Living with cacophony

The effect and potential of acoustic environments on the growth of mycelium.

Master Thesis
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INDEX

CHAPTER 1
PROJECT OVERVIEW

I will adopt a material–driven design approach which entails tinkering with materials next to systematic studies for technical and experimental characterization.

CONTENTS

GOAL

The goal of this chapter is to provide an overview of the project, get a definition of the scope and identify the overall structure and method used during the discourse of this project.

- Identifying the relevance of this project with regards to growing design and determining the overall goal of this project.
- Determining the general structure that is used for this project by identifying the different phases and activities.
- Defining the scope with the main fields of research.
- Describing the methodology that will be used as a framework to guide the direction of this project and maintain a focussed scope.

METHODS

- Literature review

Abstract

The potential and development of mycelium based materials has been increasing over the last years. This material is proving to be a green alternative for a variety of commodity materials like plastics, wood, leather, etc because of its ability to upcycle waste streams. A lot of existing research performed is focussed on the environmental factors required for mycelium cultivation and don't look beyond these minimal environmental conditions. This graduation project contributes to the development of mycelium based materials by looking beyond the realm of these growing requirements.

In this research the effect and potential of acoustic environments on the growth of mycelium is explored according to the Material Driven Design Method by Karana et al. (2015). The goal of this project is to provide first insight into the relation between the acoustic environments and mycelial growth. First by looking into physiology of mycelium and the required growing conditions needed for effective growth of the material. Additionally the physics of sound is explored to get an understanding of the factors that define an acoustic environment.

Image 1: Agar petri dish colonized by mycelium

A setup was created to host the required environmental conditions needed by designing three modular incubators. These incubators provide a multifunctional growing chamber where different stimuli systems can be placed precisely within while maintaining the required growing conditions for the mycelium and dampen external sounds.

Three different conditions were created for this research, a silence chamber, a mono speaker and a stereo setup to see the response of the mycelium to the different types of stimuli. The response was captured by doing a grayscale analysis to quantitatively analyse the change in growth.

In this research the key findings of the research provided first insights into the negative correlation between this acoustic environment and mycelial growth regarding the growth rate and textural qualities at higher volumes. In addition it provided that this type of sonic stimuli has no effect on the textural qualities and spatial distribution of mycelium.

1.2 Project Brief

This graduation project will contribute to the field of biodesign and more specifically growing design. Growing design incorporates organisms in the design and designers work together with these organisms in order to create a new product or material. By developing this field of design, industrial and mechanical processes could be replaced with biological processes which could offer new ways for cleaner production (Camere and Karana, 2018). This project bolong to growing design because it gets involved in and tinkers with the growing process of the fungal mycelium.

PROJECT OVERVIEW

This graduation project will focus on making initial explorations of the acoustic-mycelium relationships. For that, I will adopt a material-driven design approach (Karana et al., 2015). First I will obtain theoretical and practical knowledge about fungi and sound in order to design and build a suitable test environment.

In the second phase of this project I will do a set of systematic studies to enhance the organism's performance during the growth, which would eventually offer new ways of tailoring the material qualities.

AIM OF PROJECT

The aim of this project is to reveal the purpose and quality of the effect of acoustic environments on mycelium that will be explored using the Material Driven Design method. First by creating an acoustic environment for the mycelium to grow in. Secondly by performing experiments to discover new unique performative and experimental material qualities enabled by this acoustic-mycelium relationship.

1.3 Scope

MYCELIUM DESIGN

Mycelium-based composites have the potential to be a green alternative for some of the commodity plastics and wood because they use waste streams as substrate, like coffee husk, sawdust or hemp fibres. The the potentials of this material are being discovered by researching and experimenting on how to improve the material properties. For example, they experiment with the living conditions of the mycelium or apply a diverse drying or forming process when the material is grown. This results in tailor-made composites for innovative and environmentally sensitive design solutions.

SOUND PHYSICS

In this project, I will first look into what sound is in order to understand what elements of sound we could use as a variable in our experiments (e.g., vibration). Therefore I need to gain knowledge about the physics of sound, what defines sound and acoustic environments and what could provide a suitable starting point.

PHYSIOLOGY

In order to see a change in growth, it is important to study the growth parameters and kinetics, these biological processes need to be understood. On a larger scale an understanding of the required conditions for the cultivations of fungi is needed. On a smaller scale knowledge of the biological processes within the fungal hypha and cells is needed. Additionally, knowing the factors that affect the growth of mycelium.

MATERIAL QUALITIES

Materials are the cornerstone of a product and define a lot of experiential qualities. This material driven design project entails tinkering with materials next to systematic studies for technical and experimental characterization for a holistic material understanding and transferring these findings to meaningful applications/ demonstrators.

1.4 Method

The mainframe for this project will be based upon the Material Driven Design method (MDD). This method emphasises understanding the material on a technical and experience level while the outcome has an emphasis on the experience of the material in a broader sense.

During the first step in this design process, the designer is required to tinker with the material in order to discover the material properties like the embodiment possibilities or affordances, this tinkering also stimulates creative thinking. Tinkering is a form of material experimenting, “an explorative process of creation and evaluation” (Karana et. al., 2015). User tests are included to find out how the material is perceived by the end-user.

The next step in this model is to position the material the designer conducts material benchmarking where the strengths and weaknesses of the material are sought and compared to similar existing materials. Here a Material Experience Vision is developed to show the unique role or experience of the material in relation to its user, context and product. All the findings and discoveries of the material qualities made in the previous step are captured in this vision. With this vision, the designer unravels these qualities further and learns how the material will be experienced and interacted with. With these qualities, opportunities are explored within society.

In the last step, all information gathered in the previous three steps will be gathered to create the final material and actual product concepts. Here last adaptations to the material or the concepts can be made as most of the time material and product have a lot of influence on one another. This results in the most viable outcome, product or material, from a technical and interactive perspective. The scope of this project mainly focuses on the first step in understanding the material. Yet, the activities and research will not focus on tinkering with the material but on maintaining the material. This control phase is one step further after the tinkering fase. The goal is to capture the material manufacturing mechanisms, in other words, creating a controlled environment in order to manufacture the tailor made mycelium material.

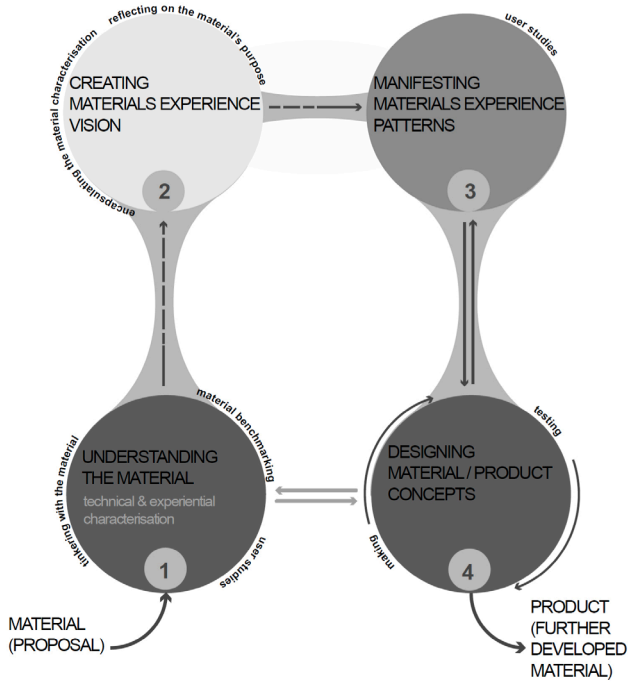
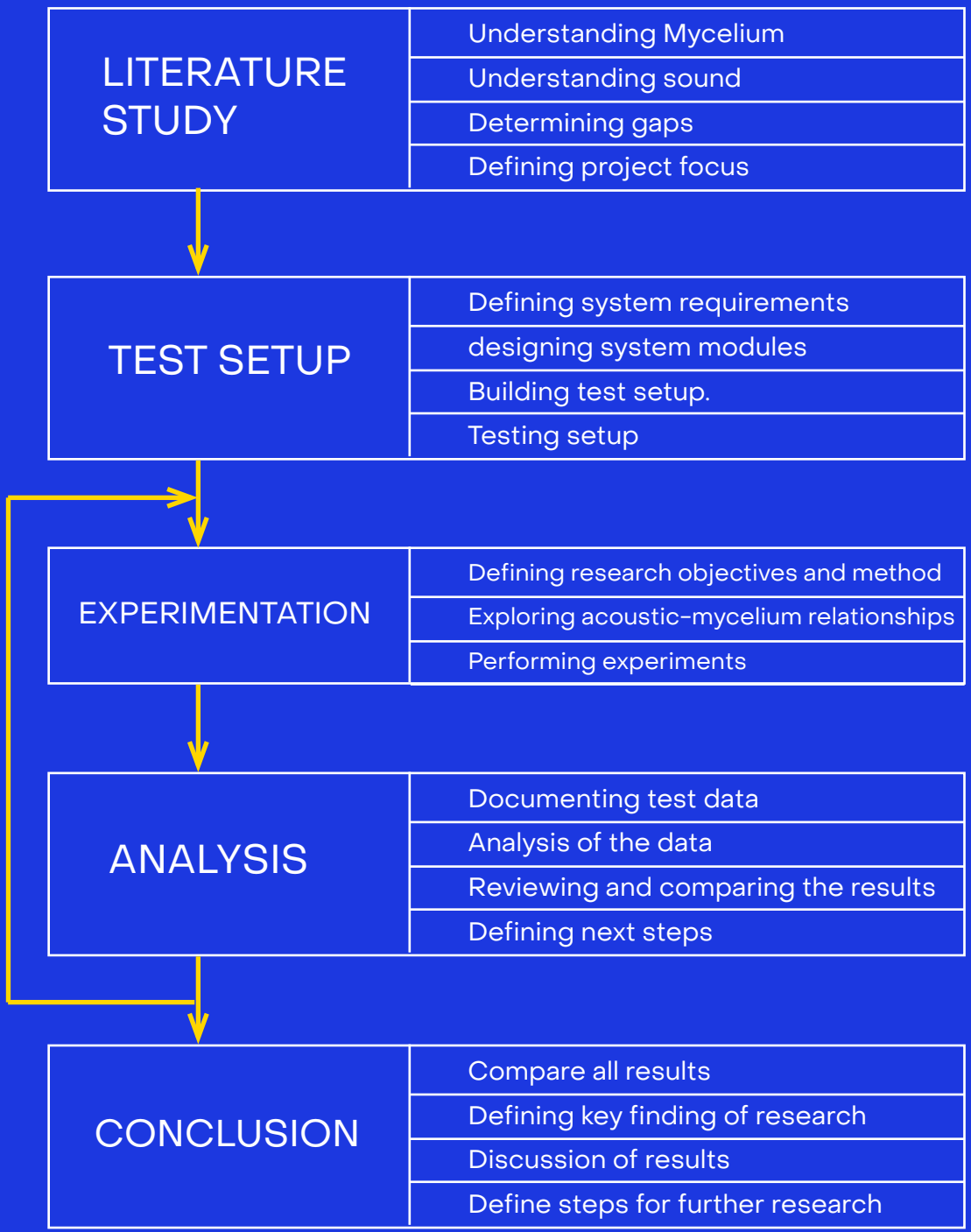


Image 2: Graphical representation of the Material Driven Design Method (Elvin Karana)

Research structure



CHAPTER 2 UNDERSTANDING MYCELIUM

Designers, artists, researchers and companies are discovering the potentials of this "new" material by trying to improve the material properties resulting in tailor-made mycelia for innovative design solutions.

CONTENTS

GOAL

The goal of this chapter is to get an understanding of the fungal organisms on a biological level but on the other hand the use and challenges of mycelium based materials. An understanding for a suitable growing setup is obtained and finally the direction is specified by determining the gaps in the current literature.

- Obtaining an understanding of fungal organisms describing their purpose in nature.
- Describing the physiology of growing hyphae, what biological processes play a role in the growth of mycelium.
- Determining the types of application of mycelium in mycelium based materials.
- Gathering information the different factors that have an effect on the growth of mycelium based materials.
- Defining the gaps in the current literature and showing design or material opportunities.

METHODS

- Literature review
- Information gathering of lab experts
- Interviews with mycelium experts (Wasabii Ng)

2.1 Mycelium

Mycelium is the vegetative part of fungi made up of a mass of hyphae. Hyphae are the long, branching filamentous structures of fungi that act as growth agents. The primary role of mycelium in nature is to decompose organic waste: with enzymes secreted from hyphae, mycelium breaks down the biopolymers to simpler bodies and then digest carbon based nutrients (Moore, 2020). The mycelium consists of a network of long thread-like fibres called hyphae. According to estimations, there are 1.5 million different species of fungi three times the amount of plant species (Hawksworth, 2001). All these different species have great differences, for example yeast is a unicellular fungi very different from an oyster mushroom. Because of this great veriety in biological differences the fungi is seen as its own kingdom.

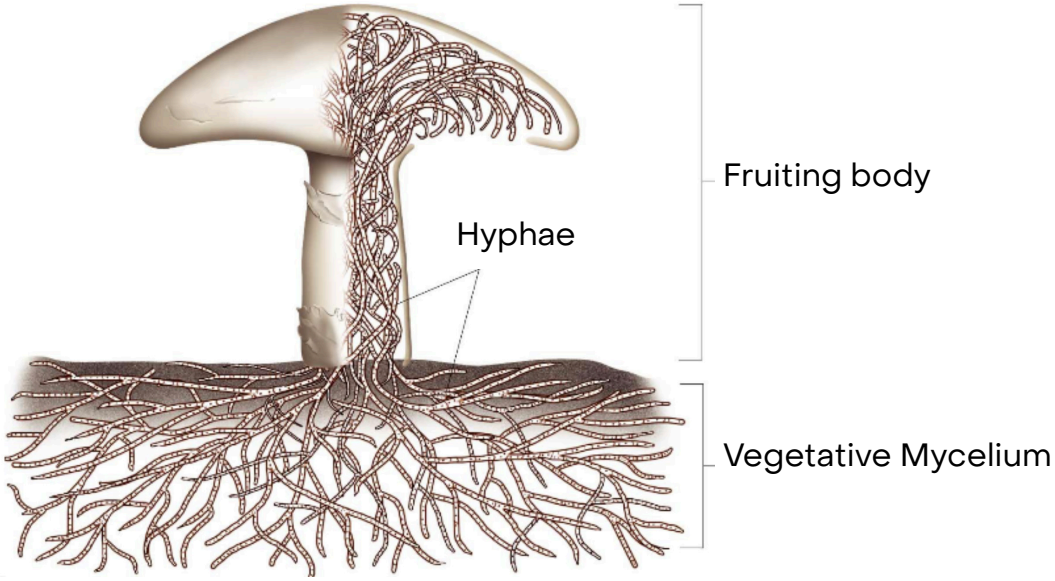


Image 3: Structure of a Typical Fungus Fruiting body Hyphae Mycelium (chegg.com)

Although it takes a while for the mycelium to grow, mycelium allows a fungus to occupy an enormously large area. A fungus, with mycelium, that lives in the Blue Mountains in Oregon USA occupies ten square kilometres, this makes it the largest organism in the world.

One of the most vital roles of fungi is to decompose organic material into inorganic materials (minerals like phosphate). These compounds will then be reused as nutritions for the current tries. They can even eradicate pollutants as petroleum and pesticides as they are also organic molecules.

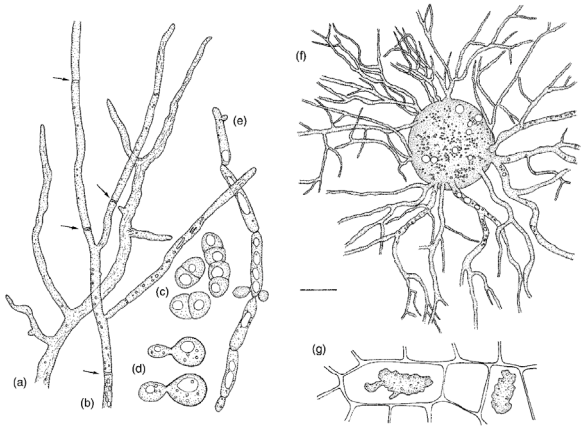


Image 4: Illustrations of different types of mycelium structures. (Introduction to Fungi by John Webste)

Top 5: Mycelium hyphae colonizing the sorgum substrate
Left 6: Mycelium hyphae growing on sorgum substrate
Right 7: Mycelium hyphae growing on agar plate

2.2 Physiology of growing hyphae

In this research, the goal is to see a change in growth. In order to change this growth, it is important to study the growth parameters and kinetics on a macro and cell level. First, the macro growth kinetics will be discussed and subsequently the cell biology. The fungi strain that will be studied is filamentous fungi, which means that they will grow by extending their apices while branching subapically which results in a network of roots called mycelium. Hyphae of lower fungi like Chytridiomycota are little to not at all septated (Peer, 2008). Fungi of a higher order like schizophyllum commune or Trametes hirsuta grow by means of filamentous extension just like many other organisms. Filament extension happens when biomass gets transported to the tip over the length of the hyphae, as long as food can be gathered tip extension continues. The rate of tip extension can be extremely rapid, up to 40 micrometres per minute (Boogert, 1994).

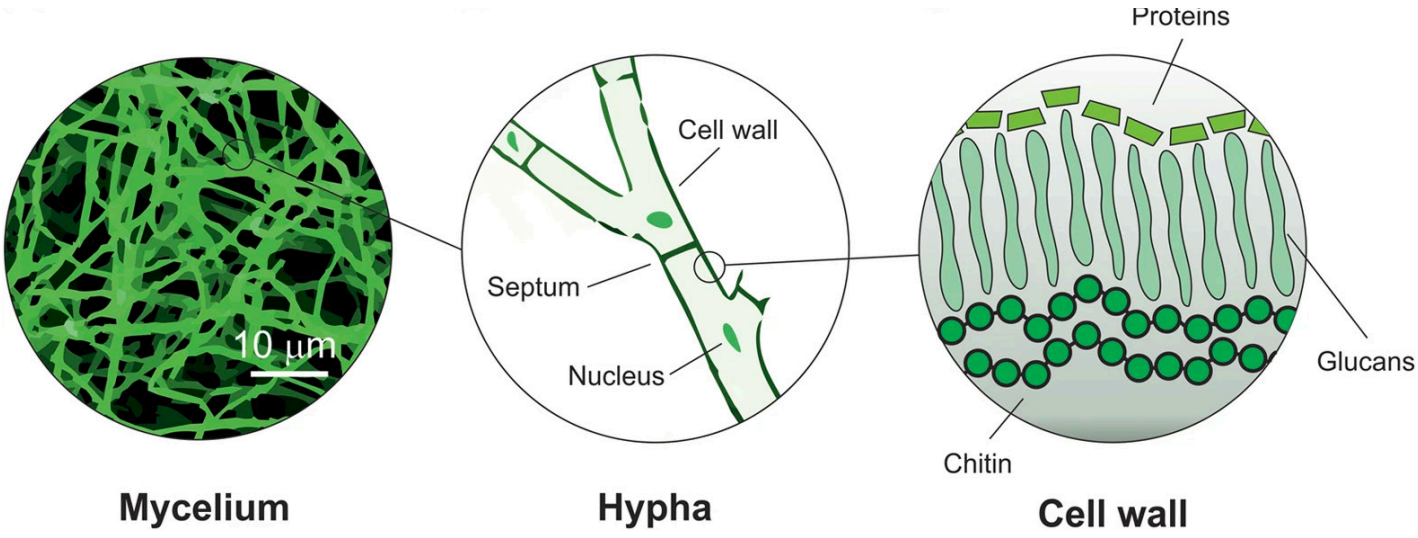


Image 8: (Left) a microscopic image of mycelium hyphae (Middle) Schematic representation of a hypha formed by cells seperated by septa (Right) Schematic representation of the cell wall with a chitin layer on top of the cell membrane and a layer of glucans. (Vega, K. & Kalkum, M.)

The obvious pattern characteristic is to find nutrients by rapidly exploring the habitat with hyphae. When a nutrition source is located extension rate outward declines and the branching rate increases. When the source is depleted it migrates to find new sources. This happens on a microscopic scale with microscopic nutrient sources and on a larger scale by extending to a different part of the landscape by means of hyphae to spores (Moore, 2020).

The extension of the tip is commonly referred to as hyphal tip growth. This is established through apical growth which is happening over the full length of the hyphae (Read, 2011). Apical growth is when the inside of the hyphae gets pushed outwards. This enables the mycelium to propagate itself and explore the habitat around itself. A lot of research has been done that confirms that this apical growth of the hyphae 'the key to the fungal hypha lies in the apex' (Robertson, 1965), yet the mechanisms that determine hyphal polarity are still not well known (Webster, 2007).

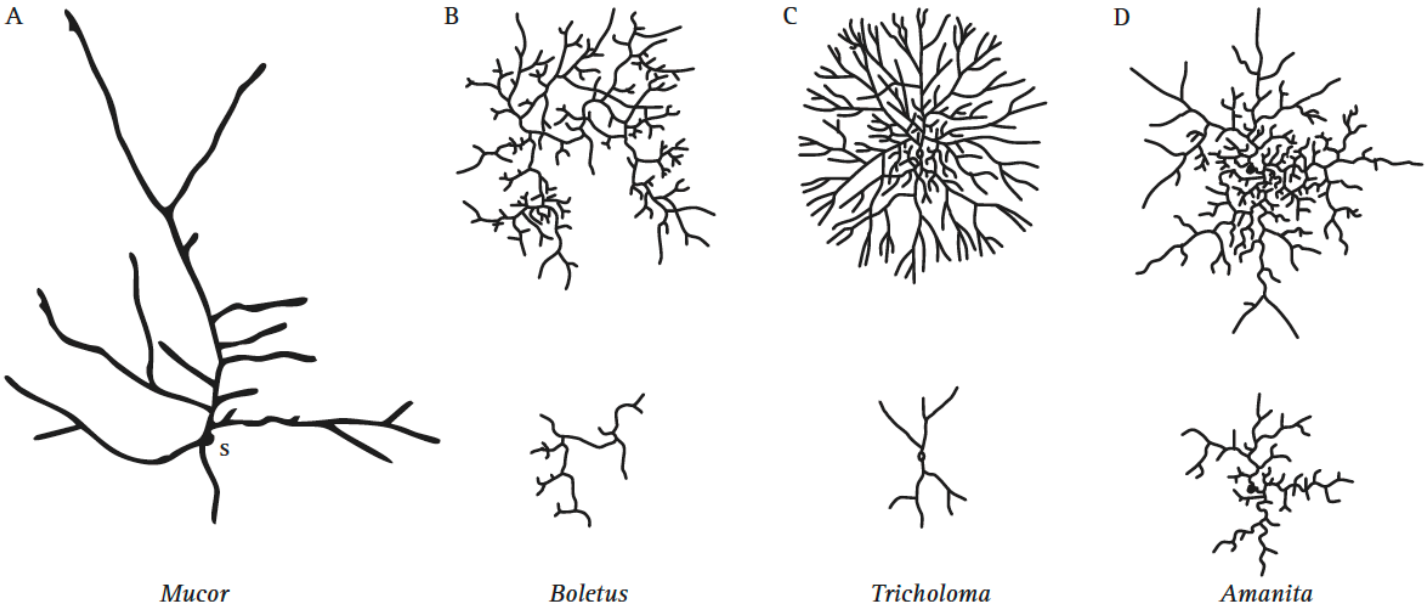


Image 9: Graphical representation of different types of mycelium propogation and networks (Australian National Botanic Gardens)

When mycelium gets older hyphen fusions occur very frequently at the centre of the colony, hyphal avoidance becomes less. According to Moore in the 21st Century Guidebook to Fungi (2020) the main growth processes that influence the distribution of hyphae in a mycelium are:

- **Polarized hyphal growth**
- **Branching frequency**
- **Autotropism (the 'self-avoidance reaction that makes vegetative hyphae grow away from the already-existing mycelium).**

Clearly, a strong relationship between hyphal extension rate, branch initiation and growth rate is evident. In this research the understanding of growth is important, thereby it is essential to make a distinction between growth and extension. Growth is the production of biomass that occurs along the complete length of the hyphae. The extension only occurs at the tip of the hyphae, therefore we only speak of hyphal tip extension instead of hyphal tip growth. Different strains and growing conditions have a different relationship between tip extension and growth, this growth could also be influenced by sound.

2.3 Mycelium & Design

GROWING DESIGN

One of the biggest challenges is to change our current economy to an eco-friendly and sustainable one. A development towards this future is the research for the use of bio based materials. The rise of these new bio based materials request a new understanding and practise where different fields of expertise intersect like design, arts and crafts, biology and material science where the designers role is changed from a passive recipient to an active maker of the material (Karana et al., 2015). This is called growing design which refers to the material that grows and makes the material. This growth is seen as a material property and is taken into account in the development of new materials and products. In growing design the designers work together with the living organisms they guide their growth by crafting the conditions that create the material or product.

MYCELIUM BASED MATERIALS

Mycelium based material is one of these biobased materials and has the potential to be a green alternative for several existing materials because it is made with sustainable resources and is fully biodegradable. Designers, artists, researchers and companies are discovering the potentials of this 'new' material by discovering how to improve the material properties of mycelium resulting in tailor-made mycelia for innovative design solutions and new concepts of sustainable materials and products. More interest is being drawn to this green alternative, there are several products being marketed in the EU and USA for construction and commerce purposes. Mycelium could eventually replace materials like synthetic foams, timber and plastics.

Mycelium based materials can be split in two categories where both categories are defined by manufacturing method. First is pure mycelium material where the mycelium grows in a liquid medium or on top of a solid medium, this can result in a pure mycelium sheets or foam. The other method is by growing it in a substrate. This is what is called a mycelium composite (Karana, 2018). The substrate is cemented by the mycelium partially replacing the substrate with tenacious biomass. It is also possible to get pure mycelium by growing it in a substrate and removing the skin that has formed on the top of the substrate (Appels et al.,2019). Mycelium based composites are made by using the whole colonised substrate material which is dried or heated to stop further colonization of the mycelium. Drying will make the mycelium hibernate so that it can grow further after the growing conditions are met again where heating will kill the mycelium and stop the growth permanently.

