the expected shape or to the expected maturity level. Instead of forming one large body of BC, the growth was predominantly made of walls of BC that connected between the layers of hose in the spiral, which was discovered during the harvest. The final form of the BC can be described as a hollow cylindrical shape which correlates with the observed air-

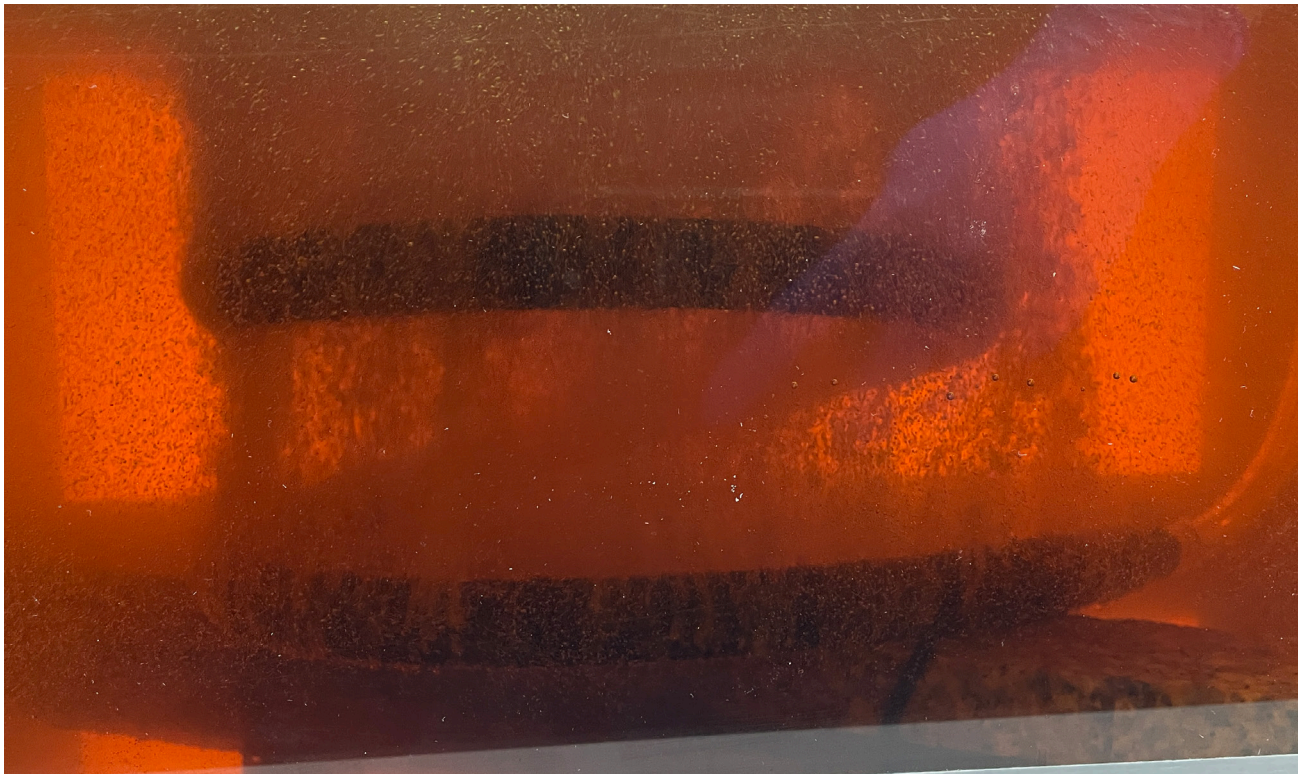


Figure 6.31: *Visibly strong aeration coming from the spiral scaffold after 48 hours*

6.32

6.33

Figure 6.32: *Sharp edge in BC growth formed inside the corner of the fish tank*

Figure 6.33: *Foam formation after 24 hours being unstable and below 20mm*

flow from the aeration scaffold during the test in water (Figure 6.31). In addition, the shape of the growth vessel had a clear influence on the formation of the BC (Figure 6.32).

The first observation after turning on the air compressor was that the air flow from the scaffold was made of much finer air bubbles coming from all parts of the hose. In the case of air stones, the bubbles don't evenly escape through the porous material but rather group together to slightly larger bubble streams. The foam only built up to a height of 40mm and did not reach the top of the fish tank (Figure 6.33). After 24 hours, the first stringy growth was visible attached to the aeration scaffold. An interesting observation was that, while the main air stream was directed upwards from the spiral scaffold, small aeration bubbles were distributed over the full volume of the medium, reaching into the corners of the rectangular growth vessel.

On day 3, the aeration scaffold and marble were covered by a thin layer of biofilm (ca. 3mm thickness) that began to reduce the airflow (Figure 6.34). However, the airflow was still significantly stronger than in previous experiments. Additionally, a free-floating piece of biofilm, ca. 10 x 10mm, was discovered. On day 5, this piece of biofilm had grown in size to 50 x 20mm. From day 6, a large active air reservoir

underneath the piece of marble was releasing bursts of air at 10 seconds intervals, resulting in slight movement of the aeration scaffold. The biofilm surrounding the spiral had increased to a thickness of 3-6mm and the first trapped air bubbles were noticeable. The airflow, however, was mainly unaffected and still created a turbulent stream of bubbles.

After 18 days, more significant changes were visible of the BC growth. A large biofilm had formed to fill the entire volume of the fish tank and it was beginning to collapse in on itself while lifting the marble piece off the bottom. Through this it was possible to see sharp edges formed by the BC, imitating the volume of the inner corners of the fish tank (Figure 6.32). The biofilm visually consisted of two parts (Figure 6.36): the outer skin which had taken on the rectangular shape of the fish tank and the interior cylindrical growth encasing the spiral scaffold. The interior part was nearly fully opaque and showed a complex system of bubble pathways, air reservoirs, and layers of overlapping thin sheets of biofilm (Figure 6.35). Overall, the aeration had minimised to only a few isolated active bubble pathways and air reservoirs releasing bursts or air. While a surface pellicle had begun to grow, it was extremely uneven (Figure 6.38).

By day 20, the marble and aeration scaf-

6.34

6.35

Figure 6.34: *First visible biofilm on day 3 around the aeration scaffold and a white feather biofilm moving with the air stream*

Figure 6.35: *Close-up of BC bubble pathways and air reservoirs*

fold were completely lifted of the bottom and floated at a height of roughly 10mm. After 26 days of growth, the aeration scaffold was detached from the air compressor and the BC growth harvested. While slowly removing the scaffold from the medium, the BC growth fully collapsed in on itself and was hanging off the scaffold. A large number of bubble pathways were visible connecting between the spiral elements of the scaffold and forming a circular ring around it. The inside of the growth was hollow, as had been previously suspected during the growth stage. Air reservoirs within the surface pellicle stayed intact and inflated the BC similarly to a pillowcase (Figure 6.39). A close-up picture of the BC growth gave a clear view on the varying levels of maturity of the BC as well as the hollow space within the spiral shape (Figure 6.37).

Developments for Next Iteration:

The results showed that the shape of the vessel can influence the shape development of the BC, however, the shape of the aeration scaffold and the air stream coming from it are more impactful. While no further upscaling of the aeration scaffold and growth vessel was tested as part of this PhD, the following ideas for further exploration emerged:

Figure 6.36: *Biofilm visibly split into two elements when looking from the side of the fish tank*

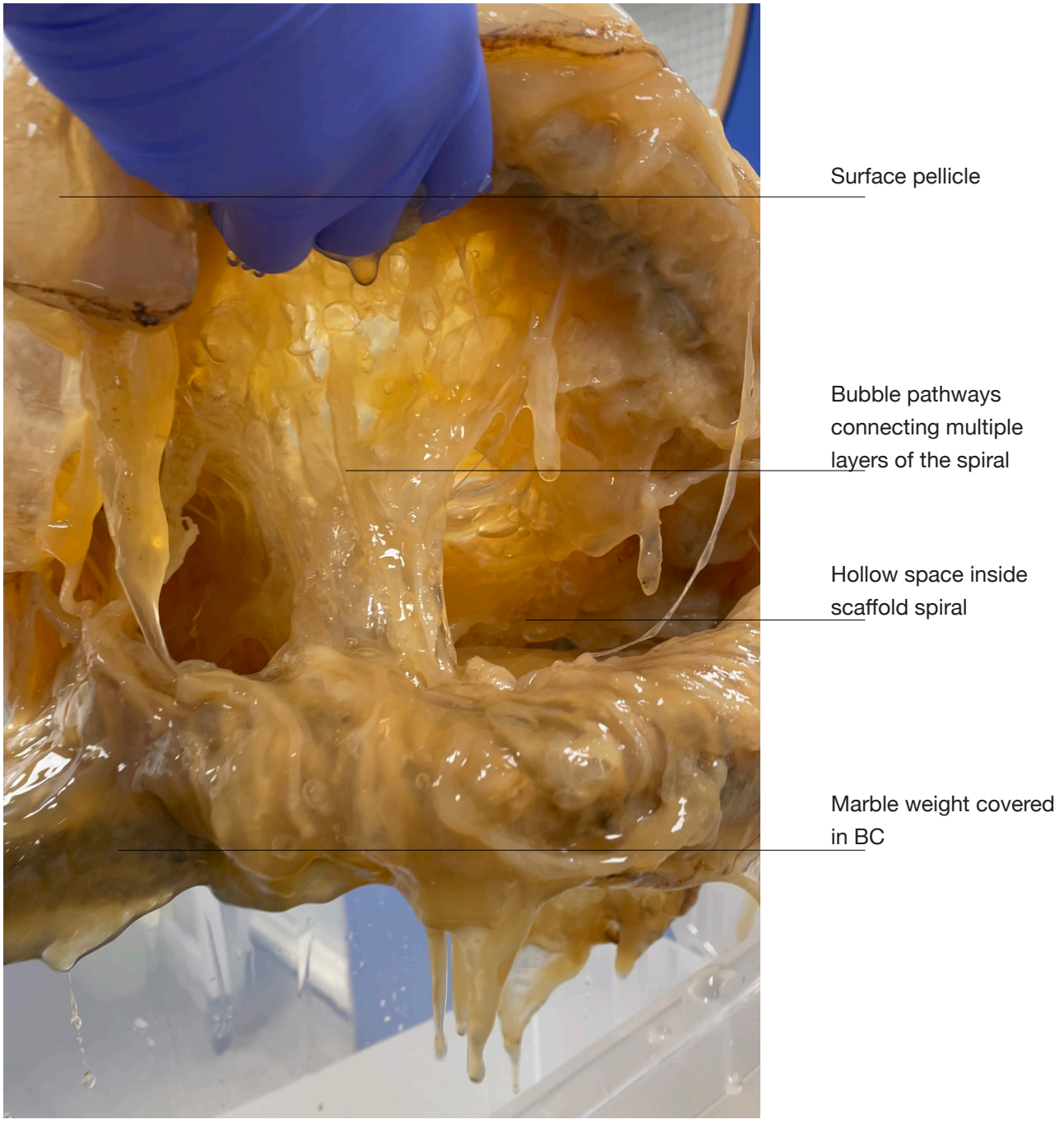


Figure 6.37: *Identification of previously observed growth features in the freshly harvested BC*

6.38

6.39

1. Following the principles of fruit moulding, could the BC be grown inside a 3D printed mould fully submerged inside the medium to grown into the desired shape? (Tweddell, 1989)

   → *Future Works (Chapter 6.4)*

Figure 6.37: *Uneven surface pellicle on day 20*
Figure 6.38: *Fully collapsed aeration scaffold with BC growth after harvest*

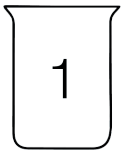
6.2. Customised Scaffolds

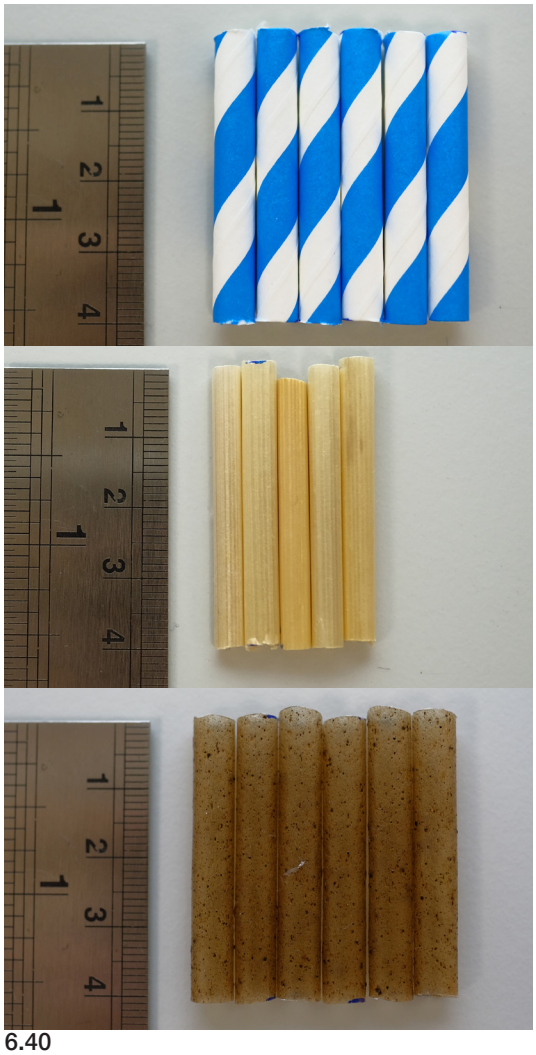
Based on the observations and analyses of the experiments in Chapter 6.1., the materiality of the aeration scaffold as well as the arrangement of porous surfaces were identified as design parameters to guide the BC growth into complex 3-dimensional forms. In order to customise the aeration scaffolds, material tests were conducted to test the compatibility with the bioreactor setup and the bacteria culture (Exp. M, N & O). Manual fabrication (Experiment P), as well as the use of digital fabrication processes (Exp. Q) were considered for the customisation of the aeration scaffolds. The additional design parameter of air flow and air pressure, allowing active manipulation and intervention during the growth stage, was also identified in previous experiments and its impact on the growth behaviour was tested further with the alternative scaffold materials.

In order to test the predictability of the BC form growing around the customised scaffolds, sketches were prepared for each scaffold prior to running the experiment in the bioreactor. The predictions of the BC forms were based on the growth stages and morphological features identified during Exp. I, and compared to the physical BC harvested, where possible.

M | Alternative Straw Materials

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			14



6.40

Figure 6.40: *Three different straw materials tested in this experiment*

Experiment Method:

The aim of this experiment was to explore the potential of using existing hollow objects to create aeration scaffolds. For this, three types of drinking straws made from plastic-alternative were chosen. The straws were made from paper (brand 'Morrison'), agricultural straw (brand "FOOGO green'), and a bioplastic made from by-products of the agave syrup production (brand 'bio agave').

Before testing the straws as aeration scaffold, their liquid absorption was tested to ensure their form stability during the prolonged submersion in liquid. Each straw type was cut into 40mm pieces (Figure 6.40) and submerged in water for up to 72 hours (Figure 6.41). During the handling and cutting of the agricultural straw, the material split lengthwise and was

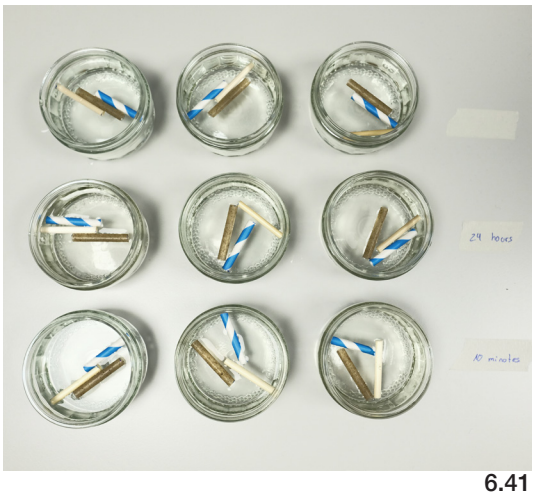
deemed unsuitable. Both other materials could be handled without breakage and retained their shape during the absorption test.

To prepare the paper and bioplastic straws for the experiment, they were manually perforated 40 times using a sewing needle and one end was sealed using silicone glue. To attach the silicone tube, a connector piece was glued into the other opening of the straw. After 48 hours of drying, the growth vessels were autoclaved, and the aeration scaffolds sterilised under UV light for 1 hour.

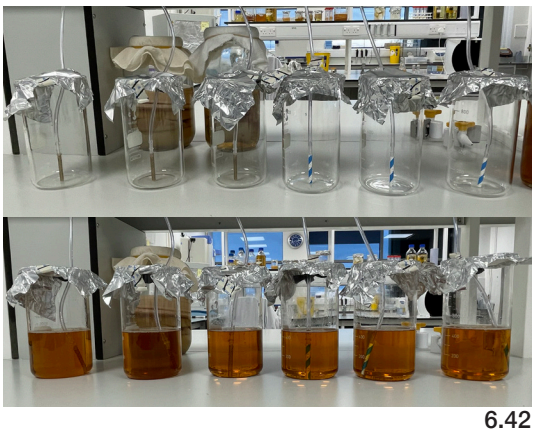
Each growth vessel was filled with 450ml tea-based medium and 22.5ml liquid inoculum from the corresponding SCOBY hotel. The straws were placed on the bottom in the centre of the vessel pointing downwards. Once the air pumps were turned on, however, the straws moved and stayed in a diagonal position pressed against the bottom edge of the vessel. Aluminium foil was used to cover the growth vessels (Figure 6.42).

Observations Summary:

The first observation was that the agave straws showed aeration only in the top half of the straw and the pores in the bottom half had a static air bubble attached. In contrast, all 40 pores of the paper straws showed active air



6.41



6.42

Figure 6.41: *Testing the absorption and form stability of the straw materials*

Figure 6.42: *Top showing straws positioned in centre of beaker; bottom showing straws in medium before air flow*

6.43

6.44

Figure 6.43: *Transparency of the agave straw showing that the yeast is growing on the outside and the inside of the scaffold after 48 hours of aeration*

Figure 6.44: *Large opening at the bottom of paper straw*

movement. One possible explanation is the behaviour of the material during the piercing process. While the holes created in the bioplastic straws remained exactly the size of the needle, the holes created in the paper straws would partly close in on themselves once the needle was removed. The smaller pore size in the paper straws could result in higher pressure inside the straw and force the air to escape through less favourable pores. Both straw types, however, created air streams of much larger air bubbles than the tested air stones (Exp. I, Chapter 5.2.1.) or aeration discs (Exp. G, Chapter 5.1.3.). This resulted in unstable foam build-up which continuously collapsed.

After 24 hours, all samples showed signs of brown yeast cells accumulating on the pores and beginning to block the airflow through them. In particular the bottom half of the agave straws was affected by this, with all pores previously holding a static air bubble now being covered in yeast. After a further 24 hours, the yeast growth was also visible on the inside of the agave straws (Figure 6.43).

After 48 hours of growth, two of three paper straw samples began leaking air through the bottom which was originally sealed with silicon. The airflow in those samples shifted to exclusively going through the larger opening at the

bottom (Figure 6.44). Following this change of airflow, the yeast growth on the affected paper straw scaffolds progressed much faster than the one with the silicone seal still intact. On the agave straws the biofilm growth could be visually divided into three areas (Figure 6.47). The upper two areas showed predominantly growth inside the scaffold while the lowest area had exclusively exterior growth. The density of the internal growth alternated with the strength of airflow in the area.

On day 4 of the experiment, a visible translucent biofilm had formed around both types of straw scaffolding but with a clear difference in shape development. The biofilm on the paper straws grew evenly along the whole length of the scaffold and the silicone tube. In contrast, the growth on the agave straws was concentrated at the bottom end of the straw with a much clearer outline than the feathery biofilm on the paper straws (Figure 6.45). This growth trend continued until the harvest on day 14. The growth around the agave straws developed into a darker brown colour and the BC on the paper straws turned into a nearly fully opaque white growth. Bubble pathways only developed on the paper straw samples and only on the ones which had the broken seal on the bottom (Figure 6.46). In the case of the agave straws, the

6.45

Figure 6.45: *Comparison of compact and concentrated growth on agave straw (top) to feathery and widespread growth on paper straw (bottom) after 7 days*

Figure 6.47: *Three zones of biofilm growth on agave straw scaffolds after 48 hours*

growth developed in a way that concentrated all airflow into one area of the straw. This resulted in the BC growth having one large opening that still exposed the scaffold.

Before harvest, minimal airflow was occuring from the paper straw samples, while the agave straw samples all had one strong stream of bubbles that created a layer of foam. None of the samples grew a BC surface pellicle.

While removing the aeration scaffold from the growth vessel, the growth on the agave straws detached fully in one piece (Figure 6.48). The consistency of the brown BC was soft yet firm with a slippery feel. The colour of the BC was unusually dark and had a marbled effect throughout. The feathery growth on the paper straws partly seperated from the aeration scaffold during harvest and showed little formstability while being handled. These BC samples showed different levels of maturity, ranging from transparent gel-like outer layers to an opaque more mature core. This difference was captured in the hand sketches (Figure 6.50). The bubble pathways were also visible in the harvested samples (Figure 6.49).

6.46

Figure 6.46: *Fully active bubble pathway forming at the bottom of a paper straw sample with broken silicone seal*

Developments for Next Iteration:

Both the agave bioplastic and the paper straws showed compatibility with the aeration

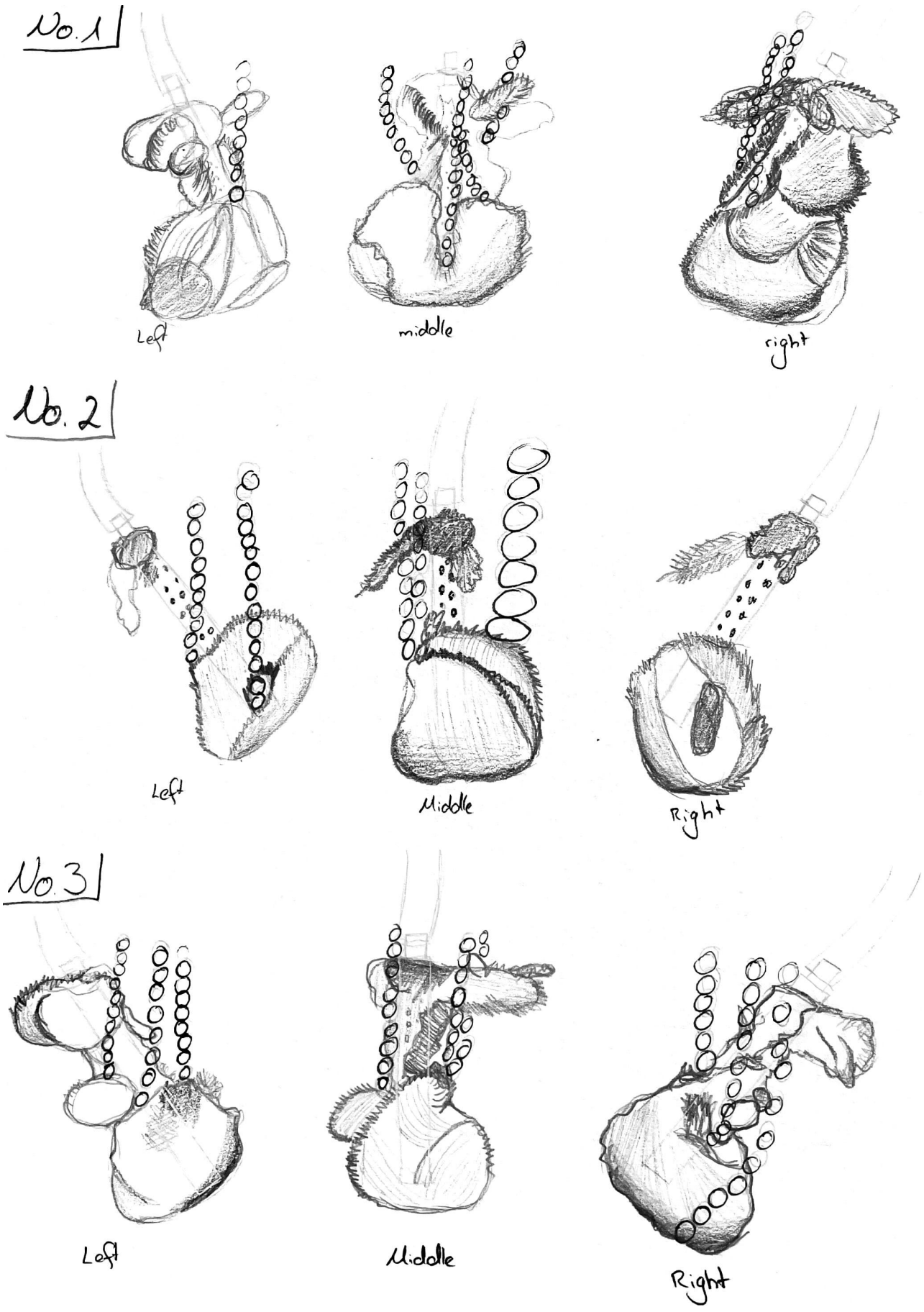
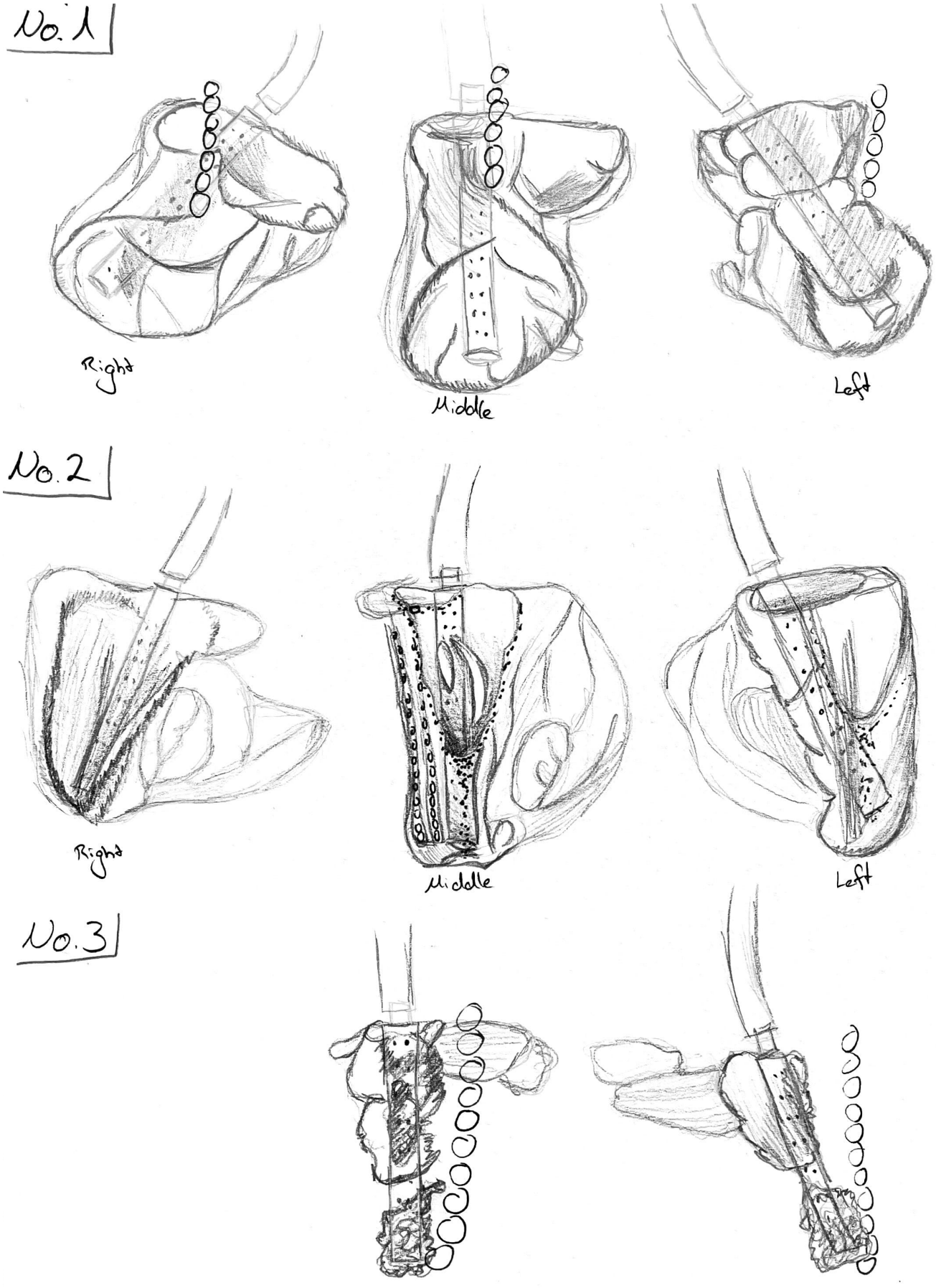


Figure 6.50: *Hand sketches of BC growth shapes on agave (left) and paper (right) straws before harvest*


No. 1
Right
Middle
Left
No. 2
Right
Middle
Left
No. 3

6.48

6.49

Figure 6.48: *Growth harvested from an agave straw setup showing marbling inside the brown-orange BC*

Figure 6.49: *Growth harvested from paper straw scaffold showing areas of transparent immature BC and an opaque core*

setup, and it was possible to grow BC on both materials. The difference in BC maturity and shape, however, led to the decision to carry on using only the agave bioplastic straws. The following ideas for further exploration emerged:

1. Can the agave straws be used to connect multiple air stones to create a larger customised aeration scaffold?

   → *Experiment P (Chapter 6.2)*

2. Is it possible to 3D print scaffolds in the shape of a straw and achieve similar or better results for the BC growth?

   → *Experiment N (Chapter 6.2)*

3. Can the agave straws be assembled into a more complex system to achieve a form-stable BC growth that does not detach?

   → *Experiment P (Chapter 6.2)*

4. Is it possible to customise a scaffold which dissolves throughout the growth process and leaves behind a 3-dimensional shape that is 100% BC?

   → *Future Works (Chapter 6.4)*

N | 3D Printed Aeration Rods

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
		120 x 170 x 60 mm & 170 x 170 x 90 mm	20

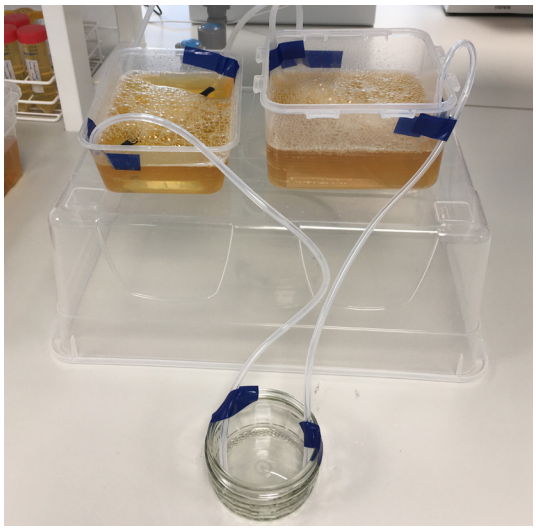
Experiment Method:

The aim of this experiment was to test the potential of using the fused filament fabrication (FFF) 3D printing process as tool to create customised aeration scaffolds. The focus was laid on the printing technique to achieve a perforated surface and the general compatibility with the existing experimental setup of air pumps, medium, and liquid inoculum.

Two straight tubes were created to imitate the previously tested straws, one with a consistent outer diameter of 7 mm and the other with an exterior diameter of 12 mm in the centre and 7 mm at the ends (Figure 6.51). Each tube had an array of 'pores' with 1mm diameter to create porous walls. The two different shapes of aeration scaffold were printed on an Ultimaker 3+

6.51

Figure 6.51: *The two types of 3D printed aeration scaffolds used in this experiment*



6.52

Figure 6.52: *Experimental setup with additional collection vessel connected to the aeration scaffold*

Extended using white PLA filament with a layer height of 0.1 mm and without support material.

To setup the experiment, both ends of the scaffold were connected to silicone tubing of which one was connected to the air pump and the other lead into an empty container. The empty container was added to collect any liquid which may enter or pass through the scaffold during the growth stage. The growth vessel was filled with 100 ml tea-based medium and 2ml liquid inoculum from the corresponding SCOBY hotel before the already aerated scaffold was submerged into it. The scaffolds were positioned horizontally in the centre of the volume of the medium (Figure 6.52). Plastic foil was used to cover the opening of the growth vessels. This set of experiments was run in parallel to Exp. I and J and prior to the development of customised lids.

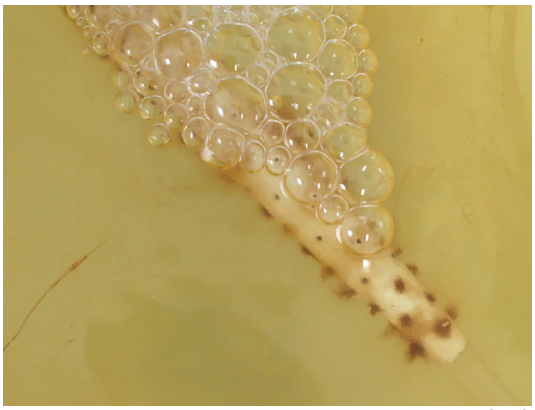
Observations Summary:

It was possible to print the tubes upright on the Ultimaker and the pores were mostly unobstructed with only a few pores needing to be cleared from surplus filament prior to use. And while the open shape of the aeration scaffold resulted in less air pressure building up on the inside, both scaffold types showed strong and continuous aeration with large air bubbles.

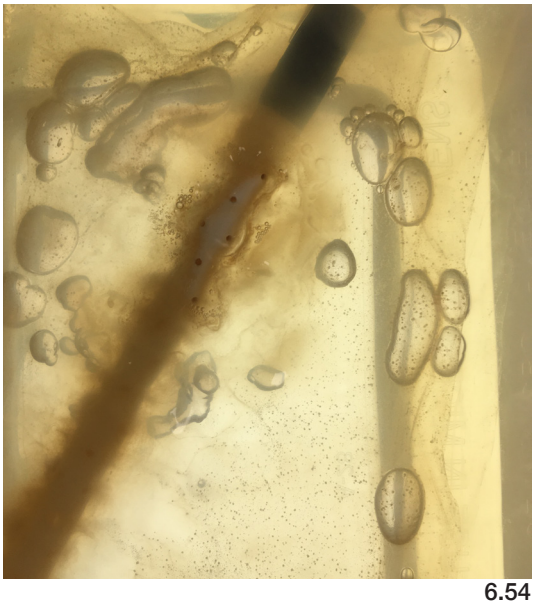
Stronger aeration was observed coming from the curved tube and this sample had a thicker layer of foam build up, even though the foam wasn't stable and collapsed continuously. However, only the top half of each scaffold showed airflow, with static single air bubbles being attached to the pores of the lower half, similarly to the agave straws in Exp. M. In the case of the curved scaffold, only the top-most pores had consistent airflow

Within the first 4 days of growth, the pores on the lower half of the scaffolds were fully covered by brown yeast growth. This also applied to the pores on the top half of the curved scaffold which had inconsistent airflow (Figure 6.53). While predominantly transparent, a thin biofilm was also noticeable at the bottom of the growth vessel through areas of white speckles within. On day 6, it began to loosely wrap around the scaffolds and on day 9, the biofilm also grew on the surface and trapped air bubbles within it. At this stage, only a handful of pores per scaffold were still allowing airflow with all others being fully blocked by brown yeast growth.

From day 9, maturing BC growth was observed directly over a section of the straight tube scaffold in the form of a shallow dome raising up from the biofilm growing on the surface (Figure 6.54). Until the harvest on day 14, the



6.53



6.54

Figure 6.53: *Brown yeast blocking unused pores of the scaffold after 72 hours*

Figure 6.54: *Doming BC forming above the functioning pores while partially exposing the aeration scaffold*

BC only matured into a white translucent colour (Figure 6.55 & 6.56).

Due to the fully hollow nature of the aeration scaffolds, similarly to the tested straws, the assumption was made that BC could grow on the inside of the scaffold and begin to block off the pores, reducing or completely blocking airflow. However, unlike with the agave straws, throughout the 14 days of growth, no liquid was accumulated inside the collection vessel and no growth was visible inside the scaffolds during the harvest process. Overall, the BC growth was limited on both types of scaffolds which can partially be explained by the low ratio of inoculum to medium. Additionally, the experiment was conducted in a shallow growth vessel that did not allow much space above the scaffold. However, in previous aeration experiments, the majority of BC growth occurred between the scaffold and the liquid surface (Chapter 6.1.).

Developments for Next Iteration:

While the 3D printed scaffolds are compatible with the general experimental setup, a few adjustments need to be made in order to continue with the exploration of customised 3D printed scaffolds.

- When designing the scaffolds, only the upward facing side needs to have perforated walls as the air will not escape through the downward facing side.
- Similarly to the commercially available air stones, the 3D printed aeration

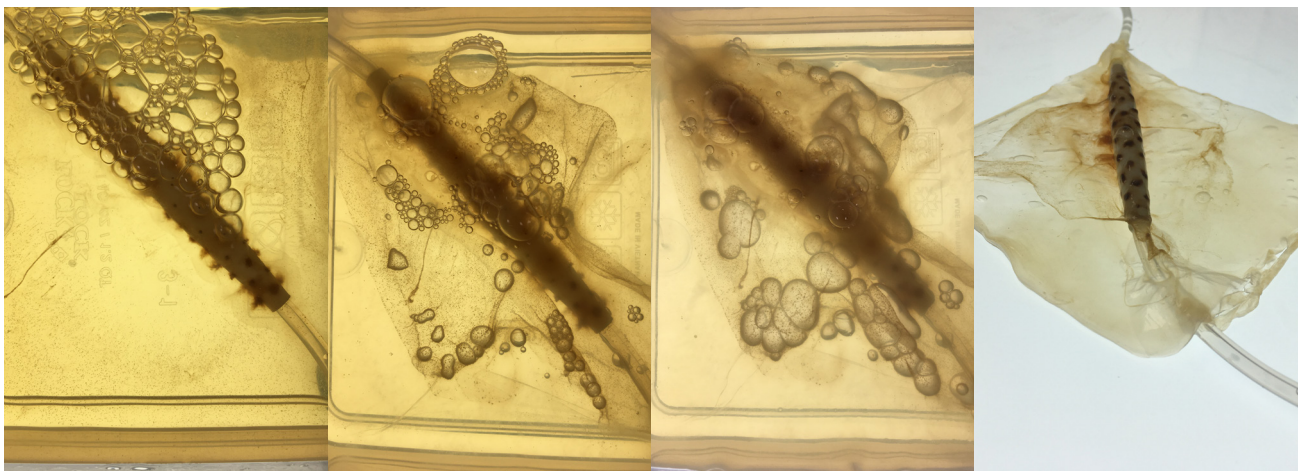


Figure 6.55: *Growth appearing on curved tube after 3, 9, and 14 days, and immediately after the harvest (left to right)*

scaffolds only need one opening to attach the silicone tube coming from the air pump. The collection vessel can be omitted in future experiments.

