
# DESIGN AND TESTING OF MYCELIUM BIOCOMPOSITE

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# DESIGN AND TESTING OF MYCELIUM BIOCOMPOSITE

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# DEDICATION

This thesis is dedicated to my group members of Introduction to Materials Science and Engineering for it was during the teamwork the mycelium composite idea originates.

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All praise is due God, the Most High, the Lord of the world, who has given me the grace to come this far, His mercy has seen me through successfully.

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## ABSTRACT

The growing global understanding of ecological footprints and environmental pollution invented by humans is gainfully affecting our material design practice, pushing the search for more sustainable alternatives, and development of natural biocomposites. In this research, the natural ability of saprophytic fungi to digest and bind lingo-cellulose is utilized to develop natural bio-composite materials for novel applications in design and architecture. This study aims to provide an insight into the production methods of mycelium-based materials and an indication of the structural performance of these bio-based materials. Several fungi species were grown on varied local agricultural-growth wastes, and different growing conditions were carefully elucidated to evaluate which pair of fungi-plant material provides the most suitable combination for product applications. The fungi; *Polyporus Squamosus, Pleurotus ostreatus, and Volvariella volvacea* were grown on woodchips of Mansonia altissima, *Terminalia Ivorensis,* Brachystegia nigerica, Combretodendron macrocarpum, Kyaya ivorensis, and Hemp. A detailed study of the mechanical behavior under compressional and flexural conditions was also evaluated. At 70% deformation, the maximum compressional stress for the optimum composition (hemp with Grey dove mushroom) was found to be 0.452 MPa. The maximum flexural stress for the optimum composition (hemp with oyster mushroom) was obtained at 0.397 MPa. The samples were also tested for selected properties including water absorption rate, density, and quality impression. By examining these fundamental materials characteristics, we aim to achieve a thorough understanding of the structural and aesthetic opportunities that this novel bio-material should offer. The current stage of the research shows that the most efficient integrations where the samples of *Polyporus ostreatus* grown on Hemp woodchips. Future work will focus on chemical treatment of the fibers, locating essential variable parameters and post-processing to achieve

desired material properties and introduce innovative characteristics and functions over existing industrial products and applications.

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# CHAPTER ONE

## 1.0 INTRODUCTION

## 1.1 BACKGROUND

The rapid growth in the economy, the continual extraction of natural resources for manufacturing, and the rate of consumers consumption are the main factors constituting environmental deterioration (Straughan and Roberts 1999). The concept of green or bio-composites sourced from natural biodegradable polymers is attracting great interest and has emerged to help society achieve sustainable consumption (Guadalupe et al. 2011; Hashim, Tanner, and Oleiwi n.d.; Lee and Wang n.d.; Nair and Laurencin 2007). Eco-design for sustainability (D4S) has been described as a design approach with special consideration for the environmental impacts and performance of a product holistically over its entire life cycle. Some examples of such impacts are in the aspects of $CO_2$ emission, energy renewability, product recycling, and toxicity. Many societies have seen this light and begin to enforce a practical demonstration of sustainability towards a green environment. For example, India has adopted a rating system known as GRIHA, the system attempts to reduce a building's resource consumption, level of waste generation and the total of ecological impact within limits of certain nationally acceptable benchmarks.

A sustainable development design strategy is a deliberate global concern towards a healthy ecology. One of the strategies involves reducing the consumption of non-renewable natural resources and encouraging the use of new bio-based materials in design procedures (Álvarez-Chávez et al. 2012; Alves et al. 2012). A known sustainable design approach referred to as *Growing Design* (Karana et al. 2018) involves growing bio-based materials from biological living organisms with attractive properties, unique functions and achieve

sustainable design solutions. Bio-based materials have been defined as “a material of which one or more of its components are sustainably grown and are fully renewable” (Lelivelt et al. 2015). These materials provide a useful solution to global solid waste issues and present an essential approach for a cleaner, more sustainable future. Composites defined as bio-based materials, often include a ductile matrix based on petrochemicals and high-strength reinforcement consisting of natural fibers(Koronis, Silva, and Fontul 2013; Faruk et al. 2012; Cicala et al. 2017). Such composites offer attractive performance at low manufacturing cost and allow great freedom in manipulating the material design to suit a specific application (Lelivelt et al. 2015).

## 1.2 PROBLEM STATEMENT

In our society, many persons have basic knowledge about edible mushroom for nutritional and medicinal values (growth of mycelium biomass) but know little or nothing about the fungal network growing underneath the surface, known as mycelium. One of the latest innovations involves harvesting the web-like network of mycelium to stick organic materials together into a rigid product (Holt et al. 2012). Growing fungus in proper conditions results in a material with properties similar to cement, engineered wood or plastic, depending on the manufacturing method. This innovative design is a biological additive manufacturing, that includes a combination of bio-engineering, structural architecture and the commercial use of mycelium bio-composites since 2007 and is slowing replacing environmentally draining materials (Travaglini and Ross 2016). However, there is limited scholarly research about this innovation, inadequate knowledge of the structural properties, potential applications and few of the mycelium products exist in the market. Therefore robust research directed towards knowing the mechanical

properties, suitable applications and possible improvements of the mycelium-based materials is needed.

## 1.3 SCOPE OF WORK

This study investigates the effect of combining different wood fibers and mushroom species on the mechanical properties of mycelium based-composites. The study examines:

1. The processing of different wood particles acting as fiber reinforcements;
2. Manufacturing of the bio-composites with different compositions;
3. The microscopic/mechanical characterization of the bio-composites, including;
    I. Scanning electron microscopy;
    II. Compressive strength test;
    III. Flexural strength test;
4. Measurement of physical properties including;
    I. Water absorption
    II. Density

## 1.4 AIM AND OBJECTIVES

This paper placed focus on a fungal mycelium-based bio-composite that is grown rather than manufactured or synthesized. This present study aims to give a robust investigation of the physical and mechanical characterization of indigenous mushroom-wood composite.

In other to achieve this goal, it was necessary to achieve the following objectives;

1. Locate the most suitable fungi-substrate combination for further exploration and development.
2. Evaluation of the microstructure of the grown bio-composite
3. Investigation of the mechanical properties of the grown biocomposites

4. Recommendation of possible applications of the developed bio-composites.

## 1.5 MOTIVATION

In nature there is practically no waste, it is altogether a regenerative system wherein all outputs become inputs; everything constitutes a recyclable loop. The concept of waste is entirely conceived by humans. Over the decades, the linear concept of produce-use-dispose has proven unsustainable in the face of limited resources, hence William McDonough and Michael Braungar created a concept known as cradle to cradle (Dougoud et al. 2018). It suggests an approach that tends to achieve waste elimination by incorporating material resources into a biological and technical cycle such that generated waste becomes nutrients for other systems and energy generation is achieved through the use of renewable resources.

The growing global understanding of ecological footprints and environmental pollution invented by humans is gainfully affecting our material design practice, pushing the search for new materials and solutions. As the environmental ecology continues to worsen, it has become a persistent public concern in developed countries and it has recent times awakened developing countries to the green movement (Bajpai, Singh, and Madaan 2014). This present global concern for sustainable ecology has sparked the emergence of new design practices, driving researchers and material scientists to find eco-friendly bio-based alternatives to traditionally used materials such as plastic and cement bricks. Emphasis is placed on recyclability and sustainability.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

## 2.1 MYCELIUM

*Mycelium* is the interwoven root-structure growing beneath the ground of the fungus-mushroom. It is a network structure constituting of thread-like tubular filaments known as hyphae. A 'hypha' is the most basic developmental unit of filamentous fungi, which grows by apical tip elongation and occasionally branch out or merge with other hyphae extending into the surrounding substrate, forming a random network-like structure of the mycelium (Walker 2009; Kavanagh, 2011; Fricker, Boddy, and Bebber 2007). Biologically active hyphae bind to and/or digest organic material by applying mechanical forces and secreting hydrolytic enzymes. The cell wall of the hypha is compose of chitin nanofibrils (Fig. 1C) which plays several physiological roles in fungi morphogenesis, protecting the hyphae (Michalenko, Hohl, and Rast 2009;Thomson et al. 2015;Papagianni 2004), providing stiffness and strength to the whole mycelium (Vega and Kalkum 2012). A typical cell wall of a hypha consists of chitin, glucans and an outer layer of proteins such as mannoproteins and hydrophobins (Bartnicki-Garcia 2003).