Several studies show that mycelium based materials show some challenges for product development. They have a relatively low density when comparing to plastics, a relatively low compressive strength when compared to conventional materials used in masonry

construction, a lack of tensile strength and a strong dependency for curing process for durability (Appels et al., 2019; Yang et al., 2017; Attias et al., 2017; Haneef et al., 2017; Islam et al., 2017; Jones et al., 2017).

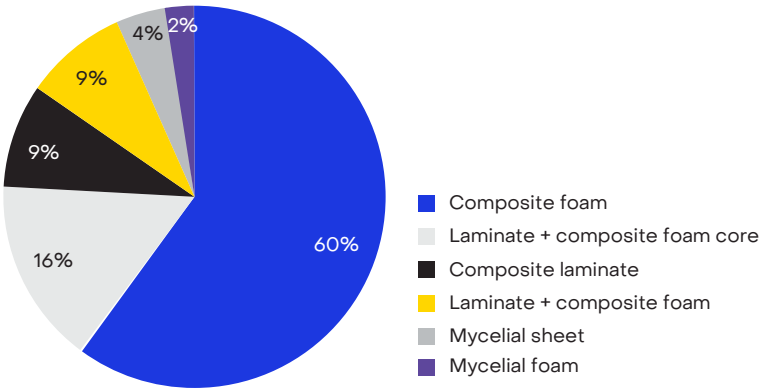


Image 10: Market share of the different mycelium based materials.

A comparative study performed by Attias et al. (2020, p. 119037) showed the common mycelium production techniques and their contribution to the overall production of mycelium based materials described in the literature. This can be seen in image 10.

2.4 Desinging with mycelium

A growing body of research dives into the manufacturing process of these new mycelium based materials by investigating the many factors that influence the growth of mycelium and conclude with the material properties and performance of the mycelium based composites. These factors are among others are: strains of fungi used for inoculation, the substrate type, environmental conditions during growth (i.e. humidity, temperature, supplementation), and forming and processing techniques (Heisel et al., 2017; Yang et al., 2017; Appels et al., 2019; Karana et al., 2018; Girometta et al., 2019; Daver, & John, 2017).

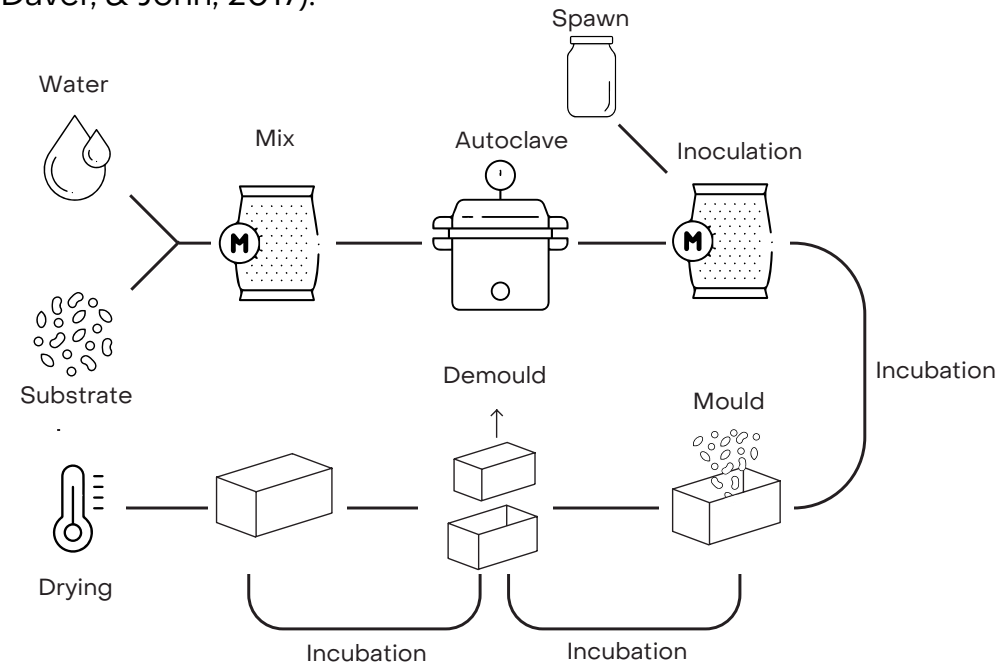


Image 11: Diagram of the general production procedure for mycelium composites

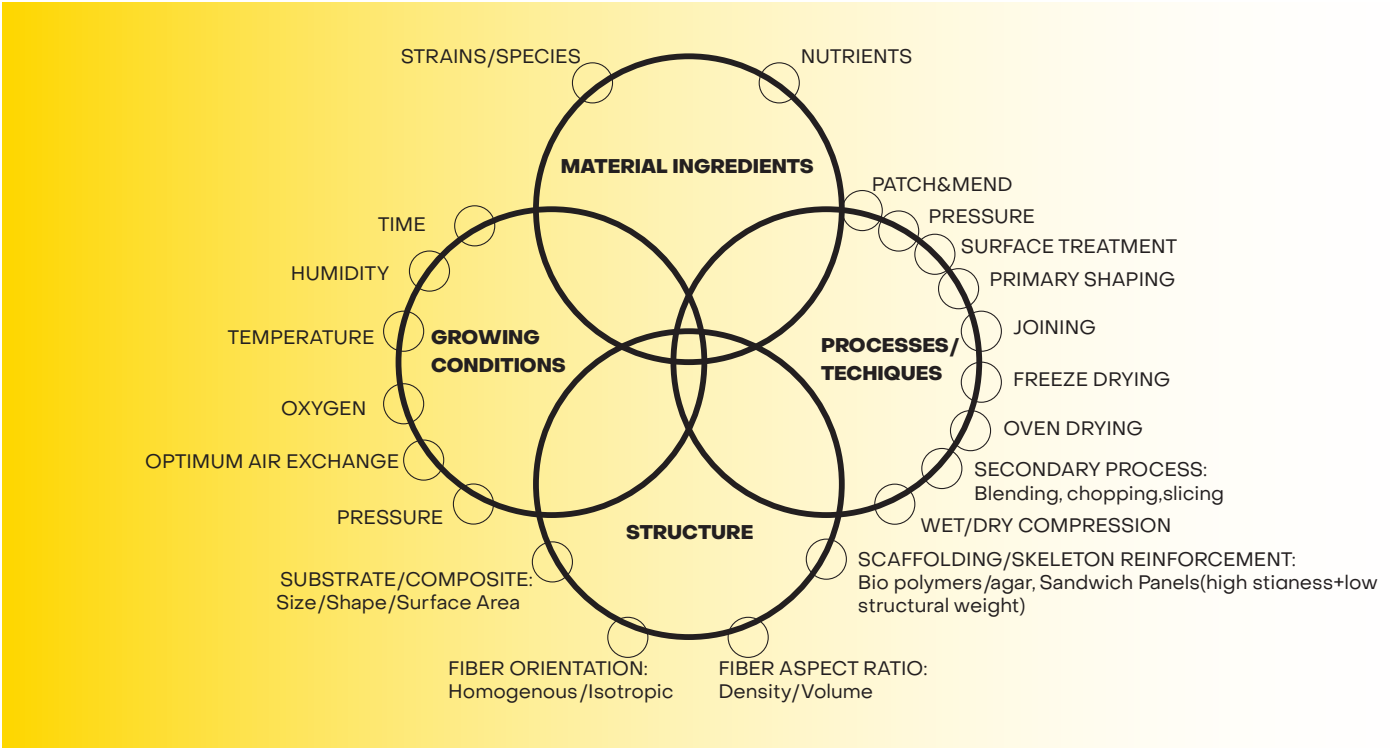


Image 12: Taxonomy of material based composites (Wasabii Ng)

GROWING REQUIREMENTS

When designing with mycelium based materials it is important to work sterile to prevent contamination and therefore the growth of other organisms (Carlile et al., 1994; Jones et al., 2017) Mycelium based materials need a controlled environment to host and stimulate the growth of the mycelium. Controlled environmental conditions for stable growth are light temperature and humidity. A complete overview of production variables for 'commodity' mycelium materials, which are known mycelium strains that have been used in research and development for mycelium based materials, are visually summarized in the taxonomy (Karana et al., 2018) see image 12.

Humidity

Next to temperature humidity has the most significant effect on the growth of mycelium according to (Yuang, 2010). If the humidity in the incubator is too low the mycelium will grow slower or even dry out below 60% which will kill the mycelium. If the humidity is too high contaminations happen more easily. For optimal conditions, a humidity of 60-75% should be maintained according to Karana et al., 2018. The moisture needed for the relative humidity is commonly added to the substrate so that the substrate has the appropriate relative humidity already in it. The incubator relative humidity prevents the mycelium from dehydration.

Temperature

Though mycelium can survive temperatures below zero and dies when the temperature is higher than 60°C. The temperature is one of the biggest influences on the rate of growth. Although it grows at a range of temperatures mycelium needs a specific temperature to thrive in. This is between 25-35 °C, when the mycelium is kept in that temperature range the growth is the highest (Karana, 2018).

When the ambient temperature reaches around 4 °C the mycelium growth is influenced in an inhibitory way so that the mycelium can be stored for up to one year under these conditions (Turković, 2015, p. 56).

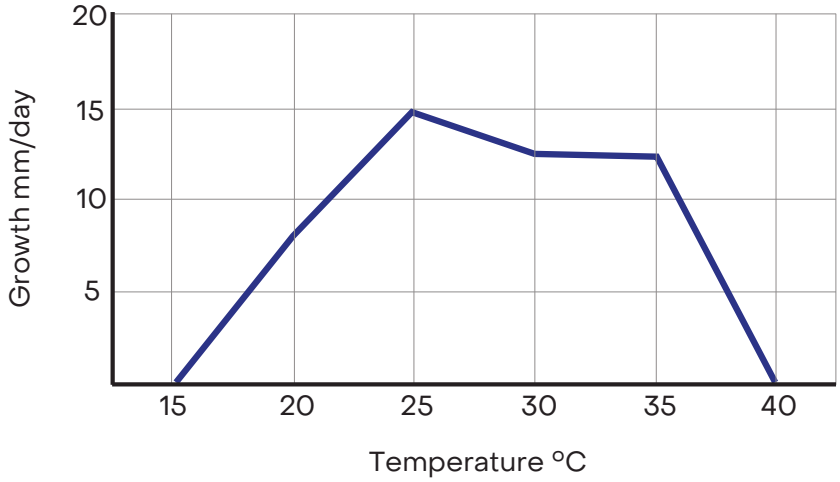


Image 13: Temperature to growth rate relation for mycelium.

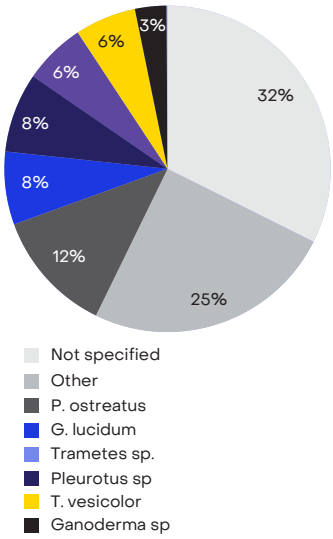
Time

The time is determined by the size of the product and the processing method. But, it is also dependent on the medium and growing conditions. But in general, complete colonization takes one week. Then the product can be remolded and grown for another week. Dependent on the end product and size the growth time will be chosen.

Strain

As discussed there are over three times more fungal strains than plant species. Many of these strains still have to be discovered. The strains mostly used for mycelium composites are Ganoderma lucidum Ganoderma R, Trametes hirsuta and Pleurotus ostreatus (Tacer-Caba et al., 2020) Their features like hyphal architecture, fungal cell wall composition, composite constituents and other growth kinetics are very suitable for making mycelium composites. Additionally, they thrive in easy to achieve circumstances and have a white colour to them. Additionally, these strains are available at the MI Lab and most research is done with these strains and therefore a lot of experience and knowledge has been gained about growing these cultures.

Image 14: Temperature to growth rate relation for mycelium.



Substrate

One way to cultivate mycelium is to grow it in a liquid medium or on a agar plate, the mycelium takes up the nutrients from the medium.

Substrate

One way to cultivate mycelium is to grow it in a liquid medium or on a agar plate, the mycelium takes up the nutrients from the medium. A different approach is to grow it in a substrate material resulting in a mycelium based composite. This growth method can upcycle various compostable agricultural waste streams used like sawdust, hemp chives and flax fibres or even city waste streams like coffee husk, recell, cacao shells, etc.

A lot of comparative research has been done on what substrates and what medium is best for the mycelium to grow on. For instance, when growing Pleurotus ostreatus mycelium on cellulose the end product is stiffer when in comparison with Ganoderma lucidum. With the addition of dextrose to the cellulose-based substrate both, fungal materials become more elastic (Haneef et al., 2017). The substrate has a lot of influence on the end product in terms of appearance and mechanical properties. The feeding substrate has different concentrations of polysaccharides, lipids, proteins and chitin. These differences are reflected in alterations of the morphology and mechanical properties of the final mycelium material (Haneef et al., 2017b).

This research will use a substrate material that is known to be a good facilitator for commodity mycelium strains. In this way when a result is found it applies immediately to mycelium composite products. According to Wasabii Ng mycelium strains described above grow very well on sorghum which is commonly used as spawn because of these properties. The high sugar contents in the sorghum provide enough nutrients for the mycelium to grow on.

Image 15: Prepared substrates in petri dishes

Image 16: Measuring the required substrate material in growing container.

Mixture

For optimal growth the right mixture of substrate, water and spawn is needed. The correct mixture is adopted from Tudryn et al. (2017).

60% water
35% substrate
5% spawn

This substrate mixture does not apply to sorghum, for sorghum a different procedure is used. The sorghum is soaked for 24 hours in water, then the sorghum is strained and is ready for autoclave. The 5% spawn is calculated from the soaked sorghum grains (Tudryn et al., 2017, p. 1481).

Skin Forming

This process let the “hyphae grow out of the substrate into the air creating a fluffy or compact layer covering the substrate”, called ‘fungal skin’ (Appels et al., 2019).

PRODUCTION TECHNIQUES

Many production techniques are discussed and evaluated in comparative studies with each technique having its own benefit. The main advantage of mycelium composite is that it can be shaped and moulded in a desirable shape. Joining utilises the growing feature of the mycelium in a way that it can heal or join other structures. When the mycelium composite is grown for a longer time it will form a layer on the outside of the composite called the skin (Jones et al., 2017, p. 246). This skin can be cultivated on its own to create a pure mycelium product like Mycoflex developed by ecovative. According to (Yang, Zhang, Still, White, & Amstislavski, 2017) the skin also improves the compressive strength and the water repellence of a mycelium composite. Additionally the skin forms a uniform layer around the product that will determine the look and feel of the composite.

Post growth the mycelium is dried at 60 degrees to stop the growing process, dry the product and kill the mycelium. After this drying the mycelium composite can be used as it is but there are several processing techniques like hot and cold pressing that change the mechanical properties and appearance of the composite. When pressed the mycelium composite resembles a woodlike or MDF structure. These post growth production techniques are out of scope of this project as the focus lays on the growth of mycelium.

MYCELIUM BASED MATERIAL APPLICATIONS

Mycelium has been used for a long time in the medical industry for the production of enzymes, antibiotics or organic acids (Wosten, 2019). The first explorations into mycelium based materials dates from the 1980's where a japanese scientist Shigeru Yamanaka who experimented with mycelium to produce paper and building materials (Girometta, 2018). Much later Stamets (2005) discovered and presented the environmental benefits of mycelium by decomposing toxic environments. Due to the environmental pressures not much later further exploration of mycelium composites done by Ecovative to explore mycelium based material as a sustainable alternative for commodity plastics and building materials.

Since then many designers, scientists and artists have been exploring the potential. These are among others, packaging materials, synthetic leather, foam, insulation materials, construction materials. A patent review by Cerimi et al. (2019) uncovered the main industries suitable for mycelium based material applications by comparing the patent requests concerning mycelium. Firstly emerged in the art sector but now concepts and products are being created in other industries as well, the most patent requests were in the following industries: automotive, construction, electrical circuit board, textile industry among others. All industries and applications for mycelium materials are growing significantly. Not only the mechanical and sustainable benefits show an advantage

for mycelium materials but their sensory properties can also present a benefit of mycelium based materials. For example a pure mycelium based leather made by MycoWorks has recently been incorporated in a bag in a collaboration with Hermes, an exclusive designer brand known for their high quality leather products. The bag is partly made out of mycelium as seen in image 19 Ecovative was the first startup that has been industrialising the mycelium based products, has been developing several materials suitable for packaging or clothing and others. They created a foam-like material Mycoflex with a wide range of applications that is very flexible as seen in image 17.

Lightweight mycelium composites enabled designers to develop furniture by cultivating mycelium. Examples of this are Erik Klarenbeek and Phil Ross who created chairs that can be seen in image 18. A very recent Delft based startup called loop creates living coffins made out of mycelium composite image 21. This coffin utilises the live properties of mycelium composite where the body is decomposed by the mycelium. These explorations of the light but strong composite material were followed up by architectural applications made out of mycelium based materials. More and more architectural applications are found because the composites are good sound insulators they are more fire retardant than petroleum based alternatives (Jones et al., 2017). For instance MOGU developed an acoustic panel made out of mycelium composite that can be placed on the wall and ceiling. Mycelium blocks have formed a biodegradable alternative for the construction of small structures. There have been several examples of the application of these blocks in architectural projects for instance: Hy-Fi Tower, the Mycotecture Alpha and the growing pavilion as seen on the Dutch Design Week 2019 image 20.

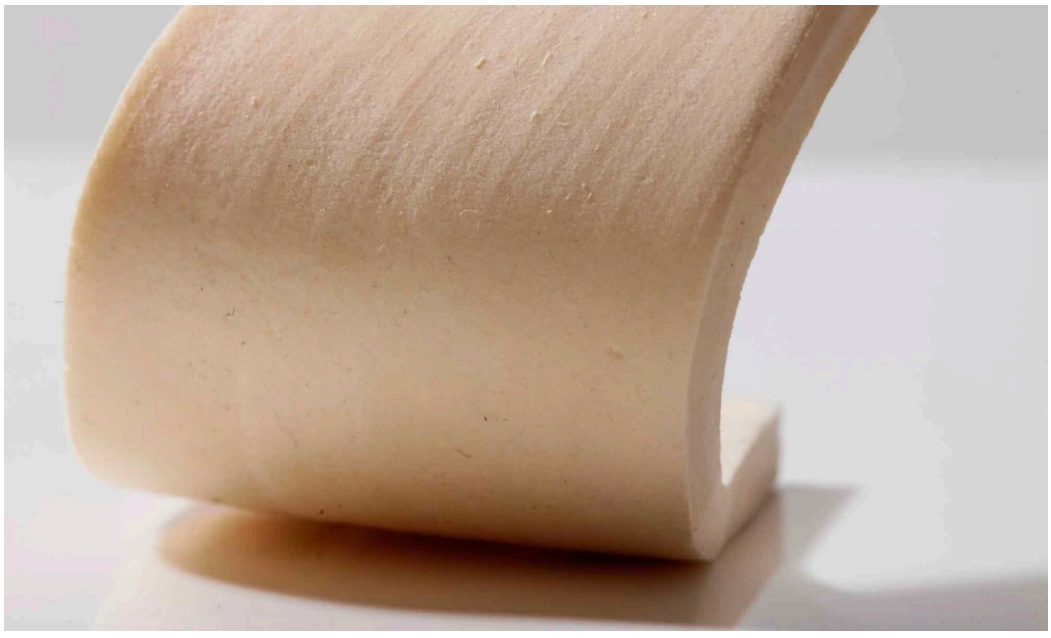


Image 17: Foam like pure mycelium material Mycoflex material (ecovativedesign)

Left page top image 18: Mycelium chair by klarenbeek. Left page left image 19: "leather" bag by Hermes. Left page right image 19: Mycelium composite packaging (Elvin Karana) Right page top image 20: Growing pavillion at the Dutch Design Week. Right page bottom image 21: Mycelium composite coffin by LOOP.


Embassy o
Safety

2.5 Defining gaps

This chapter will indicate and explain the knowledge gaps existing in the current literature concerning mycelium based materials.

INSUFFICIENT KNOWLEDGE OF LIVE MATERIAL TAILORING POSSIBILITIES.

The understanding of the effects and variables of the substrate composition and environmental conditions during the colonization and incubation are essential to obtain the desired material properties. Yet, no studies were found that present a complete overview of the significance of these factors on the material properties of the material. Additionally, no current research investigates outside the boundaries of the known growing conditions of mycelium.

INSUFFICIENT COOPERATIVE RESEARCH DONE BY DESIGNERS AND BIOTECHNOLOGICAL PARTIES

Too little research performed by designers showed the utilization of biotechnological knowledge of mycelium. Thereby the studies often do not utilize the full potential of the mycelium based material. Biotechnological methods and models can provide a great tool for designers to assess the material potential and challenges. On the other hand, many biotechnological research lacks the adaptive and creative attitude that designers and artists have in order to identify application potentials for mycelium based materials.

INSUFFICIENT KNOWLEDGE CONCERNING THE TEXTURAL QUALITIES

Current research mostly concerns the physical/mechanical properties of mycelium based materials. A lot of the suggestions made focus on the mechanical advantages of mycelium like the sound insulation or density in relation to young's modulus (strength). There are examples of the pure mycelium materials that focus on the sensory qualities of mycelium like MycoTex leather. For mycelium composites the sensory quality is not often the focus in material or product development. In order to expand the potential of these materials a comprehensive study regarding the sensory qualities of mycelium is needed. Yet, these sensory qualities are very important for the esthetics of the final material and therefore the product experience. If these qualities can be enhanced the potential for mycelium based materials will increase.

The sensory properties and therefore esthetic qualities of mycelium based materials can be altered with the previously described production processes but all these processes focus on the growing conditions or stimuli that are also related to mycelium in nature. These processes and variables are vital to the growth of mycelium. How do living organisms react to sound and process its information? What if an extra stimuli like sound could change the esthetic qualities of the mycelium material by changing the growth kinetics and morphology? How can we tailor the acoustic environment to influence the experiential qualities of a living material? This research will research the potential and effect of the sound stimuli as part of the growing conditions on mycelium based composites. The sound stimuli could provide more control over and more possibilities for mycelium based materials.

Image 22: Mycelium composite test panels (MOGU)

CHAPTER 3 UNDERSTANDING SOUND

The sine wave is the most basic form of sound and is a perfectly periodic wave that creates a single frequency or a pure tone.

CONTENTS

GOAL

The goal of this chapter is to get an understanding of the physics of sound, defining factors that determine the acoustic environment. Finally the opportunities for the sonic stimuli is determined.

- Obtaining the basic understanding of the physics of sound be dissecting all the variables that are at the foundation of sound
- Understanding acoustics and determining the propagation of sound within an environment.
- Determining the effect mechanics of sound interference.
- Defining the relation between sonic stimuli and the effect on living organisms.
- Describing the direction for the type of stimuli and describing its potential.

METHODS

- Literature review
- Interviews with acoustic experts (Dr. Spagnol S.)

3.1 Physics of sound

In this research we focus on sound as stimuli for the mycelium. Energy will be introduced to the mycelium with sound which is not the same as vibration. Sound is a type of vibration whereast vibration is the intermittent motion of a particle or body. In this experiment it is not the goal to vibrate the mycelium but to stimulate it with pressure waves of the sound wave. More specifically there will be a focus on acoustics and interference patterns. To understand acoustics we first need to understand what sound is. According to the Encyclopædia Britannica the definition of sound is:

"Sound, a mechanical disturbance from a state of equilibrium that propagates through an elastic material medium (gas, liquid or solid). A purely subjective definition of sound is also possible, as that which is perceived by the ear, but such a definition is not particularly illuminating and is unduly restrictive, for it is useful to speak of sounds that cannot be heard by the human ear, such as those that are produced by dog whistles or by sonar equipment."

Sound propagates itself through a medium with longitudinal waves, where the wave occurs in the same direction as the movement of the wave. In the image below you see a mechanical equilibrium at A (absence of sound), B represents a longitudinal wave where you see the areas of high pressure and areas of refractions. C shows a transverse representation of sound, this is how sound often is represented where you can see the amplitude (A) and wavelength (**λ**).

WAVELENGTH PERIOD AND FREQUENCY

The wave seen at C is a representation of a sound made up of a single frequency. The sound made up from one frequency is considered a pure sound, all sounds can be made up of these frequencies. The wavelength (**λ**) is the distance where the wave repeats itself through space. When this wavelength moves through space it takes time for the whole wave to pass a specific point in space. This time is Called period (T). Frequency (f) is the number of wavelengths that can pass through per second and is usually measured in Hertz. (Berg, 2020) The higher the frequency the higher pitched the sound. The healthy, young auditory system can detect tones in quiet with frequencies ranging from approximately 20 to 20000 Hz. (Dobie, 2004) Soundwaves exist in different shapes and sizes, for instance a single frequency exists of a sinus wave, saw tooth or boxed. The

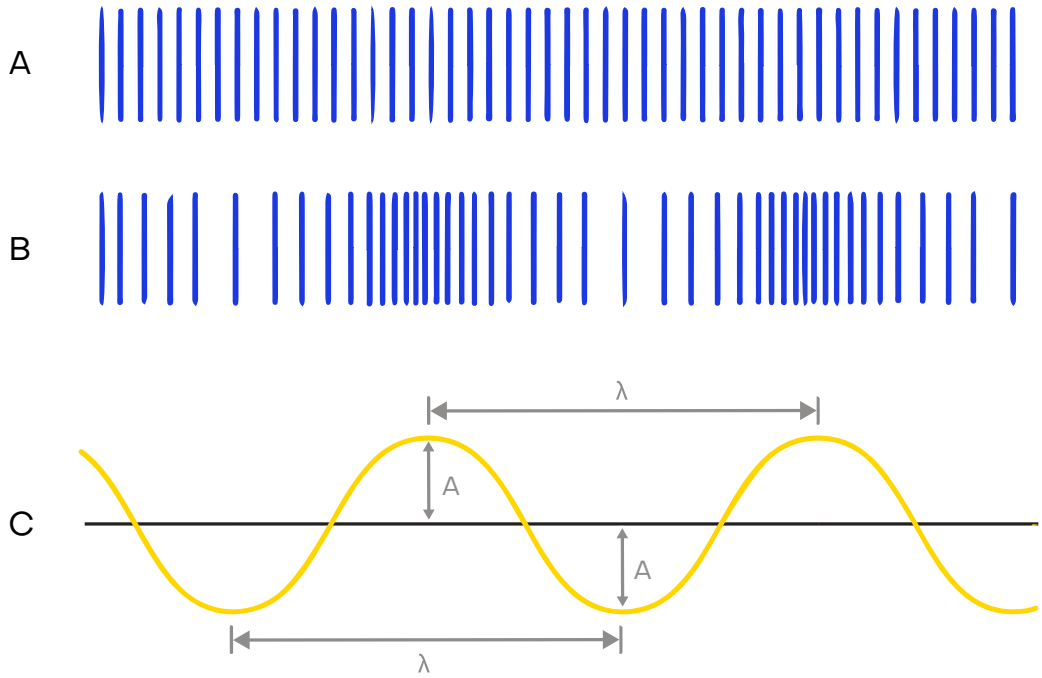


Image 23: Graphical representation of sound waves and its dimensions.