- Following the results of Exp. H, HS medium should be the preferred nutrient source for experiments with larger aeration pores due to the lack of yeast formation.

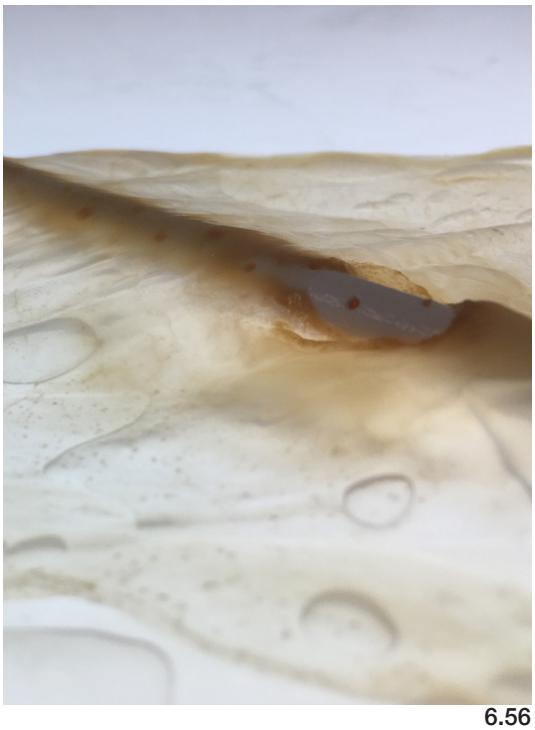
Additionally, the following questions arose for the continued exploration of 3D printed aeration scaffolds:

1. Is there a notable difference in BC attachment to the aeration scaffold depending on the filament type used to 3D print it?

   → *Experiment O (Chapter 6.2)*

2. Is it possible to fabricate aeration scaffolds with smaller pore sizes on the Ultimaker 3+ Extended 3D printer?

   → *Experiment P & Q (Chapter 6.2)*

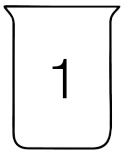


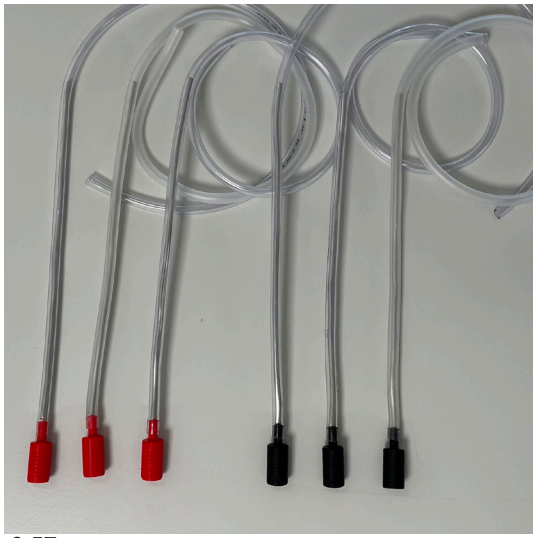
6.56

Figure 6.56: *Freshly harvested straight tube with close up of the hole in the biofilm*

O | Comparison ABS and PLA

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			16



6.57

Figure 6.57: *3D printed aeration scaffolds in the shape of a single air stone from PLA filament (red) and ABS filament (black)*

Experiment Method:

Aim of the experiment was to test whether there is a noticeable difference in the BC growth behaviour based on the filament type used to 3D print the aeration scaffold. In particular, the initial attachment of the BC to the scaffold and the following blocking of the pores by the growing BC were of interest. The shape of the scaffold was designed to mimic a single air stone with the dimensions 20 mm (height) and 10 mm (diameter).

To print the aeration scaffolds, the two most commonly used filament types for rapid prototyping, PLA and ABS, were tested. The PLA samples were printed in a red filament on a Prusa i3 printer and the ABS samples in black on a Ultimaker 3+ Extended. The design of the

air stone was modelled in Rhinoceros 7 (Figure 6.57).

Due to previous observations that the orientation of the air stones within the medium influences where the air streams form on it (Exp. N), aluminium wire was inserted into the inside of the silicone tubing to be able to straighten it and position the air stones in a fully vertical position. No difference in the strength of the air stream was observed during a preliminary test in water with and without the wire inserted.

The aeration scaffolds and lids were UV sterilised for 1 hour with regular turning and the vessels were autoclaved for sterility. Inside the laminar flow hood, 400 ml tea-based medium and 4 ml liquid inoculum were added to the beaker. The silicone tube of the aeration scaffold was threaded through the centre hole of the lid before placing it inside the medium. The printed air stone was positioned to hover 30 mm above the bottom of the growth vessel (Figure 6.58).

Once the setup was moved to the work bench and connected to the pumps, the airflow was started on the lowest setting. Over the first 7 days, the air pressure was increased incrementally whenever a significant change of airstream from the scaffold was noticed, until it reached 100% strength.

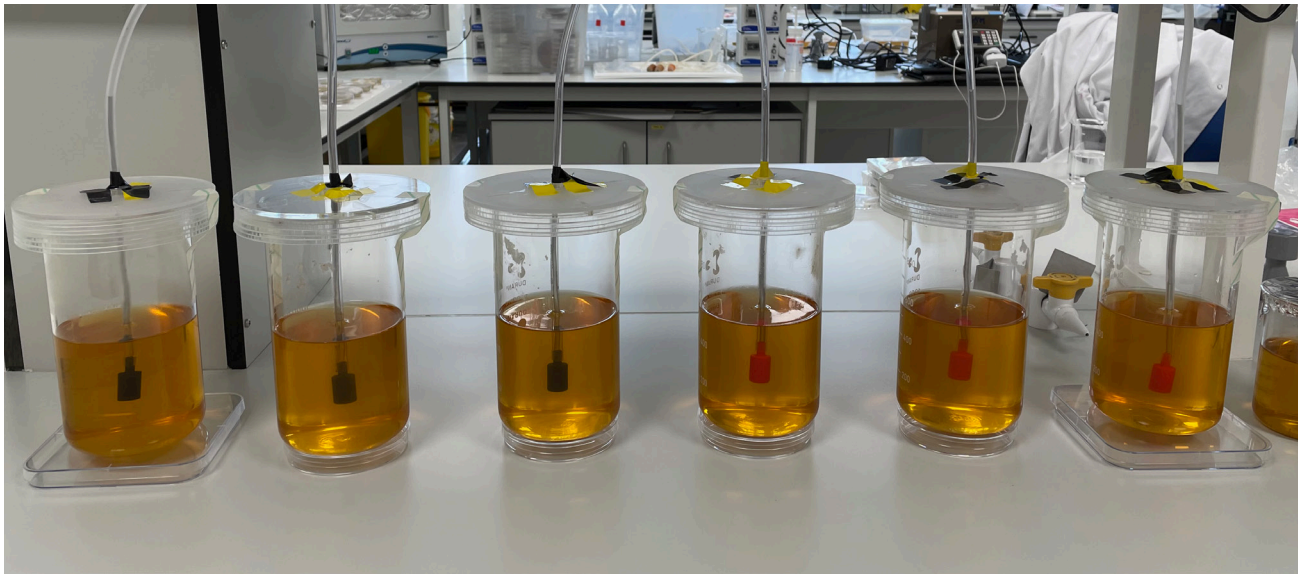


Figure 6.58: *Straight position of aeration scaffolds before starting the airflow*

6.59

Figure 6.59: *Comparison in foam formation of ABS (top) and PLA (bottom) aeration scaffolds immediately after beginning the airflow*

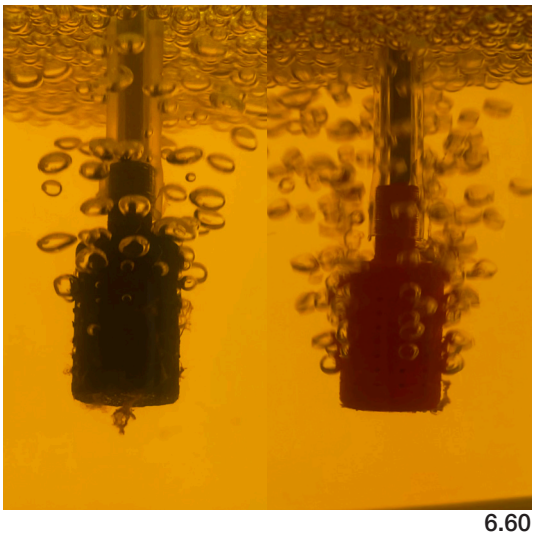
Observations Summary:

The first observation immediately after starting the aeration was a difference in airflow from the two scaffold materials. While the black ABS scaffold predominantly showed airflow from the pores in the top half of the scaffold, the red PLA scaffolds had an evenly distributed airflow from all pores across the whole length. However, neither of the scaffold materials resulted in stable foam formation on the surface of the medium (Figure 6.59).

Within 10 minutes, the first stringy biofilm formation was observed on the ABS scaffolds and within 24 hours the unused pores on the bottom half were completely covered in biofilm. The position of the pores was visible through the localised biofilm growth on each pore (Figure 6.60). In comparison, the red PLA scaffold only showed fine individual speckles of biofilm evenly distributed at this stage. On day 3, the first appearance of the fuzzy opaque biofilm was observed and was fully encapsulating the scaffolds of both material types. In the case of the ABS scaffolds, the fuzzy biofilm extended from the scaffold to the surface of the medium (Figure 6.61). A detached thin layer of biofilm also formed at the bottom of the growth vessel in both material scaffolds.

From day 4 to 8, the biofilm around the

scaffolds rapidly expanded in volume until it reached the sides of the growth vessel. The air stream was significantly reduced and formed larger individual air bubbles. From day 8, the surface pellicle began to grow and formed around the continuing air stream. In addition to larger air reservoirs under the pellicle (Figure 6.62), this resulted in the formation of small crater-like openings within the pellicle (Figure 6.63) through which air escaped in irregular intervals. Throughout the overall growth time of 16 days the medium levels decreased in all growth vessels, however the decrease was more noticeable in the ABS samples. Measured on day 10, the ABS samples had reduced liquid levels to 400ml while the PLA samples measured at 450 ml. During harvest, all samples were slowly removed from the growth vessel and documented via photography. All samples, apart from ABS no.3, split into two pieces, an immature bottom half and more matured top half attached to the scaffold, while being lifted upwards (Figure 6.64). No significant differences in the morphology of the harvested shapes were observed (Figure 6.65).



6.60

6.61

Figure 6.59: *Yeast blocking the bottom half of ABS scaffolds (left) after 24 hours but not PLA scaffolds (right)*

Figure 6.61: *Growth on day 3 on ABS (top) and PLA (bottom) scaffold with visible difference in the expansion to the surface*

6.62

6.63

Figure 6.62: *Large air reservoir formed under the surface pellicle in PLA sample on day 14*

Figure 6.63: *Crater-like surface pellicle forming around the still active airstreams from the aeration scaffolds on day 8*

Developments for Next Iteration:

The results of the experiment show that both types of filaments are compatible with the BC and do not hinder the organism's activity.

1. When designing the scaffolds in 3D, there could be a tapering of pore size with smaller diameters towards the surface of the scaffold and larger diameters towards the bottom to encourage the airflow to distribute evenly

→ *Experiment Q (Chapter 6.2)*

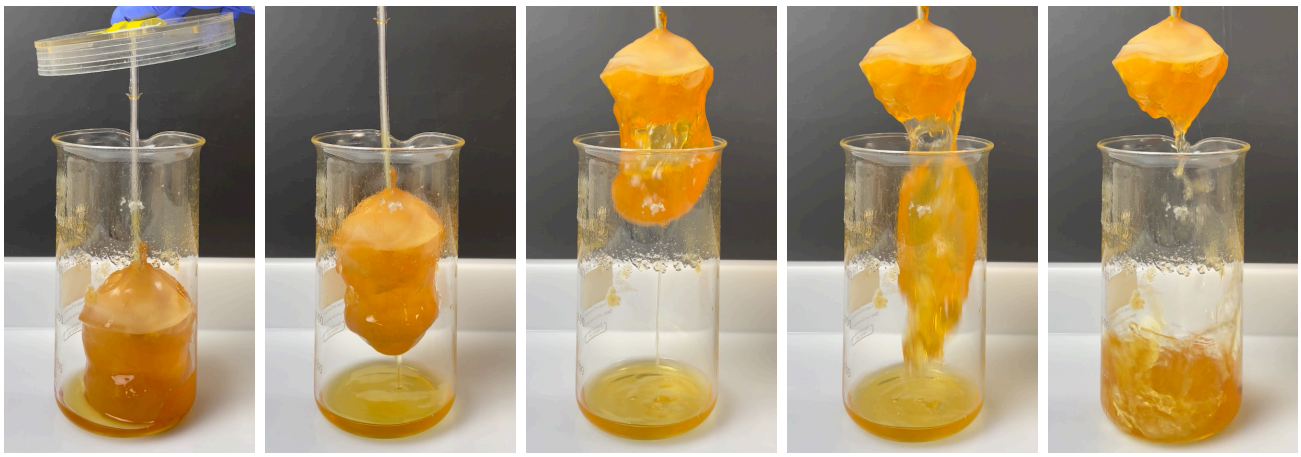


Figure 6.64: *Large element of immature BC detaching from the BC shape during the harvest process of a PLA sample*

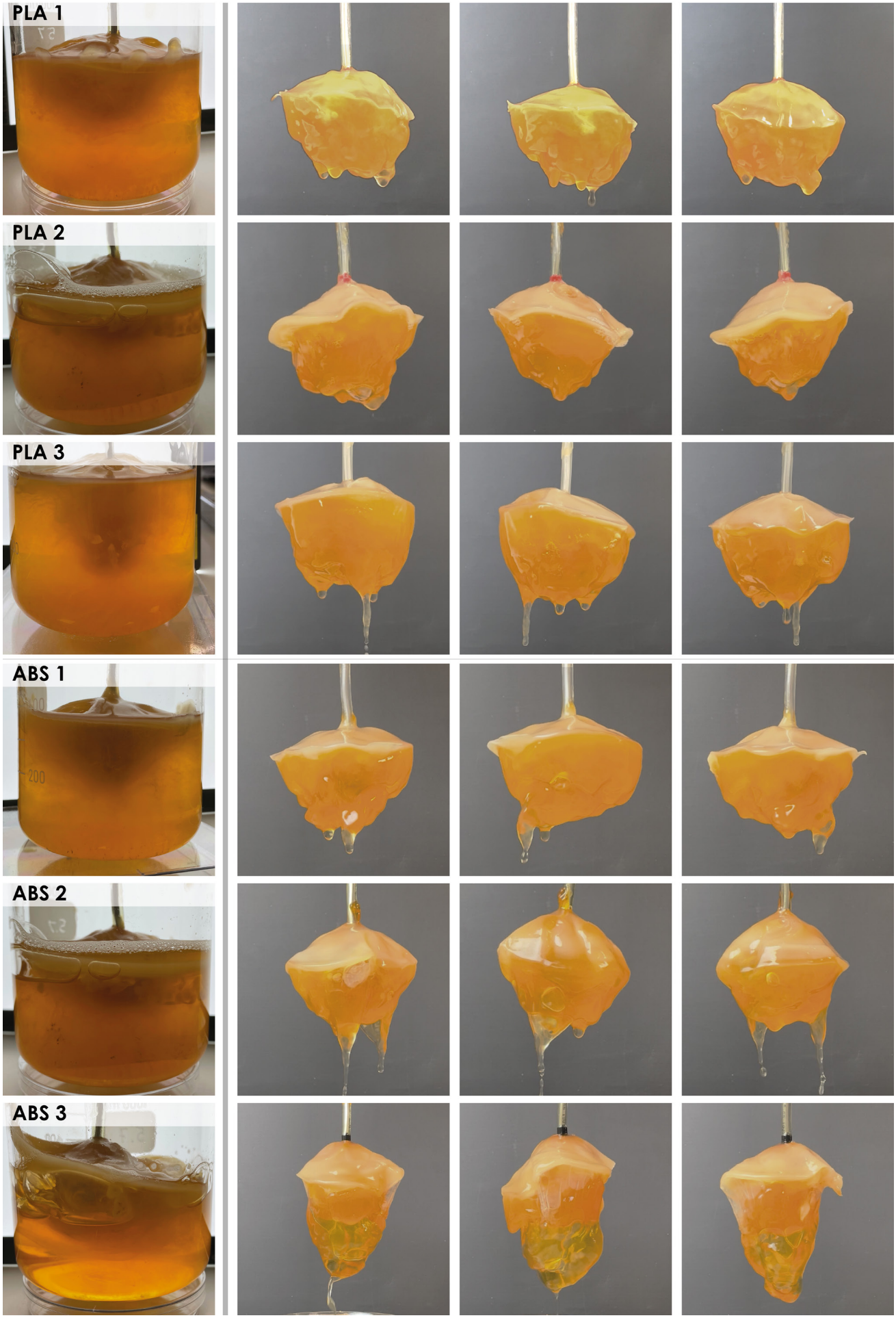
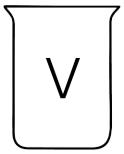


Figure 6.65: *Comparison of PLA and ABS samples on the day of harvest submerged (left) and once harvested (right)*

P | Material Combinations

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			14 / 21

6.66

Figure 6.66: *Combination scaffolds for set 1 (top row)and set 2 (bottom row) of this experiment*

Experiment Method:

The aim of this set of experiments was to test how far the BC growth can be predicted based on the shape of the aeration scaffold. An additional manipulation factor, or active design parameter, of adjustable airflow was integrated into the experimental setup to influence the BC shape formation. Various different types of aeration scaffolds were fabricated in two stages and the anticipated BC shape was sketched based on principles and behaviours observed previously (Exp. I & J & K). Where possible, the final shape of the submerged BC was documented for comparison and the samples shock-frozen for further shape preservation (Chapter 7).

The first set of aeration scaffolds was fabricated by combining individual spherical air

stones (diameter 17 mm) and pieces of agave bioplastic straw that were manually perforated with a sewing needle. Plastic tube connectors were used in two of the three designs. To join the components together, clear silicone glue was used (Figure 6.66, top). The three scaffolds were assembled as followed:

(1) Two air stones with 15 mm agave straw attached, joined at a 90-degree angle with additional connector piece.
(2) Single air stone connected to 40 mm agave straw, and a cylindrical air stone attached in parallel with a gap of 35 mm in between.
(3) Two air stones connected in line with 40 mm agave straw.

After the first set of experiments showed issued with BC attachment during harvest stage, a second set of iterated scaffolds was designed (Figure 6.66, bottom). This second set also integrated 3D printed components made from PLA filament and did not have perforations in the agave straws when used as connector pieces:

(4) One spherical commercial air stone and one 3D printed cylindrical diffuser element with elongated pores connected with 15 mm agave straws and joined at a 90-degree angle. The 3D printed component positioned on the vertical and the air stone on the horizontal axis. The overall dimensions are 95 x 70 x 20 mm and a weight of 18.6 g.
(5) Combination of only perforated agave straws and plastic corner connections to create a rectangular shape. The overall dimensions are 70 x 80 x 8 mm and a weight of 5.9 g.
(6) One 3D printed air stone (see Exp. O,) connected to 15 mm agave straw and one disc-shaped commercial air stone connected to 20 mm agave straw, joined at a 90-degree angle. The 3D printed component positioned on the horizontal and the air stone disc on the vertical axis. The overall dimensions are 95 x 70 x 40 mm and a weight of 40.8 g.

The airflow of all scaffolds was tested in water to test for blockages of pores. In set 2, this test was also done to optimise the position of the scaffold by bending the aluminium wire

6.67

Figure 6.67: *Aeration scaffolds of set 1 in unpredicted orientations within the growth vessel due to twisting silicone tubes*

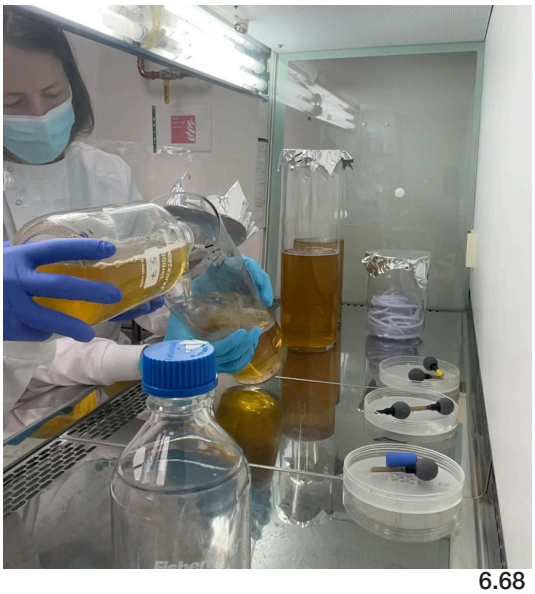
inside the silicone tube (Exp. O). This change in setup was decided on after the scaffolds in set 1 continuously changed orientation, despite the weight of the scaffolds, which influenced the BC growth unexpectedly (Figure 6.67).

To set up the experiment, each scaffold was suspended into 2,000 ml HS medium inoculated with 200 ml from the HS SCOBY hotel. In preparation, the aeration scaffolds were UV sterilised and the growth vessel autoclaved. For sterility reasons, each setup was assembled inside the laminar flow hood before being moved onto the lab bench. Each scaffold was attached to an individual air pump with adjustable airflow. To begin the experiments, all pumps were set to the lowest setting. An incremental increase of air pressure was decided on individually based on the observed growth of the specific setup.

The observational data for set 1 is incomplete due to contracting COVID on day 10 of the experiments and not being able to continue daily documentation. These experimental setups were harvested prematurely after 14 days by colleagues and, where possible, shock-frozen for future shape preservation steps. Because enough data was collected to develop a second iteration of the material combination scaffolds, the experiment was not repeated but further developed with set 2.

Observations Summary:

The first observation made before starting the experiment was the difficulty in assembling each setup inside the laminar flow. The design of the sterile working space requires the glass panel on the front to be lowered at least halfway to ensure a controlled airflow inside. This restricts the overall height that can be reached within in (Figure 6.68). To fill the tall growth vessels, a second pair of hands was needed to hold them in a tilted position. This subsequently limits the amount of liquid that can be safely filled into the vessels. While a Bunsen burner would allow working with taller vessels, the sterile surface area created would not suffice for the large opening of the vessels and the sterile scaffolds. It was therefore not used for this experiment.

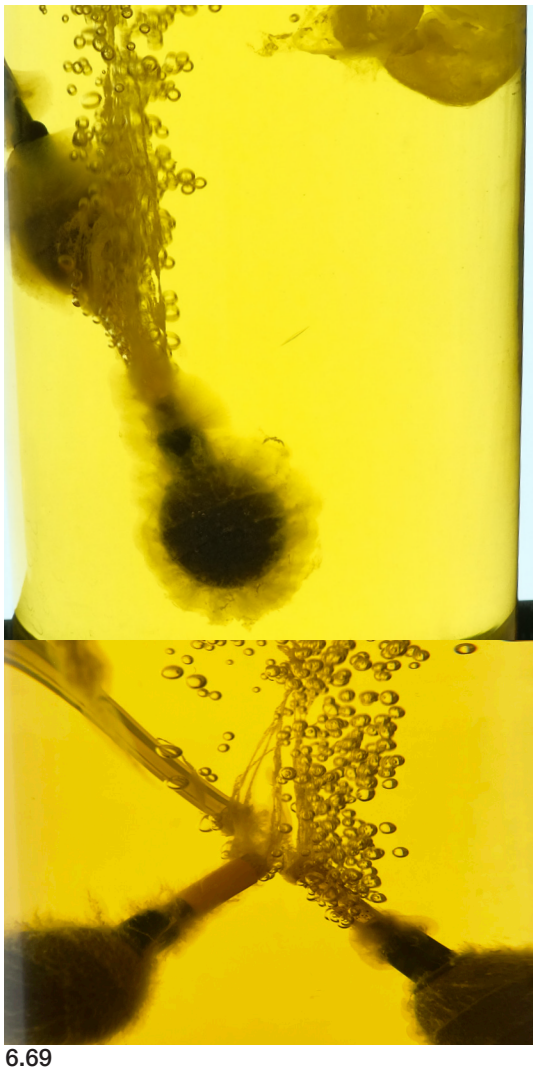


6.68

Figure 6.68: *Filling the tall growth vessels required tilting them with the help of another researcher due to restricted arm height*

Scaffolds Set 1

Due to the twisting silicone tubes in set 1 of the aeration scaffolds, the airflow from the scaffolds did not develop as expected. Instead of staying vertical inside the vessel, all three scaffolds tilted into a diagonal position. This meant the air predominantly escaped through the highest and upward-facing surfaces. In the case of scaffold (1) the air only escaped through the agave straws and not through the air stones (Figure 6.69, bottom).



6.69

6.70

Figure 6.69: *White stringy growth on scaffold (3) and (1) forming where the strongest airflow is occurring on the agave straws*

Figure 6.70: *Free-floating biofilm on day 2 inside setup (3)*

The biofilm development in the first 72 hours followed the expected pattern of stage 1 (Exp. I) with the formation of a white fuzzy layer of growth covering the aeration scaffolds and feathery stringy growth attached to areas of strong air flow (Figure 69). In setup (3), a free-floating biofilm occurred on day 2 which re-attached to the main growth on the scaffold on day 6 (Figure 6.70).

On day 5, growth behaviours of Stage 2 (Exp. I) were observed in two of the three samples. Bubble pathways began to form on scaffold (2) and (3) (Figure 6.71) while scaffold (3) also developed air reservoirs. At this stage, the air pressure was increased to enhance this developmental step. In scaffold (1) the biofilm continued to grow in thickness without showing any additional growth behaviours. The medium in scaffold (1) began turning cloudy on day 5. By day 8, the biofilm had matured to a fully opaque white and the medium had reached the medium began turning translucent again.

On day 10, the last day of observations, scaffolds (2) and (3) had continued to develop air reservoirs and bubble pathways (Figure 6.71). Because the airflow from the scaffold had visibly reduced, the air pressure was increased to 100% on the pump. This resulted in air streams breaking free from the previously carved out

pathways and active air reservoirs. The BC had begun to mature and turn translucent in colour around those form features while the overall biofilm encasing the scaffolds remained with undefined outline. No compacting of the BC was noticeable at this stage. Scaffold (1) showed no further development (Figure 6.72). All three shapes were successfully frozen during the harvesting process (Figure 6.73). However, the growth on scaffold (3) partly detached and only more matured growth directly attached to the air stones remained.

Scaffolds Set 2

An immediate difference to set 1 was that all aeration scaffolds stayed in their intended upright orientation once placed in the growth vessel due to the strengthened silicone tube (Figure 6.74, top). The air escaped from the air stones rather the perforated connecting elements and the agave scaffold (5) had an even airstream coming from all sides. Within the first 10 minutes, thin stringy growth appeared on all three scaffolds and moved with the air streams (Figure 6.74, bottom). For scaffold (4) the pump had to be adjusted to a medium airflow in order to achieve aeration from both air stones. All scaffolds produced an increasing amount of foam

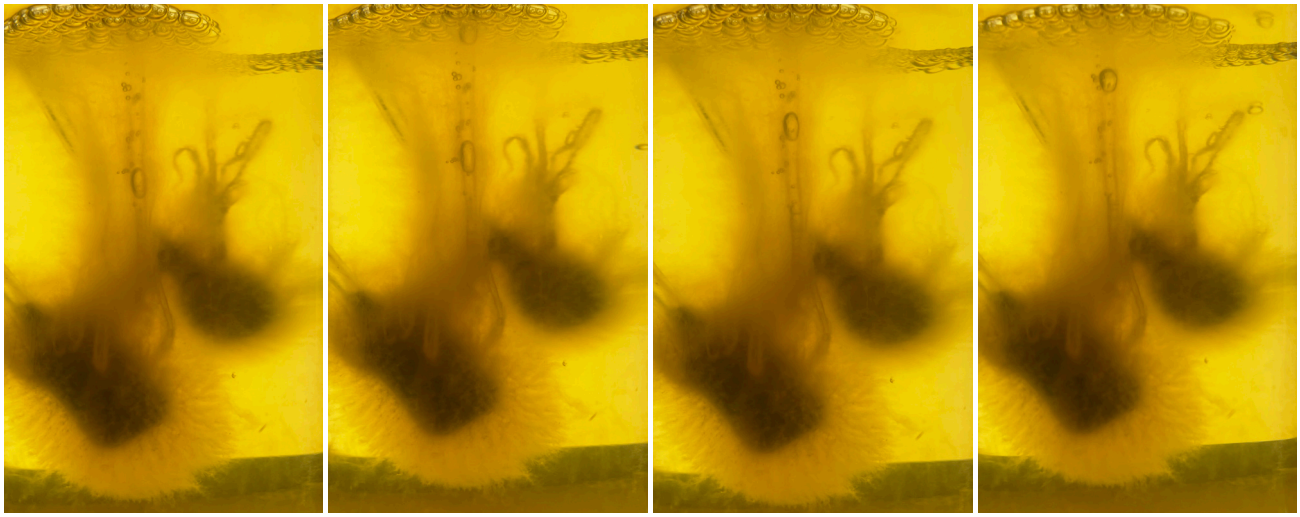


Figure 6.71: *Movement of a single air bubble through a bubble pathway on scaffold (2) on day 5*

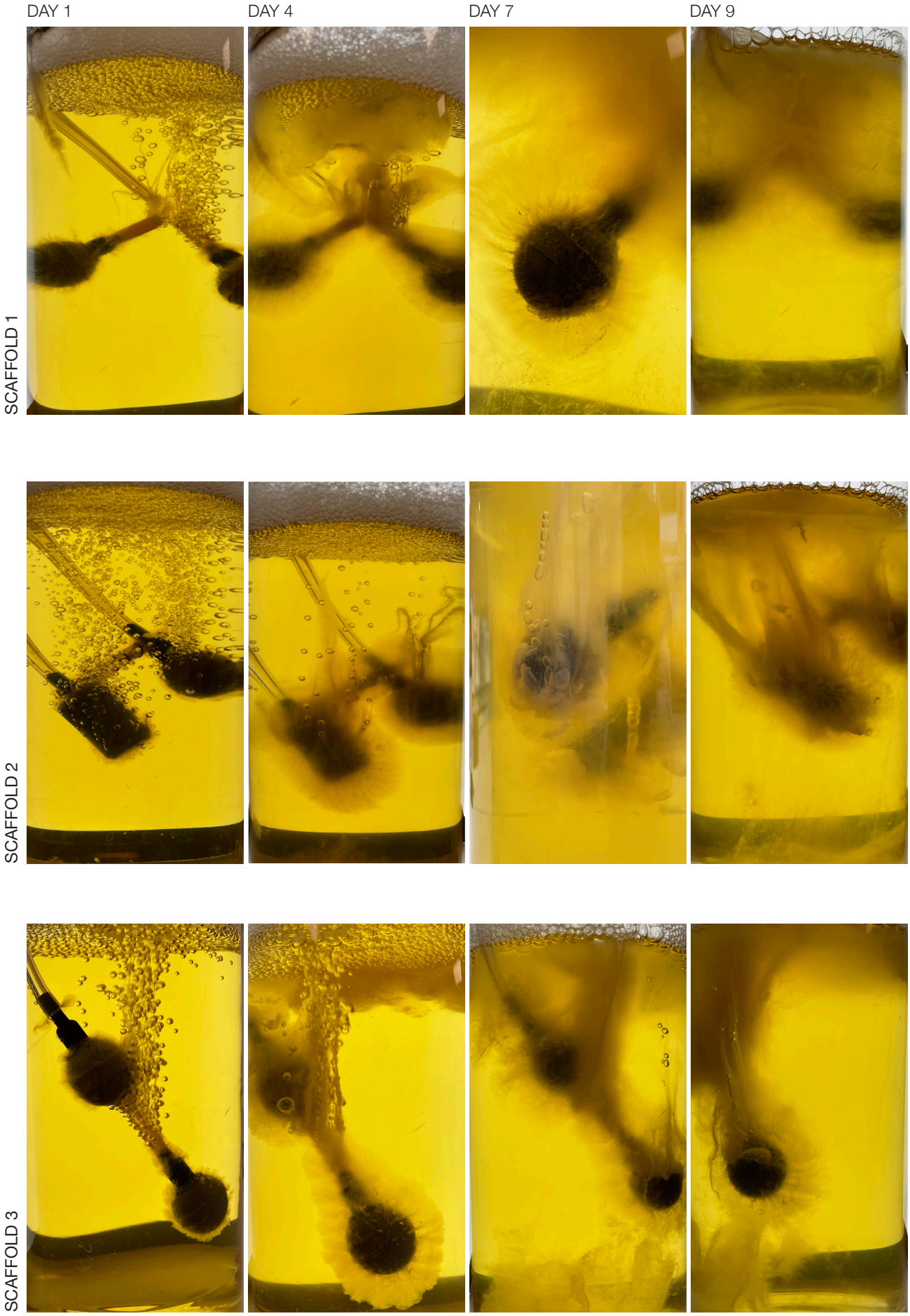


Figure 6.72: *BC growth development on scaffold (top to bottom) 1 to 3, on day (left to right) 1, 4, 7, and 9*

which reached a maximum height of 100 mm after 24 hours, before settling down again. With each increase of air pressure, the foam briefly began to rise again.

For the first 3 days, the growth around the aeration scaffolds developed as expected (Figure 6.75). A fuzzy undefined growth encasing the scaffolds grew in thickness and the white stringy growth grew longer and thicker, partially getting caught in the biofilm. The medium in set-up (4) begun to turn darker and cloudy from day 3, making it more difficult to identify the aeration scaffold within the biofilm. Additionally, the set-up of scaffold (5) began to form loose free-floating piece of biofilm which grew in thickness until it attached to the main growth on the scaffold on day 4. By day 13, this additional biofilm was fully integrated into the main growth.

On day 4, the air pressure on scaffold (4) was increased to 100% which enhanced the airflow coming from the 3D printed component. At this stage, the commercial air stone was fully blocked by the biofilm and it was not possible to regain aeration of it through the increased air pressure. In contrast to the other two setups, the biofilm on scaffold (6) did not mature at the same speed and grew a veil-like biofilm (Figure 6.75, bottom row). This free-floating growth began to wrap around the scaffold on day 6 and continued to form new layers until the harvest. The disc-shaped air stone lost all aeration on day 1 which was not regained after an increase

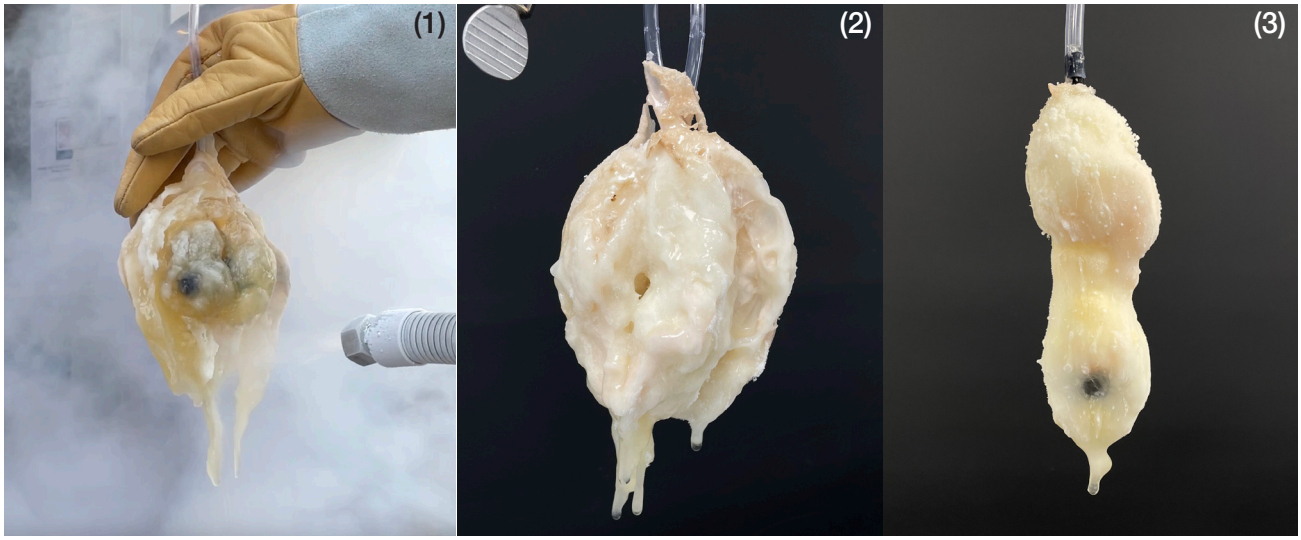
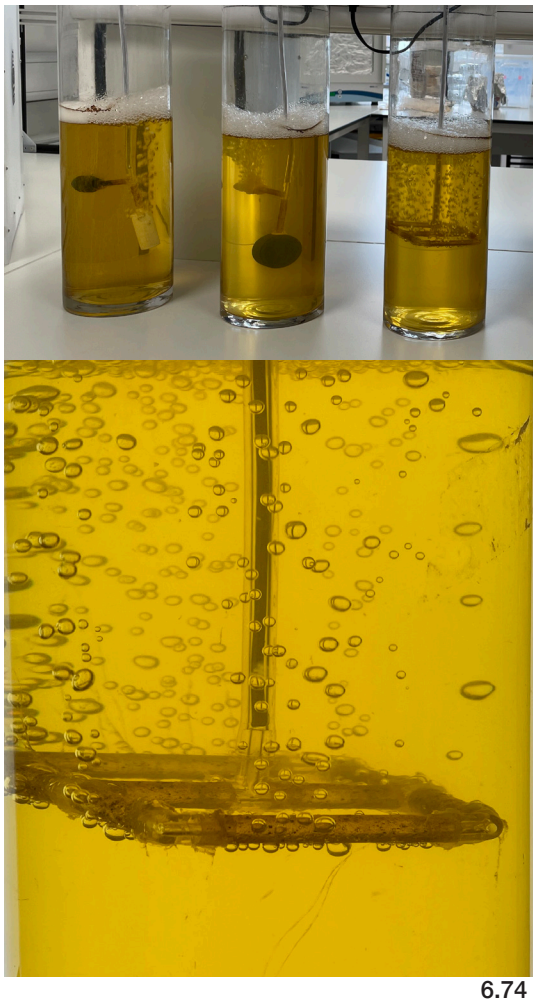


Figure 6.73: *Frozen samples of set 1, with scaffolds (1) to (3) from left to right*



6.74

Figure 6.74: *Set 2 scaffolds on the first day of the experiment (top) and close-up of the airflow coming from scaffold (5) (bottom)*

of air pressure. By day 6, the airflow from the 3D printed air stone had also decreased to a minimum with only 1 bubble bathway still intact.

