**DOKUZ EYLÜL UNIVERSITY**

**GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES**

# A BIODESIGN COLLABORATOR IN ARCHITECTURE: MYCELIUM

**by**

**Onur KIRDÖK**

**September, 2020**

**İZMİR**

# A BIODESIGN COLLABORATOR IN ARCHITECTURE: MYCELIUM

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**by**
**Onur KIRDÖK**



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**İZMİR**

**M.Sc THESIS EXAMINATION RESULT FORM**

We have read the thesis entitled **“A BIODESIGN COLLABORATOR IN ARCHITECTURE: MYCELIUM”** completed by **ONUR KIRDÖK** under supervision of **PROF.DR. TUTKU DİDEM ALTUN** and co-advising of **PROF.DR. E. ESİN HAMEŞ TUNA,** we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

………………………………

Prof.Dr. Tutku Didem ALTUN

Supervisor

………………………

Assist.Prof.Dr. Fatma Feyzal ÖZKABAN

(Jury Member)

…..……………………

Assist.Prof.Dr. İlker KAHRAMAN

(Jury Member)

Prof. Dr. Özgür ÖZÇELİK

Director

Graduate School of Natural and Applied Sciences

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# A BIODESIGN COLLABORATOR IN ARCHITECTURE: MYCELIUM

## ABSTRACT

While the cities grow bigger and buildings go higher, results of human interventions through rapid deforestation, exploitation of natural sources, and massive land use of human settlements exceeded nature's self-repair capacity. Accordingly, the global construction industry is responsible for around 40% of global GHG emissions in various phases; manufacturing the raw materials, construction, usage, and demolishing. Therefore, the building industry is facing unprecedented challenges to reduce the environmental impact of current consumption practices. In this direction, interdisciplinary studies, which are inspired by nature and related to the fields of science and biology/biotechnology, have a prevalent place in today's design strategies. Among them, a new approach that includes living organisms into the design goes beyond inspiration from nature is biodesign via bio-collaboration.

In bio-collaborative design, bio-based materials and composites are an issue of interest in the quest over replacing the late centuries' unsustainable practices and materials used in the industries. This new approach has a beneficial potential and would probably lead to a great change through the future of the design and construction industry. In this context, this study focuses on discussing the potentials of mycelium, which are microscopic cellular fibre network of fungal cells that acts as a natural binder and recycler with variety of wastes, in the design and construction sector to create a bio-based material.

Considering that material and design are inseparable, this thesis study aims to try different possibilities in the production phase of a mycelium-based biocomposite and to discuss how this product can be reflected and used in the path of final design.

**Keywords**: Biodesign, biocomposite, biomaterial, mycelium, future of architecture

# MİMARLIKTA BİR BİYOTASARIM İŞBİRLİKÇİSİ: MİSELYUM

## ÖZ

Şehirler genişler ve binalar yükselirken, doğal kaynaklarının bozulması, hızla azalan orman arazileri ve insanların aşırı toprak kullanımı gibi insanların doğaya olan müdahalelerinin sonuçları doğanın kendini yenileme kapasitesini aşıyor. Yapı endüstrisi; hammadde üretimi, yapı, kullanım ve yıkım gibi farklı fazlarda Global GHG emisyonlarının (sera gazı emisyonlarının yaklaşık %40'ından sorumludur) yaklaşık 40%'ndan sorumludur. Bunların sonucu olarak, yapı endüstrisi mevcut tüketim uygulamalarının çevresel etkilerini azaltmak için benzeri görülmemiş zorluklarla karşı karşıyadır. Bu yönde, tasarım alanında, doğadan ilham alan ve biyoloji, biyoteknoloji gibi farklı bilim dallarını kullanarak disiplinler arası çalışmalar üreten günümüz tasarım stratejileri önemli bir yere sahiptir. Bunların arasında, canlı organizmaları tasarıma dahil eden yeni yaklaşımlar gücünü doğadan alarak biyo-tasarım ve biyo-işbirlikçilik yoluyla tasarımı daha ileriye götürüyor.

Biyo-işbirlikçi tasarım kapsamında, biyo-bazlı malzemeler ve kompozitler, geçmiş yüzyılların sürdürülemeyenliği kanıtlanmış uygulamalarını ve bu endüstrilerde kullanılan malzemeleri değiştirme arayışında ilgi çekici bir konudur. Bu yeni yaklaşım yararlı olacak bir potansiyele sahiptir ve muhtemel olarak, tasarım ve inşaat endüstrisinin geleceğinde büyük değişimlere yol açacaktır. Bu kapsamda, bu çalışma ile tasarım ve inşaat sektöründe biyo-bazlı bir malzeme oluşturmak için doğal bağlayıcı ve çeşitli atıklar için geri dönüştürücü olarak fungi alemi üyesi olan bir şapkalı mantarın güçlü miselyal yapısı biyo-bazlı tasarım ve inşaat sektöründe kullanımı açısından tartışılmıştır.

Malzeme ve tasarımın ayrılmaz olduğu göz önünde bulundurularak, bu tez çalışması, miselyum bazlı bir biyo-kompozitin üretimi ve farklı faz aşamasındaki olasılıkları denemeyi ve bu ürünün tasarım yolunda nasıl yansıtılabileceğini ve sonuç olarak nasıl kullanılabileceğini tartışmayı amaçlamaktadır.

**Anahtar Kelimeler**: Biyotasarım, biyokompozit, biyomateryal, miselyum, mimarlığın geleceği

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# CHAPTER ONE
# INTRODUCTION

## 1.1.Problem Statement

There has been a significant growth over the world`s population beginning with agricultural revolution, which made it possible to support larger capacities of population within a more controlled environment then that the gatherer societies had (Harari, 2016). By the advancement in technology and the industrial revolution this growth made its peak in both ratio and number. According the data from World Bank, in two hundred years the number of people on the planet grew more than 7 times from 1 billion to 7.7 billion by 2019 (World Bank, 2019). This significant increase, which seems like a success for the survival of the *Homo sapiens*, brought serious problems which affect the resilience of the entire planet (Seto et al. 2011) and challenging biophysical planetary boundaries of our world (Rockström et al. 2009) over the limits that will not support todays life as we know it in the future.

The rapid developments in technology enabled humans to manipulate the environment they live in and to change its shape according to human self-interests and will. While humankind has played a significant role in the change of the face of the earth through the progression of world history, this role was relatively harmless until the stage of industrialization. Yet afterward, long-lasting, cheap, and fast production to provide for the growing human population and their needs became the focus. The resulting environmental problems continued to accumulate and were observed throughout the Earth, such as chemicals affecting the microbial and pesticidal chains, toxic chemicals, and non-controlled gasses poisoning the air, land, and water, and slow decomposing packaging. The results of human interventions through rapid deforestation, exploitation of natural sources, and massive land use of human settlements exceeded nature's self-repair capacity.

What are the limits of life? Life’s Principles summarize repeated patterns and principles embodied by organisms and ecosystems on earth. These patterns and principles are thought to support a sustaining biosphere (Kennedy et al., 2015) What we call biosphere is a global ecological system of all living beings and their interaction within the lithosphere, hydrosphere, atmosphere and cryoshpere in a sustained cycle that feeds all, as a mechanism of maintained harmony. This harmony of living is built upon many ingredients. Carbon is the most abundant element between those that brew and manifest the structure of life. All living organisms on earth are carbon-based. It is a key component of all known life on Earth therefore, the ‘carbon cycle’ in environmental terms equates to ‘life cycle’ (Sun, 2017). This cycle consists of the balance of the active and stored carbon in the biosphere. Hence, all living beings within the biosphere of the world can be referred to as; “carbon based” life forms, which makes this balance vital to sustain on-going life on earth.

As organisms get bigger, the stored carbon amount increases, as well as all other nutrients necessary to nourish life. Hence restoration of these constituents is very crucial for the cycle of life to continue. Decay is the mechanism of life to gradually decompose organisms into its building blocks (Merriam-Webster, n.d.). This makes the decay cycle in the soil one of the important processes in the carbon cycle (Figure 1.1). There is a lot to learn from this natural cycle; nature does not extract waste materials, but rather utilizes what is available, upcycles materials, does not utilize toxic elements, and uses the same material over and over. In this cycle, nearly all plants in natural ecosystems have a symbiotic relationship with fungi. Fungi are a kingdom of organisms, one of the oldest inhabitants of the earth, with a primal role in natural ecosystems as decomposers (Cossio et al., 2012). Particularly mycelium, a chitin based fungi biopolymer, plays a natural role in the decomposition of plant material. This process is vital to restoration of nutrients back into the soil, which in turn facilitates the plant to growth. Furthermore, mycelia grow and conserve nutrients within their systems, allowing for relocation and transportation of these resources through the soil, along vast distances. Thus, nutrients can be conserved, transferred to other living organisms or the soil (Leake et al., 2004).

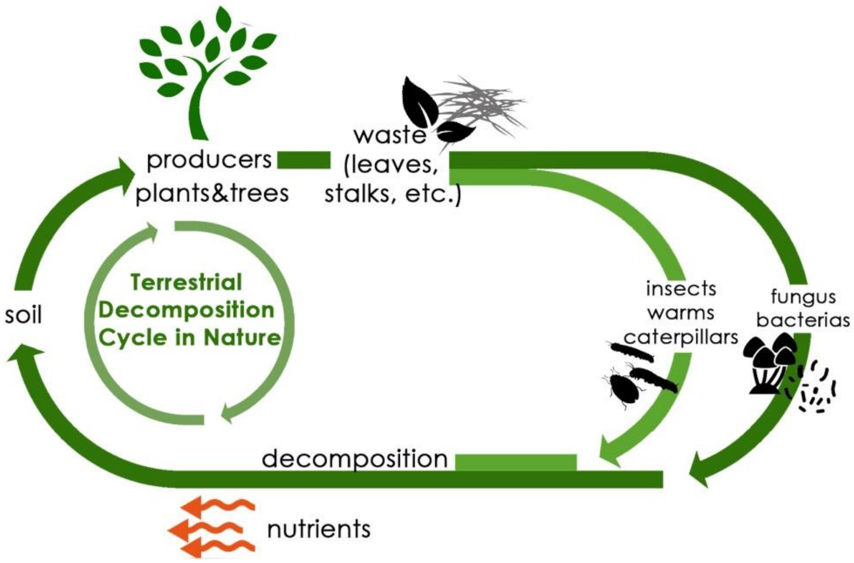


Figure 1.1 Recycle/upcycle in nature.

They have been a part of human life for millennia and have been used as food and medicine sources and to achieve spiritual experiences. Today, they are predominantly used in sectors such as bread and alcoholic beverage production, as well as a direct food source, i.e. mushrooms. In addition, some fungi metabolites are used as active pharmaceutical ingredients and fungi enzymes are abundantly utilized as catalysts in various industrial processes. Besides their role as one of the most important elements of nature´s digestive system, their fast and aggressive growth capabilities through the food sources makes them promising candidates for designing biodegradable and bio-collaborative materials. Recent developments show that, they can also be used to produce cruelty-free leather-substitutes, building blocks, furniture, and many other products. Utilization of this easy to grow bio-based materials for creating a sustainable circular economy can be possible by scaling up to mass production techniques and integration of such analogies in the design thinking.

Today, humans keep exploiting the natural resources and the land to maintain human growth by any means. While the cities grow bigger and buildings go taller, deforestation over the globe has been speeding up. Thus a way to address this issue can only be realized by urgent acknowledgement of the negative environmental impact of humans, and use of the production of new buildings with the highest standards. When we start to realize this concept, our complete dependence on ecosystems becomes more apparent (Pedersen Zari, 2009). However, all organisms are part of a "biome" that is in turn part of the "biosphere". As such, every organism's continued prosperity is dependent on the health of the biosphere. Under these circumstances, the biosphere`s cycle could change and not continue to support the known life on earth (Andersson et al., 2014). From this perspective, the construction industry is one of the main locomotives over the disturbance of the biosphere's balance with its harmful effects on the environment.

Approximately 40% of global energy is estimated to be used by the building sector. Moreover, 50% of raw material consumption by weight over the globe is used within the scope of construction industry (Pacheco-Torgal, 2015). Massive energy utilization and associated emissions are shaping raw materials into the desired construction materials. These materials generally tend to be heavy in proportion to the massiveness of the final product. This means they cause a considerable amount of emissions between their transports, since heavy materials are generally used by the conventional construction methods. However, a wide range of waste materials and chemicals pollute the land and water during the construction process while changing the land itself. Finally, upon construction, the residential demands in heating and cooling cause a significant amount of greenhouse gas emissions through the whole planet. Most building components become waste after the economic life of a building and their disposal requires energy, causes air pollution, water contamination, and biodiversity losses. Very few building materials can be reused or recycled in the same quality. Recycling usually ends up as downcycling, which means the material would not be recycled after another use. Non-biodegradability and long lifespan of these processed building materials prevent them from returning to nature. Accordingly, the global construction industry is responsible for around 40% of global GHG emissions in various phases; manufacturing of the raw materials, construction, utilization and demolishing (Figure 1.1). Therefore, the building industry is facing unprecedented challenges to reduce the environmental impact of current consumption practices (Adamatzky et al., 2020). Yet, it also presents low-cost and high impact opportunities.

From the second half of the 20th century, the questioning of the relationship of engineering and architecture -in fact all design disciplines- with nature has been one of the most important issues. As Kennedy et al. (2015) point out, design might be lacking in terms of how it fits within the larger ecosystem. Therefore, the term “sustainability” has gained importance on a global scale and has dominated academic research, and it has become a mega concept that regulates production practices through agreements between countries and development of certifications.

Within this context, many architectural theories were raised over the last century from eco-architecture to bio-architecture. The goal behind these ideologies is to construct with consideration towards the environment or at very least significantly decrease the environmental impact in comparison to conventional practices. Hence, the design of the built environment is becoming gradually more important in order to maintain quality of life and biodiversity. This situation led the designers to answer the question of ‘is it possible to design buildings which have positive effects on the environment?’. If the inspiration for architectural designs comes from the living world, then the buildings can be considered and designed as parts of a living system. Therefore it is possible to design buildings with abilities to produce energy, materials, clean air, and water while creating a circular relationship between the inorganic and organic, as to create a complex, adaptive, and cyclic system acting in the same manner as the ecosystem (Pedersen Zari, 2009).

Architecture and engineering disciplines keep searching for solutions to many problems addressing environmental responsibility today. This perspective has led to (1) increasing emphasis given to nature-based knowledge and nature-inspired/biomimetic approaches, (2) intensifying interdisciplinary relationships; particularly engineering, design, and science. Alongside many disciplines, integration of architecture and biology has great potential, since the developments in biotechnology and biology lead to understanding of the way nature works. In this direction, interdisciplinary studies, which are inspired by nature and related to the fields of science, biology/biotechnology, have a prevalent place in today's design strategies.

Among them, a new approach that incorporates living organisms into the design, goes beyond inspiration from nature, namely **biodesign** via **bio-collaboration.**

This idea leads to the perception of architecture as part of nature by integrating living organisms, either into the design process or the final product through bio-collaborative relationships; such as the examples of protocells (Beesley & Armstrong, 2011), calcifying bacteria (Gündoğdu et al., 2019), silkworms (Kırdök et al., 2019) and fungi (Karana et al., 2018). Bio-collaborative design processes focus on production of complex living and non-living biological products from raw materials like living cells or biomolecules. Adamatzky et al. (2020) stated that the "use of a living substrate in architectural systems states that innovative architectures co-exist / co-evolve with living substrates".

In bio-collaborative design, bio-based materials and composites are an issue of interest in the quest over replacement of the late centuries' unsustainable practices and materials used in the industry. This new approach has a beneficial potential and has a plausible potential to lead to a great change through the future of the design and construction industry. In this context, **this study focuses on** assessment of the potentials of **mycelium, which are** microscopic cellular fibre network of fungal cells, acting as a natural binder and recycler active towards a variety of wastes, **in the design and construction sector to create a bio-based material.**

### 1.2 Aim of the Thesis

The modern architecture has many materials to realize the desired design expectations. However, many of these materials cannot be sustained over their production cycle since they are not biodegradable and consume more resources than the Earth can replenish. Therefore, cost-competitive healthy and sustainable alternatives are necessary. Cast on-site or precast installations from mycelium based biocomposites show high potential to replace many surfaces in buildings from inner walls to building panels. They are also light-weight, which is a desired property and expected to reduce dead loads on the building structure. Mycelium acts as a binding agent, which leads to many desired properties in thus bound biocomposites; ranging from heat insulation to mechanical robustness. The rapid growth and other potential properties of mycelium-based biocomposite materials made them an interesting research area. As they can be cast to achieve nonallergic supportive environments for human health, mycelium based composites are a good alternative to today's choice of materials, ranging in many industries from construction to fashion. Therefore, many researchers today try to understand physical limits, properties, and potentials of mycelium based biocomposite products in their work. Still, most of these applications are at the laboratory stage and require further research, before becoming conventional elements in the architectural design or construction sector. However current environmental conditions require immense action and rapid change in current architectural practices. Most of the studies in the literature currently find mycelium to be too weak as a material, in order to be used structurally. For this reason, in order to become a structural element, increasing structural strength and stability of mycelium based materials and understanding of life cycle of such material is required. The production of composites that can be re-used after completing their life cycle or that can be completely degrade in nature is very important in terms of replacing today’s harmful practices. Cooperation with this living organisms and their life cycle, which we are still at the stage of recognizing, will pave the way for numerous new research and possibilities, and will be an exemplary study for understanding of the design in harmony and cooperation with living things, within the scope of biodesign.

**The main aim of this research** is to find a way to collaborate with the living organisms to replace former, unsustainable construction methods, to achieve paradigm change in architectural design. In this direction, within the scope of this study, a sustainable design process with low carbon emissions, based on the inclusion of mycelium of fungi in design practice, is experienced. In most of the studies, mycelium based bio-composites are grown in moulds, by inoculating fungi into supporting and nutrient supplying lignocellulosic substrates. The mycelium colonizes inside the mould, digests a part of the substrate and binds the remaining parts. The resulting fibrous materials have low-density and are highly anisotropic.

The material properties change mainly according to the type of fungi, the composition of the substrate and the production method, in addition to post-processing operations. Many researchers employ a similar production methodology, which begins with moulding the substrate into desired forms that would constitute a base to feed the inoculated fungi and to generate mycelium networks. According to the design and material expectations, forms are generated either by growing monolithically or by assembling units. Majority of the studies proceed by killing the fungal tissue via heating or keeping in a stasis state by drying, since the dry mycelium solidifies its connections and acts as a binder that holds substrate particles in both cases. All of these processes require certain environmental conditions and sterile work, to form a healthy mycelium network.

### 1.3 Literature review

To keep the cost of mycelium composites low and facilitate the recycling of wastes, low-cost agricultural plant-based (Karana, 2018) or forestry by-products or wastes are used as fibrous substrates, particularly straw or granular substrates, such as sawdust (Jones et al. 2020). Substrate selection and composition proportions vary depending on the type of the desired product. Different substrate combinations significantly affect the mechanical performance, strength, and toughness of the mycelium-based biocomposite (Karana et al. 2018). In addition, substrate type, particle size and processing method, depending on the chemical composition, affect the strength of the material (Lelivelt, 2015; Karana et al. 2018). Lelivelt (2015) evaluated different substrates (i.e. hemp varieties and wood), fungi species and different sterilization conditions, and demonstrated that non-woven hemp is compatible with fungi and provides high strength in compression. Ghazvinian et al. (2019) aimed to increase the mechanical and chemical properties of mycelium-based composite blocks in wall construction, and the results of compressive strength tests on block samples showed that straw-based biocomposites could not bear sufficient loads and that chip-based biocomposites could be used in place of conventional stacking units, when sufficient reinforcement was used.

Girometta et al. (2019) provide a comprehensive review of the physical, mechanical and thermal properties of mycelium-based biocomposites and the factors affecting these properties. Mycelium-based composites have been developed into a wide range of products such as packaging, construction and insulation materials, leather-like textile materials and transparent renewable films (Attias et al., 2019). Mycelium-based composites with different structural or optical properties can be produced by varying the fungi type and substrate; this allows the composite to be used in different functions.

There are also studies that examine strength and durability of mycelium-based biomaterials, as compared to other plastic foams (Attias et al., 2020; Jones et al., 2017b; Girometta et al., 2019). A few of them are about packaging applications (Abhijith et al., 2017; Holt, 2012). In the market, there are some mycelium packaging materials being used as end products. These materials are used instead of EPS (Expanded Polystyrene) to protect commercial products like computers (Dell), furniture (IKEA) from transit, handling, and storage damage. Although we know a few special cases, the use of mycelium packaging is not as common as could be desired in industry. There is a number of challenges related zo increasing the industrial use of mycelium packaging. One of these challenges is the cost of mycelium composite. It is known that the raw material cost of mycelium composite material is higher than that of EPS. In addition, the speed of the production technique of mycelium composite material is low compared to EPS.

The studies that focus on fabric reinforcement are outstanding (Jiang et al., 2013; Jiang et al., 2017; Jiang et al., 2019; Elbasdi ve Alaçam, 2017). Jiang et al. (2013) and point out that the bio-based core and reinforcement can offer enhanced structural and thermal performance, since the mycelium would act as a binder, like a polymer resin matrix. In addition, better acoustic performance is a possible outcome of this application, due to relatively low density of the material. The main advantages of these composite materials made using natural fibers (e.g., jute, flax, cellulose fiber, etc.) over traditional synthetic composites include low cost, low density, competitive strength, tensile and impact properties, reduced energy consumption, the potential for

$CO_2$ sequestration, if applied on a large scale, and perhaps most important of all - tailorable biodegradability (Jiang et al., 2019).

In summary, laboratory-supported scientific studies in the literature focus on investigating ways to produce bio-based composites by varying the type of mycelium, growth environment and conditions, meanwhile using different techniques or additives during the process.  As Attias (2020) stated, these studies are quite scattered and still contain shortcomings. In fact, most of these studies focus on a scientific effort in order to find out more sustainable, economic and easy ways to produce these mycelium based composites, rather than application types of the result and final products in the design field.

On the other hand, many of the unique qualities and potential implementations of mycelium seem to be under the radar of designers. There are also a few projects, aiming to demonstrate utilization of mycelium in architectural design and as building materials (Heisel et al., 2017; González, 2009; Camere & Karana, 2018; Adamatzky et al. 2020). However, the overall goal of these studies is to raise awareness about the future potential of mycelium in the design discipline. For this reason, it is possible to say that they are trials that underline the excitement of the use of living organisms in the field of design, while production processes remain on the background.

Considering that material and design are inseparable, this thesis study aims to assess different possibilities in the production phase of a mycelium-based biocomposite and to discuss how this product can be reflected and used in the path of final design. Therefore, the unique aspect of the study is that; it combines both laboratory and design work. To this end, within the scope of the thesis, development of a building material from a chosen type of fungi, with experimentally identified best combination of 2 substrate and 3 different fabric types in laboratory conditions, is proposed. Further on, optimization of extra nutrients and final colonization times for the identified best combination of fungi, substrate and fabric under given conditions is examined. In addition, there are limited studies in the literature focusing on fabrication of a building material and characterization of structural strength, by measuring compressive and tensile strength. However, in line with its potential as building materials of the future, mycelium-based biocomposites should be investigated in this sense with more extensively.

On a national scale, the use of mycelium in the design or construction sector is a novel topic and remains mainly in research stage (Elbasti & Alaçam, 2017; Kırdök et al., 2020). In this direction, this thesis aims to make an important contribution to the national literature in the field of architecture and design.

Designing and building with mycelium-based composites has the potential to create exciting work in the interdisciplinary research with engineering, architecture, computational design and biodesign.

### 1.3 Methodology & Scope of the Thesis

This study includes an extended literature, pavilion exposition and commercial development review summarizing the overall efforts achieved in this novel area, followed by the experimental and computational study, characterized via qualitative and quantitative methods, with an emphasis on quantitative methods. The said review is used in the context of grouping and categorization, while drawing a general but comprehensive framework for the literature. The quantitative method, the main focus of this thesis, comprises the laboratory experiments that include preliminary research for the use of mycelium fungi in the design field.

Main frame of the experimental studies has been performed with constant assessment of repeatability and the experimental setup has evolved progressively via continuous optimization. This portion of the thesis was conducted in Ege University microbiology labs between February and September of 2020. First experiments have been conducted in order to understand the growth dynamics and behaviour of mycelium. Second phase of the experiments focused on increasing the structural strength of mycelium grown over recycled substrate via fabric reinforcements. In accordance to the findings within the previous phase, last stage of lab experiments were conducted to optimize the production phase. The aim, scope and methodology of the thesis are explained in the first section of this thesis. In the second section, the damage caused by the construction industry to the environment, the importance of biodesign and potential of biofabrication as a construction tool are detailed. The third section then spotlights the potential of mycelium of mushroom as a construction material and as a design tool for future of architecture. In the fourth section author demonstrates several stages of experiments in order to determine a potent Mycelium based biocomposite combination and its optimization, in accordance to design expectations, and outline the effect of mechanical behaviour on the design process. The preliminary experiments held under ambient condition within a common household that reproduce the common applications described in the literature, in order to estimate an outline to progress through the main experiments. Several structural tests have been performed in Ege University Matal Lab on the samples produced via these experiments. At the end of this section, a design proposal has been formulated according to the experimental outcomes and correlation between material production process, material properties, structural viabilities and the design. The main objective of the efforts is to be an assessment of this potentially plausible material and methodology as to further serve better understanding with respect to nature and positive environmental impact. Last chapter reviews the conducted work as whole in the form of conclusion and provides predictions with regard to the future research related to this pioneer topic.

# CHAPTER TWO
# BIODESIGN AND ARCHITECTURE

## 2.1 Harder, better, faster, stronger; Industrialization

Over the progression of world history, humanity has a significant role over the change of the face of the earth. But this change was rather harmless until the industrialization. Our crafts and productions were only affecting its surroundings and generally based on limited processing capability. Production and preservation of the products were based on general minerals and polymers which were collected from nature, like; wood, stone, silk etc. These were generally handcrafted simple crafts. With time, human population kept expanding over almost every corner of the earth. That meant more demand over shelter, food, water, medicine etc. Yet with these simple tools and knowledge of that time were not sufficient to support such population growth, which resulted in many catastrophic events like masses deaths of starvation, plagues and many other health issues, by winds and tides of the history. To answer these demands our tools and crafts had to advance in technique.

Beginning with industrialization, production line started to seek long lasting, cheap and fast production to maintain the growing population of humans and their needs. Our techniques evolved into technology. Through technological advancements humanity kept crafting new and more complex tools. Utilizing these tools we crafted and collected better performing materials to create our products. However, it was yet unclear, how these resilient durable products would comply with the nature. After nearly two centuries of experiencing this way of life, we are able to observe the results of such aggressive approaches that hold the humanity in the centre of everything. The slow decomposing packaging, plastics and many other waste materials, overflow landfills and oceans, while poisoning the life more severely every day. Use of excessive agricultural chemicals and wrong farming methods destroy the microbial and pesticidal chains, while damaging the core of the land fertility. Meanwhile, our industries and the machinery keep producing toxic wastes, chemicals and gasses, altogether poisoning the land, air and water. With fast deforestation, exhaustion of natural resources and massive land utilization by human settlements, nature’s self-repair cycle was disrupted and the importance of our relation with our habitat has been neglected by the humanity reign.

As a result, today we have started to figure out the consequences of our progress over industrialization and capitalism. Broken chains in the cycle started to ring serious warning bells. Currently one of the most severe issues, climate change, has been named upon the World Climate Conference in 1979. Following the conference, many researches have been assigned to this topic to understand the magnitude of damage. While many others focused on figuring out ways to limit this disruption and facilitate its regeneration. All the findings point out the need for a rapid change in our industrial methods and choice of materials and our life habits. Despite the fact that, since the first conference in 1979, there have been series of scientific and political conferences held over many regions of the world, but not many significant steps have been taken forward (Gupta, 2010).

Researches point out that the release of gasses, which kept accumulated, are the main reason behind the increasing temperature over the globe. UV rays that reach earth unfiltered, because of the gaps over the ozone layer of our atmosphere, are another consequence of this broken cycle. Even minor changes over the temperatures have serious outcomes. North Pole and South Pole of our world maintains a cycle of melting and freezing massive amounts of ice in a delicate balance over the course of a year, however calculations show that every year these water reserves held in these giant ice masses are decreasing. This resulted in the rise in temperature of oceans, which harms largest reserves of life on earth. Even minimal changes in the water temperatures pose danger to the marine life cycles, ranging from corals to fish populations (Fuentes, 2016). Further the consequent rise in water levels endangers inhabited land to floods. (Zhang et al., 2004)

The heat increase does not only affect the water masses, it affects all areas and life on earth. According to the Australia`s Governmental Bureau of Meteorology`s calculations there has been nearly 2.5°C rise in the average temperature of Austria over a century. In 2019 Australia recorded to break its all-time temperature record twice in December. While an average maximum of 40.9℃ was recorded on 17 December, this record was broken a day later by 41.9℃, both beating 2013's record of 40.3℃ (BBC, 2019). As a result of this temperature rise, Australia has reported a huge increase in numbers and sizes of bushfires, witnessed lately. As a result of these devastating events many habitats have been lost, while several species were declared to face extinction. These disasters have become a spotlight of Climate Change discussions.

These most recent and obvious examples lay in front of the eyes of the world. All these examples show that our unsustainable methods to support the growing population and its demands bring out many serious side effects. These unconscious choices are the main causes behind the latest catastrophe our planet is currently facing, and we are the orchestrators of it. The rapid development in technology let us manipulate the environment we live in and bend its shape in accordance with our self-interests and will. Growing cities, spreading wide settlements, factories and production facilities to support this growth and construction of high rise are the latest trends of the century, which relates to architecture and architectural design in core. One may call these the human progress; however this human orientated attitude has been neglecting other species in terms of design thinking. According to Kennedy et al. (2015); "The design might still be lacking in terms of how it fits within the larger ecosystem. All organisms are part of a biome that is part of the bio-sphere. As such, every organism's continued prosperity is dependent on the health of the biosphere." Fall of even one kin means loss of this balance. According to an UN report (2019), at least 680 vertebrate species have been driven to extinction by human actions since the 16th century. In order to prevent such catastrophes from aggravating; a new design thinking collaborative with nature is required.

Unsustainable methods such as use of imported, toxic and hardly recyclable materials, wrong positioning with insufficient analysis is disrespectful to the habitat and fails to fit into the ecosystem. Besides, most of these applications banishes the local species from the land, poisons the land, air and water and dries out the resources that are crucial for other species. As a matter of fact, construction sector, and hence todays architectural design, is responsible for the disturbance of the environment, which will be explained further in the following section. Under these circumstances, biosphere`s cycle could turn in a direction that the existence of life on earth would no longer be supported.

### 2.2 Higher, Bigger, Supreme; Construction

Today by any means, to maintain the population growth, we keep exploiting the natural resources and the land. While our cities grow bigger and buildings go higher, our quest for the supremacy over nature continues ferociously. However, producing new buildings with highest standards brings the urgency for understanding the negative environmental impact of humans, and when we start to acknowledge that, our complete dependence on ecosystems becomes more apparent (Pedersen Zari, 2009). Researches claim that, 40% of $CO_2$ emissions, with nearly 20% of total greenhouse gas emissions releases to the atmosphere, are the result of building industry. (Pacheco-Torgal, 2015).  In this regard we can refer to the building industry and urban development as one of the main locomotives over disturbance of our biosphere´s balance, due its harmful effects over carbon cycle and the environment.

Big majority of building industry works in a continuous process to achieve a massive final production. From the beginning of the process the emission releases start to rise and continue rising throughout installation of final product, as well as all the other waste materials.

Another major problem is the continuous exploitation of natural resources, minerals and other materials used and stored in the constructions. Although, these resources are all limited, in order to comply with the needs of growing market, 50% of raw material consumption by weight over the globe is used within the building industry (Pacheco-Torgal, 2015). Long life span and negligible biodegradability of these processed building materials prevents them to return to nature back.

Generally, industries follow the same energy depleting processes causing greenhouse gas emissions. Biologist Janine Benyus (2005) points out the schematics of this process as; heating of the materials, compression with high pressure, and chemical treatment. She refers to the process from the point of view of material scientists which is; “carving things down from the top, with 96 percent waste left over and only 4 percent product” In addition to this general waste, collection of the raw materials and then transportation of the goods are other energy consuming steps, also resulting in release of toxic waste.

Even though our constructs appear to be stable in the location where they are built, construction industry transports and relocates required materials and machinery, which is an extremely resource depleting process. Most of the construction materials life begins with the excavation of the raw material, which generally requires huge amounts of energy and logistics depending on the size. Following that, massive energy use and emissions come out within the step of shaping these materials into a desired construction tool. These materials generally tend to have heavy weights, proportional to the massiveness of the final product. This means, corresponding emission releases during transfer and logistics over given distances. Construction process in the site mostly requires heavy machinery in processes like excavation of the land, relocation of the materials and tools throughout the process. Most of the current construction techniques depend on heavy energy use. Production and postprocessing of construction materials on-site in order to fit the desired function in design is another leaking step. Additional to those, building industry keeps creating pollution ranging from common wastes to toxic chemicals that pollute the land and waters through the process of construction, while changing the land itself. Intervention to the land to create the desired construction site generally means movement of the huge loads of land with heavy weight. Moving those requires machinery and tools that releases more emissions to support these loads. All these different phases of the construction industry lead to GHG emissions (Figure 2.1).