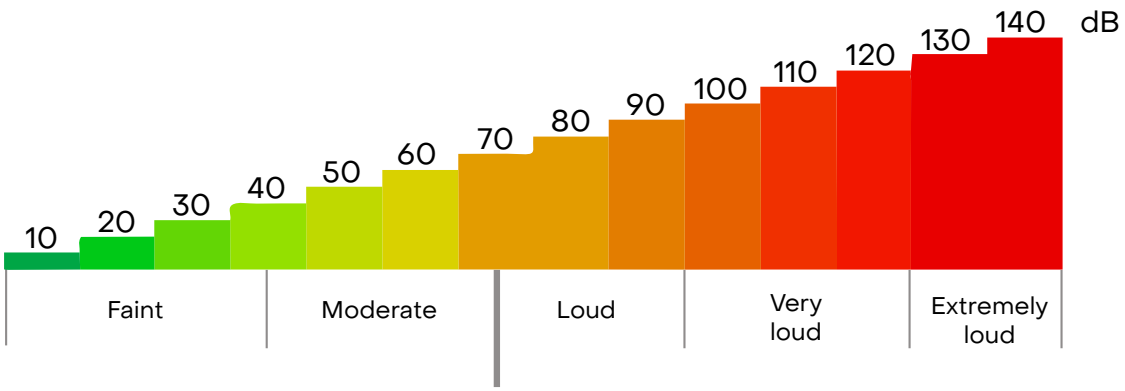
AMPLITUDE AND INTENSITY

In C an equilibrium of pressure can be seen all air particles represented by the lines are equally spaced throughout the space. The equilibrium value of pressure, represented by the evenly spaced lines in Image 23A and by the axis of the graph in image 23C, is equal to the atmospheric pressure that would prevail in the absence of the sound wave. With the passage of the compressions and rarefactions that constitute the sound wave, there would occur a fluctuation above and below atmospheric pressure. The magnitude of this fluctuation from equilibrium is known as the amplitude of the sound wave; measured in pascals, or newtons per square meter, it is represented by the letter A. The amplitude of a sound wave determines its intensity, which in turn is perceived by the ear as loudness. Acoustic intensity is defined as the average rate of energy transmission per unit area perpendicular to the direction of propagation of the wave. (Berg, 2020)

DECIBEL

The ratio of intensities between silence and 'ow that hurts my ears' is about 1:100 million. It makes things easier if a logarithmic scale is used; this is what the decibel scale is. In decibel terms, a doubling in loudness corresponds to roughly an increase in 10 dB. It does not matter whether that increase is from 40 dB to 50 dB or 70 dB to 80.

Image 24: Decibel scale



TYPES OF SOUND AND TIMBRE

There are a lot of different types of sound that do not always represent a sine wave. Most sounds are called noise, this type of sound creates an irregular and chaotic waveform that shows no identifiable patterns. examples of noises are someone talking, a tree falling, a lightning strike or construction sounds. With musical sounds the waves are periodic which means that the waves are ordery, patterned at regular intervals. (Sound & Synthesis, 2020) A complex tone exists of a combination of different frequencies known as partials. The combination of these partials is called the timbre of a musical sound (Berg, 2020). According to the Fourier theorem all sounds can be deconstructed into individual sine waves. Noises are also constructed of different frequencies that have no pattern and are completely random, noises can have a focus on a certain frequency range high pitched or low pitched noises (Fourier Series - Encyclopedia of Mathematics, 2012). The sine wave is therefore the most basic form of sound and is a perfectly periodic wave that creates a single frequency or a pure tone. The sine wave is the most straightforward to use in research because it is easy to isolate the sounds that could have effect.

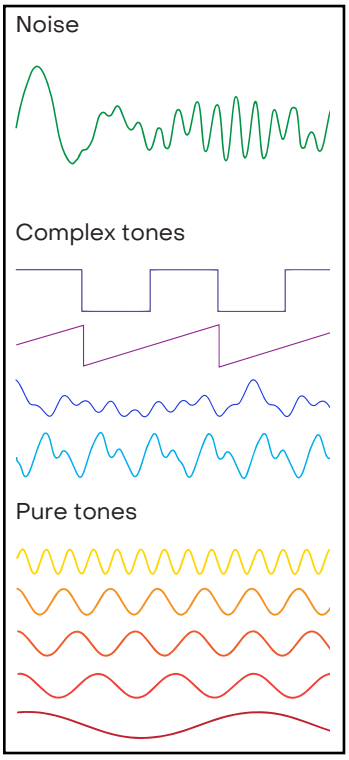


Image 25: Types of sound and their waveforms.

ACOUSTICS AND INTERFERENCE PATTERN

When a space is occupied by two or multiple sound sources interference will occur. They move through each other and affect the sound field and the resulting sound wave will change according to the distance of sources and frequency. Two waves can interfere with each other in two ways: constructive and deconstructive. With constructive interference the waves line up with each other so that the period is the same, this means that the belly and the knots of the wave are at the same location. This will result in a sound wave with an amplitude twice as high as the original sound wave which means the sound will be twice as high.

Deconstructive amplification happens when the waves line up with a period difference of half a wavelength. The sound waves have equal amplitude but reversed peaks and troughs. This will result in the cancellation of the sound wave and near silence. Sound is produced from a point source and propagates through space with a pattern comparable to the shells of an onion. The interaction of two point sources can result in interesting patterns, these patterns are called interference patterns. Where some spots will have constructive interference and some spots will show deconstructive interference. Pure tones create very distinguishable and predictable interference patterns other than noises or complex sounds. With pure tone waves it is possible to predict the interference pattern and identify the hotspots and dead spots. When the frequency or the distance is changed the patterns will change accordingly as seen below.

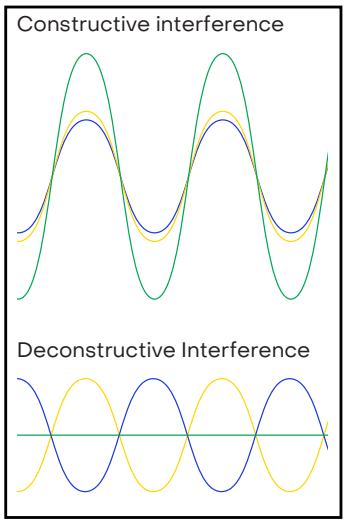


Image 26: Graphical reprisentation of sound interference.

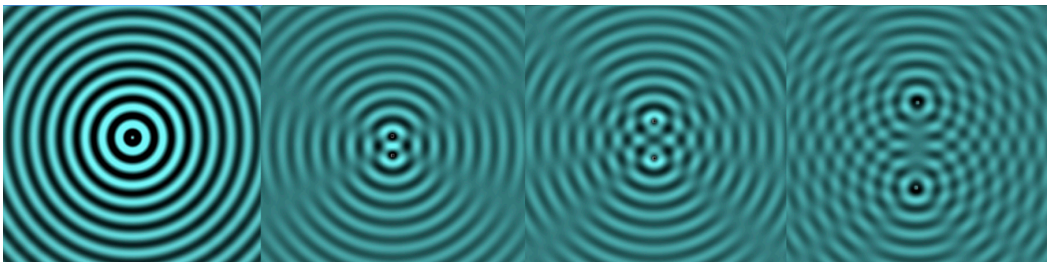


Image 27: Interfering pattern of two point sources.

So if two sound sources or speakers produce a single frequency some areas will have less stimuli and other places will have more stimuli or energy introduced. With this interference the stimuli for the mycelium can be localised and patterned where some areas of the mycelium will be in silence and will be extra stimulated.

Inside a box reflection of the soundwaves can occur which will change the interference pattern and will make a new unpredictable pattern. Reflection of sound waves mainly occurs with mid to high frequencies according to Dr. Spagnol, S. who is an expert on acoustics at the TU Delft. The higher the frequency the more reflection of the sound waves will occur. This can be reduced by placing sound absorbing materials on the sides of the container.

3.2 Sonic stimuli and living organisms

In animals sound can be captured by auditory cells and transformed into electrical signals towards the central nervous system that will analyse the sound which will influence the behaviour of the animal. But the energy present in sound can also have an effect on non auditory cells in a variety of different types of organisms like plants, cells, bacteria and in this case fungi. Lots of scientific works have been performed on the effect of non-auditory cells. The effects on growth morphology, apoptosis, proteine activities in animal, plant and bacteria cells have been proven (Exbrayat & Brun, 2019, p. 2). The effects on these cells are of physiological nature meaning that the energy of the sound is affecting the cell physically on a microscopic level.

PLANTS

For example sound can have an effect on plant cells, it has been proven that sounds can increase germination rates, plant growth and production (Vicient, 2017; Mishra et al., 2016, p. 4490). Additionally it can increase the tolerance for drought. One research concluded that the roots of plants use sound to locate water sources (Gagliano et al., 2017, p. 154). From all these findings we can conclude that plants show acoustic communication with their environments with the absence of auditory cells. Even to such an extent that some bat-dependent plants have changed their physiology in a way that

they use acoustic reflectors to attract the bats (Gagliano et al., 2017, p. 154). Sound also has an effect on the cellular processes of plant cells. These researches concern both the auditory frequency range and the non auditory frequency range (Qin et al., 2003, p. 410; Hongbo et al., 2008, p. 332).

UNICELLULAR ORGANISMS

Sonic stimuli also has an effect on unicellular organisms like bacteria and yeast in a way that the chance of survival and activity of these microorganisms is changed. Indian music with a frequency range of 38hz and 689hz had a significant positive effect on the growth of yeast and bacteria (Shaobin et al., 2010, p. 370). Another research showed tones of 1Khz, 5Khz and 10Khz were able to promote the growth of E. Coli bacteria in particular 5Khz (Lee Ying et al., 2009).

FUNGI

Unlike the variety of studies concerning the plant-sound raelation or cell-sound the relation between fungal growth and sound has not been investigated very often. Several studies investigate the effect of music or noises while others investigate frequency specific sound. A research performed by Jiang et al. (2011, p. 302) showed that by mixing classical music and cricket sounds promoted the growth of mycelium and accelerated the fruiting of six different fungal species. One study concerning the mycelium growth rate of Pleurotus Sajor-caju showed that 5 different musical treatments at 75 dB had a positive effect on the growth (Ibrahim et al., 2017, p. 1054). According to a recent study the mycelium growth of Pleurotus sajor-caju was positively affected by stimulating the mycelia with ultrasound for 1.5m (Ibrahim et al., 2020, p. 32090). One study found that 75dB is the most optimal sound level for the promotion of the growth of mycelia, only this research is hard to replicate because the frequency or type of sound waves is not specified (Firdaus et al., 2015).

A study performed by Jeong et al (2013, p. 379) studied the effect of frequency specific sounds on the spore germination. B. cinerea mycelium was treated with 5Khz sound waves at 100 dB, they found that this treatment significantly inhibited the mycelia growth. Additionally the hyphal septa were significantly shortened in the sound treated group.

3.3 Sonic stimuli for mycellium composites

Clearly sound has an effect on a multitude of organisms including individual cells and fungi. A clear correlation between sound and the growth and morphology of fungi has been proven but the research is only concerning the promotion or inhibition of the mycelium growth in order to increase yield or as a sustainable pesticide and not on the potential for mycellium based materials.

This correlation can be further investigated with a focus for the development of mycelium based materials. The sonic stimuli could provide more opportunities and control for mycelium based materials. How can mycelium learn to “like” and thrive in certain soundscapes suggesting adaptive behavior in sound environments? Allowing a change in surface texture or/and observe how sound can contribute to a pattern change. Therefore three main parameters for adaptive behaviour are identified.

- Change in growth
- Pattern change
- Change in surface texture

These changes will be facilitated by an custom built acoustic environment in order to precisely tailor the sonic stimuli for the mycelium and block out external sounds. Pure sound waves of 1000hz at 75 dB will be the source of sonic stimuli because pure tones are the simplest form of sound and the effective stimuli can be isolated. Additionally this frequency can produce a visible interference pattern and will affect the mycelium with sound and not vibration, a complete overview of the test setup can be seen in the next chapter.

Eventually this will result in the the first steps of understanding the influence of acoustic environments on mycelium composites from both a theoretical and practical point of view. Resulting in a suggestion of further implications of the funghi-human interactions, reflecting towards the new challenges for further development of mycelium cased materials.

CHAPTER 4
TEST SETUP

An incubator is an device with a insulated container that can accurately control environmental conditions like temperature, humidity, air, etc.

CONTENTS

GOAL

The goal of this chapter is to design and build a test setup needed for the execution of the experiments.

- Creating a system overview and the functionalities.
- Setting the requirements for the overall system and individual incubators.
- Designing the incubator system components.
- Building a prototype in order to test the system components and design choices.
- Building the complete testsetup including three incubators.

METHODS

- Literature review
- Information gathering at incubator companies
- Benchmarking of current commercial incubators
- Cost price estimation
- Function analysis
- List of requirements
- Weighted objectives

4.1 System overview and function

In order to perform consistent tests and create different stimuli a test new test setup had to be built. This chapter describes the design and development of the test setup and the incubators. First a function analysis as described by Roozenburg & Eekels (2013) was performed to reveal the system components with their hierarchy including functions and subfunctions. The result of this function analysis is shown below and provided a clear overview of the to be designed system.

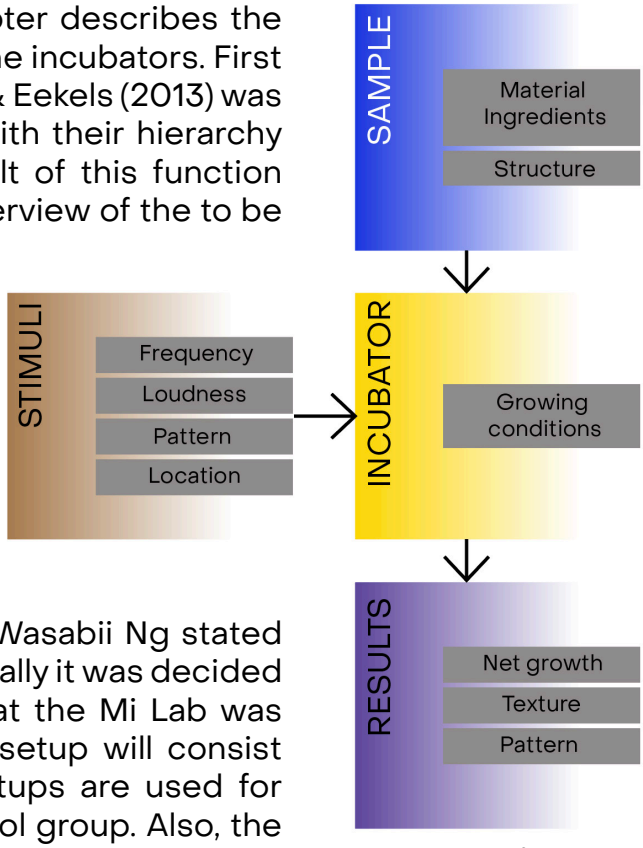


Image 28: Diagram of test setup components.

The project brief provided by Elvin Karana and Wasabii Ng stated that a new test setup needed to be built. Additionally it was decided that the incubator (Heratherm incubator 170L) at the Mi Lab was insufficient in order to perform the tests. The setup will consist of three separate environments where two setups are used for different stimuli and one environment as a control group. Also, the different environments should be suitable for further research into mycelium; investigating different types of stimuli like light and other elements that could be introduced to the system. Additionally, it should function as a demonstrator to show the work to the public and therefore it should look interesting and inspire others. During the design a cost price estimation was performed to get an overview of the budget needed for the build. This budget can be found in appendix E.

To make a successful test setup it is important to get a clear understanding of the setup function. The test setup should be able to:

- Maintain the correct growing conditions
- Isolate the samples from the environment
- Introduce different types of stimuli into the system
- Observe the samples in the incubator
- Be easily used by the researcher

List of Requirements

On this page a complete list of system requirements is shown sorted per category. This list will be used to design and test the setup.

GROWING CONDITIONS

The test setup should be a suitable environment for the mycelium to thrive in. These conditions are according to the growing conditions determined in chapter 3.4. These growing conditions should be consistent over the three different environments.

- The incubator should be able to reach a temperature of 30 °C and keep the temperature constant with a deviation of 1 °C of the set temperature.
- The heating should be evenly distributed in the growing container.
- The incubator should be able to reach a humidity of 75% with a maximal deviation of 3% of the set humidity.
- The humidity and temperature should be set by a control centre.
- The inside of the incubator should be completely dark.
- The Inside of the incubator should have the least air gaps or holes to prevent air and sound entering from outside.

ISOLATION

The isolation of the mycelium is important in order to obtain reproducible, effective and scientifically underpinned results. The isolation will block out most of the external influences. This will ensure the to be researched agitation is the only agitation that has an effect on the mycelium, in this case, sound.

- The incubator has sufficient insulation so that the temperature can stay within the temperature range of 1 °C deviation.
- The sound insulation should be sufficient to block most of the ambient sounds of 40dB thus a reduction of 40 dB is required.

STIMULI

Inside the incubator, a system should be in place that can facilitate the different types of stimuli. The sound actuators should be able to be placed dynamically and precisely.

- The stimulators should be able to be placed at any point in the incubator.
- The actuator location can be changed for later experiments.
- The actuator should be placed precisely inside the incubator by the mm.
- Different kinds of stimuli systems like lights or high directive speakers should be able to be attached to the system.

OBSERVATION

During the test, the incubator should be observable without opening the cabinet. A camera inside the incubator could act as a surveillance camera to check the status of the experiment. Possibly, this camera could provide valuable data on the growth of the mycelium.

- It should be possible for the researcher to monitor the growth of the mycelium from the inside of the incubator.
- The observation system should make pictures at intervals that can be set by the researcher.
- The camera should be able to focus on the samples.

GENERAL

The incubator should run on its own automatically for a whole week without the need for maintaining it.

- A person should be able to open and close the door with one hand.
- The inside of the incubator should be made of easy to clean material on which mycelium cannot easily manifest itself.
- The incubator should be able to fit through a door.
- The incubator should have a high quality appearance suitable for an exhibition.

4.2 Incubator design

An incubator is an device with a insulated container that can accurately control environmental conditions like temperature, humidity, air, etc. These can be adjusted according to the required growing conditions. There are many different types of incubators but they all build upon the same principle, creating a specifically controlled and steady environment. An incubator has different functionalities for different research goals and requirements. Below different types of incubators are listed. See appendix D. for the comparison and benchmark of different commercial incubators.

Image 29: Types of incubators

TYPES OF INCUBATORS

General purpose only temperature control
- microbiological test
- Colony counts
- Virology
- Toxicology

Humidity controll incubators
- Plant Incubators
- Same as general purpose
- Fungal cultivation

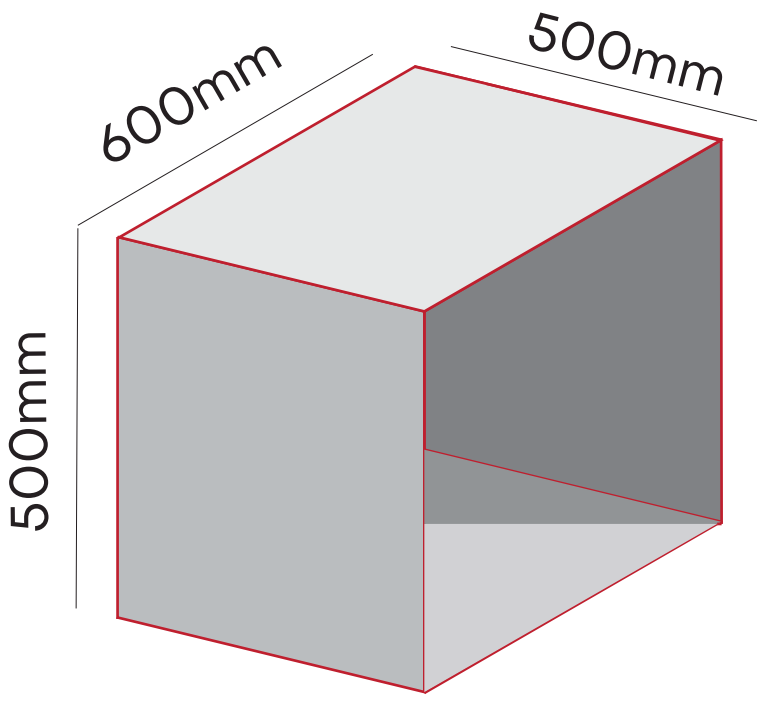
CO2 incubators
- Same as humidity incubators
- cultivation of tissue
- fungal cultivation
- IVF
- Gene expression

Specific Incubators
- Hybridisation incubators or hybridisation ovens
- Cooled Incubators
- Shaking incubators

MODULES

The Incubator can be divided into several modules, all these modules combined form a suitable and complete incubator for the test setup. From the taxonomy, growing requirements and research goal, four modules are identified: growing condition, isolation, agitation and observation. The size of the incubator should be sufficient in order to perform the different tests. The inside dimensions of the incubator were used as a starting point for the design of the incubator. The size of the container is large enough for four large petri dishes of 250x250x20mm.

Image 30: Dimensions of the growing container.



GROWING CONDITION

Temperature

In the benchmarking of the commercial incubators all incubators used a PID system for temperature control. The temperature controller will have a sensor inside the growing container that will register the temperature inside the cabinet. For the heating system two systems are commonly used: a still air system and a convection system see appendix D for more details

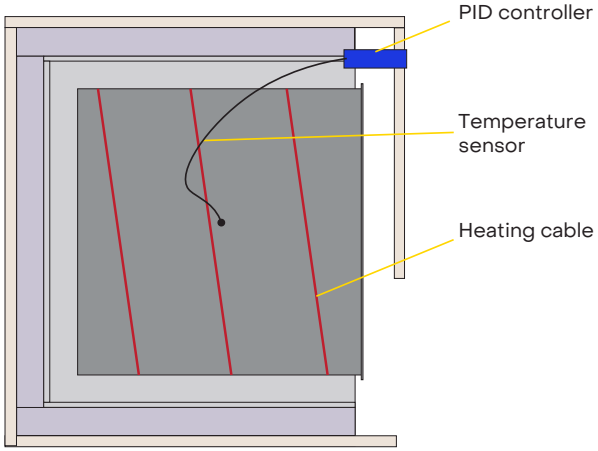


Image 31: Still air system

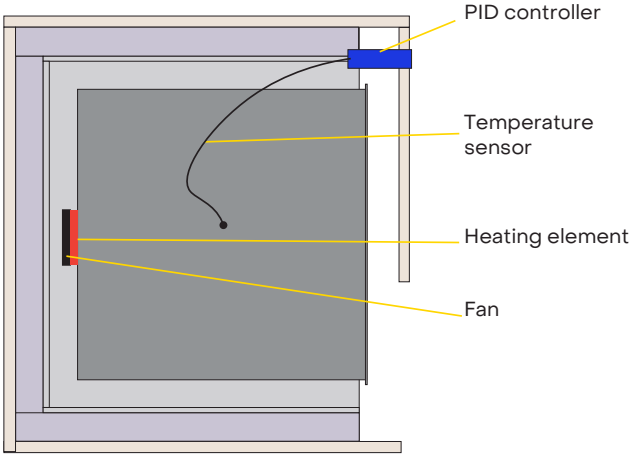


Image 32: Convection system

A still air system has been chosen because it is the most simple solution and the humidity system has a fan that will facilitate the air circulations for an even air distribution.

The heating element will be a 3m 75W heating cable around the growing container with aluminium tape for heat dissipation. This wire has been chosen in order to evenly distribute the heat along the sides of the container. The cable has been selected with the expertise of Junai, a company specialized in DIY incubators. The temperature system is also compared in a weighted objects method see table 1 where the still air system scored the highest.

		Still air		Convection	
Criteria	**Weight**	**Value**	**Total**	**Value**	**Total**
Competency	30	8	240	7	210
Safety	25	7	175	8	200
Durability	20	8	160	7	140
Cost	15	9	135	7	105
Complexity	10	8	80	6	60
			790		**715**

Table 1: Weighted objectives of the temperature system.