In the following days, the growth on scaffold (4) and (5) began to compact and pull together, beginning to reach a defined shape of the BC. In particular the growth on scaffold (5) gained an increasingly solid appearance, and by day 13, it had grown to the full diameter of the vessel (Figure 6.75, middle row). On this day the BC growth had also reached the surface, exposing roughly 20mm above the air-liquid interface. A large air reservoir had formed in the centre of it, making up just under 50% of the volume, which inreased the buoyancy and resulted in the growth rising to the surface. The scaffold and attached growth were manually pushed back below the surface via the air tubing and taped into the new position. A pulsing occurred inside the growth every time a large air bubble was released from the air reservoir in the centre. On day 16, setup (5) was harvested prematurely due to first signs of contamination. While the BC had not fully matured at this stage, the frozen shape maintained the round shape of the growth vessel (Figure 6.76, middle).

Scaffolds (4) and (6) were left to grow until day 21 at which stage both showed either no or minimal airflow. The veil-like growth around

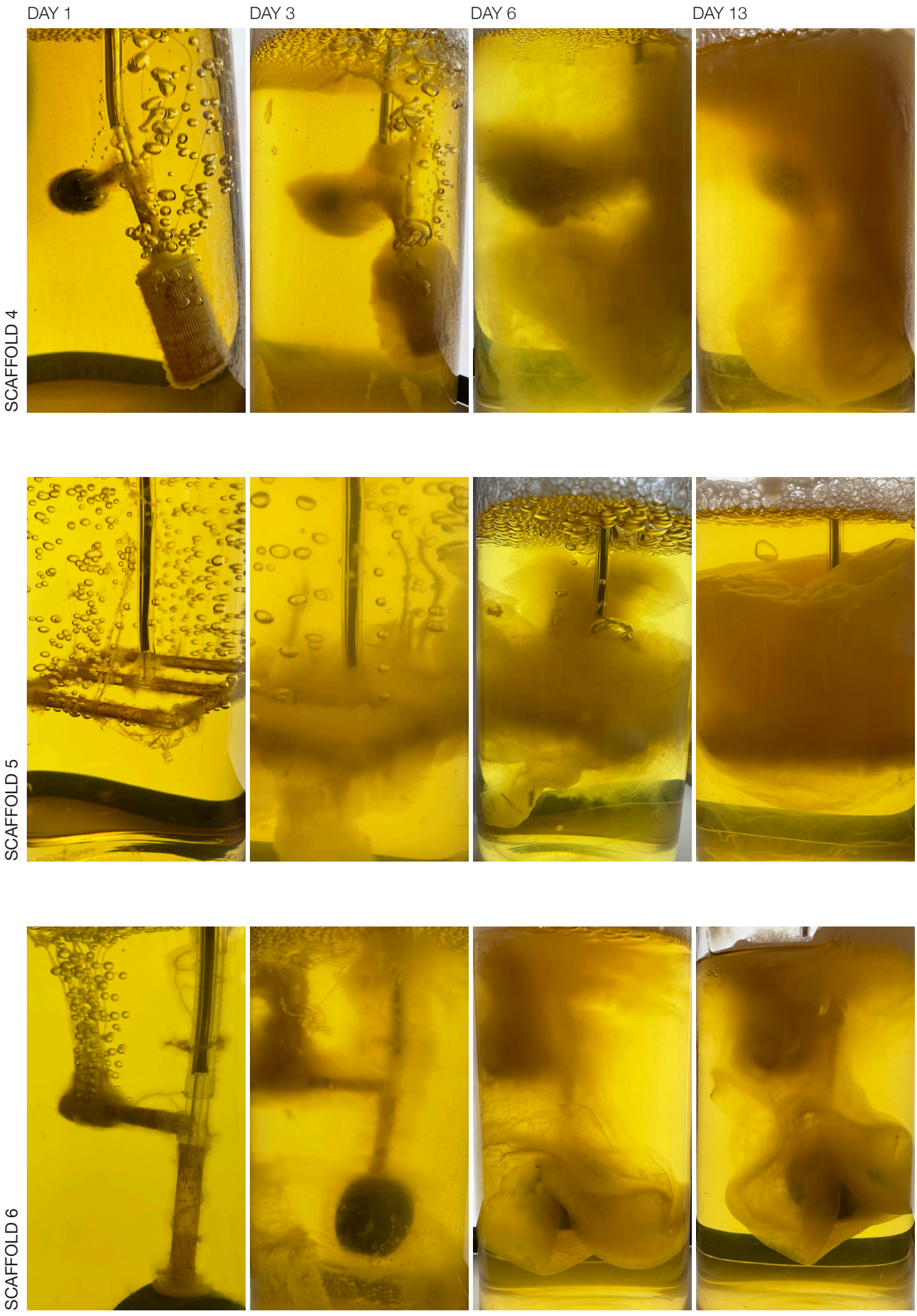


Figure 6.75: *BC growth development on scaffold (top to bottom) 4 to 6, on day (left to right) 1, 3, 6, and 13*

scaffold (6) had matured slightly into a darker colour and the blocked airflow of the air stone resulted in one irregular air stream opening on the lower disc-shaped stone on day 17. The released air collected inside the veil and was released in bursts every 5-10 minutes. The BC grown around scaffold (4) had compacted itself onto one side of the scaffold and reached the surface on day 13, at which point a surface pellicel began to grow.

The BC shapes of scaffold (4) and (6) were successfully frozen, however, the final shapes do not resemble the one observed during the active growth stage (Figure 6.76, left & right).

A generalised observation made across both sets of scaffolds was that the BC growth matured faster when located above the lowest point of aeration outlet. BC growing underneath this area tended to stay more translucent and malleable, especially during the harvesting process.

Developments for Next Iteration:

This extensive experiment tested the possibility of creating a customised scaffold to predict the final BC shape. The following principles and methods were identified as improvements of the experimental setup:

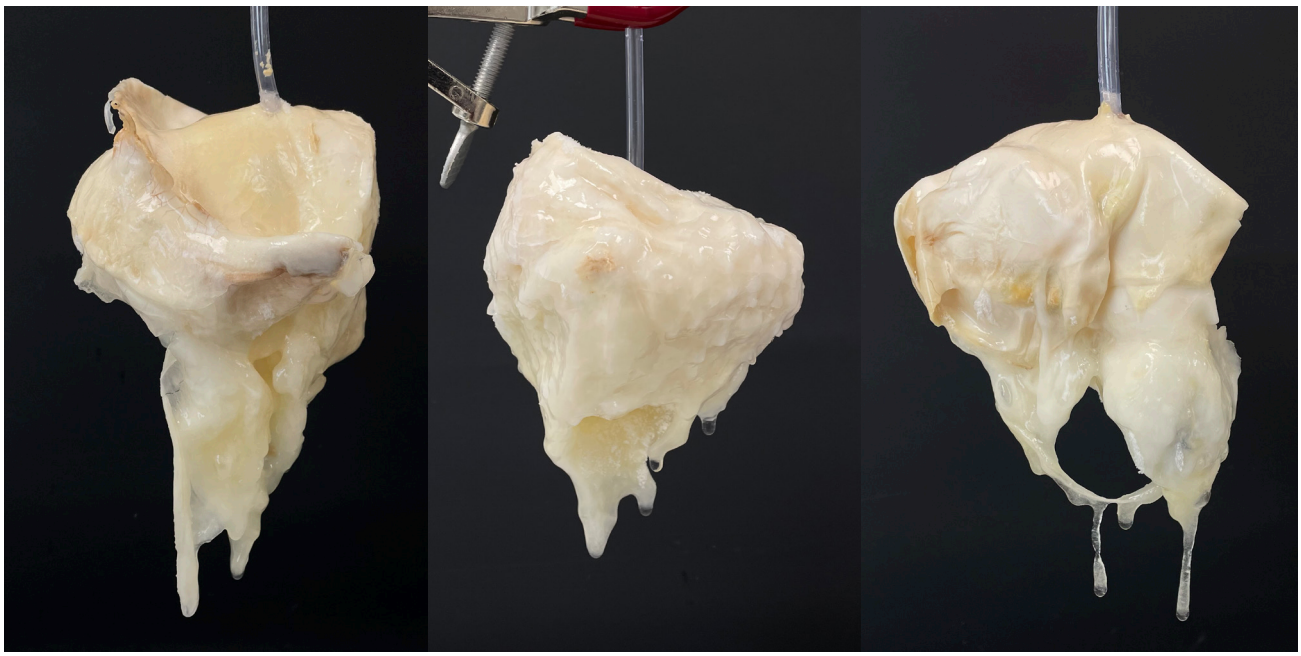


Figure 6.76: *Frozen BC from scaffold 4,5, and 6 (left to right)*

- In order to better anticipate the BC shape, it is crucial for the aeration scaffolds to stay in the intended position and orientation during the aeration. Inserting strong wire into the silicone tubing allows to counteract its natural twisting motion and position the aeration scaffold with precision.
- Floating of the scaffold due to large air reservoirs needs to be anticipated. This can result in the lid lifting off and exposing the medium and growing BC to contaminants. To prevent this, the lid needs to be secured to the growth vessel.
- Commercially available air stones require varying levels of air pressure in order to produce the intended air stream. When combining different types, the air pressure needs to be adjusted to accommodate the lower porosity stone. Otherwise, the airstream will predominantly occur through the higher-porosity component.
- Increasing the airflow on the pump soon after pores were blocked due to growing biofilm can unblock the aeration device either fully or partially. However, the effect of this is stronger on 3D printed scaffolds than commercial air stones.

The following questions were formulated based on the observations and results to be tested in the last aeration experiment:

1. Will a rounded and organic shape of the aeration scaffold influence the attachment of the BC?

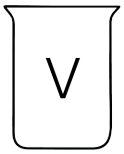
   → *Experiment Q (Chapter 6.2)*

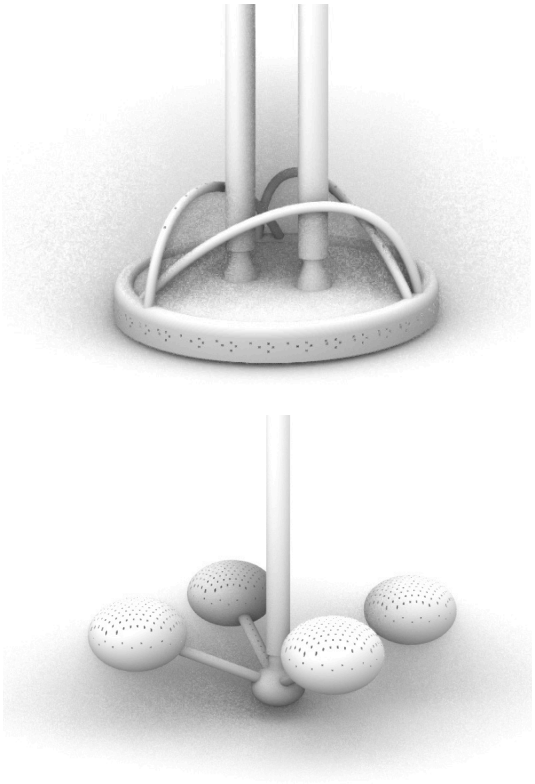
2. Can a difference in BC development be observed based on whether the aeration scaffold is made of one or multiple materials?

   → *Experiment Q (Chapter 6.2)*

Q | Customised 3D Printed Scaffolds

Bioreactor Summary:

Nutrient Media	Inoculation	Vessel	Duration (days)
			21



6.77

Figure 6.77: *3D model of customised aeration scaffolds printed in ABS filament*

Experiment Method:

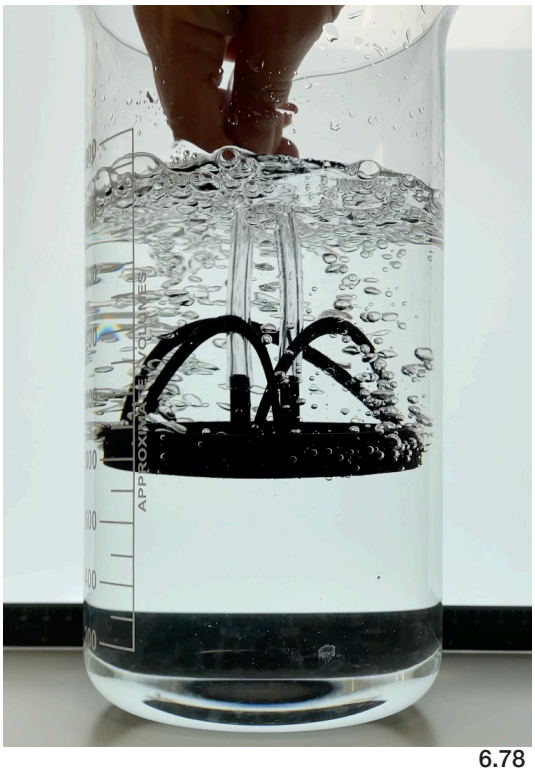
The aim of this last experiment was to design and fabricate an aeration scaffold that would encourage a predicted BC growth based on all observations made in the previous aeration experiments. One bottom-based and one half-suspended scaffold were designed in Rhinoceros and 3D printed in ABS filament on the Ultimaker 3+ (Figure 6.77). The chosen colour of the scaffolds was black with the intention to increase the visibility of them once the BC biofilm begins to mature.

The two following scaffolds were designed based on previously tested shapes, made with improvement based on observations. They were designed to incorporate organic-shaped elements and consist of only one material:

(1) A hollow disc-shaped scaffold with aeration pores on the side and the top surface and three overlapping arches along the rim. The arches are also perforated along the highest point of each with pores of smaller diameter than on the rest of the scaffold. Two air tubes attach to the centre of the scaffold (Figure 6.77, top). The shape is inspired by the aeration discs utilised in Exp. G with the addition of the arches to aid the BC attachment.

(2) A cross-shaped scaffold with a spheroid at each end of the cross. Only the upward-facing surface of each spheroid is perforated and the air inlet from the silicone tube is positioned lower than the spheroids (Figure 6.77, bottom).

The porosity of each scaffold was tested in water prior to setting up the bioreactor (Figure 6.78). The setup of the experiment was identical to Exp.P, using tall growth vessel with 2000ml HS medium and 200ml liquid inoculum. All components of the setup were either autoclaved or UV sterilised before being assembled inside the laminar flow hood. The metal wire inside the silicone stube was used to position scaffold (1) at the bottom of the vessel and scaffold (2) at



6.78

Figure 6.78: *Aeration test of scaffold (1) prior to sterilisation*

6.79

Figure 6.79: *One-sided airflow and thin biofilm forming on scaffold (2) after 10 minutes of aeration*

a horizontal position for even airflow through all spheroids. Before starting the air pumps at the lowest air pressure setting, the lids were secured to the vessel with tape to counteract floating of the growth.

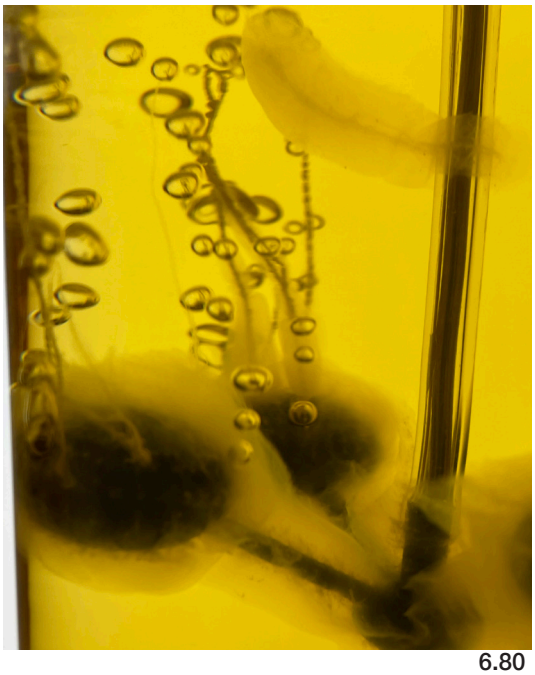
Observations Summary:

Immediately after beginning the aeration, the first observation was made about the airflow of the scaffolds. While it was possible to fully straighten the wire in the preparation stage of the experiment, the small space within the laminar flow meant that it wasn't possible to insert the scaffold into the growth vessel without slightly bending the wire. However, once the lid was attached to the vessel and a sterile environment was created, it was difficult to correct the position of the scaffolds by manually bending the wire. This resulted in scaffold (2) not being fully positioned on a horizontal plane and airflow only appeared through two of the spheroids (Figure 6.79). Re-positioning scaffold (1) was easier due to having two air tubes and wires to adjust it with from outside the vessel and because it was positioned flat against the bottom of the vessel.

The first stage of the biofilm formation occurred as expected on scaffold (2) and formed an even white layer fully encasing the 3D print with white feathery strings. Additionally, one of

the strings detached from the scaffold on day 2 and wrapped around the silicone tube where it began to grow (Figure 6.80). The same occurred on scaffold (1), however, the detached growth consisted of multiple strings that had turned into a larger knot (Figure 6.81). In general, the aeration of scaffold (1) was stronger due to being connected to two air pumps and the biofilm on the scaffold showed more stringy growth than expected. The translucent biofilm began to grow around the strings, forming one large growth. On day 3, a veil-like biofilm began to form at the bottom of the vessel in setup (2) and by day 6, it began to lift off the bottom and slowly attach to the main growth on the scaffold (Figure 6.82, top). The appearance of the biofilm resembled an upside-down jellyfish. At this stage, the biofilm around the scaffold had matured into an opaque colour and only the outlines of the spheroids were visible due to their black colour. By day 10, the veil-like biofilm had fully attached to the growth on the scaffold but remained visible as component due to the different BC maturity. Throughout this period, the airflow from the scaffold was minimal with only 3-4 air streams being active at a time. However, the position of the air streams changed depending on where the biofilm was blocking the pores.

The BC in setup (1) began to develop at



6.80

6.81

Figure 6.80: *Detached stringy growth wrapped around the silicone tube on scaffold (2) and beginning to grow in size*

Figure 6.81: *BC growth on scaffold (1) after 72 hours of aeration*

two separate components starting on day 4, one at the bottom forming around the aeration scaffold and one expanding in size around the loose strings that attached to the top of the silicone tubes just beneath the surface (Figure 6.82). While the BC forming on the scaffold grew in height, the other component predominantly grew in width until it covered the whole surface on day 7. By day 10, the top and bottom growth joined together into one large BC shape that covered nearly the entire volume of the medium. The airflow from the scaffold had reduced to only one outlet on the side by day 7 and remained the only active one until the harvest on day 21.

During the third stage of the growth (Ex-

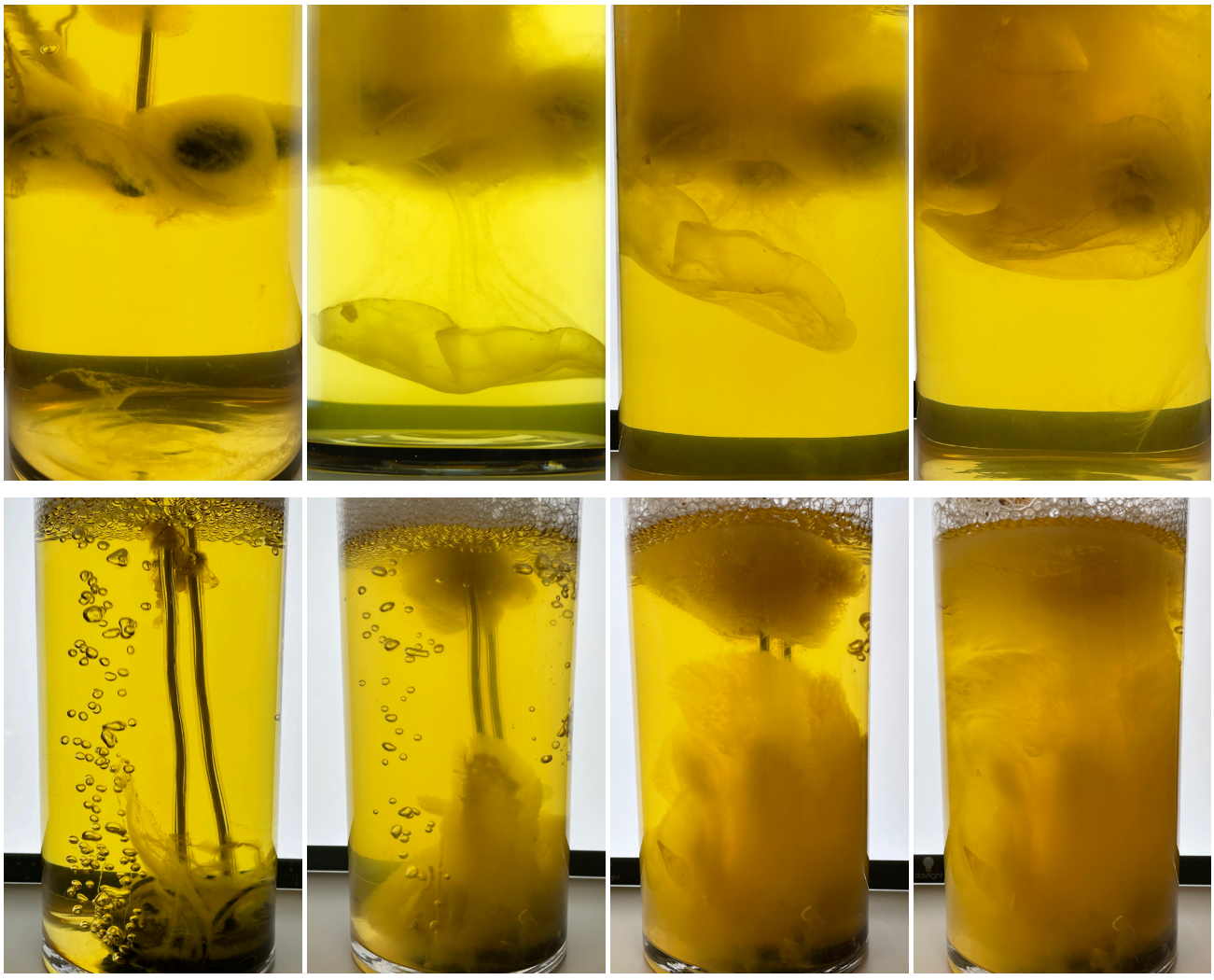


Figure 6.82: *(Top) Veil-like biofilm forming and moving in setup (2) on day 4,6,7, and 10 (left to right), (bottom) Growth of biofilm in setup (1) on day 2,4, 7, and 10, starting out as two separate elements that join together*

periment I, Chapter 5.2.1.) the BC in both setups continued to mature, gaining a darker and opaquer colour, and compacting the overall shape. Static and active air reservoirs inside the BC of scaffold (2) created high buoyancy which pushed the scaffold and growth above the surface of the medium. On day 12, the scaffold was pushed back below the liquid-air interface, similarly to scaffold (5) in Exp. P. On day 16, an active air reservoir opened up on the side of the vessel and exposed the full extent of the hollow space inside the BC (Figure 6.83).

The growth in setup (1) continued to compact upwards and eventually began to lift the scaffold off the bottom of the vessel. On the day of the harvest, the scaffold was lifted with a 25-40mm gap (Figure 6.84).

It was not possible to preserve the BC shape of scaffold (1) because the silicone tubes detached from the scaffold while trying to lift it out of the medium due to the weight of the BC. However, the BC compacted into a more solid shape with only one large air reservoir right before it detached (Figure 6.85). Similarly, the BC of scaffold (2) was not frozen due to contamination which occurred in the last 2 days of the experiment.

Compared to the sketches of anticipated BC shape, scaffold (1) grew larger than expect-

6.83

Figure 6.83: *Hollow space inside BC visible through opening of an air reservoir in setup (2) on day 16*

6.84

6.85

Figure 6.84: *Scaffold (1) lifted off the bottom through compacting BC growth on day 21*
Figure 6.85: *Large air reservoir exposed inside BC of scaffold (1) immediately before harvesting*

ed (Figure 6.86, left). The second biofilm growing at the top of the medium was unexpected, triggered by a detached biofilm string, and influenced the overall shape significantly. When only considering the BC grown from the bottom on the scaffold, the sketch and final shape are similar. However, no clearly visible bubble pathways formed. In the case of scaffold (2), the sketched prediction showed much resemblance, with the main difference being the jellyfish-like growth that occurred midway through the growth stage and remained as veil-like addition on the lower side (Figure 6.86, right). The occurence of large air reservoirs was not predicted in the sketch and neither was the lifting off from scaffold (1).

Developments for Next Iteration:

This experiment concluded the experimentation with aeration scaffolds and no further iterations of scaffolds or experimental setups were developed. Shapes preserved through shock-freezing during the harvesting stage were used to develop a shape preservation process which allowed the detailed documentation of their exterior shape (see Chapter 7). The following final thoughts offer potential for improvements of the experimentation in future developments (see Future Works, Chapter 6.4):

- The wire inside the silicone air tube

can offer sufficient rigidity to slightly readjust the position of the scaffold from the outside once the growth vessel has been setup as a sterile environment, especially when two air tubes are connected to the aerations scaffold.

- Depending on the weight and size of the BC, the scaffold can detach from the air tubes during the harvest process. Without the air tubes, it is not possible to shock-freeze the BC for shape preservation. A redesign of the tube connector on the scaffold should be considered.
- If an early-stage stringy growth detaches from the main biofilm it can initiate the growth of a second separate biofilm anywhere in the growth vessel. If a specific BC shape is desired based on the scaffold's form, these detached strings may need to be removed from the vessel as soon as they appear. Alternatively, the scaffold can be designed in a way that intentionally catches detached strings to encourage the separate growth.

6.86

Figure 6.86: *Comparison of anticipated BC form in sketch and a photo of the actually grown form on day 2 with scaffold no.1 (top) and no.2 (bottom)*

6.3. Discussion

The extended experimentation and exploration of BC shape emergence grown with the guidance of aeration scaffolds allowed a more in-depth understanding of growth behaviours by the organism in response to environmental changes actuated through the scaffold. The implementation of the research approach developed for this thesis (Chapter 3.3) led to the development of successional experiments through which multiple direct and indirect design parameters could be identified. These include characteristics of the scaffold, including the dimension, materiality, size and distribution of pores, as well as the setup and placement of the scaffold within the growth vessel and the targeted manipulation of air pressure (see Chapter 8.1.2).

This discussion extends on the findings of Chapter 5.3 and elaborates on the underlying biological principles that enable the morphological development of the 3-dimensional BC forms (see Chapter 5.4.3). Similarly to the first aeration experiments, the accurate documentation of the shape development throughout the growth phase, specifically the volumetric expansion of the biofilm, remained challenging. Removing the scaffold and attached BC from the nutrient medium once a day to quantifying the growth would have resulted in the delicate and immature shape distorting and potentially splitting. In favour of observing the BC shape emergence based solely on the implemented design parameters, the documentation was limited to photography and videography of the submerged BC only.

6.3.1. Identified Growth Patterns

The observations made during the explorations in Exp. H to L support many of the hypotheses made in Chapter 5.4.3. about the parallels between biofilm formation in aquatic environments and the 3-dimensional BC growth. In addition, the observations of repeated experiments allowed a further distinction of unique growth behaviours into patterns that could be divided into three developmental stages of the BC shape (Figure 6.8). These stages occur in every aeration setup irrespective of varied design parameters, such as vessel size or scaffold type, but the duration of each stage can vary greatly. The level of foam formation was identified as visual indicator for each of the stages because of the direct correlation to the volume and maturity of the biofilm influencing the blockage of aeration pores (see Chapter 5.4.3).

The first stage of biofilm formation includes the development of a conditioning layer with the feathery appearance of yeasts on the porous surface of the air stone as well as the interlinking of yeast cells into strings in areas of strong airflow (see Chapter 5.4.3). Depending on the type of nutrient medium used, the colour of these yeast-dominated biofilms can be changed, similarly to influencing the colour of BC pellicles grown in a static culture by using fruit dyes (Bloch, 2019). A darker colour of tea translates into a darker biofilm colour (Coskun & Kayisoglu, 2020) (Figure 6.87) and in the comparison of HS medium and tea medium, the BC grown in HS medium was near colourless. As no significant difference in BC maturity was observed between the two me-

Figure 6.87: *(Left) Biofilms formed in the kombuchas and kombuchas produced from black, green, mint, linden and sage teas at the end of fermentation; (Right) Biofilms formed in the kombuchas at the end of the fermentation (Coskun & Kayisoglu, 2020). Showing comparison of BC colour based on the tea it was grown in.*

dium types, the choice of medium can be considered an aesthetic decision. The main concern for using HS medium for the aeration experiments was the increased contamination risk. The development of contamination spreading through the BC in Exp. H and J, however, revealed the intricate network of air reservoirs by developing a distinct white-pink colour and highlighting the otherwise hidden long yeast strings before showing discoloration of the surface pellicle (Figure 6.88). This could indicate that the contaminating organism entered the growth vessel through the air pumped into the scaffold which could be addressed by integrating a filter in between the air pump and silicone tube. However, the setting of the experiments inside a biodesign lab in which multiple types of organisms are handled by researchers with varying experience in sterile work needs to be considered as potential risk enhancing factor for contamination.

Following the hypothesis that the yeasts are forming a conditioning layer on the rough surfaces of the aeration scaffold (see Chapter 5.4.3), the stringy biofilm could be explained through a detachment from the surface before the monolayer can mature and be considered irreversibly attached (Alonso *et al*., 2023). The position of the stringy biofilms in direct proximity to active air outlets of the scaffold where the strongest turbulence occurs supports this hypothesis. Likewise,

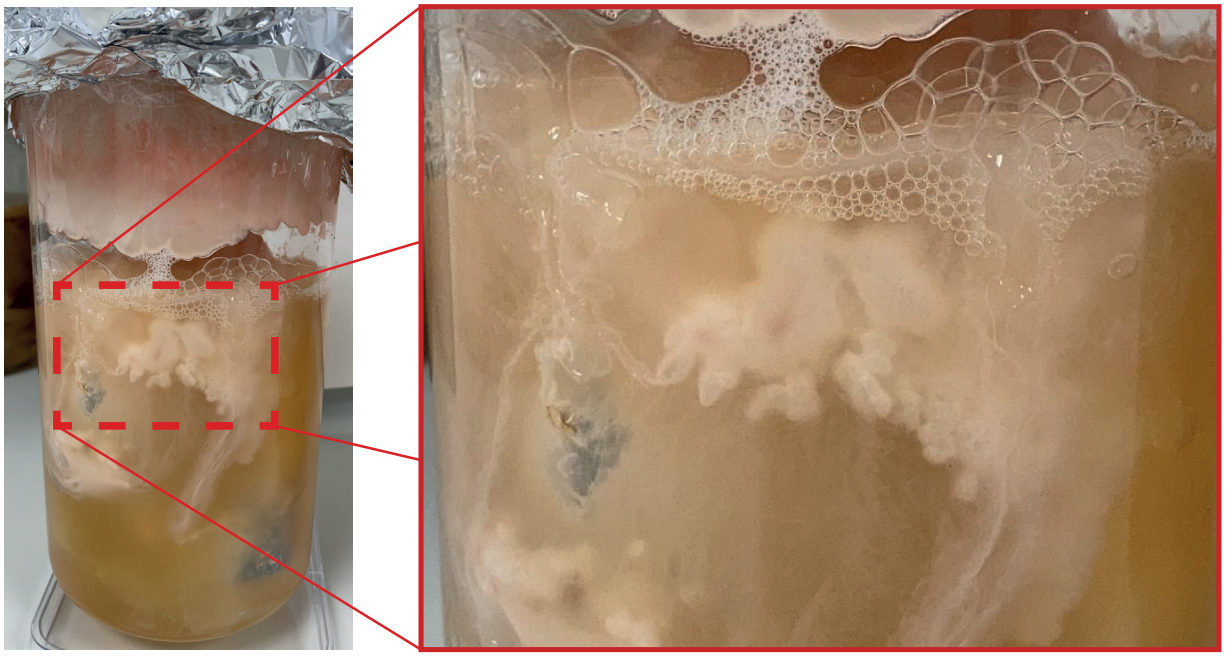


Figure 6.88: *Close up of the white-pink colouration of air reservoirs and yeast strings in experiment J through contamination, grown in 1500ml of medium.*

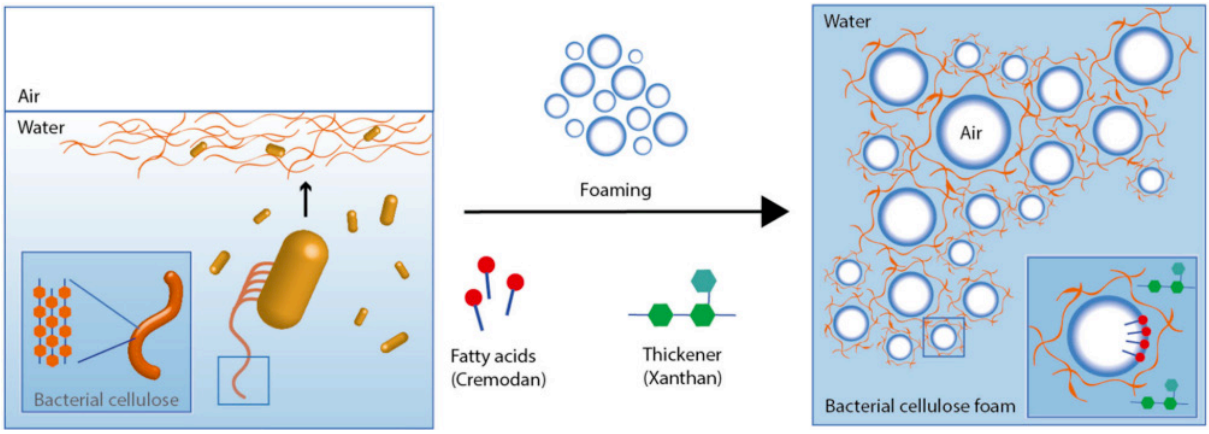


Figure 6.89: *Schematic of the bacterial cellulose foam formation process. G. xylinus extrudes bacterial cellulose as a function of oxygen and migrates toward the air–water interface. To construct a bacterial cellulose foam, a suspension of G. xylinus in growth media is foamed. The air bubbles are stabilized through interfacial stabilization by Cremodan. To avoid water drainage and to enhance stability of the foam Xanthan is added as a thickener. After bacterial growth, the foam was increasingly stabilized by BC formation leading to stable cellulose foam structures after 4 days (Reproduced from Rühs et al., 2018)*

the second vertical feature within the biofilm, identified as bubble pathways, also forms in those areas of strongest airflow. In contrast to the yeast strings, which become invisible once the BC matures, the visibility of bubble pathways increases until the point of harvest. This is due to a two-stage maturation process of the BC in those areas. The initial pathway walls mature while air bubbles move through it, until the BC has expanded to the point where it closes off the pathway

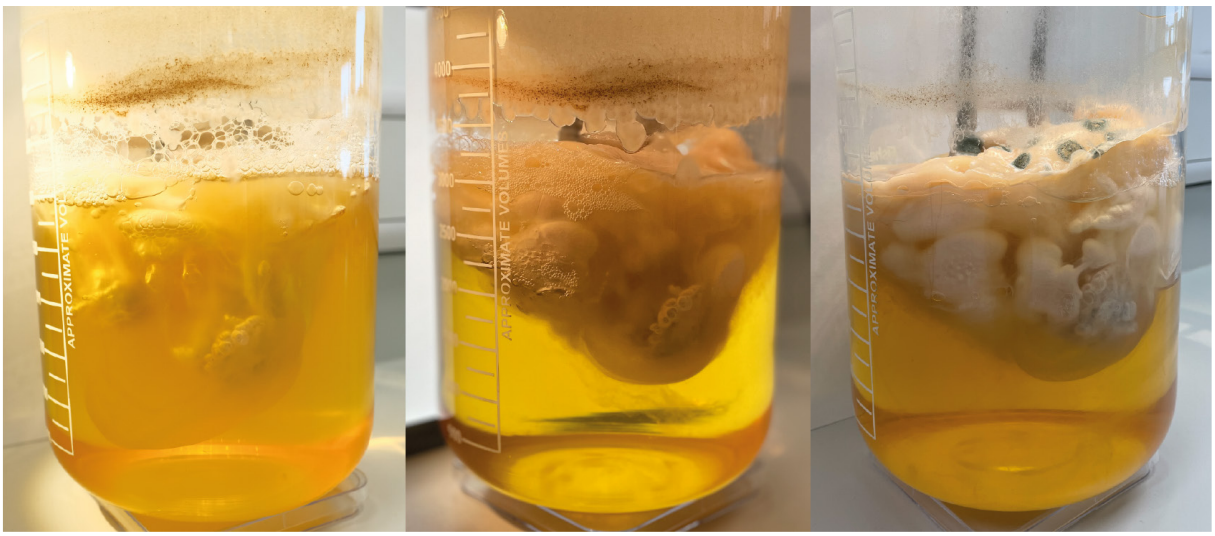


Figure 6.90: *Development of network for air movement from individual air reservoirs on day 7, over day 11 to day 18 (left to right). Vessel with*

and traps air bubbles inside it. The now stationary air bubbles continue to provide oxygen to support the BC growth, which is then similar to the formation of individual air reservoirs throughout the gel-like biofilm. This is comparable to the principle of growing BC inside a foam medium, as the foam is created with small individual air pockets that provide a locally high concentration of oxygen (Figure 6.89) (Rühs *et al*., 2018).