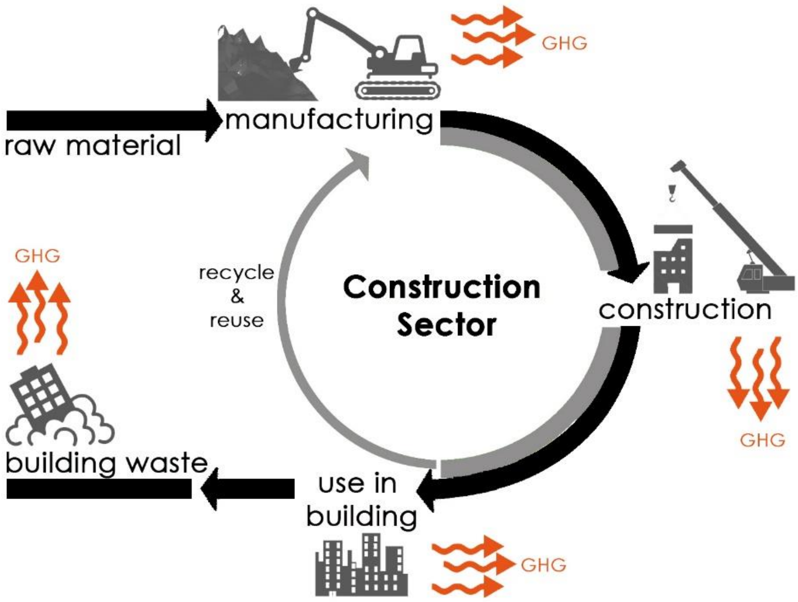


Figure 2.1 GHG emissions in different phases of the construction industry

Constructing with no consideration for the environment results in a product, that remains idle towards the ecosystem and its inhabitants. During this process of producing a massive product for global and local economy cycles, mankind keeps changing the land, bending it according to their will, therefore applications generally tend to disrupt the balance of the local ecosystems on micro scale, and continuous applications like this effects the global ecosystem on macro scale.

Design effects everything through construction to demolition. Efficient designs will reduce the wasteful use of energy and materials, while reducing carbon foot print at first.

Today's architecture discipline keeps searching for solutions to these problems. With this ideology in mind, many architectural theories have been raised over the century from eco-architecture to bio-architecture. Main goal behind all of these ideologies is to create a building analogy that respects the environment with minimal impact. This concept gave birth to the well-known, yet new marketing terminology; "Sustainability". However, the change over the industry and more precisely over our lifestyles all over the world began a slow operating action under the term of sustainability, because of today`s economic chains that bind the politic stance before action. With this terminology, new materials and technologies emerged to feed the market and the economic chain, which kept exploiting the natural resources, but are somewhat improved in general process. These situations lead architectural designers to the question; is it possible to design our buildings with positive feedbacks and effects to the environment? As a result, the idea of regenerative design began to emerge in research and industrial applications. The smart use of intelligence, advancements in technology and science has opened up the possibility to achieve such designs. So far, our buildings are enhanced by the abilities to clean air, produce energy and food, recycle wastes etc. Also, these advancements seem to be limited only by our imagination and time. Many on-going experiments are focused on creating artificial ecosystems on different scales; from search of a utopian residential complex to an interstellar expedition base construction. Ecosystems are resilient, resourceful and opportunistic. They adapt and evolve according to given situations while condensing the capacity to healing themselves (Pedersen Zari, 2009). As our understanding over ecosystems expands, creating artificial ecosystems became a viable option to heal current urbanisation practices. Today built environment, which is shaped to serve human needs, has already replaced the nature. As a result, designs that introduce interactions, connections and qualities from the nature to sustain quality of life and biodiversity, became more important. To support life, architecture as a discipline, can be reformed to create spaces that have positive effect on life that surrounds us in artificial, cultural and social ways (Naguib & Hanafi, 2013). From this perspective, architecture can be elevated to a state of art to become a regenerative tool for resolution of both environmental and humanitarian problems we are facing today.

Biologist and one of the founders of Biomimicry institute, Janine Benyus (2005) outlines this clearly in her TED speech; “Life creates conditions conductive to life. It builds soil; it cleans air; it cleans water; it mixes the cocktail of gases that you and I need to live.” If the derivation for architectural design comes from the living world, our buildings can be considered and designed as parts of a living system. Not only by mimicking the environment but also trying to be a part of it in function and purpose, it is possible to design buildings with abilities to produce energy, materials, clean air and water while creating a cycle between the waste and organic to create complex, adaptive and cyclic system which acts in the same way with the ecosystem (Pedersen Zari, 2009).

The problem of current building industry lies within the irresponsive attitude of the constructors neither to the end users nor to the nature. The fear of unknown and the veil of beauty pursued us to invent dwellings, like forts to keep out any alien force and beings other than our kin. To aid this issue, we should step aside and make peace with the nature, we should seek its intelligence, learn from it, receive its assistance, re-join with our habitat. That is, in terms of design, designing in line with the biology, a complete union of our structures with the bones of our ecosystem would result in an evolved, resilient, adaptive architecture to develop.

### 2.3 Biological tendency in architecture

21$^{th}$ century brings out many new scientific and philosophical discussions to the table, mainly on the course of life. One of the most significant examples from these discussions is between the theory of intelligent design and Darwin`s evolution. The theory of intelligent design supports the idea of all things in the universe being created with a genius behind it to support the life as we know it. Such genius crafted the universe with its ultimate algorithm rather than a random process of natural selection. On the other hand, evolution theory claims that from the origins, it took generations as the tides of choice done by the occasional conditions to fit the best way for a living organism to survive. Today we can refer to these phenomena as the genius of nature, keeping the tides coming on the shoreline of life. Even though both theories oppose each other with regard to the origins of life or what is behind them, we can find an elegant intelligence behind the act of survival of organisms, creating the very best scenario in many tides of trial and error and when we look upon the nature today, we see a grand design going on inside, that keeps the circle of life.

What lies within the nature is therefore an issue of design. It is the way of human response to the fierce and dangers threatening our survival. We must protect, show resilience, enhance to manufacture development and carry an adaptation process for the civilization we build.

As the on-going intellectual organisms, the design thinking is also evolving with us. Our buildings have evolved in many steps from the *primitive hut* and will keep evolving, parallel to the needs corresponding to the conditions of time. Gilder & Clements-Croome (2010) refers to our constructions as the artefacts of the human conscience on the route to change and reflecting these fraction points of human experience. It has been evolving alongside with us. Thus, the survival of today`s creations of humanity has to evolve in order to sustain its existence in the face of evolution step we face today, to be compatible with the current environment. However, the researches show that our designs are not supporting the environmental challenges they face today. In general, main goal of any habitable design is to build a working microenvironment as a living organism, from the inhabitants to its connection with immediate surroundings.

Biological evolution is a result of adaptation to the surrounding challenges of habitats, followed by the development of skills and properties as to enhance the resilience of life by genius of nature. Living organisms have developed specific functions in order to survive and to guarantee continuity of its kind. Our buildings require such evolution and living functions, in opposition to its inorganic solidity today. Within the framework of evolution, our buildings have resilience of sandcastles within the floating time. This also means adaptation and development of the current architecture, remains to be a fortress against the life yet struggles to keep up. In this disconnected state, the evolution of architecture in the future should reconnect all of its vital veins with life and it is essential to think out of the box and within the sphere we all dwell. We have the chance to give birth to many possible scenarios through understanding the algorithms of nature. The evolutional algorithms have already been on sight of architectural design to process a life like structure and to answer the needs of the environment and the society alike.

Therefore, integration of living organisms into design and collaboration with biology is a pioneer topic with a great potential for the future of architecture.

## 2.4 Terminology in Biodesign

The prefix "bio" originates from the Ancient Greek word "bios" (βίος) which means "life" (Lucibello et al., 2018). During prehistoric times, nature was a guide that we can mimic and learn from. The relation of any discipline that mimics a lifelike state or creates artefacts that interact with life, is an issue ought to be observed under the spotlight of the science of life.

Hence the term Biology, which is the science of life, kept evolving through branches, to create new biocentric methodologies with different analogies. Bio-technology, Bio-engineering, Bionics, Biomimetics, Bio-design, Bio-architecture are some of these branches that became new disciplines in order to specialize on a specific topic. Although these terms are relatively new, the idea of deriving intelligence from nature is as old as dawn of the humankind. Very first humans started to make tools by imitating the nature to help themselves. In the current literature, this kind of derivation of ideas is generally referred as "biomimetics".

Otto Schmitt an American inventor coined the term "biomimetics" in the 1960s to describe the transfer of ideas from biology to technology. However, the popularization of the term began with Janine Benyus (1997), with her famous book, named "Biomimicry: Innovation Inspired by Nature". Understanding the mechanics of nature`s design, and not only its form, process or relations, enabling to use all of these principles together to find solutions to our design problems, is the goal of biomimicry (Kennedy et al, 2015) Nature represents the most delicate and sophisticated design and engineering principles in complex forms and structures. To design and produce within these principles, therefore rising technology age will keep enriching the tools of humanity. Naguib & Hanafi (2013) address that, as the digital technologies and techniques advance, we are able to "analyse, create, fabricate, and simulate architectural forms inspired by nature" to apply the aesthetics, materials, structure, and environmental controls found in nature to the architectural design.

Figure 2.2 Left; Lightweight structures of Frei Otto for the 1972 Summer Olympics in Munich Olympic Park 1968–1972 Munich, Germany (Dezeen, 2015), Right; Sagrada Familia Roman Catholic church by Antoni Gaudí in Barcelona, Spain (Wikipedia, 2011)

Biomimicry is not a sole solution of design, yet it is a path of thinking. It observes and studies the knowledge of the nature to solve human problems. From Frei Otto to Gaudi, there are many famous designers who used this path to experience new possibilities for humanity, laid their interests which have already been inscribed inside the sacred within the nature (Figure 2.2). Today's digital design and calculation methods, combined with scientific findings in biology and ever progressing advanced technologies, opened a new chapter for all kinds of design and perception. Integration of state of art technologies such as artificial intelligence, information technology, smart materials and robotics have increased the potential of novel research areas. Bio-design, biomimicry, green chemistry, nanotechnology and biotechnology created new dimensions of scientific cooperation (Lucibello et al., 2018). As these developments led us to understand the way nature works, we can do more than mimicking the nature, we can labour the nature as a collaborator to produce and build.

Advancing the design by biological processes to achieve nature's adaptive products is an issue of interest held by a wide range of research groups in numerous disciplines. Yet, the integration between these groups is still missing the unity. While a bio-tech researcher misses the tools of a designer, designer lacks the arsenal of biotechnology. Therefore, the lack of unity between disciplines limits the potential of multi-disciplinary approach to achieve superior biomaterials and unique bio-designs that would enlighten the way of the future. So that; "…to unveil new ways of designing with biological systems, there is a need to bridge between research paradigms and tailor an interdisciplinary research method for a circular bio-production future." (Attias, et al.,2019).

Alongside many other disciplines, integration of architecture and biology would show great potential. Building industry bares the key role in the climate change, as dump creating blocks, acting as a separator of urban life and nature. Bio-architecture, a term for intersection of these disciplines, makes it possible to change irresponsible attitude of contemporary built environment. In this manner our buildings will become particularly or totally organic, intelligent surfaces that sustain the exchange of necessary ingredients to create comfortable living conditions, cooperating with the nature by interacting as a part of it. Dollens (2009) refers to this "growing" of the built environment as "acotones", transition zones between the habitats, which supports the locality and economics. But how can we employ living organisms, their algorithms and abilities, within the architecture? Can we labour the nature as a collaborator to construct in harmony with the environment to achieve positive, regenerative effects?

According to Pedersen Zari (2009) it is possible to achieve, yet to do so, design must integrate and relate in order to form systems in a bigger perspective, rather than applying current technology without understanding. It is a key component of regenerative design in architecture.

The aim is reached not by building using bio-inspired materials or mimicking natural principles on different scales. Rather, bioarchitecture employs the solutions and utilities presented in nature for advantage of humanity. According to Ripley & Bhusan (2016); "It is an approach that is evident in the theory behind the overall design, the careful choice of materials for construction or creation, the extraction and adaptation of principles, and the cohesion of the parts in the whole."

## 2.5 Biodesign and Bioarchitecture: Integration of living organisms into the design

Use of vegetation and manipulation of the latter to answer the needs of an architectural dwelling, plants and trees have been a tool of architecture since the beginning of times. Yet these applications became a novel movement with John Krubsack who can be recalled as the pioneer of the living architecture movement as the designer/grower of the first "living chair" in 1914. The process took 11 years and 32 young seedlings to build the chair by shaping living trees as they were growing. The chair was entirely built by shaping living trees as they were growing (Vallas & Courard, 2017). That achievement encouraged other "tree shapers," such as Axel Erlandson with his famous "Tree Circus" in California, where he displayed more than 70 "tree sculptures". Richard Reames, the finder of the term "arborsculpture", wrote reference books on the subject (Reames et al., 1995; Reames, 2002); and the inspiration he brought to the topic raised the interest of many architect like Konstantin Kirsch, Laura Spector, and Aharon Naveh. Marcel Kalberer. His "Sanfte Strukturen" team built the Auerworld Palace in less than a month in 1998; this palace is made only by living, bent and shaped willows (Rocca, 2009). In 2005, Oliver Storz, Ferdinand Ludwig, and Hannes Schwertfeger started building what they called "Baubotanik buildings" (Ludwig, 2012). With the idea of using an industrial structure to guide trees to development into the desired structure, the group succeeded in building "Baubotanik Tower" (completed in 2009) and "Plane-Tree-Cube Nagold" (completed in 2012). Mitchell (2006) notably contributed in the field by considering an entire house made entirely from living materials. The tree structure would be shaped in a similar fashion to an igloo, using the shaping techniques developed by Reames (Reames, 2002) on several living trees (Vallas & Courard, 2017).

While many scientists focus their study on understanding the mechanics defined by the algorithms of life, our technology keeps evolving around that progressing knowledge. Hence the veil between artificial and natural disappears with each development. That leads to an era, where the creativity of the mind has the opportunity and tools to venture further and deeper than ever before. Designers start to resemble a conductor of the bridge between the material and digital, bringing an adaptive and responsive creation to the reality of this world. Adamatzky outlines this very clearly in the following statement; "Use of living substrate in architectural systems states that innovative architectures co-exist/co-evolve with living substrates" (Adamatzky et al, 2020).

Today with the developments in technology, our knowledge of biology has become wider, enabling us to lead the procedures / and follow the recipes of nature to maintain a sustainable and mutually beneficial symbiotic cycle.

Figure 2.3 Terreform ONE Projects (1. Fab-Tree Hub, 2. Genseat, 3. In vitro meat habitat) (Terraform ONE, n.d.)

Symbiosis is a mutual collaboration between different species of organisms living together, beneficial for the both sides of this unity. The bacterium (like E.Coli) living in our digesting system is one of the examples of symbiosis. While our bodies support optimal conditions for these organisms to live, by constant supply of nutrients to the bacteria, they in return help us to digest and break the ingredients within the food. There are many other examples of symbiosis in our body, which we host ourselves. Then why cannot we be in symbiotic relations with our crafts and tools, residents, the places that we dwell? This is one of the main questions the bio-design thinking embraces. Assoc. Prof. Joachim Mitchell from NYU, an architect and a researcher in the area of bio-design, points out that today we can grow our homes instead of building them from differentiated components of inorganic matter (Mitchell, 2010) Buildings made from biological components and processes and use of potentials of genetic engineering methods in this manner is the issue of his research group’s focus in Terreform ONE. Their works and design proposals demonstrate of use of mycelium, living trees, vegetation and even lab grown meat (Figure 2.3). Neri Oxman and her team of researchers at MIT media Lab (Figure 2.4) refer to the potentials of using the nature in the design with the metaphor of 'Fruit bearing Fruit tree" a cycle that sustains its existence and continuity with its ability to give birth to itself. In her TED speech Oxman (2015) points out how we have always seen nature as Mother and today we have to mother the nature to shield it from our increasing threat to the environment. Their magnificent study of Silk Pavilion is one of the most innovative works utilizing biodesign, demonstrating such delicate combination of machinery and labour of the nature (Oxman et al., 2015, 2020). *Bombyx mori* silkworm’s life cycle and abilities to produce silk is adapted as an autonomous agent in processes of design and production (Oxman et al., 2013).

Figure 2.4 Silk Pavilion I, MIT Media Lab & Silk Pavilion II, MoMA (Oxman, 2013, 2019)

According to Dollens (2009), it is possible to involve nature in design within the scope of biodesign, thinking differently than ever by emphasising the hybrid buildings of biomechanical and biological systems. Such designs will bring up new opportunities, new organisations and combinations while creating self-regenerating cycles and changing our understanding of material and source. He also quotes Deleuze’s definition of “Our Nature”, by emphasizing that “thinking is part of nature and that design is part of thinking, therefore consciousness and thought are environmentally co-dependent.”

Today many institutions, companies and individuals conduct research with new ideologies, that seek to cooperate with nature, Wide range of organisms ranging from silkworms and mushrooms to bacteria and algae, have been employed in pioneering works of many visionaries, like Neri Oxman (2015b), Joatchim Mitchell (2006) to Vincent Callebaut (Naguib &Hanafi, 2013; Dade-Robertson, 2017; Vincent Callebaut Architectures, 2020), have proposed several projects to be constructed and bio-fabricated by living vegetation and organisms within this context.

All of the project teams working with biologists and professionals in the linked areas, asked questions such as “How can I make this stronger and lighter?”, “How can I use less material and have less losses?”, “How can I shorten the product line?” and “How can I make use of machinery and codes to comply with how nature, drawing for the experience of thousands of years, processes?” While they show a novel way to answer these questions, these questions still need more research (Kırdök & Tokuç, 2018).

Adamatzky et al. (2020) defines biofabrication "as the production of complex living and non-living biological products from raw materials such as living cells or biomolecules." This production occurs using mechanisms of living organisms to self-assemble materials with specific purposes on a molecular-level and currently cannot be achieved with any mechanical tool. (Attias et al., 2019). Therefore, developments in biofabrication would lead a paradigm change through the future of traditional industries.

Nature has been the major source of materials for designers with limitless possibilities for centuries. On the other hand, the ways to manage the properties and shape of matter in order to functionalize and produce materials as desired has been advancing increasingly fast in the last centuries (Attias, et al., 2019). To that end, as one of the novel production methods, use of bio-fabrication is expanding within the industry. Currently main application areas of such approach are mostly developed for medical uses; however, nowadays new examples of bio-fabrication in different sectors are promisingly and progressively appearing (Mironov et al., 2009).

Elbasti & Alacam (2017) refers to this progress in architecture as; understanding the principles of biological material systems and mechanics that organisms employ to accommodate their special needs, and labouring of this knowledge became a new trend; "a field of play for architects (designers) to explore further". More than a playground; “Biological architecture is not a science fiction” Dollens (2009) suggests; but the progress will be dependent on radical ideas, as these fiction demonstrates if we are to learn from microbiology and reflect these findings to architecture. In this manner, for example, we may achieve constructs with photosynthesizing walls or membranes.

Humanity has been in mutual connections with vast number of other species since its dawn. Michael Pollock points out in his book, “Desire of botany”, these interactions have been shaping the genetics of species like potatoes, apples, tulips and hemp for many centuries. These genetic alterations occur just because of our selection of qualities of these mutual species, in order to obtain the most useful, tasteful, strong, mesmerizing variant. So long humanity has been shaping the genetics of many botanical species, they interact with. We were choosing from the genetic pools of these vegetation’s, the adaptive ones to human will, long before we discover how to manipulate genes.

With the advancing techniques in molecular biology and genetics, Alberto Estévez points out how genetic modifications may lead to a great change. He refers to Eduardo Kac’s genetically engineered bioluminescent rabbit Alba and asks what if this technique is used in architectural design to create such places like furry bio-illuminating rooms? He envisions a potential of genetic architecture, with genetically manipulated fur grown in desired shape, texture, and colour to form architectural units, without harming any animal, but instead creating it *in vitro* (Cogdell, 2011). But is it possible? Perhaps not today, but advancements in biotechnology are evolving rapidly. A patent study by Venter on versatile synthetic genome with lengthy amino acid sequence was first created on a computer and assembled at a biotechnology company that synthesizes DNA, in order to be inserted into a bacterial cell in May 2010. The cell accepted the genome and began expressing the proteins coded by the synthetic genome (Cogdell, 2011). This is one of the lead steps in coding living organisms to utilize in production. Rothmund (2007) outlines our will to "learn how to program self-assembly so that we can build anything" that one day we may gain ability to create complex structures like living and functioning advanced organisms. All these scenarios are possible, yet there are still many steps to be achieved in order to master such technologies. Genetic architecture relies on controlled production of living cell or tissues. Potential of such applications in architecture have been for a topic of ideas like "Protocell Architecture" (Armstrong, 2009). Although genetically modified Organisms remain questioned by many people and health organisations, potential applications of this research area have already established that they will be a part of our future. Yet, these ideas are not currently realisable or couldn't be achieved on commercial levels, because of their hard dependency on lab conditions and technical requirements. However, finding ways of collaboration with the nature and adaptation of our methods in a mutual way with the life cycle of the other organism' will be an important step towards this future. The potential of fabrication with living organisms, by leading these living organisms to cooperate with us, can become a win-win situation for both species.

Within this perspective, use of mushroom mycelium as a bio-fabrication tool to achieve different properties yield great potential, therefore it is an issue of many pioneer researchers. Can it be combined with natural ingredients like clay? (Sheinberg & Gönül, 2019) Can living mycelium tissue act as an analogue bio-computer that responds to environmental changes passively? (Adamatzky et al., 2020) Can we grow vegetative tissue over mycelium layers and design parametric structures? (Mayoral González, 2009) Can it be designed to form self-repairing and self-growing structures? (Vallas & Courard, 2017). These are some of the questions raised over potential use of mycelium in architecture (Kırdök et al., 2020) (Figure 2.5). This topic will be discussed further in the third chapter.

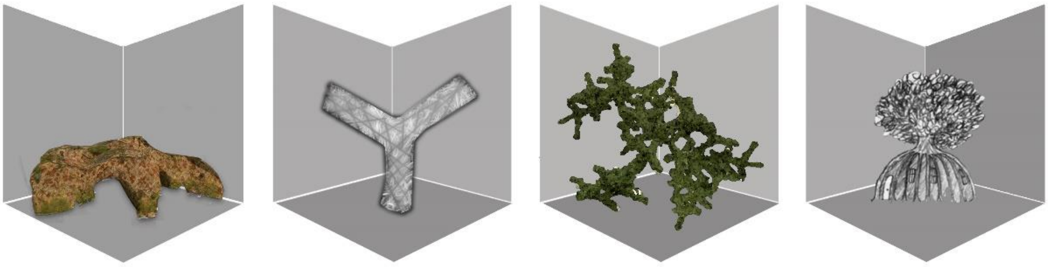

Figure 2.5 Use of mycelium of mushroom as a bio-fabrication tool (a. Claycelium, b. Fungal Computers, c. Growing architecture through mycelium and agricultural waste d. Building a living house with mycelium and trees) (Kırdök et al., 2020)

# CHAPTER THREE
# MYCELIUM OF MUSHROOM

## 3.1. What are Fungi?

Fungi are a kingdom of organisms, one of the oldest inhabitants of the earth with a primal role in natural ecosystems as decomposers (Cossio et al., 2012). They consist of single-cell, multicellular, or spore-producing organisms, and feed on organic matter. Although, most of their life cycle is cryptic, hidden underground in soil and dead matter, the most common vision coming to mind is generally mushrooms, molds and yeast. Commonly, the term mushroom is used to identify the edible sporophores, the umbrella-shaped fruiting body (sporophore) of specific type of fungi (Encyclopædia Britannica, 2020b). As these mushrooms sprouts above the ground, they begin to form specialized cells on the gills or the pores, to produce spores to be released into the air. Except the growth range, spores are the way mushroom producing fungi widen its region and move in different places. Any ground, that supports the growth conditions of the landed spores, would become the base for germination and colonization. Generally called as "substrate", this base is the food, moisture and heat source, while acting as a scaffold for a fungus to form. Substrate can be soil or bark of a tree in nature, as long as it contains the resource that fungus needs to form. If the environmental conditions are within the required range of the given species, spores will begin the forming phase of the fungus. When spore sprouts, it starts to form "hyphae"; 2–10 μm-wide microscopic cellular fibre generally based on chitin or cellulose, or in some cases both. This is basically the primary unit of the fungi that forms the general biomass. Secreted enzymes, released by these fibres, break down the polymers in the substrate that serve as nutrients for the fungus. These fibres multiply and form three-dimensional networks within the substrate in time, as they decompose the complex molecular structures they are attached to. As a result, fungal biomass replaces the organic matter within the substrate particles and forms bounding structures within the substrate (Attias, 2020). In nature, fungi preferably digest lignin and cellulose, the primary structural components of wood (Grimm & Wösten, 2018). On the other hand, their ability to decompose complex molecules makes them a great tool for environmental bioremediation of agricultural waste and reclamation of pollutant chemical compounds like textile dyes (Salame et al., 2013), therapeutics (Cruz-Morató et al., 2013), explosive residues and many others in the soil (Pointing, 2001; Attias et al., 2020). These networks of hyphae keep multiplying through the food source to become the fungal tissue called "mycelium". Depending on the availability of food source, mycelium can live up to hundreds of years or die in few months. As the initial older parts cannot reach for nutrients, these cells begin to die and become a base of growth for bacteria. These bacteria digest the mushroom in order to release the nutrients back to feed the soil, while spores released from fruiting bodies of mushroom form new hyphal networks (Karimjee, 2014). As long as there is sufficient nourishment and environmental conditions are suitable, mycelium will form new spawn of mushrooms in order to sustain its existence and growth (Encyclopædia Britannica, 2020b). As a result, mycelium body can enlarge within the underground in time using resource veins to form massive biomasses.

The largest known example is a single genus *Armillaria*, which is identified to have colonized more than 1500 hectare of soil in Oregon (Wilson, n.d.). This alone makes them the largest organisms living on earth (Ferguson et al., 2003). Mycelium can be referred to as the building/forming stage of the mushrooms life cycle. This stage of the fungal cycle is the main source of most commercially used mycelium based materials. The main goal is to use mycelium forming stage to biofabricate new biomaterials from fungal tissue of hyphae. These hyphal networks form 3D interlocking fibres to fill the space within the substrate, eventually becoming the material integrated with the substrate. As a result, the final material forms with the properties arising from both elements; substrate as the base and the mycelium as the binder, which can be referred as a biocomposite already. Type of fungus and substrate, growth conditions and post processing are the main factors influencing the final properties of the biocomposite (Attias et al., 2020).

### 3.2. Patents, Startups and Mycelium Industry

Our relationship with fungi dates back millennia. Many capped mushrooms can be found easily in the nature and have been known and collected since the hunter-gatherer communities as a rich nutrient source of human diet. From wine, to bread, many of our foods require fungal activity in order to be produced. In addition, it`s been known that several mushroom types have healing properties, with a wide range in medicine. *Ganoderma lucidum* is one of the examples of this kind of fungus. It has been in use for nearly 4000 years in ancient China as a medicine. However, the commercial use of mycelium-based materials is quite young. The first patents on mycelium have originated in the last decade and have intensified over the past few years. All of them reveal that mycelium is a strong binder, which can be grown by inoculating cells on waste materials and binding them. They emphasize that the end-product of the binding process does not have as many harmful environmental effects, compared to many materials we use today, causing high carbon emissions. The mycelium-based materials can be produced simply and decomposed in the soil after their expected lifetime has expired.

Early patents focus on how mycelium growth develops on lignin-based wastes or agar and intend to identify the organism’s development process and the necessary environmental conditions (e.g. US20130202855A1; US20130202855A1; US20150033620A1; US20160264926A1; CN1711885AA; CN1711885A). Among these, research about the growth of the fungi without producing its fruit is of particular importance (e.g. US20150033620 A1).

Many following patents focus on the production of biodegradable composite materials from mycelium and research different species of mushrooms, different types of wastes [e.g. hemp, wool, cotton, sawdust, wood, coconut, sisal, cereal straw, rice straw, corn, barley, oats, sorghum, kapok, flax, hemp, jute, ramie, cotton, stinging nettle (i.e. Urtica dioïca), animal fibers, said fibers, cardboard, etc.], different moulds and production mechanisms (e.g. production process by pressing in

WO2014195641A1; production process by pressing in roll form in US10537070B2; production by weaving the hyphae on a grid-frame in US 20180146627, or forming on the grid frame US20200055274A1; by moulding for block production in US2012O135504A1). Some of these patents contain recommendations for the use of the composite material as a packaging material in small-scale industrial products, or in the manufacture of thermal/acoustic insulation panels or fire-resistant panels, as well as some textile products. The resulting product is stated to be resistant to external environmental conditions, a good insulator, and fire-resistant (KR101571043B1; CN110713370A), it can also be produced quickly and economically.

Nearly 100 patents deal with licensing rights in America and China, and there is a small number originating from Korea and Canada. Ten patents about mycelium based composite materials are still valid. Three of them (WO2018068456A1, WO2019099474A1, WO2019226823A1) encompass all over the world and seven of them (CN108699507 A, US20190322997A1, US20190338240A1, US20190390156A1, US20200024577A1, US9879219B2, US9914906B2, US20200055274A1) encompass European countries.

One of the pioneers in research and industrial application of mycelium-based products is the firm Ecovative. In 2007, Ecovative started to commercialize its mycelium-based products, such as chipboards wherein glue is replaced by mycelium, insulation, or packaging mycelium foam, or even a do-it-yourself kit to grow mycelium. Their range of products include packaging products, which are being produced for different partner firms that use and develop this technology, such as Dell, Ikea, Biomason, Gunlocke, Bolt Threads, and Sealed Air. Mycelium packaging is a powerful competitor against the conventional non-biodegradable plastic-based products, which have been an issue of land and air pollution from the beginning of their crafting phase. Mycelium offers a nearly carbon clean production process and a fast biodegradable alternative. Besides, countries like the USA offer tax reductions, due to the use of biodegradable packaging products, to such firms. Therefore, mycelium products become a valid choice in this industry. Ecovative also produces their products using genetically modified mushrooms according to its patent (US20150033620 A1). Owing to the modification, fruit formation is not observed in this fungus, thus it is convenient to use for designers. However, there is no production continuity of this mushroom, since it does not produce spores due to the lack of fruiting bodies.

Another potential use of mycelium comes from its surface matrix and technique of craft as a leather-like vegan material. Today`s leather industry is a combination of animal cruelty and leather-like textile industries, which use many toxic chemicals during the production process. One of the leading firms in this area is MycoWorks, which manifests their mission as; “to impact the world with new materials that have superior performance to animal leathers and plastic”. They claim their products to “currently match or exceed animal leathers in their performance”. MycoFlex is another product of Ecovative, manufactured as an alternative bio-based material for the textile industry on a range of crafts, with its foam/leather-like surface.

The former MyCoPlast; Mogu currently has two innovative architectural products focused on interior production. Mogu Acoustics is an acoustic panel made from mycelium materials and of upcycled textile residues. Their second product; Mogu Floor is a collection of bio-based resilient tiles for interior design and architecture. The firm claims the tiles consist of a mycelium composite core, coated with a proprietary formulation of 90% bio-based resins. (www.mogu.bio)

There are several other startup firms like Fungalogic, focused on creating building materials out of agricultural waste streams, using fungi to transfer waste into valuable resources (www.fungalogic.nl). One of the current products of the company is an acoustic panel made from mycelium and tomato stems. The company offers furniture designs, like phone booths, made from these acoustic panels for use in the work/office environment.

### 3.3 Experimental research and the potential of mycelium in architectural design and construction sectors

By labouring the fungi life cycle we are able to form mushroom tissue in a mould as required. Most common industrial production method is to fill plastic-based moulds with an organic substrate that is incubated with mycelium to form a desired shape. Few other projects experiment with different methods, such as growing mycelium on a fibrous scaffold (Tabellini, 2015) using fibrous mats (Lelivelt et al., 2015), 3D printed cellulose scaffold (Klarenbeek, 2014) or growing mycelium on floating mats without a fibrous content (Hoitink, 2016). Most of the commercial patents on production of mycelium-based products pioneered by Ecovative and MycoWorks are based on similar concepts of moulded production processes. Final composite materials, produced in this manner, show properties similar to expanded polystyrene or other foams. Much research in this area is focused on developing a variety of products; packaging, building and insulation materials, leather-like textile and transparent edible films (Attias et al., 2017).

Drying out, heat and pressure, terminating the living activity of a biocomposite, is a way to freeze the state of mycelium forming to further point (Appels et al., 2018). Further, if production of fruiting body is restrained during this stage, there won`t be a production of spores, which can form an undesirable allergen. Drying without killing the fungus puts it in a steady state until conditions become favourable again. Under the right environmental conditions, the fungus would resume growing. However, heating would kill the fungus and would not allow fungus to grow further. There are several studies that question whether termination is necessary or not (Adamatzky et al., 2020). The fruiting body; the mushroom is one of the positive outcomes of our collaboration with fungus, since many of those known to be a nutritious part of the human diet, while many others have been used for their medicinal and spiritual properties.