Humidity

Also for the humidity all commercial incubators had A PID controller for the humidity system. An alternative for a PID system is the use of a container with salted water. This option does not give the control and stability needed to meet requirements. Therefore a PID system was adopted to ensure the level of control and stability needed across the incubators to perform the experiments

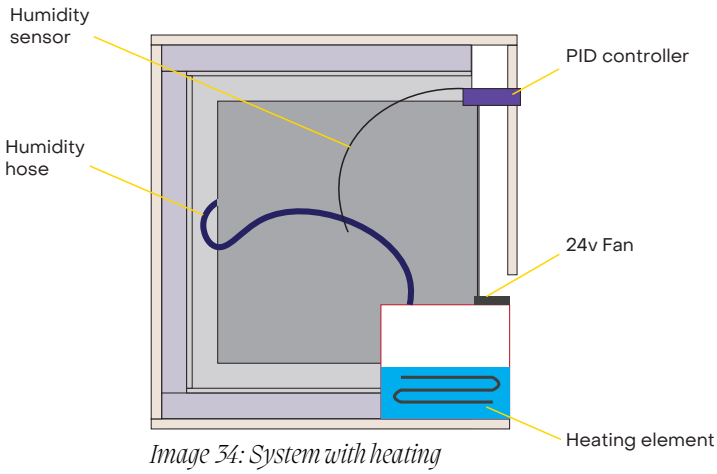


Image 34: System with heating element

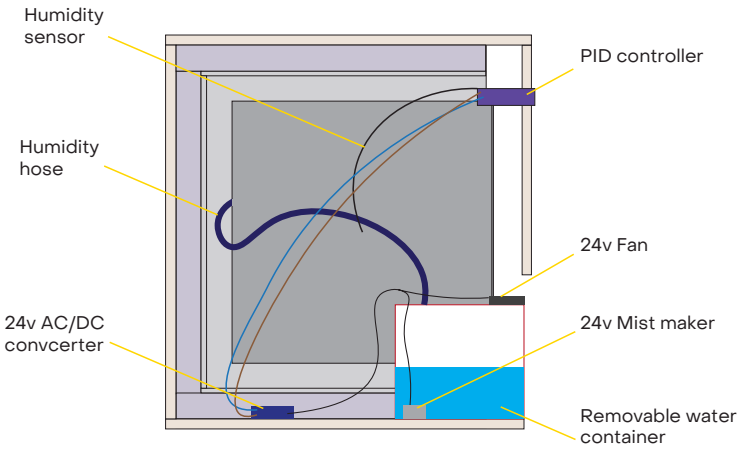


Image 35: Ulstrasone system

The humidity can be produced by heating water with a heating element or with a ultrasone humidifier. Ultrasone humidifiers are also often used in medical equipment and can vaporize nutrients or medicines, this creates an opportunity for future research performed in the incubator. A fan with a tube will blow the humidity inside the incubation chamber. In table 2 a weighted objects method of the system can be seen where the ultrasone concept won by a large margin but mainly because of the safety and the immidiate response or efficiency.

		Ultrasone		Heating	
Criteria	**Weight**	**Value**	**Total**	**Value**	**Total**
Competency	30	9	270	6	180
Safety	25	7	175	5	125
Durability	20	7	140	9	180
Cost	15	8	120	8	120
Complexity	10	7	70	7	70
			775		**675**

Table 2: Weighted objectives of the humidity system.

The downside for this active system with a fan is the introductions of air inside of the container which increases the chance of contamination. This is not a problem when growing in petri dishes or growing bags. But when growing open the system could be improved with a UV light at the water container and a hepa filter. The upside of a fan is the dissipation of heat and humidity throughout the growing container.

ISOLATION

Sound

The most common way of soundproofing is a box in box principle. In this case it is a box in a box in a box for extra insulation. The box in box principle means that a material that contains a lot of air is placed between two dense materials. The dense materials refract part of the sound waves thereby losing energy which results in a dampening effect. Materials with their characteristic impedance similar to air are regarded as best soundproofing materials thus foamed or loose fibres are preferred.

The double box in a box refracts the sound three times and dampened twice in order to block most sounds. The refraction has most effect against high frequencies and absorption is most effective against low frequencies according to Dr. Spagnol S. For the sound insulation complete silence is not the goal because this is nearly impossible to create. An existing study performed by Jeong et al (2013, p. 379) grew the control group inside a chamber where sound levels not exceeded 40dB. Therefore the goal is to not exceed the 40 dB during the experiments.

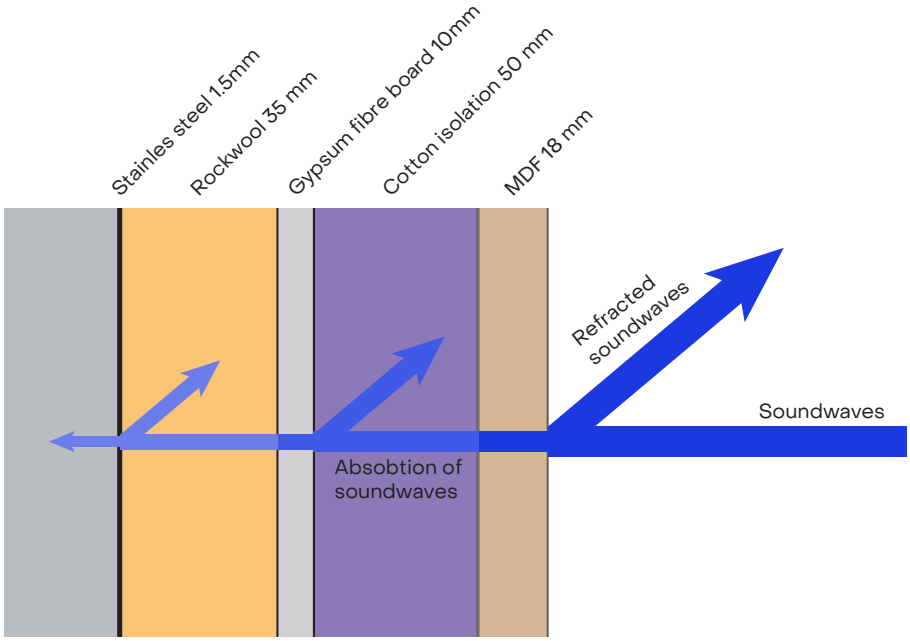


Image 36: Graphical reprisentation of the sound insulation.

Heat

The two materials close to the heating wire (max temp 60C) need to be fire retardant and heat insulative. Not because the heating wire will reach the ignition temperature of wood but to ensure the safety of the incubator. Rockwool and gypsum fibreboard are chosen for these materials. These materials are officially rated as fire retardant and are often used in construction as heat insulation materials. Rockwool or glasswool is also often used in commercial incubators because of their low thermal conductivity.

STIMULI

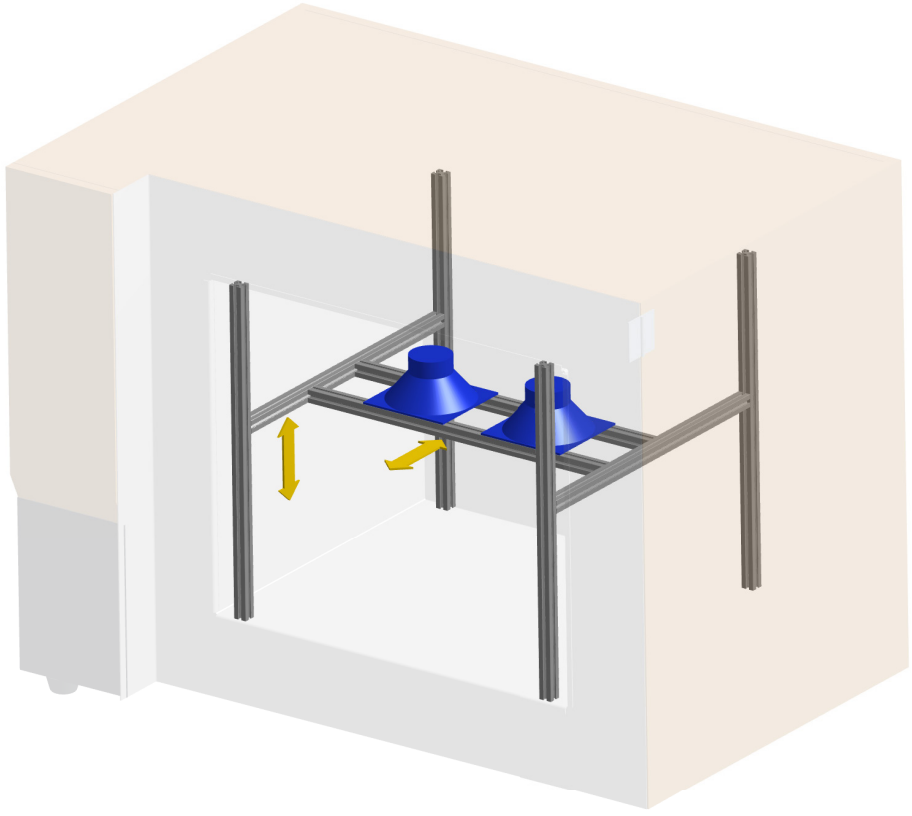
Sound

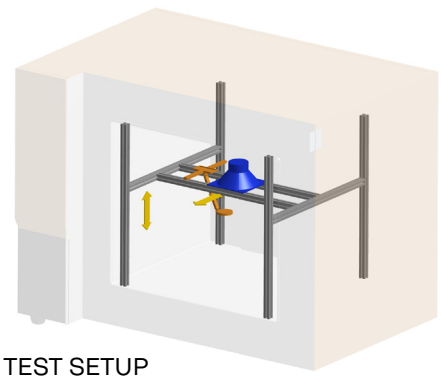
Sound is an important part of this research therefore sound should be produced precisely and effectively. Thes sound actuators are PA speakers because the sound generated is not complex and the PA speakers provide a good solution to generate these types of sound. The sound will be produced with an audio and visual editing program called Max MSP. It is a very flexible program to create specific interactive software where the soundminipulation possibilities are far beyond the scope of this research. With this program, the frequency volume and duty cycle of the sound can be changed. The patch can be found in appendix G. This program will send a signal to an audio interface with multiple outputs to the amplifier which will send the signal to the PA speakers inside the growing container.

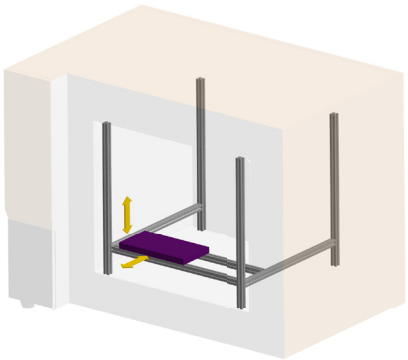
Modular Frame

A frame with similar requirements as described can be found in 3D printers there an adaptable but precise frame. An adaptation of such a frame is used for these incubators. Aluminium extrusion profiles of 20x20mm form the structure of the frame slide nuts enable the frame and objects to be moved and changed.

Image 37: Stereo speaker setup.







Left Image 38: Mono speaker setup with camera. Right image 39: Led light close setup.

4.3 Prototype and test

The following chapter describes the building and testing of the prototype for the incubators. The Indicated parts of the incubator design like sound insulation and growing conditions are tested.

GOAL OF PROTOTYPE AND TEST

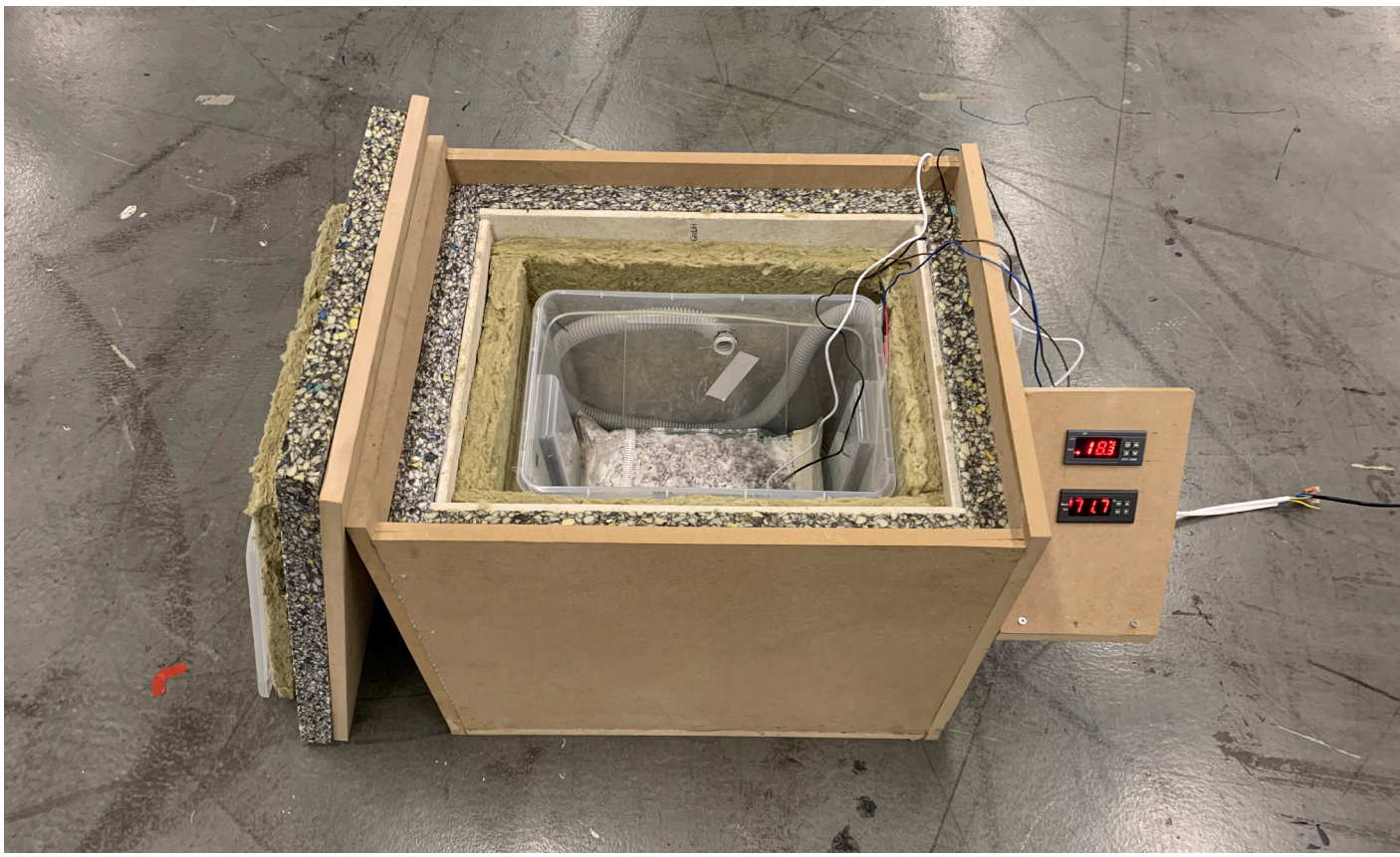
The prototype will fulfill two main purposes, one of a test setup where the different modules can be verified and tested and secondly, the prototype will suit as the control environment if the prototype performs as expected. This incubator will be a simple version of the other incubator design; it will be smaller and does not have the modular system inside.

The goals for the build of this incubator are the following.

- Test the temperature control system.
- Test the humidity control system.
- Test the sound insulation.
- Test the growth performance.

The prototype was built according to the system modules determined in chapter 4.2. The inside dimensions of 250x300x400mm this is the minimal dimension to hold three large petri dishes or three mycelium growing bags.

Image 40: Prototype and its insulative layers.



TEMPERATURE AND HUMIDITY CONTROL TEST

The goal of this test is to see if the temperature system and the humidity system are working as needed.

The prototype was switched on and the temperature was noted every 15 minutes. This resulted in the following graphs.

The temperature kept fairly constant throughout the test and stayed within the 1°C deviation that was set on the controller. The humidity was very irregular and not at the preset 70 percent humidity. The fan for the humidity was running constantly because of the low value. When I opened the box at 2.5 hrs I noticed that the inside was very wet and condensed. I waited until the condense evaporated and closed it again but with the same results.

Before this test, I changed the holes for the fan so that less air can flow through and additionally I changed the location of the sensor so that the air is blown against it, this resulted in smaller puffs of air being introduced in the container. Both values stayed within the deviation set on the controllers: 0.5 degrees Celsius and 1% humidity. In a later experiment the humidity sensor was again changed and this had little effect on the humidity, the humidity problem of test 1 did not occur any other time. After some research on the internet it was found that this was occurring more often with this sensor when installed for the first time.

Image 41: Prototype humidity and temperature test 1: 03-12-2020

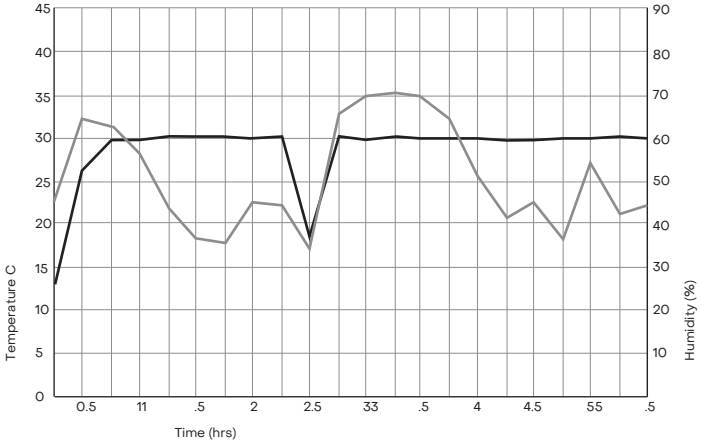
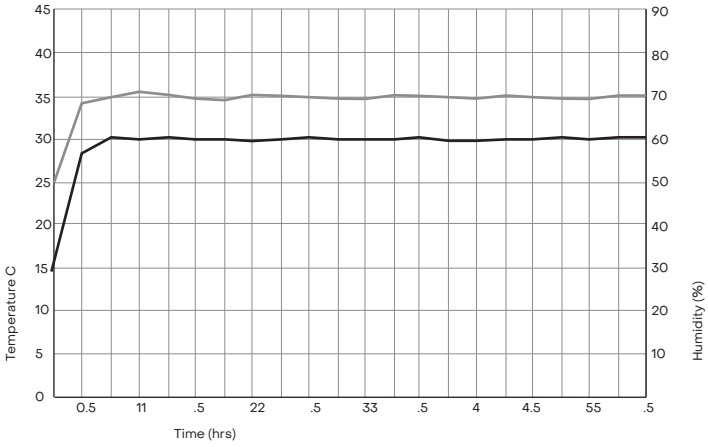


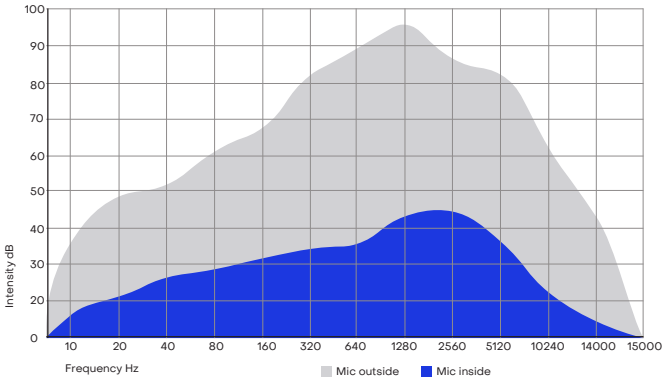
Image 42: Prototype humidity and temperature test 2: 04-12-2020



Sound insulation test

The box is also tested for its sound insulation qualities. The test setup and method is adopted from Tascan and Gaffney (2012). The computer was used to produce different frequencies starting at 40Hz and doubling every time. The values for both microphones were written down and this resulted in the following graph.The sound insulation is significant as it halfs the decibels for each frequency. As a reference, the peak outside the box was 95 decibel which is equal to a motorcycle or power tools while 45 dB is equal to whispering or a library. (Stevens, 1936, p. 406)

Image 43: Results of sound insulation test.

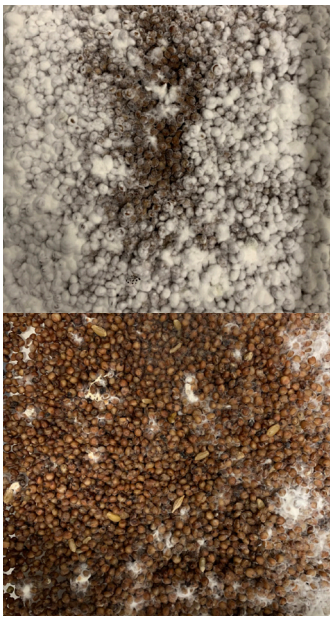


GROWTH PERFORMANCE TEST

As a benchmark for the prototype and its systems, a test for growth was done comparing the prototype with the professional incubator in the MI Lab. The comparison will be done by looking at the dry weight at the end of the growth. The inoculation and incubation conditions are done according to protocol described in appendix B.

The images below are after one week of growth. The bottom image is the sample from the professional incubator and the top is from the prototype. The little growth on the left is mainly due to the fact that the humidity in the professional incubator was significantly lower than in the prototype. This was monitored with a datalogger. It clearly shows a lower humidity level where mycelium does not thrive in and starts to hibernate.

Image 44: Samples of growth rate test.



4.3 Final design

The following chapter shows the final design of the incubators and setup and deccribes points of improvement. An overview of the build can be found in appendix E.

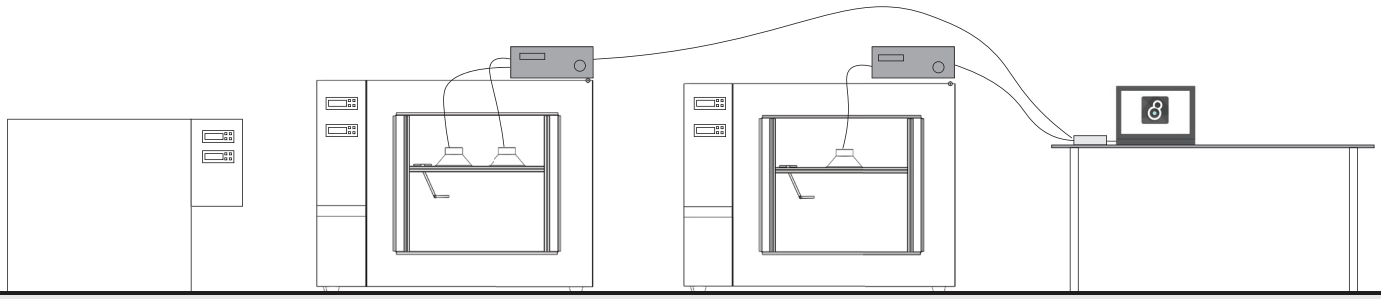
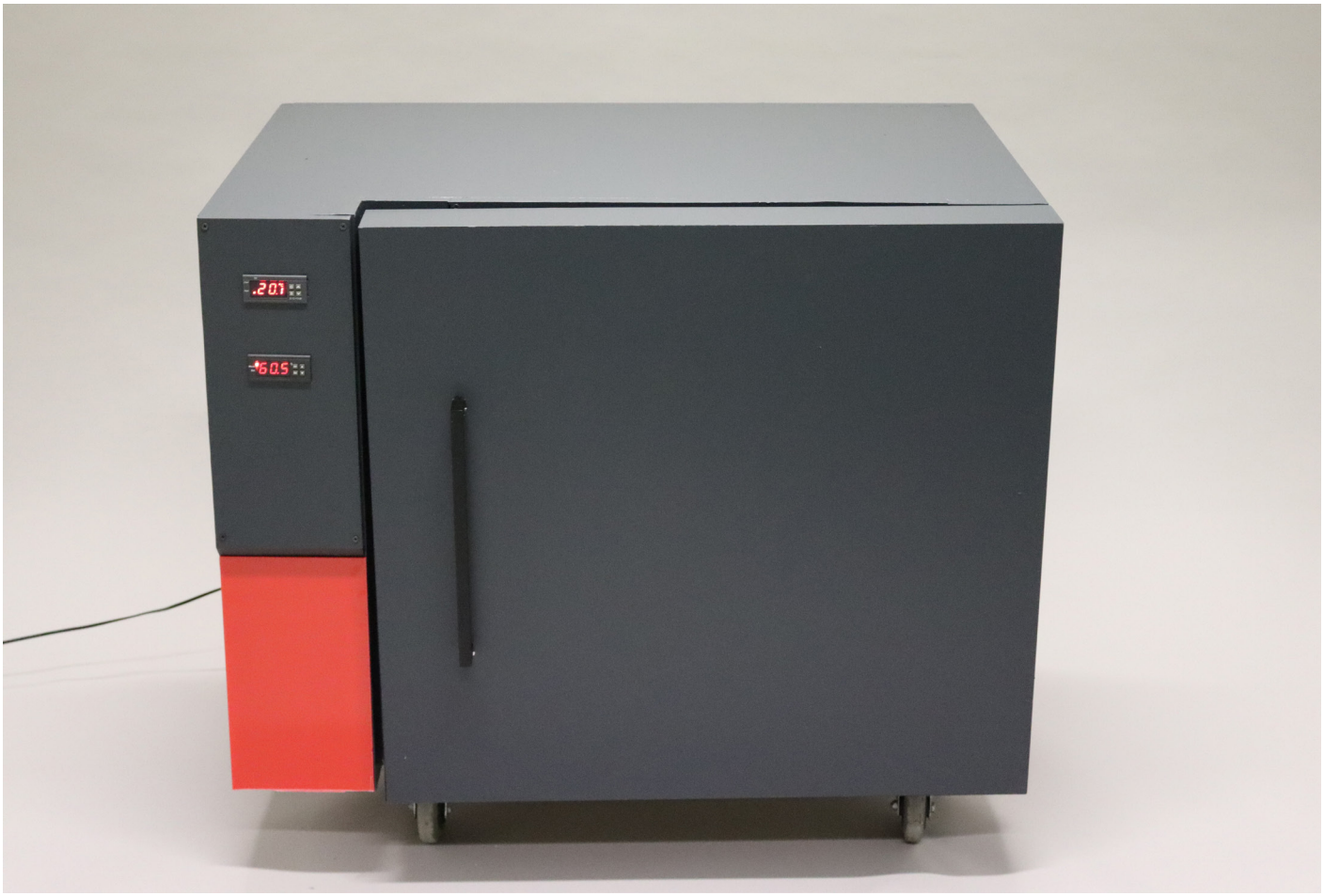
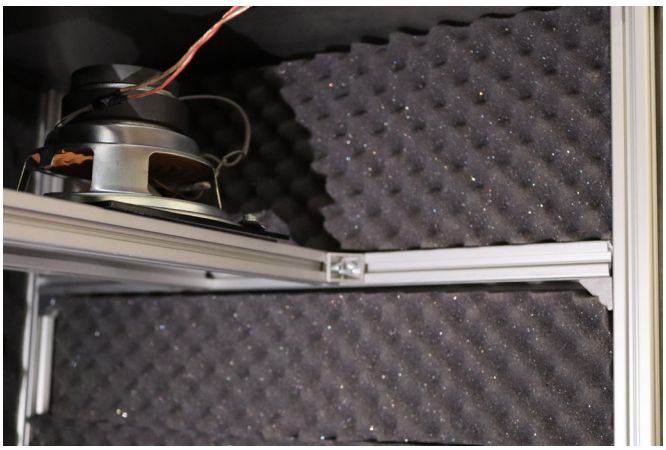
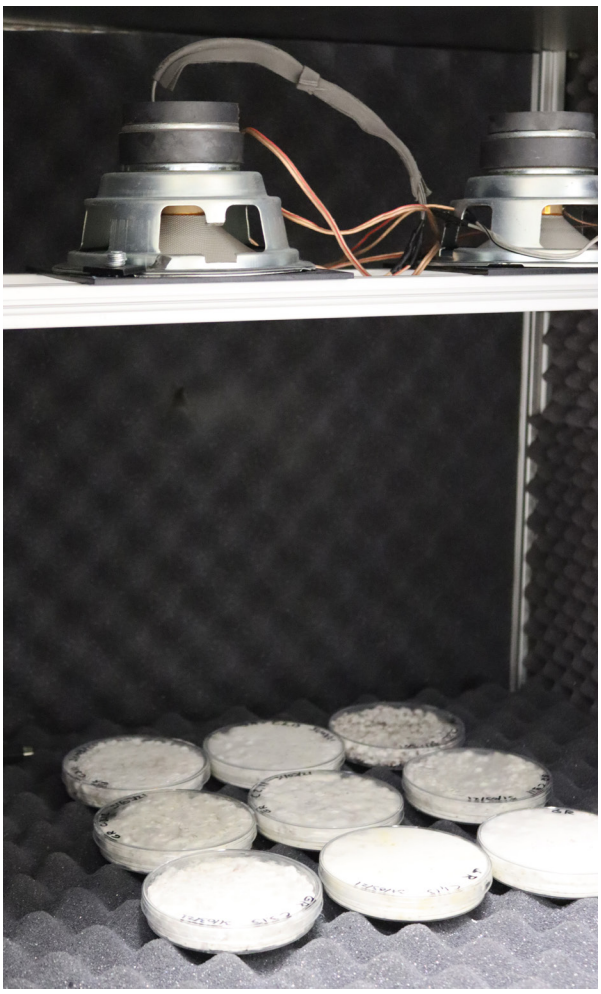


Image 45: Overview of the complete test setup.