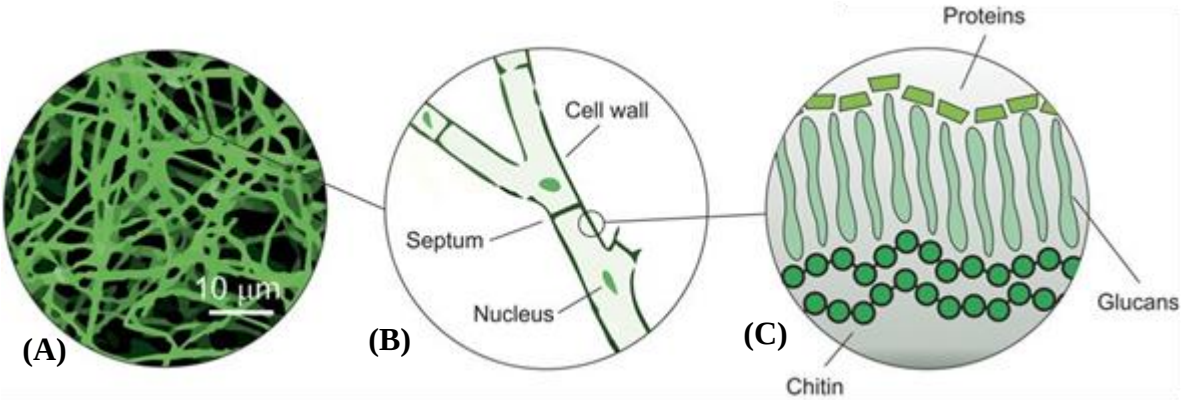



Figure 2. 1: (A) Optical microscopy of mycelium film (B) Schematic representation of a hypha (C)Schematic representation of the cell wall, (Vega and Kalkum 2012).

Many potential applications of mushroom fungi have long been explored in human history, cutting across the production of food (such as cheese, bread, and beer) to medical biotechnology (such as antibiotics and antivirals) (Wainwright 1992). However, only lately have fungi been considered and explored as sustainable alternative resources for bio-based materials (Holt et al. 2012; Karana et al. 2018). Mycelium-based materials have been grown by two alternative methods: either exploiting the abilities of mycelium to interlock other substances within its network to form a bulk material, thus acting as natural self-assembling glue (mycelium-based composites; Figure 2A), or cultivating a liquid culture of mycelium (pure mycelium; Figure 2B) (Haneef et al. 2017;Holt et al. 2012).

Figure 2. 2: (left) Mycelium composite, (right) pure Mycelium

### 2.1.1 MYCELIUM – FUNGI

This section presents fundamental knowledge about mycelium, how it feeds, reproduces, grows to maturity and behaves. These are the necessary information needed to understand the underlying process of manufacturing mycelium materials.

### 2.1.2 FUNGI – CLASSIFICATION AND CHARACTERIZATION

The fact that Fungi are heterotrophic organisms means they feed by decomposing organic substances. Yeasts and Moulds are examples of fungi. Of all the biological classifications of the fungi subtaxa, two are of interest for generating mycelium materials; the **Ascomycota** and **Basidiomycota**.

One of the very useful characteristics of Basidiomycota is hyphal Anastomosis (or hyphal fusion). An Anastomosis is the ability of two adjacent or encountering vegetative hyphae to form a cross-connection and fuse together when they (Chagnon 2014). Two important functional consequences of anastomosis necessary for creating mycelium materials are worthy of note. A fast-growing mycelium is a direct consequence of anastomosis and it is crucial for the creation of large networks. Larger networks allow uniform and wide distribution of nutrients from regions sufficient in nutrients to regions poor in nutrients. This permits a more homogeneous multidirectional growth of the hyphal colony giving rise to faster colonization of the substrate. Another benefit of anastomosis is the fact that it generates a stronger and denser mycelium. As more hyphae are crosslinked the resulting mycelial network becomes much more coherent and able to safely dissipate stresses more efficiently than a mycelium that lacks anastomosis (Carlile, Watkinson, and Gooday 2001).

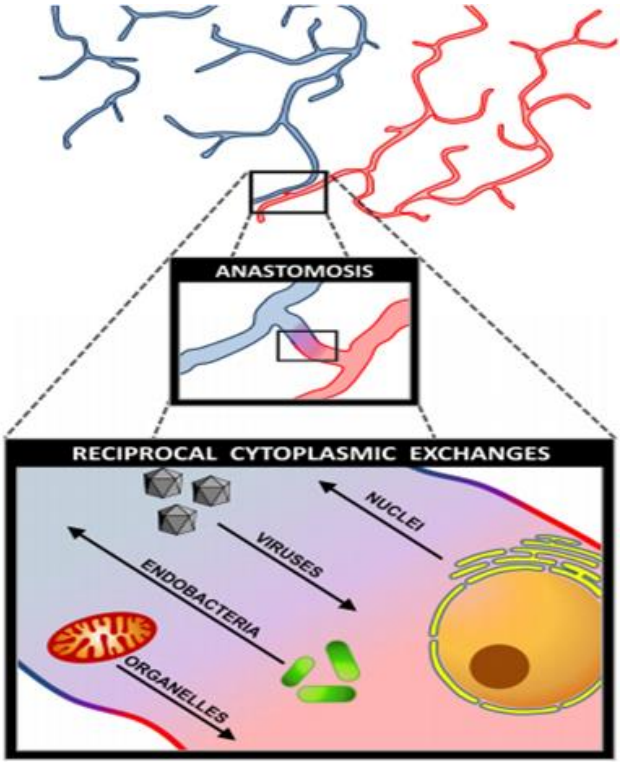



Figure 2. 3: Inter-individual exchanges during anastomosis events (Chagnon 2014).

The Basidiomycotus starts by inoculation of its spores on a suitable growth medium or substrate such as in soil, (dead) wood, or debris such as dead leaves. The spores start to grow upon germination apically (at the tip) into long tube-like filaments known as hyphae. Such hyphae have a diameter of 5-15 μm (Geitmann and Emons 2000). The hyphae grow into dense structures through the substrate to find nutrients and/or to 'mate' with other compatible hyphae, creating a network known as mycelium. A developing fertile mycelium breaks down an organic matter of the substrate absorbing nutrients from its surroundings and expands at an exponential rate. (Stamets, 2005). When two adjacent mycelia meet, their cells can fuse by the process of anastomosis, condensing into hyphal a knot, a larger and stronger organism. When the organism has sufficiently grown strong, it starts to develop denser networks of certain inflatable cells at a stage where it finds a free surface. At this point, under suitable conditions, it will begin to sprout into fruiting bodies. The special cells are known as primordia and the process of generating primordia is known as "pinning". When these special cells of primordia are completely developed under suitable environmental conditions such as humidity and temperature, they then grow exponentially by absorbing water rapidly from the environment through the extensive network of the hyphae. Consequently, these primordia develop into the fruiting bodies of the basidiomycete, known as the mushrooms. The growing organism selects the most promising few from the multitudes of primordia, channeling all its energy and available nutrients to these few to develop into mature fruiting bodies of the mushroom, which then generate new spores. Spore generation is the sexual reproduction phase of the mushroom life cycle. Most mushroom strains are often shaped like a high pillar with a cap over the top. The cap houses the new spores that will periodically be released into the environment for propagation by airflow. The height of the mushroom ensures that the spores are

elevated to be appropriately dispersed over a large area. The spores that settles on suitable habitats can germinate and start producing mycelia of their own, beginning the basidiomycete life cycle anew!