Some futuristic research, search for a way to use a live mycelium network with mushrooms, as a bio-computer in order to manage the growth without a mould, while controlling the conditions over the whole structure, by examining and manipulating the electrical current and chemical activities of the fungus (Adamatzky et al., 2020). Olsson and Hansson (1995) made several interesting tests on stimulation of the fungus with an electrode to observe the information transfer over the mycelium, as an electrical output. Taking these as a base, Adamatzky et al. (2020) propose their concept design at the interface of biofabrication, functionalizing and computing of living substrate, for a monolithic living fungus based building, assembled by living fungal mycelium, enhanced by nanoparticles and polymers in order to create a fungal computer. They envision this fungal building to self-grow into desired geometries, as conducted by the architect, while presenting the properties like self-repair and waste management, as an environmentally adaptive structure that self-responds to the stimuli as a “biological" intelligent building paradigm. The group outlines potentials of biofabrication to control the growth and branching scheme of the mycelium network with a range of chemical and physical stimuli. To operate the growth, chemoattractants such as oxidised lipids, carbohydrates, peptones and some amino-acids and chemo-repellents, like sucrose, tryptophan and salt, are proposed for this purpose. Stimulation with light and volatiles is also another potential method to achieve the aim. Computing and functionalizing of the electrical current over the mycelium network are the key elements for these processes. In order to examine the outlying potential of mycelium to create such a structure Adamatzky et al. (2020) observed the electrical current through the fungus cells, as a response to thermal and chemical stimulation, by High Resolution Data Logger, in order to test the electrical properties of mycelium networks to serve as analogue computers. Meanwhile this work demonstrates the potentials operating mycelium networks as bio-computers that could be designed to act as a passive computer, which maintains itself. Their aim is to use this easy-to-grow biomaterial, and its biological information network to create a whole self-sustaining system.

The experiments show that electrical activity recorded on fruit bodies might act as a reliable indicator of the fungi`s reactions to thermal and chemical stimulation, since these fruit bodies react like input ports of the fungal computers.

Attias et al. (2019) points to the potential of fungi to react to changes of environmental conditions during its growth, like every living material. Hence natural features of mycelium come up during its colonization phase. This allows for a dynamic fabrication process, which is affected by biological and biochemical mechanisms of the living fungal tissue in order to modify final product's material properties**.**

Controlling the environment during growth is already within the range of our industrial technology. Today it is possible to build self-automated systems with the aid of several mechanical systems and technologies for clean production, which only require regular control and maintenance. The technologies like self-automated servant observation and response robotic systems have already been experimentally optimized and employed in several innovative farming industries. Therefore, it can be expected to have a self-sustaining, automated mycelium building/farms in a mutual relationship with the architectural designer's agenda. It's been a topic of research for most of the novel experiments regarding industrialization of mycelium design, which aims to use on-site fabrication and installation potential of this fast growing and resilient biomaterial. This will be discussed in following sections.

### *3.3.1 Design with mycelium (Mycodesign)*

As outlined in previous chapters, architecture must be taken into account as a process. This brings the necessity for underlining the lifespan of constructions. In their book, "Cradle to Cradle: Remaking the Way We Make Things" (2002), architect William McDonough and chemist Michael Braungart outlined the circular relation between design and material, eliminating the concept of waste via repurposing. The framework of Cradle to Cradle design thinking is a biomimetic approach deriving the idea of; "Everything is a resource for something else. In nature, the "waste" of one system becomes food for another." Additionally, as nature builds and decomposes simultaneously, everything can be designed to be degraded into subunits (Braungart et al., 2007). Hence, in this case, mycelium-based materials have many benefits which ultimately fit the Cradle to Cradle design principles, given their high biodegradability and recycling potential of the substrate it binds to (https://mcdonough.com/cradle-to-cradle/). While there are many environmental pros of construction by mycelium composites, there are still several steps needed to be taken into account before commercializing a whole performing mycelium-based structure.

There are also events like workshops, exhibitions and educational programs which encourage academicians, investors and individuals to think further in design. The Fungal Futures exhibition which took place in 2016 (http://www.fungal-futures.com/) is one of those events. Within the scope of exhibition design projects focused on mycelium have been showcased. One of them is the mycelium-based furniture design by Erik Klarenbeek. In this case 3D printed biodegradable materials were used as a shell scaffold for mycelium colonization. (Klarenbeek, 2015) (Figure 3.1a) One other interesting project is a dress fabricated with mycelium, developed over flexible textile and attached by a special treatment process by Aniela Hoitinc, who aims to change the way we use textile (https://greenstitched.com/tag/fungal-futures/, 2016) (Figure 3.1b). ”Mycelium tectonics” by Gianluca Tabellini also points out another design potential by mycelium, namely grown hemp-fiber scaffolds in 3D (Attias et. al., 2020) (Figure 3.1c).

Figure 3.1 a. Mycelium Project 1.0 & 2.0 (Klarenbeek, 2014), b. MycoTex Dress (Morby, 2016), c. Mycelium tectonics (Tabellini, 2015)

“Biofabricate” is an annual summit of the emerging world of cultivated products, taking biofabrication into focus. First of their Creative in Biotech series hosted pioneer designers, using mycelium as a biofabrication and design tool, like Maurizio Montalti from Mogu and Grace Knight from Ecovative design.

Biodesign Team Turkey also focused on the issue on national level and several novel design and ideas have been produced during the workshops organized & mentored by the team. During the 3rd workshop event in Studio-x Istanbul (2018) and 4th workshop event in Design Week Turkey (2018), teams of designers have been formed to work each on an organism type (Mushrooms, Calcification bacteria, Algeas & Slime moulds) in order to promote biodesign thinking (Figure 3.2).

Figure 3.2 3rd Biodesign Team Turkey Workshop (Biodesignteam, 2018)

Many institutions, ranging from Stuttgart University (Germany) to Dokuz Eylül University (Turkey), have hosted several experimental, structural and design courses in different departments of architecture.

Today there are many research groups, individuals and companies, demonstrating their progress and craft online in their websites, blogs or social platforms like Instagram and Facebook (Table 3.1). Advancing technology led general public to share their experience and production with each other in a more rapidly and open source fashion, as opposed to the conventional academy. This enhanced the communication and information transfer within the mycelium producers/designers community and the professionals. Hence the community grows daily, by the people`s increasing interest on social media, while more people are sharing their stories and crafts with each other. As a result, many new entrepreneurs can follow these production processes and experiments. Such open-access knowledge transfer leads people to become more enthusiastic and enhances further progress. This means an ever-growing data pool for further applications and shows the potential future of the mycelium industry. Therefore, designing with mycelium and its common acceptance in design field develops faster. When we consider architecture discipline, where the same forms and materials have been used over and over again for many centuries, such progress on a short time scale is a significant achievement of mycelium of fungi.

Table 3.1 List of researchers and corresponding online platforms

| Researcher | Link |
|---|---|
| Turkiye Biodesign Team | https://www.instagram.com/biodesignteamturkey/ |
| Biodesign LAB | https://www.instagram.com/biodesign_lab/ |
| Mogu | https://www.instagram.com/mogumycelium/ |
| Mycelia BVBA | https://www.instagram.com/myceliabe/ |
| Mycotech Lab | https://www.instagram.com/mycl.bio/ |
| Institute for Advanced Architecture of Catalonia (IAAC) | https://www.instagram.com/mycocrete / |
| Grown.bio | https://www.facebook.com/grownmaterials/ |
| Mycoworks | https://www.mycoworks.com |
| Ecovative | http://www.ecovativedesign.com |
| Amanda Morglund | www.myceliummade.com |
| Institute for Advanced Architecture of Catalonia (IAAC) | https://www.instagram.com/mycocrete / |
| Janis De Vogelaere | https://biofabforum.org/t/update-on-bioluminescent-mycelium-project/427/3 |

### *3.3.2. Architecture with mycelium (Mycoarchitecture)*

The 21st century has conceived many new architectural trends. Among all; the pursuit of building higher, constructing sustainably and designing via integration of biological disciplines, stands most prominent, as Naguib & Hanafi (2013) outlines. Therefore “a new balance will be struck between scientific-technological advancement and human development” in order to replace previous ecological design visions with modern biocentric inspirations and designs. However, rising century brought along its own problems to be solved. Hanafi defines algorithm as a series of methodological step used to calculate problem solving and decision making. Today from creation of artificial intelligence to the production line, all technology relies on algorithms, which must be adapted to survive and improve along with today's needs. Therefore, architecture and the building industry should update its algorithms too. With the warnings the global warming poses, the biggest problem in today's world is our disconnected status from the nature. And to solve this issue it requires a series of calculations and serious decision making. We must define a new algorithm for architecture. In that manner observation of nature, understanding of its algorithms and creation of mutualistic connections with nature becomes a primary approach for the upcoming future of architecture.

Even with these advancements, we barely scratch the surface of perceiving the magnificence of life cycles. The true potential, to enhance and maintain the possible scenarios of chains to happen and adapt, still, lies behind the curtain. Knowledge has been one of the greatest tools in our arsenal. However, the knowledge itself is just a mere tool in sight. The way we use this tool defines the outcome. Collaboration between the different fields of science yields a great potential for research and application in architectural design, to use these tools efficiently.

In this manner, while biology is not a new area of research, with the new decade humankind's library of knowledge have been doubled many times already and keeps growing every day.

This thesis aims to use the growth cycle of mycelium in line with today's technological advancements, forming a multidisciplinary study bridging the fields of building and natural sciences. Several of innovative and pioneer studies on this topic will be addressed in the following text.

There are very a few examples of structural applications realized by mycelium composites in building constructions. Adamatzky et al. (2020) categorizes these examples into two different areas, according to design and structural approaches, in order to classify the distinctions between these pavilions. First group is the “production approaches that seek to grow monolithic structures and those based on discrete-element assemblies" and the "production approaches that kill the mycelium during the production process and those that seek to maintain the mycelium as a living organism" is the second group. Several of these architectural pavilion designs will be assessed with regard to their structural qualities and the state of the organism.

First recorded architectural pavilions based on mycelium were built upon brick-like basic compression elements, integrating discrete-units in order to perform structurally. To be exhibited at MoMA PS1, Ecovative produced mycelium bricks for 13m tall structure, Hy-Fi tower, was reported by the Living to be “the largest architectural structure made of mycelium so far” (Benjamin, 2014; Dessi-Olive, 2019; Attias et al., 2020). The structure of pavilion is designed as mycelium bricks, aligned to give the form of a barrel vault shape with three large chimneys over a structure made of steel and timber for support (Attias et al., 2020; Adamazky, 2020) (Figure 3.3a). Bricks used in Hy-Fi tower were cultivated in plastic moulds and terminated in desired form by heat treatment. Another smaller pavilion designed by mycelium brick units grown on an oak sawdust substrate, Mycotectural Alpha, have been revealed by Philip Ross the same year (Ross, 2016) (Figure 3.3b), In this case he kept mycelium tissue alive throughout the duration of exhibition.  This way mycelium kept colonizing and this growth integrated the bricks by fusing with each other. In this case mycelium acted as a natural binder. Integrity of the form and strength of the pavilion have been enhanced without a need for any adhesive, by labouring its biological abilities. Moreover this structure was able to produce mushrooms in order to be harvested freshly, and the group served the visitors tea made of this mushroom, which is known for its medicinal properties; *reishi* (Ross, 2016; Attias et al., 2020; Adamazky et al., 2020).

Figure 3.3 a.Hy-Fi Tower (Frearson, 2014) b.Mycotectural Alpha (Inhabitat, 2014)

Shell Mycelium in Karela is a design by Rahman, Arredia, and Yassin’s, a group of Indian and Italian architects (Frearson, 2017a; Attias et al., 2020) (Figure 3.4a). Design forms as a shell-like canopy structure. In order to obtain the structure, the group connected wooden beams in triangular matrix and closed the bottom of these triangles by boards as to form boxes where colonizing mycelium was to be placed. The aim of this pavilion was to create a lightweight coating layer, which can attach itself to the pavilion. Upper layer of the colonizing mycelium is dried, while the rest of the parts attach itself by mycelium to the wood structure. While mycelium is not utilized in this design as a structural element, but rather issued as a layer within the design, allowing the live mycelium form over the built structure, forms a promising idea. Yet, control over colonization is limited and risks like contamination are fairly higher than those of a building based on pre-produced units, which are already terminated or strongly colonized.

On the other hand, MycoTree project at Seoul Biennale 2017 is a very interesting design by Dirk E. Hebel and Philippe Block from ETH Zurich (Switzerland), and Karlsruhe Institute of Technology (KT, Germany) assesses the structural potentials of mycelium (Frearson 2017b; Attias et al., 2020) (Figure 3.4b).  The construct is made of computationally designed mycelium blocks and simple timber joints to support its own structure in a tree form. While this self-supporting tree-shaped structure shows a brand new approach, as oppose to other compression structures by design, it also is built upon mycelium units, which are thicker and higher in volume.

Figure 3.4 a. Shell mycelium (Frearson, 2017a), b. MycoTree (Frearson, 2017b)

A student at London’s Brunel University, Aleksi Vesaluoma developed an innovative technique to mould growing mycelium. He filled tubular cotton bandages with mycelium mixed with cardboard in order to form “mushroom sausages” (Morby, 2017) (figure 3.5a). He worked with a studio to create a Grown Structures series based on this innovation. After filling these sausages, he positioned these in an artistic layout, as juxtaposed buckles in a mould and let them colonize for a four-week period inside a greenhouse with ventilation. At the conjoining points mycelium colonization merged these sausages together like “glue”. Their vision is to use these designs to realize contemporary restaurants that are grown from and meanwhile serves mushroom, as an outcome of using live mycelium

Another larger scale pavilion, namely Growing Pavilion, is designed as a collaboration between Pascal Leboucq and Erik Klarenbeek’s studio Krown Design (Pownall, 2019)(figure 3.5b). On a circular layout, the group used timber as the structure that was enclosed with mycelium panels, in a fashion allowing for removal and reuse, if necessary. The rest of the materials are also composed of biomaterials, which is one of the goals of the design by Leboucq. “He outlines that; he wanted to make a bigger statement, so that a lot of people can discover this fantastic material”. Pavilion was then placed Ketelhuisplein for the Dutch Design Week in Eindhoven. The group aims to keep their experiment on this pavilion, with a goal to build a pavilion “that will last outdoors for a few years, or even longer.” Mayoral also reported that the people at Ecovative Design focused on developing a procedure using essential oils, enabling it to be built and grown outside (Mayoral, 2009), which is one of the most crucial limitations of designing with mycelium constructs outdoors. Yet, this issue still requires further research before achieving a commercial stage. The designers of Growing Pavilion used a natural waterproof coating acquired from Mexico and used to protect these panels. Although mycelium panels demonstrate plausible qualities in terms of sound and heat insulation, they are mostly susceptible to environmental effects.

One other important aspect of the project is that the mushrooms grown from the panels were daily harvested in front of visitors and freshly cooked meals made from these mushroom were sold from a food truck nearby. (Pownall, 2019) Such demonstrations, as Ross and his team did with Mycotectural Alpha, are very important to attract public attention and defeat public's prejudice, which is vital to commercialisation of mycelium materials.

Figure 3.5 Mycelium façades (a. Sousage structure of mycelium (Morby, 2017), b. Growing pavilion (Pownall, 2019)

On a small scale, the first exploration of monolithically grown mycelium designs can be can be assigned to the artist, Erik Klarenbeek, who has achieved numerous inspiring ways of structural experimentation in combination with adapted 3D printing technologies.

Requirements to build monolithic mycelium structures are similar to other production techniques; namely sterile, dark environment with moisture and sufficient air circulation. However, increasing thicknesses prevents sufficient mycelium colonization and premature death due to lack of air access, which requires a specific mould design to properly manage growing conditions for a monolithic mycelium structure.

In quest of designing self-supporting monolithic mycelium structures, begins with “Mycoarch”, a monolithically grown, almost a person tall twisted arch made of approximately 0.4 $m^3$ mycelium, produced by Ecovative (Figure 3.6a). This very first experimental structure collapsed before fully colonizing because of structural instabilities and effects of rain. However, such application encouraged other researchers, suggesting that growing monolithically was possible. An experimental architecture course “Thick and Thin” at the Georgia Tech School of Architecture carried this encouragement further and designed a double curved monolithic mycelium arch, to work as a compression structure, due to the material weakness (Figure 3.6b). Form-finding and cardboard core to support structure, was computationally designed, using Rhinovault and internal formwork scheme. The group decided to use cardboard here, in order to let mycelium to fuse in this core, which also forms an additional food source for the mycelium. Growth took place indoors due to the relatively small size, which allowed more control over the environment and reduced the risks of contamination and low colonization. The voids left on the surface after the first growth periods, were patched by students via application of fresh mycelium. Several months upon drying of the structure, it achieved strength sufficient to support a person’s weight of approximately 75kg.

After the success of this pavilion, Group attempted to scale up their technique in order to create a bigger structure; “Monolito Micelio”, a bio-pavilion which served as a performance structure for a barbershop quartet at an exhibition in the School of Architecture at the Georgia Tech (Figure 3.6c). For the design of the pavilion, same compression-only principle has been used as in “Thick and Thin”. The form comes from a mushroom column concept, split in four similar parts, arranged in order to create a vault-like pavilion in a cubic space of 2.5 x 2.5 x 2.5 m. Approximately 800 kg of live mycelium material was employed to produce the volume of the vault design of 2.75 $m^3$ volume.

The group outlines the importance of the mould/formwork design;" Structurally, the formwork must be robust enough to resist the influence of the wet and densely packed fibrous substrate. Large quantities of mycelium produce quantities of carbon dioxide that are damaging to its growth, so the formwork materials should be semi-porous and breathable. To achieve outdoors growing structure at this scale, they have designed a formwork that has "rigid internal 'lost-work' reinforcing skeleton, and a hybrid removable formwork system that combines a highly controlled plywood exterior with a highly flexible geotextile interior."

The pavilion was installed by three working groups; Breakers - to break the pre-cultivated bag of mycelium ordered from Ecovative, Feeders to mix this pre-cultivated mycelium with additional food source and moisten it homogenously in a clean cement mixer as Ecovative suggests for their product, and lastly; Packers, installed the formwork and filled the mycelium in. Installation was completed by an average of six people working for twelve hours. Group then removed the formwork gradually, in six days, to finalize the pavilion. To achieve such structure from mycelium, the group points out that "a combination of correct form, internal reinforcing systems, and robust yet breathable formwork materials are crucial to the success of grow structures with monolithic mycelium techniques." (Dessi-Olive, 2019).

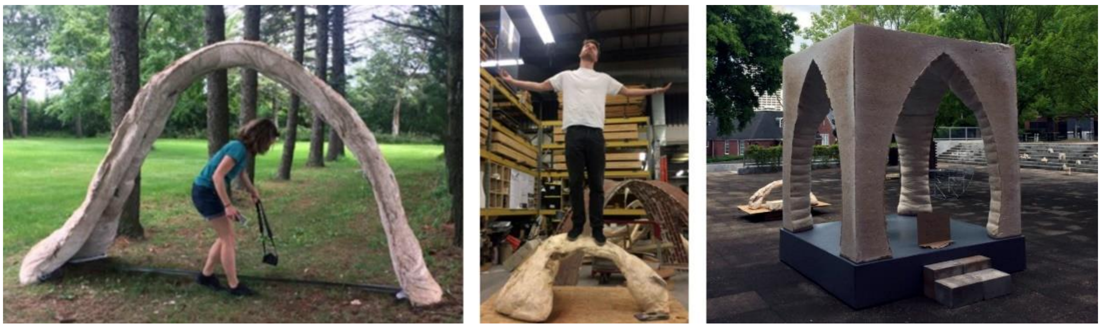

Figure 3.6 Monolithic Mycelium: Growing Vault Structures by Dessi-Olive (a. Mycoarch, b.Thick and Thin, c.Monolito Micelio) (Dessi-Olive, 2019)

While pavilion installations are very important as the stream line of progression of public recognition, as they demonstrate realized applications, conceptual designs are the roots and branches of commercialisation and introduction into the market.

Although academic studies on the use of mycelium in architecture and design are limited, lately the interest over this novel research area has increased. By the aid of tools of biotechnology, researchers are able to observe, experiment, analyse and alter properties of mycelium-based composites more precisely. However, the majority of studies in the literature on mycelium-based materials are still missing the basic information, such as fungal strain, type of substrate, environmental conditions and production techniques (Attias, et al., 2019). This attitude leads to many conceptual designs to become abrupt, preventing realization of the projects. However, such imaginative thinking without the boundaries of material, pushes the architectural design forward in a way that cannot be ignored.

Such example can be observed in a Master of architecture thesis work written by Munira Z.Karimjee in Azrieli School of Architecture and Urbanism. She proposes a cast on site production cycle with mycobricks and a continuous factory that supports this biodegrading infrastructural technology, to build a landscape design as a park, envisioned to host music festivals (Figure 3.7). The main idea behind the design is to heal the land by growing fungi on the polluted soil, to clean the land while using this soil to produce biodegradable mycelium bricks supporting the desired infrastructure construction, which will form a part of the soil, cleaned by the mycelium’s digesting properties (Karimjee, 2014). Inspiration behind this project comes from a design and patent held by mycologist Paul Stamets and artist Philip Ross, a brick shaped mycelium building element to produce fungus structures. Although proposal carries a lot of conceptual qualities, study shares very limited information related to mycelium, like the type of substrate, environmental conditions etc. The rest of the information on possible risks and how these will be avoided, which is a major design problem when working with mycelium, is blurred, as the concept spine is built upon a patented design.

Figure 3.7 Park design proposal by Karimjee (1. Park design, 2. Grow room) (Karimjee, 2014)

In a experimental work, Elbasdi and Alacam (2017) used a growing kit from a local supplier containing wheat grains and sawdust inoculated with *Pleurotus ostreatus*. Although provider suggests 12-21 days of growth over the substrate for fully grown mycelium, only 6 days of growth was monitored for this study, since goal of the research is to observe the adaptability of the material to the fabric formwork (Elbasdi & Alacam, 2017). However, the test conditions were not properly provided and the mycelium mixture in the growing kit wasn`t homogenous. These kits tend to be intrinsically contaminated or get easily contaminated under suboptimal conditions. Therefore the results of this experiment can only be taken into account from the perspective of aesthetic and conceptual potentials. Their observation on solidification of the biocomposite on the fabric formwork over time, however, can be scientifically valid. According to their observation, the first 2 days is the best time to change the formwork, while the ability to adapt falls drastically upon five days after inoculation.

Mayoral (2009) explains the term biocomposite as composite materials made of organic components showing enhanced properties, as compared to each component's standalone performance. In this manner he points out the potentials of programming the living matter, in order to achieve biocomposites with special utilities, such as genetical modification or use of intrinsic bioluminescent properties. His proposals, aiming to achieve a grown cultivated habitat and designed by parametric 3D CAD modelling, rose from units made out of Greensulate product of Ecovative Design, such as grass grown on top of glowing mycelium biocomposites. He claims that by using such biocomposites it is possible to produce fully disposable, biodegradable and compostable products and designs, ranging from inhabitable structures to insulation.

His proposals are focused mainly on the concept design prioritizing aesthetic properties and structural potentials over material properties and their effect on final product. First proposal presents a modular 3D structure as a branching pavilion, second one is a wall prototype, reinforced with additional tree branches, placed in the core to become a unit, projecting an inhabitable, growing dwelling. In order to achieve a parametric design, Mayoral used 3D Design program Rhinoceros with grasshopper, as to create a modular design with several algorithms facilitating a unity between the modules, to respond the needs of an urban pavilion. Mayoral used several relations between the space and the proposal in order to compare porosity, density, friction, solar access and lighting. Therefore, he was able to observe the different outcomes of computer aided parametric module manipulation scenarios.

Figure 3.8 Module and design proposals by Greensulate (González, 2009)

In the second proposal, Mayoral offers a variety of potential scenarios for use of mycelium wall, which would be built using mycelium blocks made from Greensulate reinforced by branches (Figure 3.8). These scenarios mainly serve as temporary inhabitable places such as extensions or complement for an existing space, because of short life span of the biocomposite, if not treated. The main idea is to create an inhabitable system that serves the city as an organic extension, which would lead to minimization of the transportation costs, hence reducing the pollution and fuel consumption. Since mycelium is an organic material, it lets the end user to have cultivations or plants as part of the structure; these structures can support food growth, oxygen generation and air cleaning (Mayoral, 2009).

Upon computational design, in order to build prototypes, Mayoral designed moulds for 2 types of modules, than 3D printed these modules. After disinfection of these moulds with hydrogen peroxide, he filled them with pre-boiled agricultural waste. Next, he spread mushroom seeds and placed the moulds in bags with humid environment to let them grow. During the growth process, the units of the mould were fixed, in order to achieve a structure with a joint point. To increase the structural strength, Mayoral placed sterilized tree branches into the core with the agricultural waste, to act as reinforcement (Mayoral, 2009). However, there is no information about material and conditions such as the type of the fungus, agricultural waste or type of branches used, nor about the length of the process, growth conditions and the production environment. The only acknowledgement underlined is the reference to Greensulate product of Ecovative. Yet all of this information combined brings out the final material property and qualities.

Vallas and Courard discussed a proposal design of a living building with a range of characteristics to grow, build and repair itself while responding both to its user and the environment by any means, from ecologic to economic aspects. In this proposal, the most obvious problem is the high risk of contamination of mycelium components during the process of growth in the outdoor spaces or environmental factors that can cause erosion and dispersion. The proposal offers to use a substrate (preferably sawdust) inoculated with *Ganoderma applanatum* mycelium, although further research on optimum substrate and fungus combination is necessary. Yet the research proposes that mycelium sheets are waterproof (with and without pine tar coating). The sheets have a good resistance to fire, as they can stay on a Bunsen flame for several seconds with no significant alteration prior to carbonizing without flame, with or without pine tar coating. Although the idea to build a whole living structure still requires more research, this kind of proposals are frontiers of biodesign path (Vallas & Courard, 2017).

Today many visionaries ranging from Joatchim Mitchell (Mitchell, 2006) to Vincent Callebaut, have proposed several projects constructed by living vegetation and microorganisms. Yet challenging the design is not the sole answer. In order to achieve design expectations, material and method must blend in harmony. Architectural designers who seek to address all the requirements of their design must also be aware of the fact that, the nature is not just there as a cheap market for materials. Design, material and form are bound to each other in an endless cycle.

### 3.4 Mycelium based materials (Mycomaterials)

Researches show that the choice of material has a huge influence on high $CO_2$ releases and pollutions, caused by building industry (Pacheco-Torgal, 2015). Therefore, new toolset of materials, that show better properties and compatibility with nature, in order to reduce the negative impact of our constructs, became one of the pioneer research areas of the past century. Biomaterials are one of the most promising of these toolsets. Some of them degrade well in the nature and doesn’t affect the habitat, one example of which is mycelium-based materials (Karimjee, 2014). While others act like a carbon sink; trapping the carbon within the materials used in construction, with long life spans and fast yielding potentials, as stated in Biorock of Wolf Hilbertz (Dobraszczyk, 2017). Using the knowledge of nature in combination with the current industrial production processes, to achieve a material for construction, as a compatible product with desired properties that go along with its environment, yet standalone they don’t answer the need of an evolved architectural perspective or a design. Material, method and design are not separable parts of architecture; hence architects must learn to cooperate with the nature.

As a novel research area, there is much on-going work focused on understanding of what mycelium is and what can be done with it. How can we shape it? How can we modify it? What is its strength? How can we improve it? Protect it? To answer these and many other questions, today many researchers from all around the world compete on an academic level. Several of them will be addressed in the following sections.

### *3.4.1 Mycomaterial Research & Preliminary Experimentations*

This chapter will survey through the literature over production process of mycelium-based materials and the factors that affect the colonization process and consequently the final product. According to those studies, which dictated author's decisions regarding the four stages of the experiment will be explained.

Haneef et al. (2017) tested two fungal species grown on two substrate types, one with potato dextrose in combination with microcrystalline cellulose (MCC) and the other with MCC alone, which is harder to digest as a substrate for fungi. MCC substrate example brings out a stiffer composite, emphasizing the effect of the substrate on mechanical properties of the composite material.

Appels et al. (2018a) studied the factors which may affect final morphology of the product and found out that parameters such as, the type of substrate, species of fungus, and post-processing methods, such as hot pressing can influence morphology, density, tensile and flexural strength, and water absorption properties of mycelium based materials. Attias et al. (2020) claims similar ideas. When compared with EPS foams, mycelium-based foams show lower density, flexural strength, and water absorbance. Hence, to compete with the conventional synthetic alternatives, progress on reduction of dried material density, increased flexural strength, and decreased water permeability is necessary, for the commercial applications to be successful.

Jones et al (2017a, 2017b, 2018, 2019, 2020) performed series of experiments on producing fire retardant mycelium-based foams. The group used rice husk, an agricultural waste and natural fire retardant, that contains high levels of lignin and phosphorous, which are favoured as nutrients by the fungi.

Study of Islam et al. (2017, 2018) points out that the mycelium matrix reacts differently to the soft and hard compression modes. This is a cause of rapid stiffening, which is known as “Mullins effect” and is abundantly observed in filled and unfilled elastomers (rubbers). This can be defined as the strain/stress curve, reflecting the load of the last affective maximum force.

To form the framework of this study, first a literature review over the current studies has been conducted in order to understand the state of the art proposals. According to their aim, these studies can be divided in three groups; first group focuses on physical properties of mycelium and its interaction with different condition sets, second group questions the ways of design with mycelium and tries to figure out new innovative approaches and the third group tries to include both goals. However, there are very few examples that begin with material testing and end with a commercial product experiment or a pavilion construction so far.

Optimization of production and enhancement of structural performance are the main requirements necessary to promote the use of mycelium in constructions. Use of mycelium as a material is merely a new issue with this respect; to enhance the structural performance, further research and experimentation is required. Yet before progressing through the upgrades, optimization of the material production and understanding the mechanics behind different combinations of this parameters become more crucial since the general process heavily depends on a living organism. Living organisms require greatly higher maintenance effort than conventional materials used in construction sector. This process begins with choosing the specific type of fungi to answer the design needs or material expectations, following with the compatible substrate type. Environmental conditions must suit this combination, supported by compatible mould design. These will be explained in following sections.

#### *3.4.1.1 Environmental conditions*

In nature colonization of *P. ostreatus* occurs mostly underground or hidden in wood-based substrates. These bases provide the necessary heat and moisture conditions for colonization and sustained formation of mycelium away from light.

During mycelium-based material production; most of the process, takes place inside moulds. There are several important points that must be considered with regard to the environmental conditions which must be controlled, in order to speed up the procedure and increase the safety against contamination etc. Lighting, temperature, moisture control and sterilization of the surfaces and where possible, of air, are the primary considerations. All of these environmental condition necessities are the facts of biological requirements of the *P. ostreatus.*

Most important of parameters to be controlled is the breach of light inside the mould. Since mycelium colonizes with negative phototaxis behaviour, which means it will colonize in the direction without light. Prevention over daylight, in order to prevent unexpected exposure to UV and visible light, would possibly enhance the speed of colonization. For necessary lighting, choice of pale/cold lighting fixtures would be appropriate; however the most optimal condition would be prevention from any light during colonization phase.

Living organisms perform at certain temperatures. For *P. ostreatus* colonize optimally between 22-27°C which is within the range of ambient room conditions, which we recall as most comfortable range of temperature for humans. Yet this is not a restriction, out this range colonization may perform, but will be slower and weaker. There are several options to maintain the required environmental temperature in growth containers, depending on the local temperature. Air conditioning, electrical heating and passive cooling are several of those.

Moisture control is an option to support the appropriate conditions of *P. ostreatus* colonization. Colonization can be performed in a locked plastic bag, which will keep the moisture inside indefinitely. However, the need for air circulation within these bags would result in moisture loss, therefore continuous tracking moisture levels and spraying extra water through the colonizing substrate, is a good way to maintain the moisture levels. Designing the mould in a manner that balances the moisture level at expected levels, would enhance the colonization speed and density.

Sterilization is one of the crucial issues. Contamination means competition, which will result in weaker colonization or undesired species forming faster than *P. ostreatus*. Beginning with preparation of the substrate and production environment, which requires highly sterile environmental conditions and labour, the most general problems occur during these very first steps. From cultivation through the colonization, high risk of contamination, unbalanced colonization of organic matter during distribution are the most common problems. To prevent these issues, understanding the mechanics between fungi, substrate, mould and environmental conditions is a must. Therefore, choice of fungus, preparation of the substrate, design and material performance of the mould, and postprocessing, form the four corners of the experimental design to begin with. All these parameters must be held under optimal environmental conditions in order to achieve the best performance. The choice over fungus, substrate, moulding and postprocessing will be discussed in the following part.

*3.4.1.2 Choice of Fungus type*

Series of studies and tests conducted by Attias et al. (2017, 2019, 2020) show the most informative study, including literature review over fungal species, substrate recipe and the conditions.

According to the literature review of Attias et al. (2020) while 22 out of 42 publication did not indicate the specific fungal species employed, all species detected in the literature review belong to the Basidiomycota division, which is characterized by their hyphae, that can split or fuse on a nano-level, create clamp connections for vegetative reproduction and nutrient transfer, and penetrate cavities on a microscopic level. Current data show that there are more than thirty thousand Basidiomycetes species on earth, however only 24 fungal species were identified in the literature review. This indicates the need for more experimentation and research over different species, which may open new horizons in mycelium products (Attias et al., 2020). On the other hand, there is not much research that projects the behaviour and dynamics of the final product providing a best case scenario for specific type of fungi. As most of the published studies use different substrate compositions and protocols, the possibility to conduct a comparative analysis or summary of the general properties of mycelium-based composites is currently limited (Attias et al., 2020) Few of these studies can be nominated for comparison of the process or results, but at the basis the strains would be different in genetic content. Therefore, optimization of a specific type of fungus for series of experiments would at least provide a basis for the continuity of the study.

The choice of the mushroom type has been made in accordance with both literature survey and ease of access of the fungus type. There are about nine fungal species which have been indicated in the literature until 2018. *Ganoderma lucidum* and *P. ostreatus* were found to be the most common over these (Attias et al., 2020). Although this information comes from a limited source, preventing a conclusion over the best alternative, *P. ostreatus* seems to be the most common, easy to find, and easy to grow option over all. The growth temperatures required by this fungus, was detected to fit the general room temperature threshold of 22-27°C. In addition to those, as a commercialized farming and recreational product, pre-inoculated *P. ostreatus* mushroom growing kits for hobby growers are easy to find both online and at local mushroom kit suppliers. Researchers' choice of the mushroom type was gathered by the ease to access in the region and popularity among the different studies, which makes *Pleurotus* family a good candidate for discussion and comparison.