The air reservoirs are distinguished between an active and passive state, depending on whether air movement can be observed inside it. During the second identified growth stage of 'shape development' (Figure 6.8, Exp. I), a fast increase of passive air reservoirs spreading throughout the full volume of the transparent and translucent biofilm can be observed. A possible explanation is the increasing density of the gel-like BC which increasingly hinders the air moving through it (Aldrich & Deventer, 1994). In the identified 'maturing' stage the formation of new air reservoirs decreases while multiple existing passive air reservoirs change into an active state. This change is accompanied by multiple air reservoirs connecting into a new network through which the air can escape to the surface (Figure 6.90). A potential explanation for this is that the air moves through the path of least resistance which, if comparing the surrounding gel-like biofilm to the

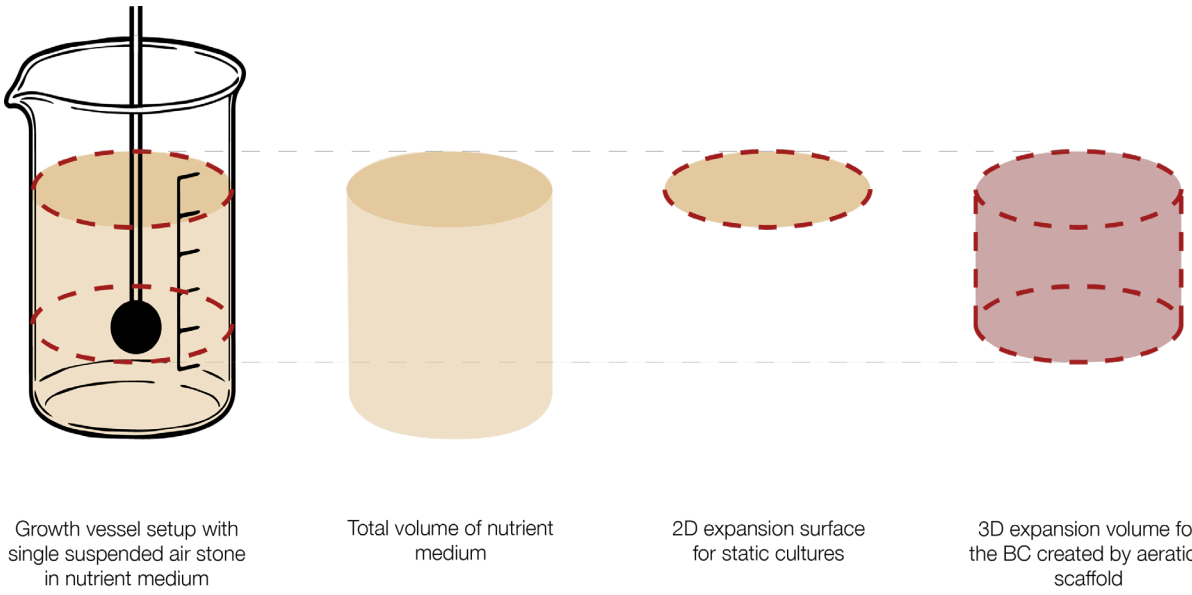


Figure 6.91: *Diagram explaining the transition from a 2-dimensional expansion space to a 3-dimensional once through the addition of an aeration scaffold*

thin and immature BC walls separating two air reservoirs, is the latter. As a result of air moving through these networks in bursts a contracting movement of the BC, comparable to the beating of a heart, is created. Due to the BC having expanded to the full volume of the growth vessel at this stage, this movement is often accompanied by an audible popping sound as the air escapes through small gaps between the glass walls and BC.

The overall volumetric expansion of the BC is directly linked to and influenced by the shape of the growth vessel. The observations of upscaling Exp. K and L showed that even an increased availability of medium and space will result in a BC shape which expands to all side of the growth vessel and even adopts volumetric features of the vessel (Figure 6.32). However, while the horizontal growth adapts to the available space, the vertical expansion is dictated by the depth of the aeration scaffold within the medium. The predominantly upwards directed aeration of the medium results in BC growth between the lowest point of aeration and the air-liquid interface. The same principle applies to airlift bioreactors and is the reason for the placement of the sparger at the bottom of the tank to achieve the most coherent oxygenation (Chistit & Young, 1987).

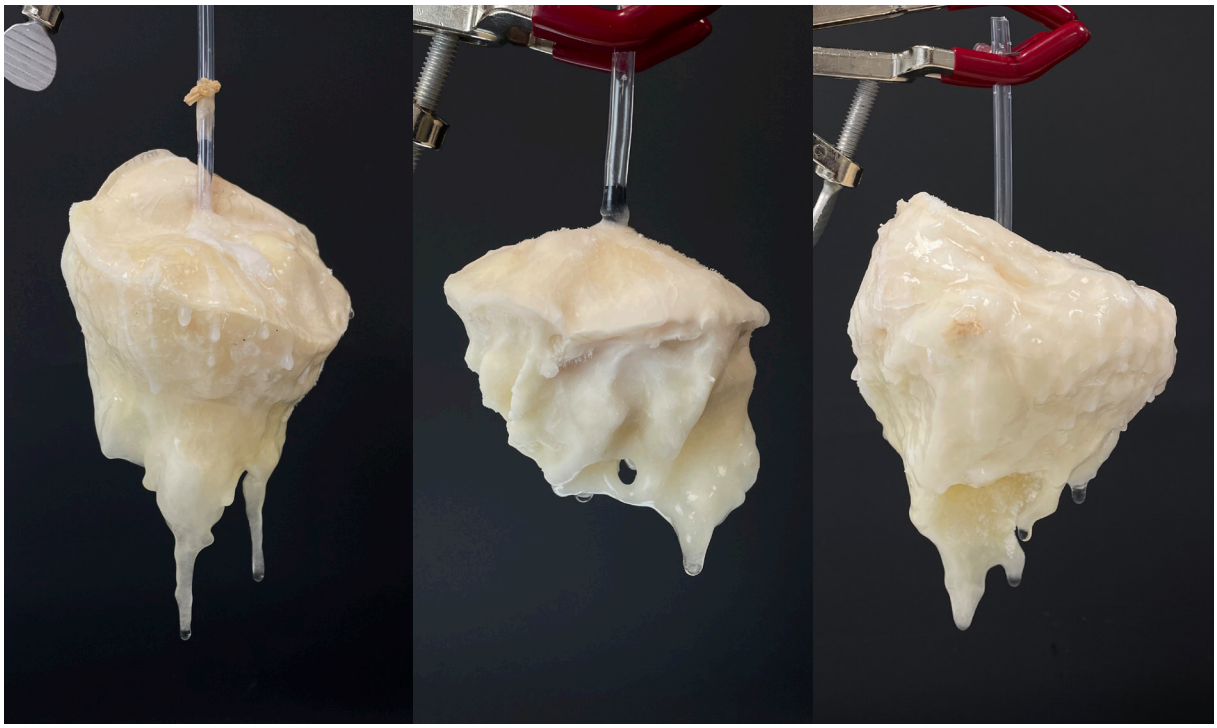


Figure 6.92: *Three harvested BC forms with visibly flatter tops and distinguishable surface pellicles*

A simple explanation for this behaviour is the difference in density of air and water which results in buoyancy and the air bubbles rising upwards. In the context of the aeration experiments, this indicates that it is possible to translate the 2-dimensional expansion area of a static culture at only the air-liquid interface (see Chapter 2.3.1) into a controlled 3-dimensional volume through the addition of an aeration scaffold (Figure 94).

While the 3-dimensional growth of BC is made possible, not every area within the possible expansion volume matures equally. The most mature and structural element of each grown shape was the surface pellicle which developed towards the end of growth period. This is made visible particularly during harvest when gravity influences how the shape develops once pulled out of the liquid, especially if a surface pellicle had developed (Figure 6.92). This behaviour can be explained through the difference in growth conditions between the submerged areas and the air-liquid interface, which provides the bacteria and yeasts with an abundance of oxygen at any given time compared to varying levels of dissolved oxygen within the medium (see Chapter 2.3). The growth period of the BC in the third 'maturing' stage can therefore be considered one of the most influential design parameters for the BC objects in a harvested state.

6.3.2. Effects of Materiality and Positioning

The material of the scaffold, in particular the surface texture, determines how well the conditioning layer of biofilm can adhere to it and stay attached throughout the growth period and during harvest. In bacterial biofilm formation, a rougher surface encourages the adhesion of organisms through a larger surface area (Zheng *et al*., 2021). A direct comparison of the effects of a smooth and a rough surface texture was made in Exp. M, utilising a smooth bioplastic straw and a conventional rougher paper straw. In addition to a uniformly rough surface, the paper straw had a shallow helical ridge running across. Throughout the growth period of the experiment, BC growth occurred across the whole length of the paper straws while only a concentrated BC growth at the bottom of the bioplastic straw developed (Figure 93, left). A possible explanation for this is the protruding silicone glue at the bottom of the bioplastic straw which created an area of with different roughness (Figure 6.93, right). Similarly, no BC attachment to the walls of the growth vessel were observed in any of the experiments which indicates that glass is a suitable material for the vessel to not interfere with the shape development of the BC. The attachment of the BC

Figure 6.93: *(left) Comparison of BC on paper straws extending the whole length of the straw and BC on bioplastic scaffold concentrated around the bottom end of the straw; (right) silicone glue sealing off the bottom end of the bioplastic straw*

to the scaffold, or the lack thereof, was also noticeable during the harvest where only the BC on the paper straw scaffolds remained attached (Figure 6.49). In the context of developing reusable aeration scaffolds, the surface texture could play a significant role to ensure the BC detachment.

Two materials not suitable for reusable scaffolds are the tested 3D printer filaments PLA and ABS (Exp. O) which both resulted in equally strong biofilm attachment. More influential than the materiality of these scaffolds, however, is the layer height at which the scaffolds are printed as this print setting significantly influences the surface texture (Ayrilmis, 2018). In addition to the overall surface texture, the layer height influences how smooth the edges of the aeration pores are formed (Figure 6.94) which were observed to be the first attachment point for the biofilm. Similarly, the process of manually piercing holes into the scaffold also affects the quality of the pores. This was particularly noticeable on the agave straws where the piercing motion pushed the material edge inwards, creating a one-sided rough surface, which could be an explanation for the observed phenomenon of primary growth occurring predominantly on the interior of the agave straws (Figure 6.47).

The process of aeration pores being blocked through the biofilm growth has previously been discussed in Chapter 5.4.3. However, not only the expansion of the biofilm influences the air movement through the pores of the scaffold. The positioning and orientation of the scaffold is equally influential, as has been observed particularly in Exp. N where a horizontal placement of the elongated scaffolds was tested. The same principles of difference in density between air and water, which creates the 3-dimensional expansion volume for the BC, and path of least resistance

Figure 6.94: *Schematic showing the difference in print quality of the aeration pores between a thicker 0.12mm (left) and thinner 0.06mm (right) layer height*

(see Chapter 6.3.1) influence which pores of the scaffold the air will escape from. The vertically highest and upwards facing pores have greater airflow through them which can result in unused pores in lower or downwards facing areas of the scaffold. The combination of irregular or rough surface texture and little to no air turbulence while being in close proximity to the active airflow resulted in a faster onset of biofilm growth in every aeration experiment. The dormant behaviour of less favourable aeration pores is not a permanent state, however, and can be reversed depending on the strength of airflow and expansion of mature biofilm across the favoured pores. Conventional air stones are designed to sparge air in two phases, with the second phase only activating once the favoured pores in one section of the air stone have become blocked. The internal design of the air stone then redirects the air pressure to utilise the less favourable pores (Willinger & Wang, 1991). The same behaviour was observed with the tested customised scaffolds, in particular when the biofilm on the less favoured pores was predominantly fuzzy yeast growth. To gain more control over the activation of this second stage of the aeration, the air pressure of the pump can be manually increased. By choosing an incremental increase spread over multiple days in Exp. K, O, P & Q, it was possible to prolong the active state of some of the pores and increase the formation of bubble pathways as new pores are activated. This indicates that the air pressure is a direct design parameter to actively influence and guide the growth behaviour of the BC. To gain more precise control over the incremental increase, air pumps with a wider pressure range could be used in addition to a display indicating the current pressure setting. The air pumps used for this research, however, did not have a display and each increase of pressure was estimated.

Towards the last aeration experiments of this thesis, it was possible to anticipate the growth behaviour and BC shape emergence based on the scaffolds' design, materiality, position within the medium, and air pressure. However, an aspect of the growth stage that could not be predicted was the detachment of stringy biofilm which was particularly impactful on the shape development if it occurred during the first days of the experiment (Exp.Q). Once detached, the free-floating biofilm either reattaches to the main body of the growth or continues to grow as an individual shape, similarly to the formation of BC in agitated cultures (see Chapter 2.2.1). Scaffold no.1 of Exp. Q was designed with upwards reaching arches to provide more opportunities for loose biofilm strings to get caught in and reattach, which was partially successful. In addition to the anticipated

BC growth on the scaffold, however, loose biofilm attached to the silicone tubes of the scaffold and grew into an additional shape. While it could be possible to manually remove detached biofilm elements from the growth vessel to maintain more controlled growth environment, they are a direct reminder of the unpredictability that is connected to working with living organisms.

6.4. Future Development of Aeration Scaffolds

During the active experimentation with commercial and customised aeration scaffolds, multiple directions for further exploration were discovered to either expand the understanding of BC growth behaviours or to improve the shape retention properties of the material.

The first area of future investigation is the development of a two-phase experimental setup in which the scaffold is first used to aerate the medium and then repurposed to distribute additives into the BC to aid in the solidification of the shape. Whether this additive is in a liquid form, such as agar or gelatine, or a culture of microorganism which can be triggered to cause the solidifying of the BC solidify based on external cues, is to be explored. Reducing the liquid loss during the first minutes and hours after the harvest could make the development of new preservation methods possible as well as offer more time to document the position of the scaffold within the BC while it is translucent in colour.

In addition to exploring the self-morphing behaviour of BC, the potential of more directly guiding the shape finding exists. The dimensions and shape of the growth vessel has previously been identified as influential parameter for the shape development of the BC by restricting the

Figure 6.95: *Mould used to shape a pumpkin into the shape of a head (Slingfisher, 2024)*

overall expansion of the living material as it grows while simultaneously creating a 3-dimensional air-liquid interface for the bacteria (see Chapter 6.3.1). To achieve a more controlled or predicted shape of the BC, the method of negative moulding could be integrated into the aeration experiments by utilising customised growth vessel. The practice of produce moulding utilises hollow plastic shapes which are tightened around the stem of an unripe fruit or vegetable for it to grow into the constricted shape (Slingfisher, 2024) and offers a starting point for developing customised growth vessels (Figure 6.95). Another possibility is the combination of existing 3D moulding techniques using superhydrophobic coatings (Greca *et al*., 2018) with the submerged aeration scaffold which could allow a level of control over the material maturity of the walls of the form, achieving a stronger shell than interior.

Another potential fabrication techniques for creating complex BC forms is through foam. First developments of foam templating with BC have shown great potential for creating volumetric and lightweight shapes with a porous interior (Rühs *et al.*, 2018). While the foam in the presented experimental setups is disregarded as a by-product of the aeration, it holds the potential of creating a second complex BC shape outside of the liquid medium by implementing foam stabilising

Figure 6.96: *Honeycomb with porous interior (left) and fast dissolving honeycomb in a jar of water after 60 seconds (right)*

practices, such as additives or wire scaffolding above the liquid medium for the foam to expand within. Furthermore, the compatibility of BC foam shapes as aeration scaffolds could be tested in the existing experimental setup with the potential of creating pure 3-dimensional BC shapes.

Another area of interest is the development of a soluble scaffolding material which can be absorbed by the bacteria or yeasts and turned into BC. The concept of absorbable or transformable scaffolding has been explored in clinical research (Gama *et al*., 2016), and has already found application in orthopaedic surgery, where a magnesium-based allow is used to produce screws that first provide stability to secure fractures and is later absorbed by the body and synthesised into bone tissue (Biber *et al*., 2017). Glucose, or another type of sugar, could be used to create this type of scaffold for BC growth. A preliminary experiment testing the possibility of using honeycomb, a sweet made of syrup and bicarbonate of soda, showed a potentially sufficiently porous inner structure to diffuse air (Figure 96, left). However, the purely sugar-based material easily dissolves in water (Figure 96, right) and the recipe requires further development to extend shape retention capabilities of the honeycomb underwater.

Looking further into the future, a prediction of the BC shape based on the scaffold shape and vice versa could be achieved through programming and training a software with observation data of multiple sets of experiments. Instead of creating a custom growth vessel for the BC to expand in, a scaffold could be created with more and less dense aeration pores to guide the growth. More complexity could be added by including multiple design parameters, such as surface texture, air pressure or local nutrient concentration. To achieve this level of predictability, however, a much deeper understanding of the material's behaviour is required.

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Close-up of freeze-dried bacterial cellulose with visible cracking throughout the material

CHAPTER 7

PRESERVATION METHODS

Chapter Summary

One of the main challenges encountered during the experimentation stage was the accurate documentation of the exterior morphological features, interior structure, and dimensions of the complex BC at the end of its growth. Each experiment created a unique BC shape of which the steps in morphological development were documented through photography (see Chapter 6). However, due to the BC being grown fully submerged it was only possible to document the growth from outside the growth vessel without causing possible disturbance. In particular the development of an understanding of the formation of interior pathways and cavities was impeded. Additionally, due to the different stages of BC maturity within the grown shapes, parts of the shape, usually the lower half, were translucent and not distinguishable from the surrounding medium while submerged. However, once removed from the liquid medium which exposed the full extent of the shape, the BC would began to lose volume and change shape rapidly. While it would be possible to collect all the liquid dripping from the growth and retrospectively analysing the volume, this option would still not allow documentation of the actual shape grown. The still mainly translucent and reflective surface of the BC also prevented the infrared-based 3D scanner 'Revopoint POP' from making an accurate reading of the exterior, not allowing documentation of the shape digitally. Hence, a method to preserve the shape as precisely as possible needed to be developed.

Two ideas for preserving the shape of the BC to enable further analysis of the shape were explored, each with different aims of the data that was to be extracted from individual samples. The preservation of the BC was identified as crucial to improve the form stability of the exterior and interior of the shape by eliminating the continuous water loss after harvest (see Chapter x) and allow the required time to document unique features. The first method concentrated on achieving a dried sample, while the second method kept the BC in a hydrated state. It was not possible to apply both preservation methods to one sample, so it was necessary to evaluate which data was needed first before choosing how to preserve it.

The first method used freeze-drying techniques in the final step to create a sample with no moisture left in the BC. The process allowed for a full observation of the exterior shape of each

sample and the digital documentation. The second method focused on exploring the interior structure of the BC. In the hydrated preservation the samples were cast into a gelling agent, such as gelatine or agar, and sliced horizontally by hand using a sharp blade which exposed the large variety of cavities hidden on the inside.

7.1. Existing Preservation Methods

The applications of BC are diverse, and the choice of preservation method depends heavily on the intended use of the material. This is because the characteristics and properties of BC change significantly between a hydrated and a dried state. While a few standardized preservation methods exist, it is common to develop a tailored approach based on specific experimental or application requirements. BC can be dried using various methods, which can be broadly categorized into evaporation drying, heat drying, and freeze-drying (Clasen et al., 2006; Indriyati et al., 2019). To halt microbial activity, the material is typically washed in ethanol or industrial methylated spirits (IMS), while further purification to remove organic residues, such as bacterial cells, involves chemical treatments followed by thorough rinsing before drying (Indriyati et al., 2019; Bodea et al., 2021; Greenhope et al., 2024). Evaporation drying is commonly performed at room temperature, in an oven at low temperatures or inside a climate chamber, allowing water to gradually evaporate from the material (Loh et al., 2025). Heat drying, which includes techniques such as using a heat press or microwave, accelerates moisture removal through direct thermal exposure and is usually combined with vacuum filtration (Srikandace et al., 2025). In contrast, freeze-drying requires the BC to be rapidly frozen, typically to –80°C using liquid nitrogen, before being placed in a freeze-dryer (Clasen et al., 2006). Unlike evaporation and heat drying, which significantly reduce the thickness of BC, freeze-drying preserves the material's volume by replacing water within the cellulose matrix with air, avoiding the collapse of the 3-dimensional fibre matrix (Meftahi et al., 2018). An alternative, less common preservation technique involves supercritical carbon dioxide ($scCO_2$) drying, which creates BC aerogels with minimal volumetric and structural changes from their hydrated state. This process exposes BC to 100 bar pressure and supercritical CO_2, producing an ultralight material while preserving its three-dimensional structure (Liebner et al., 2010).

In applications where BC is intended to remain moist or hydrated preservation typically involves storing the material in purified water, glycerol, or other aqueous solutions to maintain its flexibility and biocompatibility (Cielecka et al., 2019). In medical applications, hydrated BC can also serve as a carrier for liquid medications, allowing for controlled drug release, as seen in burn wound treatments (Portela et al., 2019). However, hydrated preservation is generally not suited for long-term storage, as prolonged hydration can promote microbial contamination over time. Each of these methods affects BC's mechanical and morphological properties differently, influencing its suitability for specific applications. However, these techniques have been developed primarily for sheet-based BC, predominantly on small scales, and have not yet been extensively tested on three-dimensional BC forms.

7.2. Freeze-Drying

The freeze-drying process was chosen after an initial test of air-drying an aeration sample in a hanging position. Similarly to air-drying a flat sheet of BC, the aeration sample significantly shrunk in thickness and lost nearly all of its original volume. In the case of the aeration samples, the BC dried to form a skin around the air stone and tubing of the scaffold which, ultimately, resulted in a 3-dimensional shape (Figure 7.1). However, it was not representative of the hydrated shape the BC had prior and was therefore not suitable to record the final morphological.

To achieve a near fully dried state of the BC in its 3-dimensional form the method of freeze-drying was chosen. Freeze-drying is an established method for preserving BC and has previously been explored in a design-focused setting (Zolotovsky, 2017). During the preservation process it was possible to measure the length and width of the complex shape, as well as a complete digital model of the exterior of the shape. The final dried state of the BC, after freeze-drying, was fragile and occasionally exposed parts of the interior structure due to breakages. While it was not possible to record the entire interior morphology in detail, parallels between features observed during the growth stage and the dried sample supported the understanding of the formation and morphology of BC underwater.

Freeze-drying, also known as lyophilization, was invented in the early 20th century and describes the process in which water or other solvents are removed from a product under a vacuum, changing from a frozen state directly to vapor and skipping the liquid phase. This process state change is called sublimation, and it is unclear when it was first discovered by humans. However, it's first intentional application was to preserve biological matter, such as blood serum during World War II in 1944 (Murgatroyed, 1997). The freeze-drying involves two main steps, the first being the original freezing of the product and the second being the sublimation drying. The freeze-dried product still contains 1-4% of moisture and needs to be stored in an airtight container with adequate desiccant materials to avoid rehydration. In the case of BC, the process removes most of the water from the sample, which has a water-holding capacity of 98%, and leaves behind the complex matrix structure of the cellulose fibres.

Method

The first step of the process involved harvesting the fresh and fully grown BC sample from the original growth vessel and to freeze them. To remove as much of the medium residue inside the BC as possible, the sample was carefully immersed into a container with purified H_2O and moved up and down for a few seconds. This process was repeated in fresh H_2O until it no longer stained yellow from the medium. Removing the media, which was rich in salts, was important to improve the freeze-drying process and avoid rehydration of the sample in areas of high salt content. After the initial rinse of the sample, it was shock-frozen using liquid nitrogen (LN_2). Initial tests showed that placing the freshly harvested BC directly in a -80 degrees Celsius freezer did not stabilise the shape sufficiently fast to avoid distortion and volume loss through liquid drainage. In comparison to this standard laboratory freezer, the LN_2 works significantly quicker in immobilising all water inside a sample with a boiling point of -196 degrees Celsius. The conventional way of using LN_2 to freeze objects involved filling a vacuum vessel, also called dewar, with the cryogenic fluid and immersing the object fully for a few seconds. This method was not suitable for the BC samples for two reasons. Firstly, due to the size of the samples, a large-sized dewar with

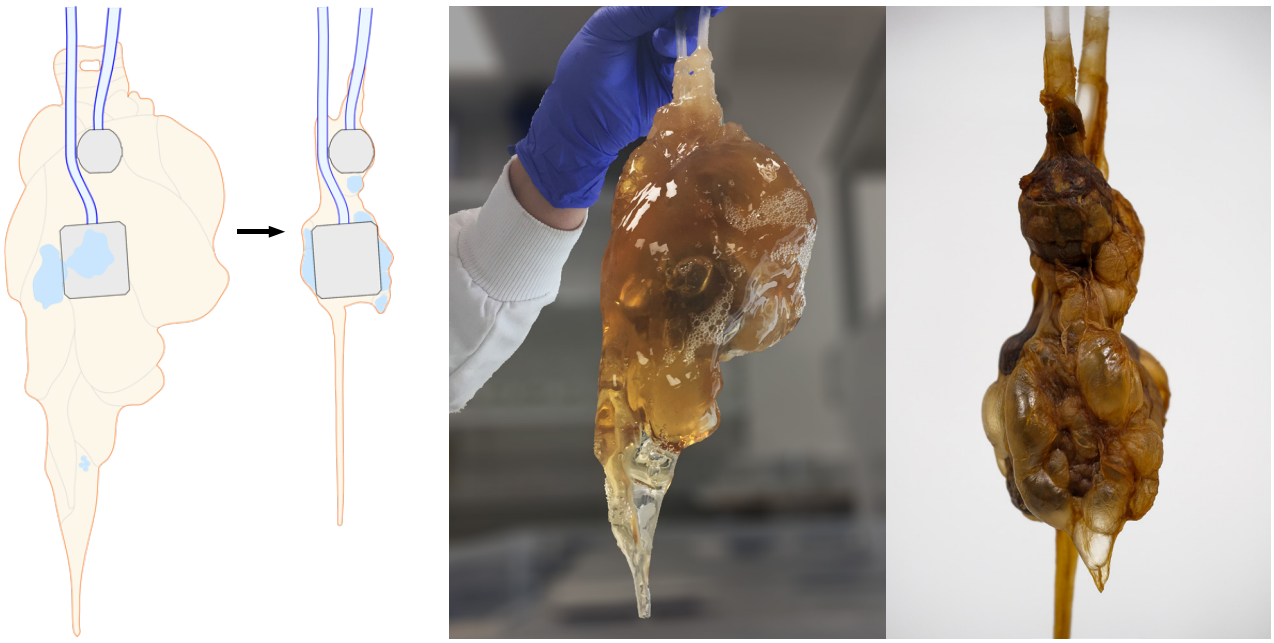
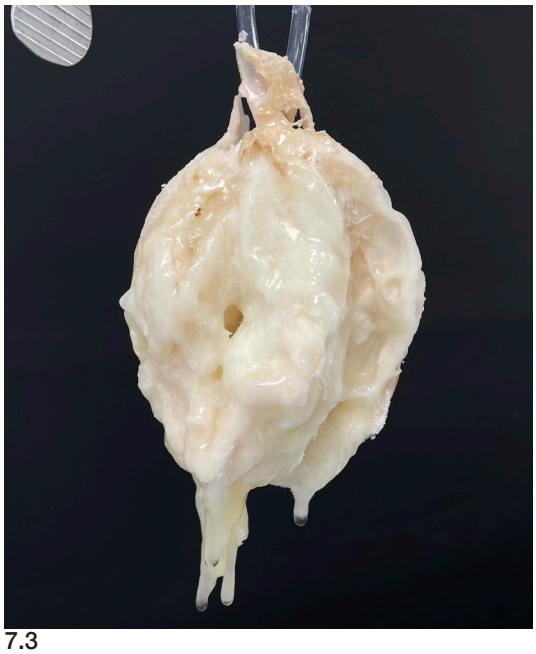


Figure 7.1: *(Left) Diagram showing shrinkage of BC around scaffold with trapped air pockets in 5 days, (right) comparison of shape and volume of sample in hydrated and air-dried state after 7 days*

7.2



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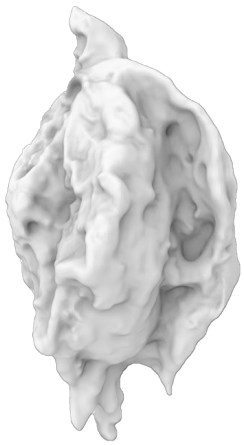


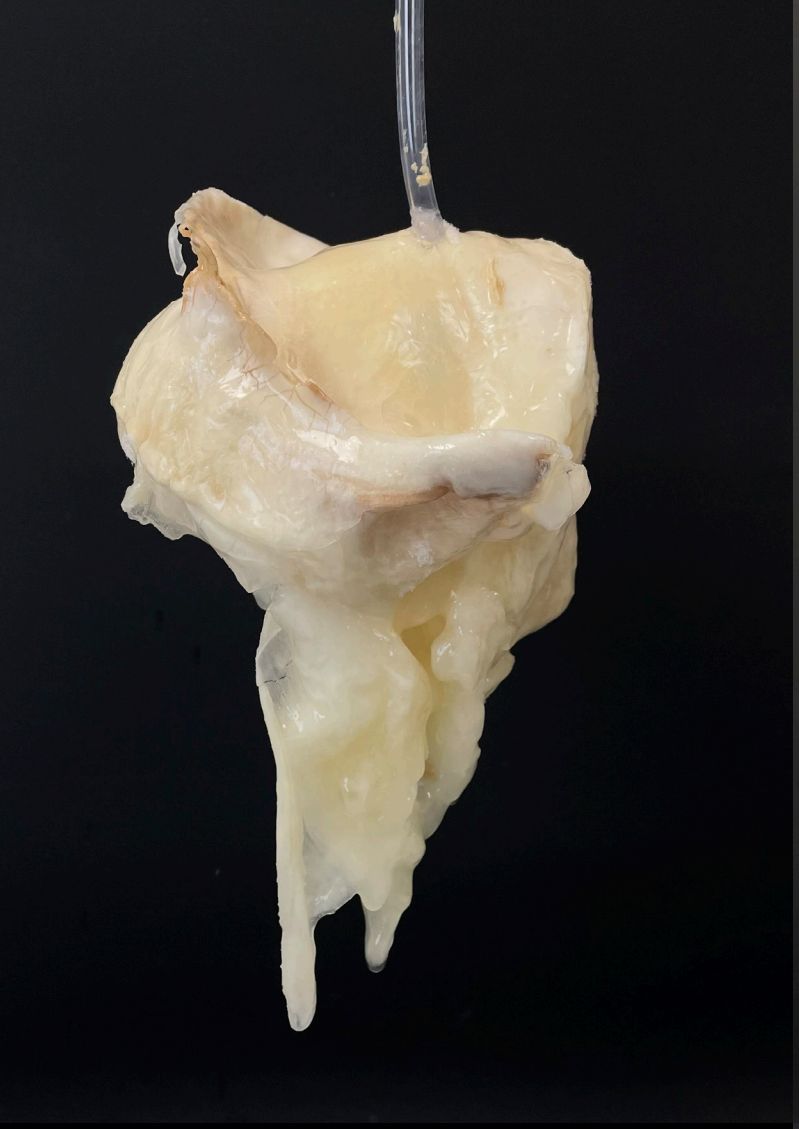
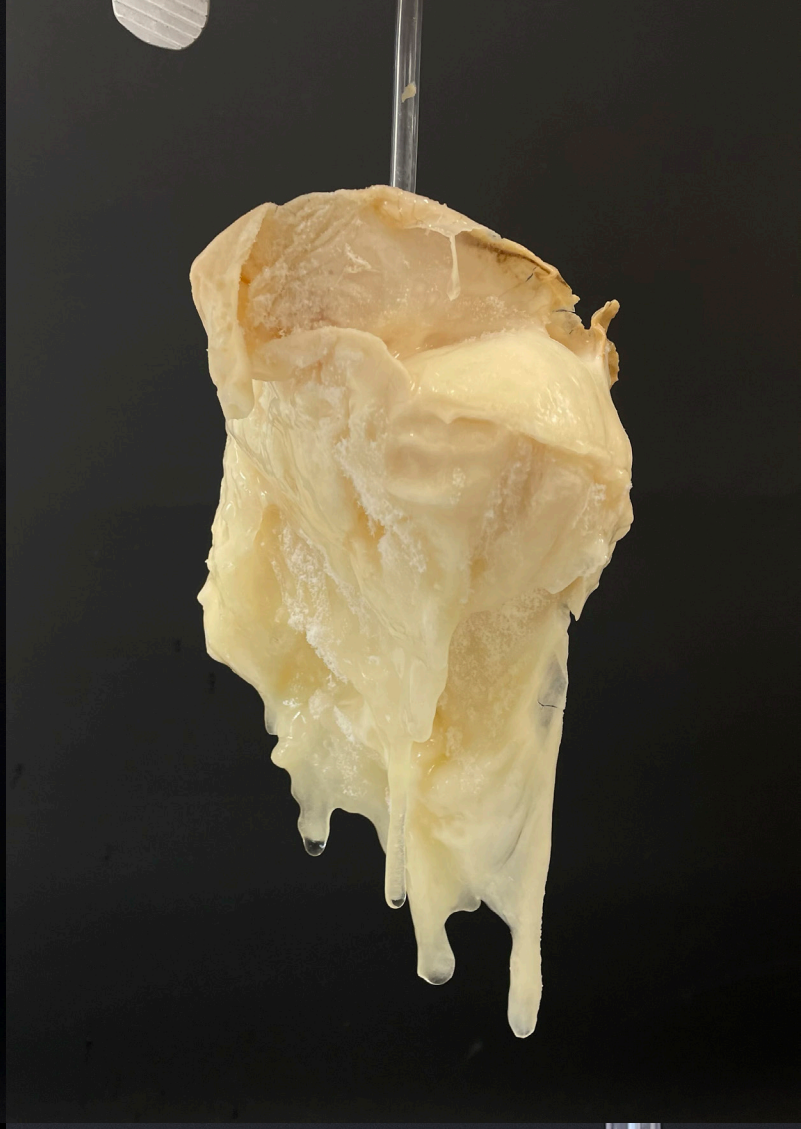
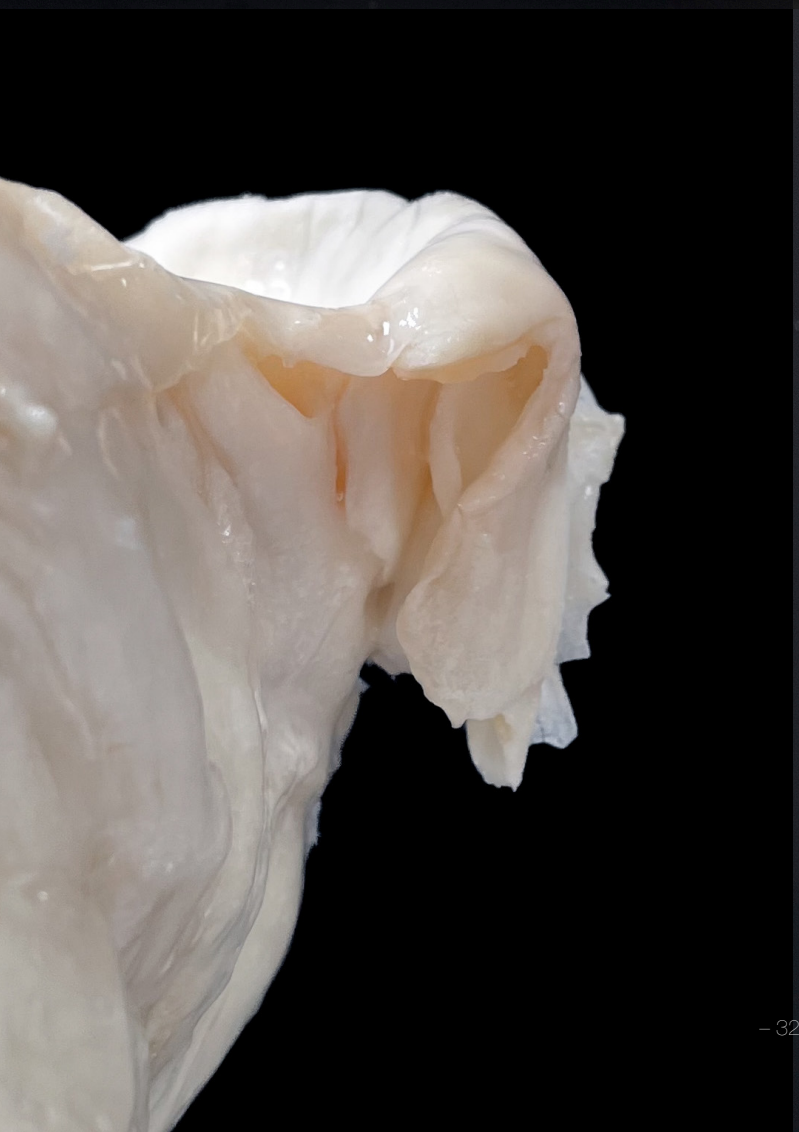
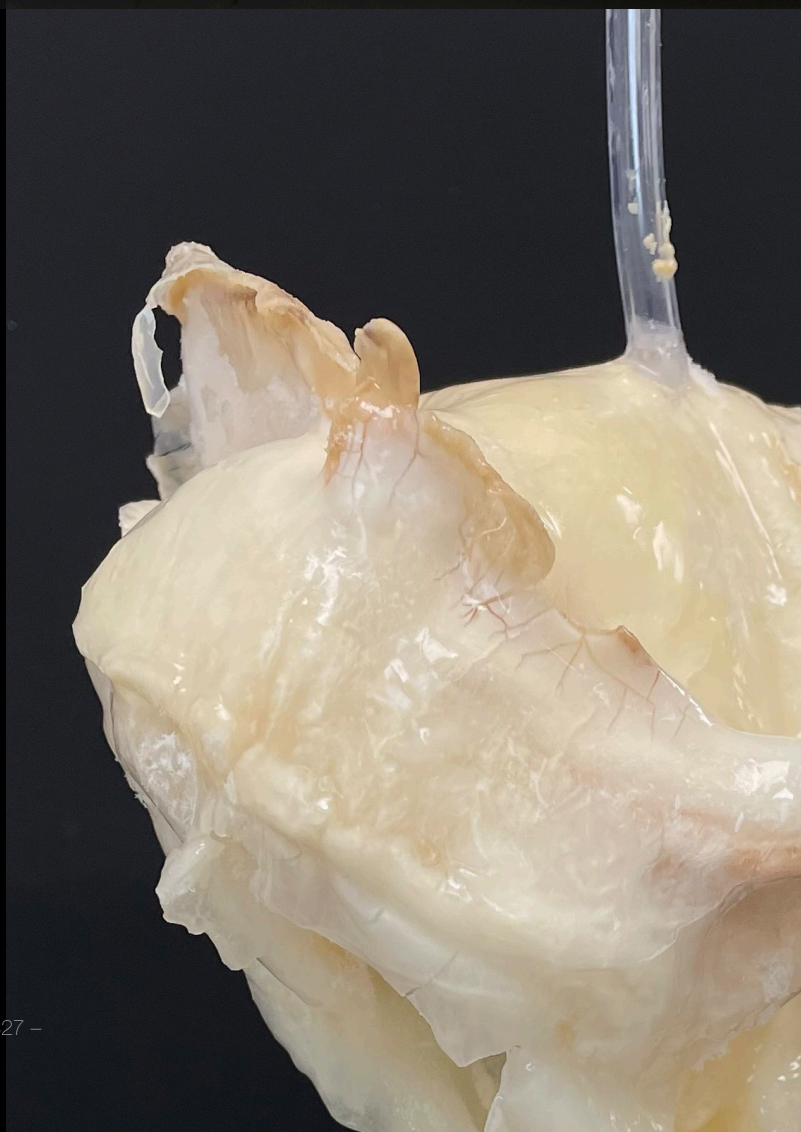
7.4

Figure 7.2: *Schock-freezing of freshly harvested BC*
Figure 7.3: *Frozen form of scaffold (2) in Exp. P*
Figure 7.4: *3D scan of frozen BC form*
Figure 7.5 (right): *BC form of scaffold (4) in Exp. P*

a minimum capacity of 15 litre and wide opening would have been needed. With a maximum of three samples being frozen at any one time, there would have been a vast amount of wasted LN_2 as a result. This method also poses the risk of 'over freezing' the sample and causing cracks and breakages of the BC. Secondly, immersing the easily malleable BC into a liquid posed the risk of distorting the shape as it freezes from the bottom up. With little to no control over how the BC freezes while being lowered into the gas, this would have added yet another variable to be considered in the final analyses of the shape.