Top Image 46: Front of incubator
Bottom left image 47: Side of incubator
Bottom right image 48: Inside growing container with modular frame, speaker and sound foam.



Left page Image 49: Incubator
Top image 50: Door and inside of incubator
Bottom left image 51: inside of incubator with speakers and samples.
Bottom right image 52: Detail of incubators with PID cotrollers.

Improvements on design

During experiment A some troubles with the humidity system occurred, the water containers were not air tight enough in order to keep the outside dry and the incubation chamber humid. These were replaced with air tight food containers. Additionally, water got in the humidity hose and blocked the airflow into the chamber; this was resolved by changing the tube path. The distribution of the humidity and temperature was tested with a data logger and showed that the humidity and temperature was similar high and low in the incubator. The humidity was 5% lower than the PID controller showed. The temperature and humidity did stay limits of deviation.

During a different test with very high and very low humidity levels a discreptiancy between the humidity value of the PID controller and the data logger was found. They both showed a consistent humidity but a different absolute value. If the absolute value is critical in a future study the humidity sensors can be replaced by more accurate biomedical humidity sensors. If future experiments ask for it another upgrade for the humidity system is a decontamination system by installing a hepa filter and UV light at the water tank.

The raspberry camera inside the incubator has appeared to be a very sensitive setup that needs knowledge about the script and how to propperly set the camera up . Aditionally the results of the camera are not suitable for scientific proof. This setup is therefore not ideal for future use.

Image 53: Incubator build with all materials

Image 54: Me working on the incubators.

CHAPTER 5
EXPERIMENTS

The test setup and built incubators have proven to be a suitable and effective way of creating the tailor made environment needed for this research and more to come.

CONTENTS

GOAL

The goal of this chapter is to discover the relation between acoustic environments and mycelium growth by looking at the growth dynamics, textural qualities and spatial distrubution.

- Defining the objectives for this research and the focus points.
- Describing the test setup, goal and methods used during the analysis of the results
- Providing an overview of the results
- Discussing the results and the points for improvement for the research method.

METHODS

- General empirical research structure
- Comparison of results to existing literature
- Grayscale analysis

5.1 Research objectives

Based upon the knowledge gaps and opportunities determined in chapter two and four the research will be focussed on the relation between the sound characteristics and the following topics that indicate change in material qualities: growth dynamics, textural qualities and spatial distribution.

The human perception of the change of mycelium material is strongly affected by the context, material and use of it. The identification of these perceptions request for qualitative user research which is out of the scope of this project. Here the focus will be on the relation of the change of growth and textural qualities by means of quantative research.

GROWTH DYNAMICS

An overview of the change in growth including the overall colonization within 7 days and 14 days of growth and the formed skin and the end of the growth. This gives an overview of the complete biomass production of the mycelium.

Growth after 7 and 14 days

Determining the mycelial growth in terms of biomass production by looking at the amount of colonization throughout the substrate.

TEXTURAL QUALITIES

An overview in the change of textural qualities caused by the sonic stimuli by looking at the mycelial skin.

Skin forming

Analysing the relation between sonic stimuli and the skin forming of mycelium. Determining the mycelial growth by looking at the skin formed on the substrate.

SPATIAL DISTRIBUTION

Determining a change in the textural qualities by looking at the distribution of mycelial growth and how uniform the growth of the mycelium is by analyzing the spots on the binary image made from the grayscale image and by looking at the color images.

5.2 Research method

This chapter describes a detailed description of the general method used for all experiments including the equipment and data analysis.

EQUIPMENT

For the experiments the test setup as described in chapter 4 was used. Additionally, the equipment used for the inoculation can be found in the protocol for the preparation and inoculation of the samples in appendix B. For the Images a Repro adjustable vertical camera stand was used with a non reflective black cloth.

Capture settings

The photos taken at the specified times were captured with a Canon EOS 250D with a Canon EF-S 35mm F/2.8 Macro iS STM Prime Lens. To ensure correct grayscale analysis, consistent imaging was secured by keeping the camera settings constant.

- Resolution: 6000×4000
- Colour profile: sRGB IEC61966-2.1
- Shutter speed: 1/125
- Aperture: f/2,8
- ISO: 800

The distance between the object and the camera was kept constant for all photos taken for each test depending on the object size.

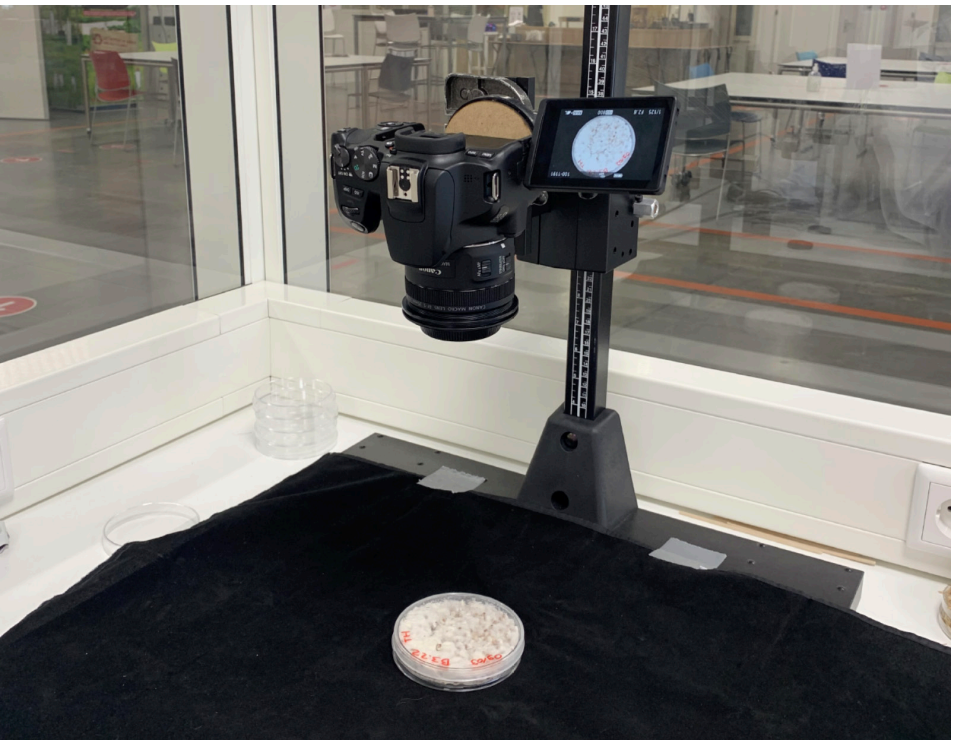


Image 55: Imaging setup used during the experiments. v

DATA ANALYSIS

The growth will be observed in four different ways during these experiments. High resolution camera images are converted into grayscale images. The grayscale analysis was performed in matlab the script can be found in Appendix G.

Grayscale

Grayscale analysis is often used in scientific research because it can analyse and describe several properties of digital images like the variations in the grayscale values the greyscale distribution and contextual features therefore providing new and scientific perspectives of an image (University of Oslo & Bjork, 2006).

The grayscale analysis starts by transforming the colour image into a gray image with the rgb2gray command. It converts the coloured image into 8-bit grayscale values using the NTSC standard for luminance by creating the weighted sum of the R, G and B components.

$$\text{gray scale intensity} = 0.2989R + 0.5870G + 0.1140B$$

The weighted sum will make sure the grayscale values have the same perceived luminance as the original images. These grayscale values can be accumulated in a histogram so that the number of pixels of all the gray values are shown. A histogram is created per image, then all the individual histograms are accumulated in one plot and divided by the total of histograms in order to get the average histogram of multiple images of one group.

Binarized image

In order to get valuable insights in the spatial distribution of the mycelium the grayscale image was converted to a binary image with the im2bw command. It replaces all pixels in the grayscale image with luminance larger than the specified threshold value by white pixels (value 1), all other pixels are replaced with black pixels (value 0).

For most details of the mycelial growth a lower threshold value is desirable, but too low value includes the substrate material as mycelial material. The threshold value is determined per substrate material by trial and error.

Visual percentage of mycelial growth

With this binarized image the percentage of white pixels in relation to the black pixels can be calculated. A copy of the binarized image with a very low threshold level is used to calculate the total amount of pixels inside the petri dish. For each image this percentage is shown above the binary image.

Image 56: Original colored image

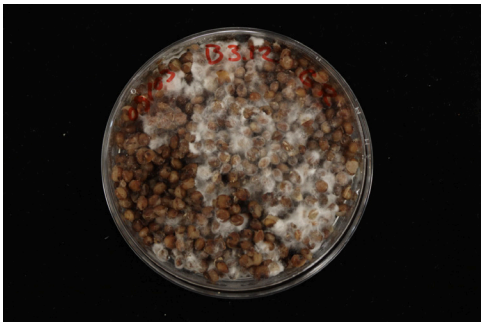


Image 57: Grayscale image

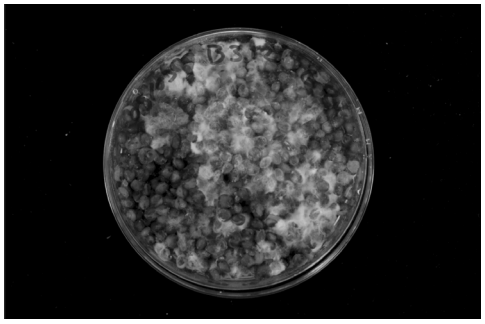


Image 58: Binarized image

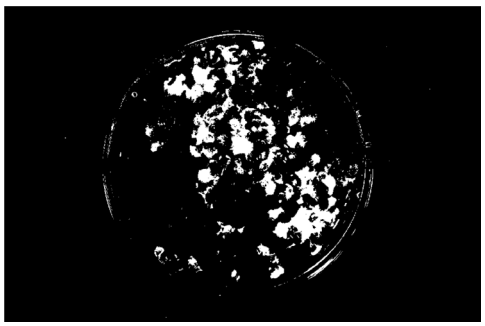
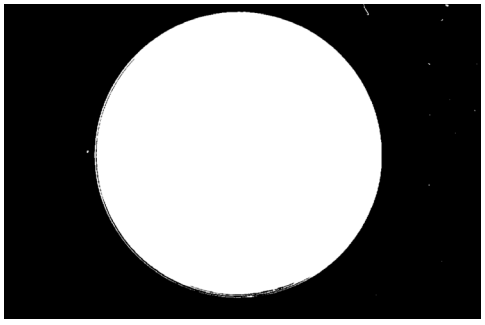


Image 59: Image used for colonization calculation



VISUAL DESCRIPTION

Because the quantitative analysis of growth with the grayscale analysis does not describe the growth completely, a qualitative descriptive analysis of the samples was done. Providing a description of the visual changes for the mycelial growth as a group and for individual samples in each sound group. In order to do consistent observation the description will be along the following aspects as described in the research objectives:

Textural qualities

Description of the textural qualities of the mycelium with a focus on the visual changes in textural qualities from close by for instance the entropy (self avoidance of the mycelium) and as a whole.

Spatial Distribution

A description of the spatial propagation of the mycelium with a focus on the hotspots and dead spots and possible representation of the nodal lines. The spatial distribution will be analysed as a group and individually by sample.

DATA VISUALIZATION

The histograms extracted from the images will be used for the analysis in the next chapters. Now the characteristics of these graphs will be described.

The X-axis describes the grey scale values with where value 0 is a complete black pixel and 255 is a white pixel. The Y-axis is the value of a measured amount of pixels of a certain grayscale. When analysing this graph the absolute values on the y axis are not leading these graphs suit the purpose of indicating trends. Important is the visual “centre of gravity” this will be different for each substrate because one substrate could be much darker than others. The x value of the graphs peak and distribution indicate the “centre of gravity” of the histogram.

The mycelium fibres have a grey value of more or less 200 depending on the lighting conditions, shade and age of mycelium but a peak with steep edges around that value indicates a lot of mycelial growth.

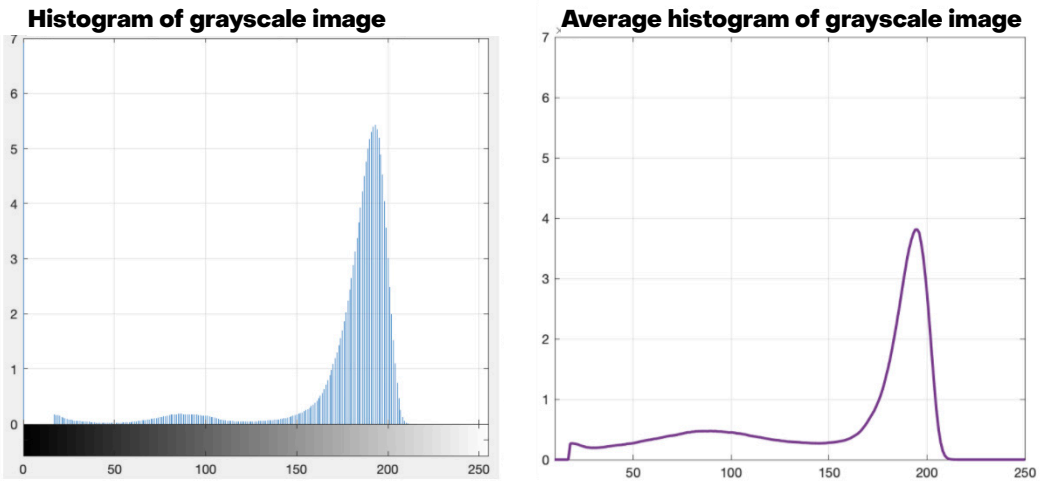


Image 60: Examples of histograms

Overview of Experiments

EXPERIMENT A: LARGE PETRI DISHES SORGHUM-GANODERMA RESINACEUM

Group 1: Mono 1000hz	Code A1.[1-4]
Group 2: Stereo 1000hz	Code A2.[1-4]
Group 3: Control	Code A3.[1-3]

EXPERIMENT B: SMALL PETRI DISHES DIFFERENT COMBINATIONS

Group 1: Mono 1000hz	Code B1
Sorghum-Ganoderma Resinaceum	Code B1.1[1-3]
Sorghum-Trametes Hirsuta	Code B1.2[1-3]
Hemp chives-Ganoderma Resinaceum	Code B1.3[1-3]
Hemp chives-Trametes Hirsuta	Code B1.4[1-3]
Group 2: Stereo 1000hz	Code B2
Sorghum-Ganoderma Resinaceum	Code B2.1[1-3]
Sorghum-Trametes Hirsuta	Code B2.2[1-3]
Hemp chives-Ganoderma Resinaceum	Code B2.3[1-3]
Hemp chives-Trametes Hirsuta	Code B2.4[1-3]
Group 3: Control	Code B3
Sorghum-Ganoderma Resinaceum	Code B3.1[1-3]
Sorghum-Trametes Hirsuta	Code B3.2[1-3]
Hemp chives-Ganoderma Resinaceum	Code B3.3[1-3]
Hemp chives-Trametes Hirsuta	Code B3.4[1-3]
Group 4: Control MI LAB	Code B4
Sorghum-Ganoderma Resinaceum	Code B4.1[1-3]
Sorghum-Trametes Hirsuta	Code B4.2[1-3]
Hemp chives-Ganoderma Resinaceum	Code B4.3[1-3]
Hemp chives-Trametes Hirsuta	Code B4.4[1-3]

EXPERIMENT C: SMALL PETRI DISHES SORGHUM

Group 1: Mono 1000hz	Code C1
Ganoderma Resinaceum	Code C1.1[1-3]
Trametes Hirsuta	Code C1.2[1-3]
Group 2: Stereo 1000hz	Code C2
Ganoderma Resinaceum	Code C2.1[1-3]
Trametes Hirsuta	Code C2.2[1-3]
Group 3: Control	Code C3
Ganoderma Resinaceum	Code C3.1[1-3]
Trametes Hirsuta	Code C3.2[1-3]
Group 4: Control MI LAB	Code C4
Ganoderma Resinaceum	Code C4.1[1-3]
Trametes Hirsuta	Code C4.2[1-3]

5.3A Experiment A

The following chapter describes the setup of the first experiment performed in the test setup described in chapter 4.

GOAL

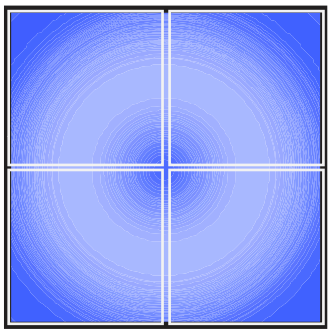
Find a difference in mycelial growth regarding textural changes, pattern changes and growth dynamics, between different stimuli groups.

SPECIES SUBSTRATE MIXTURES AND FABRICATION METHOD

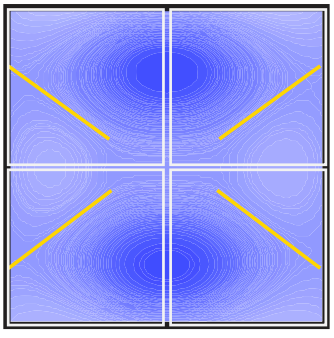
The fungal species used for this first experiment is schitzofillum comune . Substrate preparation, inoculation and growing conditions are based on chapter 2 and a detailed description of procedure is described in appendix B. The substrate used is soaked sorghum that is prepared in filter bags and autoclaved in batches of 1 kilo at 120°C for 45 minutes. Next the substrate is placed in Sigma square sterile petri dishes 250x250x25mm in batches of 250 grams (95%of total weight). These are inoculated on 19/02/2021 with 5% or 12.5 gram sorghum spawn (spawn inoculation 03/01/2021). For group 1 and 2 four petri dishes were prepared and for group 3 three petri dishes, three is the minimal amount required for the results to be scientifically grounded. All species have the same required growing conditions as described in chapter 2; at 30°C, 75% humidity and complete darkness.

Image 62: Theoretical interference patter n image projected onto the samples.

A1 Mono



A2 Stereo



STIMULI

For the first test a frequency of 1000 hertz is used due to its wavelength and energy introduction into the mycelium (see chapter 2.4). The 4 petri dishes are placed in a rectangle next to each other on the bottom of the incubator for group 1 and 2. For group 3 the three petri dishes are stacked on top of each other.

Group 1

The mono speaker is placed in the middle at a distance of 300mm to the samples. The volume was set at 75dB at 300mm with a duty cycle of 100%

Group 2

Stereo sound with a distance of 200mm between the PA speakers and the same distance of 300mm between the speaker and the mycelium. The volume is set at 75dB with a duty cycle of 100%. The yellow lines in image 62 indicate the nodal lines which are areas where there is constant deconstructive interference and therefore little stimuli.

Group 3

This group functions as the control group and there is no speaker placed inside.

GROWTH TIME

For this experiment a total growth time of 2 weeks is adopted. During the first week the mycelium will have the chance to colonize the petri dish. After this first week the mycelium will be remodeled inside the petri dish and compressed so that a flat surface is created. This is done to promote the growth of a smooth mycelium skin. After this remould the samples are grown for another week under the same circumstances before taken out.

Left page Image 63: Closeup of sample [A3.LT]
Top image 64: Closeup of sample [A1.RB]
Bottom left image 65: Me putting samples in the incubator.
Bottom right image 66: Sample [A2.LB]

5.3B Results experiment A

RESULTS DAY 7

This chapter will describe the results obtained from the first experiment and will draw conclusions from these results.

During the first week there were some issues with the humidity system in incubator one and two, this means we can not draw clear conclusions about the effect sound has on the growth. A lot of condensation on the petri dishes which made it impossible to do the gray scale analysis. The images are only qualitatively analyzed because of the humidity problems and the condense on the petri dishes.

Spatial distribution

The control group showed normal growth where the mycelium evenly colonizes throughout the petri dish. The bottom sample [A3-B] opened in the chamber which caused it to dry out and not grow properly. This sample is considered as an outlier and not taken into account with the results.

In incubator one and two containing sample groups A1 and A2 it can be seen that near the edges the growth is less and more concentrated on other places, these spots with little mycelium occured at places where the parafilm showed tears and appeared to be less moist. This indicated that the growth was affected by the difference in humidity.

Textural qualities

For all the three groups the skin forming and textural qualities of the skin appeared to be the same.

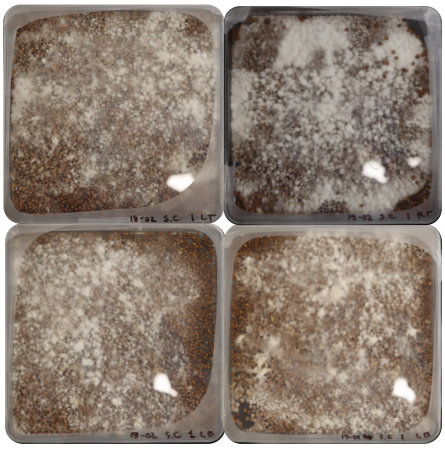


Image 67: Samples A1

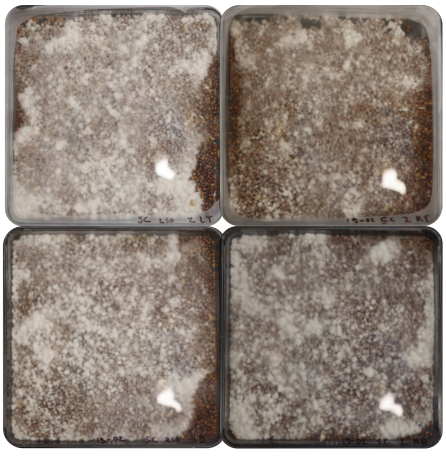


Image 68: Samples A2

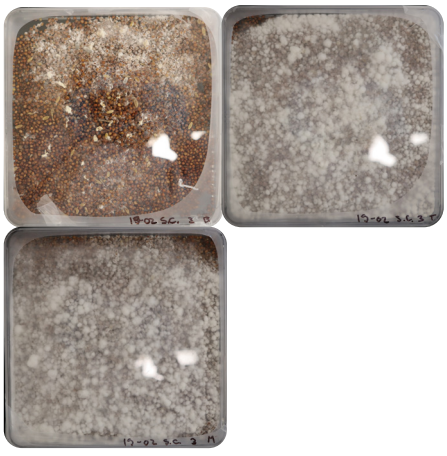
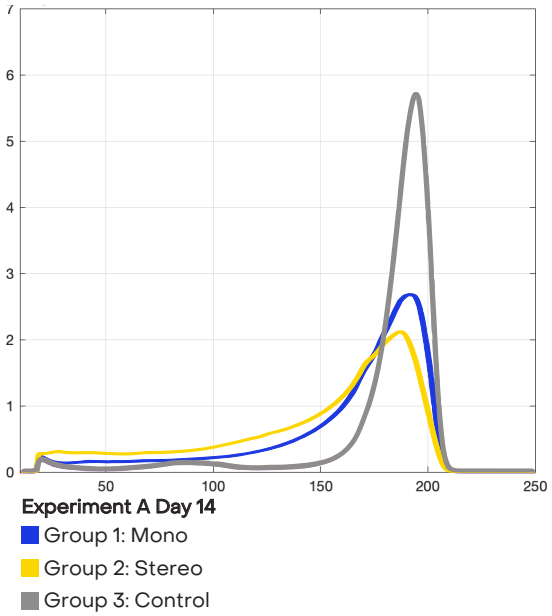


Image 69: Samples A3

RESULTS DAY 14

Comparing Average histograms

When looking at the average histograms of the three groups all the curves have a similar shape, they all show a peak at around 180. This indicates that there is a significant amount of mycelial growth. Curve A3 has a higher peak than A1 and A2 meaning that more pixels with the mycelium gray value are present. Curves of A1 and A2 show a less steep incline on the left side of the peak. This indicates that the mycelium is less thick. When the mycelium is very thick grown over the sorghum there are not many shadow spots and the color of the sorghum does not shine through the mycelium. A second peak on the left would indicate spots with no mycelium.