#### *3.4.1.3 Mould design requirements*

Most of the studies in the literature currently focus on optimizing these conditions in order to achieve products with special properties. In a study published by Islam et al. (2017, 2018) it is pointed out that the network density, dimensions, elasticity, branching, and entanglement, influence the mechanical properties of the mycelium composite products. This means that the conditions during colonization directly affect the quality of the final product. Most of the colonization happens inside the mould, which renders the mould design one of the most important points in this case.

To cultivate a mycelium-based material, mould must meet several crucial requirements, in addition to giving the design its final form. Protection from environmental effects and contamination is the first and most important issue.

Contamination is “the state of containing unwanted or dangerous substances” in the cultivation space (Cambridge Dictionary, n.d.). These uninvited organisms would populate inside the mould, while limiting the desired organism to colonize and spread as intended.

Moisture and heat requirements are specie specific markers, that determine the most suitable conditions for the organism to form. As a reflection; maintaining the moisture and heat levels required by the fungi, while protecting the structure from external interruptions is the key challenge of moulding to be overcome within the scope of the design.  If moulding system cannot control the moisture balance or the heat, general distribution of organic matter over the product would be unpredictable and non-uniform. Therefore, postprocessing failures and other technical errors may occur because of the failure of mould design.

*3.4.1.4 Finishing applications*

After the colonization reaches the desired degree, the decision of using the composite alive or not must be aligned with the material expectations. While live fungus could be used to produce mushrooms to support human diet, its structural performance is reported to be weaker. Most living organisms have to maintain a certain amount of water in the system to keep its functions and survive. As the main source of life, water forms the major content of all living organisms. While humans are approximately 60% water, mushrooms show a magnificent performance with nearly %90 water content (Boztok, 1990). Hence, when dried out remaining organic matter, shrinks as the water evaporates and acts as an organic glue, that keeps the bindings intact, formed during cultivation by fungi through the substrate, forming a 3D fibrous glue mash.

With the above discussion in mind, majority of the researches focus on a drying process.

*3.4.1.5 Enhancing Mycelium-Based Materials (Mycomaterials)*

Mycelium-based materials are relatively strong in compression however, lateral forces and flexural strength is its weakest point, resulting in fast decomposition and failure of structural integrity. Based on similar production methods, all studies report stronger structure, when dried out and compressed. To fix this issue, a form of reinforcement seems to be necessary.

Studies covering this topic are divided in two groups. One group mainly focuses on enhancement of the fungi by physical and genetic manipulations. Although fungi based materials are common as industrial products, manipulation of its biological properties by the aid of biotechnology and molecular biology will unlock many great potential uses, that need to be studied interdisciplinarily (Hyde et al., 2019; Attias et al., 2020). An exemplary study of the mycelium of a *Schizophyllum*, reports a strain with hydrophobin gene sc3 inactivated, which show a 3–4- fold higher maximum tensile strength, as compared to non-modified samples (Appels et al, 2018b). Genetically modified strain is reported to retain more water than the wild type strain. This is explained by the fact that the encoded protein coats the aerial hyphae with a hydrophobic coating.

Appels et al. (2018b) pointed out the potentials of optimization of growth conditions and applying gene modifications to control mycelium composite to show the desired properties, such as deletion of hydrophobin gene, which would result in increase in density and branching of the fungal hyphae. Meanwhile the second group conducts their research through reinforcing the mycelium based materials structurally by creating composites. With this aim at hand, Jiang et al. (2014, 2016, 2017, 2019) has been performing a progressing study on mycelium-based sandwich biocomposite production. They used the natural textile fabrics, fixed with a bio-glue, to create laminar fabric layers that would be used as a preform shell for the cultivated substrate. To finalize the fabrication, bio-resin infusion into the preform has been tested to increase mechanical properties dramatically. Areas of substrate bound by more mycelium were stronger, and upon resin infusion rendered the coating significantly stronger, however the core appeared to be the weakest point in the sandwich biocomposite, that showed deformation under stress.

The idea of using fabrics with mycelium materials has been the topic of several researchers including Appels et al. (2018). However none of them aims to use natural fabrics directly, for the scaffold formation. Uses of natural fabrics bring out two major solutions to the moulding techniques. First, most of the natural fabrics are based on agricultural materials, which are favoured by the fungi as nutrition sources. This will allow the mycelium network to form bindings with the scaffold as a skin, moreover if fungal skin is allowed to be formed, its combination with the fabrics would resemble a soft shell, which would in turn enhance the surface integrity further. Second benefit would be the better air circulation through the surface by the woven structure of fabrics, than the closed plastic alternatives with air holes. Today`s tailoring technologies allow us to weave the fabric structure by desired pattern in order to optimize and direct the fungal growth. Also, this fabric would hold moisture and might work as a drainage system.

This thesis aims to use **mycelium of fungi** to **bio-fabricate** organic structures using natural fabrics as the scaffold. In this manner we aim to **increase the structural integrity of mycelium composite and enhance its flexural strength**. Bio-fabrication requires understanding of what is favoured by the utilized organisms. Therefore, understanding the **fungi`s life cycle** and the **mechanics** behind current **mycelium-based composite production techniques,** will be the first step through the construction of an architectural research pavilion, which is the final goal of this study.

Keeping with the discussion above, series of experiments have been conducted by the Author. These experiments have been run step by step in accordance with the preliminary results. These steps can be grouped up in three main stages. First stage is the preliminary experiments, conducted by pre-mixed substrate bags ordered from local shop, to observe the growth process and formability of mycelium with basic moulding. Second stage is to observe the dynamics of the chosen type of fungus under lab conditions with different combination of substrate and fabric in order to form test samples for structural strength and optimize the growth conditions of this combination. Third stage is the optimization stage for moulding, ingredients and colonization time. Following these experimentation stages, the design stage of the unit that will form the pavilion in accordance with the findings of all previous stages finalizes the study. All the main experiments have been conducted by the Author in collaboration with Biodesign Team Turkey (TBT) in bio-engineering and civil engineering labs of Ege University.

# CHAPTER FOUR
# EXPERIMENTS AND DESIGN

## 4.1 Preliminary experiments (Mycomaterials & Basics)

Three preliminary tests have been decided to be the first of a series of experiments. In order to be guide through a pavilion design by mycelium based materials and composites. These very first tests mostly set in order to find out the basic principles and understand the mechanics of general myco-material production process. All the preliminary tests have been conducted together simultaneously.

First preliminary experiment setup intends to observe the changes in growth speed of the mycelium by an additional food source. For this test, four similar small plastic box have been used. Food colouring and flour have been added to the first, only food colouring have been added to the second, only flour have been added to the third and nothing have been applied to the fourth to be the control group.

Second preliminary experiment samples have been set in order to see the effect of forming by round moulds. Therefore, two different sized bowl shaped plastic containers have been used to observe the difference the form of the mould may result in the process.

Third experiment samples have been prepared in order to make pre-trial of main experiments issued in following sections. Layers of fabric added to the composition, in order to see how the fabric and mycelium will interact through the process.

### *4.1.1 Preparations*

All three preliminary tests were set up using the “oyster mushroom growing kits for hobby growers” ordered online from a local firm Hayger (Figure 4.1).


Figure 4.1 Oyster mushroom growing kits for hobby growers by Hayger (Personal archive, 2019)

#### *4.1.1.1 Requirements & Conditions*

This experiments at this stage were held under household conditions at the author’s residence. Surfaces used during the experiments were sterilized by %96 alcohol solution. Alcohol kills microorganisms by altering the protein structures and dissolving the lipids of the peripheral cell wall/membrane. Therefore, it is an effective tool as a disinfectant against bacteria, fungi, and viruses. Room conditions were generally held between 24-28°C as favoured by the *P*. ostreatus (Hoa & Jang, 2015). The test samples were placed in a big plastic box in order to control the environmental conditions. A Versatile Vtc-1 temperature & humidity meter was placed in the same box in order to observe the temperature & humidity fluctuations regularly (Figure 4.2).

Figure 4.2 Growth box & growth conditions (Personal archive, 2019)

#### *4.1.1.2 Commercial source of the mycelium*

In order to understand the growth dynamics of *P. ostreatus*, two similar growth kits were ordered from a local company Hayger (www.hayger.com). The choice of the firm was made according to the authors previous experience with the kits ordered from the same firm. These kits consist of pre-inoculated substrate from agricultural waste like cotton and wheat straw. However the ingredients used as substrate are not disclosed by the manufacturer. Within several days after kits arrived, formation of mycelium could be observed on the substrate bags.

#### *4.1.1.3 Preparation of the moulds & Sterilization*

Researchers suggest that breaking the forming mycelium prior to moulding, results in a stronger bonding mycelium to form on the substrate. This process will be explained later in this chapter. For this purpose, these pre-inoculated substrate bags were crushed by hand under aseptic conditions before moulding in plastic storage boxes

Before moulding, textiles and heat resistant objects were placed in a pressure cooker for 90 minutes. All the surfaces and plastic food storage boxes were sterilized by rubbing with alcohol. All precautions, such as use of sterile gloves to prevent contamination have been taken.

### ***4.1.2 Production of Mycomaterial***

#### *4.1.2.1 Filling the moulds*

The first experimental samples were set up as four similar plastic food storage boxes to be filled as moulds during the mycelium colonization. Main intend of this experiment was to observe the changes in growth speed of the mycelium with an additional nutrient source as a parameter. Box one was only filled with the crushed pre colonized substrate of Hayger, as a control unit without any addition. A spoon of wheat flour was added to the substrate and mixed prior to filling of the second sample. Three drops of liquid food colourant were mixed with water and added to the third sample. Both wheat flour and food colourant were added to the fourth sample (Figure 4.3).

Figure 4.3 Filling the moulds by crushed pre-colonized substrate (Personal archive, 2019)

Second set of experimental samples were chosen set in order to observe the effect of round moulds on mycelium formation. In this case two different sized circular plastic food storage boxes were used as the mould to achieve a basic pot shape; big one as the outer shell, and small one as the inner shell of the mould (Figure 4.4).

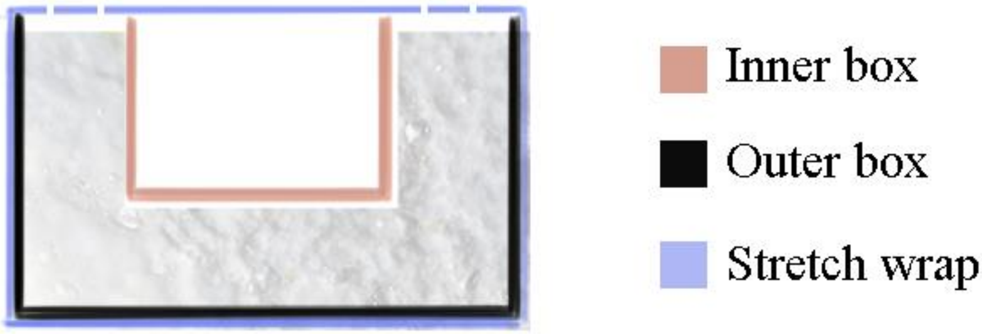


Figure 4.4 Section scheme of round sample moulding.

Third set of samples were prepared in order to form the preliminary reference for the next phase of the main experiments of this thesis; bio-composite production grown in form of mycelium sandwich. Brick shape has been chosen in order to be able to conduct compression and flexural tests and compare the results with similar materials. Plastic food storage boxes were filled as a sandwich; one layer of textile laid on the ground surface, over that 2.5cm of substrate placed, a layer of textile laid over that, placed to be approximately in the middle of final product. Other this construct 2.5cm of substrate with the final layer of textile was laid at the top surface in order to achieve a mycelium based sandwich brick with 5cm thickness, which is the optimum for the mechanical testing process for bending and compression. Two sandwich bricks were installed with different types of natural textiles; Jute, and cotton.

*4.1.2.2 Incubation-Cultivation*

During the growth phase the plastic boxes were placed inside a bigger sterile plastic box with cotton air filters. (Figure 4.5) Bottom of the bigger test box was filled to 1/8 with water, to act as a pool that evaporates to sustain the moisture level above %95 with 24-26 °C room temperature range. Moisture and temperature levels were observed regularly for 40 days by a Versatile Vtc-1 temperature & humidity meter placed in the test box.

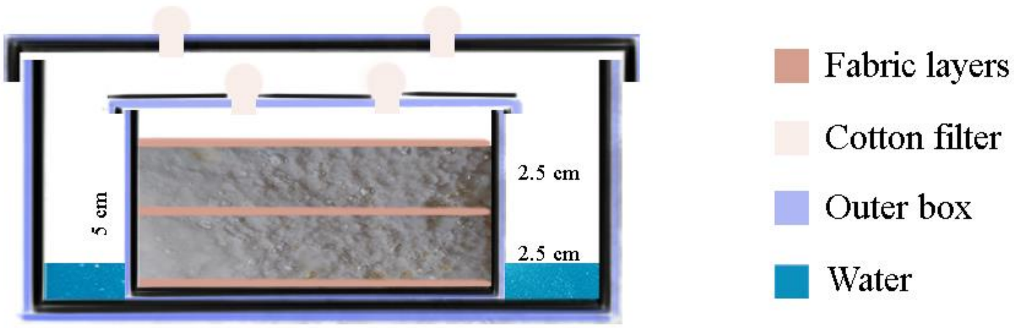


Figure 4.5 Section scheme of growth conditions of third set of test samples

*4.1.2.3 De-moulding*

After 40 days of growth in the moulds, inner boxes were placed in a plate and the fluffy upper parts were compressed by a metal spoon. In this way the flattened leather like surface was achieved. After flattening, with an aid of a regular cardboard knife, samples were released easily from the sides of the boxes. Then the boxes were rotated upside down, to take the sample out of the box, by simple tapping, without any damage. The samples were then placed on a metal oven plate for drying (Figure 4.6).

Figure 4.6 Preliminary test samples (Before drying) (Personal archive, 2019)

*4.1.2.4 Drying*

In order to stop organic activity and vaporize the water content, all samples were placed into a metal oven plate and dried in an oven. First 24 hours; oven was set at 90°C, then switched to 120°C heat for 90 minutes.

### *4.1.3 Observations & Findings*

Amongst the first set of examples, the ones with additional food supply, showed contamination during growth, still mycelium formation was sufficient to solidify the substrates. However, these were obviously mechanically weaker and readily broken apart, with loose appearance. The wheat flour and food colouring packs used in this test were unsealed earlier. As a result, during colonization, microorganisms arising from these sources could be the reason of the observed contamination. Sterilization of all the elements may be the most important directive for mycelium-based material production.

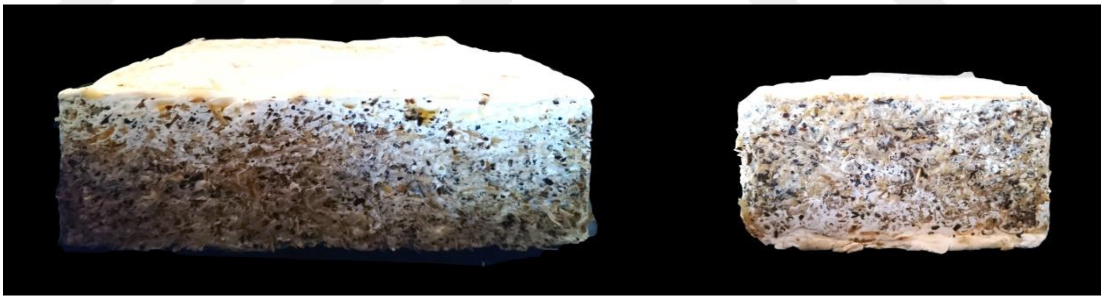

Figure 4.7 Colonisation over the side views of third set of preliminary test samples (Before drying) (Personal archive, 2019)

Under these conditions, growth period took nearly 40 days in total to completely wrap the observable surfaces. The colonization wasn`t unified, as can be seen from the side views (Figure 4.7) mycelium fibers predominantly wrap upper parts, which is more accessible to air circulation. According to these observations, it was concluded that 99% moisture level in the test boxes may have limited or blocked the air penetration through the deeper layers. Also, moisture and humidity are related, yet are present in different contents. This very high humidity resulted in accumulation of water droplets on the top layer, which may also prevent the air penetration, while increasing the colonization on these surfaces. Hence, in further experiments, test setups were prepared under prefixed moisture levels in samples and lower environment humidity levels. To achieve that, new moulds were digitally designed and produced by 3D printing and laser cut machine assist. This will be explained in following sections.

Food colouring altered the final colour of the material; however, it was not a homogenous colour change and created a pattern of wavy colour veins on the fungal coating. Also, different ratios and distribution over the substrate with food colouring addition may be used to create patterns. This aspect should be further investigated with regard to design and pattern relations of mycelium production with additional colouring methods in future studies.

Round samples were achieved by using one big outer mould and one placed in the centre, to give form of a bowl. Inner box was removed during the growth, in order to ease the air flow within the substrate; however this caused a fluffy mycelium skin to form on the open surfaces, in direct with air and with no limitations by the mould. When this fluffy mycelium was compressed by a metal spoon, random portions of surface became light brownish, possibly due to the substrate underneath (Figure 4.8).

Figure 4.8 Colonisation over bowl shaped sample (Personal archive, 2019)

The successfully colonized samples were observed to grow a fluffy cloudlike mycelium skin on the open upper faces inside their moulds. To achieve such surface, moulds can be rotated during the mycelium forming period to let the skin develop on the surface. This method was applied throughout the next stages of this study. When this fluffy skin is compressed it forms a leather-like surface. Compression may increase the number of interlocking fibres with the fabric layer. However, compression requires extra machinery and it is not suitable for every application, especially in case of non-unified forms. On the other hand, compressing as a method to achieve denser material requires further investigation.

The process of drying in the oven resulted in colour change for all the samples from ivory to brownish, which also gave the fungal skin further leather-like appearance (Figure 4.9). On the other hand, with the moisture loss, the shape of the samples was changed and distorted. Further moulding and filling experiments were to be examined, as to prevent the deterioration in form.

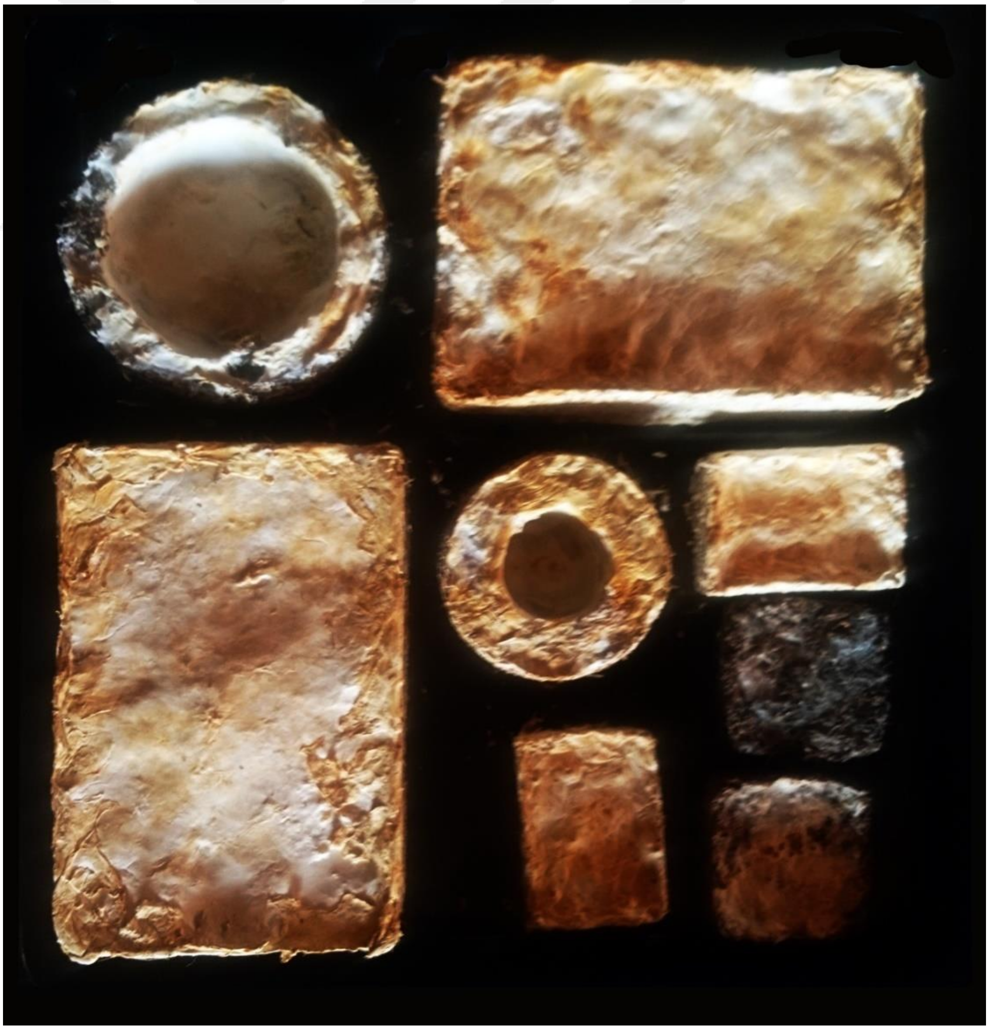

Figure 4.9 Preliminary test sample (Before drying) (Personal archive, 2019)

After drying, all successful samples were observed to retain their integrity even after 6 months, particularly portions around the textiles. Samples were bent in order to observe the strength loss and determine if the samples will break after a period of time. Samples could still preserve their integrity with increased elasticity. However, this elasticity also showed that the substrate should be compressed further during moulding, in order to achieve minimal amounts of air gaps throughout the structure, which was another reason of non-unified colonization. Researcher also decided to remove the middle layer of the fabric, since this layer might have limited the elasticity over the general body of the sample. Also, addition of an extra fabric layer in the middle added another time-consuming step to the production process and didn't seem to increase the integrity.

### *4.1.5 Discussion & Decision*

Visual observation during the colonization period, revealed contaminations of different samples which were placed in different boxes which had no contact during the colonization phase (Figure 4.10). This might be due to two reasons; first, used pre-cultivated cultures were already contaminated prior to inoculation, and second, disinfection processes might not have been sufficient.

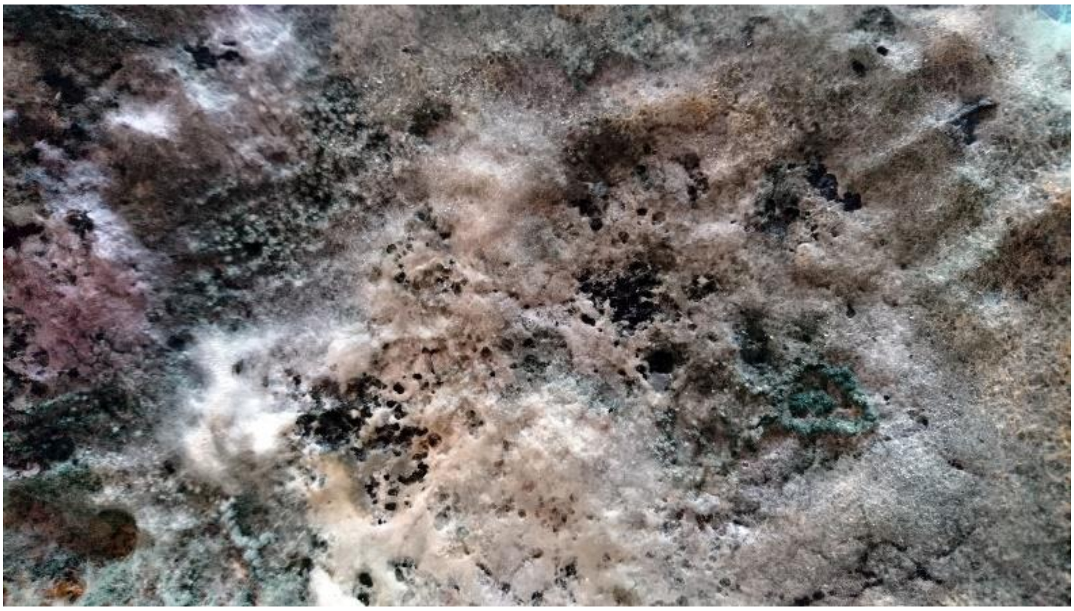

Figure 4.10 Contaminated sample surface (Personal archive, 2019)

In order to prevent such failure the following precautions were taken;

1- To continue the next phases of the experiments in a sterile and controlled environment, experiments were relocated to lab of Prof. E. Esin Hameş Tuna at Bioengineering Department of Ege University.

2- Prior to the next phase, obtain and stock the pure culture of the *P. ostreatus was obtained and stored,* for use in all further studies.

Preparation of the substrate mix, with a recipe compatible with the new fungus culture was a necessity. Therefore, a series of experiments with regard to the new substrate recipe was a part of the next stage.

Visual and physical observation of this phase also showed that, a specific mould setup design with controlled airflow for colonizing new samples is required in order to perform the structural test.

Moulding and retaining moisture are some of the key problems, observed during the preliminary experiments. Limited airflow from the top surface through the bottom, due to the mould restrictions, and high moisture levels prevented the overall homogenous growth. On the other hand, low environmental humidity levels would result in faster moisture loss within the samples, which would slow down the mycelium development drastically. For the following experiments pre-set moisture level of %75 was decided upon.

### 4.2 First set of Main experiments: Mycelium based bio-composite combination

In order to determine the structural qualities of the scoped bio-composite for this thesis; which is produced by *P. ostreatus* cultivation on a substrate formed with waste enhanced with fabrics, two sets of experiments were held in order to have an analogical comparison on similar material and structures.

To determine the best performing combination of each step of experiments; visual observations, results of tensile and compression strength tests, and moisture retaining capacities were performed and results were taken as a basis for comparison. All structural tests were conducted in MATAL laboratories in Ege University, under supervision of TBT team member Assoc. Prof. Özge Cakir.

The following section describes the process and subsequent findings with regard to the choice of substrate types, production of the first set of test samples of this stage, structural and material tests run over these samples.

### ***4.2.1 Preparations***

First a set of test samples was designed to understand which combination of substrate and natural textiles performs better with cultivated *P. ostreatus* in comparison.

#### *4.2.1.1 Preparation of conditional requirements*

General structure of the growth process found in the literature, providing details about the production phase, is similar to the guide publically shared by Ecovative on their website. Process begins with sterilization of the substrate and incubation of this substrate with the desired type of fungi. After letting fungi to colonize for a few days in the dark, fabrication continues with breaking of the colonized substrate and filling into the moulds, to grow into the desired shape. The process is completed with a drying process, in order to evaporate the water to deactivate the living fungus cells. To ensure the comparable environmental conditions and establish controlled environment; experiments were conducted in microbiology lab of Prof. Esin Hameş at Bioengineering Department of Ege University. Here due to the ongoing COVID-19 quarantine, the author had to move the test samples to complete final colonization within the moulds under household conditions. No problems were observed during this process, since all examples were sealed carefully in plastic bags with cotton filters, that prevented contamination to a sufficient extend. Samples were placed inside a laminar flow cabin, reserved for the test setups in order to provide even environmental conditions, required for comparison of the final samples. Room temperature held between 24-27°C and both humidity of the environment and moisture levels on the faces of the samples were monitored regularly.

#### *4.2.1.2 Purification of fungal strain*

Several growth kits were ordered from different local companies, after the first stage of experiments. 1kg bags of Aras Misel (www.yalovaarasmisel.com) were chosen, because of the single substrate type (wheat grain) they chose for inoculation. However, new packs were already contaminated, which could be visually detected even before opening the sealed bags; as a result the purification of the strain became a necessity before further processing.

The purification of the culture was performed by inoculating the petri dishes containing Potato Dextrose Agar (PDA). For this purpose, 39g / L PDA was mixed with distilled water and sterilized at 121°C for 15 minutes. After cooling to about 55°C, 13-15 ml of PDA was transferred into the petri dishes and allowed to solidify by cooling. Then, the mushroom kit was opened in a laminar airflow cabinet under aseptic conditions and 3-5 wheat grains wrapped with mycelium were placed on PDA and incubated for 7-10 days at 27°C. At the end of the incubation period, while superior to the previously obtained kits, several different bacteria and fungi could still be observed (Figure 4.11). At this stage, the white mycelium fragment (~2mm) of *P. ostreatus*, uncontaminated by other microbial species nearby, was sub-cultured under aseptic conditions into another PDA medium. This process was repeated until there was no other growing organism detected, upon 7-10 days of growth within the medium. The purified culture was stored at + 4°C until further experiments.

Figure 4.11 The purification process of the culture (Green and yellow dots are the invasive species) (Personal archive, 2020)

Then, two methods were tested to obtain a sufficient amount of inoculum before starting the experiments. In the first, 0.5 kg of wheat grains containing 70% moisture was sterilized in a heat resistant plastic oven bag, equipped with a cotton filter for air circulation, and *P. ostreatus* was inoculated and incubated at 27°C for 7-10 days. In the other method, Erlenmeyer (1L) containing 200 ml of 17.0g/L Malt Extract Broth (MEB) was inoculated and incubated for the same period and temperature under 150 rpm shaking conditions.

#### *4.2.1.3 Substrates & natural fibers*

This part of the experiments was conducted in collaboration with Sevdenur Sertkaya`s MSc Thesis, supervised by Assoc. Prof. Ayça Tokuç (TBT), on suitable substrate characteristics and selection of best mushroom types. Five substrate types; namely, wheat straw, cardboard waste, textile waste, beech chips and vine stem, were chosen with regard to their recyclability potentials and comparability to similar materials used in the literature. Agricultural waste based substrates; wheat straw, beech chips and vine stem were obtained from local farms. Textile waste was obtained from chopped down old cotton t-shirts pieces. Cardboard waste was collected from the regular cardboard waste found around Bioengineering Department.

For biocomposite enhancement, several natural fabrics were assessed according to the locality and price; namely, Jute, Bamboo and Hemp. Only hemp fabric was an exception, which is relatively new on Turkish market. However its exceptional qualities and fast and cheap production possibilities, which is spreading all over the world quite astonishingly lately, made it a good competitor against Jute and Bamboo textiles. While Jute and Bamboo textiles could be obtained easily from local textile firms in İzmir, Hemp fabric was provided from Institute of İzmir Kız Olgunlaştırma, as a waste material from wedding dress production (Figure 4.12).

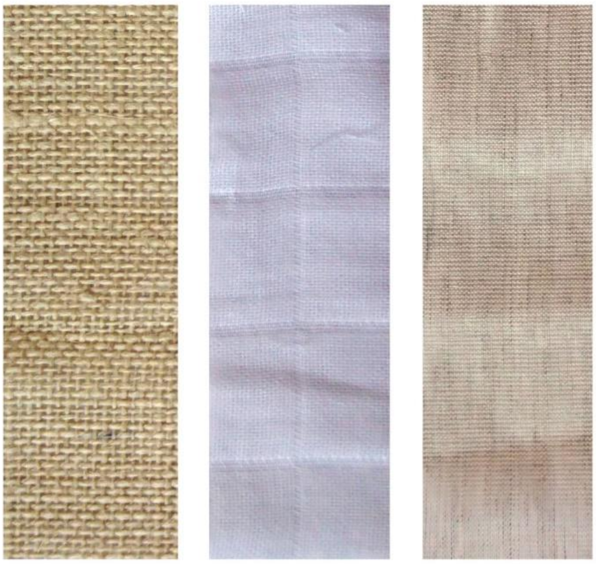

Figure 4.12 Natural fabrics used to enhance the bio-composite production (1.Jute 2.Bamboo 3.Hemp) (Personal archive, 2020)

#### *4.2.1.4 Choice over the substrate types & substrate size*

To observe the effect of particle size of the substrate granules and address the adaptability between substrate type and chosen fungi, series of preliminary experiments were conducted in Bioengineering Department of Ege University. Five different substrate types and two different particle sizes were tested for their compatibility with *P. ostreatus* to support its growth.

To down-size the substrate particles as needed for this research; an industrial grinder was used. To determine the granule sizes, sieve analysis was conducted in Ege University, Civil Engineering labs.


Figure 4.13 Five different substrate and two different particle size with *P. ostreatus* growing over (Personal archive, 2020)

A crosscheck between two lists; a list of general substrate choices in the literature and a list that research team nominated according to their locality and ease of access were made.

With those manner 5 types of substrates; “Wheat Straw, Cardboard waste, Textile waste, Beech chips and Vine stem” have been decided with their recyclability potentials, locality and comparability of similar material use in the literature (Figure 4.13).

Both bigger granules and smaller particles were placed in separate cultivation jars. To speed up the growth process, wheat bran added in defined percentages to the substrate composition as additional carbohydrates. Before inoculation, all cultivation jars were sterilized in an autoclave. After sterilization all jars were opened in lamin air flow cabin and inoculated by addition of similar amounts of wheat grains with pre-colonized pure *P. ostreatus* mycelium. After that the jars were placed in incubator (Memmert IN 450) at 27˚C and the colonization processes was observed and photographed for 30 days.

After preliminary experiments, it was decided to continue with the cardboard and wheat Straw waste, which facilitated better growth than vine stem and beech dust during observations. In addition to their mechanical properties, these wastes were chosen because of their recycling potentials, accessibility and ease of processing.