An alternative method was chosen in which the BC shape was pulled out of the liquid and held up using the aeration tubing to be sprayed with LN_2 directly from the pressurised tank (Figure 7.2). Slow turning of the sample allowed an even freezing of the surface, creating an ice shell locking in the liquid on the inside. After the exterior of the sample was visibly frozen and no longer malleable, it was transferred to the minus 80 degrees Celsius freezer in a hanging position. The combination of shock-freezing the exterior with LN_2 and subsequent placement in the lab freezer allowed the preservation of the shape with little to no loss of volume (Figure 7.3 & Figure 7.5).

Once the sample was fully frozen, it was

7.6

Figure 7.6: *Process of 3D scanning the frozen BC*

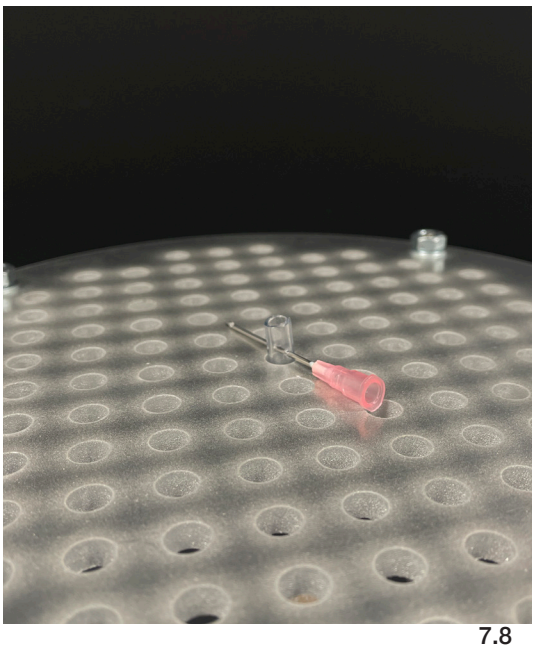
possible to remove it from the freezer for a short amount of time without melting. For larger-scale samples, such as the one grown in a 5L beaker, this was roughly 15 minutes while smaller samples with a single air stone started to melt after 10-12 minutes. This time frame was sufficient to create a digital 3D scan of the exterior using a Revopoint Pop handheld scanner (Figure 7.4 & 7.7). It was also possible to weigh the frozen BC and take manual measurements of the sample at this stage of the preservation process. To scan the sample, the scanner was positioned on an even surface and kept statically while the samples were slowly turned in front of it until a point cloud of all sides had been created (Figure 7.6). Examples of the digital 3D models can be found on the following pages.

The last step of the process involved the transfer of the sample into the freeze-drying machine and removing all the liquid inside it. The standard way of freeze-drying samples is by placing them flat on an open wired shelf within the machine. Due to the nature of the samples grown from aeration experiments, a new method of placing them in the machine was needed to not have the sample crush under its own weight. A modular frame was designed which allowed the samples to hang freely during the process (Figure 7.7). The frame consists of two

plates on the top and bottom, and 6 screw rods. The plates were waterjet cut using 6 mm aluminium, and the threaded rods made of stainless steel. The bottom plate is cut out to a ring which was required for the freezer-dryer layout which pulls the water out to the bottom through suction. To not block these vents, the bottom part of the frame had to have minimal surface area. The top plate was designed with a grid of holes cut out in the size of the air tubes. This allows the samples to be hung by pushing the end of the tube through the top plate and securing it with a needle to not fall back out (Figure 7.8). The whole frame was suitable for autoclaving in the case of contamination occurring and full sterilisation being necessary.

For optimal results of the freeze-drying, it is necessary to have a fully frozen sample that goes into the machine. If the sample has already begun to defrost on the surface, chances of not fully drying are high and the result is a still wet material that is again malleable. This could lead to a distortion of the BC shape. Due to the freeze-dryer being in a different building to where the BC was grown and frozen, the sample needed to be re-frozen before being put into the machine after transportation. Depending on the size of the sample, two different methods were used:

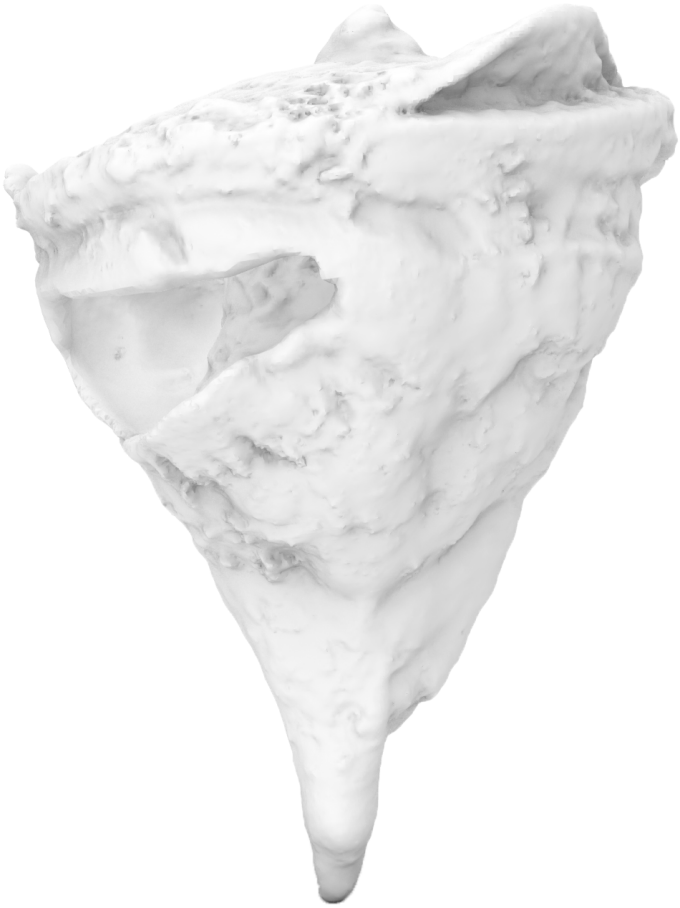
7.7



7.8

Figure 7.7: *Custom built scaffold to freeze-dry the frozen BC forms*

Figure 7.8: *Using a needle to hold the BC form in place by piercing the silicone tube*



Experiment I

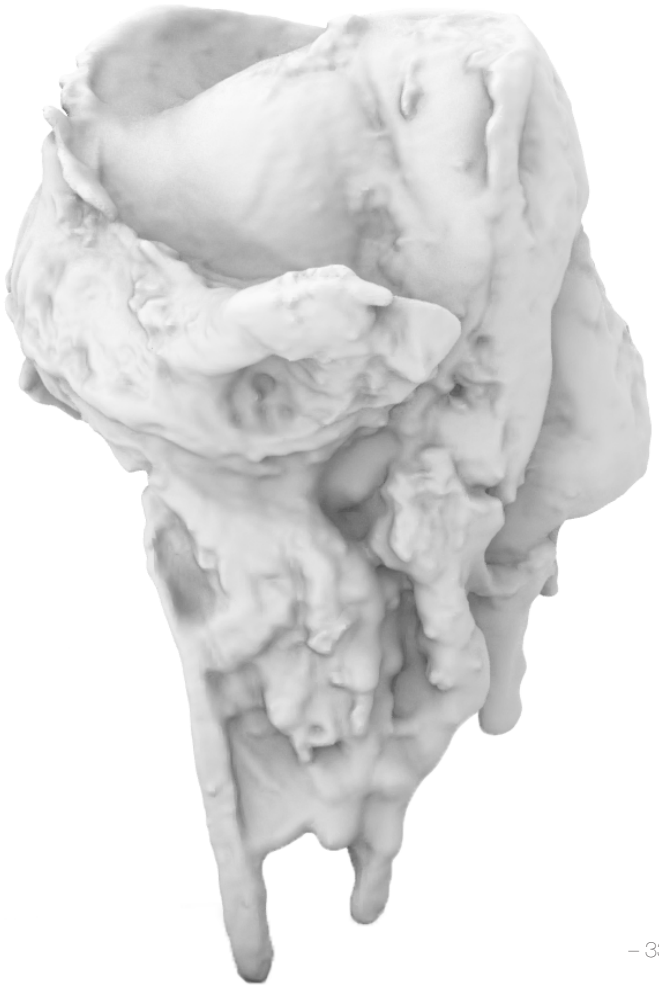
Medium: *Tea-based*

Scaffold: *Single round air stone*

Dimensions: *94 x 93 x 124 mm*

Special features:

Large internal air reservoir under mature surface pellicle



Experiment P

Medium: *Hestrin-Schramm*

Scaffold: *Combination (4)*

Dimensions: 125 *x 104 x 191 mm*

Special features:

Irregular shape of surface pellicle and multiple vertical foldings in lower half

Experiment I

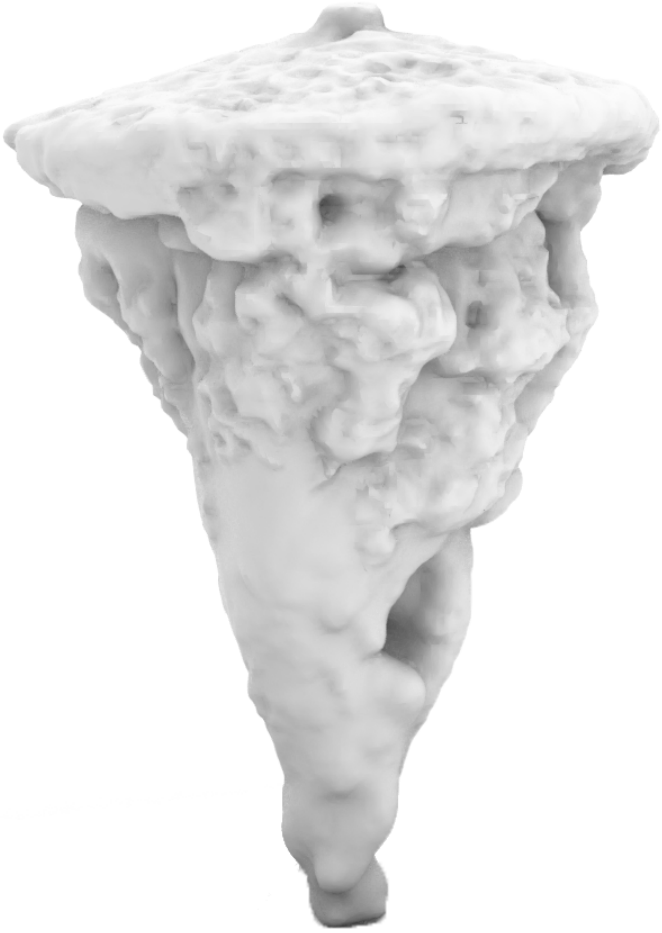
Medium: *Hestrin-Schramm*

Scaffold: *Single round air stone*

Dimensions: *93 x 95 x 137 mm*

Special features:

Uniform shape with clear distinction between surface pellicle and submerged BC growth



Experiment P

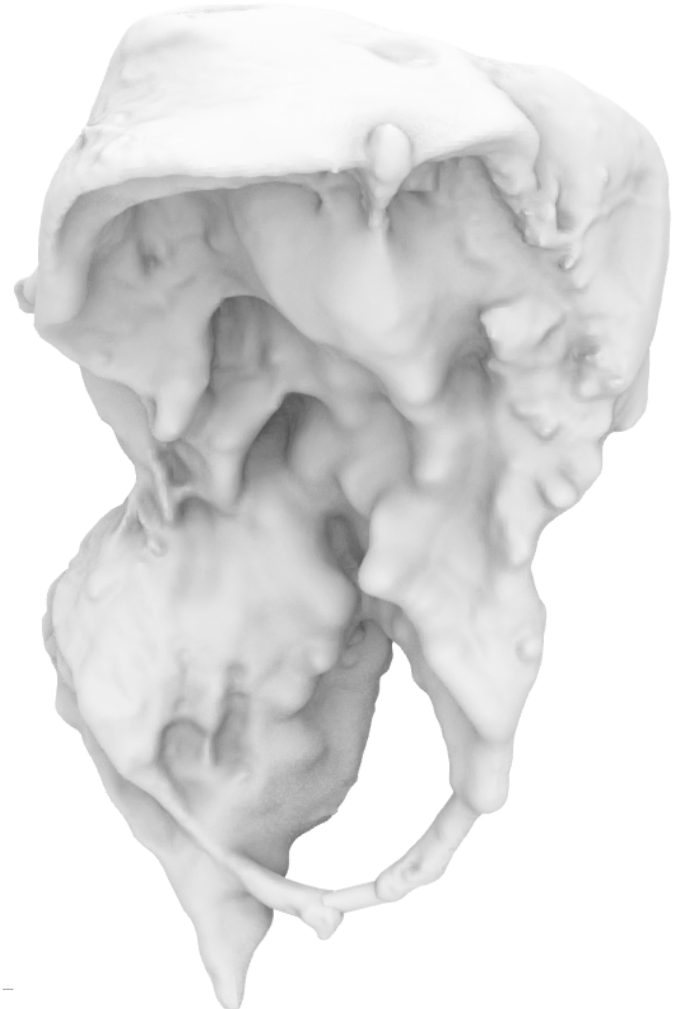
Medium: *Hestrin-Schramm*

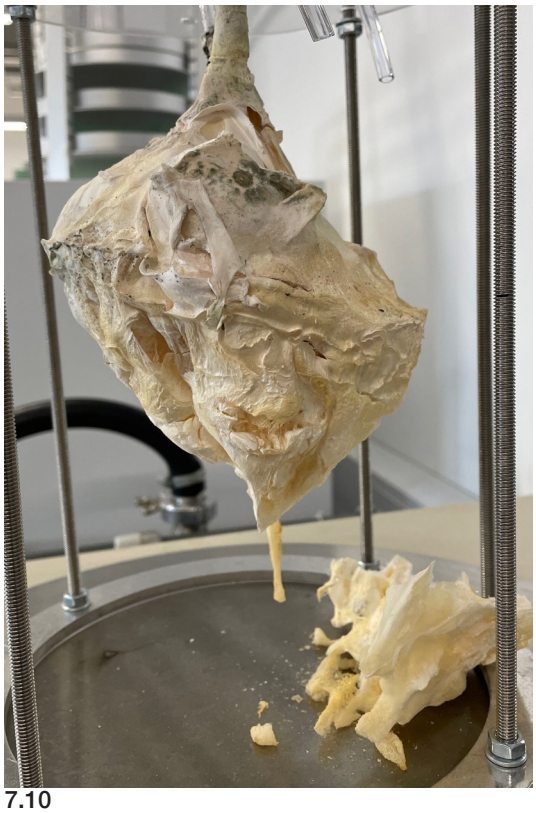
Scaffold: *Combination (6)*

Dimensions: *78 x 117 x 140 mm*

Special features:

Loop created by BC at the lower submerged end and a overall more rectangular in section.





7.10

7.11

1. Smaller samples were dipped into LN_2 for a few seconds until a cracking sound of the material surface breaking was audible;
2. Larger samples were hung inside the frame and transferred back into a local freezer for a few hours. Before placing the refrozen sample in the freeze-dryer, the machine was started and cooled down to the desired temperature. Depending on the size of the sample, the freeze-drying process took between 4 and 6 days. The colour of the BC was used as indicator for when it was fully dried. To remove and store the dried sample, it was carefully taken out of the frame and transferred into an airtight box in a hanging position with desiccant inside the box.

Results

During the drying process it was already noticeable that the BC became very brittle, with protruding elements cracking and falling off (Figure 7.9 & 7.10). The first signs during the drying process were fine cracks at the surface (Figure 7.11) and the appearance of the BC changing

Figure 7.9 (right): *Close-up of freeze-dried BC pieces*

Figure 7.10: *Fully dried BC, indicated by the light colour, with a large piece broken off*

Figure 7.11: *Visible cracking of the BC as it dries*

Figure 7.12: *Freeze-dried BC from experiment K, grown in 5000ml beaker, partially rehydrated*

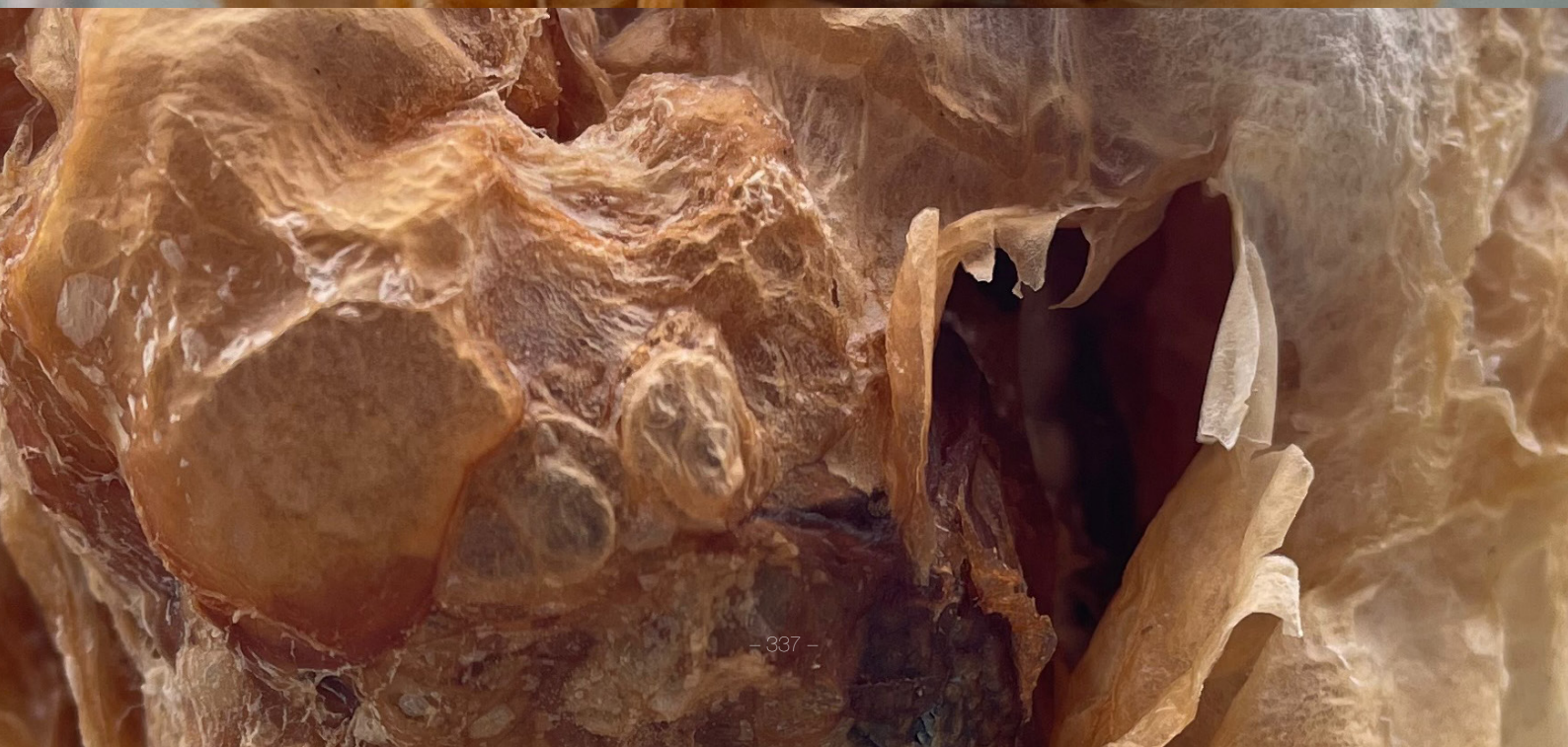
to a porous, flake like, material. Once the sample was visually fully dried, indicated by a flaky texture and evenly light colour, it was carefully removed out of the freeze-dryer with the metal frame. The slightest movement caused the dried sample to break and by the time it was transferred into its storage box, only the BC directly attached to the aeration scaffold was still in place. This observation indicates that the BC develops with gradient maturity around the scaffold, with mature BC near the air source to immature BC furthest away. It was also noted that the BC would rehydrate even when stored in an airtight container with desiccant gel inside. A possible cause for this could be the quantity of salts from the medium still inside the sample when being frozen, as salts are hydrophilic.

Elements of the sample breaking of either during the drying process or through the immediately handling afterwards made it not possible to 3D scan the full freeze-dried sample and compare it to the previous scan taken at a frozen stage for changes in form and shrinkage. However, there were no visible changes in the volume the samples took up inside the metal freeze-drying frame before and after. Prominent features of the form, such as loops and folds, were still visible after the freeze-drying (Figure 7.12 & 7.13).

An unforeseen benefit of the breaking of the freeze-dried samples was the exposure of the interior structure of the BC samples. Prior to this, the understanding of the interior had solely been based on visual observations during the growth phase in the glass vessel. Broken off pieces exposed that the BC formed differently depending on how close access to oxygen was. The natural air-liquid interface of the medium, the aeration scaffold, and trapped air pockets were the main providers of oxygen for the bacteria. Between air pockets the BC grew into a fine interwoven matrix while the BC grown at the air-liquid interface and around air pockets grew more sheet-like. The overall appearance of the BC once dried was fragile.

Overall, the process of freeze-drying the BC shapes allows for the documentation of shape, dimensions, and weight. However, it does not provide a suitable option to store the samples for a prolonged period of time due to the fragility and hydrophilicity of the BC.

Figure 7.13: *Close-up images of material surface of freeze-dried BC from Exp. K*



7.3. Hydrated Method

While the freeze-dried method allowed for the documentation of the overall shape and dimension of the sample, it was not able to gain a clear image of the interior structure and the distribution of the BC in direct proximity to the aeration scaffold. An approach which allows the handling of soft tissue was developed based on processes found in the medical field to address these shortcomings. The method developed consists of two parts, including the manual slicing using sharp blades and Computer Tomography (CT) using x-ray technology. For each, the BC sample had to be cast in a gelling agent to stabilise the form and allow handling with minimal to no deformation.

7.3.1. Manual Slicing

Method

The consistency of freshly harvested BC can be compared to the one of tissues found in animals and humans, such as brains and other organs. In medical research, mouse brains are regularly analysed for various studies and a variety of processes and tools have been developed for the handling of the delicate organ. To analyse the interior structure of the brain it is sliced into thin, even slices after being treated with a variety of chemicals to stabilise the tissue. To achieve an accurate thickness of each slice a customised mould is used. The brain matrix is such a mould and is made from a solid piece of material, often stainless steel for sterility. It has a customised cavity in the shape of the brain and parallel slots on either side to guide the knife or razor blade through the brain in a straight line. While a regular razor blade is sufficient for smaller organs, such as the mouse brain, a brain knife has a longer blade with a sharpness of a razor blade and matches the size of the BC samples.

Using the method of the brain matrix, a similar mould was designed and fabricated for the BC samples. Due to the unique shape of each experiment output, a standardised size

Figure 7.14: *Custom-built slicing mould, Sliceatron 3000, to cut hydrated BC forms into slices*

7.15

7.16

Figure 7.15: *Double strength gelatine holding the shape during cutting after being fully set*

Figure 7.16: *Buoyancy of BC sample on liquid gelatine*

was chosen. As the majority of samples was grown in tall form 1000 ml Duran beakers, the slicing mould was designed around the interior volume and shape of this beaker. The slicing guides on the side were customised to fit the blade of a disposable 12-inch brain knife of the brand MacroKnife and the final slicing mould, lovingly named Sliceatron 3000, was fabricated using a combination of techniques. Stainless steel was cut by waterjet to form the slicing guide walls and 3D fused filament printing was utilised to create the bed of the mould in the shape of the beaker. One side of the mould was closed off using a laser cut part and all parts were assembled on sealed plywood board (Figure 7.14).

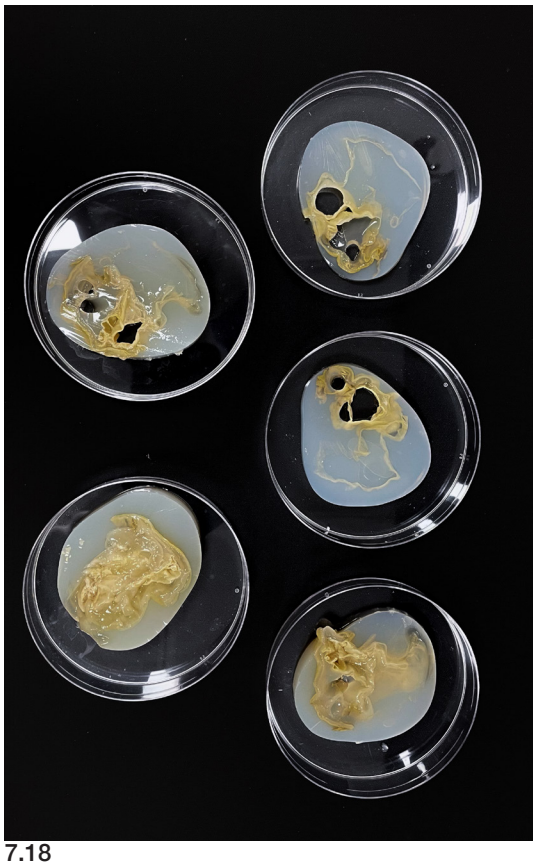
The hydrated BC is soft, malleable, and porous once removed from the growth medium, and therefore requires to be prepared into a more dimensionally stable state. Two gelling agents, gelatine (12g or 24 g per 100 ml) and double strength agar (1.8 g per 100 ml), were tested to cast the BC sample into the shape of the 1000 ml tall form beaker (Figure 7.15). To prepare the sample for the hydrated preservation, 1% Virkon was added to the growth beaker 48 hours prior to harvesting the BC. This was to ensure all activity of the organism was ended prior to preservation. After harvesting

the BC following the standard procedure (see Chapter 4.2.2), the BC was treated and washed using the same technique as for the freeze-drying. It was then placed in a 1000 ml tall form beaker with roughly 300 ml of warm and liquid gelling agent. As the BC floats due to its buoyancy from multiple air pockets, the casting had to be done in layers (Figure 7.16). The beaker was placed in the fridge until the first layer of gelling agent had set, then the next layer of liquid gelling agent was poured onto and around the BC. This process was repeated until the BC was fully covered. Care was taken to not fill up the beaker too quickly, as the still warm liquid could reheat the already set gelling agent and release the BC from its grip. Each sample was cast in 3-6 layers, depending on the shape and size of the BC. Samples with larger air pockets required multiple more shallow layers.

Once fully set, the BC was removed from the beaker and placed inside the slicing mould to be cut into pieces of either 5 or 10 mm thickness using the brain knife guided by the moulds slots. To help remove the sample from the beaker without force, it was placed in a container with warm water.

7.17

Figure 7.17: *Sliceatron 3000 cutting mould in use with a BC sample cast in gelatine*



7.18

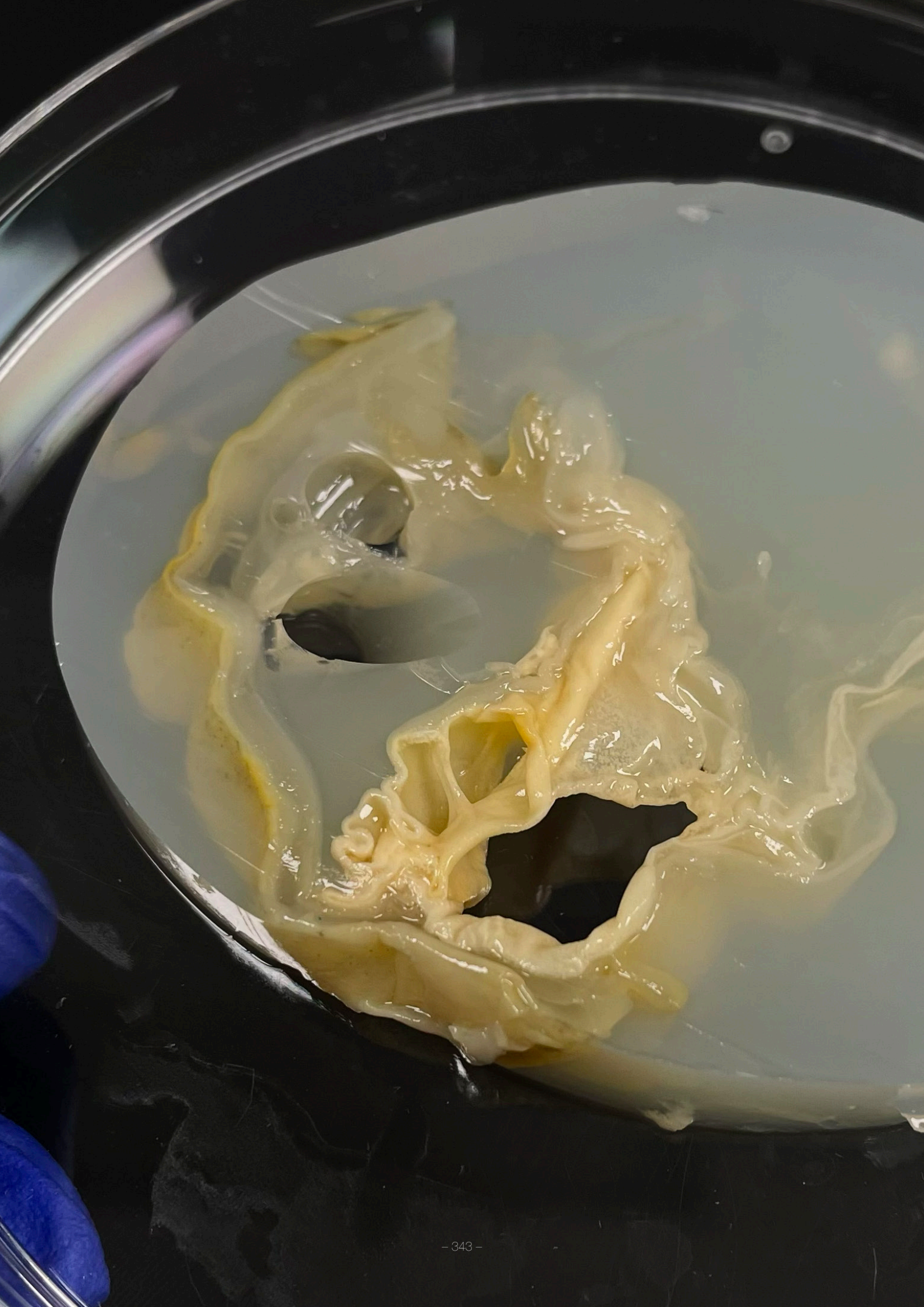
Figure 7.18: *Examples of hydrated BC cast in agar and sliced into 10mm thick slices; BC grown in 500ml bottles*

Figure 7.19 (right): *Close-up of hydrated BC slice cast in agar*

Results

The BC set in double strength agar showed very good shape retention during handling as well as slicing and did not detach from the BC. The consistency of the agar was rubbery and rather than breaking, the agar would crumble away under force. The gelatine samples, in single and double strength, did not provide enough strength and began to break and fall apart while being placed into the mould (Figure 7.18). Breakage was especially noticeable in the areas where the gelatine was attached to BC. This could be due to the BC releasing extra liquid during the setting process and preventing the media to fully set by diluting the gelling agent-to-liquid ration.

While the brain knife was sharp enough to slice through the agar and BC with minimal pressure, it was not possible to cut through the air stones from the aeration scaffolding on the inside of the sample. Both the porous stone and the hard plastic connector part were too hard for the knife to pass through. Cutting through the silicone tubing was possible by applying slightly more pressure with the knife. This increase in pressure, however, could easily lead to distortion of the BC as the tubing is pushed downwards from the knife. In the case that the knife reached an uncuttable element, the BC around



it was cut in circular motion and carefully pulled off the scaffold element afterwards. This left a visible hole in the BC slice (Figure 7.18 & 7.19). These sliced BC samples were kept in individual petri dishes in the fridge at 4 degrees Celsius when not being used for analysis under the microscope or on the light table for photographic documentation.

Concluding, while it was possible to slice through the samples, it was not possible to gain a full image of the interior which included the scaffolding and intricate BC connections. Casting the BC in double strength agar allowed for precise cutting and preservation but distorted the shape of the BC nonetheless, especially in areas where air pockets had formed. When developing this method further, it can be investigated whether the agar can be injected manually into the air pockets to provide more stability while cutting.

7.3.2. Computed Tomography

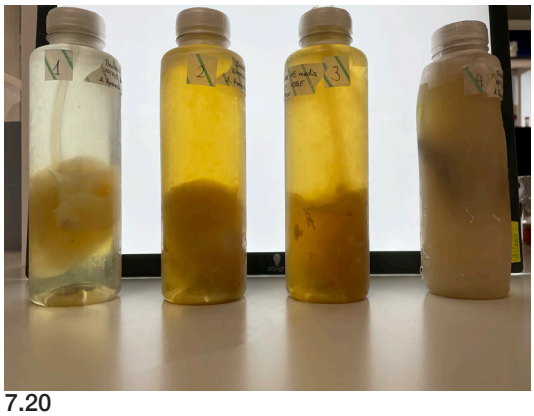
While the manual slicing allowed a closer inspection of the texture and state of the BC in certain areas of the sample, it was not possible to recreate a complete image of the complex interior shape with all cavities. Additionally, once the sample was cut, it was no longer possible to reassemble and position cuts in other areas of interest within the sample.

Another possible method to keep the sample undamaged but gain information about the interior is the use of computed tomography, also called CT scanning. CT scanning is commonly used in clinical settings or for medical research of soft tissue and utilises specialised x-ray equipment to create sliced images of an object either horizontally or vertically. The x-rays do not leave any visible marks on the material imaged which allows for repetitive imaging as well as combination with the manual slicing method. Magnetic Resonance Imaging (MRI) is also a method conventionally used in the medical field for imaging of internal organs and is less harmful to living organisms compared to x-rays. It also provides a clearer and more detailed picture of tissue and tissue abnormalities. However, CT technology offers clearer results in areas where air is present, such as lung imaging. As the interior structure of the BC consists of many small and large air pockets, CT scanning was chosen as the preferred method.

Pre-clinical imaging describes the visualisation of tissue in small living animals in medical research. The pre-clinical machines have a higher resolution compared to ones used in hospitals but can only accommodate a smaller testing volume. As the only available CT machine was pre-clinical, smaller BC samples had to be grown to match the maximum cylindrical volume of 68mm diameter and 350mm length.

The BC was grown in PET bottles of 500ml volume using the same method as described in Chapter 6 (Figure 7.20 & 7.21). The aeration scaffold consisted of a PLA printed double walled air stone with singular air tube connection. The 3D print was scaled down in size to the dimensions of 12 x 12 x 32mm to maximise the space in the bottle for the BC to grow. Nonetheless, the ratio of scaffold volume to medium was significantly smaller than in previous experiments and needed to be considered as potential inhibitor in the shape development.