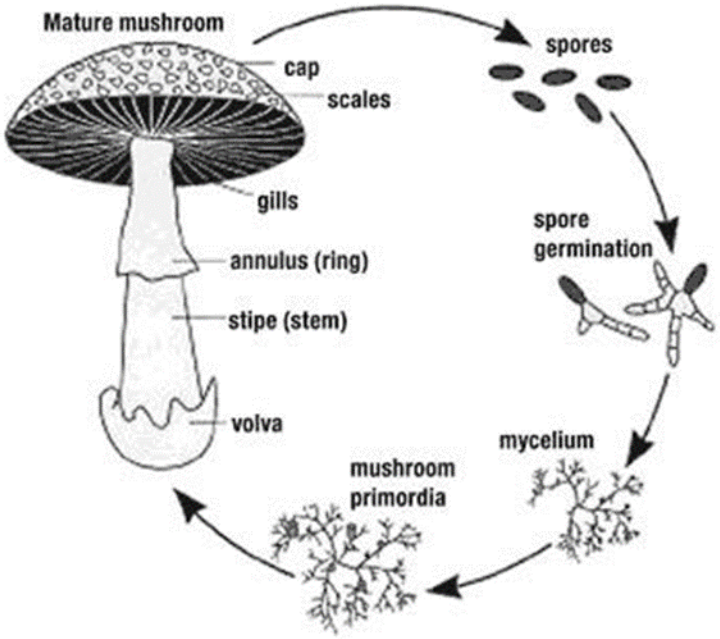



Figure 2. 4: Basidiomycete life cycle

## 2.2 OVERVIEW OF GROWING METHODS

Predominantly, Basidiomycota are only agriculturally cultivated for their mushrooms. It is therefore, expected that we learn how to mushroom cultivate a mycelium from the mushroom industry. The pinning process however is unwanted and thus needs to be prevented. For a mycelium-based material it is more productive to allow the organism focus on creating a strong and dense mycelium than letting it waste its nutrients on developing biologically expensive fruiting bodies. The cultivating process of mycelia consists of four steps and to produce mycelium-based composite materials additional three steps are required (Lelivelt et al. 2015). The flow-chart process is shown in Figure 3. The first step involves producing a suitable habitat for the fungus known as the substrate. The substrate can be any natural fiber material rich in cellulose such as hemp, wood or straw.

The purpose for which the fungus is cultivated is important but also the nutrient composition of the substrate varies with the strain of fungus. For example, if the goal is to harvest bulk mushroom, a cheap but nutritious (high cellulose content) substrate, like straw, is preferable. If the fungus is cultivated for genetic research in a biological laboratory it is necessary to have a very clean and controllable substrate like a sugar solution.

When the appropriate substrate has been selected and mixed, there is a need for the substrate to be adequately sterilized to guide against other malicious organisms from competing with the fungus during growth. Several methods have been identified to do this and they will be discussed in detail in section 2.2.1**.**

After sterilization the substrate can be inoculated with the spawn of the desired fungus. It is expected that working tools and environment are sterilized similarly to prevent contamination. Preferably pre-grown spawn cultivated by specialist companies that work under specific conditions to create very pure and reliable spawn are used.

After inoculation the fourth step, which is the final step for production of mycelium, begins. Now, it is expected that the fungus colonize the substrate by growing through the mass. This is the most crucial step and it is important to provide the correct growing conditions, which once again vary according to the type of species and depend on the goals of the cultivation. The growing methods and conditions are highlighted in section 2.2.2.

To finally produce a mycelium material out of the colonized substrate, the growing process needs to halt. The termination of the growing phase of the mycelium is important else the fungus would still be alive and would ultimately consume the entire substrate and/or start to develop fruiting bodies which is not the goal. The sample material is preferably

demolded before terminating the growth which can be done by prolong heating at an elevated temperature. Usually to enhance the surface properties of the material a coating might be added as a surface finish phase.

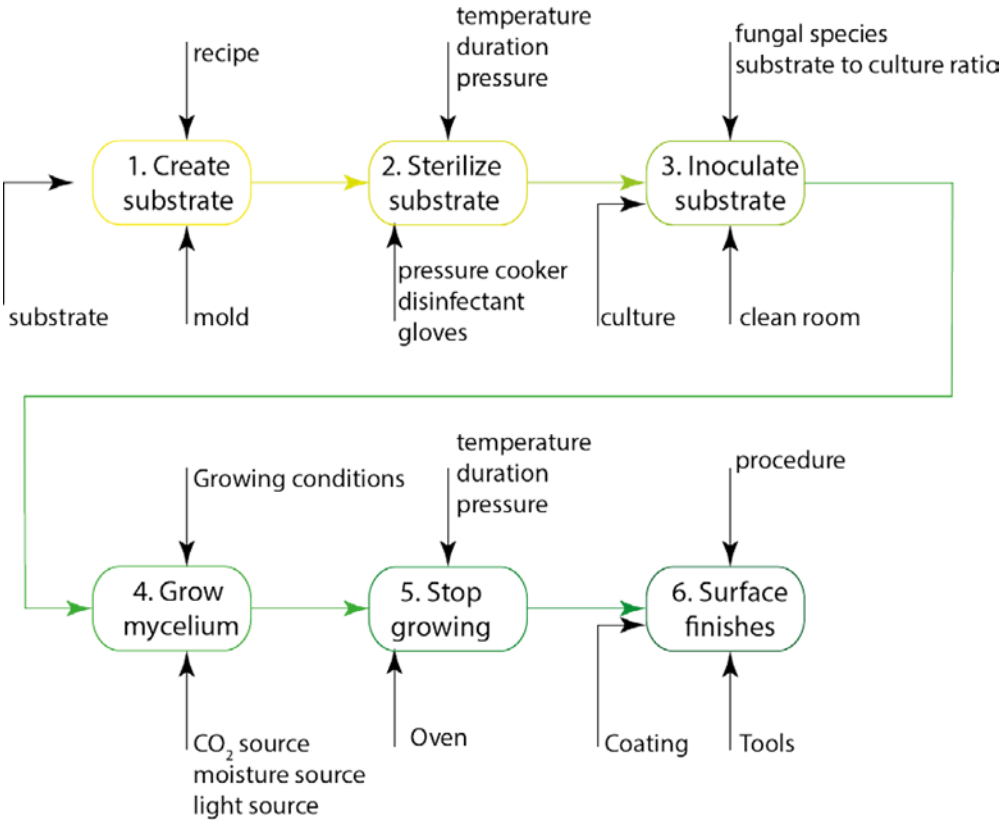



Figure 2. 5: Schematic of mycelium-based material production process (Lelivelt et al. 2015).

### 2.2.1 PRETREATMENT OF THE SUBSTRATE

Usually, it is expected that a substrate is initially inhabited by many other undesired organisms such as bacteria, insects or other fungi that will compete with available nutrients needed by the desired fungus, inhibiting its growth. Therefore it becomes important to ensure the substrate is clean beforehand. Four different methods have been discussed in this session: sterilization, pasteurization, hydrogen peroxide treatment and natural composting.

Sterilization is often the most drastic pretreatment method. The recommended conditions to sterilize a substrate includes heating to a temperature of 123 ˚C and a pressure of 100 KPa (1 bar) for about 30 minutes. The advantage of this treatment is that it has the potential of killing all organisms and to a reasonable degree one is assured that the substrate is completely inert. The challenge is that it involves a great deal of energy and specialized equipment such as autoclaves or pressure cookers. Another downside is that sterilization often eliminate some micro-organisms which are actually helpful to basidiomycetes during their growth. This treatment is generally not a prerequisite to breed most fungi but can be useful if one needs to be absolutely certain to have an inert substrate (Boeck 2012)

Pasteurization involves heating the substrate to a temperature of 60-80 ˚C for 60 minutes. Most harmful organisms cannot survive at this temperature but the helpful organisms can. This method is easier to perform, less technical, less energy requirement and will not kill helpful microorganisms but less secure than sterilization.