#### *4.2.1.5 Pre-cultivation: First stage incubation in plastic bags*

The first cultures from pre-cultivated bags were inoculated on two different substrates. After 10 days of hyphae formation, these cultures were placed in moulds designed for the test setup. After moulding, growth rate of the Mycelium was observed daily, in order to see approximate time needed for full colonisation at 27˚C temperature and %75 moisture level with specific substrate mixes.

Figure 4.14 Colonizing substrate in heat resistant oven bags (Personal archive, 2020)

In order to achieve the moisture level of %75, which been decided upon according to previous observations, distilled water was added with the calculated water/solid substrate content ratio prior to sterilization of the substrate in an autoclave. All prefixed substrates were placed in heat resistant plastic oven bags, with cotton filter caps, in order to resist the heat and pressure during the sterilization process in the autoclave.  The pressure inside the autoclave can be expected to penetrate the water inside the substrate and consequently homogenize the moisture distribution over the substrate. The growth media included a white filter; a cotton based air filter system, which prevents different organisms to reach the substrate, while allowing for air circulation within the media, as required.  (Figure 4.14)

A balance of 25% substrate, 5% wheat bran and %70 water, to provide optimal moisture and nutrient levels for mycelium formation was maintained. To achieve a denser and stronger connection between hyphae and the substrate, first cultivation was allowed to colonization for 10 days in plastic oven bag.

Every sample was placed open for 10 minutes every two days in an incubator under a controlled environment in order to increase the air circulation within the bags.

*4.2.1.6 Design of the test setup & moulds*

In order to determine which combination are better in comparison, two types of substrates (wheat straw and cardboard waste) and 3 types of natural textiles (hemp, bamboo and jute) were cultivated with the same *P. ostreatus* using the same procedure, growth periods and conditions.

Test setups with four identical sample moulds were designed using SketchUp. The dimensions for the moulds were chosen in accordance with the requirements of the mechanical testing equipment. Also, as observed in previous experiments the colonization of 5 cm thick brick took longer time than expected. In order to shorten the colonization phase, the volume of the samples was reduced. For the purpose of these tests, ratio of dimensions is more important, than absolute dimensional change. According to the advice of Assoc. Prof. Özge Andiç Çakır and the requirements of test equipment, 4x4x12cm sample for tensile tests and 4x4x4cm cube samples for compression tests, were determined to be optimal. To reduce the moulding costs and to achieve colonization of both samples under equivalent conditions, both samples were grown in one piece; 4x4x16cm quadrangular samples. After samples were ready for testing, these have been cut by a cardboard knife slowly in order to obtain two separate samples with required dimensions for testing. Each sample group for each substrate type was produced three times simultaneously under the same environmental conditions.

#### *4.2.1.7 Preparation of the moulds & Sterilization*

The experimental setups and the working environment were sterilized by rubbing with alcohol.

Sampling boxes used to cultivate the test samples, with 4cmx4cmx16cm were designed in SketchUp, 3D design program. Six 1mm wide circular holes per face, were distributed uniformly on each face to achieve better air circulation from each side of the mould (Figure 4.15a). 24 of these sampling boxes were then 3D printed using semi-transparent polylactic acid (PLA) filaments to observe the cultivation process better. Ultimaker and Ultimaker Plus 3D Printers were used to fabricate these boxes.

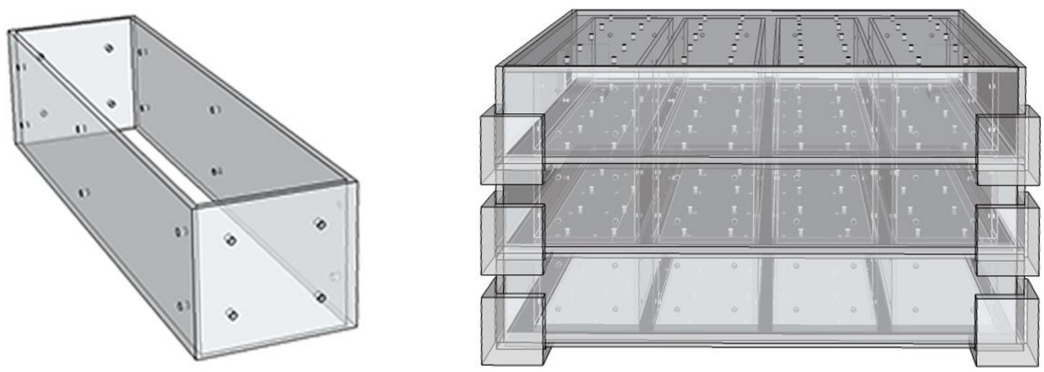

Figure 4.15 Colonization moulds for structural test samples designed in SketchUp (a. Sample unit b. General test setup) (Personal archive, 2020)

Following this process, in order to keep all samples under the same condition during cultivation; a transparent box, holding four different samples, 1cm apart from each other were designed by an AutoCAD 2020 student licenced drawing program. This containment box then was laser cut by Epilog M2CO2 75 Watt laser, cut from transparent plexiglass. In addition to that, to observe the difference in performance between 3D printed PLA growth moulds and plexiglass, several plexiglass growth moulds were also laser cut. The production phase of these experimental setups was carried out by FabLAB İzmir maker laboratory of İzmir Municipality by the aid of Ceyhun Çubukcu and Melis Başkonuş Demirci. Plexiglas was chosen as primary material, because of its flat and clear surface, allowing for the product to be easily de-moulded after growth and the process observation due its transparency.

As the last parts of the mould setup, a cap plate with a frame layer, that locates the place of the growth pods, with a twin that fits the bottom of the outer box, were laser cut (Figure 4.15b). These frames were cut to be 1mm in total larger than the growth pods. This design was used to easily rotate and remove a growth pod out of test setup, without losing the integrity of the composite. The test setup was designed to allow for rotation and movement of growth moulds separately. In addition, the layered box structure design was expected to aid preventing moisture loss within the system. To improve the moisture retainment, the whole system was then placed in a sterilized autoclave bag with cotton air filter gap.

### *4.2.2 Production of Mycomaterial (second stage incubation)*

#### *4.2.2.1 Filling the moulds*

After 10 days of incubation; pre-cultivation bags were opened on the sterilized transparent glass plate and the mycelium wrapped substrate was crushed by hand. Crushing down of the substrate allows re-shaping and as claim in the literature increases the overall strength of mycelium structure.

Layers of fabrics, to be placed on top and bottom of the mould, were sterilized in pressure cooker for 90 minutes wrapped in aluminium foils. After cooling, for each sample mould unit, a rectangular layer of fabric with dimensions of 4x16cm was placed on the bottom. Over that, the crushed pre-cultivated substrate was placed, in an amount necessary to fill the space in sample unit mould uniformly by softly pressing by hand. Another rectangular layer of fabric with same dimensions was placed on top of this construct. Three of these container units were loaded with natural fabrics; hemp, bamboo and jute, in addition to those one unit without fabric reinforcement was placed as the fourth unit, in order provide a control group for the effectiveness of fabric enhancement for biocomposite production.

This process was repeated three times on three sets of colonization moulds for each substrate type, namely wheat straw and cardboard waste. Pre-cultivated substrate for one of the cardboard control sample was lacking as a result of a calculation error. A total of 23 specimens were setup, with one cardboard control unit missing. The experimental sets were then placed in sterile autoclave bags with cotton filtered mouth openings, in order to prevent extra moisture loss and protect from contamination.

#### *4.2.2.2 Incubation-Cultivation*

*P. ostreatus* inoculated Erlenmeyer (1L) containing 200 ml of 17.0g/L Malt Extract Broth (MEB) were placed in a shaker with 150 rpm shaking conditions to form pellets. Upon 10-day incubation the grown pellets were inoculated into the heat resistant plastic bags containing sterilize substrates. After inoculation, these bags were further incubated for 12 days, placed in the dark environment at 27˚C. After the first incubation period, the substrates were removed from plastic bags and transferred into the moulds, which were sterilized by rubbing with alcohol. Then, incubation was continued under the same conditions for 18 days to let mycelium re-colonize in the moulds prior to de-moulding (Figure 4.16). During this period, each of these plastic bags was opened in a lamin air flow cabin regularly for 10 minutes in a 5 days sequence, to provide air circulation over the surfaces to the increasing mycelium skin formation on both faces. This practice also would be beneficial to remove the accumulation of carbon dioxide from the colonization environment, in order to reduce the acidity of the air.

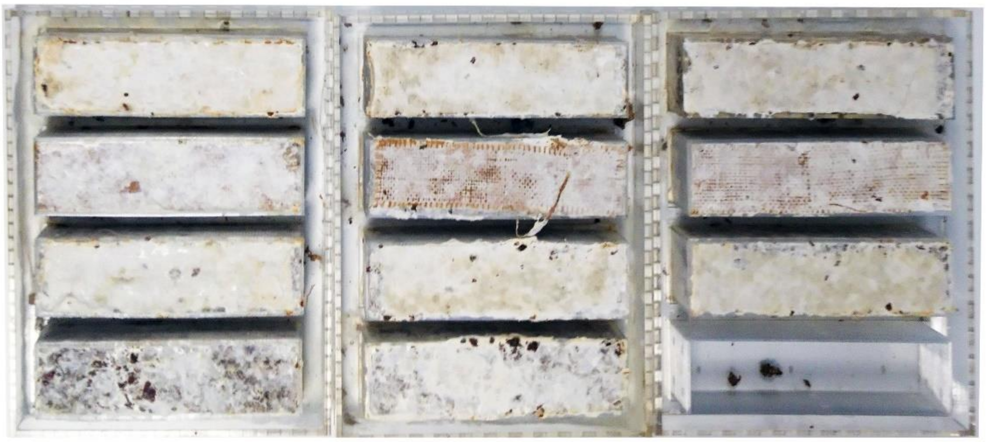

Figure 4.16 Fully colonized cardboard test samples (Personal archive, 2020)

*4.2.2.3 De-moulding*

Before de-moulding moisture levels on the surfaces of the samples in the moulds was controlled with MD46 Digital Moisture Meter, in order to determine the final surface moisture. Observation showed that; wheat straw based samples show 42-43% moisture levels on the surface, while cardboard samples were ranging between 40% and 42%, indicating that there was an undesired moisture loss inside the mould at the end of the incubation period.

First wheat straw samples were de-moulded. Edges of the samples connected to the mould were cut gently with an aid of a utility knife in order to separate samples from the moulds. The samples were further pushed out of the moulds by applying soft pressure over the surface. Although wheat straw samples had weak colonization, all samples remained intact upon removal out of the moulds. However few deformations were observed around the corners of several samples (Figure 4.17). Each sample was weighed after de-moulding

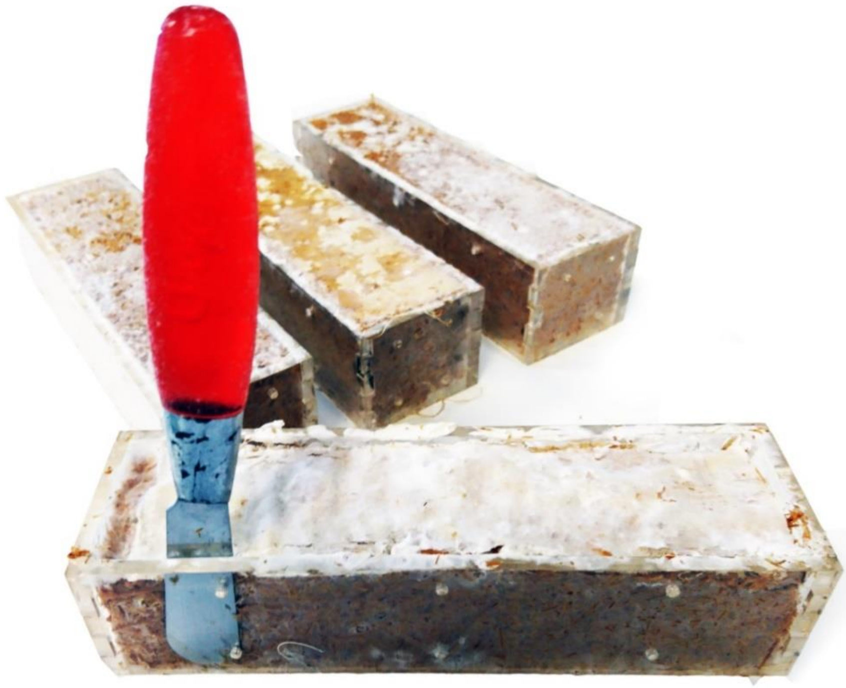

Figure 4.17 De-moulding of wheat straw samples from plexiglass moulds (Personal archive, 2020)

#### *4.2.2.4 Drying*

De-moulded samples were placed in Memmert brand oven with 120 ˚C for 1.5 hours, to eliminate further fungal cell production. Then the samples were placed to be dried again at 60 ˚C for 24 hours twice. In each process of drying, every sample was weighed again, until no weight change was observed. All specimens were completely dried out prior to structural tests.

### *4.2.3 Experiments*

#### *4.2.3.1 Conditional examinations*

Each sample was weighed before and after complete drying, in order to observe the final moisture levels after the colonization. In order to detect the general size of the particles; grain-size analysis conducted by sieving these particles in Civil engineering labs of Ege University. After drying the samples in an oven completely, moisture capacities of the samples were determined by measuring their weight.

### *4.2.3.2 Structural tests*

In order to make a comparison with the literature references and the conventional products of the structural materials such as common brick, the similar compression and bending tests with Jiang et al. were successfully performed in Ege University Central Research Test and Analysis Laboratory Application and Research Center (Ege MATAL). For the compression tests; compressive strength (MPa) of each specimen according to maximum force (kN) was tested until 75% maximum deformation. During bending tests; flexural strength (MPa) of each specimen was recorded according to maximum force (kN). Compression rate was set at 10 mm/min and bending rate of 0.5 mm/sec. Support clearance for bending test is 80 mm. For compression tests, a movement limitation of 23 mm was applied. Total 23 specimens were tested; three samples for each combination of substrate and the fabric reinforcement and one as control unit without fabric reinforcement (Figure 4.18).

The samples were coded according to their combination. Wheat Straw (S), Cardboard Waste (K) as first digit, the order of the triplicate samples (1-3) as the second, and the type of the fabric; Hemp (K), Bamboo(B), Jute (J) and control group (0).

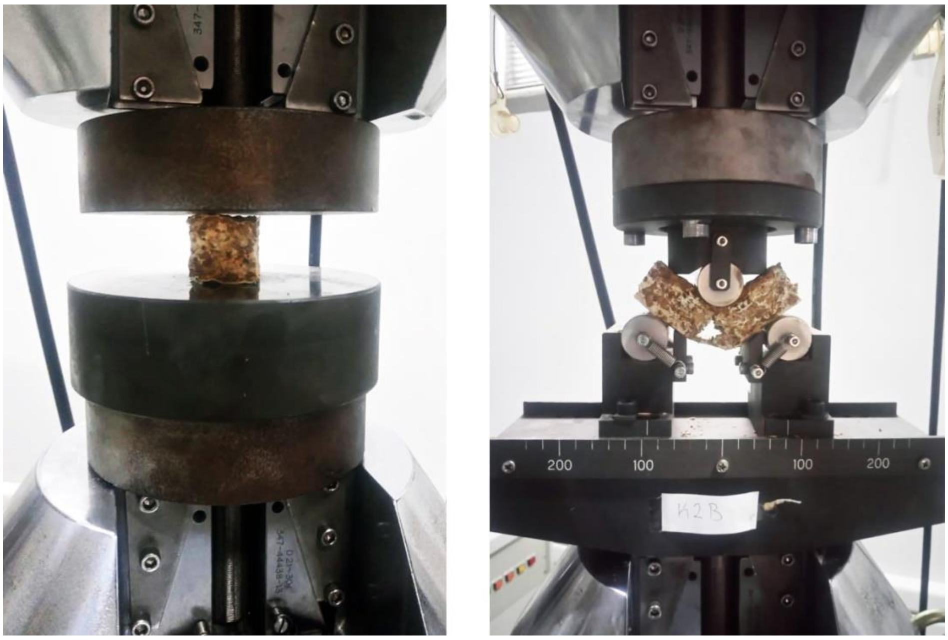


Figure 4.18 Structural tests (a. Compression b. Bending) (Personal archive, 2020)

### *4.2.4 Observations & Findings*

Substrate choice is important here from two aspects. Firstly, the substrate supports the growth of the relevant organism, as the organism is fed by digesting the substrate. The organism will hold the material together by spreading its colonisation between the substrate particles, showing better mycelium growth, when the proper nutrient medium (suitable substrate) is provided. Attias et al. refers to the main goal behind the choice of the substrate as follows: "toward the development of cleaner production technologies, where waste residues with no current disposal solution are upcycled to form natural bio-composites". Hence recycling potential is a virtue expected from such material combination. In addition to that, chosen substrate type and its material properties would affect the final products properties. The second choice is the particle size of the substrate used. Research suggests that use of grains to inoculate the substrate or as nutrition to fungi to feed on, increased the general density two to three times compared to wood particles (Girometta et al., 2019).

Air circulation is one of the most crucial factors during the forming phase of mycelium matrix. According to observations, the reason behind the slow or low growth using substrates in very small forms, such as granules, could be because of water molecules filling the space among the particles of the substrate and preventing air penetration necessary to support growth.

Observations of the substrate particle size from preliminary experiments show that; use of larger pieces limits mycelium formation and effective penetration of the substrate, since the bio-chemical process of decomposition requires more time as the substrate particle size increases. Also, if the particle size of the substrate is too large, the mycelium cannot penetrate the particles and distribute homogeneously. On the other hand, if the particle size of the substrate is too small, penetration of air will be limited, which will limit the growth of the aerobic organism. The type of substrate, in this case, is a very effective parameter. Not micron but small particles visible to the eye were observed to be the most effective size. However, a generalization couldn`t be made because of the changing properties of different substrates, like wheat straw or fabric waste. Further, the properties of the substrate will affect the final properties of the composite, since the whole material of the substrate will not be digested. Therefore, the substrate type will affect the stiffness, strength, thermal and acoustic properties of the composite.

The substrates for this test were grinded using an industrial grinder. In order to determine the average size of the particles, grain-size analysis was conducted by sieving in Civil engineering labs of Ege University. Results indicate that cardboard waste substrate mostly consists of 1-4mm particles. Wheat straw samples consist of 0.125-2mm particles. However, sieving was not a consistent method to determine the particle size of wheat straw, due to its rod-like elongated structure.

Several substrate types were cultivated to begin this stage of experiments: Textile, cardboard waste, wheat straw, vine stem and beech sawdust within a list of candidates. Textile waste was chosen because of its fibrous, woven structure, which is expected to let the mycelium root and glue itself within this fibrous structure during the incubation. Also different textiles may lead increase in acoustic and thermal insulation and enhance the mechanical properties (Briga-Sá, 2012) Cardboard is the last chain of the recycle cycle of paper products, hence use of cardboard in mycelium products adds another valuable step to this cycle. In addition, composites made with cardboard incorporation show significant enhancement in acoustic and thermal properties. Many insulation methods employ cardboard to increase their utilities. Moreover, it is one of the most common wastes that can be obtained easily. Wheat straw is one of the favourable substrates used in *P. Ostreatus* farming, because of its high cellulose (34–40%), hemicellulose (20–25%), and lignin (20%) content (Rodriguez-Gomez et al., 2012). It also is an abundant and inexpensive product, which makes it a good candidate. Vine stems and beech sawdust were chosen due to their woodchip properties and compatibility with the fungus type. Researches show that woodchip composites bring out better results attributed to their high water absorptivity with minimal swelling and compression strength (Attias et al., 2020).

Visual observation during the substrate examination showed faster and denser colonization over cardboard waste, cotton textile waste and wheat straw cultivation jars, which could be considered to have higher performance. However, textile waste is hard to cut down in smaller pieces and require extra machinery and effort. With the above discussion in mind wheat straw and cardboard waste were chosen as optimal for further experiments.

One of the main problems of mycelium production processes is the limited air flow which was observed in preliminary experiments. As Attias at al. (2020) mentions as well, the mould characteristics and the conditions within the mould play significant role on the incubation process. Appels et al. (2018) refers to the importance of increasing oxygen levels in the material centre as the means to increase colonization. Air flow, moisture retention and surface texture are several of these properties, which seem to be dominantly determinative of the incubation period.

Although both test sample sets were incubated and preserved under approximately same conditions, the difference between two substrate types was very apparent to the naked eye. Even prior to moulding for the final colonization, the cardboard waste-based specimens already appeared colonized better, compared to the wheat straw-based ones. Formation of mycelium skin was not complete in straw-based samples and mycelium tissue could be barely seen from the transparent mould faces after colonization. Many conventional mushroom farmers widely use wheat straw or similar content in their substrate mixture. From an industrial concern perspective, some of these farmers tend to share their recipes publically. Several of these people have demonstrations and informative videos uploaded in online platforms such as Youtube, Vimeo etc. or online mushroom farming forums. Since not many of these people have autoclave in their backyard, some of these conventional mushroom farmers boil the wheat straw beforehand for sterilization or use pasteurization method which takes almost twice longer to finalize the production. Majority of these farmers add limestone to the substrate before boiling to enhance sterilization. This action might be speeding up the breaking down of wheat straw to the nutrients and enhancing the mycelium formation and colonization. As observed by the author, pre-treatment of wheat straw with autoclave results in poorer performance. When we compare the performance of wheat straw and cardboard waste from this perspective, card board waste is a product which is already pre-treated several times. As a result, cardboard waste might already be in a state, which results in easier access to the nutrients, necessary for the hyphae to form mycelium colony. From this perspective, if we compare suitability of wheat straw with cardboard in order to utilize production steps and cost, the cardboard waste bares better performance, since wheat straw requires boiling and extra ingredients. From industrial perspective, these requirements translate to extra energy, material and time, which defeats the purpose of this works main objective.

Another reason for the different levels of colonization might be the chosen grain type and its interaction with water. While the wheat straw substrate kept its integrity loose after autoclaving with the calculated water content of 75%, the cardboard waste substrate grains became more like dough.

Mycelium colonization on wheat straw substrate samples appeared weak. During de-moulding some of these samples showed minimal integrity and resulted in material loses around the corners. On the other hand, the fabrics placed on top and bottom surfaces were attached fairly well with the substrate by mycelium during final colonization in the moulds. The main goal behind forming a sandwich structure with fabrics is to increase the structural performance of the composite. The forming mycelium is expected to act as a natural glue in this process. Proliferating hyphae would create millions of bridges that bind and knot the substrate and the fabric. Visual observations of the samples show that, forming mycelium established integrity by binding the general substrate and the fabrics placed on the surfaces. These properties indicated that formation of bio-composites was achieved successfully as expected.

Two types of sampling moulds were assessed (Figure 4.22). 3D printed one performed superiorly compared to Plexiglas ones because of its flexibility, which allowed for de-moulding easier. Flexibility is one of the important qualities for mould design; however, if the mould is too flexible problems may occur in the final form.

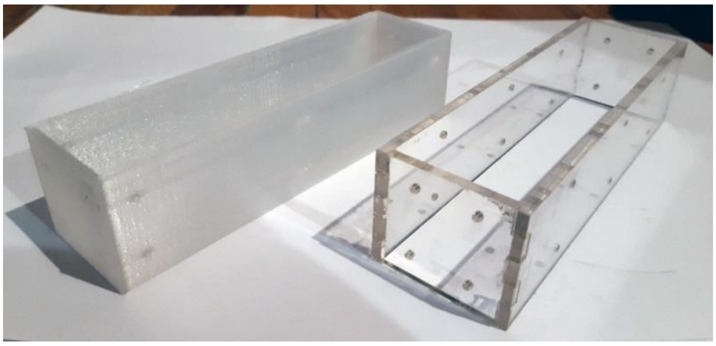

Figure 4.19 Sample moulds a. 3D Printed b. Laser-cutted (Personal archive, 2020)

Smell is another tricky point at this stage. Contaminations generally smell heavier than fresh mushroom. This smell is easily recognizable as the smell of the forest ground after rain, arising from the mycelium network colonized underground of the forest.

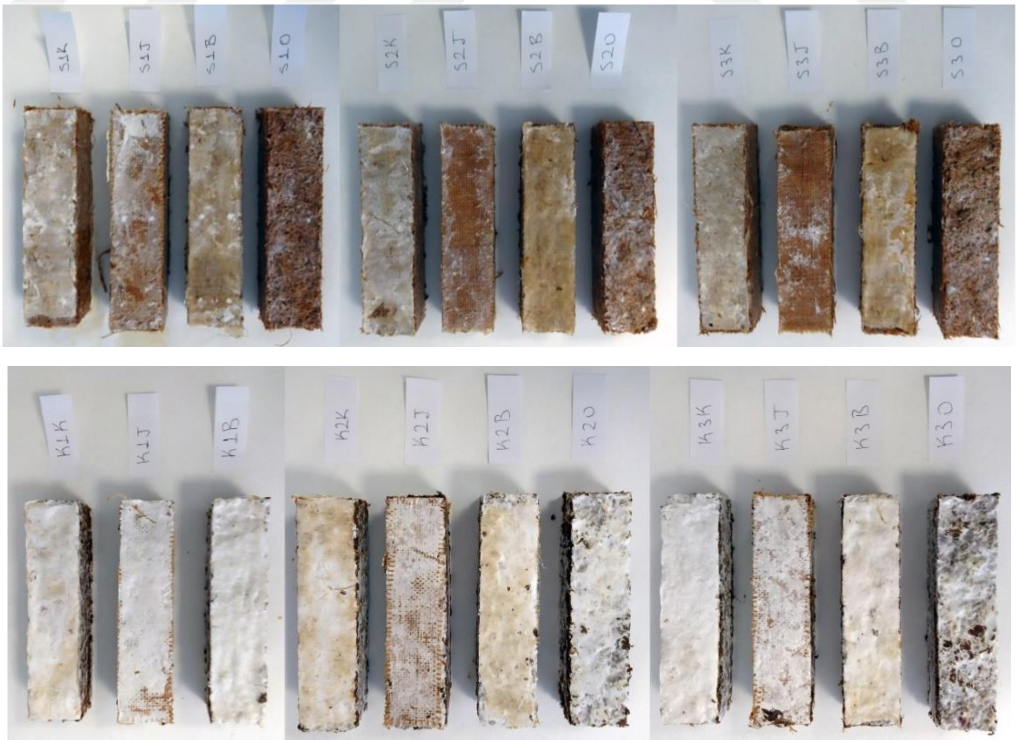


Figure 4.20 All the samples before drying (Upper: Wheat Straw, Below: Cardboard waste substrates) (Personal archive, 2020)

No contamination was observed on the test samples in this phase of the study. Prior to the drying process, all samples had soft and spongy appearance, as the fungi remain alive. (Figure 4.20) Cardboard samples were nearly twice heavier as compared to wheat straw samples. Composites came out showing substrate colours and filamentous mycelium network over those.

After the drying process, all samples were hardened and decreased in weight due to drying process, as expected (Figure 4.21). While straw based composites became brittle with low integrity, cardboard samples came out strong and solid. Cardboard samples particularly resulted in non-uniform deformations over all surfaces. This might be explained by the dough-like state of the cardboard substrate with moisture and vaporized water content during drying. The shape of wheat straw samples was less deformed, however this might be because of earlier mentioned low colonization contend and loose formation of the substrate.

Figure 4.21 All the samples after drying (Personal archive, 2020)

All samples acquired brownish color upon drying. Mycelium observable on the surface became light milky brown, while cardboard and wheat straws became chocolate brown and chestnut brown respectively. All samples show different colorization distribution. This might be because of the substrate type properties and non-uniform organic content distribution over the samples.

During the colonization phase, temperature and humidity levels of the colonization environment were monitored regularly. The conditions were recorded to range between 24-27°C and humidity levels of 40-50%. Moisture levels on the faces of the samples were recorded as ranging at  35-45%, using a digital temperature and moisture meter (Figure 4.19).


Figure 4.22 Moisture, temperature & humidity observations (Personal archive, 2020)

In order to determine the final moisture retained by these samples after 30 days of colonization in the moulds, every sample was re-weighed until there was no weight change as observed after each drying cycle. The difference between surface moisture and the bulk moisture retained within the substrate  can be a result of higher evaporation rate on the surfaces in direct contact with air, After the drying process, specimen showed dimensional variations, as they shrink with evaporated water content. Yet the drying may also result in increase in the integrity of cardboard samples due to lamination with mycelium. Table 4.1 presents the weight, density and dimensions of the test samples before and after drying process.

The results of bending (Table 4.2) and compression tests (Table 4.3) are presented on tables. The first step of the sample coding indicates the type of substrate used (S =

Straw, K = Cardboard). The second step of the coding indicates the set group indicating the triplicate of the simultaneously produced sample group (1, 2, 3). The last step of coding refers to the type of fabric placed on the top and bottom surfaces to strengthen the biocomposite (0 = No-fabric-control group, B = Bamboo, J = Jute, K = Hemp).