The samples were grown for 16 days and then detached from the air pumps to stop the growth. Out of the 4 samples grown, three were



7.20

7.21

Figure 7.20 & 7.21: *Samples growing in 500ml bottlesfor testing in CT machine*

chosen for imaging. To minimise disturbance in the image due to air and to reduce the chance of the BC deflating, the BC was suspended in different media for testing before sealing the bottles watertight with parafilm wrap:

1. Left in the HS media it was grown in;
2. Growth media replaced with same amount of distilled water;
3. Growth media replaced with liquid double strength agar and cooled until the agar was fully set.

Each sample was imaged inside the Bruker Skyscan 1176 in-vivo Microtomograph with 19µm pixel size (Figure 7.23).

Results

None of the samples tested gave clear imaging of the BC inside the media. While the sample in agar gave no results apart from a fully grey image, the two samples in liquid provided images indicating the outline of air pockets and the aeration scaffold. The rounded bottom of the bottle was also clearly visible (Figure 7.22).

It was not possible to clearly distinguish whether the air pockets in the image were inside the BC sample or in the media surrounding

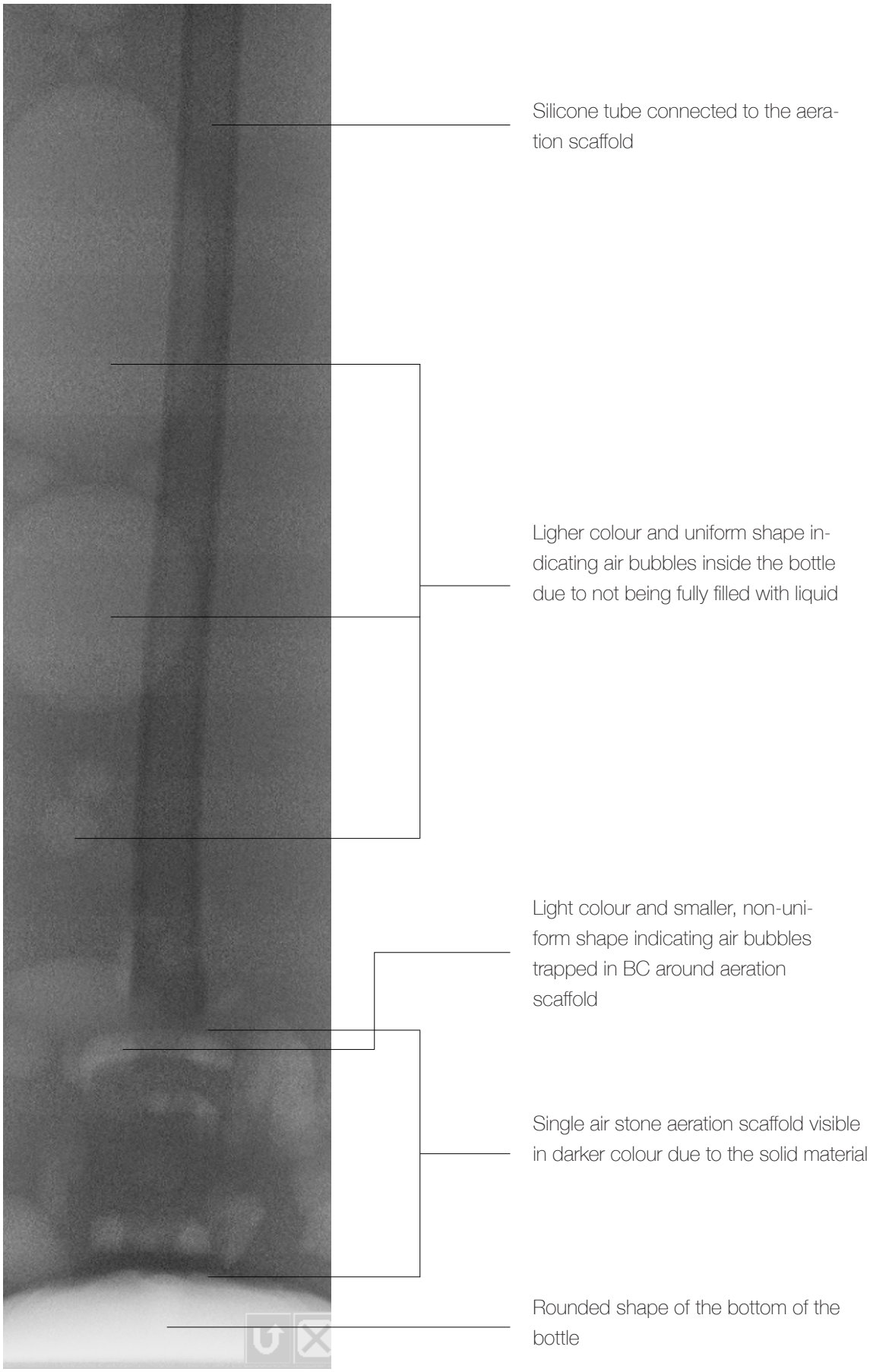


Figure 7.22: *CT image of hydrated BC in water and 500ml bottle, showing only limited features of the form*

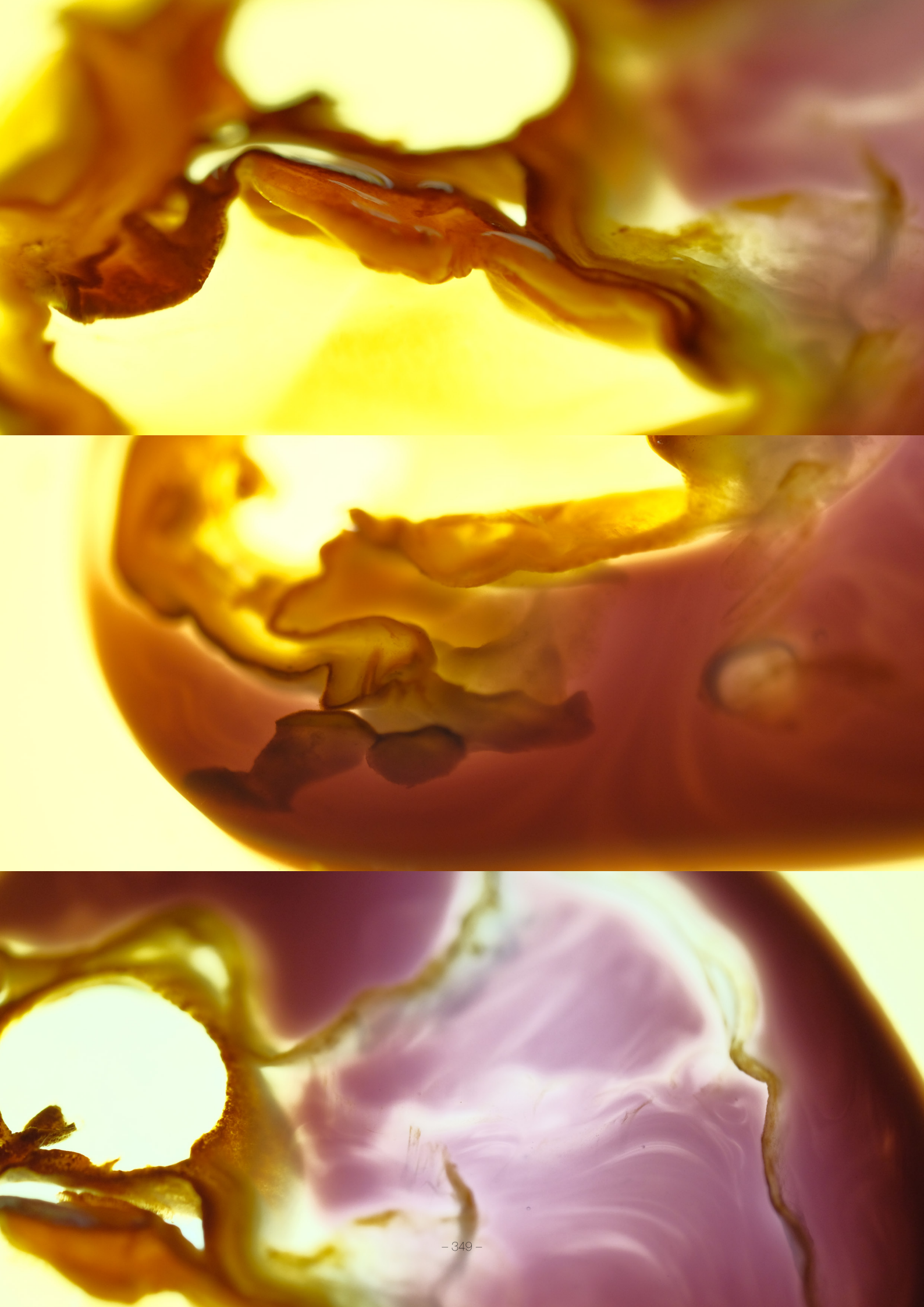
it. The air pockets inside the medium occurred while closing the bottle with an approximately 2mm gap at the top of the medium to the rim of the bottle neck to avoid spillage. While changing the pixel resolution and the contrast increased visibility of the aeration scaffold, it did not improve the visibility of the BC growth.

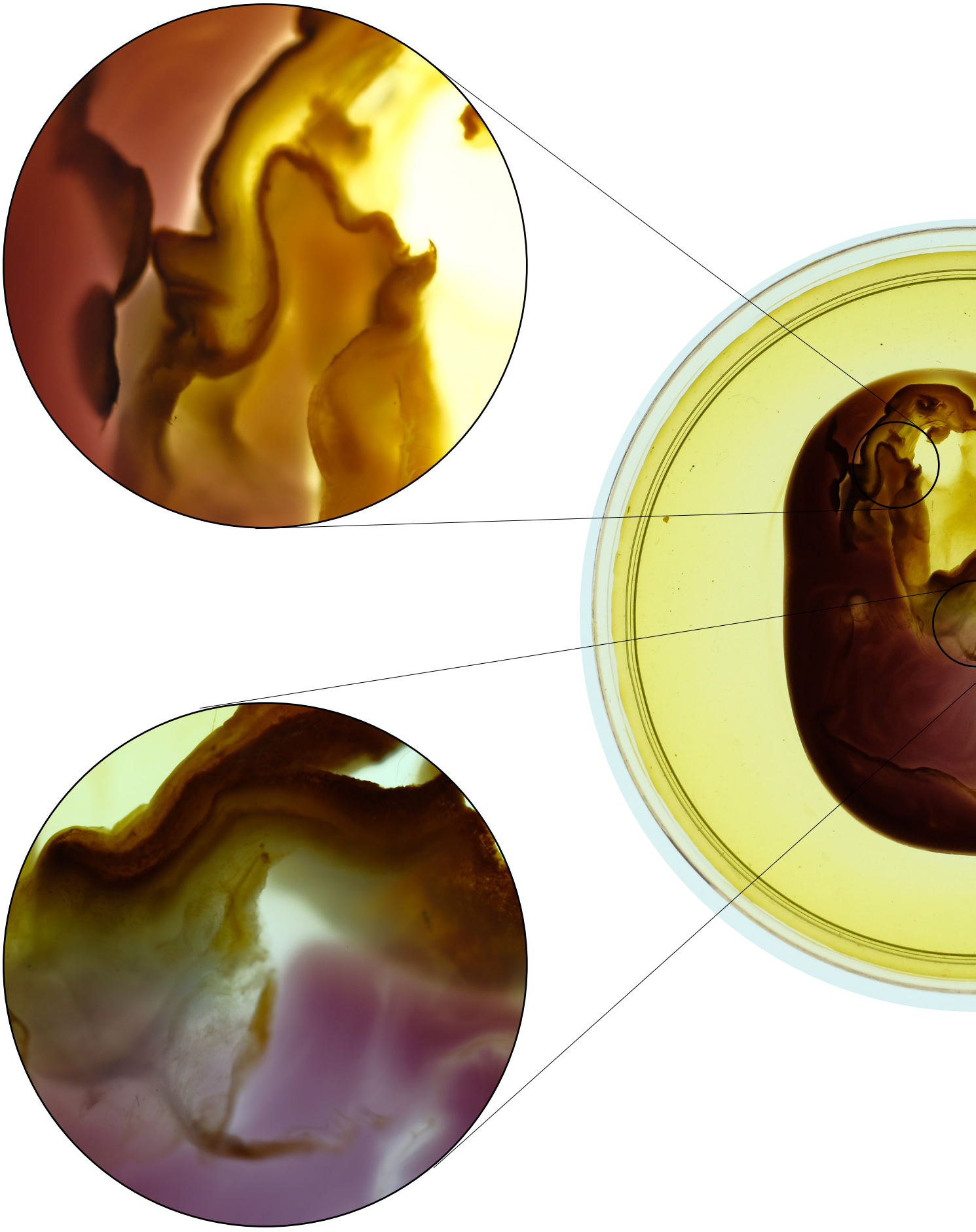
Iodine is a commonly used contrast agent used in x-ray imaging and was tested with a sliced BC sample, to determine whether it could be used in further experiments. The iodine solution of 30% stained the agar around the BC and enhanced the visibility of the yeast cells inside the BC. The BC, however, remained unchanged (Figure 7.24 & 7.25) and the staining did not increase the visibility in the CT imaging.

Overall, using CT technology to analyse the BC was not successful and did not add to the analysis of the BC shapes. However, improving the method of preparing the BC through trialling different types of stains to enhance material visibility as well as changing the method of containing the BC during the scan hold the potential of making CT a valuable analysis method.

Figure 7.23: *BC sample being tested inside the SKYSCAN CT machine for digital X-ray imaging*

Figure 7.24 (right): *Three close-up images of the hydrated BC cast in agar, sliced and stained with iodine solution*





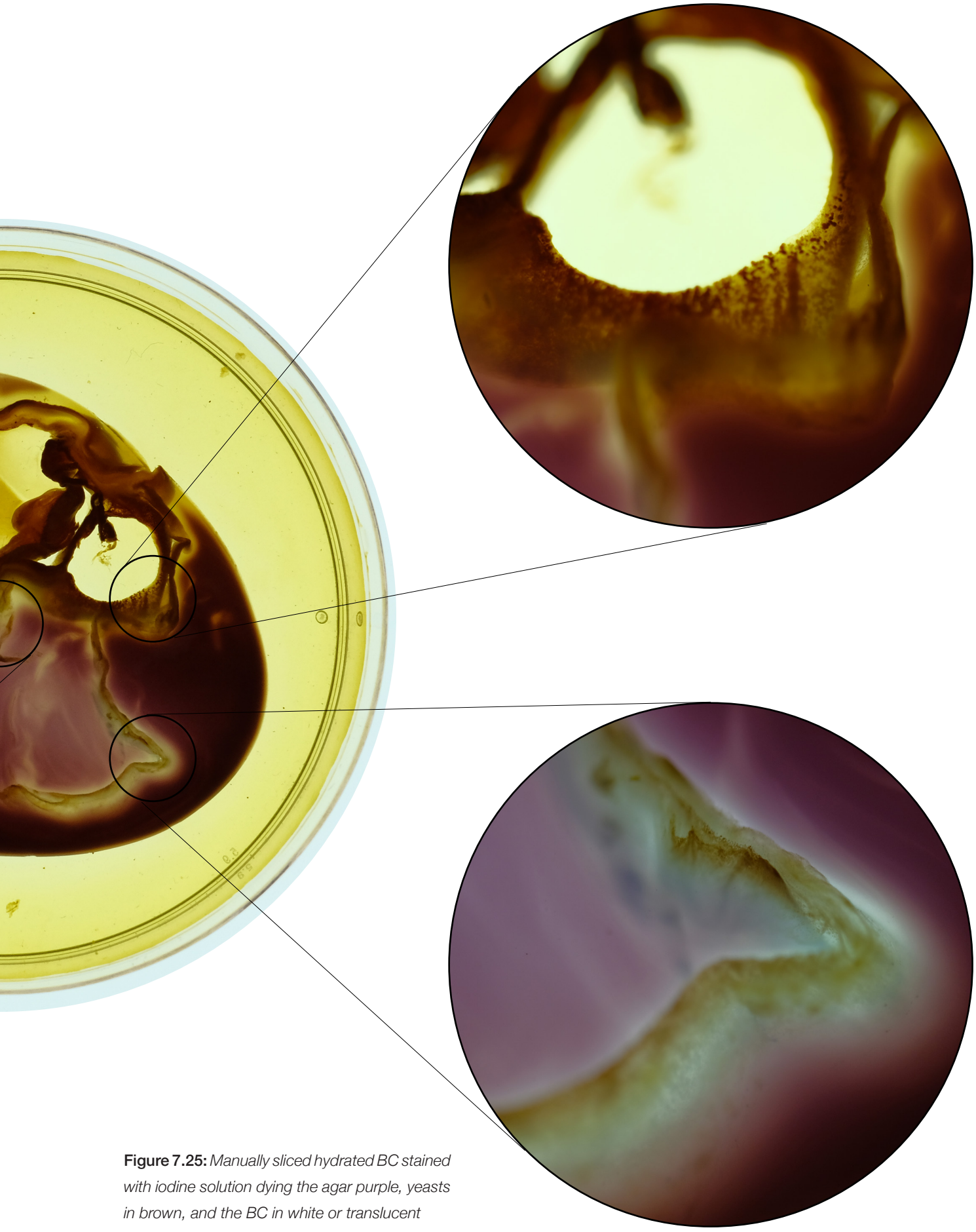


Figure 7.25: *Manually sliced hydrated BC stained with iodine solution dying the agar purple, yeasts in brown, and the BC in white or translucent*

7.4. Challenges and Limitations of the Preservation Methods

The harvested BC shapes pose the challenge of preservation due to their novel fabrication technique and varying levels of material maturity, which result in a fast-changing morphology once harvested due to water loss. In order to acquire data about the volumetric expansion of the BC as well as the development of internal features it was necessary to achieve a state of form-stability of the BC. The initially tested air-drying of the BC shapes followed the same pattern observed during the air-drying of flat BC sheets (see Chapter 4.2.1) and showed a significant decrease in volume over a timespan of multiple days. While this method was suitable to identify the accurate number and size of air reservoirs within the BC, information about the full shape while hydrated was lost during the process.

First attempts to 3D scan the freshly harvested and hydrated BC with the 'Revopoint POP' failed due to the translucent nature of the BC skins. The infrared technology of the scanner requires the surface to reflect projected light back to sensor cameras, which detect any distortion in the light beam caused by the surface topology using triangulation (Bernardini & Rushmeier, 2002). The shock-freezing and following full freezing of the BC resulted in a change of opacity and enabled successful scanning while also being form-stable state. The speed at which liquid is frozen influences the formation of small air bubbles within the ice (Carte, 1961), with more bubbles forming the faster it freezes. The immediate shock-freezing with liquid nitrogen results in a high concentration of these bubbles in particular in the outer layer of the shape, which is beneficial for the scanning process.

While freeze-drying is a common procedure to preserve the 3-dimensionality of the cellulose fibre matrix (Clasen *et al*., 2006; Zolotovsky, 2017) the fragility of the dried material is dependent on the maturity of the BC that was reached during the growth stage. A fully matured BC sheet dries into a coherent soft material that is sponge-like but does not return to its original shape after compression. The 3-dimensional BC shapes, however, have varying degrees of maturity throughout with predominantly immature BC towards the outer shell. Immature BC is made up of less dense fibre matrices and can result in a more fragile dried material which can crack under its own weight depending on how the mature and immature BC are aligned. The difference

of BC maturity was also visible in broken freeze-dried samples which showed varying thicknesses of the material throughout (Figure 7.26 & 7.27). In addition to material thickness, morphological features, such as air reservoirs and bubble pathways were distinguishable through the clear formation of more mature surrounding walls, which can be traced back to the increased presence of static or near-static oxygen in these areas. Even though it was possible to link the presence of these features to observations made during the growth stage, the location of the features did not always accurately correspond. A possible explanation for this displacement of features is the effect of gravity on the liquid-filled shape as it is pulled from the liquid during harvest. The sagging behaviour is particularly visible on the top pellicle which is flat while being in the liquid and folds downwards from the centre when harvested. Due to its ability of manipulating the BC shape, gravity could be considered an additional static design parameter of the experimental setup.

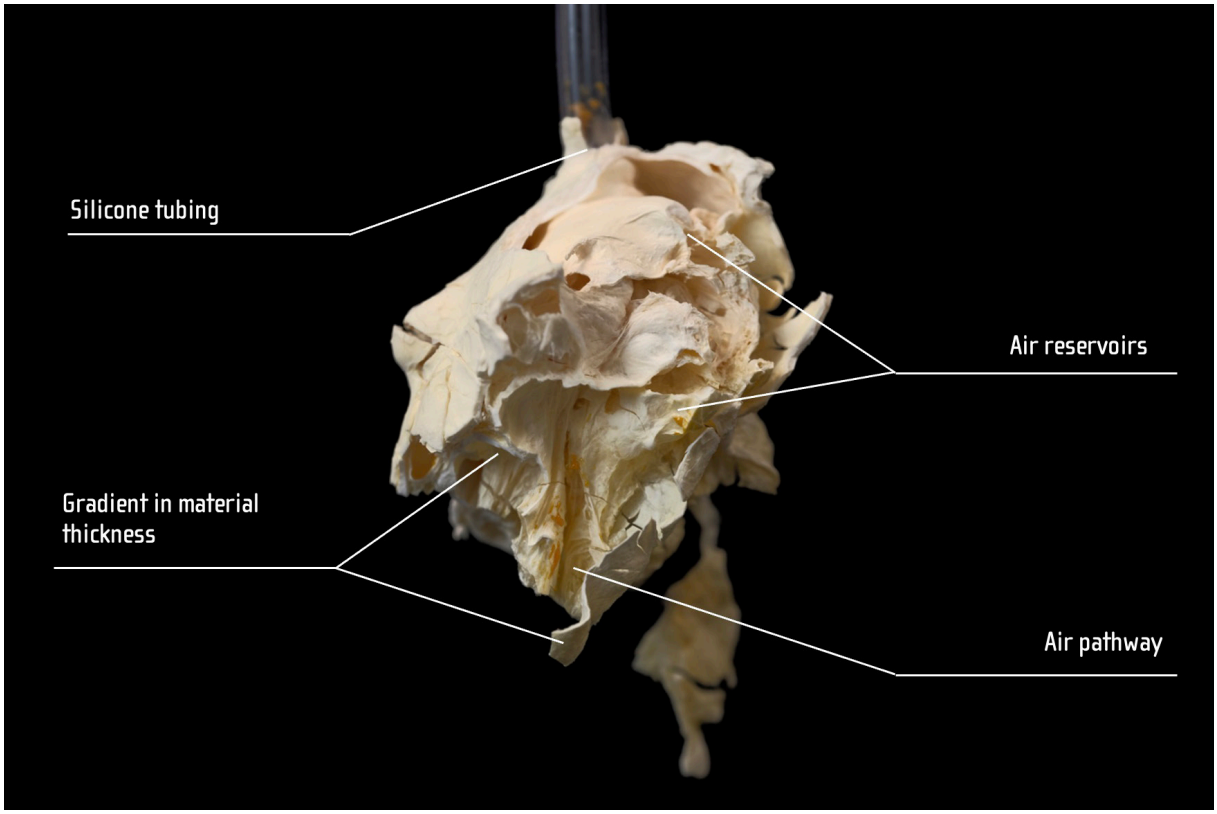
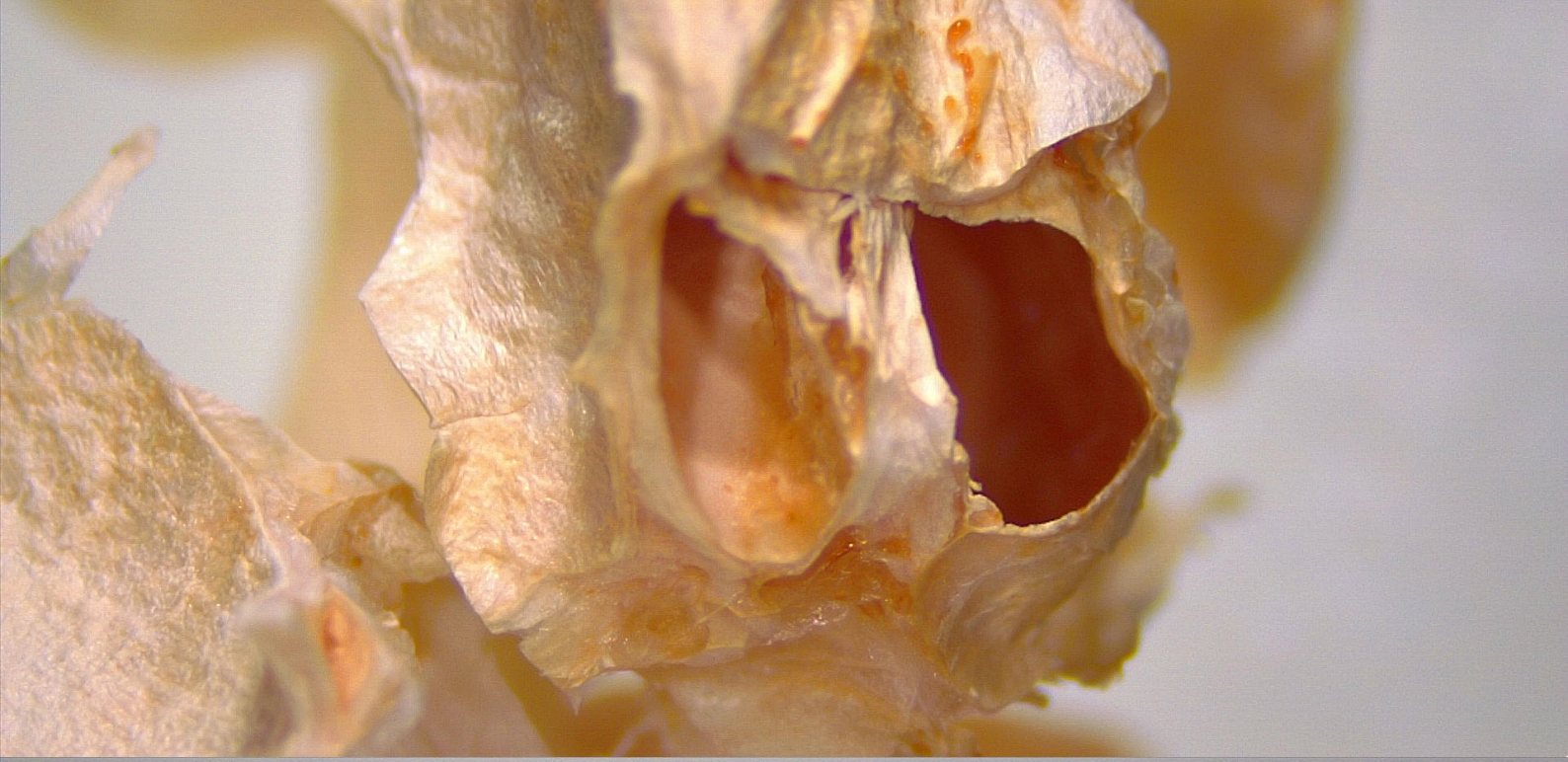


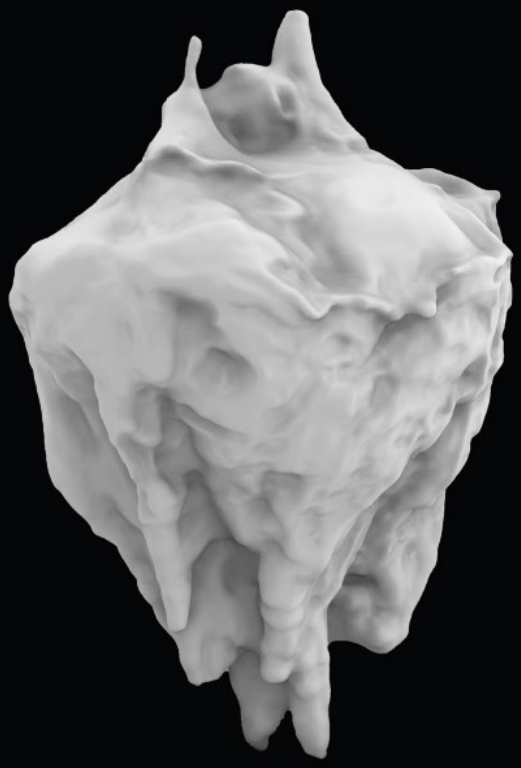
Figure 7.26: *Broken freeze-dried sample showing growth-features on the inside*



The process of freeze-drying allows for the documentation of the BC form post-harvest and a partial analysis of the location of morphological features within the overall volume of the material. However, only the freezing is necessary for the digital documentation, and it can be argued that the following freeze-drying step does not provide any further benefits due to the fragility of the final material. A clear limitation of this method is the lack of documentation of the aeration scaffold on the interior. As the BC matures, the position of the scaffold can adapt and adjust to the material growth, which means a shift of solid material within the soft BC. As the frozen and freeze-dried BC is opaque, the positioning of the scaffold has to be approximated based on observations during the growth stage. Another limitation of this preservation method lies in the longevity of the material. Freeze-dried BC is extremely hydrophilic, indicating a rehydration through a darkening of the material (Figure 7.12), and requires storage inside closed containers with desiccants. Combined with the fragility of the material, only limited handling of the sample is possible once it is fully dried. Nonetheless, each step of the preservation process offered valuable insights into the formation of the BC shapes and the digital documentation of the frozen shape made it possible to collect a form of comparable data across all aeration experiments (Figure 7.28).

The developed hydrated preservation method was developed based on existing preservation techniques for soft tissues but adapted to accommodate the unique shapes and varying material properties of the BC shapes. Agar is a commonly used substance in research to increase stiffness and form stability of soft samples by surrounding the sample in a semi-soft cast (Armise & Latatas, 1987). Gelatine is more often used in baking to support the setting of liquid. In the case of the BC, both gelatine and agar were tested as casting material to aid the cutting of the BC inside the slicing moulds, with the agar showing significantly better results. While the agar formed a firm support around the BC, the gelatine split and separated from the BC before the sample could be placed inside the cutting mould. While the outer part of the gelatine appeared firm, the inner material that was adjacent to the BC was soft and partly liquid. This indicates that the gelatine was not able to set in those areas around the BC. A possible explanation is the water loss of the BC which diluted the gelatine mix to a point where it was not able to solidify. A limitation of

Figure 7.27 (left): *Detail images taken with microscope of freeze-dried BC samples*
Figure 7.28 (next page): *Comparison of BC shapes grown in various aeration experiments with the corresponding scaffold and experiment parameters*

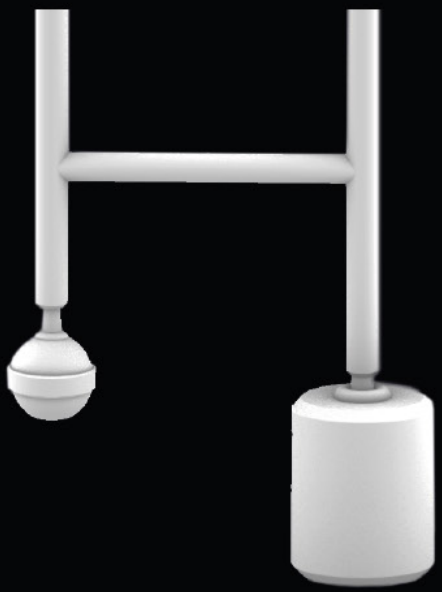


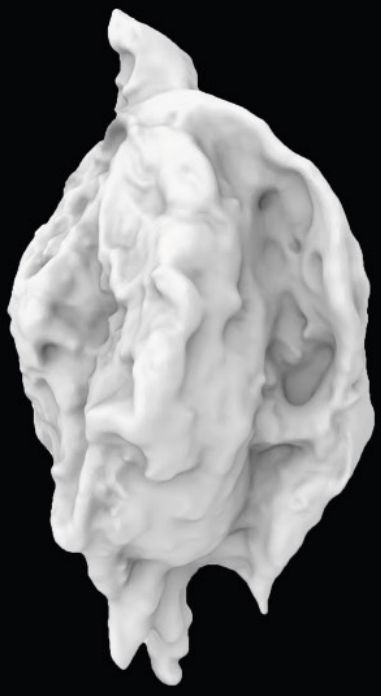
MEDIUM
> Hestrin-Schramm, 3000 ml

GROWTH TIME
> 21 days

DIMENSIONS
> 121 x 150 x 234 mm

SCAFFOLD



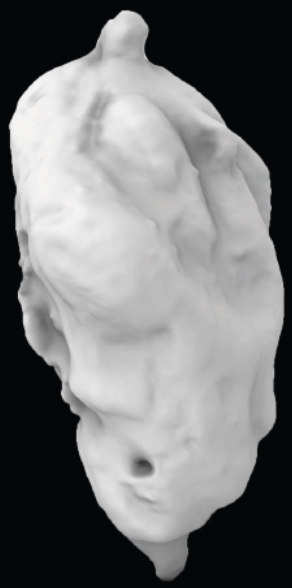


MEDIUM
> Hestrin-Schramm, 1500 ml

GROWTH TIME
> 23 days

DIMENSIONS
> 80 x 113 x 209 mm

SCAFFOLD

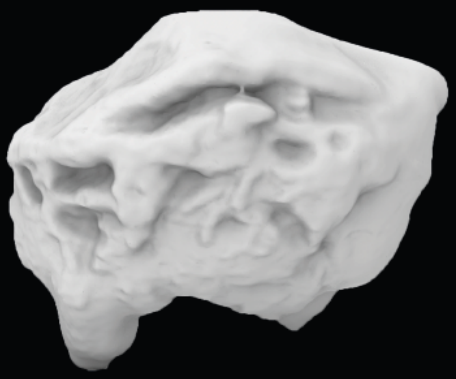


MEDIUM
> Tea-based, 700 ml

GROWTH TIME
> 28 days

DIMENSIONS
> 79 x 60 x 134 mm

SCAFFOLD



MEDIUM
> Tea-based, 400 ml

GROWTH TIME
> 21 days

DIMENSIONS
> 64 x 54 x 59 mm

SCAFFOLD

this casting method was the need to add the gelatine in layers around the BC instead of casting the whole shape at once due to the buoyancy of the samples. This allowed the still exposed BC to leak liquid and dilute the freshly poured gelatine mix. In contrast, the setting time of the double strength agar solution was significantly faster and, based on observations, remained unaffected by the draining liquid from the BC. Another benefit of the agar is its higher plasticity which allows the handling of the sample without breaking. While the exterior walls of the BC shape were held in place through the agar, the cutting with the brain knife still resulted in the distortion on the interior, such as the position of bubble pathways and the shape of air reservoirs. The need for additional pressure on the knife while cutting can be linked to the non-directional assembly BC fibrils (see Chapter 2.1.2), which results in cutting against the grain in every direction. However, it was possible to slice whole BC samples in a horizontal orientation and create an overview of the interior structure by arraying each piece in order (Figure 7.29). A side-by-side comparison of the arrayed images and the fully hydrated shape during the growth stage makes a visible correlation between the expression of morphological features on the inside and the outside possible.

A significant limitation of the hydrated preservation method is that the BC begins to decompose inside the cast material and can therefore not be stored for prolonged periods of time. A possible reason for this is the presence of environmental bacteria inside the encapsulated air reservoirs and a lack of ventilation in those areas which creates favourable conditions for certain bacteria and fungal spores. This is enhanced through the presence of nutrients which are retained within the BC fibre network and support microbial activity. Similarly to the freeze-drying samples, hydrated BC samples are not suitable for long-term storage. However, they are beneficial for the immediate analysis of materiality and extend on the understanding of shape formation from the interior outwards.