Hydrogen Peroxide ($H_2O_2$) is a suitable chemical for killing harmful organisms in the substrate. It damages all organisms in a substrate, but is more damaging to harmful micro-organisms than to the mycelia of a fungus. The treatment involves immersing the substrate in a 0.3 % solution of Hydrogen-Peroxide which is just enough to keep the harmful organisms away, but allowing the mycelium to colonize the substrate. The advantages of this method are that the peroxide have a lasting effect after treatment thus the substrate remains protected, no equipment is required other than a mixing bowl, it is much simpler and requires no energy. In the case of heat treatment, the substrate will simply cool down and eventually becomes susceptible to reentry of malicious organisms. With treatment of

hydrogen-peroxide, the chemicals remain in the substrate and provide ongoing protection against new organisms.

Natural composting is a method preferred by industrial companies that create substrate at a large scale. The substrate is expected to be partially moist, it is thoroughly mixed and then placed in a closed space. Through natural process of composting toxic gases, such as ammonia, do build up in high concentrations and the temperature increases significantly, up to 90 ˚C. Without further treatment, these conditions are aggressive enough to kill the malicious organisms. A big advantage of this method is that the environment can gets so toxic that the waxy outer layer that shields most plants from fungi is weakened or even completely destroyed, thus making it easier for the mycelium to penetrate the substrate. The downside of this treatment is that toxic gases are created during composting. These toxic gases are often hazardous to the environment, make processing more difficult to control and consequently require extra safety measures for the workers (Yadav and Tripathi 1991).

### 2.2.2 GROWING METHOD (CLOSED OR OPEN)

After pretreating the substrate, what follows is inoculation with pre-grown spawn. After the inoculation, the mix needs to be kept in a controlled environment where the optimal growing conditions can be created, monitored and maintained. Each species of the fungi requires specific cultivation conditions. Generally, fungi do not possess chlorophyll and do not perform photosynthesis, therefore exposure to sunlight is not exactly mandatory. However, it does not mean fungi necessarily require a dark environment to grow. One advantage of cultivating the fungi in the darkness is that dark areas often provide the moisture that the spores need to reproduce (Ahmadi 2016). Because fungi are unable to

retain the moisture, an environment with a high humidity is preferable to prevent water loss. The optimal growing conditions vary for each species but according to different authors, most wood-inhabiting basidiomycota thrive at the following conditions:

- Temperature: < 30 ˚C (heat is produced during growth)
- Humidity: 90-100% (moist to the touch)
- Light: None
- $O_2$: Necessary for growth
- $CO_2$: High

*Growing conditions for mycelia according to Maurizio Montalt* (Lelivelt et al. 2015)

- Temperature: 30 ˚C
- pH: 5.5.
- Humidity: 55%
- Urea: 1.5-3%
- Duration of growth: 21 days
- Turning frequency: once at mid-incubation
- Superphosphate: 1%

*Growing conditions for C. Versicolor according to Yadav et al*(Yadav and Tripathi 1991)

- Temperature: 20 - 25 ◦C
- pH: 5 - 8
- Humidity: 80 - 100%.
- Light: darkness.
- Time: generally, between 2 to 4 weeks are ideal for mycelium running

- Ventilation: mushrooms breathe and exchange gases, so air circulation and gas exchange is required.
- Nutrients: cellulose, lignin, fibre content of substrate, husk rice, straw, and corn.

*Growing conditions for Pleurotus* species (Baysal et al. 2003; Kim et al. 2001; Mandeel, Al-Laith, and Mohamed 2005)

Medium temperature and a high humidity are maintained as these are the natural growing conditions for wood-inhabiting fungi. The fungi also require oxygen and generate carbon-dioxide during its growth. Intensity of light and concentration of carbon-dioxide are special conditions as they act as signifiers to commencement of pinning. Pinning is a growth process where the fungus generates the primordia on its surfaces that will eventually grow into mushrooms. Wood-inhabiting fungi will only start to pin when they get to a free surface so that mushrooms can grow in the open to facilitate the dispersion of spores. The fungus perceives it reaches a free surface when it senses light. Also, inside the substrate the carbon-dioxide that is generated during growth can't escape freely and this therefore leads to a high concentration of $CO_2$. When the fungus reaches a free surface, it is expected that the $CO_2$ concentration will drop significantly which is another trigger for the fungus to start pinning. Growing conditions are usually determined to optimize fruiting body production. However when the sole purpose of growing fungi is for their mycelium, it is preferable to avoid the spawning of mushrooms. The fungus can then focus its entire resources on growing a dense and homogenous mycelium instead of growing biologically expensive fruiting bodies. To prevent pinning, light intensity needs to be kept to a minimum and the $CO_2$ concentration needs to be kept high.

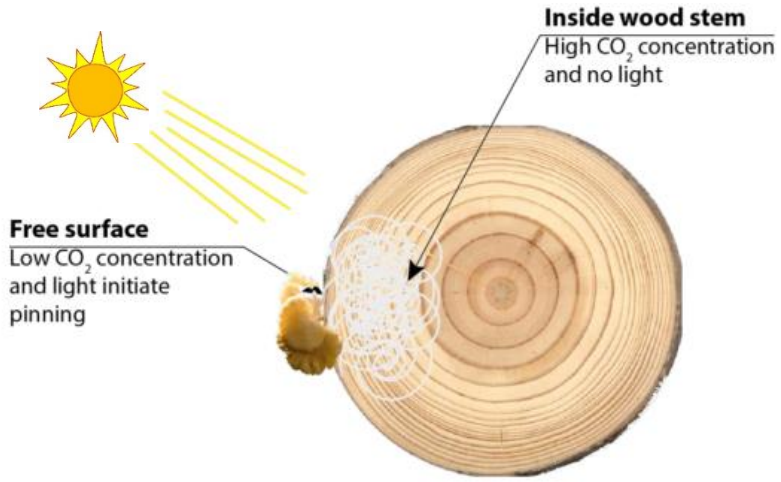



Figure 2. 6: Conditions of pinning

## 2.3 CONCEPT OF COMPOSITES

Composite materials are engineered materials made from two or more constituent materials with significantly different physical and/or mechanical properties that, when combined, produce a material output with characteristics superior to the individual components (Pourang 2007). There are two major categories of constituent materials: matrix and reinforcement. The matrix surrounds and supports the reinforcements by maintaining their relative positions, while the reinforcement material ensures the desired mechanical properties. An enormous lists of materials are used today as matrices and reinforcements, depending on the application (Belgacem et al. 2008). A mycelium composite material consists of two components; fungus and substrate. The fungus acts as a natural glue that holding the substrate together which functions as the reinforcing fibers.