Table 4.1 Weight density and dimensional change calculations before/after drying the samples (Personal archive, 2020)

| Group Code | Weight before drying (g) | After Drying I 120˚C 90min | After Drying II 50˚C 48h | Remaining dry weight % | Width (mm) | Thickness (mm) | Height (mm) | Volume cm3 | Dry density g/cm3 |
|---|---|---|---|---|---|---|---|---|---|
| S1K | 101.12 | 79.03 | 29.83 | 0.294996044 | 40 | 40 | 40 | 64 | 0.46609375 |
| S1J | 120.88 | 97.79 | 39.56 | 0.327266711 | 40 | 40 | 40 | 64 | 0.618125 |
| S1B | 107.3 | 84.6 | 35.2 | 0.32805219 | 40 | 40 | 40 | 64 | 0.55 |
| S1O | 104.11 | 83.43 | 33.14 | 0.318317165 | 40 | 40 | 40 | 64 | 0.5178125 |
| S2K | 105.37 | 83.86 | 29.94 | 0.284141596 | 40 | 40 | 40 | 64 | 0.4678125 |
| S2J | 102.7 | 82.31 | 32.31 | 0.314605648 | 40 | 40 | 40 | 64 | 0.50484375 |
| S2B | 107.53 | 85.81 | 34.6 | 0.321770669 | 40 | 40 | 40 | 64 | 0.540625 |
| S2O | 109.86 | 90.29 | 36.95 | 0.336337156 | 40 | 40 | 40 | 64 | 0.57734375 |
| S3K | 111.32 | 92.26 | 38.88 | 0.349263385 | 40 | 40 | 40 | 64 | 0.6075 |
| S3J | 118.08 | 93.06 | 37.14 | 0.31453252 | 40 | 40 | 40 | 64 | 0.5803125 |
| S3B | 116.14 | 95.45 | 42.38 | 0.364904426 | 40 | 40 | 40 | 64 | 0.6621875 |
| S3O | 119.78 | 95.39 | 40.34 | 0.336784104 | 40 | 40 | 40 | 64 | 0.6303125 |
| K1K | 183.66 | 152.57 | 59.28 | 0.322770336 | 40 | 30 | 30 | 36 | 1.646666667 |
| K1J | 170.6 | 150.31 | 67.52 | 0.395779601 | 40 | 30 | 30 | 36 | 1.875555556 |
| K1B | 171.18 | 149.53 | 65.61 | 0.383280757 | 40 | 30 | 30 | 36 | 1.8225 |
| K1O | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K2K | 201.24 | 169.28 | 66.33 | 0.32960644 | 40 | 30 | 30 | 36 | 1.8425 |
| K2J | 221.2 | 196.28 | 92.81 | 0.419575045 | 40 | 30 | 30 | 36 | 2.578055556 |
| K2B | 188.94 | 165.56 | 77.06 | 0.407854345 | 40 | 30 | 30 | 36 | 2.140555556 |
| K2O | 190.74 | 166.03 | 76.72 | 0.402222921 | 40 | 30 | 30 | 36 | 2.131111111 |
| K3K | 193.73 | 172.04 | 72.04 | 0.37185774 | 40 | 35 | 30 | 42 | 1.715238095 |
| K3J | 193.27 | 171.46 | 69.91 | 0.361721943 | 40 | 35 | 30 | 42 | 1.66452381 |
| K3B | 205.13 | 184.18 | 76.31 | 0.372007995 | 40 | 35 | 30 | 42 | 1.816904762 |
| K3O | 198.8 | 176.47 | 71.13 | 0.357796781 | 40 | 35 | 30 | 42 | 1.693571429 |

Table 4.2 Bending test results (Personal archive, 2020)

| Code | Substrate | Fabric Reinforcement | Maximum Force (kN) | Flexural Strength (MPa) | Group Code | Average Flexural Strength (Mpa) | Flexural Strength Standard Deviation (Mpa) | Support Clearance (mm) |
|---|---|---|---|---|---|---|---|---|
| S10 | STRAW | CONTROL GROUP | 0.0621 | 0.15208 | | | | 80 |
| S20 | STRAW | CONTROL GROUP | 0.11586 | 0.28374 | S-0 | 0.290382 | 0.009393206 | 80 |
| S30 | STRAW | CONTROL GROUP | 0.13568 | 0.297024 | | | | 80 |
| S1B | STRAW | BAMBOO | 0.05411 | 0.11857 | | | | 80 |
| S2B | STRAW | BAMBOO | 0.10647 | 0.26074 | S-B | 0.190103333 | 0.071089241 | 80 |
| S3B | STRAW | BAMBOO | 0.0736 | 0.191 | | | | 80 |
| S1J | STRAW | JUTE | 0.06674 | 0.16344 | | | | 80 |
| S2J | STRAW | JUTE | 0.07798 | 0.21481 | S-J | 0.208946667 | 0.042876738 | 80 |
| S3J | STRAW | JUTE | 0.10151 | 0.24859 | | | | 80 |
| S1K | STRAW | HEMP | 0.00896 | 0.02195 | | | | 80 |
| S2K | STRAW | HEMP | 0.07985 | 0.19556 | S-K | 0.256635 | 0.086373093 | 80 |
| S3K | STRAW | HEMP | 0.12973 | 0.31771 | | | | 80 |
| K10 | CARDBOARD | CONTROL GROUP | - | - | | | | - |
| K20 | CARDBOARD | CONTROL GROUP | 0.06374 | 0.25264 | K-0 | 0.316645 | 0.090516739 | 80 |
| K30 | CARDBOARD | CONTROL GROUP | 0.10278 | 0.38065 | | | | 80 |
| K1B | CARDBOARD | BAMBOO | 0.38078 | 1.403 | | | | 80 |
| K2B | CARDBOARD | BAMBOO | 0.40915 | 1.51537 | K-B | 1.398573333 | 0.119071729 | 80 |
| K3B | CARDBOARD | BAMBOO | 0.37849 | 1.27735 | | | | 80 |
| K1J | CARDBOARD | JUTE | 0.48828 | 1.69366 | | | | 80 |
| K2J | CARDBOARD | JUTE | 0.1627 | 0.55624 | K-J | 1.264793333 | 0.618142005 | 80 |
| K3J | CARDBOARD | JUTE | 0.41701 | 1.54448 | | | | 80 |
| K1K | CARDBOARD | HEMP | 0.78909 | 2.92254 | | | | 80 |
| K2K | CARDBOARD | HEMP | 0.41002 | 1.51859 | K-K | 2.38426 | 0.75707137 | 80 |
| K3K | CARDBOARD | HEMP | 0.68415 | 2.71165 | | | | 80 |

Table 4.3 Compression test results (Personal archive, 2020)

| Code | Substrate | Fabric Reinforcement | Maximum Force (kN) | Compression Strength (MPa) | Group Code | Avarage Compression Strength (Mpa) | Compressive Strength Standard Deviation (Mpa) | Maximum Deformation (%) |
|---|---|---|---|---|---|---|---|---|
| S10 | STRAW | CONTROL GROUP | 0.1516 | 0.09475 | | | | 37.0028 |
| S20 | STRAW | CONTROL GROUP | 6.80687 | 4.25429 | S-0 | 4.76409 | 0.720966074 | 74.9939 |
| S30 | STRAW | CONTROL GROUP | 8.43822 | 5.27389 | | | | 74.9905 |
| S1B | STRAW | BAMBOO | 6.31213 | 3.94508 | | | | 74.9949 |
| S2B | STRAW | BAMBOO | 6.79913 | 4.24945 | S-B | 4.318423333 | 0.412181149 | 74.9932 |
| S3B | STRAW | BAMBOO | 7.61719 | 4.76074 | | | | 75.0001 |
| S1J | STRAW | JUTE | 10.298 | 6.43627 | | | | 74.9864 |
| S2J | STRAW | JUTE | 6.5292 | 4.08075 | S-J | 5.074653333 | 1.220052638 | 74.9902 |
| S3J | STRAW | JUTE | 7.53111 | 4.70694 | | | | 74.9983 |
| S1K | STRAW | HEMP | 0.09771 | 0.06107 | | | | 32.6808 |
| S2K | STRAW | HEMP | 0.12688 | 0.0793 | S-K | 4.56792 | 0 | 23.8779 |
| S3K | STRAW | HEMP | 7.30867 | 4.56792 | | | | 74.9956 |
| K10 | CARDBOARD | CONTROL GROUP | - | - | | | | - |
| K20 | CARDBOARD | CONTROL GROUP | 5.52932 | 4.60777 | K-0 | 5.72647 | 1.582080712 | 74.9867 |
| K30 | CARDBOARD | CONTROL GROUP | 9.58324 | 6.84517 | | | | 74.9894 |
| K1B | CARDBOARD | BAMBOO | 5.14287 | 4.28572 | | | | 74.9966 |
| K2B | CARDBOARD | BAMBOO | 4.72895 | 3.94079 | K-B | 5.039276667 | 1.613162285 | 74.9949 |
| K3B | CARDBOARD | BAMBOO | 9.64785 | 6.89132 | | | | 74.9908 |
| K1J | CARDBOARD | JUTE | 3.62749 | 3.02291 | | | | 74.9956 |
| K2J | CARDBOARD | JUTE | 6.92499 | 5.77082 | K-J | 5.104426667 | 1.841109144 | 74.9898 |
| K3J | CARDBOARD | JUTE | 9.12736 | 6.51955 | | | | 74.9854 |
| K1K | CARDBOARD | HEMP | 9.7685 | 8.14042 | | | | 74.9872 |
| K2K | CARDBOARD | HEMP | 12.9182 | 10.7652 | K-K | 9.185283333 | 1.391789878 | 74.9898 |
| K3K | CARDBOARD | HEMP | 12.1103 | 8.65023 | | | | 74.9847 |

As can be observed from structural test results demonstrated on Table 4.2 and 4.3 the best performing candidate amongst the samples in both compression and bending tests are the samples with **cardboard waste as substrate** and **hemp fabric as the reinforcement aid**. Mycelium performs weak upon bending, and better upon compression. Results show that fabric enhancement resulted in obvious change in cardboard based sample behavior. K-K samples performed with 2.38 MPa average flexural strength, which is more than 7 times that of the control group with 0.31 MPa average. Performance of K-K samples are followed by K-B samples with 1.40 MPa, and K-J with 1.26 MPa detected flexural strength. However, K2J reduces the average value by 0.56 MPa. This could be a result of weaker connection between the fabric and colonization. Other two samples performed with detected 1.69 MPa and 1.54Mpa, exceeding the average of all bamboo-based samples. Hence the bending performance within the cardboard group can classified as follows: hemp-based samples demonstrate the best performance, followed by jute, than bamboo and control group being the weakest. On the other hand, wheat straw based group performed even weaker than the control units of cardboard based group. However, these results were expected after visual observations, which showed lower colonization, weaker connections between fabric and mycelium and consequently weaker structure. These results show that a certain level of colonization is required and flexural strength is increased when fabrics are integrated with mycelium.

No strong correlation was found between fabric reinforcement and densities of the dry samples. This can be explained by the non-homogeneous colonization within the substrate. On the other hand, general dry densities of all the cardboard samples were significantly higher than those of the wheat straw based ones, as they performed better in flexural strength.

Compression test results show similar outcome for all samples except K-K combination that nearly doubled the performance of others, with 9.19MPa average compression strength. However, the rest of the results do not indicate any significant enhancement by fabric reinforcement on compression strength. *P. ostreatus* seems to favor hemp, as evidenced by the increased colonization rate in these samples, as well as the compression strength.

Apart from the results of the compression tests, the states of samples upon the compression tests were intriguing. Particularly the cardboard samples became strong, without any distortion with flattened smooth surfaces upon the pressure application. Wheat straw based samples, on the other hand, showed signs of crumbling upon mechanical testing, yet even on these the surfaces became flattened and smooth. This indicates that the pressure application, as a post-processing process might solidify the general structure, increasing the load bearing capacity and solve the deformation issue upon drying in oven.

### *4.2.5 Discussion & Decision*

First stages of these experiments were conducted in order to analyse which substrate could become a base to form a construction material and how to optimize be the incubation. Both grain-transfer and inoculation by liquid culture were tested. At the end of the incubation, mycelium growth on the wheat grains in the plastic bag became visible (Figure 4.23a). In liquid culture, the filamentous structure of the mycelium was wrapped on itself effected by shaking, forming spherical structures with a diameter of 2-5mm, called pellets (Figure 4.23b). Pellets allowed for more homogenised colonization then the grain transfer method. When the results were evaluated, since homogeneous mycelium growth could not be achieved on each grain of wheat, the use of pellets was determined to be optimal for the future studies to ensure the homogeneous distribution and standardization of the inoculum.

These cultures were stored in a fridge at 4˚C during the COVID quarantine for two months in sealed jars and petri dishes. After the two months, new cultures could be easily reproduced from wrapped wheat grains.

Figure 4.23 a.Mycelial growth on the wheat grains, b mycelial pellet formation in liquid culture (Personal archive, 2020)

Substrate is one of the main parameters effecting the final material. According to their compression test results Attias at al. (2020) outlines that; if the substrate is weakly compatible with the digestive enzymes of the fungus, this would limit the mycelium colonization rate. This will result in a denser core with higher compression strength and lower water absorption in the final product. This incompatibility may also change the fungal cell-wall composition. So far, in order to accurately construct experiment, detailed information over the ingredients and their amounts in the substrate is required in order to conduct repeatable experiments and assess the relationship between fungus and the substrate. Researches show that, the type of substrate, particle size and method of processing directly affect the various properties of the final bio-composite (Elsacker et al., 2019).

Utilities of mycelium composites are generally tested by several physico-mechanical tests, ranges from density of dried composite, compressive and flexural strength, thermal resistance, water absorption, water vapour permeability, dimensional stability and the textural qualities like skin formation and colour changes under circumstances. There are also several researches over acoustic insulation properties (Pelletier et al., 2017) and antibacterial benefits (Haneef et al., 2017; Ziegler et al., 2016) of mycelium composites. (Attias et al., 2020). In order to understand the potential uses of fabric reinforced mycelium based biomaterials in architecture; the second part of this stage of experiments designed in order to find a correlation between natural fabric reinforcements, *P. ostreatus* and the pre chosen combination of substrate to perform a building block with more stiffness and bending qualities. Compression and bending tests have been issued with that sense over the produced samples. Jiang also have conducted similar structural tests with this thesis (Jiang et al., 2017). According to ASTM C393 and D7250 standards, 30 specimens have been produced and tested, 10 times for each kind of reinforcement to have accurate and comparable results. However, even though the production procedure and tests are similar, both test conditions and mycelium spawn are different, so the results will just demonstrate a view of how these materials perform even grown or produced differently.

Mycelium-based materials show good performance against compression, but fast biodegradation properties limit the outdoor use and hence require extra processing in order to be used as a structural element of compression. Venturing the potential of different fabric reinforcement combinations would result in different characteristic properties, ranging from water retention, hydrophobicity, antibacterial properties, heat and sound insulation etc. depending on the chosen fabric type, forming the scaffold for the production of such composites.

Normally mycelium-based materials show weak tensile strength and can easily disintegrate on impact and therefore require tensile reinforcement. From this perspective, the test results obtained at this stage of investigation were very promising; representing that the fabric reinforced mycelium biocomposites can be a solution and even perform better against conventional competitors indoors. Fabric reinforced mycelium-based composites will be sufficient for indoor applications, used as separator prefabricated boards and fillings, in order to replace non-structural elements such as inner walls, plasterboards, acoustic panels etc.

Specific mould design and post processing were observed to be very effective on the final product. For example to achieve a light/air gap inside the final pavilion design, shaping the mould in order to create holes on mycelium composite`s substrate part while keeping the textile portion intact, to enhance the tensile strength is one of the issues to be further examined in future studies. ´

There are several articles, such as that by Jiang et al. (2019), focusing on industrial mechanisation and optimization development of the mycelium-based composite process. The team uses more advanced toolsets of machinery and the techniques such as thermal press and vacuum resin infusion. After compression tests, samples became more stiffened instead of scattering. This means that by compression, strength and stiffness of the produced bio-composites may be increased. Yet this must be still further researched.

By calculating the energy cost, use of tools and the time the process takes in an equation to compare the potential drying scenarios Jiang et al. assessed several potential methods. Their experiments show that the thermal press has a drying time cycle of around 20 min at 250˚C, followed by an air drying process up to 7 days (if convection oven is not used), while the convective oven requires one day drying cycle in the oven at 82˚C for 12 h and 93˚C for 8 h (standard cycle for Ecovative`s mycelium packaging products) (Jiang et al. 2016). Thermal press shows fastest results; however it employs an expensive piece of equipment from a manufacturing perspective (Jiang et al. 2014). With this findings and cost calculations in mind, the group has decided upon drying in a convective oven as the most effective method of drying, however air drying is more suitable in order to keep mycelium alive or in steady state, which will be further investigated.

## 4.3 Second set of Main experiments: Mycelium based bio-composite optimization

Previous tests show that hemp fabric with cardboard waste show the best results amongst the various assessed combinations with *P. ostreatus*. This portion of experimentation was performed in order to optimize the final colonization time and additional nutrient source within the substrate, with an upgraded mould design.

According to observations of previous experiments, a new test mould was designed in SketchUp and produced in FabLAB İzmir by the author.

### *4.3.1 Preparations*

Second set of tests was designed and conducted with regard to the results of first set of main tests to determine the optimal growth condition for the best performing combination; cardboard waste as substrate and hemp fabric as reinforcement.

#### *4.3.1.1 Preparation of conditional requirements*

Similar with the previous phase of experiments, production of cultures and test samples at this stage was conducted in lab of Prof. E. Esin Hameş Tuna at Bioengineering Department of Ege University.

#### *4.3.1.2 Pre-cultivation: First colonization in liquid culture*

For previous experiments, grains wrapped with mycelium were used as inoculum. However, the growth in the first incubation period, bags were not homogenous as expected. Therefore to conduct optimization experiments, a liquid culture of mycelium in forms of pellets, as described in previous chapters, was prepared. two Erlenmeyer flasks (1L) containing 200 ml of 17.0g/L Malt Extract Broth (MEB) were placed in a shaker incubator with 150rpm shaking at 27˚C temperature for 10 days. (Figure 4.24)

Figure 4.24 Pellets forming using Malt Extract Broth (MEB) (Personal archive, 2020)

*4.3.1.3 Inoculation*

Heat resistant plastic oven bags, filled with pre-mixed substrate required for each sample production (total of 13 samples), were placed in laminar air flow cabin for inoculation. Each plastic bag inoculated with approximately 1.5 g of pure mycelium pellets. The plastic bags were cut with sterile scissors from upper surface, to transfer the pellets inside, after rubbing with alcohol. These holes were then buckled and sealed by a paper tape from outer surface in order to prevent contamination. Following the inoculation, substrates were mixed by hand from outside of the bags, in order to homogenise the pellet distribution and colonization.

*4.3.1.4 Pre-cultivation: First colonization in plastic bags*

Incubated plastic bags were placed in an incubator (Memmert IN 450) at 27˚C in the dark for 10 days of colonization. Colonization processes was observed and photographed twice a week. All plastic bags displayed a successful and homogenous colonization without any visual contamination.

*4.3.1.5 Design of the test setup & moulds*

The porous design of the first mould increased the air penetration as intended, however it also increased the moisture loss during the colonization, which is an undesired occurrence. Therefore, additional methods to keep the moisture at optimum levels, while allowing for the air circulation became a necessity.

Figure 4.25 Mould design v2 (Personal archive, 2020)

For this step of experiments the porous structure was preserved, and, to prevent the unintended moisture loss, an extra layer of space on the bottom of the new test setup was designed, acting as a moisture pool. (Figure 4.25) Sterilized, wet perlite was placed into this layer, to keep moisture intact inside the test setup during the final colonization. Perlite is a glassy volcanic rock with a rhyolitic composition and 2–5% of bound water (Doğan & Alkan, 2004) (Figure 4.26). The most of the known world's perlite reserves, with nearly 70 %, are located along the Aegean coast in Turkey. It is very porous, has a strong capillary action and can hold up to 3–4 times its weight in water (Papadopoulos et al., 2008) therefor it is widely used in different local farming activities.

Figure 4.26 Perlite (Personal archive, 2020)

Sampling moulds were designed as four parts; two growth pods; one intended for compression tests with inner dimensions of 4x4x4cm and the other for bending tests with inner dimension of 4x4x12cm (Figure 4.27). Two caps were designed to cover these pods and keep the laid fabrics intact, in order to prevent the risk of separation of fabric from the pre-colonized substrate during colonization. Top and bottom parts act as a cap to keep the corners, while hollow part in the center increases the air access through the composite surfaces. Height of these caps creates a 1cm gap over the fabric surfaces, in order to allow more air contact, hence improved mycelium skin formation on this faces are expected. This would also increase the binding between the fabric and composite, hence, will increase the structural performance of the final product. Again, to enhance the mycelium skin formation on both faces, samples were rotated twice a week.

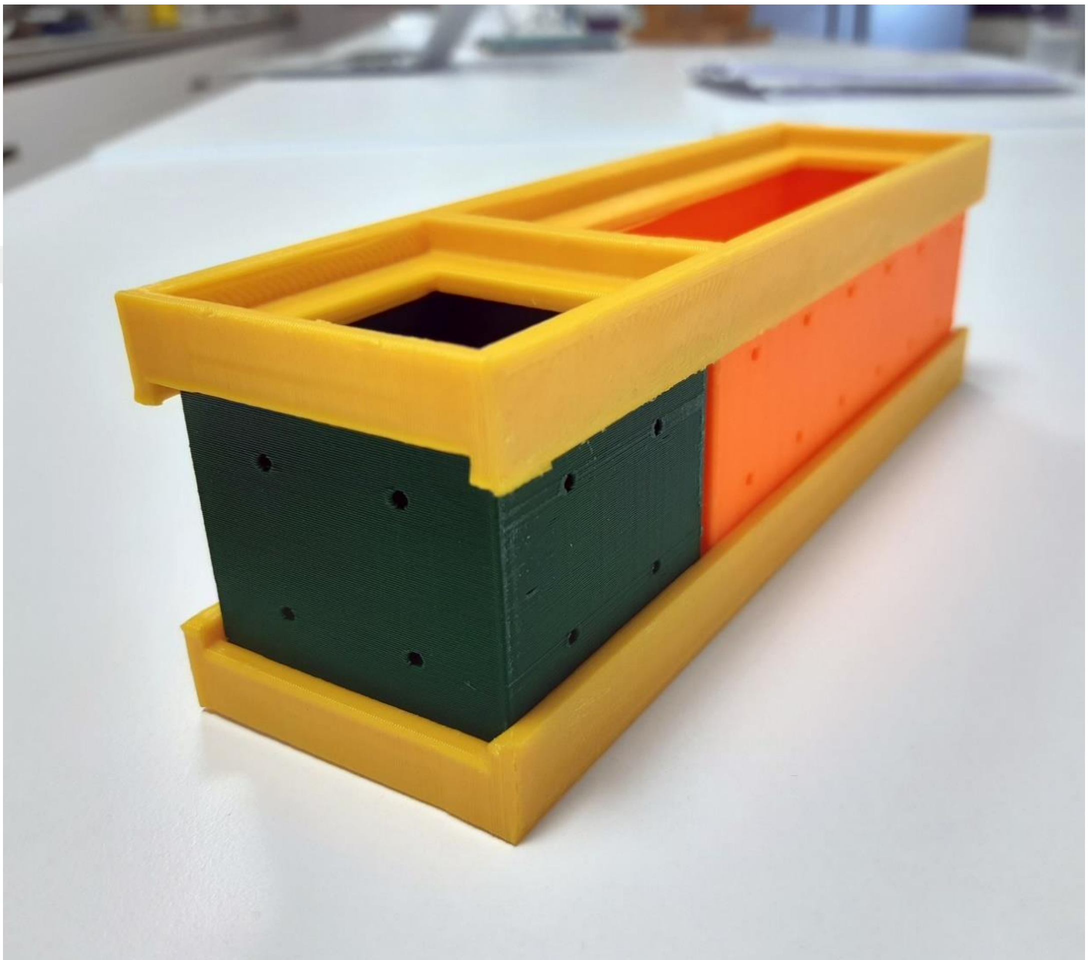

Figure 4.27 Sample mould design v2 (Personal archive, 2020)

Like previously manufactured moulds, sampling units were printed with Ultimaker 3 Extended and Ultimaker 2 Extended Plus 3D printers and all the other parts were laser cut by an Epilog M2CO2 75 Watt laser cutter from transparent Plexiglas in FabLAB İzmir. For this design, , coloured PLA filaments were used for 3D printed parts, in order to observe the spread of mycelium within the mould better, since previous transparent PLA printed moulds did not perform as expected, to facilitate observation of the mycelium development on the substrate.

#### *4.3.1.6 Preparation of the moulds & Sterilization*

All parts were prepared in FabLAB workspace. Laser-cut pieces were attached to each other using the fast-drying glue, in order to form the casing that sample colonization moulds would be placed into. Each unit was sterilized by rubbing with alcohol prior to moulding.

### ***4.3.2 Production of Mycomaterial***

#### *4.3.2.1 Filling the moulds*

After 10 days of colonization, growing mycelium was crushed by hand before opening the bags' seal. The bags were opened in a lamin air flow cabin, sterilized and pre-cut hemp fabrics were laid on the bottom of the sampling moulds. Crushed pre-colonize substrate was filled into these sampling moulds one by one by hand for each bag, until they were full. On top of that another layer of prepared hemp fabric was laid and the caps of the moulds were locked to keep fabrics attached and stretched during colonization. After all the sampling moulds were filled and prepared for colonization, were placed in the new test setup with an order corresponding to the final incubation period determined by Design Expert (Version 7.0).

#### *4.3.2.2 Incubation*

*P. ostreatus* pellets from liquid culture were used for inoculation into the substrate bags. These bags were incubated for 10 days, placed in the dark environment at 27˚C (Figure 4.28). At the end of the incubation period, the substrates were removed from the plastic bags and transferred into the moulds. Incubation was continued under the same conditions, changing between 7 to 21 days according to colonization times determined by Design Expert. According to that the order; 5 (7 days) is the first sample to be removed from the setup, 11, 13 (9 days, 1 hour and 12.5 minutes.) are the second group, 2, 3, 4, 6, 7, 8, 10 (14 days) are the third, 1, 9 (18 days, 22 hours and 4 minutes) are the fourth and 12 (21 days) is the last sample, for the five different colonization time points.

Figure 4.28 Colonization in heat resistant plastic bags (Personal archive, 2020)

*4.3.2.3 De-moulding*

Similar approaches with previous test were followed during de-moulding. Each mould was taken out from the test setup at given time by the Design Expert program. All units were removed preserving the smooth surface and well colonized, significantly superior to the previous samples (Figure 4.29).

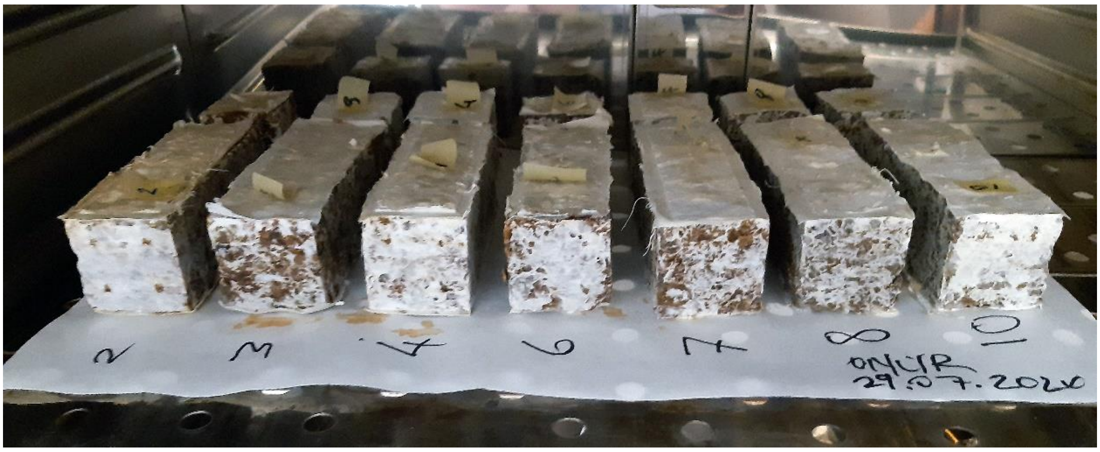

Figure 4.29 Second main test samples (Before drying process) (Personal archive, 2020)

*4.3.2.4 Drying*

Previous post-processing procedure was killing the organism, by placing in an oven with 120˚C heat for 90 minutes first and 60˚C for 48 hours to completely dry out the samples. The author has decided to remove the killing process step from the procedure, since drying of the samples would be sufficient for the testing. All samples were placed in a refrigerator at 4˚C for 5 days, until drying process. These precautions were decided upon, because of the lab availability during Covid-19 conditions. The samples were then placed in an oven with 55˚C heat for 48 hours initially, and to ensure no further weight change, this time was increased by 24 additional hours after inspection of the samples. Different degrees of bending was observed on the samples after the drying process (Figure 4.30)

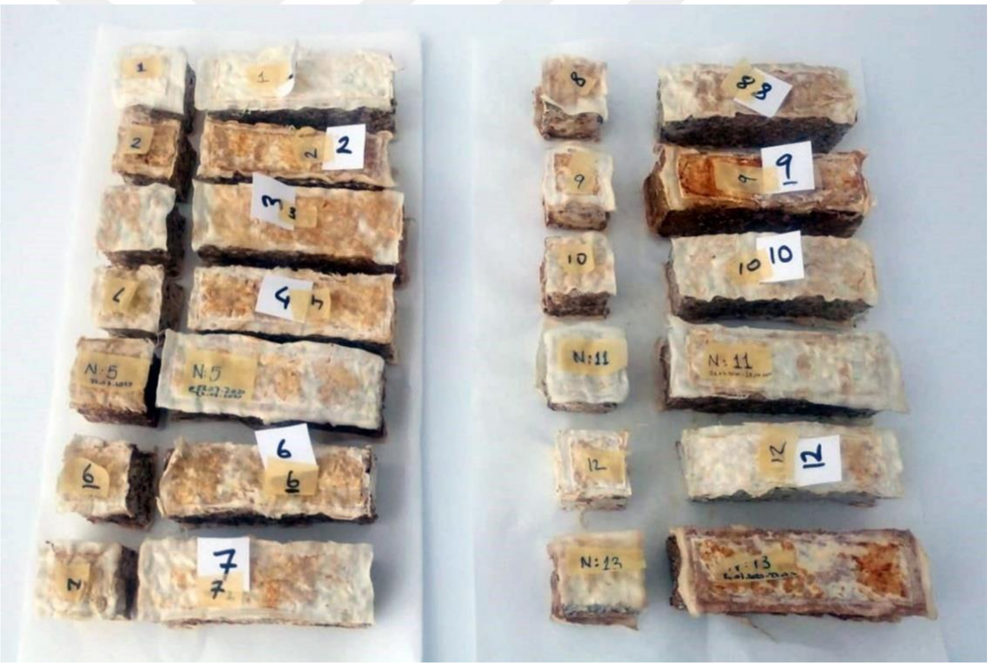

Figure 4.30 Second main test samples (After drying process) (Personal archive, 2020)

*4.3.2.5 Structural tests*

Each sample was weighed before and after drying, in order to determine the final moisture levels retained in the samples after the colonization period and assess the performance of the new test setup design.

Same procedure was followed as previous structural tests, under same conditions and using the same equipment. For the compression tests; compressive strength (MPa) of each specimen according to maximum force (kN) was tested until samples showed deformation. During bending tests, flexural strength (MPa) of each specimen was recorded according to maximum force (kN). Total of 13 specimens were tested. For the tests, compression rate was taken as 10 mm/min and bending rate as 0.5 mm/sec. Support clearance for bending test was set to 80 mm. In compression tests, a movement limitation of 23 mm was applied.

### *4.3.4 Observations & Findings*

New test setup performed noticeably better than previous. This seems to be because of more free space allowing for air circulation and better humidity control. Mycelium colonization abundantly increased through the perlite bed. Units were rotated twice a week again, this time mycelium skin did not form only throughout the upper part, but beyond it as well and the magnificent mycelium skin formation was observed on both layers which was more accessible for skin formation, due to the separation from the test setup by the height of the caps (Figure 4.31).

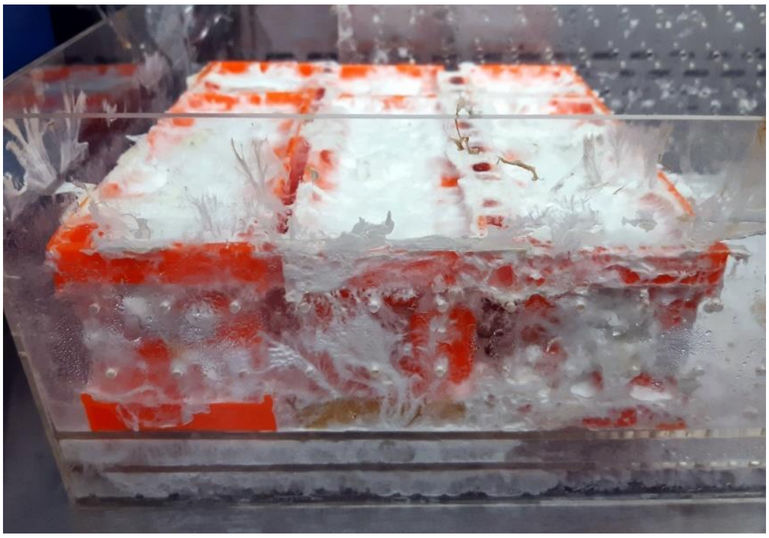

Figure 4.31 Aggressive mycelium growth through perlite bed (Personal archive, 2020)

Visual inspection showed different levels of growth, staring from the first days of incubation. Visual inspection after 10 days of growth in plastic bags were noted through best to weakest in order of; 12, 13, 11, 6, 4, 9, 10, 5, 2, 8, 7, 1, 3. After 7 days of incubation in sampling moulds; this order remained same.

After drying process, samples were weighted. Dry weights range between 25-30%, which means that moisture levels, during colonization, remained almost unchanged and stable within the desired range of 70-75%, , as an indicator of performance of the new test setup design with perlite bed, which performed significantly better than the previous experiments.

After the structural tests, reanimating the growth by dumping the samples was to be examined, in order to see if it was possible to keep mycelium alive.