Comparing the colonization

When looking at the different colonization percentages it can be seen that A2 achieved the least growth with 8.5% colonization less than A1. This is mainly caused by A2 RT which showed the least growth in day 7 because of the humidity problems. A3 has a very high colonization of almost a 100% and was growing a thick skin when looking at the samples.

Sample	Colonization %
A1 LT	89.1
A1 RT	89.8
A1 LB	88.7
A1 RB	89 .0
A1 average	**89.2 %**
Sample	**Colonization %**
A2 LT	85.9
A2 RT	71.0
A2 LB	88.3
A2 RB	77.7
A2 average	**80.7 %**
Sample	**Colonization %**
A3 T	(34.1)
A3 M	97.0
A3 B	94.3
A3 average	**95.7 %**

Table 4: Colonization percentages experiment A day 14

Textural qualities

When comparing these three photos of the samples it can be concluded that no significant change in the textural characteristics of the skin has been found. The three images appear to be of the same sample or group.

Image 70: comparrison of textural qualities experiment A day 14

Spatial distribution

When comparing the spatial distribution by looking at the binary image it becomes evident that A2 has more mycelial growth near some of the edges the same occurred for A1 but to a lesser extent. After discussion with Wasabii NG it was concluded that this was probably caused by the gap in the foam near the edges. The most mycelial growth was where the gap was the biggest. This gap caused warmer areas near the edges because the warmer bottom of the incubator was exposed. This pattern does not correspond with the interference pattern

CONCLUSIONS

There are no hard conclusions that can be drawn from this experiment but valuable lessons were learned.

There was no significant change in textural qualities of the mycelium under influence of the sonic stimuli. There was a difference in growth but this can be caused by several other aspects. Before diving into the textural qualities and focusing on one strain and substrate it is decided to do a more explorative experiment that focuses on the effect of sound on net growth on different strain-substrate combinations.

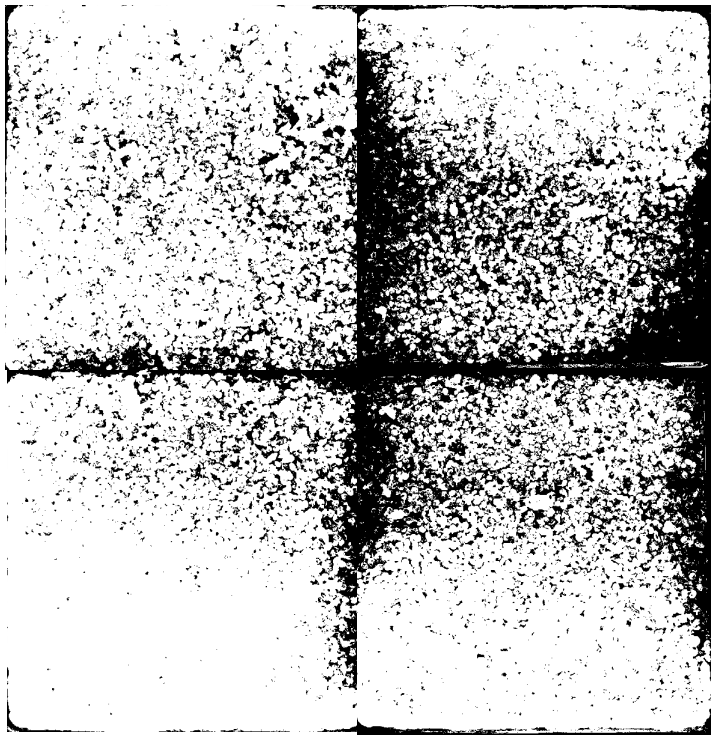


Image 71: Binary images of samples A2

5.4B Experiment B

GOAL

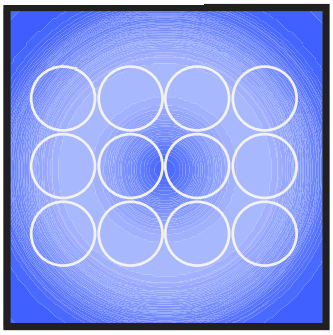
Find a difference in mycelial growth focussing on net growth but including observations on textural changes, pattern changes between different stimuli groups and different strain-substrate combinations.

SPECIES SUBSTRATE MIXTURES AND FABRICATION METHOD

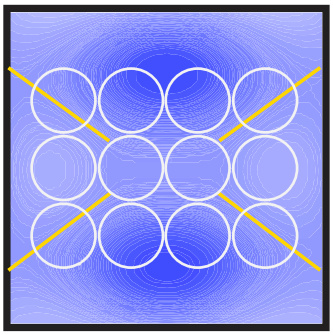
The fungal species used for this experiment are Ganoderma Resinaceum and Trametes hirsuta. Substrate preparation, inoculation and growing conditions are the same as in experiment A The substrates used are hemp chives and soaked sorghum that are prepared in filter bags and autoclaved in batches of less than 1 kilo at 120°C for 45 minutes. The substrate is placed in standard round sterile petri dishes with 50mm radius 10mm thickness. The sorghum was filled with 35 grams substrate (95%of total weight) while the petri dishes with hemp chives are filled with 14 grams. These are inoculated on 09/03/2021 with 5% spawn or 1.8 grams for sorghum and 0.7 grams for hemp chives (Trametes hirsuta spawn inoculation 15/01/2021 and Ganoderma Resinacum 29/01/2021). This resulted in four categories with different substrate-strain combinations per incubator group with three samples each. An extra control group was added as a control for the control group. All incubators have the same required growing conditions as described in chapter 2; at 30°C, 75% humidity and complete darkness.

Image 72: Approximation of interference pattern projected at the mycelium.

B1 Mono



B2 Stereo



STIMULI

The stimuli has remained exactly the same as in experiment A with a frequency of 1000 hertz For B3 and B4 the three petri dishes are stacked on top of each other.

Group B1

The mono speaker is placed in the middle at a distance of 300mm to the samples. The volume was set at 75dB at 300mm with a duty cycle of 100%

Group B2

Stereo sound with a distance of 200mm between the PA speakers and the same distance of 300mm between the speaker and the mycelium. The volume is set at 75dB with a duty cycle of 100%. The yellow lines in image 72 indicate the nodal lines which are areas where there is constant deconstructive interference and therefore little stimuli.

Group B3

This group functions as the control group and there is no speaker placed inside.

Group B4

Same conditions as group B3 but in the incubator of the MI lab.

Growth Time

For this experiment the same total growth time of 2 weeks is adopted. One week for colonization and another week after remould under the same circumstances before taken out.


T. hirsuta
15/1

Left page top image 72: Overview of samples experiment B
Left page bottom image 73: Measuring of the required spawn
Right page top image 74: Sample [B1.32]
Right page bottom image 75: Samples inside the incubator

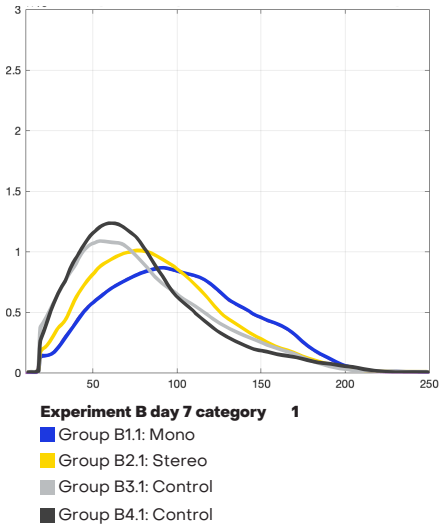
5.3B Results experiment

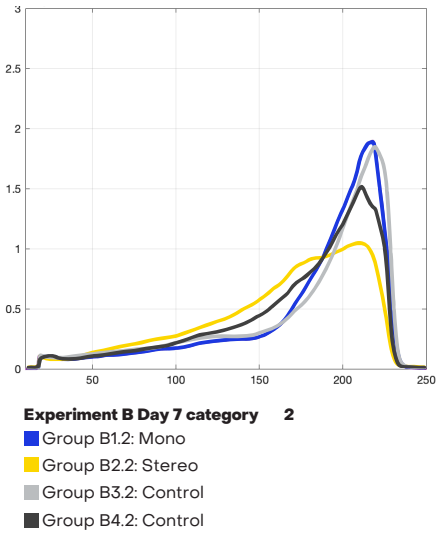
RESULTS B DAY 7

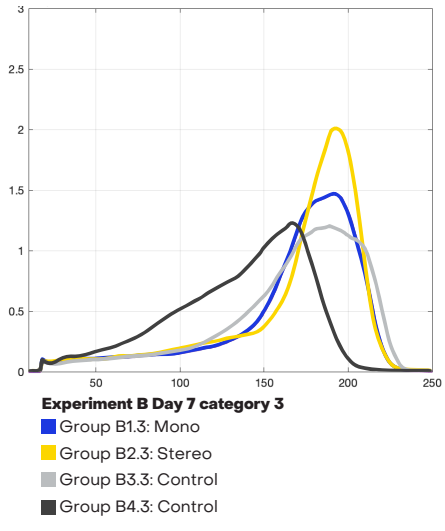
This chapter will describe the results obtained from experiment B and conclusions from these results will be drawn.

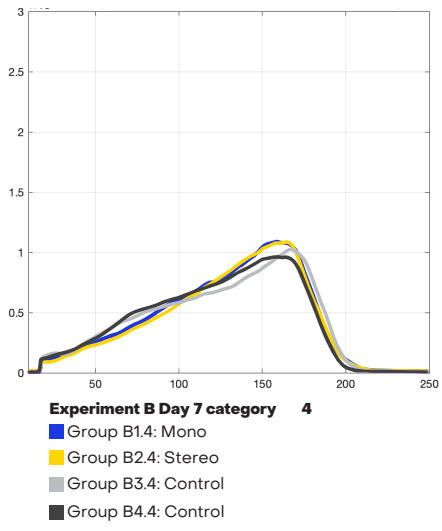
Comparing average histograms

Each graph shows all the average histograms for one category in every incubator. The graph of category 1 (ganoderma-sorghum) has most peaks on the left indicating many pixels have the gray value of sorghum. It can be seen that samples [B1.1] has a line that is shifted to the right indicating more growth. Samples [B2.1] is in between the control incubators and mono incubators. Category 2 shows tall and steep peaks on the right indicating good mycelial growth. [B2.2] shows a second peak on the left that is caused by one sample [B2.22] that showed significantly less thick skin. For category 3 group [B2.3] shows a tall peak indicating a thicker skin than group B1.3. The peak of group [B4.3] is shifted significantly more to the left than [B3.3] which is strange because the conditions were the same for both groups. Category 4 shows no differences in gray values because this whole category did not show any growth, the gray values have stayed very similar since inoculation.









Comparing colonization
When comparing the colonization percentages per group it can be seen that category 2 and 3 performed the best over all the different incubators. This is a result that shows that the strain-substrate combination has a big effect on the growth. There are no big differences between the incubators except for category 1 where [B1.1] shows 19.3% more growth than other groups. Another interesting result is the difference between group [B3.3] and [B4.3] these two groups are identical with the same mixture, growing conditions and no stimuli, yet they show a difference in colonization of 32.4%. This difference in growth can be explained by slight changes of the growing conditions because of the opening of the MI Lab incubator.

Spatial distribution
Because there were many different samples laid out in incubator 2 it is hard to assess the spatial distribution on a larger scale. On sample scale the difference between samples [B2.11, B2.13] that are on top of a nodal line and [B2.12] can be observed by looking at the binary images but there was no difference in the spatial distribution between these three samples.

Textural qualities
No significant changes in the textural qualities of the mycelium between groups has been found.

Group	Colonization %	Group	Colonization %
B1.1	34.7	B1.3	75.1
B2.1	15.4	B2.3	76.9
B3.1	13.0	B3.3	71.3
B4.1	12.3	B4.3	38.9
Group	**Colonization %**	**Group**	**Colonization %**
B1.2 8	5.8	B1.4	31.6
B2.2	78.5	B2.4	32.7
B3.2	81.2	B3.4	33.2
B4.2	82.7	B4.4	28.8

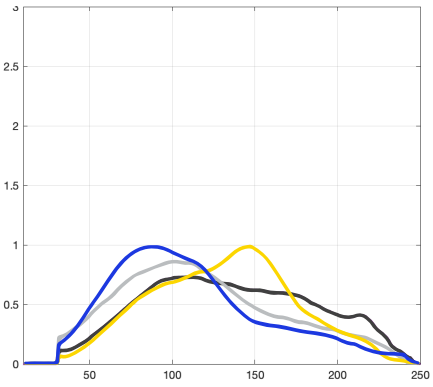
Table 5: Colonization percentages experiment B day 7

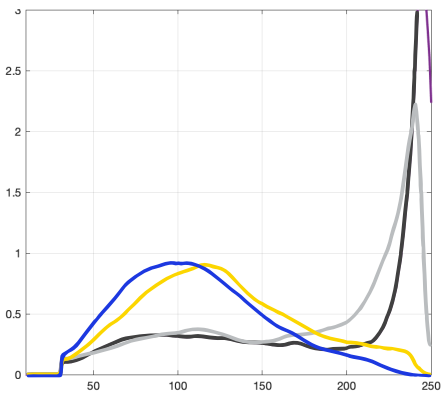
RESULTS B DAY 14

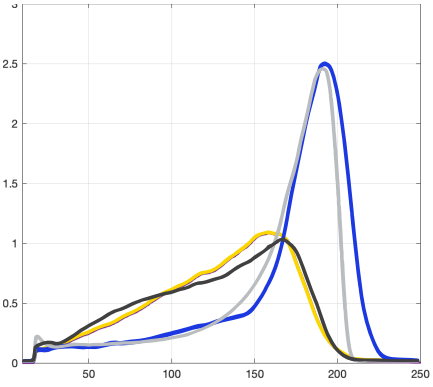
Comparing average histograms

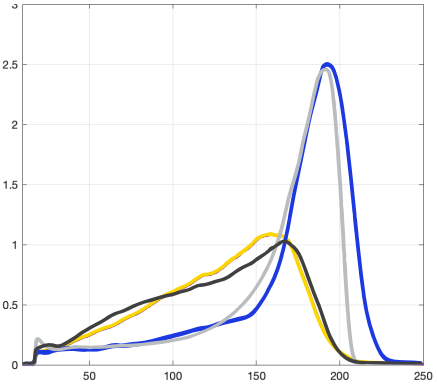
When comparing the histograms it can be seen that category 4 is still inactive similar to day 7. When looking at the average histograms of category 1 it can be concluded that the graphs are lower and more to the left than at day seven indicating less growth overall. The peak in the middle of group [B2.1] is caused by a contaminated sample. But when looking at category 2 high steep peaks to the right can be seen in the control groups [B3.2] and [B4.2], the sonic groups [B1.2] and [B2.2] show curves characteristic for sorghum color thus the sonic stimuli induced the mycelial growth. In groups [B3.2] and [B4.2] the petri dishes were stacked where the bottom sample showed very little growth. Looking at category 3 histograms it can be seen that group [B1.3] and [B3.3] have high peaks on the right thus more mycelial growth. Group [B4.3] shows a curve indicating no growth, while this group is identical to group [B3.3] that has a lot of growth. Group [B4.2] was stacked on top of group [B4.3] and also this bottom group showed very little growth indicating that the stacking suffocated the mycelium and stopped it from growing.

From the average histograms of category 2 a relation can be seen, that the growth of Trametes Hirsuta is induced by the sonic stimuli. In category 3 a possibility for limiting the growth is found.









COMPARING COLONIZATION

When comparing the colonization percentages per group it can be seen that again category 2 and 3 performed the best over all the different incubators. The colonization percentages show similar results like the histograms, lower colonization numbers of group [B1] and [B2]. The difference in growth is less clear in the coloniztion numbers also because of the many outliers that make it hard to analyze.

Group	Colonization %	Group	Colonization %
B1.1	34.7	B1.3	75.1
B2.1	15.4	B2.3	76.9
B3.1	13.0	B3.3	71.3
B4.1	12.3	B4.3	38.9
Group	**Colonization %**	**Group**	**Colonization %**
B1.2	85.8	B1.4	31.6
B2.2	78.5	B2.4	32.7
B3.2	81.2	B3.4	33.2
B4.2	82.7	B4.4	28.8

Table 6: Colonization percentages experiment B day 14

CONCLUSIONS B

A suggestion for a effect on the growth rate has been shown in this experiment. But when doing research with living organisms the variances in results can be caused by biological differences. Therefore a repetetive study is needed to see whether these results hold up and are repeatable.

5.4.3 Experiment C

GOAL

As cocluded in experiment B a control experiment is needed in order to validate the findings found during experiment B. The goal for this experiment is to find a difference in mycelial growth between different stimuli groups and different strain-substrate combinations.

SPECIES SUBSTRATE MIXTURES AND FABRICATION METHOD

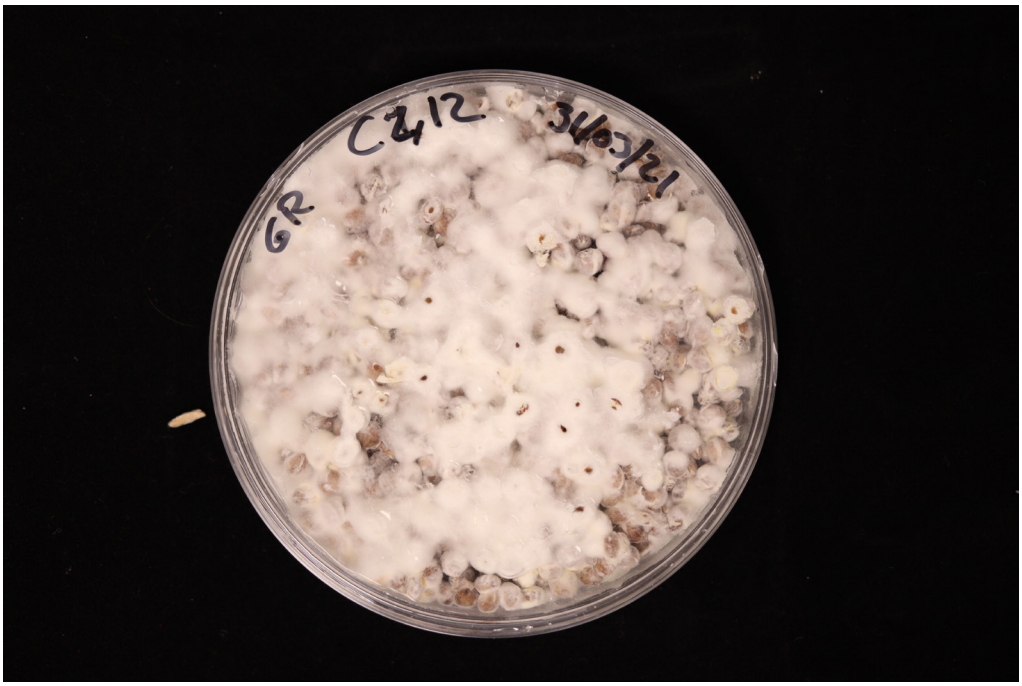
The fungal species used for this experiment are Ganoderma Resinaceum and Trametes hirsuta. Substrate preparation, inoculation and growing conditions are the same as in experiment A and B. The substrate used is soaked sorghum that is prepared in filter bags and autoclaved in batches of less than 1 kilo at 120°C for 45 minutes. The substrate is placed in standard round sterile petri dishes with 50mm radius 10mm thickness. The sorghum was filled with 35 grams substrate (95%of total weight) and were inoculated on 31/03/2021 with 5% spawn equal to 1.8 grams (Trametes hirsuta spawn inoculation 15/02/2021 and Ganoderma Resinacum 29/01/2021). An extra control group was added as a control for the control group. All incubators have the same required growing conditions as described in chapter 2; at 30°C, 75% humidity and complete darkness.

STIMULI

The stimuli has remained exactly the same as in experiment A and B.

GROWTH TIME

For this experiment the same total growth time of two weeks is adopted. One week for colonization and another week after remould under the same circumstances before taken out.



Top image 76: Samples [C2] inside the incubator
Bottom image 77: Sample [C2.12]

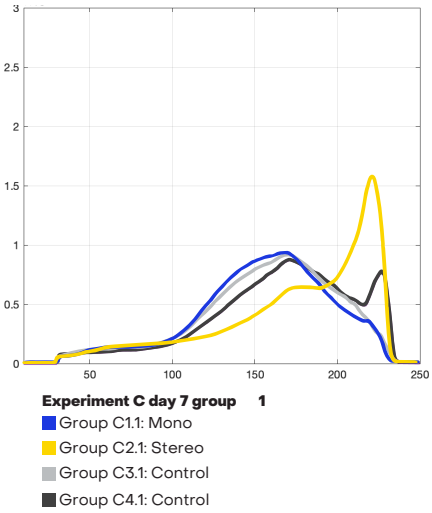
5.4B Results experiment C

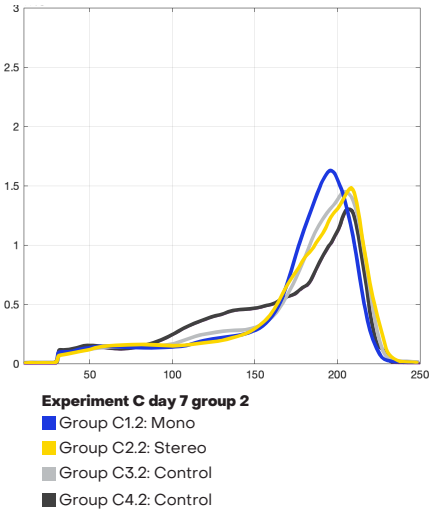
RESULTS C DAY 7

This chapter will describe the results obtained from experiment C and will draw conclusions from these results.

Comparing average histograms

The graph of category 1 Ganoderma Resinaceum shows very similar graphs except the Group [C2.1] which shows a higher peak that indicates more mycelial growth. All the other groups show similar growth. The graphs of the trametes Hirsuta all show very similar curves indicating all the groups had similar growth thus no significant difference in growth.





Comparing colonization

When comparing the average colonization percentages per group it can be seen that category 2 and 3 performed the best over all the different incubators. For all the Ganoderma Resinaseum the average colonization is around 80% with no significant difference between the groups. For the Trametes Hirsuta the differences are smaller and also no significant difference is found.

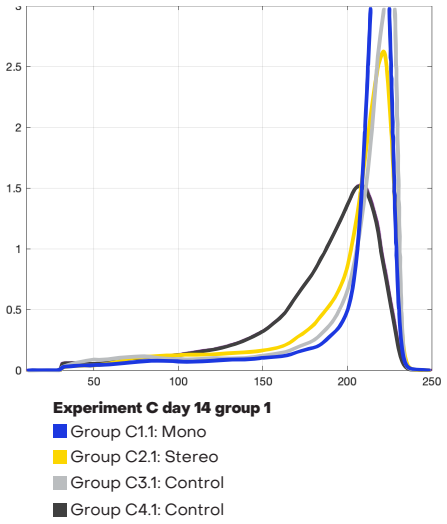
Group	Colonization %	Group	Colonization %
C1.1	77.5	C1.2	84.8
C2.1	83.3	C2.2	84.3
C3.1	78.0	C3.2	83.0
C4.1	81.7	C4.2	77.3

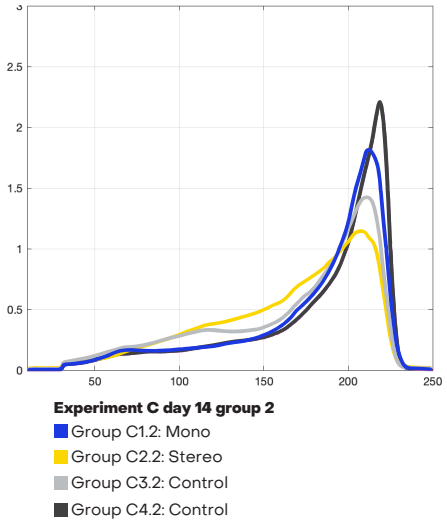
Table 7: Colonization percentages experiment C day 7

RESULTS C DAY 14

Comparing average histograms

When looking at these histograms we see that group [C4.1] shows a very different curve compared to the others while group [C4.2] is higher. The relative humidity in incubator 4 is generally lower than incubator 3 because it is opened often. According to (Maryam & Mehrdad, 2018) Trametes Hirsuta grows best at 65% humidity while ganoderma resinaceum grows best at higher air humidities of around 80% to 90% (Okamoto & Mizuno, 1997). This explains why the ganoderma shows a lower graph in incubator 4 and the Trametes higher. In both graphs it can be seen that the group C1 shows a higher graph than the control C3 and C2 is lower than C3. The volume in C2 is higher at certain places, this higher volume can have caused the inducement of the growth while lower volumes can stimulate the growth as in incubator 1. The negative effect found in experiment B in week two is repeated for incubator two while incubator 1 the growth is stimulated. Yet these differences are small therefore one should be careful drawing hard conclusions.





Comparing colonization

The results shown in the average histograms are again represented in the colonization percentage. It can be seen that [C1.1] and [C1.2] had high colonization but with small differences to the other groups.

Group	Colonization %	Group	Colonization %
C1.1	93.1	C1.2 8	4.5
C2.1	89.2	C2.2	78.5
C3.1	89.6	C3.2	77.5
C4.1	87.4	C4.2	85.2

Table 8: Colonization percentages experiment C day 14

5.6 Conclusions of all experiments

In this chapter all the conclusions of the results will be discussed according to the research objectives described in chapter 5.1. The effect the sonic stimuli has had on growth dynamics, textural qualities and spatial distribution.

GROWTH DYNAMICS

Evaluating the effect of the sonic stimuli on the growth of the mycelium composites, it can be determined that the differences are modest. Yet some differences were found, during experiment B and C a negative effect of sound on growth has been found. This proves there is a relation between acoustic environments and mycelial growth. The results of the samples from the stereo incubator from experiments B and C showed less growth then control and mono group. The stereo incubator has more volume than the other incubators that could have caused the bigger effect on growth in comparrison with the mono incubator.