As part of the documentation methods trialled on preserved hydrated shapes, imaging of the interior structure via CT was tested. Based on the principle of x-ray imaging, a CT imaging creates a 3-dimensional visualisation of an object based on differences in density within it (Hermena & Young, 2023). While CT imaging is often used in preclinical research to identify changes and abnormalities in soft tissues, it was not possible to achieve an image of the BC. A possible explanation for this is the high water content of BC, in particular of immature BC (see Chapter

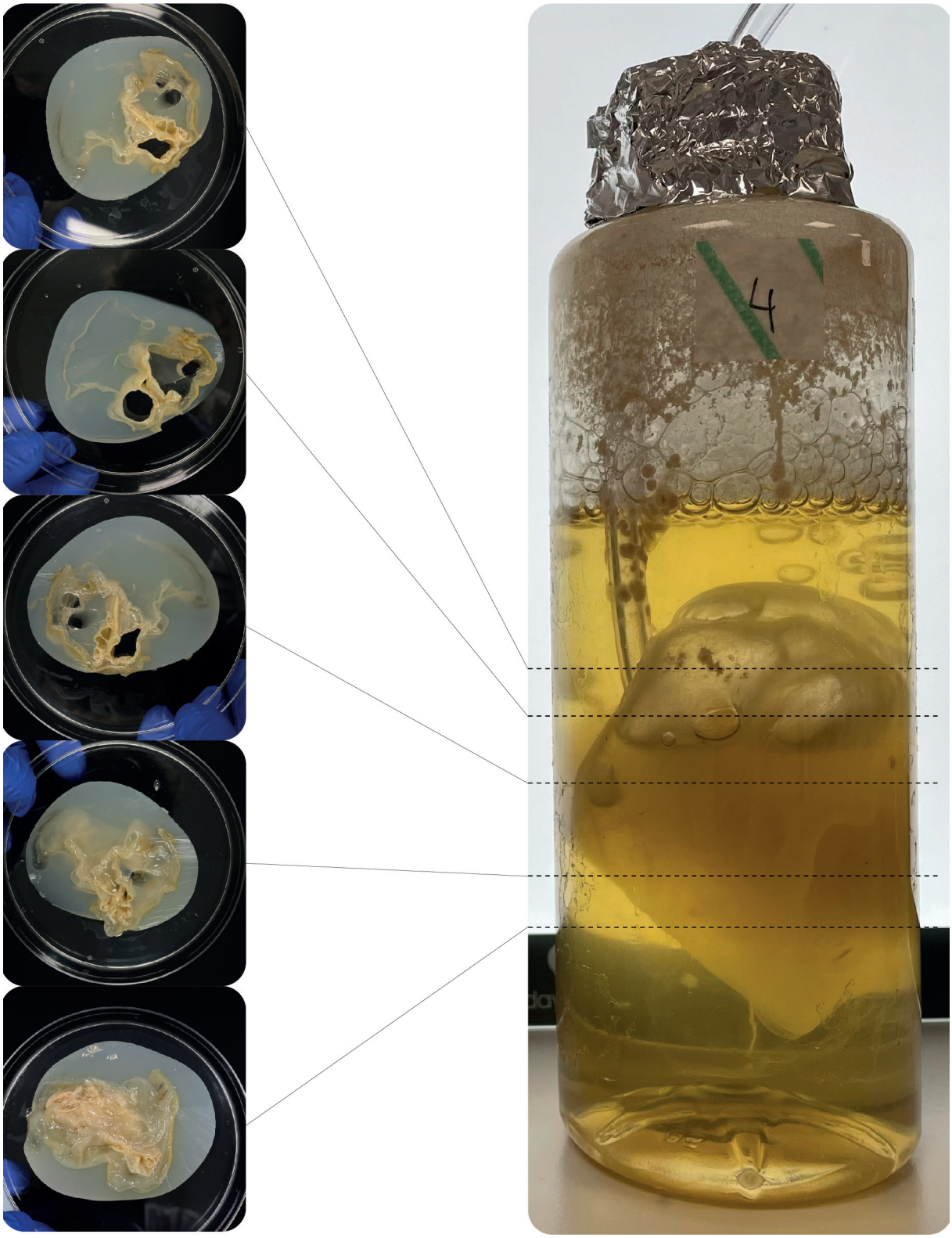


Figure 7.29: *BC cast in agar and horizontally sliced with indication of the location of each layer in the previously BC shape prior to harvest*

5.4.3), which results in a near identical density to the medium surrounding it and the liquid trapped inside different cavities of the shape. For this reason, additive are required to enhance the visibility of the BC (Shi *et al*., 2022). Iodine is a possible staining agent for CT imaging. However, BC is not receptive to iodine staining due to not containing complex helices that are able to hold the iodine molecule (Barrington & Barron, 1960) and remains a white-yellow to translucent colour (Figure 7.23). While methods of increasing the BC density exist, such as the integration of metal nano-particles or other radiopaque materials (Gupta *et al*., 2023), this in-depth exploration was outside the scope of this thesis.

In summary, the two explored methods of preservation enabled the documentation of specific characteristics of the complex BC shapes but the requirements for each material preparation prevented the holistic documentation of the interior and exterior of a single sample. In addition, the results of each preservation method were influenced by the sample's maturity and morphology, which makes a direct comparison of results difficult and instead offers multiple hypotheses for successful documentation.

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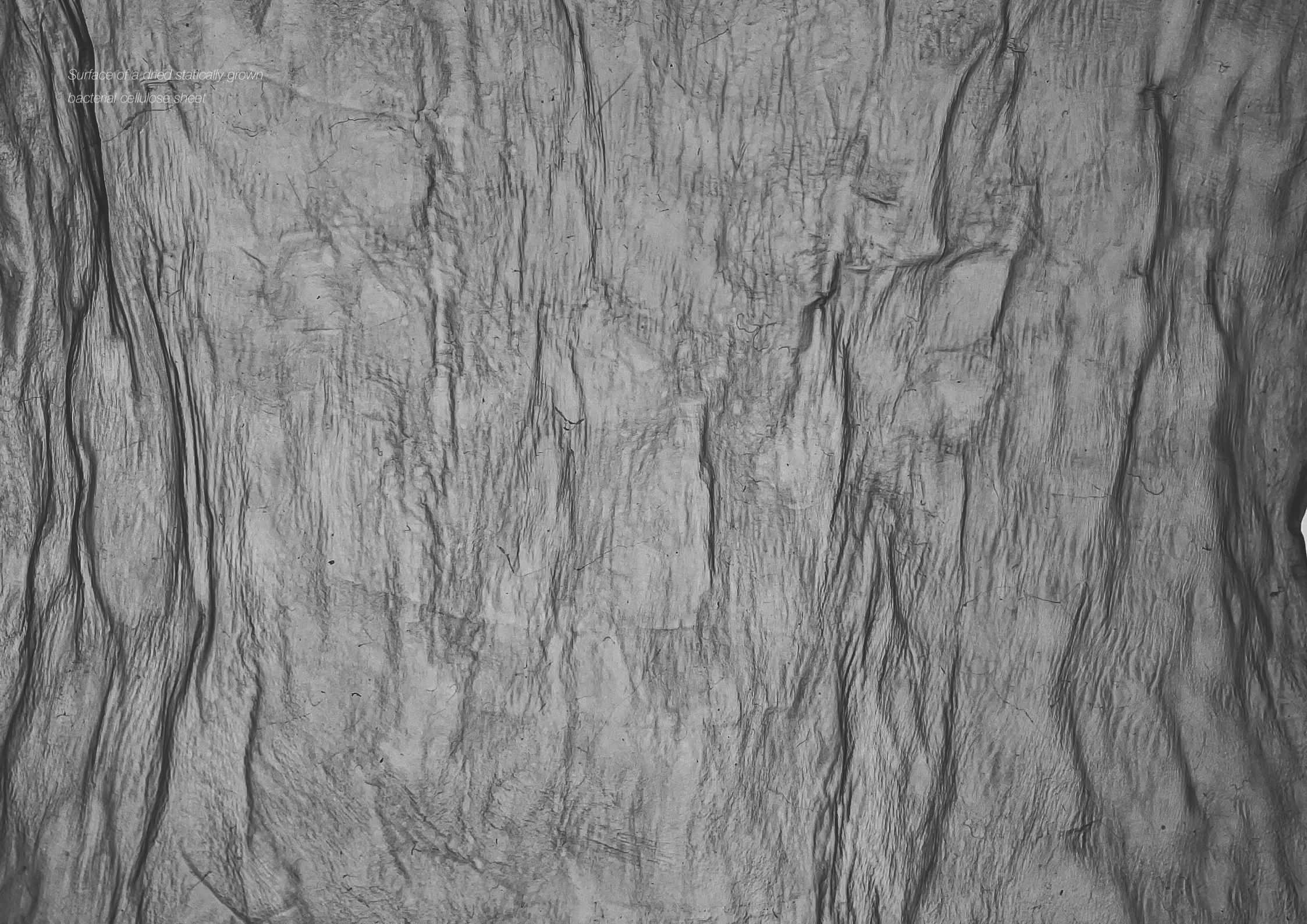


Surface of a dried statically grown bacterial cellulose sheet

CHAPTER 8

DESIGN PERSPECTIVES & CONCLUSION

Chapter Summary

In this chapter, the discussion steps back to look at the broader challenges in Biodesign with living systems that were identified during the experimentation with BC and highlights how the developed research approach can facilitate a structured but flexible investigation to mitigate some of these challenges.

The first part discusses the key challenge of unpredictability when working with living systems due to their inherently complex nature of underlying chemical, biological, and physical mechanisms. The different aspects contributing to the degree of unpredictability are summarised together with possible approaches to mitigate or control their extent. Furthermore, the implementation of the methodological approach developed for this thesis is broken down into three parts to analyse how it allowed a systematic and rigorous but simultaneously explorative and open-ended research. Lastly, the developed design parameters to guide the fabrication of 3-dimensional BC are summarised and the extent to which they can predict the morphological outcome is discussed.

The second part of the chapter suggests the presence of digitality within living systems and elaborates on how this digital component can be defined. It emphasises the role of non-standard communication between human designer and the organism through the principle of persuadability. This part concludes by introducing the term 'biodigital' to highlight the integration of the agency of living systems into data sets for digital additive fabrication and to further define the work of this thesis in the broader field of biological fabrication.

The last part concludes the research of this thesis. It recaps the aim, research questions and objectives that guided the research development and summarises the work, before going into detail about the key contribution it offers to the Biodesign community. Lastly, limitations of the research are briefly discussed, and the chapter concludes with a future perspective.

8.1. (Un)Predictability of Biomaterials

The approach to working with living biomaterials is inherently different to existing material practices for non-living materials and the development of new methodologies has been established as a key challenge in the field of Biodesign. The challenge lies in understanding the increased complexity of the living materials stemming from the organism's ability to exhibit its own agency through responsiveness to environmental and internal influences in real time (Armstrong, 2014). This agency can take on many different forms on many scales, such as cells secreting enzymes and plants orientating their leaves towards the sunlight (Fankhauser & Christie, 2015). BC is fabricated as part of the metabolism of certain bacterial strains or symbiotic cultures of bacteria and yeasts and secreted as individual fibrils which dynamically self-assemble into 3-dimensional matrices (see Chapter 2.1). The process of self-assembly has been defined as "the autonomous organization of components into patterns of structures without human intervention" (Whitesides & Grzybowski, 2002 p. 2418) and within the self-assembly behaviour lies a hierarchy of processes that connects the nano to the micro and to the macro scale. In Chapters 5.4 and 6.3 a multi-scale and mechanistic analysis is presented to establish the link between the observed growth behaviours of the BC on a macro scale and changed metabolic conditions for the organism on the micro scale. This deeper understanding of the hierarchy of the BC's self-assembly required an initial investigation of its natural growth behaviours before introducing various environmental triggers to evoke change, which are discussed as developed design parameters in Chapter 8.1.2.

Materials harvested from nature can exhibit variability in their materiality throughout which has to be acknowledged and can be used by the designer to guide the fabrication process. An example of this are natural features within wood, including the direction of grain, knots, colour differences, and growth rings, which make it heterogenous and also unique. Not only does this variability influence the application of the wood based on appearance, but it also influences how the material will behave as it ages exposed to different environment. Because humans have utilised wood for over 400,000 years extensive knowledge about its materiality and responsiveness exists today (Barham *et al*., 2023). This knowledge is summarised in material guides to teach new generations of craftspeople as well as applied in research, for example in the form of

wooden hygromorphs for natural ventilation in high-humidity locations (Hoadley, 1980; Pynirtzi *et al*., 2024). To achieve similar levels of sophisticated knowledge and control over living materials foundational research, such as Living Morphogenesis, are essential contributions. However, distinct differences exist between non-living heterogenous materials, such as wood, and materials created by living biological systems of organisms. The former has been devitalised to remove any kind of agency from it with the responsiveness directly linked to its now finalised material state, but thelatter is still in its living cycle with real-time responsiveness and the ability to adapt to their environment. While patterns in their responsive behaviour can be identified, a degree of unpredictability in their behaviour needs to be acknowledged (Uludağ, 2014). Multiple factors can contribute to this unpredictability, and the ability to minimise unpredictable behaviour often depends on the availability of resources. Biological variability is the first key factor and can exist within the organism. It refers to genetic and developmental differences that can occur due to mutation or varying ages of the cells. In BC fabrication, spontaneous mutation of the bacteria *K. xylinum* can occur through exposure to shear force which significantly reduces or fully eliminates its ability to produce cellulose fibrils (Krystynowicz *et al*., 2002). Each bioreactor presented in Chapters 5 and 6 creates a unique environment which may cause this mutation to occur during the growth period of the BC. Without time-intensive and regular testing of each culture to count the number of cell mutants present, the presence of equal numbers of cellulose-producing cells can only be approximated based on the volume of liquid inoculum used. Similarly to genetic mutation, the age of the bacteria within the culture plays a role in its reactivity and ability to produce cellulose which both decrease with increasing age (see Chapter 2.1.2) (Sushil, 2018). Within the SCOBY hotel, which is used to create the liquid inoculum for the experiments (see Chapter 4.1.2), a mixture of young and older bacteria and yeasts is present due to varying ages of BC pellicles used to create the culture. This implies that individual samples of inoculum taken can contain various percentages of young and old cells. Certain measures can be taken to reduce the age variability of the liquid inoculum, including regularly feedings the SCOBY hotel, fully exchanging the medium every 3 months, and waiting at least 6 weeks for the culture of a new SCOBY hotel to ferment before use (see Chapter 4.1.2). More nuanced differences in culture age, however, remain. For the aim of this research, however, these nuances in growth speed of the BC were of secondary importance compared to the occurrence of novel growth behaviours.

The second key factor driving the unpredictability of the living materials is the complex interconnectivity between the multiple biological systems in the kombucha SCOBY. It involves not only understanding the metabolism of the bacteria but also the yeasts', and how they mutually influence each other. While Chapter 2.1.2 provides an overview of the general understanding of the symbiotic interaction between the yeasts and bacteria, the specific microbial composition of a kombucha SCOBY can vary widely (La China *et al.*, 2021; Coskun & Kayisoglu, 2020). The age of the culture and the origin of the tea used for cultivation are two of many factors influencing what strains of bacteria and yeasts are present, which has led to new areas of interest in research regarding the specific function and role of each individual component of the SCOBY (Tran *et al*., 2020).

The uncertainty about the culture's composition also implies that the overall environmental sensitivity cannot be predicted with full accuracy as the reaction of each component to environmental changes remains unknown. This sensitivity goes beyond changes in ambient temperature that can be monitored during the fabrication process and includes subtle differences in light or chemical exposure, such as the pH of the medium (Aswini *et al.*, 2020). The sensitivity of the organism to oxygen and its influence on the metabolism also contribute to the unpredictable behaviour. While oxygen is one of the two key requirements for the bacteria to fabricate cellulose fibrils (see Chapter 2.1.2), limited research about its effects on BC yield exists (Hwang et al., 1999) and no quantification of the correlation has been documented in detail. For this reason, approximations are used to design the aeration scaffolds for the bioreactors presented in Chapters 5 and 6, which intentionally create variability of oxygen concentration within the medium and function as design parameter for the BC growth (see Chapter 8.1.2).

Establishing the desired function of the living biomaterial allows the designer to develop approaches to mitigate, or exploit, certain aspects of the unpredictability (Uludağ, 2014). Potential measures include creating highly controlled synthetic environments by integrating an array of sensors, working with single strain cultures, or conducting extensive preliminary studies to quantify environmental sensitivity. Minimising the unpredictability is essential for some applications of living materials, in particular in those fields linked to healthcare and biomedical sciences, where full control over the nano- and microstructure of the material are crucial for its biocompatibility with the host (Torres *et al.*, 2012). In contrast, the work of this thesis focuses on the manipulation on the

macro scale and the part of the material that is observable without magnification. The aim was not to control or manipulate the organism's inner metabolism, but to develop a holistic understanding. This understanding could then be used to create a workflow that leverages the organism to fabricate 3-dimensional BC while meeting its basic needs for survival. Nonetheless, the existing unpredictability on the nano and micro scale can translate into unexpected behaviours of the material on the macro scale and need to be acknowledged for the design process.

The principles of 'form follows function' and 'function follows form' represent two philosophies within the broader field of design that reflect on how to approach the creation of objects, or in the context of Architecture, how to design buildings and spaces. While the former can be described as a minimalistic inside-out approach in which the functionality and efficiency of the building are at the forefront, the latter values the aesthetic and appearance of the building and implements an outside-in approach with the functionality as secondary concern. The presented design approach for 3-dimensional BC follows neither principle. The aesthetic of the BC is not decided on by the designer but instead the result of a series of environmental manipulations interpreted by the living material-fabricating organisms. The biological process of developing form is coined 'morphogenesis' and is derived from the Greek words "morphḗ" (form or shape) and "génesis" (origin or creation). In a strict biological understanding it refers to "the molding of cells and tissue into defined shapes" (Roudavski, 2009, p.433) but because parallels can be drawn between nature's way of building and construction in architecture, such as a blueprint to direct assembly, the term is also used in an architectural context when generative digital tools are implemented to derive form (Roudavski, 2009). In addition, the term has gained traction in the field of Biodesign with living organisms to emphasise their multi-scale existence (Zolotovsky, 2017). Implementing the broader concept of morphogenesis has the ability to bridge between the 'form follows function' and 'function follows form' principles. It can emphasize a design approach equally driven by functional optimisation and inspired by natural forms through the integration of responsive feedback systems, such as living organisms (D'Uva, 2018). In this research, designing with morphogenetic principles went beyond the concept of bio-inspired and towards a material "with features un-matched in nature" (Uludağ, 2014, p.1) through the implementation of an explorative experimentation research approach.

8.1.1. Implementation of Explorative Experimentation

The methodological approach developed for this thesis (see Chapter 3.3) is based on exploration through an iterative process of interpreting parts within the context of the whole, and the whole within the context of its parts. Applied to Biodesign research with living materials, this translates into understanding the biological system in its entirety through a holistic view on its functionality on a macro scale while simultaneously developing an understanding of the inner workings of the organism on a nano- or microlevel. The methodological approach offers flexibility that allows for an intuitive development of the research which has been showcased in Chapters 5 to 7 through three different implementations, each choosing one aspect of the conceptual iteration circle as the most impactful (Figure 8.1).

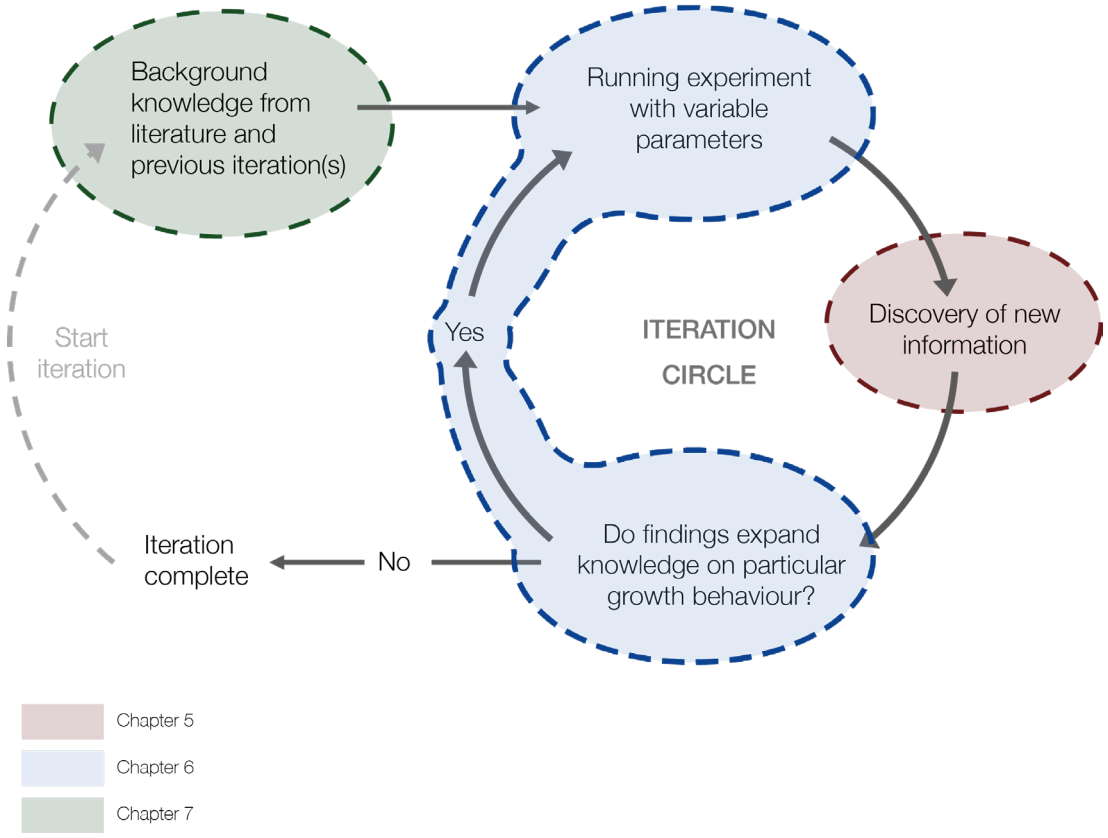


Figure 8.1*: Key aspects of the iterative methodology for the experimentation in each core chapter*

The aim of the initial experimentation (Chapter 5) was the exploration of various methods to create scaffolding that encourages the 3-dimensional growth of the BC, with a focus on three different scaffold materials identified through literature. One iteration circle was started for each material experiment to explore its compatibility with the organism. Instead of aiming to complete the iteration by achieving full understanding of the BC's growth behaviour in context to the scaffold, the aim of these experiments was to discover potential new branches of investigation. The discovery of new information about the BC's growth behaviour was collected in the form of questions about why the behaviour is occurring and how the behaviour could be further developed into a design parameter. This discovery was utilised as tool to compare the three material options and identify the one with the most potential for further in-depth exploration, based on trans-disciplinary literature review of existing BC fabrication methods and experimental composite materials. The questions of the chosen scaffold material of air then formed the background for the iterations of the next phase of the research.

The second phase of the experimentation (Chapter 6) explored the extent to which air can be utilised as design parameter to guide the BC growth through porous and hollow scaffolding and was developed in two parts. The first part focused on completing iteration circles to answer the questions compiled about the BC's behaviour. The second part was dedicated to the development of design parameters to achieve a level of control in the form emergence of the BC (see Chapter 8.1.2). The completion, or near completion, of iterations was at the core of both parts. However, the overall aim to work within the constraints of solely manipulating the physical environment of the organism, and not its internal mechanisms, occasionally resulted in branching of the iteration acknowledged for future works. This phase almost exclusively utilised information and ideas generated within the immediate or previous iteration circles for further refining of the experimental setups through which comprehensive knowledge of the organism's behaviour was achieved. The results of this phase were the identification of a set of repeatable and novel growth features of BC and methods of guiding the emergence of these features through specific characteristics of the bioreactor setup.

For the last phase of the experimentation (Chapter 7) the acquisition of knowledge from existing literature played a key role to identify methods of preservation for the novel forms of the

well-researched material. The additional aspect of form stability for the intended digital documentation was considered when two final material states, hydrated and dried, were identified. The iteration process was then implemented to develop the most suitable protocol for achieving those states through heuristic testing. Because the availability of comparable material samples to test protocols on was a clear limitation, due to each BC form grown during the second phase having unique features or material characteristics, the full completion of an iteration according to the hermeneutic principles (see Chapter 3.3) was not considered necessary. The possibility of multiple suitable protocols was recognised through open-ended branching of the iteration (see Chapter 7.3).

The developed methodological approach for the explorative experimentation of this thesis has proven as flexible and adaptable to the complexity of living material research. It provides a framework to develop an intuitive progression of the research by allowing the designer to learn about all aspects of the living organism while simultaneously pursuing the overarching aim with a level of systematic rigour. This allows the shift of design work into the scientific field not only through the adaptation of scientific methods but through aiding the targeted cross-disciplinary learning of the designer.

8.1.2. Design Parameters for 3-dimensional Bacterial Cellulose

The developed fabrication method for 3-dimensional BC through an aeration bioreactor moves away from traditional computational design and additive fabrication tools for non-living materials that aim for precise outcomes and repeatable results. Instead, a physical space to guide emerging form is created through a set of design parameters, established through the analysis of various growth behaviours (see Chapter 5.4.3 and 6.3). Through this approach it questions the "well-known mentality in architectural-design whereby materials are typically selected, not designed" (Oxman *et al*., 2010, p.72).

These design parameters can be divided into two groups based on when the designer implements them (Figure 8.2). Passive design tools are implemented to finalise the setup of the bioreactor prior to starting the growth phase, which creates the basis for the BC's growth environment. The following passive designer parameters were identified:

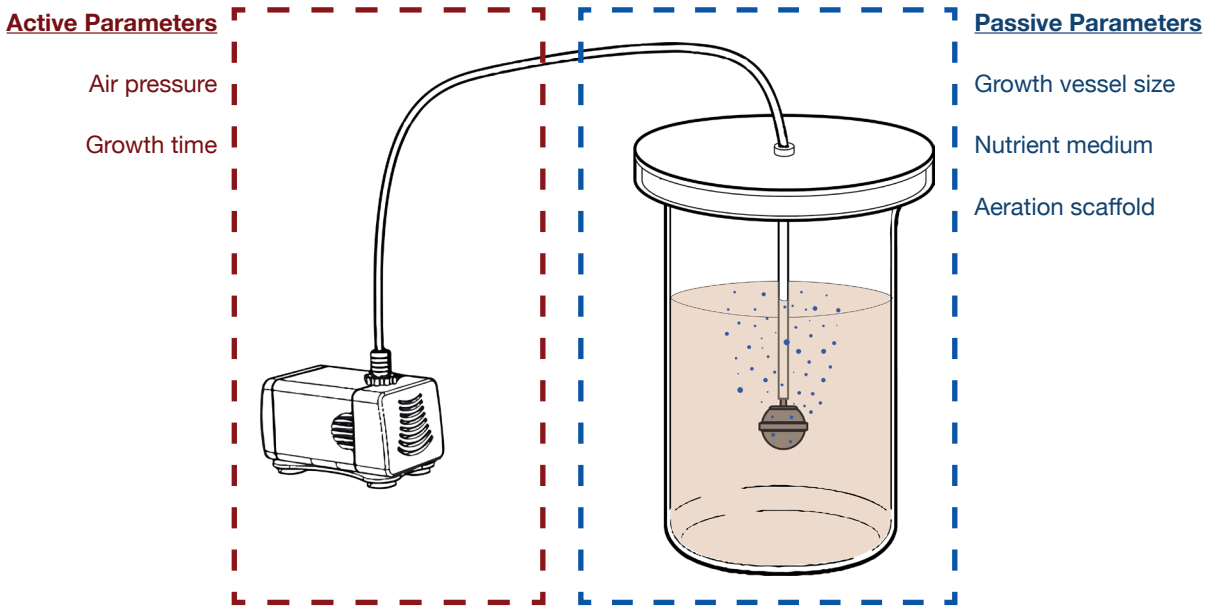


Figure 8.2*: Location of passive and active design parameters in bioreactor setup*

Nutrient Supply: The type of nutrient medium used determines the quantity of visible yeast growth and appearance of the BC. A tea-based medium results in dark orange BC with defined outlines and visible brown yeast strings, while HS medium results in thinner white yeast strings and light-coloured BC with less defined outlines. The choice of nutrient medium also decides the level of sterility required for the experiment which implies an upscaling limit (Exp. H).

Growth Vessel: Referring to the dimensions, shape, and volume of the vessel in which the BC is grown. This vessel holds the nutrient medium which is transformed into a 3-dimensional space of higher dissolved oxygen content through the Aeration Scaffold (see Chapter 6.3.1). Inner geometries of the vessel, including sharp corners, rounded edges, and foreign objects attached to the wall, function as negative mould for the BC as it grows and expands to the edge of the vessel. Depending on whether the vase is more tall or wide, it determines the growth area for the BC as either vertical or horizontal (Figure 8.3).

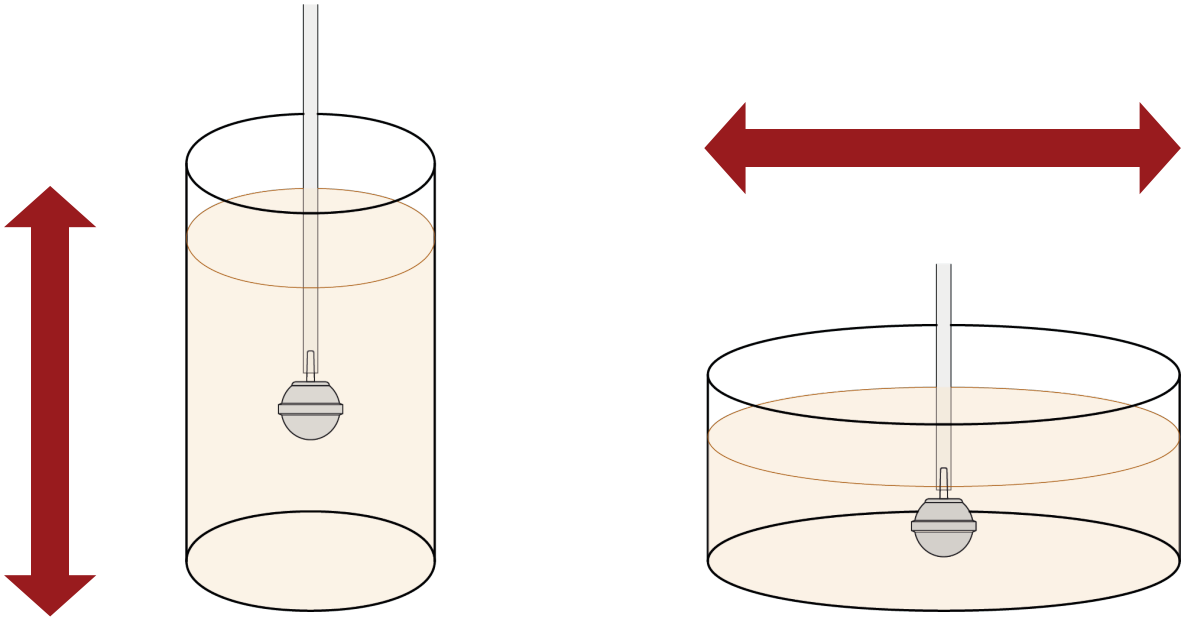


Figure 8.3*: Comparison of BC growth volume expansion in tall and in wide growth vessel*
Figure 8.4: (right): *Comparison of the emergent BC forms grown inside bioreactors with HS medium and various different aeration scaffold (Exp. p & Q).*

Scaffold Material
> agave bioplastic
> silicone

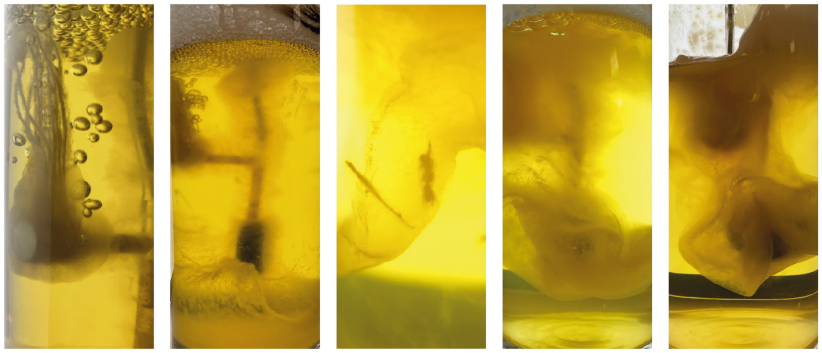
Growth Period
19 days

Scaffold Material
> PLA print
> agave bioplastic
> porous stone
> silicone

Growth Period
20 days

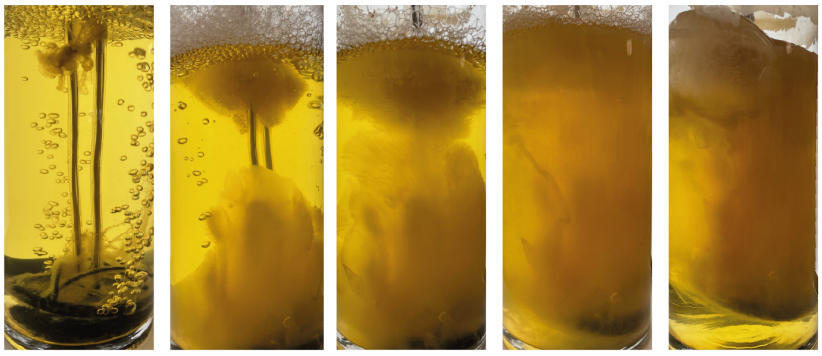
Scaffold Material
> PLA filament print
> agave bioplastic
> porous stone
> silicone

Growth Period
23 days



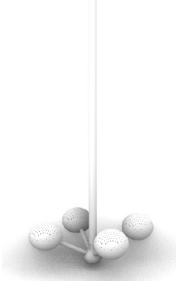
Scaffold Material
> ABS filament print

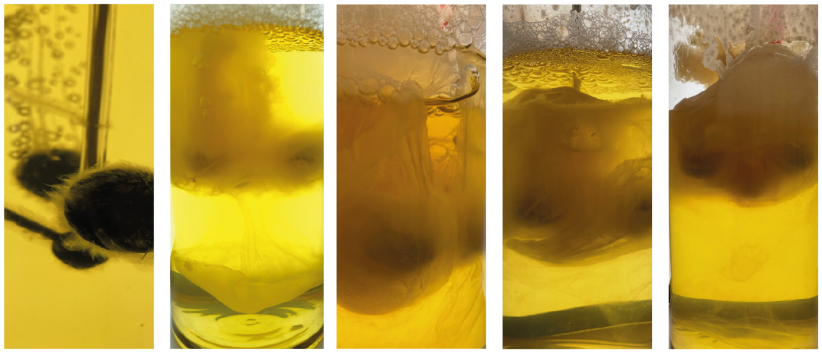
Growth Period
21 days



Scaffold Material
> ABS filament print

Growth Period
25 days





Aeration Scaffold: This element of the bioreactor is the most influential on the geometric development of the BC (Figure 8.4). The materiality of the scaffold determines the ability of the BC to attach to it during the submerged growth and stay attached during the harvest. The form of the scaffold directs the distribution of dissolved oxygen through the movement of air bubbles within the overall volume of the medium, which directly influences the organism's ability to produce BC (Figure 8.5). The size and distribution of the pores influences where yeast flocculation occurs during the early stages of growth which decreases airflow and reduced the BC maturation in those area. The position of the pores also makes them more or less receptive to the active design parameter of air pressure. Lastly, the design of the scaffold determines how much or little rigid structure will be left within the BC once harvested, due to being considered a lost scaffold.

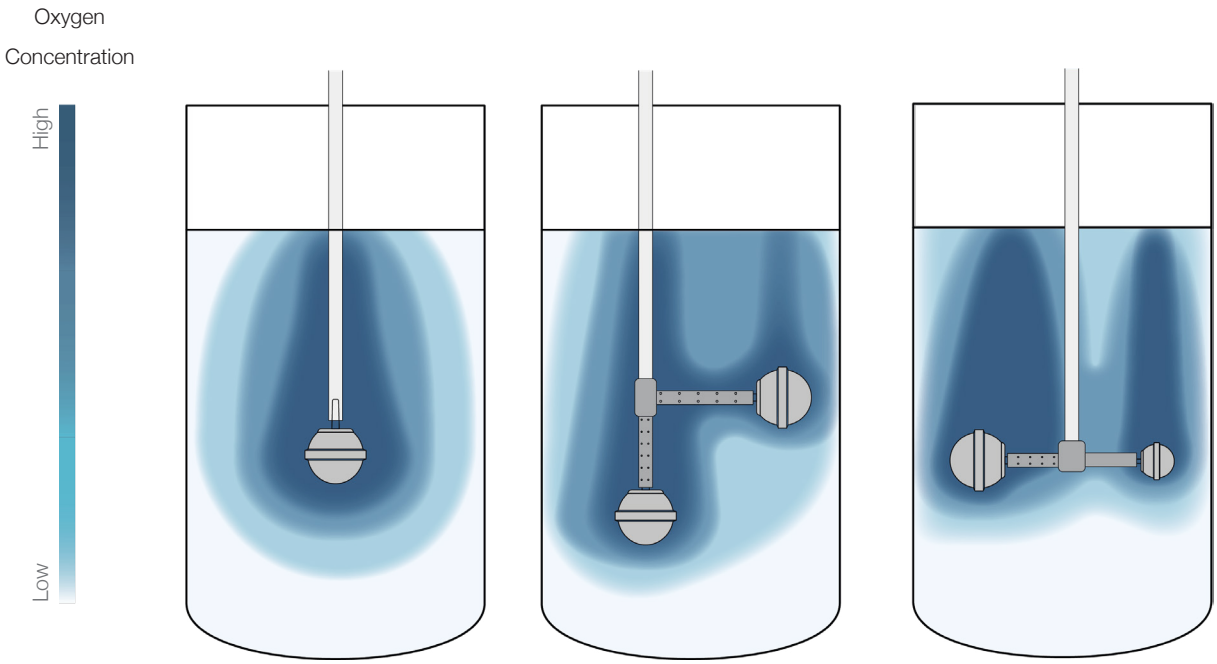


Figure 8.5*: Predicted varying oxygen concentrations within the medium based on the shape of the aeration scaffold and the produced air bubbles, ranging from high oxygen concentration near the scaffold and low concentration toward the edge of the growth vessel (not considering the oxygen at the natural air-liquid interface at the surface).*

One direct and one indirect active design parameter were identified for the guidance of emerging BC forms:

Air Pressure: The force with which the air is escaping from the aeration scaffold has direct influence on the degree of disturbance that occurs in those areas as well as the availability of oxygen and is connected to the position and size of the pores. Areas of high disturbance show delayed BC attachment and increased yeast flocculation which is linked to the metabolic process of the kombucha SCOBY (see Chapter 2.1.2). The presence of strong airflow from particular areas of the scaffold also enhances the probability of bubble pathways forming in later growth stages (see Chapter 6.3). A sudden high increase of air pressure can unblock pores in some areas of the scaffold to trigger bubble pathway formations. The timing and intensity of air pressure changes decide on the effect they will have on the BC growth, with increasing time having decreasing effects.

Growth Time: The timing of the harvest, which ends the growth stage of the BC is an indirect design parameter that the designer can utilise to determine the maturity of the final BC form. Extended growth time results in more mature BC which contracts in shape and pull the individual growth features together spatially. It also prolongs the period of flat BC growth on the undisturbed surface which influences the final form especially in the harvested state (Figure 8.6).

While these developed design parameters enable a prediction and degree of guidance for the morphological emergence of the growing BC and the presence of distinct growth features, the agency of the organism cannot be predicted to the same extent to which geometric code (G-code) determines the material deposition of a 3D printer. The reason for this is that “design principles that form biological materials often result in non-linear responses of stress to strain, or force to displacement” (Patteson *et al*., 2022, p.1) and only very limited theoretical understanding for the nonlinear behaviour exists to date. An example of nonlinear behaviour in the context of this

research is the formation of spontaneous secondary air scaffold created through bubble pathways or air reservoirs. This can enhance material maturation in unexpected areas towards the outer edge of the growth vessel and away from the intentional aeration scaffold. Digital measures could be integrated into the bioreactor setup to collect observational data, such as sensors to measure the pH and dissolved oxygen content in specific areas within the growth vessel. However, while the additional data can enhance the knowledge about the influence of the aeration scaffold on the oxygen distribution, the possibility of spontaneous behaviour of the organism would remain.