Compression test results and final dimensions after drying process are presented below for each sample (Table 4.4);

Table 4.4 Compression Samples Sizes and Test Results (Personal archive, 2020)

| Sample | Width (mm) | Total Thickness (mm) | Coating Thickness (mm) | Core Thickness (mm) | Max. Force (kN) | Flexural Strength (MPa) | Core SS (MPa) |
|---|---|---|---|---|---|---|---|
| 1 | 35.82 | 30.92 | 0.5 | 29.92 | 2.04048 | 7.15006 | 0.9363 |
| 2 | 33.28 | 30.37 | 0.5 | 29.37 | 2.66904 | 10.4343 | 1.3424 |
| 3 | 36.28 | 32.4 | 0.5 | 31.4 | 1.60966 | 5.07174 | 0.6954 |
| 4 | 33.96 | 33.2 | 0.5 | 32.2 | 1.45844 | 4.67549 | 0.6566 |
| 5 | 35.54 | 34.39 | 0.5 | 33.39 | 1.62537 | 4.64037 | 0.6747 |
| 6 | 32.81 | 30.4 | 0.5 | 29.4 | 0.43834 | 1.73474 | 0.2234 |
| 7 | 35.37 | 31.05 | 0.5 | 30.05 | 0.77894 | 2.74112 | 0.3604 |
| 8 | 34.26 | 32.61 | 0.5 | 31.61 | 1.42402 | 4.69038 | 0.6472 |
| 9 | 34.75 | 33.93 | 0.5 | 32.93 | 0.39112 | 1.17319 | 0.1683 |
| 10 | 32.43 | 29.58 | 0.5 | 28.58 | 0.56168 | 2.37533 | 0.2977 |
| 11 | 34.94 | 34.72 | 0.5 | 33.72 | 0.74661 | 2.12713 | 0.3122 |
| 12 | 33.9 | 30.48 | 0.5 | 29.48 | 0.56282 | 2.14449 | 0.2768 |
| 13 | 36.46 | 36.82 | 0.5 | 35.82 | 0.46915 | 1.13895 | 0.1771 |

Compression test graphs of the best three performing samples (12, 10, 9) also presented below;

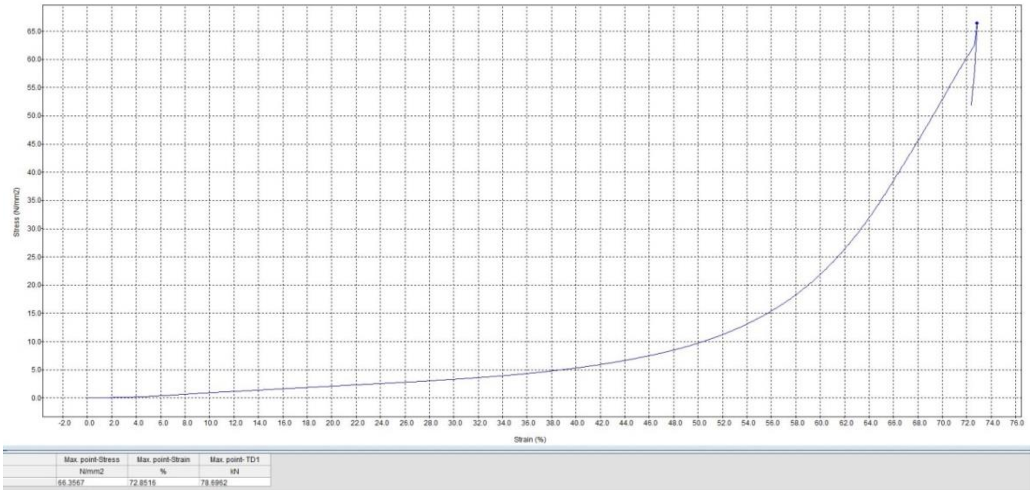


Figure 4.32 Sample 12 Compression graph (Personal archive, 2020)

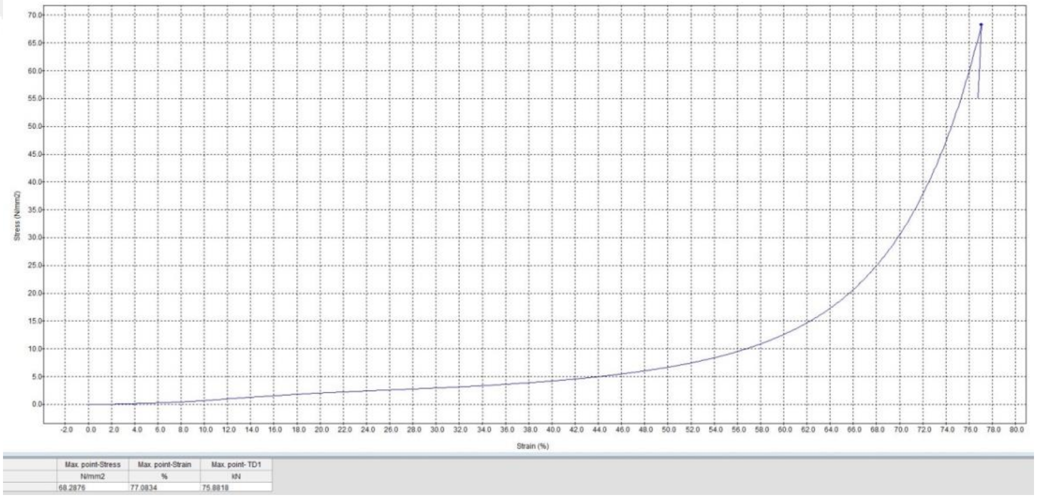


Figure 4.33 Sample 10 Compression graph (Personal archive, 2020)

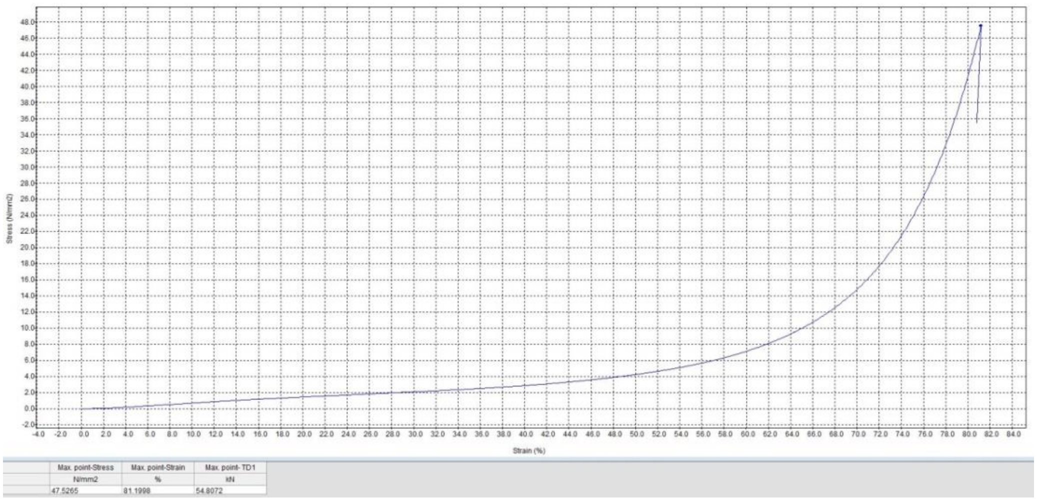


Figure 4.34 Sample 9 Compression graph (Personal archive, 2020)

As can be seen from the graphs above, depending on the structure of the composite material; samples were compressed over time until final compression was achieved. The compressive strength was measured as 72.01713 Mpa for sample 12, 68.2876 Mpa for sample 10, 47.5265 Mpa for sample 9, presenting the best three performance samples obtained so far. (Figure 4.32, Figure 4.33, Figure 4.34)

Bending test results and final dimensions after drying process are presented below for each sample (Table 4.5);

Table 4.5 Bending Specimens Sizes and Test Results (Personal archive, 2020)

| Sample | Width (mm) | Thickness (mm) | Height (mm) | Press Force (kN) | Stress (MPa) | Max. Deformation (%) |
|---|---|---|---|---|---|---|
| 1 | 35,43 | 36,33 | 31,73 | 25.5576 | 19.8556 | 72.4545 |
| 2 | 33,13 | 34,8 | 30,24 | 30.3378 | 26.3138 | 76.0184 |
| 3 | 37,66 | 36,96 | 32,84 | 13.0846 | 9.40043 | 70.0365 |
| 4 | 35,99 | 33,88 | 29,56 | 52.0933 | 42.7225 | 77.7733 |
| 5 | 37,08 | 35,82 | 34,77 | 19.2274 | 14.4762 | 66.131 |
| 6 | 33,7 | 36,71 | 29,91 | 35.9966 | 29.0969 | 76.8925 |
| 7 | 34,93 | 35,95 | 32,96 | 20.1611 | 16.0553 | 69.395 |
| 8 | 34,84 | 34,76 | 29,85 | 100.922 | 83.3353 | 77.18 |
| 9 | 33,31 | 34,62 | 28,32 | 54.8072 | 47.5265 | 81.1998 |
| 10 | 33,22 | 33,45 | 29,83 | 75.8818 | 68.2876 | 77.0834 |
| 11 | 36,72 | 36,31 | 31,43 | 12.4566 | 9.34266 | 73.1423 |
| 12 | 33,53 | 32,59 | 29,58 | 78.6962 | 72.01713 | 77.7552 |
| 13 | 34,85 | 35,37 | 31,57 | 18.4974 | 15.0063 | 72.8113 |

Not: Core SS = Core Share Strength (ASTM C393)

Flexural test graphs of the best three performing samples (2, 1, 5) also presented below;

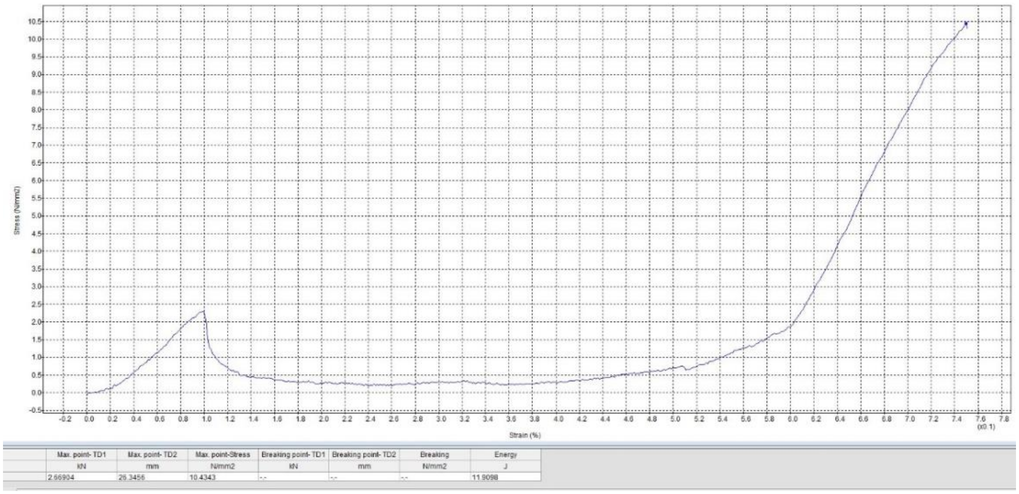


Figure 4.35 Sample 2 bending graph (Personal archive, 2020)

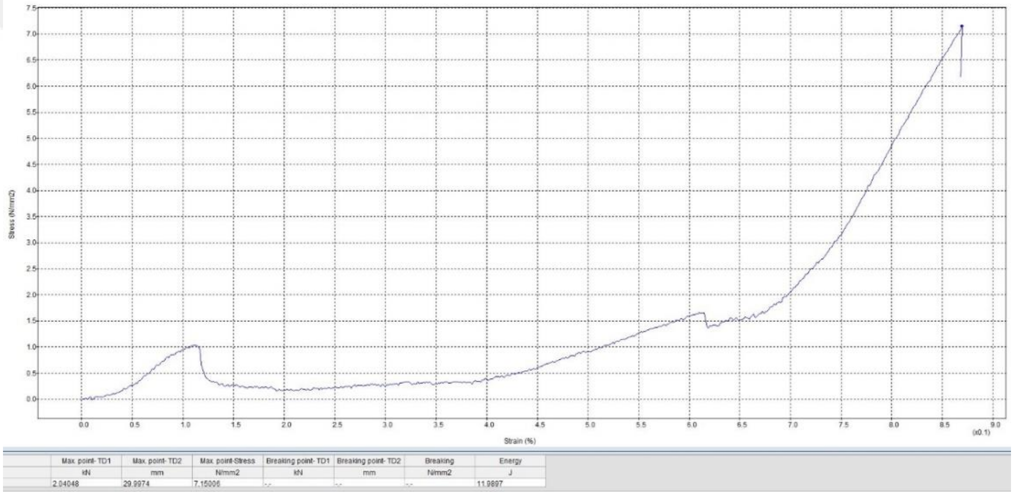


Figure 4.36 Sample 1 bending graph (Personal archive, 2020)

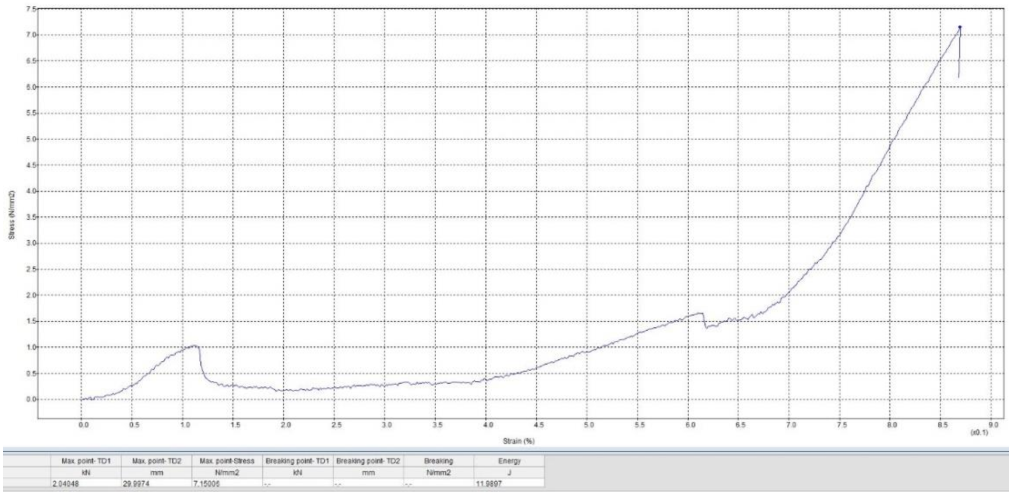


Figure 4.37 Sample 3 bending graph (Personal archive, 2020)

The flexural strength was measured as 10.4343 Mpa for sample 2, 7.15006 Mpa for sample 1, 5.07174 Mpa for sample 3, also demonstrating the best three performing samples. (Figure 4.35, Figure 4.36, Figure 4.37)

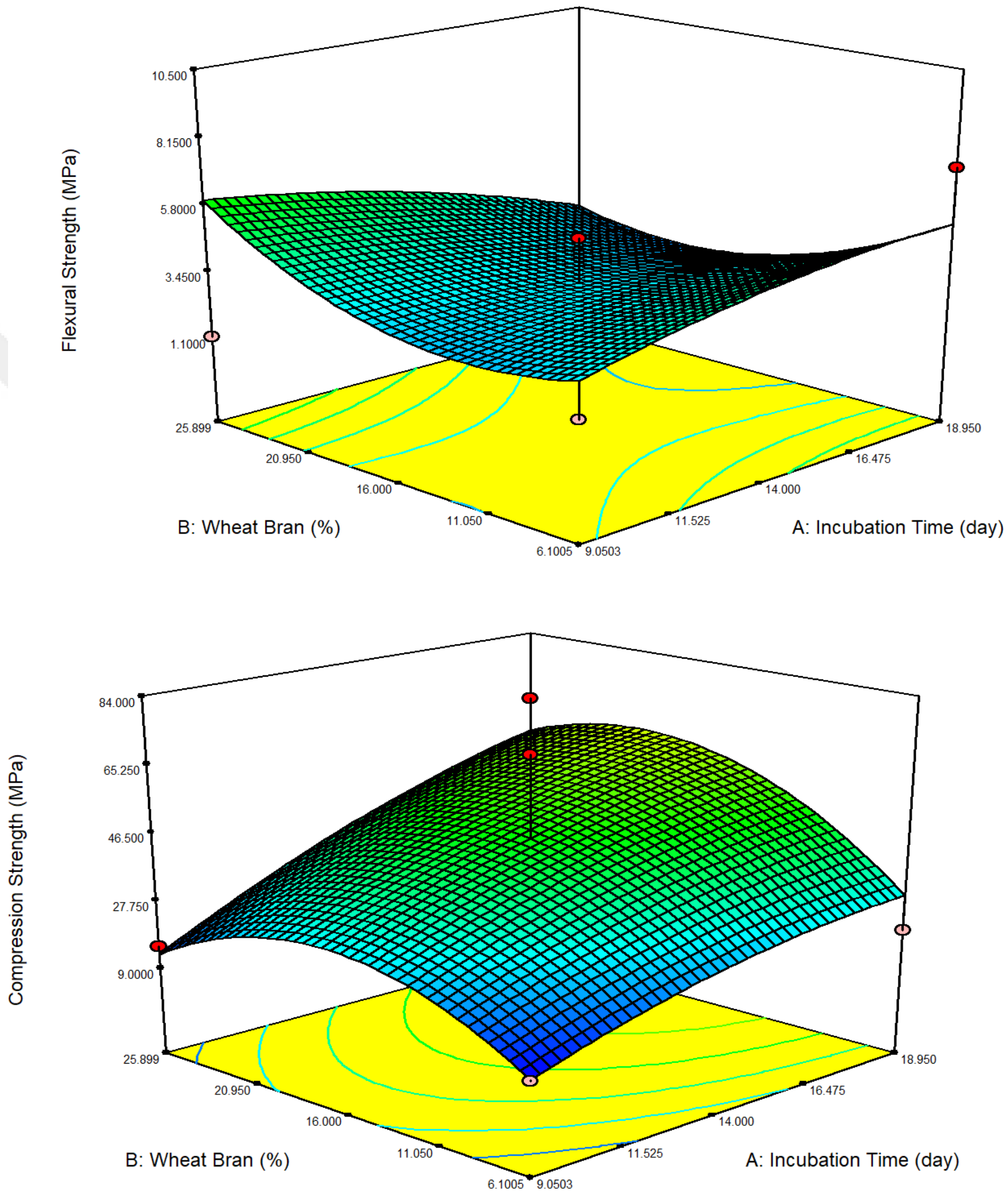


Figure 4.38 Design Expert graphs (Personal archive, 2020)

When the compression and bending tests of the samples obtained using different wheat bran and different incubation times were evaluated within Design Expert graphs above were obtained (Figure 4.38). These results, which will be confirmed in future studies, show us that the incubation period (14-15 days) and the addition of wheat bran (15-17%) are effective in the compression strength of the samples. In the tensile tests, no difference was found in the range of work

#### *4.3.5 Discussion & Decision*

At this stage the study was initiated to examine the effects of additional nutrition and final incubation time in moulds, on the final product by using statistical experimental design.

Literature survey of Attias et al. (2020) showed that only in 30 out of the 47 featured papers provide information about the substrate composition. Most of the researchers mainly focused on agricultural crop waste, like cotton, corn, wheat, hemp, kenaf and flax residue, yet 11 of the papers described the hardwood-based substrates, which are generally used as a mix with annual plant residues and carbohydrates as a nutritional supplement. Also, Jiang (2015) reports that substrate mixtures are often enriched with carbohydrates and calcium, to stimulate mycelium development and bonding, however there is limited to no information about the growth schedule. (Xu et al., 2013) It is possible to conclude that the tendency of these structural components to degrade depends on the fungal type and carbohydrate composition of each plant material in the substrate (Hori et al., 2013). To optimize the production process, effects of additional nutrients are as important as the substrate type, since these will form the substrate mixture together with the water content.  Previous phase of the study contained the same amounts of carbohydrates (wheat bran), added as nutritional supplement (5%), with two types of substrate. In this phase to optimize the nutrition addition, different ratios were applied according to the Design Expert program.

The test results were in many ways much better than previous examples, but; It was determined that the moulding stage should be done in a much more standardized way in order for the program to create a model. Although the samples obtained were used in the same moulds, their width, length, thickness and weight showed some differences after the drying stage and caused differences in the results of the tests made on these samples.

Secondly non-homogeneous distribution of the matter and the mycelium pellets upon first incubation, which indicates that use of a homogenizer is required for future sampling. Further research for the production optimization will be held in the future studies.

Compression test results (Table 4.4.) shows a performance chart; 8, 12, 10, 9, 4, 6, 2, 1, 7, 13, 5, 3, 11, from best to weakest. Yet sample 8 was reported to have an error during testing due to the bending bars, hence this sample reports might unreliable. However, the rest of the tests and results are consistent, consequently the result of the sample 8 will be excluded from further this discussion. Herewith, sample 12 demonstrated the compression strength of 72.01713 MPa, which is outstandingly higher than previous results. Sample 12, followed by sample 10 with compression strength of 68.2876 MPa. Even the weakest sample; 3 demonstrated 9.40043 MPa compressive strength, which is similar to the strongest sample obtained from the previous setup. This might be a result of new test and mould setup and the incubation by pellet method, used during the first colonization process, since the rest of the environmental conditions were kept same with the previous experiment stages.

Results indicate that short incubation period resulted in lower compression strength. (5; 7 days, 3, 11; 9days) This is followed by the sample 3 with the lowest additional nutrition ratio of 2%, yet no other clear correlation between nutrient composition and the results could be found. Therefore, further experimentation on this issue is necessary. Colonization in different growth bags might have changed the conditions,, as mentioned above. Therefore, for future research the same ratio of nutrients in the same growth bags will be used.

According to the results presented on the tables 4.4 and 4.5, as the compression strength fails, flexural strength increases. However this performance was not consistently opposite. Bending test results show an order of performance through 2, 1, 3, 8, 4, 5, 7, 10, 12, 11, 6, 9, 13. Highest performing sample; 2 showed 10.4343 MPa flexural strength, while majority of the samples demonstrated nearly half of that value. Lowest result in this case is 1.13895 MPa reflected by sample 13.

Performance of the samples in decreasing order can be projected as given below;

Performance observations during growth: 12, 13, 11, 6, 4, 9, 10, 5, 2, 8, 7, 1, 3.
Compression performance: 8, 12, 10, 9, 4, 6, 2, 1, 7, 13, 5, 3, 11
Bending performance: 2, 1, 3, 8, 4, 5, 7, 10, 12, 11, 6, 9, 13.

Inhomogeneous colonization amongst the samples might be the reason of inconsistencies observed on the test results. Since mycelium composite production employs a living organism, such organic distribution of not-fully-colonized samples will be inevitable. In addition to that, the final forms of the samples were not rigid, which might have been caused by the weak points, that effected the test results. Further studies over strength distribution within the samples will be addressed in future studies.

Although the results are not clearly repeatable; considering that these values are exceeding most of the current building materials, it is planned to continue these experiments in a more comprehensive manner in the future studies.

Dry density of the specimens was determined to be approximately 0.22 g/cm3, which is much lighter weight than that of many conventional building materials. Lightweight means better performance against forces such as earth moves. In addition to that, use of such composites to replace elements like tiles, seperating walls and coating boards etc. will reduce the dead loads over the structure.

## 4.4 General Results & discussion of the main experimentation

General aim of the experimental plan within the scope of this thesis is to explore and optimize the process if utilization of mycelium-based biocomposites in a stepwise process. Consequently, the process of exploration of the mechanistic aspects, involved in fabrication and the corresponding governing parameters, can be observed throughout the study in increasing depth. The fabrication process is explored in depth, starting with mushroom growth kits through the final structural performance tests. The improved performance of the product upon each optimization step could clearly be detected indicating that the study plan is efficient and bares fruitful potential towards the final product development.

General structure of the study can be referred to as series of experimental studies and observations, to understand each aspect of the mycelium material performance from a wide perspective that examines different substrate combinations and fabrics under prescribed growth conditions. For this purpose, upon preliminary trials regarding mycelium formation and growth, a specific culture of fungi was produced under lab conditions from the commercially obtained mushroom growth kits. This culture was used as the backbone of the mycelium material production experiments and allowed for a comparative study. As the next step preliminary experiments for determination of the optimal substrate type amongst five plausible candidates were conducted. To achieve that, samples from each substrate type inoculated with the said cultures were analysed placed in Petri dishes (in triplicates) and glass jars. Intrinsic properties of the substrate would affect the final qualities of the composite, since the compost digestion rate and efficiency would directly influence the stiffness, strength, thermal and acoustic qualities of the composite. According to the observations cardboard waste and wheat straw were chosen as target test groups for further mycelium-based bio-composite sample production, in combination with 3 chosen fabric type. The total of 23 samples was produced at this stage, forming a myco-composite improved by fabric cladding. Even though the main goal of these experiments was to compare the benefits of enhancement of mycelium-based materials with fabrics in order to increase structural performance, numerous predictions on different properties and potentials of mycelium as a material toolset were acquired during the course of this assessment. The general methodology employed in production of the material remained similar for different types of substrate and reinforcement types. Changes were incorporated into moulding and additional processes.

Main idea behind using natural textiles, as the surface component of mycelium-based composite, is providing a fibre surface that mycelium can attach to. As the second parameter, type of the natural textile to be used would show different strength properties. Moreover, this would prevent the composite from dispersing, during the increasing general tensile forces acting on the composite. Also enhanced thermal and acoustic properties are the possible outcomes. Similar research also points out that the bio-based core and reinforcement can offer enhanced structural and thermal performance, since the mycelium would act as a binder like a polymer resin matrix (Jiang et al., 2013).

Jiang et al. examined a similar idea on fabric reinforced mycelium biocomposite, to assess the capacity of textile fabric and mycelium together as a biocomposite. The group developed a five-step manufacturing process for mycelium based biocomposites with a single or multi-layered laminate skin (woven textiles in this case). They used cut/print skin preforms made from a wooden male mould and thermoformed plastic female mould, to re-grow pre-colonized mycelium substrates binding laminate skins into a composite with the core. The study was conducted within the scope of collaboration between Rensselaer Polytechnic Institute and an innovative bio-materials company, Ecovative Design, LLC. To demonstrate and manufacture bio-composite laminate and sandwich parts made with mycelium-bound agricultural waste core material, natural textile reinforcement, and vegetable-oil based resins series of experiments were held. Collaboration aimed the creation of a mycelium sandwich composite, using mycelium to bond the natural woven textile acts as reinforcements, agricultural waste from farms as core, and natural bio resin to provide strength. The aim was to create a new sandwich structured material, which is

100% recyclable or biodegradable after its service cycle that would performance wise to be comparable or superior to its unsustainable alternatives (Jiang, et al., 2014). During these studies researchers tested the performance of three types of textile with Ecovative mushroom colonized over hemp-based substrate (Jiang et al, 2016).

To create better alternative to its competitors, such as mid-density polyethylene (MDPE) and ABS, the group added resin as an on-going series of experiments complementing their previous work with resin-less sandwich beams. They claim that resin containing biocomposites exhibit better performance in all mechanical properties. Group reported the core shear ultimate stresses of resin cured beams to be increased 3-5.5 fold, compared to the corresponding resinless samples, core shear yield stresses increased up to 2-4 fold of the original values, skin ultimate stresses increased up to 1.5-2.5 fold and flexural strengths increased up to 4-6.5 fold. The group compared their effective flexural strength results of bio-beams manufactured using flax reinforcements with mid-density polyethylene and ABS. While their myco-composites show 30 MPa, the flexural strength, the mid-density of polyethylene (MDPE) is 40 MPa and ABS is 75 MPa. Yet the researchers believe that these would form a good alternative and can be further improved with better production process and materials. Hence myco-composites have a potential to replace their competitors such as MDPE or ABS plastic panels, as interior panels for transportation vehicles, sports, and entertainment goods, as well as building panels for decoration and insulation (Jiang et al., 2019). In addition to that, when compared against composites on the market, myco-composites are structurally weak but resistant to pressure, which can be seen as an advantage or disadvantage depending on the targeted application.

The following equation is used for flexural strength calculations to compare with the results obtained by Jiang et al. (2016);

$$F^{ult}_{s} = P_{max}/(d+c)b \tag{4.1}$$

P = maximum force, d = total thickness, c = core thickness, b = width, c = d-2t, t= coating thickness (ASTM C393)

Results produced by Jiang et al. show that jute containing composites perform with a maximum average flexural strength of 14.1 MPa, which is almost 10 fold the corresponding test sample 2, with 1.3424 MPa (Table 4.3). This indicates that bio-resin infusion shows great potential to increase flexural properties of mycelium based biomaterials. On the other hand, while the team improved structural performance and mechanical properties by bio-resin infusion significantly, costs and required processing have also increased. In addition, the group reports that the most common failure mode observed during the tests was the tensile failure of the core material, meaning that the core is the weakest part in the structure. Which brings out the question if myco-materials are the right choice for the core of such resin containing production? This process requires numerous additional steps in order to maximize the strength of the final product.

Consequently, there are many different variables between two studies and therefore results can only be discussed in terms of qualitative comparison of performance.

Experiments conducted by the author herein, question the potential production method with minimum need for machinery in the production phase. Production of the moulds eliminates this concern since these moulds can be reused many times. Yet more nature-friendly alternatives of mould production remain necessary.

The focus of this thesis research is not on achieving the maximal strength, but rather is to determine the effectiveness of using different natural textiles to form a soft shell and compare these textile types with regard to the resilience of the composite. Also this study aimed to generate structural strength data to support this novel approach. Yet, further research on coating and integrity enhancement of mycelium based composites should be researched. Such enhancement may be used with future advancements that may facilitate the mycelium bio-composites improvement with fabrics to form both structure and the enclosure of an architectural design

The reliable way to increase the structural performance of mycelium based composites is to optimize the substrate mixture and fungal strain combination. This mixture both serves as the base for mycelium formation and the source of nutrients and water for the organism. In their work Tudryn et al. (2017), tested the mechanical properties of mycelium composites with carbohydrate addition at homogenization phase of fabrication. The group found that nutritional addition of carbohydrates after a homogenization step contributed to a larger and more continuous hyphal network forming, positively impacting the strength of the final composite. This situation is possibly a result of extra and easy to break nutrition addition for the fungi to the mixture. As a result organism gains more energy to grow which leads a stronger hyphal network formation. To optimize the process and allow mycelium to form into a more rigid matrix, addition of an extra carbohydrate source was examined, through each phase of the study. The first stage of main experiments included addition of %5 wheat bran. In the second stage, optimization of the additional carbohydrate nutrient by testing different additive ratios ranging from %5 to %20 produced. Although no strong correlation could be observed, samples with higher percentage of wheat bran performed better and showed faster colonization. The author will continue the optimization of these tests in the planned future works.

One other important findings of this study is that the amount of the inoculated mycelium pellets seems to increase the colonization ratio and found to be more effective than additional carbohydrate source on small samples. Most of the research in the literature does not provide information on the amount of inoculated organism used for the production. While very rich source of ingredients is in excess to the few inoculated fungal cells, mycelium formation will start from these initial contact points and produce new cells utilizing the nutrient sources. However, if the aim is to completely cover the space, using more initial points to start this dispersion, would be much more effective with regard to both speed and density of colonization. In this case, where food source is provided in excess for full colonization, using more mycelium pellets for inoculum would increase the colonization speed and surfaces wrapped by mycelium. A smoother skin-like surface can also be achieved through full colonization (Jiang et al., 2013). To homogenise the division of mycelium colonization, homogenizers and mixers will be assessed together with the ratio and speed optimization of inoculation and colonization in future studies.

Keeping environmental conditions such as humidity and temperature constant is one of the key factors in mycelium production. After each step of the study, a different moulding strategy was developed making use of the previous findings. While very high moisture levels resulted in low colonization, loss of humidity slowed the colonization down. Best performance was achieved by using a bed component, perlite, to retain the moisture levels in the colonization environment. Other than humidity, air circulation is another key parameter, optimized by the porous layered and filtered moulds, which increased airflow and facilitated removal of the undesired $CO_2$. Mushrooms do not require strong air circulation, but accumulated $CO_2$ increases the acidity of the environment, hence effects the environmental pH levels. Further on to increase the air access a porous design that facilitates penetration inside of the colonizing substrate, would be very effective to accelerate the colonization, as observed.

The main advantages of myco-composite materials produced using natural fibers (e.g., jute, flax, cellulose fiber, etc.) over traditional synthetic composites are low cost, low density, competitive strength, in terms of both tensile and impact mechanical properties, reduced energy consumption, the potential for $CO_2$ sequestration if translated to a large scale, and perhaps most important of all, tailorable biodegradability (Jiang et al., 2019). However, for marketing of such products formed as myco-composites; energy and material consumption of the process is one key component, while manufacturing time and quality of the final product are amongst other important considerations. Jiang at. al. defines manufacturing of the mycelium-based composites similar to traditional advanced composites. Myco-composites with a sandwich construction consist of two main parts: a single or multi-layered laminate skin (woven textiles) and loose core material as substrate mixed with mycelium. Myco-composites are also adjustable and offer a wide variety of options for the designers. Different substrate types bring different qualities ranging from fire protection to sound insulation. This implies that myco-composites can potentially serve as insulation elements, which can be a good replacement for many unsustainable construction elements. Furthermore, additional applications to increase the structural strength of the material can conjoin these as the structure itself offering many potential improvements and research areas. Today many researchers focus on adjusting myco-composites to a wide range of application. Use of clay, textile, branches, 3D printing are some of the applications already mentioned in previous chapters. MycoComposite™ of Ecovative, ground tiles and acoustic panels of Mogu (mogu.bio), provide a reference of successful examples of commercial applications. Depending on application type they are generally light-weighted, nature-friendly and easily biodegradable.

Testing kits were designed in a manner to require minimum energy during the production. After designing and crafting the negative mould that answers needs of fungi to grow (air circulations, contamination protection, moisture retention etc.), the only energy consuming step would be keeping the mould under the optimal heat and moisture conditions. However, in many cases it is not challenging to maintain the required conditions, since the organism tends to grow optimally under ambient room temperature conditions. For the scenarios of different geographical regions a different type of mushroom can be chosen responding to the changing temperature levels. Apart from the environmental condition maintenance there is no extra energy consumption, until the drying phase. This, in fact, renders the product cost competitive with its low-embodied-energy production process. However, the process is still dependent on hand layup method for manufacturing, which limits the production speed and increases the cost (Jiang et al., 2013). Aid of machinery specific to produce myco-composite units would minimize the failure and accelerate the production speed, however will increase the cost.

As another way to increase the performance; post-processing may be effective against cost competitiveness. Although additional steps of production would cause longer production times and extra costs, samples pressed during compression tests were observed to be denser, more rigid and smooth. Meaning that the applications like cold pressure could be quite effective in achieving a clear form with higher density. During previous compression experiments, samples compressed to 75% volume, resulted in very rigid surfaces with increased strength. With this respect for future works, cold or hot pressing, as another post-processing step, can be tested to create rigid surfaces and increase structural strength, as proposed by Appels (2018a). In this manner the final product would be smoother and compact, which is a desired architectural quality for most of the surfaces. In addition to that, different densities can be achieved, since the material can preserve its integrity up to almost 75% of compression without deformation. The final samples generated in this study show an average of 0.22 $g/cm^3$ dry material density, which is considered to be lightweight with good structural performance. Production of such units could be beneficial for filling elements. On the other hand, pressed samples can reach up to 0.88 0.22 g/cm3, which is still considered to be very lightweight. Such composites can replace indoor separators, fillers and furniture. In addition to that, the production method seems very potent for pre-cast board production. Also, to eliminate the painting process, food colouring can be used where necessary. Meanwhile surfaces achieved by combination of fabric, substrate and mycelium also brings out different white, latte colour schemes depending on the drying process, chosen substrate and fabric.

Table 4.6 Comparison of the best results of final tests with the products in market (Personal archive, 2020; Gürdal & Acun, 2003; Özeren, 2016; akg-gazbeton, 2017, CSB, 2019; Kudret, 2017; Kalaycıoğlu et al., 2012)

| Definition | Unit type | Dry density (gr/cm³) | Compression Strength (MPa) | Flexural Strength (MPa) |
|---|---|---|---|---|
| Sample 12 | | 0.22 | 72.01 | 2.14 |
| Sample 2 | | 0.22 | 26.31 | 10.43 |
| Additive Drywall 50% water / cement | PANEL/BOARD | 1.65 | 16.5 | 9 |
| Additive Drywall 60% water / cement | PANEL/BOARD | 1.78 | 15.5 | 6 |
| Additive Drywall 70% water / cement | PANEL/BOARD | 1.88 | 9.5 | 5.5 |
| Fiber Gypsum without Perlite 0% | PANEL/BOARD | | 8.8 | 5 |
| Fiber Gypsum without Perlite 1% | PANEL/BOARD | | 14 | 13 |
| Fiber Plaster without Perlite 2% | PANEL/BOARD | | 13.5 | 11 |
| Perlite Fibrous Gypsum 2% fiber | PANEL/BOARD | | 2.5 | 4 |
| G3 Aerated Concrete | FILLING | 0.5 | 3.84 | 1.05 |
| Vertical perforated brick with M10 Mortar (space <35%) | FILLING | 1.5 | 5.08 | |
| Vertical perforated brick with M5 Mortar (space <35%) | FILLING | 1.5 | 5.4 | |
| M2.5 Mortared vertical perforated brick (space> 35%) | FILLING | 1.3 | 3 | |
| M10 Mortar Filled Block brick | FILLING | 2.2 | 3.82 | |
| M5 Mortared Block Brick | FILLING | 2.2 | 3.6 | |
| M2,5 Mortared Block Brick | FILLING | 2.2 | 3.04 | |
| Gas Concrete (thin mortar) | FILLING | 0.95 | 3.1 | |
| Gas Concrete (Thick mortar) | FILLING | 0.95 | 2.62 | |
| M1 Mortar Cut stone | FILLING | 2.5 | 3.7 | |
| 22,5 Hollow brick | FILLING | 0.47 | - | 0.026 |
| 30 Hollow blocks | FILLING | 0.5 | - | 0.048 |
| Masonry Brick | FILLING | 0.6 | 6 | |

Table 4.7 Comparison of the best results of final tests with wooden structural materials in the market (Personal archive, 2020; Sun & Li, 2020; Lam, 2001)

| Definition | Dry density (gr/cm³) | Compression Strength (MPa) | Flexural Strength (MPa) |
|---|---|---|---|
| Sample 12 | 0.22 | 72.01 | 2.14 |
| Sample 2 | 0.22 | 26.31 | 10.43 |
| Southern pine | | | 19.98 |
| Canadian hemlock | | | 20.2 |
| Glulam | 0.484 | 26.1 | 53 |
| Timberstrand 1.3E | | 4.7 | 11.7 |
| Timberstrand 1.5E | | 9.4 | 28.7 |
| Microllam 1.9E | | 9.4 | 33.1 |
| Parallam | | 9.4 | 36.9 |

Results of the final tests were discussed and compared to several groups of materials used in construction industry (Table 4.6). First group of products is the separator panels. Main goal of the panel use in architecture is to cover surfaces rapidly and efficiently. From the test results and experiments, it is foreseen that myco-composites offer cheaper production, with potent insulation properties against heat and sound, even though production process remains slower than that of conventional panels.