> **These results can suggest a new hypothesis: Vegetative mycelial growth is induced by sonic stimuli of a 1000hz at higher volume levels than 75dB.**

Another recurring result is that the inducement of growth happens during the second week of growth. After the first week of growth during all experiments the groups showed similar histograms and no distinctive change in growth.

Interference pattern

The hypothesis at the start of the experiments was to see if it was possible to project the interference pattern discussed in chapter 3.3 onto the mycelium composites and create patterns. Unfortunately this research has not succeeded in doing so this could have been caused by multiple factors caused by either the mycelium or the test setup. The mycelium cannot be affected on specific spots because it is a network of interconnected hyphae and the stimuli at a hotspot would affect the whole network. It can be that the mycelium is not sensitive enough for higher frequencies that can create interference patterns. On the other hand the test setup could have caused that the pattern was not properly projected onto the mycelium. These causes will be discussed in the reflection of the method.

TEXTURAL QUALITIES

Now a conclusion of the change of textural qualities caused by the sonic stimuli will be drawn. The textural qualities were only looked at in experiment A. During this experiment no significant differences were found in the textural qualities.

Skin forming

The skin in experiment A showed no differences between all groups. During the other experiments the textural change was not the focus but also in these experiments no clear differences in textural qualities of the skin were found. The only difference between these skins was the thickness of the skin. Where the groups with induced growth B2 and C2 showed a thinner skin.

SPATIAL DISTRIBUTION

Another goal of this research was to determine a change in the textural qualities by looking at the distribution of mycelial growth and how uniform the growth of the mycelium is by analyzing the spots on the binary image made from the grayscale image. This is only analysed in experiment A. Where no differences have been found in the spatial distribution of the mycelium.

5.7 Discussion & reflection on method

This chapter evaluates the results of the research, reflecting on the internal and external factors that could have had an effect on the outcome of this research, outliers and relevance of the results found.

REFLECTION OF METHOD

The test setup and built incubators have proven to be a suitable and effective way of creating the tailor made environment needed for this research and more to come. Yet, several issues of the research and setup can be improved.

Capturing stability

For the grey scale analysis it is of utmost importance that the images taken are taken under the same circumstances. The images should have the same amount of light circumstances for every test. The images should be taken in a closed environment with a controlled light placed inside for the most stable lighting conditions. These lighting conditions should be most optimal that the camera can be set at a low ISO to minimize the noise and thereby fluctuations in luminance.

Textural qualities

The analysis of the textural qualities was now done with the greyscale analysis and color images. Yet, this method has not proven to be very successful. Due to the nature of the composites having textural qualities itself it was hard to analyse these qualities this way. Also, the way of imaging from the top could not show the textural qualities efficiently. For the textural qualities a different method should be adopted. This could be done by making detailed images of the hyphae structure through a microscope or macro lens. Another method for a later stadium could be a qualitative analysis by performing a user research.

Interference pattern

The interference pattern was not projected onto the mycelium composites; this could have multiple causes, by either the nature of mycelium or the test setup. Now the factors caused by the test setup will be discussed. The first factor could be the petri dishes that contain the mycelium composites, the lid of the petri dishes could effect the sound in such a way that it disturbs the pattern. Another cause could be that the pattern with its nodal lines were not clear or distinguished enough caused by reflection in the growing container. When the pattern was tested with a decibel meter a difference in volume was evident but not with a larger difference then 20dB. Optimizing the inside of the growing container could result in a more clear interference pattern.

VARIANCES IN RESULTS

Growing Conditions

The growing conditions have a big effect on the growth of mycelium as described in chapter two. During the first experiment the incubators showed some issues with the humidity system, which had an effect on the results. During experiment C incubator one had run out of water for 2 hours which caused the humidity to drop for that period of time. Besides this the data logger showed that the humidity and temperature inside the incubators were kept stable. When the humidity is set at very low and high settings the sensor becomes less reliable in maintaining the absolute value.

Composites

Performing the experiments with composites was done to make a shortcut to a mycelium composite product instead of first performing the tests on agar plates. Though because of this choice the substrate material could have had an effect on the results. First of all by the density and acoustic performance of the substrate (sorghum) the way sorghum is packed has an effect on the acoustic properties. The same density has been tried to maintain but this could not be ensured like with agar which has the exact same acoustic properties every time.

Additionally, the substrates had an effect on the images used for the grayscale analysis. The irregular surface of sorghum created shaded parts and over exposed parts, this had resulted in some variance in the results.

The textural qualities are hard to capture with the composites before a well developed skin has formed. When the skin is not needed to capture the textural qualities the process can be shortened significantly to have more results in shorter time. Also for the textural qualities a cross section is a good method to see the thickness or “fluffiness” of the mycelium which is not possible with composites.

CHAPTER 6
CONCLUSION

A valuable next step is to research the effect of physical vibration of sound caused by different and lower frequencies and possibly reveal a new material potential.

CONTENTS

GOAL

The goal of this chapter is to conclude the results found and the potential of the test setup and make a recomendation for further research.

- Determining the key findings of this report
- Defining the potential of acoustic-mycelial relationships and the testsetup
- Making a recomendation for steps to be taken in further research.

METHODS

- Comparing results to known research.

6.1 Conclusions

This chapter wile evaluate the key findings of this research and reflecting on the potential of the developed test setup and potential of acoustic environments for mycelium based material development.

KEY FINDINGS

This research has provided first insights into the correlation between acoustic environments and mycelial growth regarding the growth rate and textural qualities. It showed a correlation between the acoustic environments and mycelial growth of trametes hirsuta and Ganoderma resinaceum. The growth of these mycelium strains have been induced at higher volumes. In addition it provided that this type of sonic stimuli has no effect on the textural qualities and spatial distribution of mycelium.

The incubators have proved to be a suitable test setup that can maintain the required growing environment for mycelium stable with the possibility of adding new stimuli or different environmental conditions.

POTENTIAL OF ACOUSTIC ENVIRONMENTS

The initial hypothesis: the effect of the acoustic environment has on the growth, textural qualities and spatial distribution of mycelium has failed. Yet, there is still potential for the acoustic environment for mycelium material development.

A correlation has been found which suggests that with tweaking of the sound parameters more effects can be found. In this research just one sound type has been used and many opportunities of effects are still to be discovered. Additionally it opens up a realm of possibilities of extra environmental growing conditions. If designers and scientists look further than the required growing conditions the material qualities of mycelium based materials can be extended and diversified to suit a broader variety of applications.

A limitation for the acoustic treated mycelium could be the durability of the textural qualities. Mycelium skin or textural qualities tend to diminish by touching it and therefore losing these qualities by using the product.

POTENTIAL OF INCUBATOR SETUP

The incubator shows good potential for further research where controlled weather conditions are needed (relative humidity, light, temperature and sound) with the option to add one or multiple systems in the growing chamber.

The incubators have a modular character that every part of this system can be changed or improved. The system is useful for more than research into mycelium based materials, it could host a variety of organisms like bacteria, algae, etc.

6.2 For further research

Now an overview of topics and matter of interest into mycelium based materials and acoustic environments for further research is described.

ACOUSTIC ENVIRONMENTS

This research is only a first exploration into the potential of acoustic environments. There are many variables like volume, frequency, vibration, volume, duty cycle, etc to be explored. In this research pure sound energy has been used as stimuli instead of vibration.

The literature has shown that mycelial growth can also be stimulated by using music as stimuli that includes high as well as low frequencies. Therefore a valuable next step is to research the effect of physical vibration of sound caused by different and lower frequencies or vibration plates. This could yield very different results from this research and possibly reveal a material potential.

DIFFERENT SPECIES

In this research only two species have been used but there are many other species used for mycelium based products and even more are still to be used for product development. Using a larger variety of strains is a valuable topic.

EXPERIENTIAL ASPECTS

The qualitative interpretation of the textural qualities have not been extensively researched yet and with the goal of manipulating their textural qualities it could be valuable to perform research into the experiential aspects of these products.

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APPENDICES

DESIGN FOR our future

TU Delft

IDE Master Graduation

Project team, Procedural checks and personal Project brief

This document contains the agreements made between student and supervisory team about the student's IDE Master Graduation Project. This document can also include the involvement of an external organisation, however, it does not cover any legal employment relationship that the student and the client (might) agree upon. Next to that, this document facilitates the required procedural checks. In this document:

- The student defines the team, what he/she is going to do/deliver and how that will come about.
- SSC E&SA (Shared Service Center, Education & Student Affairs) reports on the student's registration and study progress.
- IDE's Board of Examiners confirms if the student is allowed to start the Graduation Project.

USE ADOBE ACROBAT READER TO OPEN, EDIT AND SAVE THIS DOCUMENT
Download again and reopen in case you tried other software, such as Preview (Mac) or a webbrowser.

STUDENT DATA & MASTER PROGRAMME

Save this form according the format "IDE Master Graduation Project Brief_familyname_firstname_studentnumber_dd-mm-yyyy".
Complete all blue parts of the form and include the approved Project Brief in your Graduation Report as Appendix 1 !

family name	Sonneveld
initials	D
given name	Diederik
student number	4270894
street & no.	
zipcode & city	
country	
phone	
email	

Your master programme (only select the options that apply to you):

IDE master(s):	IPD ★ DfI SPD
2nd non-IDE master:	
individual programme:	(give date of approval)
honours programme:	Honours Programme Master
specialisation / annotation:	Medisign
	Tech. in Sustainable Design
	Entrepeneurship

SUPERVISORY TEAM **

Fill in the required data for the supervisory team members. Please check the instructions on the right !

** chair	Elvin Karana	dept. / section:	SDE
** mentor	Wasabii Ng	dept. / section:	SDE
2nd mentor			
organisation:	TU Delft		
city:	Delft	country:	Netherlands
comments (optional)			

Chair should request the IDE Board of Examiners for approval of a non-IDE mentor, including a motivation letter and c.v..

Second mentor only applies in case the assignment is hosted by an external organisation.

Ensure a heterogeneous team. In case you wish to include two team members from the same section, please explain why.

Procedural Checks - IDE Master Graduation

APPROVAL PROJECT BRIEF

To be filled in by the chair of the supervisory team.

chair Elvin Karana date - - signature

CHECK STUDY PROGRESS

To be filled in by the SSC E&SA (Shared Service Center, Education & Student Affairs), after approval of the project brief by the Chair.
The study progress will be checked for a 2nd time just before the green light meeting.

Master electives no. of EC accumulated in total: 27 EC

Of which, taking the conditional requirements into account, can be part of the exam programme 27 EC

List of electives obtained before the third semester without approval of the BoE

★ YES all 1st year master courses passed

NO missing 1st year master courses are:

name date - - signature

FORMAL APPROVAL GRADUATION PROJECT

To be filled in by the Board of Examiners of IDE TU Delft. Please check the supervisory team and study the parts of the brief marked **.
Next, please assess, (dis)approve and sign this Project Brief, by using the criteria below.

- Does the project fit within the (MSc)-programme of the student (taking into account, if described, the activities done next to the obligatory MSc specific courses)?
- Is the level of the project challenging enough for a MSc IDE graduating student?
- Is the project expected to be doable within 100 working days/20 weeks ?
- Does the composition of the supervisory team comply with the regulations and fit the assignment ?

Content: APPROVED NOT APPROVED

Procedure: APPROVED NOT APPROVED

comments

name date - - signature

Personal Project Brief - IDE Master Graduation

TU Delft

Living with Cacophony: project title

Please state the title of your graduation project (above) and the start date and end date (below). Keep the title compact and simple. Do not use abbreviations. The remainder of this document allows you to define and clarify your graduation project.

start date 05 - 10 - 2020 26 - 02 - 2021 end date

INTRODUCTION **

Please describe, the context of your project, and address the main stakeholders (interests) within this context in a concise yet complete manner. Who are involved, what do they value and how do they currently operate within the given context? What are the main opportunities and limitations you are currently aware of (cultural- and social norms, resources (time, money,...), technology, ...).

Subtitle: The effect and potential of acoustic environment on the growth of mycelium.

This graduation project will contribute to the field of biodesign. Biodesign incorporates the living organisms in the design instead of only using it as an inspiration (Myers, 2012). By developing this field of design, industrial and mechanical processes could be replaced with biological processes which could offer new ways for cleaner production (Camere and Karana, 2018). A development towards this future is the research for the use of mycelium for material development. Mycelium is the vegetative part of a fungus that consists of thread like hyphae (Read, 2011). Mycelium-based composites have the potential to be a green alternative for some of the commodity plastics because the use waste streams as substrate, including coffee grounds, sawdust or wood fibres. Designers, artists, researchers, producers are discovering the potentials of this "new" material by discovering how to improve the material properties. For example, they experiment with the living conditions of the mycelium or apply diverse drying or forming process when the material is grown. This results in tailor-made composites for innovative and environmentally sensitive design solutions.

In a recent publication (Karana, 2020), Wasabii Ng (PhD candidate, IDE TU Delft) proposed a novel approach to the enhancement of habitat viability for mycelium by understanding and designing the acoustic qualities of a habitat. Building on this idea, in my project, I aim to explore the acoustic-mycelium relationships in a set of systematic studies to enhance the organism's performance during the growth, which would eventually offer new ways of tailoring the material qualities in biodesign.

As described in Soundscape Ecology by Pijanowski et. al. (2011), sounds are a perpetual and dynamic property of all landscapes. The sounds of animals and the nonbiological entities like the running water of a creek and wind blowing through a forest emanate from natural landscapes. All these sounds determine the "soundscape ecology" which are part of the whole ecology. This soundscape is affected and effects all entities within this ecology. For instance, plants can detect vibrations to find water by sending out acoustic emissions or a butterfly is affected by the sounds of the leaf it sits on. Research in what ecological role the soundscape plays is called ecoacoustics (Farina, 2017). Next to biodesign research and practice, This research will build upon and contribute to this new field of research on ecoacoustics.

In my project, first I will look into what sound is in order to understand what elements of sound we could use as variable in our experiments (e.g., vibration). I will design and build an acoustic test setup, which will also act as a viable habitat for mycelium organism. In control studies I will explore the individual and collective effects of these different sound elements on the growth of mycelium-based materials and their ultimate material qualities. Eventually I will build an installation suitable for an exhibition that will communicate the research findings and inspire other designers and researchers. For that, I will adopt a material-driven design approach (Karana et al., 2015), which entails tinkering with materials next to systematic studies for technical and experimental characterization for a holistic material understanding and transferring these finding to meaningful applications/demonstrators.

I see a lot of opportunities for me to learn in this project because I will gain deeper biological knowledge and I will be challenged to build on scientific research and transfer my findings to design communities through meaningful demonstrators. This will be a unique opportunity to work on the brink of these two disciplines and move forward for innovative solutions.

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introduction (continued): space for images

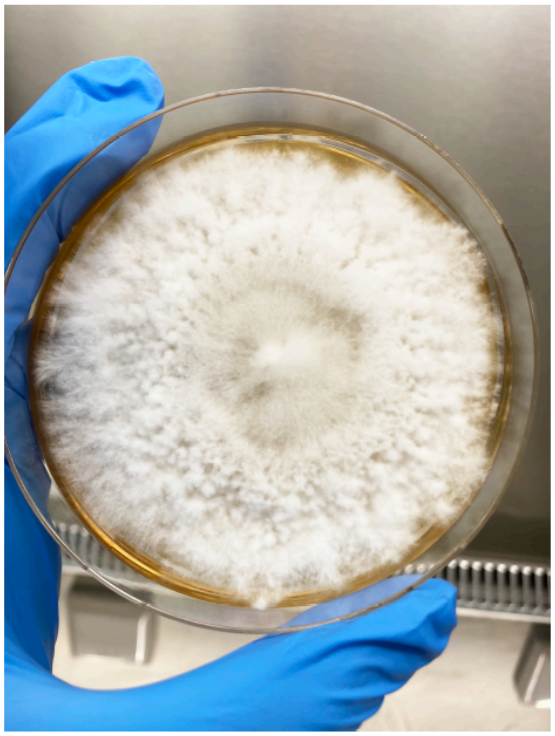


image / figure 1: Growing Mycellium

image / figure 2: Silent room

Initials & Name D Sonneveld Student number 4270894

Title of Project Living with Cacophony:

PROBLEM DEFINITION **

Limit and define the scope and solution space of your project to one that is manageable within one Master Graduation Project of 30 EC (= 20 full time weeks or 100 working days) and clearly indicate what issue(s) should be addressed in this project.

The effect of sound is often underestimated. As humans we often take sound for granted, yet sound plays a big role in the ecology. Research like Perspectives in ecoacoustics by Farina (2017) shows that sound and organism have a big influence on each other. For example, plants can absorb and resonate to specific external sound frequencies (Hou et al. 1994a, b; Hou and Li 1997a, b). Sound waves can change the cell cycle (Wang et al. 1998). It is evident that acoustic biology is becoming increasingly more popular. However, there is a big knowledge gap in this field when it comes to mycelium-based materials. In this research I want to discover what effect the acoustic habitat can have on the growth of mycelium. How does mycelium react to and process the energy of sound? What are the sounds that have a desired effect on the mycelium? Is it possible to tailor the technical and experiential qualities of a living material by careful crafting of the acoustic qualities of a habitat? Is it possible by carefully designing these acoustic environments to affect the growth physiology of the mycelium or activate stress induced genes that will suggest adaptive behaviour in these environments? The effect sound has on the mycelium will be documented by looking at the different modes of growth: Overall growth, pattern, hyphal branching, hyphal tip extension, hyphal length. This will result in a different surface texture and mechanical material properties. The biggest challenge is practical: How to design and build a suitable test setup? What type of controlled environments is needed? How to build these environments? The combination between hands on prototyping and scientific research are important for the development of this test setup. Secondly the challenge will be to balance my role as a designer, between science and design aspect of this project. I will act as a scientist in conducting systemic material research yet I will be keeping an eye out for aesthetic and design potential.
This project aims to:
1. Discover the acoustic-mycelium relationship that can lead to new insights for further research or contribute to develop innovative design solutions and new concepts of sustainable materials. 2. Inspire other people to contribute to the development of a future where mycelium plays a role inside an eco-friendly and sustainable economy.

ASSIGNMENT **

State in 2 or 3 sentences what you are going to research, design, create and / or generate, that will solve (part of) the issue(s) pointed out in "problem definition". Then illustrate this assignment by indicating what kind of solution you expect and / or aim to deliver, for instance: a product, a product-service combination, a strategy illustrated through product or product-service combination ideas, ... In case of a Specialisation and/or Annotation, make sure the assignment reflects this/these.

In this project, the effect of acoustic environments on mycelium will be explored using the Material Driven Design method. First an acoustic environment for the mycelium to grow in will be designed and prototyped. Secondly, a set of experiments will be conducted to discover new unique technical and experiential material qualities enabled by this acoustic-mycelium relationship.

This research project will provide a deep understanding of the acoustic-mycelium relationship. This understanding will be theoretical supported by a literature review and subsequently a more experience-based understanding will be developed with research experiments. This will be achieved by dividing this graduation project in three separate assignments.
1. Conducting a literature review in the field of sound. I will explore what the effecting parameters of sound are. This will result in a scientific dissection of sound so I can use this knowledge in the practical part of my research. Additionally, I will research the physiology of mycelium to get an understanding of the material. I will research how to design with mycelium and how to grow it. This will provide the knowledge base in order to conduct well designed and valuable tests.
2. Building a test setup and conducting experimental research. I will design, prototype and finally build a test environment where the experiments can be conducted. This environment is on one hand technical/theoretical (designing and delivering sound) on the other hand it is physical. Subsequently in controlled experiments I will try to understand the material on a technical and experiential level, discover new possibilities or affordances while maintaining the emphasis on the experience of the material in a broader sense as an outcome.
3. Finally I will create an object suitable for an exhibition that displays the findings. This will inspire other designers to continue or further develop the research and contribute to the field of biodesign. It also functions as a way to show the research to a broad public which can have a positive influence on the public discourse of biodesign.

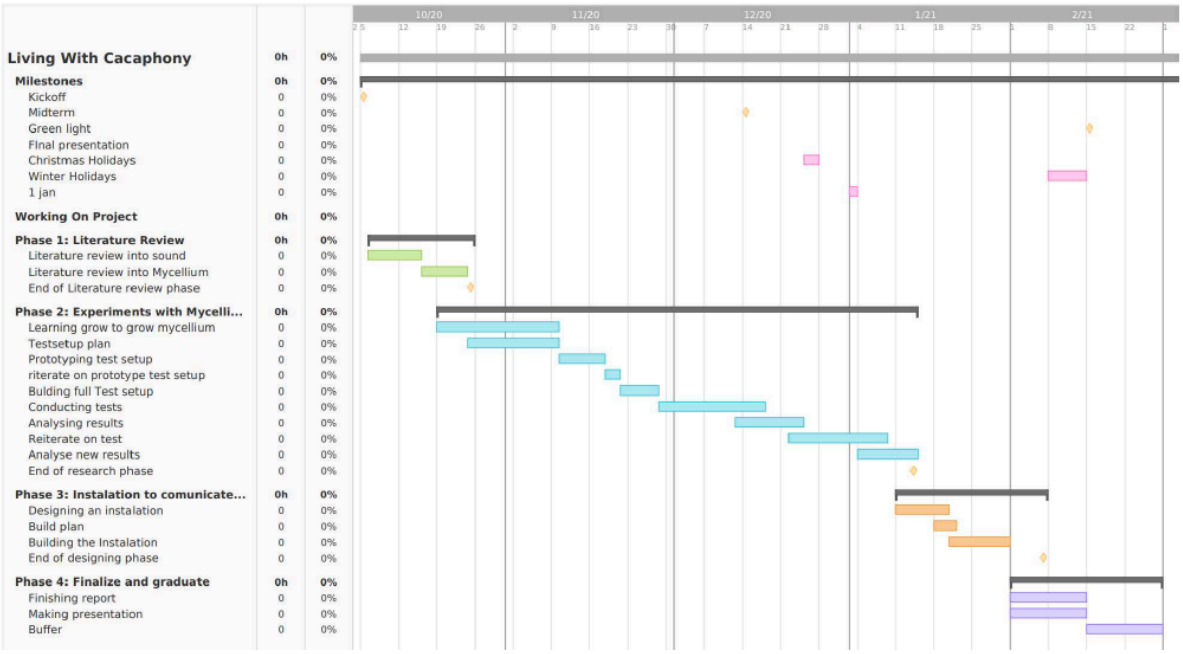
Initials & Name D Sonneveld Student number 4270894

Title of Project Living with Cacophony:

PLANNING AND APPROACH **

Include a Gantt Chart (replace the example below - more examples can be found in Manual 2) that shows the different phases of your project, deliverables you have in mind, meetings, and how you plan to spend your time. Please note that all activities should fit within the given net time of 30 EC = 20 full time weeks or 100 working days, and your planning should include a kick-off meeting, mid-term meeting, green light meeting and graduation ceremony. Illustrate your Gantt Chart by, for instance, explaining your approach, and please indicate periods of part-time activities and/or periods of not spending time on your graduation project, if any, for instance because of holidays or parallel activities.

start date 5 - 10 - 2020 26 - 2 - 2021 end date



Above the rough planning. Things to note is that I don't want to spend too much time on literature review because this is not of most importance for this project. The literature review will be embedded throughout the project over the entire length of it.

Secondly, I want to note that I don't know how much time a experiment or test with mycellium takes. This will heavily effect Phase 2. Preferably I want to do as many iterations as possible, thereby we can improve as much as possible and gain a lot more valuable results. I will discuss this together with Wasabii what is possible and define the planning better.

Initials & Name D Sonneveld Student number 4270894

Title of Project Living with Cacophony:

MOTIVATION AND PERSONAL AMBITIONS

Explain why you set up this project, what competences you want to prove and learn. For example: acquired competences from your MSc programme, the elective semester, extra-curricular activities (etc.) and point out the competences you have yet developed. Optionally, describe which personal learning ambitions you explicitly want to address in this project, on top of the learning objectives of the Graduation Project, such as: in depth knowledge a on specific subject, broadening your competences or experimenting with a specific tool and/or methodology, ... Stick to no more than five ambitions.

Us humans and the world we created around ourselves are becoming more derived from nature while the discussion on what is nature could state other but thats a whole other research on its own. This brings life to new challenges for designers like reconnecting humans and its processes with nature in a practical and philosophical way. In my opinion design should be natural, by this I mean that it should have a natural fit in our world, it should be natural on a sustainable level, usability level, aesthetic level and its meaning and place should become naturally. I envision a future where humans, nature and technology live together and merge.

This project is a great contribution or step in that direction it will yield me the biological, technical and theoretical skills that can help me achieve goal of merging nature and technology as a designer. The process of creating beautiful, applicable and desirable materials or insights from scientific research and natural phenomenon is something I want to develop further.

Creating something aesthetically pleasing in a sustainable way with a technological approach is what makes my blood flow and that is exactly what this project entails.

FINAL COMMENTS

In case your project brief needs final comments, please add any information you think is relevant.

Initials & Name D Sonneveld Student number 4270894

Title of Project Living with Cacophony:

Detailed growing procedure

STEP 1: GATHER MATERIALS FOR SUBSTRATE PREPARATION

1. Gather substrates needed.
2. Gather growing bags that contain semi permeable patches or a container like a flask.
3. Gather other materials: water container, scale, autoclave tape, painters tape water resistant marker.

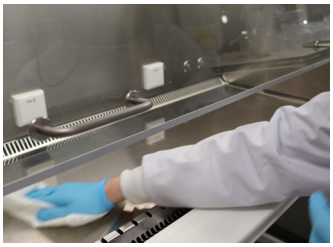
STEP 2: SUBSTRATE PREPARATION

1. Carefully weigh down the amount of substrate you want in the growing bag. Make sure the bag is not too full to prevent problems with sterilization.
2. Add the measured water to the bag according to mixture composition.
3. For sorghum let soak for 24 hours and strain excess water.
4. Close the bag with the painters tape and label the date, mixture and name. Also add a piece of autoclaving tape to the bag.