### 2.3.1 FUNGUS

According to different sources, a number of mushroom fungi have been identified to be suitable for use in a structural material. The selection criteria for a suitable fungi are that it should relatively grow fast, easy to cultivate and it is important that it creates a dense and homogeneous mycelium. For instance the mycelium of Oyster mushrooms grows under

relatively simple conditions while Champignon mushrooms are difficult to produce without special equipment and expert knowledge (Lelivelt 2018). In the selection process of the suitable choice of fungi various experts, professionals and reputable journals were consulted. From researches, P*leuratus Ostreatus and Polyporus squamosus* proved to be the most promising fungi as they have a dense mycelium, grow fast and they grow in easy to obtain conditions (Lelivelt 2018).

| | **Possible Fungi** | **Source** |
|---|---|---|
|  | P. Ostreatus (Grey Dove mushroom) | Recommended by Fields and Forest |
|  | P. Ostreatus (Oyster mushroom) | Recommended by designer Maurizio Montalti |
|  | P. Squamosus (Dryad's saddle) | Used by packaging US company Ecovative |
|  | G. Lucidum (Reishi mushroom) | Used by artist Philip Ross |

Table 2. 1: selection of viable mushroom (Bayer et al. 2012; Lelivelt 2018)

### 2.3.1 NATURAL FIBERS

A natural fiber basically is a hollow tube with progressively smaller tubes within the perimeter. At the molecular level natural fibers are composites with rigid and high strength cellulose embedded within a lignin matrix (Pettersen 1984). Therefore, high cellulose content corresponds to a high tensile strength. Some fibers in addition contain a waxy outer layer that provides a natural protection that guides against bacteria and other potential sources of disease. Natural fibers can be classified according to their origin and grouped into leaf: abaca, cantala, curaua, date palm, henequen, pineapple, sisal, banana; seed: cotton; bast: flax, hemp, jute, ramie; fruit: coir, kapok, oil palm; grass: alfa, bagasse, bamboo and stalk: straw (cereal) (Kalia, Kaith, and Kaur 2009). Some natural fibers together with their contents are highlighted in table 2.

| Fiber | Cellulose (wt %) | Hemicellulose (wt %) | Lignin (wt %) | Waxes (wt %) |
|---|---|---|---|---|
| **Bagasse** | 55,2 | 16,8 | 25,3 | - |
| **banana** | 60-65 | 11_21 | 19-24 | - |
| **Bamboo** | 26-43 | 30 | 21-31 | - |
| **Flax** | 71 | 18,6-20,6 | 2,2 | 1,5 |
| **Kenaf** | 72 | 20,3 | 9 | - |
| **Jute** | 61-71 | 14-20 | 12-13 | 0.5 |
| **Hemp** | 68 | 15 | 10 | 0.8 |
| **Ramie** | 68,6-76,2 | 13-16 | 0,6-0,7 | 0.3 |
| **Abaca** | 56-63 | 20-25 | 7-9 | 3 |
| **Sisal** | 65 | 12 | 9.9 | 2 |
| **Cotton** | 90 | > 8 | < 2 | - |
| **Coir** | 32-43 | 0,15-0,25 | 40-45 | - |
| **Oil Palm** | 65 | - | 29 | - |
| **Pineapple** | 81 | - | 12.7 | - |
| **Curaua** | 73.6 | 9.9 | 7.5 | - |
| **Wheat Straw** | 38-45 | 15-31 | 12-20 | - |

| | | | | |
|---|---|---|---|---|
| **Rice husk** | 35-45 | 19-25 | 20 | 14-17 |
| **Rice Straw** | 41-57 | 33 | 8-19 | 8-38 |

Table 2. 2: Contents of some selected natural fibers (Faruk et al. 2012; Satyanarayana, Arizaga, and Wypych 2009)

When making selection of substrate, some factors are important to consider. First the substrate requires to be rich in cellulose content. The nutrition of a fungus consists of glucose. A fundamental difference between other organisms and fungi is that fungi can breakdown cellulose into glucose. This means that substrates with high cellulose content allows fungi to grow rapidly, whilst other organisms cannot. Therefore it is practical to use cellulose-rich substrates when growing fungi to prevent contamination by other organisms. Another advantage of using cellulose-rich materials is that cellulose is present as a structural compound in many agricultural crops thus adding structural integrity to the material.

Secondly the substrate materials need to be locally available and in abundance. It would be counterproductive to create a fully biobased material when the constituent resources are not sustainable and needs to be shipped large distances. Thirdly, the substrate needs to be compatible with fungi. Some plants, such as hemp have a natural anti-infectant waxlayer that makes them less susceptible to malicious micro-organisms and lowers the demand for sterilization (Li and Pickering 2009). Other plants contain special compounds inside them to prevent the growth of fungi.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 MUSHROOM SPECIES.

To evaluate which pair of fungi-plant material features the most suitable combination for product applications and composite development, a variety of fungi species were grown on different local agricultural substrates and different growing conditions were monitored. Locally available mushroom varieties, *Pleurotus ostreatus* (white oyster) and *Volvariella volvacea (Paddy straw) mushroom*, were sourced from Mycofarms and Allied Synergy Limited (Edo State, Nigeria). Two exotic strains *Pleurotus ostreatus* (Grey dove oyster) and *Polyporus Squamosus(*Dryad's saddle) were sourced from Field and Forest Products (Peshtigo, WI, USA). Two Grow-It-Yourself kits and two already made mycelium tiles (15 cm) purchased from Ecovative Design (Green Island, NY, USA) helped serve as the control for this research. Plastic moulds for growing mycelium bricks were sourced from Ecovative Design (Green Island, NY, USA). Mushroom species were in the form of guinea corn and wheat spawn grains. They were conserved in a refrigerator at 4°C prior to use.

### 3.2 SUBSTRATES

Selection of substrate inputs were based on some important factors including biodegradability, availability, cost, textural and structural properties, and nutritional contribution. To fulfill mushroom's cellulosic needs, flour was incorporated as per the Ecovative Design grow kit instructions to the substrate mix as nutritional supplement. The inclusion of flour also ideally enhances mycelial bonds and fosters a strong root network. For the first group substrates used consist of loose hemp fiber and local available wood

chips including Mansonia altissima - Mansonia (Okhuoya, Akpaja, and Oghenekaro 2005); *Terminalia Ivorensis* - Black Afara (Edwin-Wosu, Omara-Achong, and Nyannayo 2013); Brachystegia nigerica (Okhuoya et al. 2005); Combretodendron macrocarpum (Okhuoya et al. 2005); Kyaya ivorensis - Mahogany (Anon 2009). These wood chips were sourced from two local sawmills (Dei-Dei and Lugbe, Abuja, Nigeria) during timber processing.

Substrate particle sizes and the ratio for each input were considered. Research from previous literature suggested that substrates with smaller particle sizes might be preferable and enhance growth of the mycelium through the particle mass. This can possibly allow for a less dense material, which could be a benefit or detriment on the application. The ratio of each substrate component was hinged on previous literature, as well as the advice of members of Mycofarms and Allied Synergy Limited, and was fine-tuned throughout the screening trials.

For the first group a spawn to total weight ratio of 30% was used. For the second group a ratio of 15% was adopted. A higher ratio was given to the first group to ensure a faster growth. Based on the results of the pre-screening trials, three mushroom species and the hemp fibre were selected for further investigation.

## 3.3 FIBRE REPARATION

The used fibres vary in size, heterogeneous sizes distribution to necessary inter-particle packing. To have the adequate fibre size for the tests, the fibres were soaked in water for 2 hours and then were rinsed abundantly, after which they were squeezed manually and spread on a plate in a warm room to allow the water to evaporate. The moist chopped fibres were then spread on trays rapped with an aluminum foils. The fibres were sterilised

to render the substrate inert and eliminate any form of microorganism present. This was done by placing the tray in an autoclave machine for 30 minutes at 136°C.

## 3.4 COMPOSITE FABRICATION

### 3.4.1 INOCULATION

The inoculation is performed in sterile conditions and clean environment. The worktop, hand gloves and all working tools used in this procedure were cleaned with a 95% alcohol solution to prevent contamination of the samples. The sterile fibres, distilled $H_2O$ and spawn necessary to fill the moulds were weighted, mixed together and put in the moulds. The added water is half times the weight of the total mix. Then, 15% spawn is added to the substrate. The moulds are filled by layers, while compressing each layer with a spoon to obtain a compact and dense sample (Figure 8). A transparent foil is used to seal the moulds with perforations at about 1 inch interval with a pin.

Figure 3. 1: (left) loading the fibers in an autoclave machine, (right) mixing the prepared fibers with spawns.

### 3.4.2 Growing process

The worktop, gloves and all other equipment used in this procedure was cleaned with a 95% alcohol solution to prevent contamination of the samples. Finally, the filled mould were then placed in larger boxes that could be closed off. The samples were allowed to grow in dark conditions at room temperature. To ensure a completion of the growth process, a long growth period of 35 days was used.