Filling products, such as bricks, gas concrete, are the second group to compare. Results show a better performance both in compression and bending against these conventional materials. Myco-composites are a potent replacement for such separator materials, for indoor and outdoor applications. In addition, it can bare beneficial features, such as sound and heat insulation. As it meets the described requirements as a natural material, that degrades in nature, by including multiple functions in a single product; can become the reason of choice in labour, cost, time and energy. When the general structural features of the final product are compared, it can be used as an equivalent product to accommodate the structural elements of the masonry structure. It can also be used in prefabricated products, after passing through certain phases.

Although myco-materials are not currently suitable for structural use; with further improvement this goal is achievable. In order to demonstrate the potential of these materials a table of comparison with the wood based structural materials in the market is presented (Table 4.7).

It is obvious that the aforementioned graphics of final the experiments will vary depending on the application areas of the desired final products. For example, if the final product is intended as a sound or heat insulation plate, the compression and bending graphics will remain as mentioned above. If the end product is a product with a high load carrying capacity requirement, it will have to be pre-compressed into the final product, such as on the structural systems. , In case of such final product the compressive strength measurement the volume compression will no longer be reflected in the compressive strength graph.

Since this study was planned as one of the pioneer studies in this field, more detailed comparison analysis is yet to be conducted in the future studies. Designing product elements according to application areas and revealing the properties of these products will form the basis of other studies in the future.

Except the described groups, myco-composites can be used in decorative products, from laminated and compacted surfaces, doors, windowpanes to tables and chairs, as a alternative with cheaper production costs. Mycelium could replace resin in chipboard-resin combinations, commonly used in such applications, to form a more environment friendly alternative to the products on the market.

That being said, although myco-composites bare high potential for improvement, their current production processes cannot yet compete with the composites on the market. Despite the relatively low production costs, production process remains time-consuming compared to the conventional composites. In addition, as these materials are quite new, their life time, durability over time and potential risk scenarios during their life time, including their resistance to different conditions, must be observed particularly in light of its biodegradability. There is no information on the durability of these products in utilization, since this may vary with the recipe of the substrate and additional applications.

Furthermore, coating of these materials must be addressed to provide protection against humidity and UV effects. The production process must also be simplified and mechanized for the marketing purposes.

Most of the findings discussed in this study are used as the bases for the development through each experiment, while some others, like addition of food colouring and postprocessing are only used within the design concept.

## 4.5 Design & construction by mycelium

In this section, a special design, which aims to demonstrate the architectural potential of experimented myco-composites, is proposed. The main idea behind the proposal is to outline the potential of non-regular approaches that can replace current unsustainable methodologies and materials used in building industry with regenerative and responsive alternatives.

To simplify the production process; a sample unit, namely a beam with the chosen combination of substrate and textile, was used as the main elements of the design. Shape of the units was converted from brick-like beams to cylinder. Brick shaped myco-composite production with fabrics requires two-step fabric placement during production, which is heavily dependent on hand work. In order to accelerate the production process, a concept of simple rolling machine was designed using Solidworks Design software. Using this specific roll machine, rolling of the substrate with fabric into a cylinder form with minimum waste is possible (Figure 4.39).

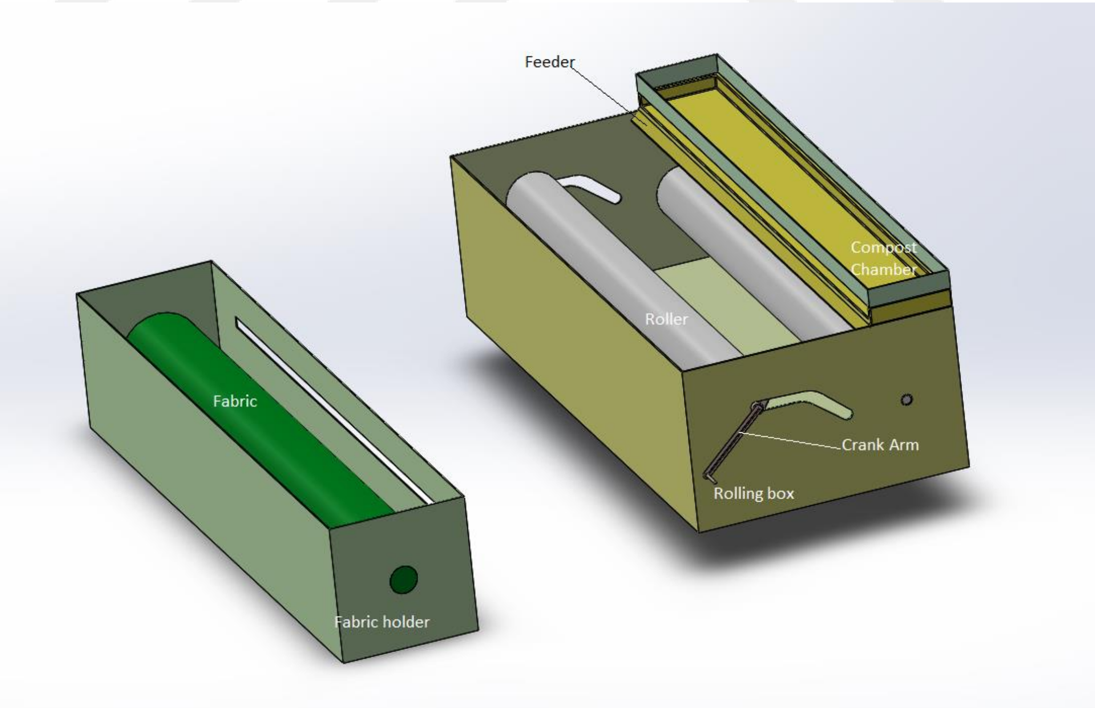


Figure 4.39 Units of myco-beam rolling machine (Personal archive, 2020)

The machine consists of two parts; a rolling box and fabric holder. Working principle of the machine is basic. Inside the rolling box there are two cylinders attached by a loose fabric, re-locatable with crank arm, are located. Fabric located in the fabric holder then will be fed from the razored mouth to the cylinders. After sufficient fabric is discharged, the razor located in this mouth will cut the fabric roll. The pre-crushed substrate in compost chamber will be then discharged onto the fabric. Cylinders will be locked onto each other by the crank arm, and these cylinders will be revolved in opposite directions by the aid of a basic electric engine. In this manner the compost will be distributed homogeneously and fabrics will wrap this compost, while compressing the air gaps. Then re-locating the crank arm in the first position, the rolled-up myco-composite beam will be released out of the system and placed in plastic bags in a colonization room. Before incubation, these beams can be bended in the desired angle, or even unified, knotted to each other, to achieve different combinations to be used in design (Figure 4.39). Further advancement of this method will be a topic for future studies.

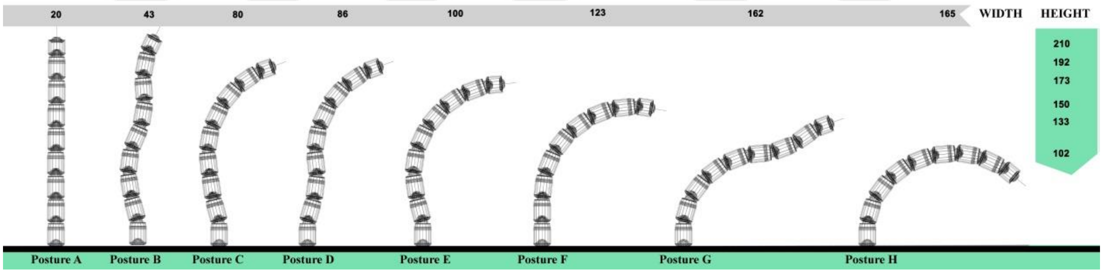


Figure 4.40 Beam scenarios; height and width of different postures

Using this methodology the author aims to achieve further strength and integrity, as fabric will be kept in one piece surrounding the entire substrate. The rest of the procedure is similar to the sample production process described in the experimental section herein. These units are designed to allow for a degree of formability upon moulding. When a unit is released out of the rolling machine, it can be bended as required by the design prior to final colonization, since it will be loose enough for formability. Also, after colonization is completed, these cylinder units can be sliced down into smaller, wheel-like pieces, to be stacked as simple masonry units as logs. During production, such cylinders can be equipped with a hollow tubular core, by placing tubular cardboards in the center during production phase; this will increase the air circulation throughout the units. As a product; these lines can be used to place cables and pipes as required by the design.

### *4.5.1 Design method*

The design program was addressed as a contemporary park, to host events. All elements are made from cylindrical myco-beams. To connect these, simple hemp ropes will be used. Vertically placed beams will be anchored to the ground, by burying 1/5 portion underground with a special footing. The beams serving as lightning fixture will be produced with a cardboard tube, to achieve a hollow core. To allow for bendability, these cardboard cores should be made of stacked cones, preferably cardboard (Figure 4.41).

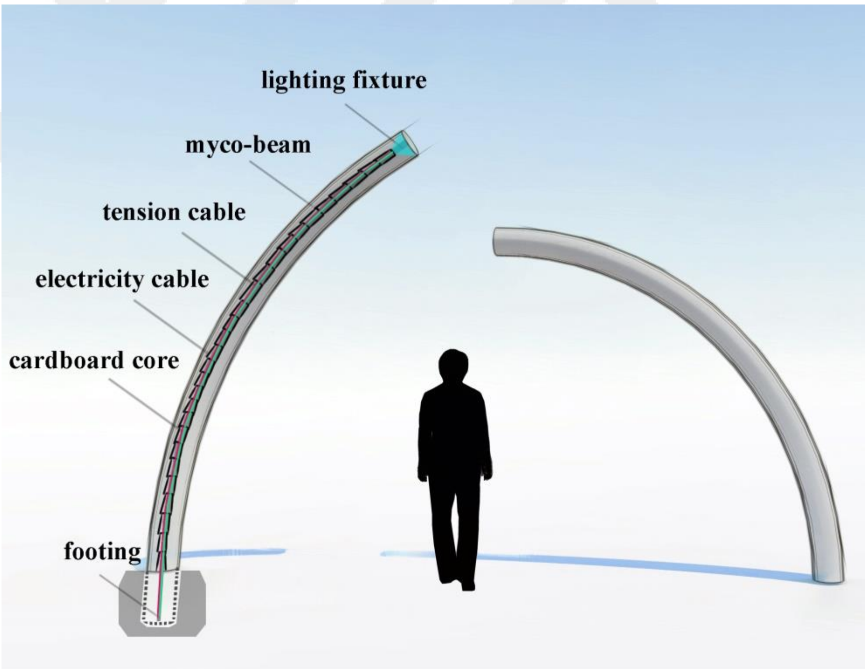


Figure 4.41 Anchored vertical myco-beam detail

This approach would increase the production costs but will also allow for extra air circulation during colonisation and speed up the production process. To increase the strength; an elastic cabling system, holding the beam intact and allowing for transfer of electricity to the lighting fixture, will be attached within the core. Park walls were produced by stacking the sliced down myco-beams, to create seating and achieve more strength, these units' cores will not be hollow. (Figure 4.42)

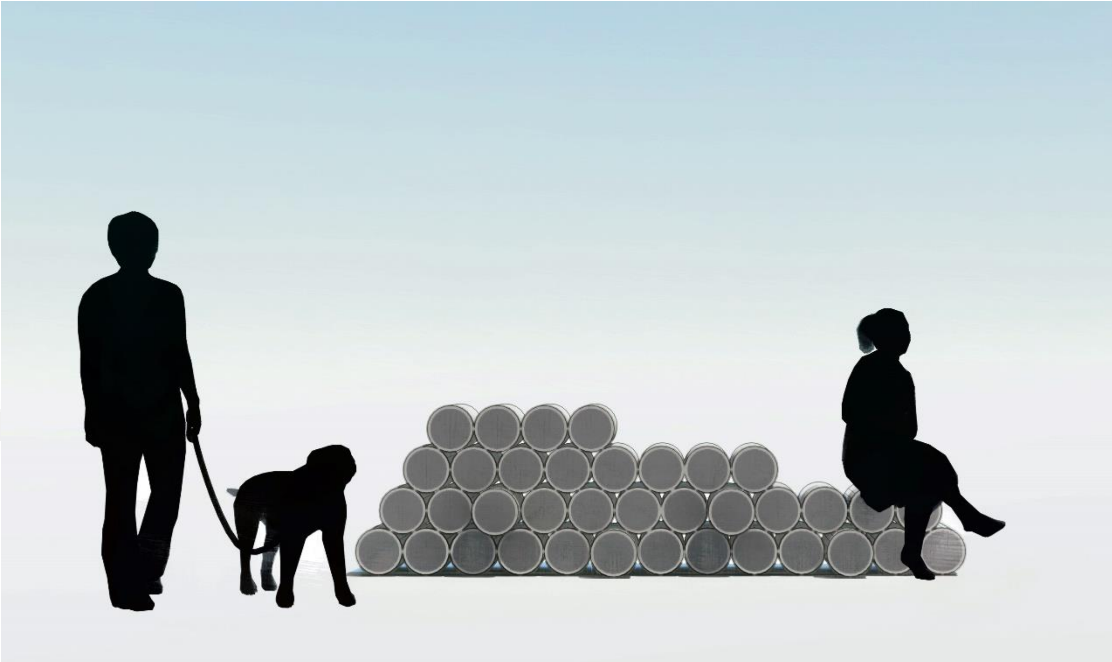

Figure 4.42 Furniture by stacked myco-beam slices

### *4.5.2 Myco-Park*

What is expected from material of park furniture or a playground toy? Main objectives can be listed as; antimicrobial and non-allergic surfaces, safe and soft components to keep children from harming themselves, easily replaceable and cheap parts are also very important, since they are generally used to their limits in different ways of imagination. The use of myco-beams as the main unit offers a solution to the described requirement. To attract attention to these potentials; a park design for contemporary outdoor events and games was proposed.

Contemporary park design is a free formed proposal and seeks to address the most basic and fast construction methods, in order to host events at rural locations (Figure 4.46). Myco-beams were used as vertical lighting elements, sound barriers and walls, to define variety of space and program element (Figure 4.44). The main goal behind the design is to obtain the readily detachable and transferable structure, therefore basic approaches were employed (Figure 4.45).

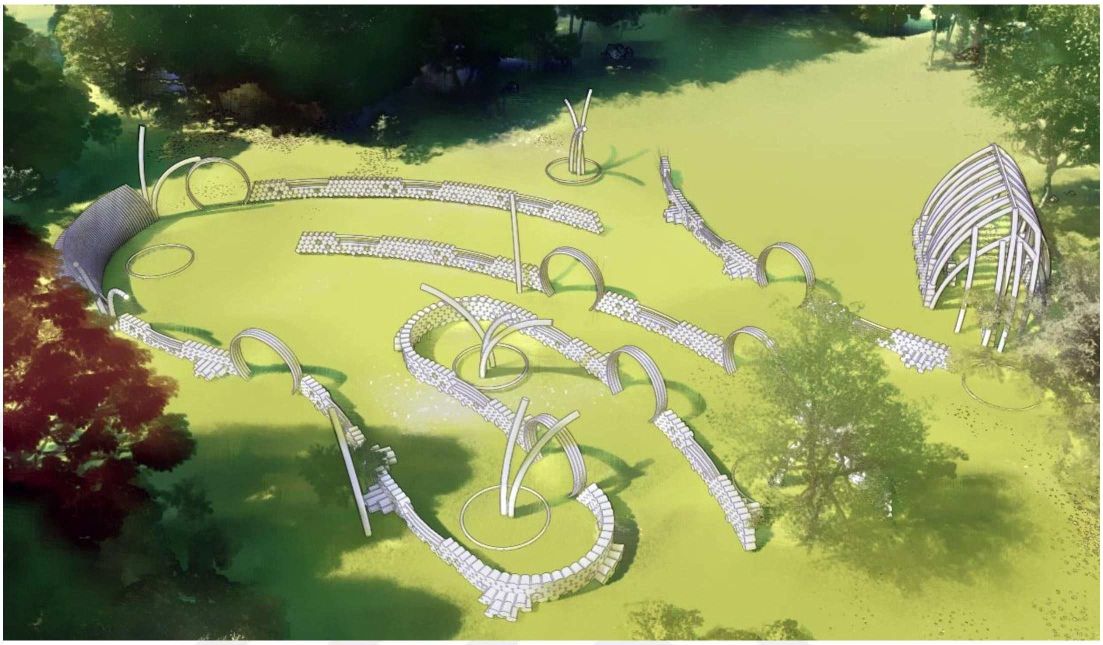

Figure 4.43 Myco-Park - Contemporary park design

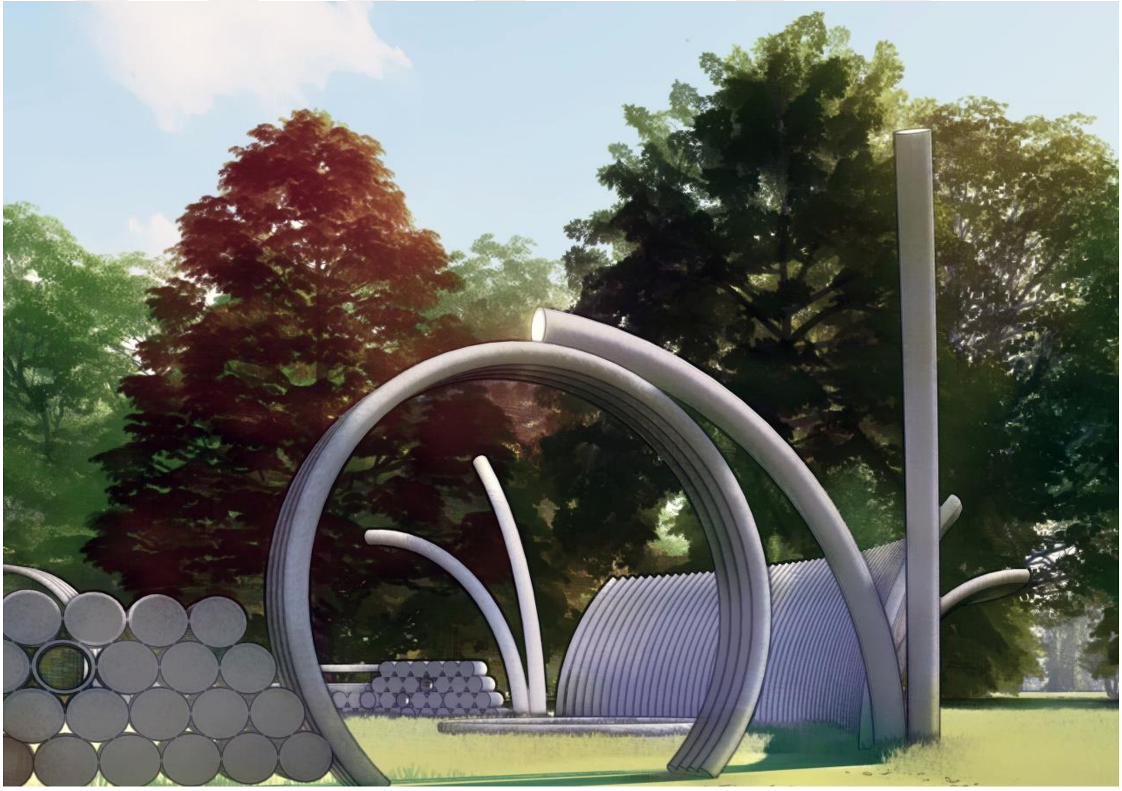

Figure 4.44 Myco-Park Front Stage

Figure 4.45 Contemporary park units

A servant unit designed as a semi-closed space was proposed. During the events, this unit will be the gathering space and a multifunctional gallery. (Figure 4.46-47)

Figure 4.46 Servant space

Figure 4.47 Servant space views

A special fabric can be placed, wrapped between the vertical beams, to achieve partial shielding from the sun, rain and wind. As a backbone of the structure, a “V” shaped beam will be placed on the connection point of these beams. All other beams will be attached to this backbone by simple joints and ropes.

The conic units offer lightweight properties and soft surfaces, against potential accidents. Even in the event of unit failure, there will not be any sharp pieces that may pose risk, since the structure will be composed entirely of mycelium, cardboard and fabrics. The damaged unit can easily be detached from its knots and the new unit can be placed. Placing these by knotting will ease the replacement. Parts that have fulfilled their lifespan will be used as fertilizer to the gardens and forest in the surrounding area. One of the main problems of contemporary events outdoors is the trash left over on the site. Use of myco-beams will be therefore very beneficial since the leftovers will be nourishing the land.

Another method to stack these beams would by placing some of them alive to encourage mycelium to spread over other beams and form connections between structural beams. This method might reduce the structural strength in the beginning and several days of pre-fabrication might be necessary before placement. To speed up this process, daily moistening with liquid nutrient source will be applied until the desired integrity. After that, living mycelium will be air dried on top, and left alive underground to spread. Footings will be designed accordingly, to supply extra nutrient source to increase colonisation. Using this method live mushroom will be a part of the system and mycelium will be inoculated into the ground to colonize the area. If we provide necessary ingredients for mycelium to spread out, the mushroom colony will heal the land from toxic pollution, produce food and support biological diversity. These live mushrooms then will be placed accordingly, to achieve and observe a ring like mushroom formation throughout the year. The design concept is based on natural phenomenon called “fairy rings”. Each year fruiting mycelium colonization spreads through underground food source, while leaving the center to decompose in time, resulting in fruiting mushrooms in ring-like formation resembling a rippling effect.

The expansion of the park will be sustained and limited by the production capacity, which will also define the efficiency of the complex. Combinations of designs, which can be produced by these readily formed beams, seem limitless.

This construction as a whole will support the research and development process, through the interaction on the site, and as the method is developed further, new design units will be displayed at the Myco-Park as a showroom (Figure 4.48-49). Likewise, the mushroom farming, such design facilitates, will run with a kick start; first installation, then care and effort will produce the fruit. To achieve further progress, one of the main goals of this proposal is to attract and educate people on the use and production of mycelium-based materials. In order to achieve an interactive platform, all elements were placed freely accessible for human activities.

Figure 4.48 Alternative scenarios - Shading

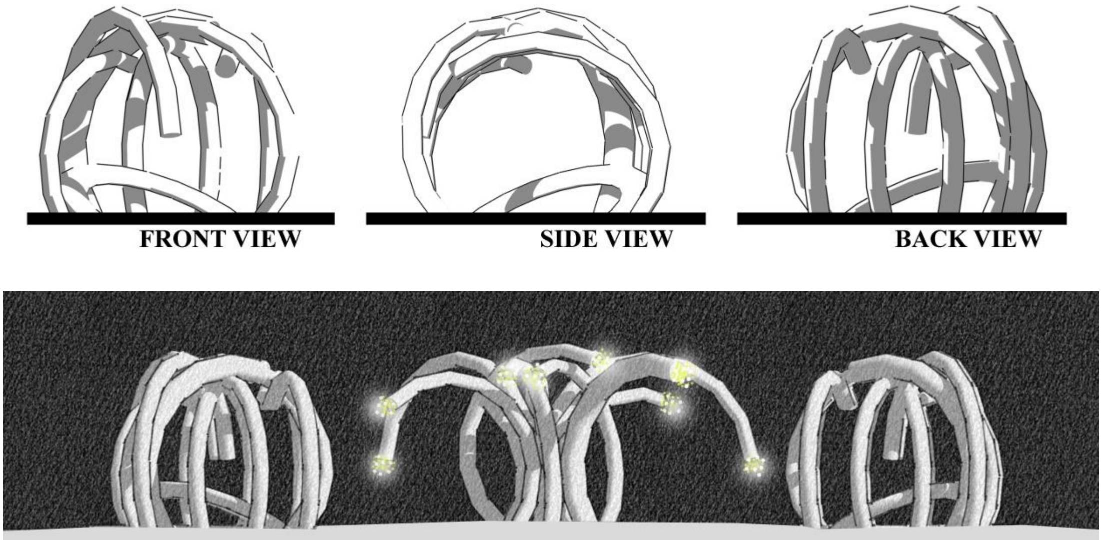


Figure 4.49 Alternative scenarios – Lighting and seating

# CHAPTER FIVE
# CONCLUSION

## 5.1 Conclusion

At the time of industrial revolution, human population has risen at an enormous rate, faster than ever before. To comply with the needs, cities have expended drastically. Consequence of this expansion, such as massive land utilization and uncontrolled massive raw material consumption, the balance of the biosphere has been disrupted. In addition to that, production techniques that create piles of wastes, use unsustainable materials, as well as transportation and construction of these, resulted in massive amount of greenhouse emission releases. Currently, construction industry become one of the main actors behind the global warming. Solutions to these problems are already existent in nature. Nature has always been an inspiration source for the designers. However, true potential of the nature's wisdom has been overlooked. Today's advancements brought new opportunities to analyse and utilize the ages of knowledge biology bares. New multi-disciplinary research areas have already been developing, as to learn how to use and adapt these principles in our technology. The knowledge nature bares within, is a result of limitless trials and errors that resulted in advanced mechanisms to maintain the living. As pointed out by Janine Benyus; biomimicry is an effective tool to understand design principles. Yet, generally it is seen as ideology to look nature as a source of knowledge. However, addressing only the methods would limit the true potential of studying the living organisms. Why mimic their processes, when they can be translated into a design and production tool by these organisms in a collaborative way? Current urbanization alienates all other living being but humans. However, from the dawn of existence a balanced coordination with different species, a harmony, has been an issue to maintain this existence. To fix that, more than understanding of the biology of living organisms, a design method that allows mutual formation would be an effective solution. Biodesign is a multi-disciplinary approach addressing design in collaboration with organisms on interface of biology and design. Adaptation of such methodology in architectural design to create novel approaches such as bio-architecture, have been questioned in a manner that responds regeneratively to the environment. Such design methodologies would advance the known architecture into the new era. To comply with the needs of today's advancements many disciplines have to redefine themselves and must unify and collaborate with other disciplines in order to survive these fast changing features of progress.

Conventional materials and methods employed today already revealed their harmful faces. A design has mastered in this period by thinking in a way that banishes all the other organisms. However, results became obviously catastrophic nowadays. In order to strengthen our bounds and increase resilience of our civilization through advancement new approaches are nourishing in unity.

Collaboration with living organisms is a novel topic addressing such unity today. In cross section of biology, bioengineering and architecture limitless opportunities await. Mycelium in this case shows a great potential to replace many conventional unsustainable elements in architecture and is an example of an active design tool of using organism to fabricate. Labouring organisms to weave bio-polymers as a part of their life cycle would result in complete unity between design and the nature. In this research, mycelium as a biodesign collaborator in architecture has been examined. To understand the colonization dynamics and behaviour during the production series of experiments have been conducted by the Author.

Literature survey showed that, there are several examples of mycelium as a design tool or a material. However, most of these studies are missing important information with regard to repeatability and basic information such as strain, substrate and environmental conditions. In order to form a reliable source for the literature, all stages of the experimental observation and findings have been discussed and explained in detail. The findings of the experimental stages have been used as an opportunity to improve the production method and outline potential future scenarios. Each stage of the experiments has been designed in steps of myco-composite production, enhanced by fabrics. Literature survey shows, mycelium materials are good in compression, but weak in tension. Hence to enhance structural properties of mycelium-based materials enhancement by fabric layer has been studied. Firstly, a preliminary stage of experiments was conducted in order to understand the requirements of the process and determine the test structure for the next stage. Following that first stage of main experiments has been conducted as an elimination process to design the next stage of the study. In this stage, specific combinations of substrates and fabrics have been examined in order to achieve a textile-reinforced myco-composite with improved structural properties, against tensile forces. Results of the compression and bending tests have been used to determine the best combination to continue testing. Hemp fabric with cardboard waste performed as the best candidate in this case. Second stage of the main experiments has been designed in order to optimize production conditions of the chosen combination. In order to begin the optimization, structural properties under different colonization periods and by different amounts of nourishment supply, were examined. Even though results are not giving specific indications, the obtained information remains valuable with better performing samples and mould design.

According to the findings of these experiments a new method to produce a myco-composite in form of a cylindrical beam has been proposed. This “myco-beam” will easily be manufactured by the aid of machinery and newly produced myco-beam will be available to be bent or manipulated before the start of solidification. To express the potential of this methodology a contemporary park design made by bending or cutting these beams in desired shape has been proposed. In general, the application areas and structural potential of mycelium, as a biodesign collaborator in architecture, have been studied and described. Author attempted to provide details of the overall process to encourage the public to study mycelium as replacement for unsustainable materials and methodologies on the market.

To continue the search for biodesign and using mycelium as a biodesign tool; several study scenarios have been proposed by the author, as continuity of this study.

## 5.2. Future works

### *5.2.1 Short term goals*

Shortening the incubation time while achieving better structural performance is one of the first issues to address further with regard to the work conducted herein. Different incubation periods and the resulting incubation times, will be assessed in future experiments. Incubation time is one of the essential decisions to be made according to the mushroom type, whereas speeding up of the process is a necessity for industrialization of mycelium-based products. Therefore, further research on coherence of different substrates and mushroom types is necessary. According to their findings, Attias at al. (2020) suggest that short incubation periods would restrict organic matter digestion. On the other hand, keeping incubation period longer would cause higher degree of digestion and alterations to lignocellulose content. According to this information, tests over different growth periods on the selected combinations of substrate and mushroom type must be conducted, in order to find out the best structural properties achieved by the conjoin levels between the substrate and hyphae. Depending on the substrate or fungus type these parameters may change. Hence there is another short-term goal – defining the best candidate of fungal strains with combination of suitable substrates responding to the different requirements of materials in architectural design, to define different elements of a structure. Future experiments will also include quality characterization by mechanical property testing under various temperature and humidity conditions. Homogenization of organic substrate is one other important issue to be examined prior to further experimentation. After these steps, optimization of experiments will be conducted.

In a study by Jiang at. al., a manufacturing cost model has been created that includes all labour, material and overhead costs on an example mycelium-based biocomposite, sandwich structured production process by a commercial firm (Jiang et al. 2016). In this case changes in machine processing times showed that the solution found is relatively robust. However, an optimization of cost is another goal planned in short term to create an application to market.

Production of proposed myco-beam and required machinery to craft these beams will be installed by the author. A pavilion design, according to these findings, is also planned in reference of designs proposed in this thesis. Meanwhile new methods to form, colonize and design mycelium will be researched further.

### *5.2.1 Long term visions*

Within the scope of future goals, experimental structure, as that suggested by Adamatzky et al. (2020), will be placed and potential methods to lead mycelium formation will be questioned. If designed accordingly, there are possible future scenarios which consider constructing mushroom buildings, such as colossal bio-computer dwellings. This might still seem far from reality, however further progress in studying such living organisms will open the path of multifunctioning and self-responding structures, sustaining its life cycle as a part of its environment. Scenarios which allow for the use of live mycelium will be addressed as the main objective of future studies. To widen the collaboration, new experiments to identify other organisms working symbiotically in this production will be researched. Fungi can be both parasitic and symbiotic.

This integration is an evolutionary step that architecture needs. Harm brought by conventional modern methods and aggression is an obvious topic of discussion. The conventional materials should be urgently and radically revaluated.

There are almost limitless opportunities, branching on the intersection of Architecture and Mycology, to create a fungal architecture; an alternative future environment, shaped by living organisms, in order to achieve complete harmony with the surroundings. These constructions collect sunshine, decompose our wastes, as its major nutrient source. They produce food and even collect water via specialized gills. Your house grows your food, collects water for you, keeps you safe and warm, and may even provide light. The concept of inorganic housing, which remains idle and dead for centuries, can instead be replaced with a living organism, that interacts, as we instinctively desire today. A living architecture may become an interactive companion in the future of humanity.

Nature always finds a way, but what if we ourselves are the way that nature found to cure our harm to this balance on this occasion? As Frei Otto said; **“The majority of architects don’t understand that there are infinite possibilities for architecture in future, there are no limits.” (Songel, 2004).** Further research on this healing process and regenerative potential of **Biodesign** and **Bio-architecture** will be studied.

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