Out of the identified parameters, the dominant factor influencing the morphology is the aeration scaffold through the artificial air-liquid interface it creates through the air bubbles. This is followed by the growth time which dictates the maturity the BC can achieve throughout the fabrication process. Because immature BC has little to no attachment to the scaffold, meaning that it cannot be harvested to expose its full morphology (Figure 6.64 & 8.7), there is a threshold of minimum growth time that needs to be passed. This threshold, however, cannot be defined

Figure 8.6*: Comparison of harvested BC forms with increasing thickness of BC surface pellicle with increasing growth time.*

as one fixed value and instead depends on many factors influencing the fabrication setup as well as internal mechanisms of the organisms, linking back to the predictable unpredictability of living materials (see Chapter 8.1).

The design parameters function as a one-directional communication link from the designer to the organism to directly influence its natural process of cellulose fibre secretion and its assembly into a material. Communication from the organism to the designer, however, is indirect and requires interpretation from the designer. The development of an interpretation guide and intuitive understanding of the organism is an essential part of the research and requires repetitive experimentation (see Chapter 8.1.1). In this research, the presence of air, visible in the form of bubbles or the lack thereof, is a visualisation tool to bridge between the micro scale of the organism and the macro scale of the designer. It symbolises the presence of both required conditions for BC growth (see Chapter 2.1.2) and allows the designer to predict the productivity of the bacteria in its proximity. This is unlike other non-invasive approaches to Biodesign with complex multicellular organisms, such as plants, which can be trained to respond to stimuli through associative learning and show direct agency (Gagliano *et al*., 2014). More recently, similar adaptive behaviour has been discovered in slime moulds, which indicates that habituation of unicellular non-neural

Figure 8.7: *Example of immature BC detachig from the scaffold during harvest from Exp. P. (Left to right) Visible BC growth on day 11, harvest on day 18, the frozen BC form, and a digital render of the 3D scanned morphology*

organisms is possible (Boussard *et al.*, 2019). Likewise, genetic modification of the bacteria or yeasts to produce more direct signals, such as colour changes or bioluminescence in response to oxygen, are a possibility (Baronian, 2004; Rogers *et al.*, 2016). These biosensors within the SCOBY could give the organism the ability to communicate more directly with the designer and signal its needs. This ability can strengthen the inter-species co-designing approach of humans and living organisms, leading to a stronger sense of predictability of the nonlinear behaviours (Camere & Karana, 2018).

8.2. Bio(digital) Fabrication

The term ‘biodigital’ is not a new invention and can be found across fields to describe hybrid futuristic processes combining aspects of the digital and the biological world (Cruz & Beckett, 2016; Mahmoud Mohamed Gomaa Ahmed, 2015; Petersen et al., 2018). Similarly to the use of biological fabrication, a level of terminological inconsistency exists in literature for the term ‘biodigital’. In a strict sense, the word ‘digital’ refers to “electronic technology that generates, stores and processes data in terms of positive and nonpositive states” (Yasar, 2023, para.1), with the latter referring to the binary language of only using the digits 0 and 1. In a fabrication context, ‘digital’ more loosely refers to the translation of objects designed in a digital 3-dimensional space into a language that can be processed by machines, which then execute the physical making of the object (Keating, 2016). Yet another more nuanced definition of ‘digital’ can be justified when placing it in the context of biological fabrication (see Chapter 1.1.2.).

The information that drives biological fabrication, can lie in three different domains, in silico, in vitro, or in vivo, and one fabrication process can draw from multiple domains simultaneously. In vivo refers to those processes that occur within the organism on a microbiological scale, including their metabolic functions as an individual cell or as part of a community (Dade-Robertson, 2021). This implies that the living organisms contribute to or are the sole supplier of the instructional data of the fabrication process. This assumption attributes the living organism a degree of autonomy, and the expression of this autonomy is foremost through a responsiveness to environmental stimuli. The responsiveness of organisms can be likened to digital computational agents, which are defined as entities capable of perceiving their immediate environment through sensors and autonomously acting upon it to achieve specific goals (Liu, 2001).

Alan Turing identified a ‘natural computing’ of living organisms, suggesting that they hold an inherent genetic code which influences how they will react to changes in their chemical, biochemical, and physical environment. In his definition “computation is not just a language of nature; it is the way nature behaves” (Dodig-Crnkovic, 2013, p.115). In computational multi-agent systems, the individual agents exchange certain information and can learn new behaviours or functions to attain a common goal. (Liu, 2001). Similarly, Davies & Levin describe “cell collectives as agential

material, with their own goals, agendas and powers of problem-solving" (Davies & Levin, 2023, p.46). In both systems, the concept of emergent behaviour exists based on the continuously developing complexity of the individual agents within the system (see Chapter 1.1.1).

Bringing the aspect of computing and digitality back to fabrication processes, the question of how the genetic code of a living organism can actuate the fabrication of a physical material arises. It is here where a clear distinguishment between digital and biodigital fabrication should be made. While conventional digital fabrication relies on the use of external agents and machines to execute the human design idea, living biodigital systems have the ability to internally fabricate in response to stimuli without the reliance on external agents. This gives them the advantage of fast response times and better energy efficiency (Davies, 2014; Dade-Robertson et al., 2023). In a comparison of technical properties of machines and natural computers, Armstrong (2014) highlights further benefits of biodigitality, including the ability to run multiple processes in parallel and possessing variable states that allow a gradient of on and off (Table 8.1). Since publication of the comparison, however, the abilities of artificial intelligence are becoming increasingly more sophisticated and may eventually be able to replicate these biodigital advantages (Górriz et al., 2020).

Table 8.1*: A comparison between machines and natural computers. Reproduced from Armstrong (2014)*

Technical constituents of machines and protocells		
	Machine	Natural Computer/Protocell
Component	Object	Agent
Order	Series	Parallel
Power structure	Hierarchical system	Non-hierarchical
Functional system	Machine	Assemblage
Energy	Extrinsic	Intrinsic and extrinsic—spontaneous operations may be prolonged with resource supply.
Control	Hard	Soft
Transformation	Binary—on/off	Variable states. Generally conservative but may behave unpredictably and collapse or transform at tipping points
Points of influence	Internal	Internal and external (environmentally sensitive)
Ability to form networks	Insular. Creates barriers between systems	Facilitates technological convergence by building networks of exchange

Based on the preceding argumentation, the term ‘biodigital’ can, in the context of the work of Living Morphogenesis, be defined as followed: The acknowledgement of the contribution of the biological living system to the data set that facilitates the fabrication of 3-dimensional BC forms through its emergent functionality, agency and ability to interpret environmental stimuli (see Chapter 1.1.1) that are provided by the human designer in the form of a synthetic growth environment. It differentiates between the mere exploitation of metabolic behaviours and the encouragement to exhibit novel expressions or the implementation of said behaviours. It implies a level of communication, or soft control according to Armstrong (2014), between the human and living system to create a co-designed fabrication process that aspires towards the goal of bringing together the computational design, its fabrication and the material development (Oxman et al., 2010).

The fabrication of BC as material on the macro scale can be compared to that of additive fabrication (AF). In the case of BC, the individual fibres assemble into a 3-dimensional matrix as they are being produced by the bacteria, with layering occurring towards the oxygen-facing side of a statically growing pellicle (see Chapter 2.1.2). In AF, 3-dimensional forms are created by successively adding material layer by layer, building up an object from the ground using an existing material, such as plastic (Keating, 2016). For the user to communicate with the AF machine, a specific type of geometric code (G-Code) needs to be written to translate the digital 3-dimensional design of the desired output object into instructions that the machine can process. The translation of these instructions, including coordinates, extrusion rate, and speed of movement, occurs within the AF machine through specific programming of motors which deposit the material with high accuracy (Garcia Cuevas & Gianluca Pugliese, 2020). Once the fabrication has started, changes to the coordinates usually require a re-slicing of the geometry and restarting the fabrication process. Similarly, the identified passive design parameters (see Chapter 8.1.2) fulfil the role of the G-code for the fabrication method presented in Living Morphogenesis. The passive parameters hold the core information about the physical output in the form of the bioreactor setup which creates the space for the material fabrication to occur within. Rather than direct coordinates that function as blueprint, however, the “morphogenetic mechanisms [of the organism] are more goal-seeking than blueprint following” (Davies & Levin, 2023, p.47), meaning that the designer creates boundaries for the biodigital fabrication to occur within. These boundaries can range from

tightly to lightly restricting, which directly influence the predictable accuracy of the final output, with consideration of a remaining degree of unpredictability (see Chapter 8.1). Changes to these boundaries, such as increasing the growth volume or changing the aeration scaffold, similarly require a rebuild of the bioreactor and restart of the fabrication. Because the living organism exhibits emergent behaviours (see Chapter 1.1.1) that contribute to the final output of the fabrication process, this biological G-Code is in part created by the human through the bioreactor setup and in part by the living organism through its interpretation of the provided environmental parameters.

While the fabrication of cellulose may or may not be an involuntary process of the bacteria's metabolism, the organism holds control over its localised occurrence and is able to halt it or enhance it through its "innate computation and […] homeostatic agendas" (Davies & Levin, 2023, p.47). In particular the control over the state of the fabrication, on/off or on a gradient thereof, is the organism's contribution to the fabrication code that is otherwise written by the designer through the provision of a synthetic environment. This implies the existence of a co-designing process because neither the designer nor the organism is able to achieve the fabrication results without the contribution of the other. Undoubtedly, it is the human designer that initiates the fabrication and incentivises the organism to participate. In this research, the concept of persuadability was implemented to harness the biological intelligence without the need of invasive manipulation through synthetic biology (Levin, 2022). Michael Levin describes persuadability as the "conceptual and practical [tool] that [is] optimal to rationally modify a system's behaviour" (2022, p.3). The persuadability of the organism researched in this thesis is summarised in Chapter 8.1.2 and presented in the form of design parameters that convey a sense of programmability of the biodigitality.

The imminence of this work to architecture does not lie in the potential future application of the 3-dimensional BC forms as building material, but rather in the way that it challenges formal architectural design approaches based on mathematical equations and computational parametric design for high precision outputs. Living Morphogenesis positions itself opposite these approaches and proposes a design process in which a new form of digitality is present and biological systems are co-designers that enhance the design through their ability of fluidly adapting morphology and materiality in response to external and internal stimuli.

8.3. Conclusion

The research in this thesis set out to develop a novel fabrication technique of BC that pushes the boundaries of the material's natural growth behaviour of forming flat sheets. The aim was to fabricate complex and solid 3-dimensional BC forms and to develop a set of design parameters to guide the emerging material form through interventions in its physical environment only. The research approach taken acknowledges and values the living organism as active design collaborator to the human designer due to its expression of agency through responsive, adaptive, and also unpredictable behaviours. The exploration of this agency was at the core of this research and formed the foundation to answer the two research questions of 'how to manipulate the physical environment to alter the organism's growth behaviour' and 'what level of control can the implementation of a designed synthetic environment achieve for the prediction and guidance of the emerging BC form' (Table 1).

The first part of the research (Chapters 2 to 4) focused on compiling the theoretical background of the biological mechanisms occurring within certain bacteria and yeasts that enable them to fabricate the physical material of BC on a macro scale, in addition to summarising existing commercial, artistic, and research-based fabrication methods for this living material. Through this comprehensive summary, it accomplished the sole initial objective and established the current gap in research regarding the controlled fabrication of BC into 3-dimensional forms. Furthermore, it developed an interdisciplinary research approach that facilitates a structured but adaptive exploration into the agential expression of morphogenesis by acknowledging the organism as co-designer. Lastly, the foundational methods to working with the biological matter of kombucha SCOBY are outlined, together with variations of each that were implemented in the next part.

The second part (Chapters 5 to 7) demonstrated the flexible implementation of the developed methodological approach resulting in an intuitive progression from broad concept exploration to the discovery of in-depth growth behaviours of the organism. The physical experimentation began with a broad view on scaffolding methods utilising solids, such as inert and other living materials, and gases, particularly atmospheric air. Air was identified to have the strongest potential for guided morphological development of BC and was systematically explored through the

development of an adaptable bioreactor. This bioreactor creates a synthetic growth environment which generates novel growth behaviours of the organism, presented in the form of complex 3-dimensional BC forms. A progressive identification of objectives based on gained insights continuously adjusted the direction of the research towards its overarching aim. The final element of the explorative experimentation was the identification of methods to preserve, document, and analyse the complex form and materiality different material states, hydrated and dried.

Table 8.1: *Overview of Research Questions, Objectives, and relevant Findings*

Research Question	Objectives	Results
How can the growth environment of BC be manipulated to alter the natural fabrication behaviour of the organism to fabricate the material into complex 3-dimensional forms?	1) Summary of comprehensive understanding of existing BC fabrication methods	Chapter 2: Literature review of commercial, artistic, and research-based fabrication processes
	2) Systematic approach of exploring and analysing behavioural responses of the organism	Chapter 3: Development of iteration-based research approach for interdisciplinary work
	3) Finding different methods of scaffolding for shaping the BC growth	Chapter 4: Identifying core lab methods for growing BC Chapter 5: Testing of three different scaffolding materials (fabric, mycelium & air)
To what extent can the implementation of a synthetic growth environment guide and predict the characteristics and emerging morphology of the BC?	4) Analyse patterns in BC growth behaviour with scaffold to identify design parameters	Chapter 6: Development of bioreactor with adaptable parameters to guide the BC growth
	5) Methods of documenting and analysing the final BC forms	Chapter 7: Testing hydrated and dried preservation for BC to document the complexity

8.3.1. Contributions

This research offers contributions to the wider field of Biodesign with living materials and to the development of BC materials in three specific ways:

1. by providing a methodological approach for the systematic and explorative investigation of the inherent agency of living materials;
2. by demonstrating the value of research without predetermined objectives for the design with emerging complexity, and;
3. by developing a novel fabrication method for 3-dimensional BC.

These contributions are the result of an interdisciplinary research bringing together methodologies of creative design practices, material science, and microbiological concepts to identify the inherent morphological capabilities of cellulose producing bacteria. The contributions can be thematically grouped:

A. Explorative Biodesign Methodology

The main methodological contribution to the broader field of Biodesign with living materials is the development of a novel research approach that offers a framework for the systematic and simultaneously adaptive exploration of living materials. It challenges the traditional perspective on working with materials on a macro scale and offers a new angle to Biodesign material research with non-engineered organisms. The methodology is based on iterations which are developed through transdisciplinary literature review on the organism's metabolic processes on a micro level and design approaches for open-ended exploration. Feeding the results and findings of previous iterations into the development of new iterations enables a fluid direction of the research that encourages the discovery of previously unknown phenomena. The methodology proposes research without predefined objectives which progressively identifies research goals through the completion of experimental iterations. This is possible due to the continuous generation of hypotheses which is a part of the iterative exploration, creating new branches of investigation.

The methodology offers a unified language for exploring living materials, providing a common set of principles that facilitate the communication among researchers across different disciplines. These principles help break down intuitive, complex, and creative design thinking into smaller, more comprehensible units, leading to clear and comparable outcomes. This also enhances the reproducibility of the explorative research. Additionally, the methodology serves as entry point for novices in the field of Biodesign, enabling them to begin unrestricted but systematic research with any living material that exhibits its own agency. It emphasises the equal importance of scientific background and novel exploration.

B. Novel Fabrication Method for BC

The main applicable contribution is a novel fabrication method for BC, developed as case study for the implementation of the above mentioned Explorative Biodesign Methodology. It proposes 3-dimensional growth of BC as fabrication method with the living material and positions itself clearly apart from existing fabrication methods which rely on flat 2-dimensional growth and material post-processing for shaping. The fabrication process places the organism's natural growth behaviours and metabolic functions at the centre of the fabrication process and offers a set of design parameters implemented through a bioreactor to guide the emergence of the material form. The information feeding the fabrication is shared between the organism and the synthetic environment created by the human designer. This enables the organism to exhibit previously unknown self-forming behaviours and uncovering inherent computational properties for responsive form-finding.

The fabrication bioreactor creates a synthetic growth environment for the BC. It consists of a growth vessel with inoculated nutrient medium, a suspended aeration scaffold, and an air pump with adjustable air pressure that feeds into the scaffold. Each of these elements guides different aspects of the BC formation and represents an adjustable design parameter. These parameters are either passive, creating the boundaries for the form emergence prior to starting, or active and relay guidance to the organism in real-time. This creates a predominantly hands-off and self-operating fabrication process for the human designer, with the implication that partial control over the final form design is given to the organism.

The fabrication method contributes to the fundamental research of BC growth and its potential for agential expression of morphogenesis without genetic modification of the organism. The setup of the method also enables further experimentation with BC to research specific characteristics or behaviours through an adaptable design. Overall, the fabrication method steps away from a common concept of Biodesign that utilises biology to find form and instead takes a teaching approach to designing with emerging complexity that is unpredictable and mostly unexplored.

8.3.2. Current Limitations

The presented material exploration is not indended to translate directly into commercial application of BC in the built environment but instead to challenge the current architectural paradigm of parametric and algorithm-based design. However, the relatively short longevity of the BC was a limiting factor in the preservation and documentation of the emergent morphology. The high hydrophilicity of BC in particular poses a challenge by making the freeze-dried BC highly receptive to environmental humidity, requiring airtight storage or further treatment to maintain its form. Both the preservation in hydrated and freeze-dried states (see Chapter 7.1 & 7.2) only offered short-term solutions, diverting the preservation from the physical to the digital realm through 3D scanning and photography. To gain a complete picture of the interior and exterior structure of one sample, more extensive research into the material qualities and ways of preserving the BC are necessary to develop methods that are complementary rather than mutually exclusive.

The behaviours of novel living organisms cannot be compared to those of inert materials due to their agential and heterogeneous nature that results in emergent behaviours. Despite being able to make assumptions about the final morphology of the BC grown inside the bioreactor based on an array of catalogued observations, each sample was unique and not replicable even under identical growth conditions. This implies that making clear comparisons between samples remains challenging, leaving room for speculation. This research cannot claim to have uncovered a comprehensive understanding of the organism's intrinsic agency, but rather offers a directed and subjective insight into the occurrences on the macro scale and their hypothesised underlying microbiological principles.

BC material research, or more generally living material research, currently lacks a standardised method of data collection, presentation, and comparison due to the high complexity and diversity of the organisms involved and their varied emergent functionalities. This is particularly true for living materials fabricated outside of controlled laboratory spaces not intended for medical application. To advance the development and eventual implementation of these new materials into applications, developing frameworks making the data more coherently accessible is necessary.

Lastly, a major challenge in the progression of this living material research is the upscaling

of the material. Access to facilities that enable the implementation of unconventional methods while offering clean and sterile environments and equipment that can operate with large volumes of medium, substrate or vessels is currently limited. To help make the jump in scale and develop the field of Biodesign further, setting up dedicated Biodesign fabrication labs or adapting existing lab facilities will be necessary.

8.3.3. Future Perspective

Drawing on the findings from this case study of developing fabrication methods for BC, incorporating living organisms and their agency into the material design process necessitates a thorough rethinking of traditional design approaches to accommodate a degree of uncertainty and unpredictability. However, continuing to question the inherent morphogenetic behaviour of organisms broadens our understanding of possibilities for future applications of these new materials. Arguably, the discovered material potential of BC goes beyond imminence in architecture. It offers knowledge on the shaping of BC to the biomedical field utilising the intricate cellulose network for tissue engineering of organs, to textile practices for grown-to-shape apparel, and the consumer goods sector for customised biodegradable packaging material.

During the experimentation phase, a multitude of promising further directions for the development of the presented BC 3D fabrication method were discovered. A continuation of the direction presented in Chapter 6 could be increasing the complexity of scaffolds by intentionally shaping airflow, such as varying pressure zones or incorporating larger air bubbles, to provide greater control over the BC form. Building on that, observations during the viva exhibition setup also suggested that temperature might influence BC growth behaviours, warranting further investigation. Additionally, introducing additives before the BC is harvested may help stabilize its form during air-drying, preventing deformation and improving the longevity of the material. These additives could be fed into the material using a reusable multi-purpose scaffold, which might also allow for localised nutrient distribution to the interior of the structure, potentially giving the human designer more control over emerging morphology. Another direction involves the exploration of dissolvable scaffolds which slowly release nutrients throughout the growth period of the BC. While initial tests with sugar indicated potential, alternative biodegradable materials could be explored. Lastly, the technique of fruit moulding suggests that the vessel shape could play a crucial role in defining the maximum expansion of BC, whether through the outer glass vessel or a secondary internal scaffold made of porous material.

In the age of digitalisation and artificial intelligence, new possibilities for the handling and processing of data are available. This thesis presents a fabrication process and provides the

necessary methods to set up a bioreactor that facilitates the formation of 3-dimension BC and to document the emergent exterior and interior form. As a next step, a more sophisticates prediction tool could be developed based on large sets of data collected through the implemented fabrication process. This could entail the training of computer software to aid the design process and strengthen the biodigital aspect of the fabrication and further harness the responsiveness of the living system: a software, much similar to current Computer Aided Design (CAD) and slicer software for 3D printing, taught through machine learning to identify the ideal bioreactor setup for a pre-defined BC form or to predict the BC form based on pre-defined bioreactor design parameters. In addition to potentially enhancing the efficiency of the fabrication process by providing more accurate predictions of the emerging form, a visual interface would support the accessibility of the process to a wider audience not familiar with Biodesign methods.

The field of living materials is gaining momentum and will certainly increase in relevance as the built environment moves towards a sustainable future. Fundamental research with a variety of organisms is a key aspect to this mind shift. In addition to this research, other examples for living material explorations on a macro scale include the theses of: Elise Elsacker with mycelium (Elsacker, 2021), Thora Arnardottir with microbially induced calcium carbonate precipitation (Arnardottir, 2024), Dilan Ozkan* with parametric mushroom formation, and Emily Birch* with bacterial spore hygromorphs. These works show that the concept of living morphogenesis, identified in this thesis, is not limited to bacterial cellulose but can can be applied as a broader concept to describe the agency of a spectrum of species and organisms.

** Emily Birch's thesis on 'Biodynamic Architecture – A transcalar, interdisciplinary exploration of bacterial spore-based hygromorphs' and Dilan Ozkan's thesis on 'Fungal Parametrics: Designing a Living Material through Bio-Digital Fabrication' are current doctoral projects of the HBBE and are expected to be published by the end of 2025.*

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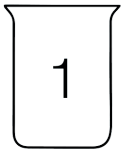
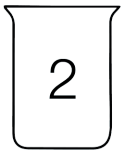
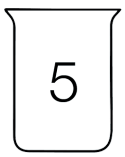
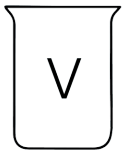
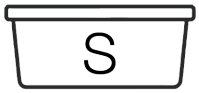
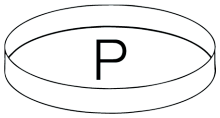
Close-up of thin bacterial cellulose film forming in the exposed loop of a monofilament fabric

GLOSSARY

Symbols

Used in Chapter 4 to 6 to describe the bioreactor setup of each experimental setup.

Category	Symbol	Meaning
Nutrient Medium (see Chapter 4.1.1)		The nutrient medium is prepared using a tea-based recipe
		The nutrient medium is prepared using the Hestrin-Schramm recipe
Inoculation Method (see Chapter 4.1.2)		A whole SCOBY culture is used to inoculate the nutrient media
		Liquid inoculum at a certain ratio is used to inoculate the nutrient media
Glass Vessels (see Chapter 4.1.3)		A tall form Duran beaker with 1000ml capacity is used
		A tall form Duran beaker with 2000ml capacity is used
		A squat form Duran beaker with 5000ml capacity is used
		A decorative cylindrical vase with 120 mm diameter, 400 mm height, and 4500 ml volume is used

Category	Symbol	Meaning
Plastic Vessels (see Chapter 4.1.3)		A plastic box with lid and the dimensions 150 x 250 x120mm
		A plastic box with the dimensions 190 x 330 x 110mm
		A plastic box with the dimensions 260 x 360 x 140mm
		A shallow plastic box with the dimensions 600 x 400x 70mm
		A standardised round petri dish with lid 100 x 15mm

Abbreviations

3D = three-dimensional

A.D. = Anno Domini

ABS = Acrylonitrile Butadiene Styrene

AF = Additive Fabrication

BC = Bacterial Cellulose

BCR = Bubble Column Reactor

CAD = Computer Aided Design

CO2 = Carbon dioxide

CT = Computed tomography

DIY = Do-It-Yourself

DR = Design Research

DS = Design Sciences

ELF = Engineered Living Manufacturing System

ELM = Engineered Living Materials

EM = Electro magnetic

FFF = fused filament fabrication

G-Code = Geometric code

HBBE = Hub for Biotechnology in the Built Environment

HCI = Human-Computer Interaction

HCl = Hydrochlorid Acid

HS = Hestrin-Schramm

iGEM = International Genetically Engineered Machine competition

LC = Living Construction group

LN2 = Liquid nitrogen

MC = Microbial Cellulose

MRI = Magnetic Resonance Imaging

NaOH = Sodium Hydroxide

Nm = Numeric measure

PC = Plant Cellulose

PCS = Plastic composite support

PET = Polyethylene terephthalate

pH = Potential of hydrogen

RDB = Rotating Disc Bioreactor

RfD = Research for Design

RiD = Research into Design

RtD = Research through Design

SCOBY = Symbiotic Culture of Bacteria and Yeasts

UV = Ultraviolet

Section of 3-dimensional bacterial
cellulose form cast into again

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Preserved freeze-dried BC forms exhibited in jars with dessicant pearls

APPENDIX

VIVA EXHIBITION

Figure A1: *Perspective view of the exhibition wall showing the three stages of experimentation, (1) Fabric as Scaffold, (2) Mycelium as Scaffold, (3) Air as Scaffold*

Figure A2*: Close up of material samples from experiments with fabric and mycelium scaffolds*

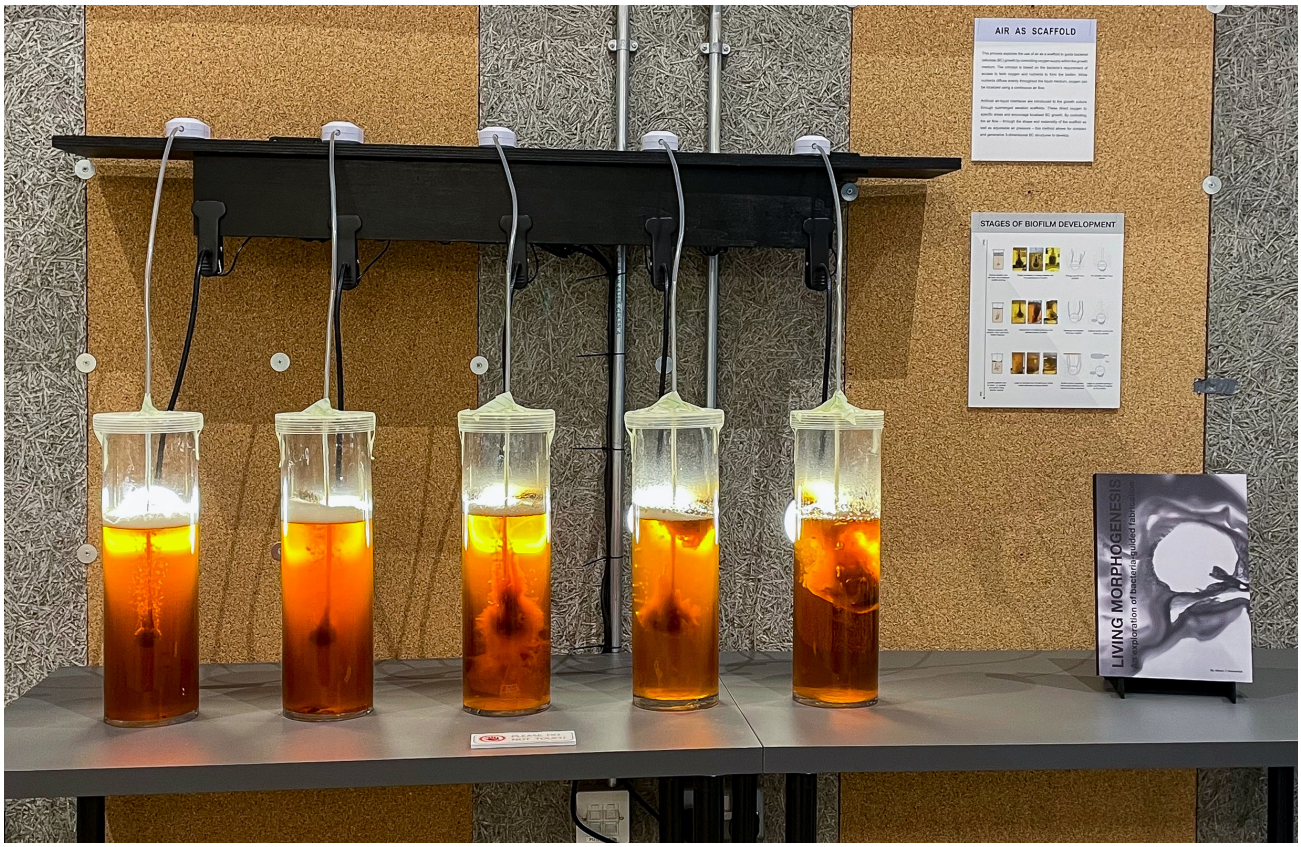
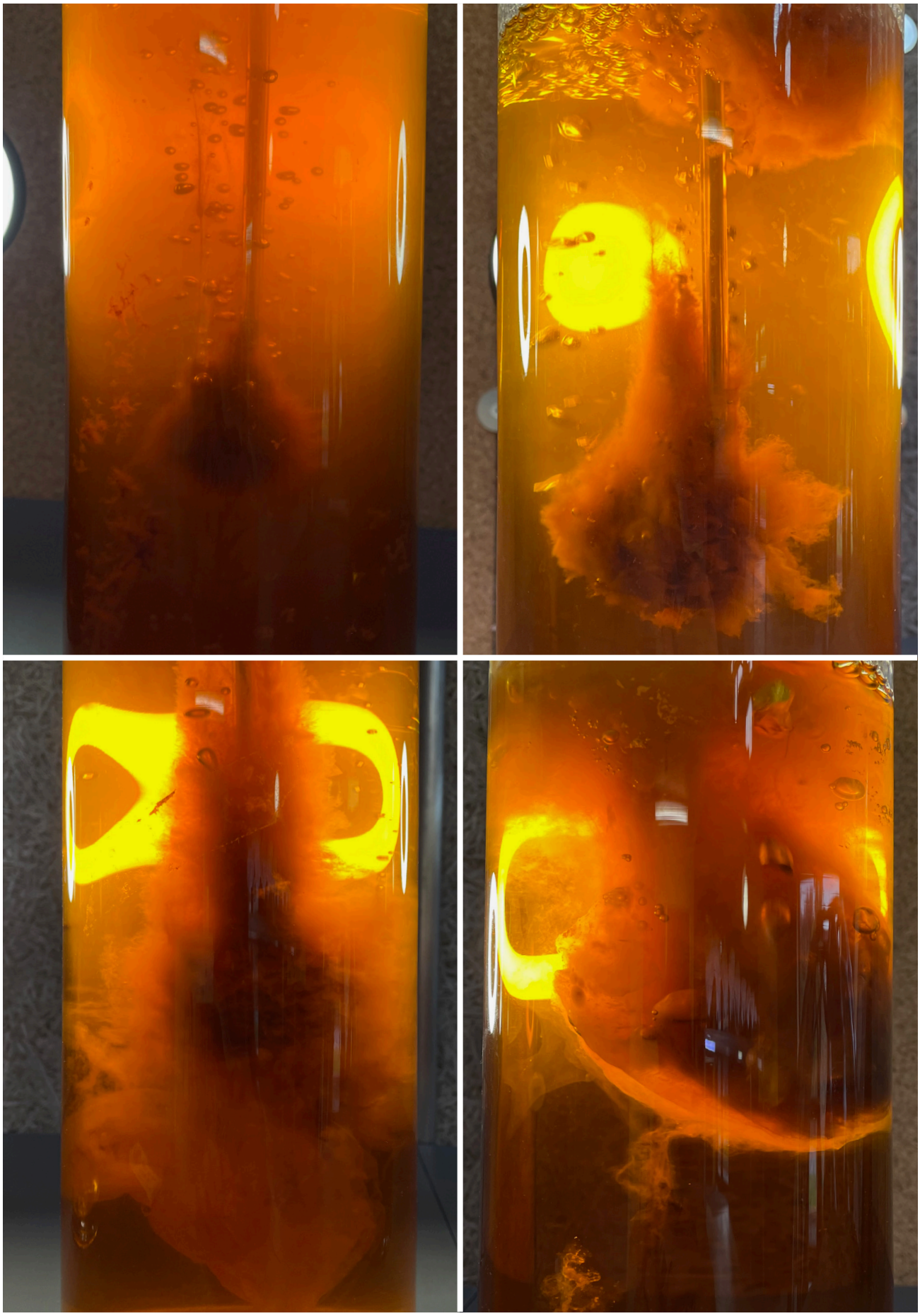


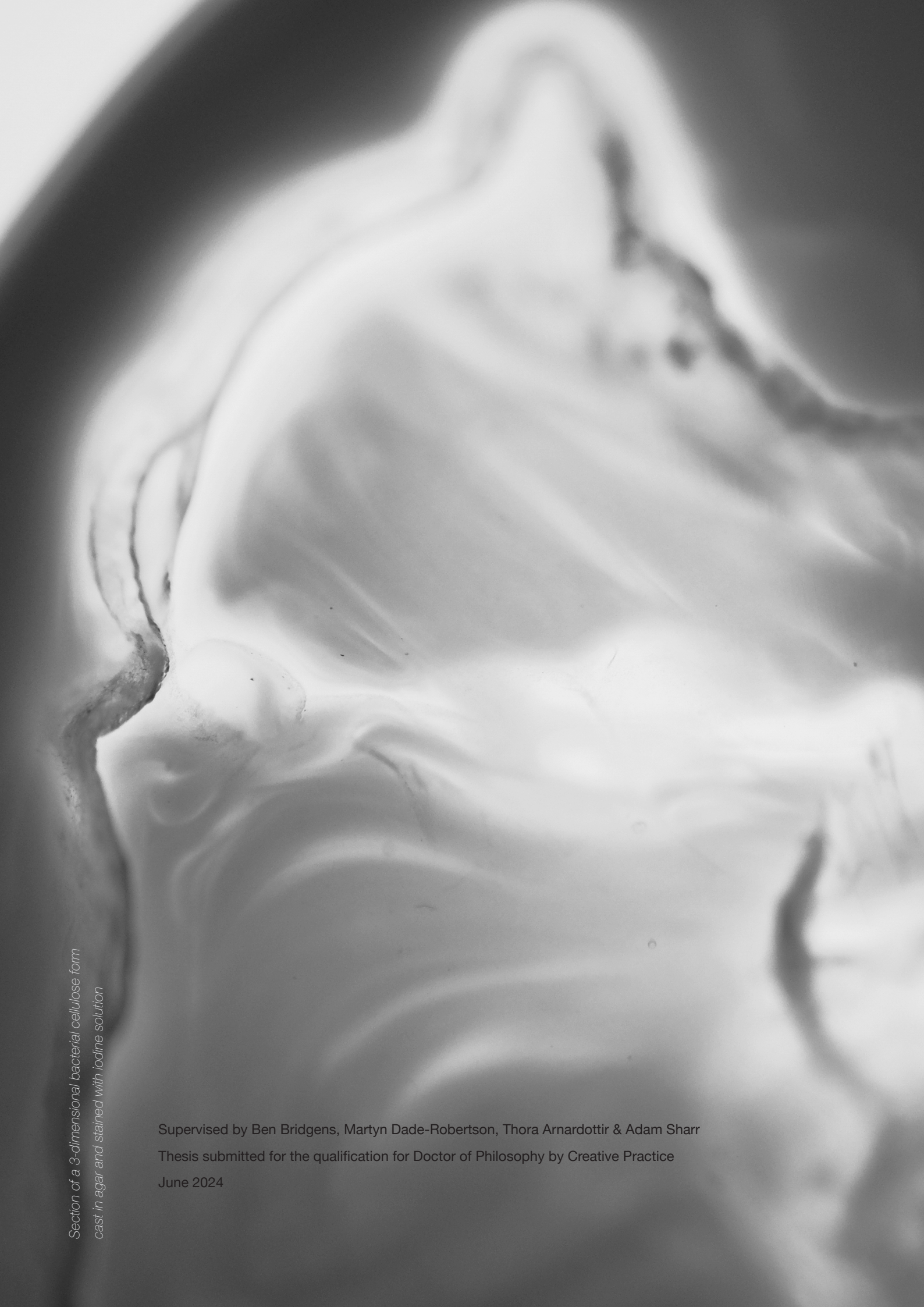
Figure A3*: Live experiment with bioreactors showing the various stages of BC grown on an aeration scaffold. From left to right, the bioreactors show growth after 1, 3, 7, 12, and 20 days.*

Figure A4: *Five different BC forms grown during experiments in Chapter 6, preserved with the freeze-drying technique described in Chapter 8.*

Figure A4: *Representation of BC forms grown around various different aeration scaffolds. The forms are 3D printed based on 3D scand made of the frozen forms and the respective scaffold is etched into the clear stand.*

Figure A5*: Perspective view of the exhibition space with the plinth showing the results of freeze-dried preservation in the foreground.*
Figure A6 (right)*: Close-up images of the BC growth in the bioreactors of day 3, 7, 12, and 20 (left to right, top to bottom).*





Section of a 3-dimensional bacterial cellulose form cast in agar and stained with iodine solution

Supervised by Ben Bridgens, Martyn Dade-Robertson, Thora Arnardottir & Adam Sharr

Thesis submitted for the qualification for Doctor of Philosophy by Creative Practice

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