### 3.4.3 DRYING PROCESS

Upon completion of the growth period the samples were demolded and allowed to dry in open air for 48 hours. Afterwards they were transferred to an oven at 70 ˚C for 18 hours until their weight stabilized and thus all water was evaporated. This is to render the material inert and ensure the fungi does not continue its growth.

Figure 3. 2: (left) loaded fiber-spawn mix in a mould, (right) drying the samples in an oven.

The combination of loose hemp fibres and spawns of *Pleurotus pulmonarius,* Pleurotus ostreatus and *Polyporus Squamosus* were selected for the second test series. The inoculation, growing process and drying conditions were repeated for the second series. After drying the samples were cut into cuboids for compressional and flexural tests.

Flexural samples were cut into rectangular cross section of 15 × 30 × 100 mm while compressional samples were cut into square cross section of 12 × 12 × 50 mm.

## 3.5.0 MECHANICAL CHARACTERISATION

### 3.5.1 COMPRESSIVE TESTS

The second group of samples were tested using an Instron testing machine with load cell of 500N cell. The tests were displacement controlled at 5 mm/min. The rectangles were tested standing on their smallest surface. The contact surface was not perfect due to the rough surfaces of the samples. The test was stopped when a fixed strain was reached in the specimen, varying between 70% and 80%. The load-displacement curve was converted to a stress-strain curve. The compressive stress was computed by dividing loading force by the cross sectional area. Strain was calculated from machine displacement by dividing the displacement by original length. For each composite formulation, 3-5 specimens were tested.

$$\sigma = F/A \qquad 3.1$$

$$\varepsilon = \delta/l \qquad 3.2$$

Where σ = stress (in MPa) ε = strain (in mm/mm)

F = Force (in N) $\delta$ = displacement (in mm)

A = Area (in $mm^2$) $l$ = original length (in mm)

### 3.5.2 FLEXURAL TESTS

A three-point bend test configuration was used to determine the flexural strength of the samples. A loading span of 80 mm was used for the entire three point bend test. The

specimens were deformed monotonically to failure displacement controlled at 5 mm/min. For each composite formulation, 3-5 specimens were tested.

### 3.6.0 PHYSICAL CHARACTERISATION

### 3.6.1 DENSITY MEASUREMENT

Dry densities were calculated from the weight after drying and the volume of each specimen prepared. For each sample the thickness, the width and span were measured using a caliper, and the weight was measured using a digital scale.

$$D = m/v \qquad 3.3$$

Where D = density
M = mass
V = volume

### 3.6.2 WATER ABSORPTION

Square specimens (40 × 40 mm) were tested in triple to determine the water uptake when submerged in water. Specimens were placed in containers filled with distilled water maintained at room temperature and weight was measured after every one hour for the first six hours. The weights were later measured at intervals of 3, 6 and 12 hours on the second, third and fourth day respectively until the weights were consistent indicating point of saturation. For each measurement, samples were taken out of water, manually removing the superficial water with filter paper and weighed within 1 min after removal from the water.

### 3.6.3 SEM IMAGING

The interactions between the mycelium and the substrates as well as the mycelium growth were observed using a scanning electron microscope (SEM) (**ZEISS**-EVO/LS10). Pictures

were taken of the top, middle, side, and bottom of the samples to examine the microstructural mycelium growth patterns and compare the structural pattern across the composites. The pictures also intended to determine the failure pattern of fractured surfaces.

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

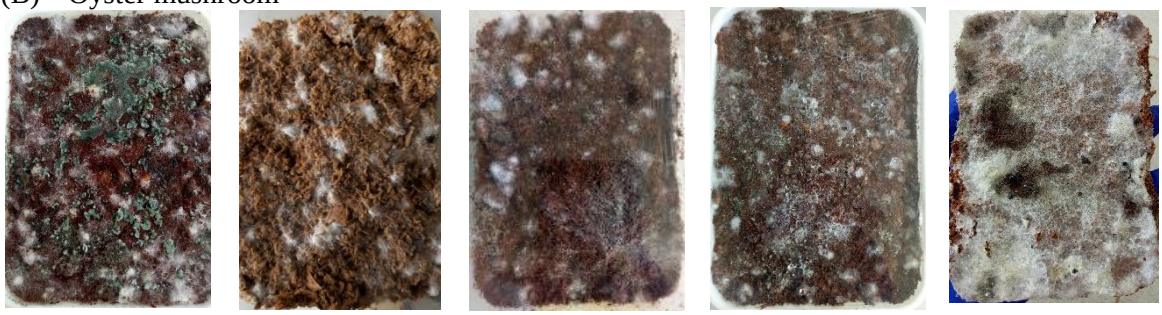
### 4.1 INFECTION RATE

The pre-screening experiments utilized five wood species and hemp fibers in a variety of forms. In parallel to the composite fabrication in moulds, samples were grown in one liter glass bottles. The growth evolution of a representative selection of the samples is presented in Figure 9. The samples with Brachystegia particles, Combretodendron particles and Kyaya dust were poorly grown after 12 days. Due to a slow growth all wood dust samples were eventually contaminated. It was found that the wood mushroom integration did not produce a sturdy composite and thus no further tests were conducted. Of all the wood substrates, the sample containing Mansonia dust showed a good compatibility with mycelia as growth could be observed to be much denser but still could not develop a cohesive composite. The samples with Black Afara also showed a comparable but slightly less dense growth resulting in a mass too incoherent to be used for testing. While samples with loose hemp fibers ultimately grew, we observe a dense white chitinois layer formed all over the hemp specimen. This constitute samples for the second stage.

| Brachystegia | Combretodendron | Kyaya | Black afara | Mansonia |
|---|---|---|---|---|

(A) Dryad's saddle mushroom

(B) Oyster mushroom



(C) Grey Dove mushroom

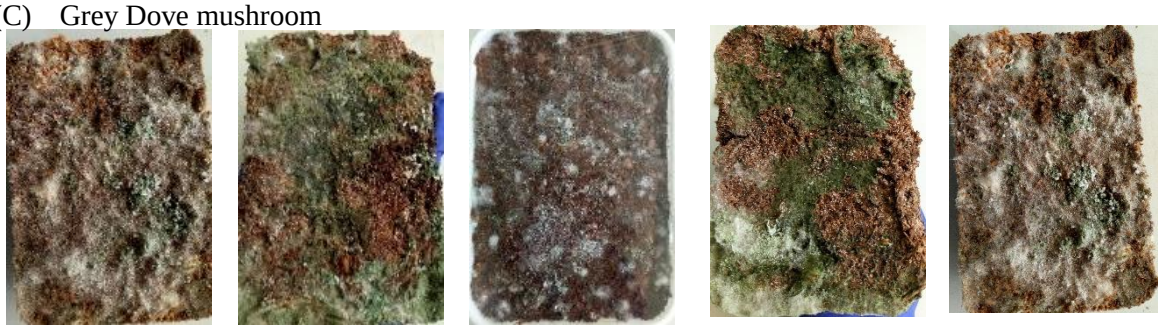


Figure 4. 1: Growth evolution of a representative selection of the samples

## 4.2 VISUAL INSPECTION OF GROWTH

Samples of the second stage showed a strong gradient in mycelial density over the depth, with stronger concentrations of mycelia network at the interfaces. The mycelium network was also stronger at the top surface than at the bottom. Figure 10 shows different views of a sample in which it is clearly observed that the bottom and top have a much denser mycelium and that the top is denser than the bottom. Figure 14-16 shows SEM images of top, bottom and side of a sample with Dryad's Saddle mycelium. Two explanations are possible for this visible effect. First of all air enters the mold through the perforations of

the lid at the top. Eventually, oxygen concentration gradient develops resulting to most oxygen available at the top and least at the bottom. Secondly, heat is generated during growth of the mycelium. The heat present at the center of the mold will not be able to dissipate as much as the heat at the interfaces. As oxygen stimulates growth and heat deters growth, this combine effect would explain why the mycelium is denser at the interfaces and denser at the top. The first effect explains also why the higher mycelium concentration is available at the top than at the bottom. If the thickness of the material is increased too much, there will be a point at which the center becomes too hot or too anaerobic to permit any growth. The implication is that mycelium-materials in a typical design should have a maximum thickness unless measures are taken to create an even distribution of oxygen and temperature.