STEP 3: AUTOCLAVE

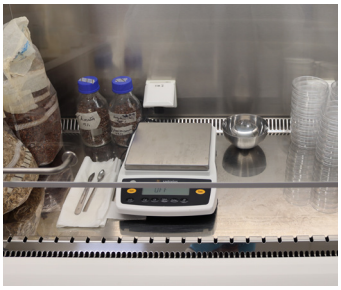
1. Open up the autoclave and check the water level.
2. Put in the containers with the prepared substrates. Make sure the containers or bags don't touch each other and the volume is not too big for full sterilization and close the autoclave machine.
3. Autoclave the substrates at 120 degrees at a pressure of 15 psi for 45 minutes.
4. Open up the autoclave machine, be careful for excess steam, and check if the autoclave tape shows black stripes.
5. Let the prepared substrates cool down to room temperature.

STEP 4: ORGANISE WORKSPACE



1. Gather all tools needed for inoculation like: scale, knife, spoon, water resistant marker, growing containers and a small bowl also gather all materials needed like isopropanol, tork roll, growing containers, spawn, prepared substrates, rubber gloves, parafilm and painters tape.
2. Thoroughly sterilize the laminar flow cabinet with isopropanol and tork roll and turn on the laminar flow, light and socket.
3. Sterilize all the tools and materials that go into the laminar flow cabinet. Be sure to minimize the amount of things inside the cabinet to prevent disturbance of the laminar flow.

STEP 5: INOCULATION



1. It is of utmost important to work very sterile during this phase. The change of contamination is the biggest during this step.
2. If growing inside the bag skip 3 and 4.
3. Label all the containers that are used to host the growth of the

mycelium composite. with the date and type of composite.

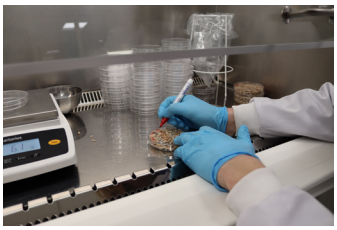
4. Cut open the substrate bag, measure the needed substrate in the growing container and close the container, repeat for all containers.
5. Precisely measure the needed spawn with a spoon in the small bowl and add to the substrate repeat for all containers. Minimise the time the growing containers are opened.
6. Write down all weights of the prepared mycelium composites, date when the spawn was inoculated.
7. Close the spawn used and left over of the prepared substrate.
8. Close the growing containers, for petri dishes use parafilm to seal them. Parafilm is semi permeable and allows the exchange of air and moisture.
9. Shake the composite containers thoroughly in order to distribute the spawn throughout the substrate.
10. Put the prepared composites in the incubator and clean workspace. Shut down the laminar flow cabinet.

STEP 6: INCUBATION

1. Incubator settings at the MI Lab are: 30 degrees, 70 percent humidity in total darkness.
2. let the mycelium colonise the substrate inside the incubator. This time differs per composition of substrates and strain.

STEP 7: REMOLD

1. When fully colonised take the composite material out of the incubator.
2. prepare workspace like with inoculation, still pay attention to work sterile but chance of contamination is lower at this phase.
3. Label all the molds that are used with the date and composite mixture.
4. Cut open the substrate container, measure the needed substrate in the mold and close the mold with for instance perforated film, repeat for all molds. Pay attention to the compression of the substrate in the mold this will affect the final material and growing time.
5. Put the molds inside the incubator and let the mycelium recover and cement the substrate.
6. For skin formation, take the composite out of the mold when fully colonised and let the mycelium grow longer covered by perforated film in the incubator.



STEP 8: DRYING

1. Take the mycelium composite and put uncovered in the over at 60 degrees for 24 hours to kill the mycelium.

Growing exploration

This chapter describes the first explorations into growing mycelium composites. The goal was to gain experience in the steps of manufacturing of mycelium composites.

Step 1 Substrate preparation
The first step of the procedure is mixing the substrate with the water inside of a mycelium bag. These bags have an air filter so air can be exchanged but the inside can not be contaminated.

As a rule of thumb, the composition of the bag should be the following for optimal growth.
- 30% Substrate
- 65% Water
- 5% Spawn

With 250gr substrate this relates to:
- 833.33 gr Total weight
- 250 gr Substrate
- 541.67 gr Water
- 41.67 gr Spawn

In this first exploration 6 bags were made:
Sorghum (new spawn)
- Hay
- Hemp fibre
- Wood fibre
- Cacao peel
- Fax

After weighing the bags were closed with autoclave tape and labelled. Labelled and closed the bags are placed in the autoclave to make the inside of the bag sterile and prevent contamination. The autoclave brings the bags up to 121 °C under pressure for 45 minutes.

Step 2 inoculation
When the bags are taken out of the autoclave they rest to be cooled down in order to inoculate the bags. If the bags are too hot when inoculated the mycelium will be killed.

Inoculation is a vital moment in growing mycelium, the chance of contamination is high therefore it is important to work very sterilely. The inoculation is done in a laminar flow cabinet to minimize the chances of contamination.

A complete and step by step description of the inoculation procedure can be found in the appendix.


VIBRA
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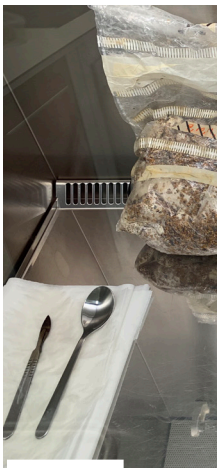
Zero/Tare



Step 3 Mold
After a week the bags should be fully colonized by the mycelium with a formed skin. At this stage the colonized substrate material can be put inside a mold for final forming. Again working sterile and inside a laminar flow cabinet.

Step 4 take out of mold
A week later the products inside the mold should be rigid and can be taken out of the mold. Now the product will be taken out so that a skin can form on the outside of the product. This will make the product more appealing and have a soft skin on the outside. A Plastic foil is put around the mycelium composites to prevent them from drying out.

Step 5 Drying
When a nice skin has formed the product they can be dried in the oven. The products are placed inside the oven for 24 hours on 65 degrees.

OBSERVATIONS WEEK 1
The first batch showed contamination within the first week. Green spots appeared in the bags. This is a different kind of fungi probably penicillin according to John. This means the bags are not useful to continue. The bags were sterilized in the autoclave and discarded.

OBSERVATIONS WEEK 2
During the first week I noticed that no contamination had occured and the mycellium was growing well. The mycellium grew particularly wel on the hemp fibre and flax seed. The wood fibre and cacao were growing less fast because it is more dense. While putting the substrates in the mold I noticed that how tightly one packs the mold with the substrate has a big effect on the end product. Packing the mold is a important step for making a consistent and desired end product. After a week in the mould I noticed that the mycellium regenerated through the mould but not on the outside. A few days out of the mould a nice skin on the outside formed especially on cacao. After a few days more i saw that the white skin had turned grey and brownish and i showed fruiting bodies which is a clear sign that the mycellium has grown to long.

GROWING CONCLUSIONS

Importance of working sterile

Working sterile during the inoculation is very important because this is when the substrate specimen is most suspectible for contamination. In others stages like molding it is also important but not as mutch. Then the mycellium is already strong and can eleminate intruders.

Time

The time for growing will be a limiting factor for the amount of experiments possible. Only the first week of growth is usefull for the experiments in order to see change.

Substrates

All substrates reacted different and had different growing times. The substrate with the highest growing efficiency will be chosen which is sorghum.

Skin

Also the skin is important part of mycelium composites and the mycelium should have room to grow this skin unlike with the molds.

Incubator benchmark

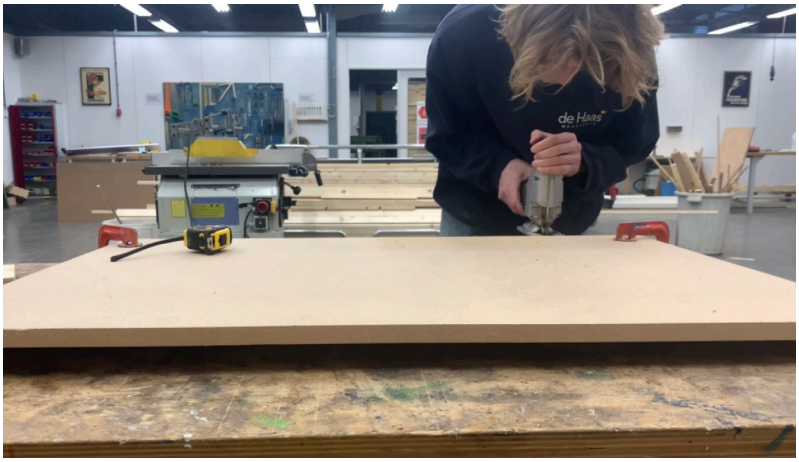
Incubator	Pros	cons	SPecial	Price	Size	Temperature	Humidity	Insulation	Material
 INCUBATOR-SERIE 636 PLUS 65 L	Compact and inexpensive	no humidity		1480,-	65L	5-80 Still air incubator	no	double wand	Steel inside and out
 Memmert incubator	High quality and preheated air supply, Full lcd screed display	very expensive	Stainles steel adjustable grid racks		160L	30-80 Still air and forced air.	no	double wand with glass wool	stainles steel inside and out
 Biolab Incubator	Very extensive User interface With automatic start, automatic stop, timed operation, clock display function Ambient temperature detection function	expensive	cooling and warming		150L	0-60 Still air	yes 30-95%	Double wand wit PU foam	stainlessteel outside aluminium inside
 Heratherm incubator	most acurate and effective incubator on the market	very expensive	nterior glass door, interior socket, access port	3297,-	104L	105 max still and forced air	yes 30-95% method unknown	unknown	stainlessteel outside aluminium inside
 Heratherm compact bio	simple and effective and cheap	no humidity controll and very small		460	15L	30-70	no	double wand with glass wool	stainlessteel inside and out
 memmert co2 icomed incubator	Humidity controll		double seal at the door, CO2 incubator		160	18-50 still air and forced air	yes 30-95% with humidifier and fan	double wall with glass wool	Stainless steel
 salvisLab Incucenter	usb connectivity		looks nice	5422,-	240	5-110 cooling and heating still air and forced air	yes 30-95%	double wand glass wool	stainless steel
 salvisLab Incucenter CO2	Double door Process data download and high temperature	no cooling	data logging		190	no cooling -200forced and still air	yes 30-95% with heat bath	double wall glass door	stainless steel
 Binder CO2 incubator	Fast recovery with preheated temperature	very complicated	water connection and possible cooling with peltier module	12000,-	240	forced heat with specific air flow	yes with water hose connection	double wall	stainless steel

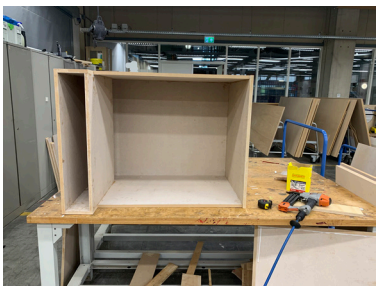
Incubator build

This appedix will provide a short discription of the incubator build and images of the process. Special thanks to the avans university and the workshop supervisers for the advice and support.

WOOD CONSTRUCTION

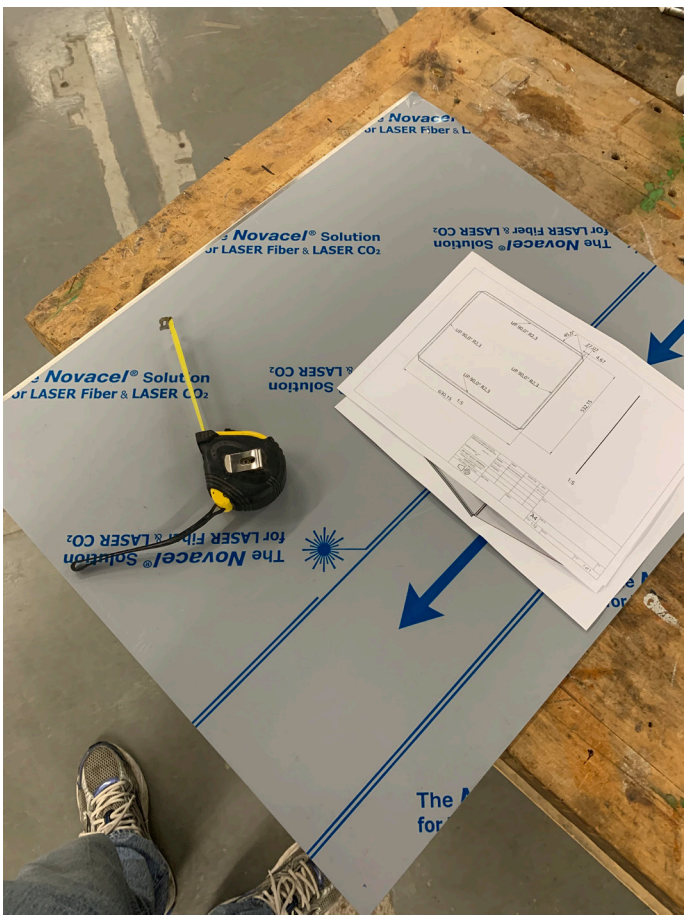
After the whole design was modelled in the computer the wood was first cut and constructed with wood glue and a nailgun.

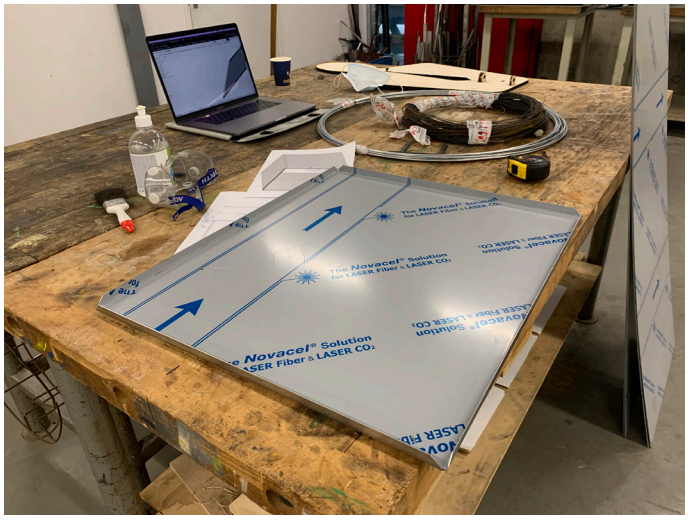




INSULATION

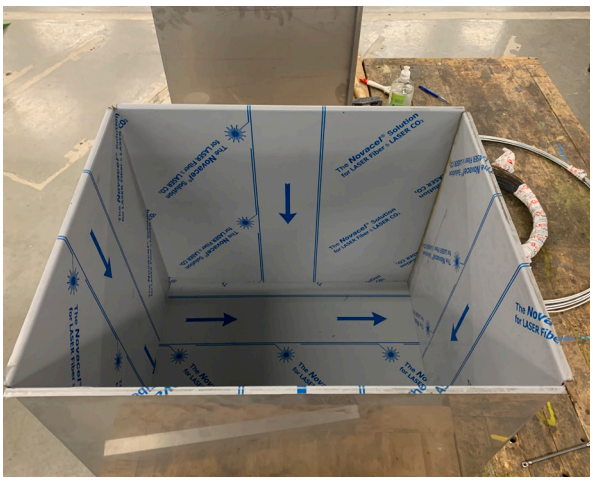
When the outside of the cabinets were finished the insulation was measured and cut to size. First the cotton insulation was put in whereafter the gypsum fiber board and last the rockwool before putting in the metal container.





METAL GROWING CONTAINER

When the insulation was done the metal sheets were cut to size and bent for assembly. The box is glued together with strong hold bison kit. This was pressed and clamped with woodbeams and left over the weekend. The stainles steel appeared to be a very tough material to work with but the result was very pleasing.



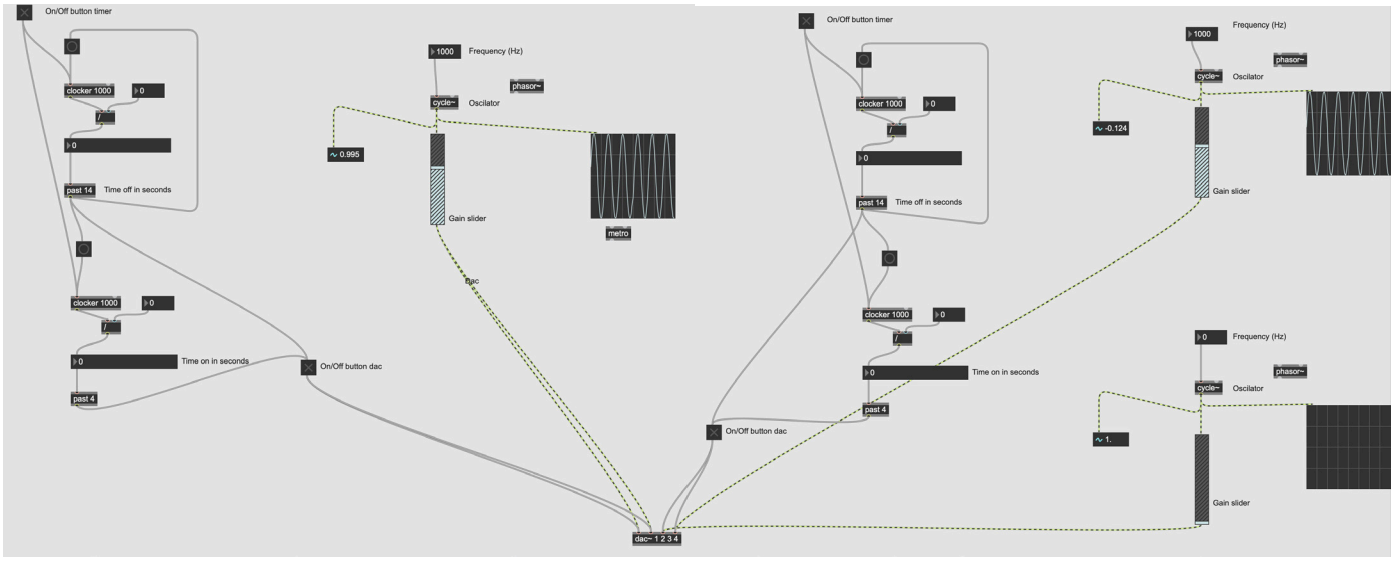
ASSEMBLY

Now that the oustide and inside cabinets were finished the assembly started. This was the hardest part of the build. All the boxed had to fit into the box and this needed some adjustments here and there. The hardest part was placing the stainless steel growing container that had to be placed precisely so that the door would seal and close properly. After that worked the frame and electronics and humidity system were installed and finally the incubators were painted with sikkens rubbol rezisto.

Incubator build

	BUDGET	Incubator					
	item	Partlink	unit	*price/piece*	amount		*subtotal*
SOUND/VIDEO							
	amplifier	Thriftshop	piece	€ 25,00	0	€	-
	High frequency speaker		piece	€ 15,00	0	€	-
	cables	Thriftshop	generic	€ 5,00	2	€	10,00
	IR camera	amazon	piece	€ 7,75	3	€	23,25
	Raspberry pi zero plus cables	https://www.kiwi-electronics	Piece	€ 15,50	3	€	46,50
	SD card 8 GB	https://www.amazon.nl/King	piece	€ 5,75	3	€	17,25
	SUBTOTAL					€	79,75
HUMIDITY AND TEMPERATURE CONTROLL							
H	Verneverlaar	https://www.amazon.nl/dp/E	Piece	€ 12,00	1	€	12,00
H	humidity controller		Piece	€ 11,00	1	€	11,00
H	fan	https://www.digikey.nl/produ	piece	€ 2,75	2	€	5,50
H	12v adapter	https://www.digikey.nl/produ	piece	€ 8,75	1	€	8,75
T	temperature controller	https://www.amazon.nl/ICQ	piece	€ 11,25	1	€	11,25
T	heating cable	https://www.junai.nl/junai-si	piece	€ 7,75	1	€	7,75
T	Power cable	Thriftshop	piece	€ 2,50	1	€	2,50
	SUBTOTAL		piece	€ 58,75	3	€	176,25
INCUBATOR HOUSING AND CONSTRUCTION							
	Outer Box (MDF)	https://www.gamma.nl/assc	2.5 m^2	€ 20,00	1	€	20,00
	Sound Insulation	https://www.gamma.nl/assc	m^2	€ 26,00	1	€	26,00
	sheet metal	https://rietcon.nl/bestelpagi	m^2	€ 53,00	2	€	106,00
	Fire retardant board	https://kunststofplatenshop	m^2	€ 12,00	1,67	€	20,04
	Rubber door seal	Thriftshop	piece	€ 15,00	1	€	15,00
	Paint	Hornbach	piece	€ 2,50	1	€	2,50
	Rivets and Small electronic parts		piece	€ 5,00	1	€	5,00
	screws and glue	gamma	Generic	€ 3,00	1	€	3,00
	noppenschuim	Wasabii	m^2	€ 35,00	0	€	-
	hinges and handles		piece	€ 20,00	1	€	20,00
	SUBTOTAL			€ 217,54	3	€	652,62
MODULAR HANGING SYSTEM							
	Aluminum frame	https://www.amazon.nl/dp/E	meter	€ 9,50	3	€	28,50
	connectors		Piece	€ 2,75	4	€	11,00
	SUBTOTAL			€ 39,50	3	€	118,50
TOTAL							
	TOTAL ESTIMATE COST					**€**	**1.027,12**
	UNFORSEEN COST				10%	**€**	**102,71**
	TOTAL ESTIMATE COST					**€**	**1.129,83**

MAX Patch, Matlab, raspbery code




```
clc;    % Clear the command window.
close all;  % Close all figures (except those of imtool.)
clearvars;
workspace;  % Make sure the workspace panel is showing.
format long g;
format compact;
fontSize = 18;


% Specify the folder where the files live.
myFolder = '/Users/diederiksonneveld/Documents/TU Delft/Graduation/Experiment result images/Experiment A
% Check to make sure that folder actually exists.  Warn user if it doesn't.
if ~isfolder(myFolder)
    errorMessage = sprintf('Error: The following folder does not exist:\n%s', myFolder);
    uiwait(warndlg(errorMessage));
    return;
end

% Get a list of all files in the folder with the desired file name pattern.
filePattern = fullfile(myFolder, '*.jpg') % Change to whatever pattern you need.
% Get a file listing of files in myFolder from the operating system:
theFiles = dir(filePattern);
hFig = figure;
hFig.WindowState = 'maximized';
allCounts = zeros(1, 256);
n = 0
% Now loop over all found files, renaming each in turn with the new desired extension:
for k = 1 : length(theFiles)
    % Get the input filename.
    baseFileName = theFiles(k).name;
    fullFileName = fullfile(myFolder, baseFileName);
    % Read in the image
    theImage = imread(fullFileName);
    % Convert to gra scale if needed.
    if ndims(theImage) == 3
        theImage = rgb2gray(theImage);
    end
    %subplot(2, 3, 1);
    %imshow(theImage, []);
    %title('Original Image', 'FontSize', fontSize);
    %drawnow;
    % Get the histogram.
    %subplot(2, 3, 4);
    %imhist(theImage);
    %grid on;
```

```
    %drawnow;
    %title('Original Image', 'FontSize', fontSize);

    % Threshold it at 120.  Mask it so that less than 120 shows up as black.
    thresholdedImage = theImage;
    thresholdedImage(theImage < 30) = 0;
    subplot(2, 2, 1);
    imshow(thresholdedImage, []);
    title('Greyscale Image', 'FontSize', fontSize);
    drawnow;
    % Get the histogram.
    subplot(2, 3, 4.2);
    imhist(thresholdedImage);
    grid on;
    ylim([0 700000]);
    drawnow;
    title('Histogram of Greyscale Image', 'FontSize', fontSize);

    threshold = 0.2;
    copyimage = im2bw( theImage, threshold);
    % Do a "hole fill" to get rid of any background pixels or "holes" inside the blobs.
    copyimage = imfill(copyimage, 'holes');
    WhitePixels = sum(copyimage(:));

    thresholdLevel = 0.5; % Get threshold.
    binaryImage = im2bw( theImage, thresholdLevel); % Do the binarization
    numWhitePixels = sum(binaryImage(:));
    numBlackPixels = sum(~binaryImage(:));
    % ROI as a percentage of Total Image
    pwp = (numWhitePixels / (WhitePixels)) * 100;
    test = '%';
    %Peremiter of binary image
    %perimImage = bwperim(binaryImage);
    %numPerimeterPixels = sum(perimImage(:));
    caption = sprintf('Binarized Image Mycelium percentage %.1f %c', pwp, test);
    % Plot the binary image.
    subplot(2, 2, 2);
    imshow(binaryImage);
    title(caption, 'FontSize', fontSize);




    theseCounts = imhist(thresholdedImage);
    n = n + 1;
    allCounts = (allCounts + theseCounts); % Add counts for this image into the running total.
    subplot(2, 3, 5.8);
    plot((allCounts / n), 'LineWidth', 2);
    ylim([0 300000]);
    xlim([10 250]);
    grid on;
    drawnow;
    title('Average histogram of Greyscale Image', 'FontSize', fontSize);

    promptMessage = sprintf('Do you want to Continue processing,\nor Quit processing?');
    titleBarCaption = 'Continue?';
    buttonText = questdlg(promptMessage, titleBarCaption, 'Continue', 'Quit', 'Continue');
    if contains(buttonText, 'Quit', 'IgnoreCase', true)
        return;
    end
end
```

```
import RPi.GPIO as GPIO

import time
import picamera

timestamp = time.strftime("%Y-%m-%d_%H%M")
variable = "/home/pi/camera/I1_" + timestamp + ".jpg"

GPIO.setmode(GPIO.BCM)
GPIO.setup(4, GPIO.OUT)

GPIO.output(4, GPIO.HIGH)

with picamera.PiCamera() as camera:
        camera.start_preview()
        # Set ISO to the desired value
          camera.iso = 800
          # Wait for the automatic gain control to settle
        time.sleep(5)
          # Now fix the values
          camera.shutter_speed = 50000
          camera.exposure_mode = 'off'
          g = camera.awb_gains
          camera.awb_mode = 'off'
          camera.awb_gains = g



        camera.capture(variable)
        camera.stop_preview()

GPIO.output(4, GPIO.LOW)
```

Wiring diagram