Figure 4. 2: from top right going clockwise; top, short side, long side and bottom view of Grey Dove oyster mushroom and Hemp fibers Composites

## 4.3 SEM OBSERVATION

Several SEM images show the differences in structure between the mycelium of different mushroom species. Figure 11 shows the mycelium matrix from White oyster mushrooms, Figure 12 shows the mycelium matrix from Grey dove oyster mushrooms, while Figure 13 shows the mycelium matrix from Dryad's saddle mushrooms. These images indicate that there is successful mycelial growth within the composite, confirming its ability to develop a strong adhesion to the hemp fibers.

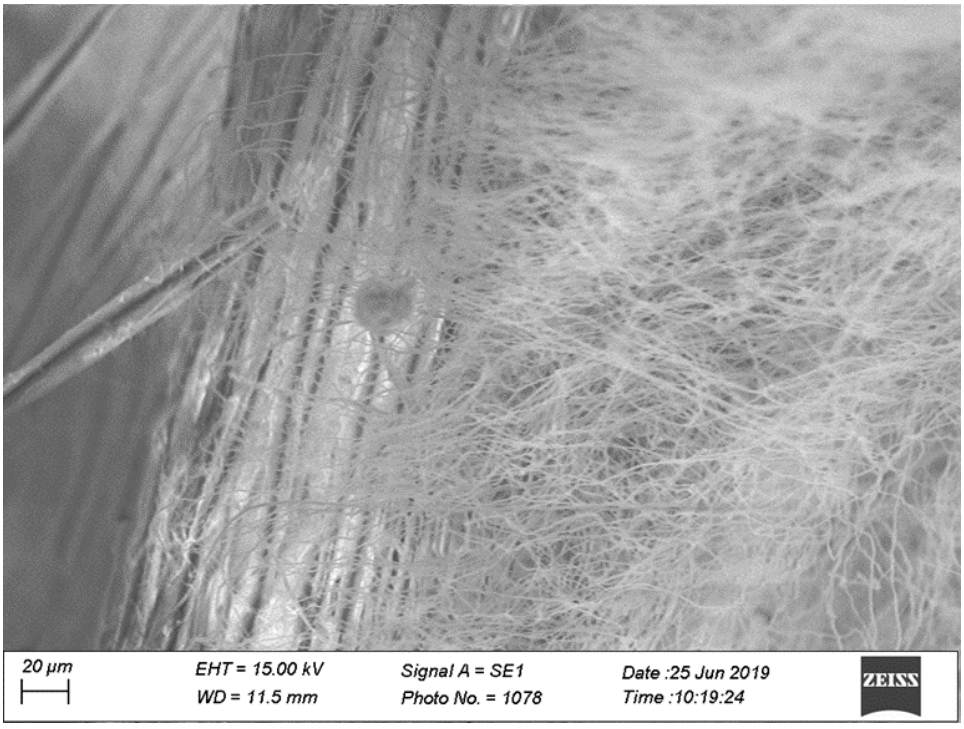



Figure 4. 3: SEM image of White Oyster mycelium matrix

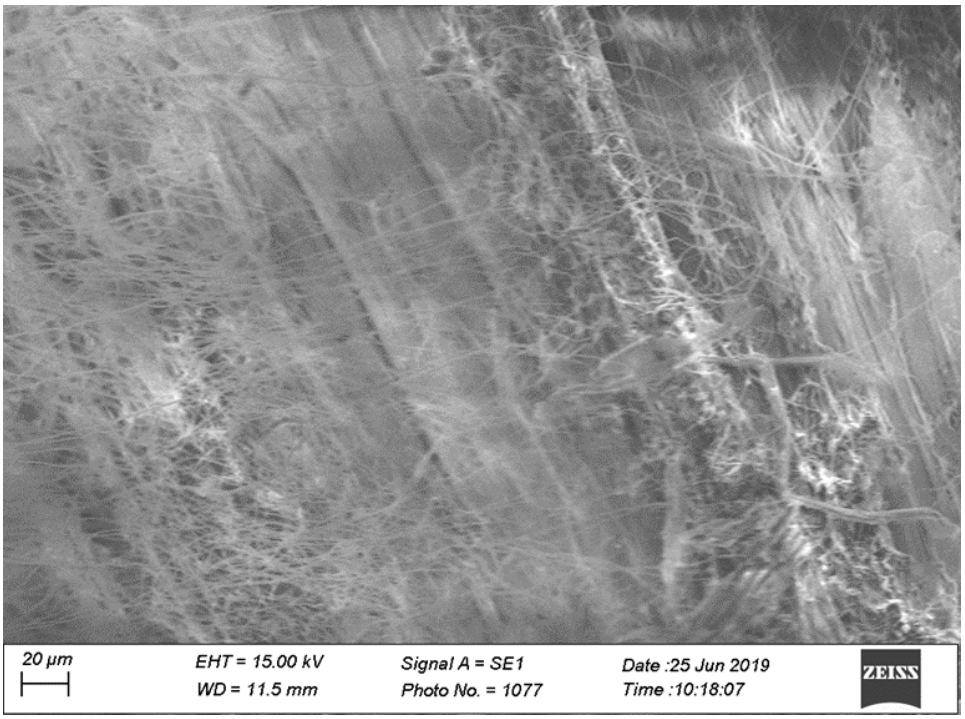


Figure 4. 4: SEM image of Grey Dove Oyster mycelium matrix

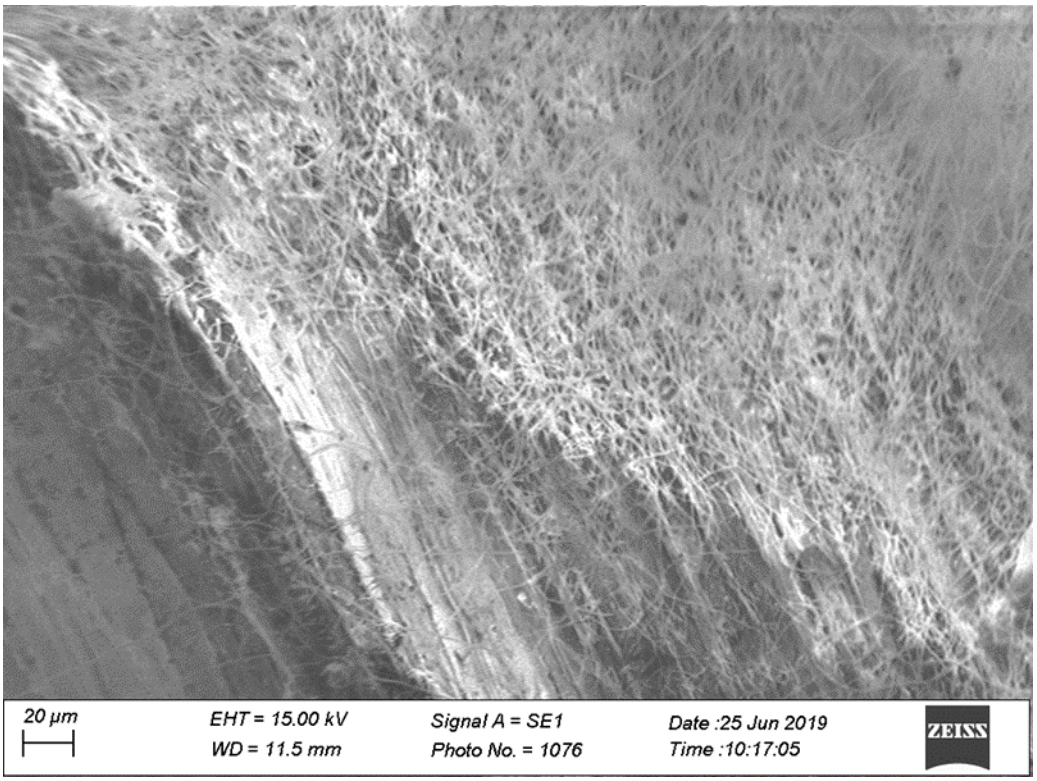


Figure 4. 5: SEM image of Dryad's Saddle mycelium matrix

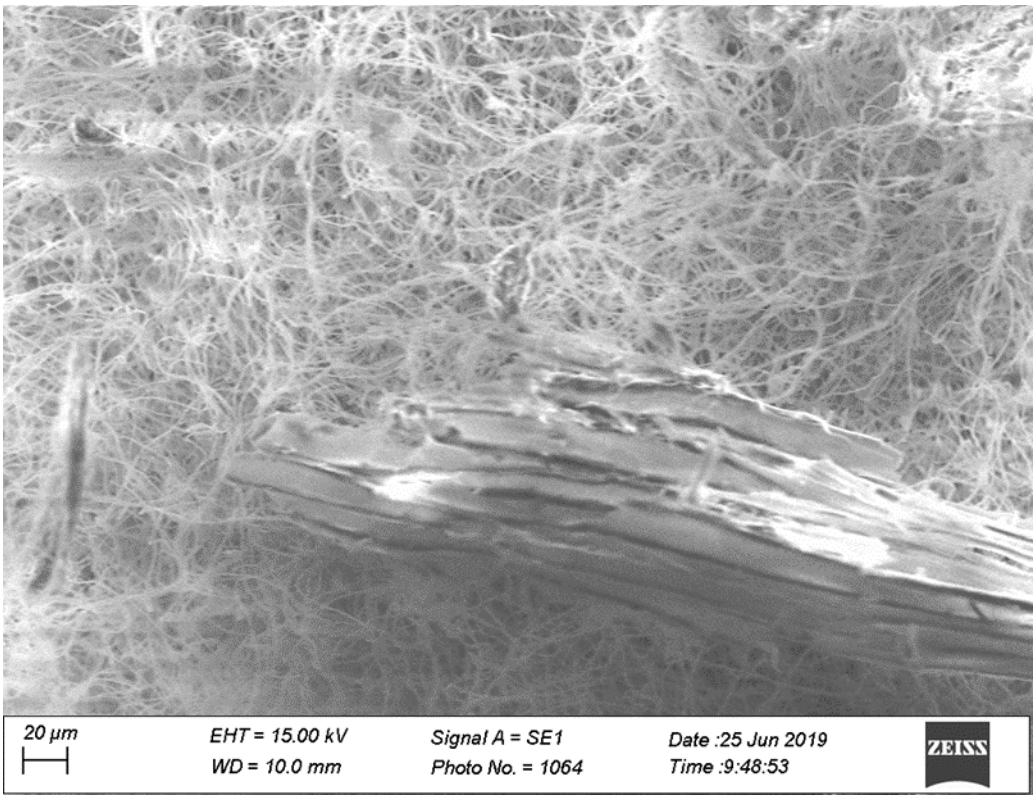



Figure 4. 6: SEM top image of Dryad's Saddle mycelium matrix


Figure 4. 7: SEM bottom image of Dryad's Saddle mycelium matrix

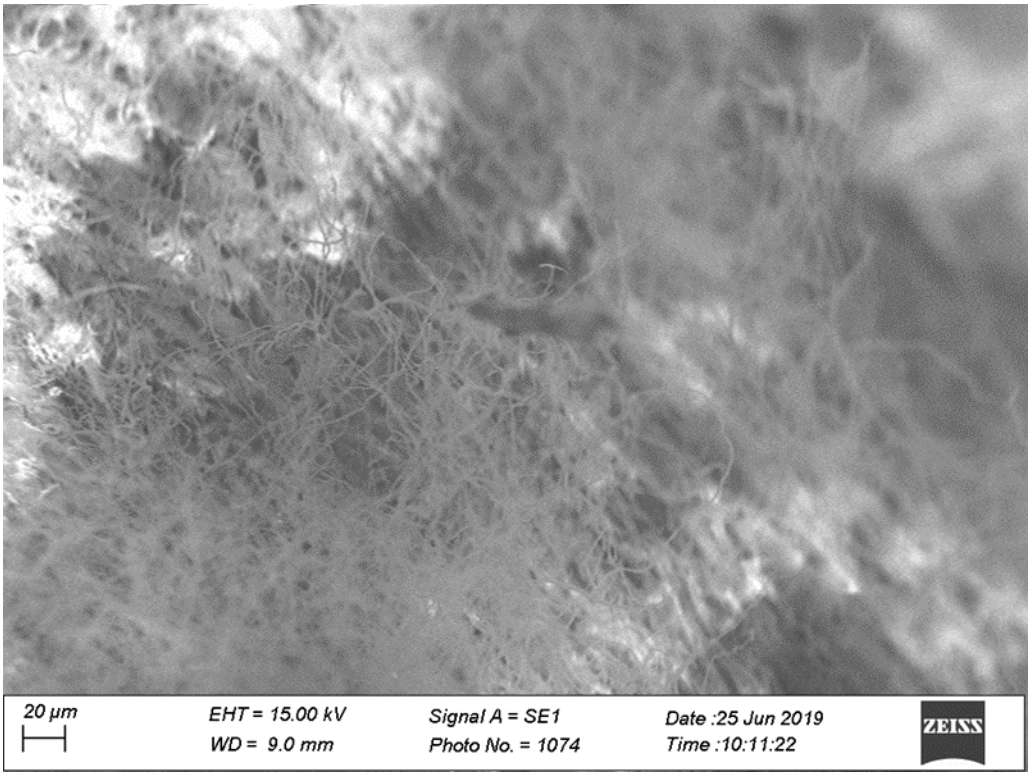



Figure 4. 8: SEM side image of Dryad's Saddle mycelium matrix

## 4.4 COMPRESSIVE AND FLEXURAL RESULTS

Results of the compressive and flexural tests are summarized in table 3 and 4 respectively. Figure 17-21 and Figure 22-26 illustrates mechanical behavior of each composites under compressional and flexural loads respectively. A common trend of stress strain behavior was observed for each composites. After a small proportional path, a definite top in stress can be observed, finally followed by a gradual or abrupt drop.

Under compression, both the stiffness and the maximum stress of the specimens with Grey Dove specie were higher than the specimens with Dryad's Saddle and specimens with Oyster mushroom appears to have the least maximum stress. While in flexural loading, the peak stress observed in composite with Oyster mushroom was higher than the composite with Grey Dove and specimens with Dryad's Saddle mushroom possess the least maximum stress.

Some literature have reported on a specifically good compatibility between hemp fibers and mycelia (Li and Pickering 2009;Li, Pickering, and Farrell 2009). This is in tune with the results shown here. Packaging Styrofoam and already made mycelium tiles (150mm) purchased from Ecovative Design were adopted as control samples. Our composites possess comparable and superior properties to these control samples. From section 4.2, variations in strength properties was observed at different portion of the composite. These site variations for each composite were compared and illustrated in Figure 27-30.

| Sample | Matric Composition | Maximum Load (N) | Area ($mm^2$) | Compressive stress (MPa) |
|---|---|---|---|---|
| CI | Hemp + Dryad's Saddle mushroom | 64.512 | 144 | 0.448 |
| C2 | Hemp + Oyster mushroom | 36.364 | 144 | 0.256 |
| C3 | Hemp + Grey Dove mushroom | 65.088 | 144 | 0.452 |
| CA | Ecovative tile | 65.232 | 144 | 0.453 |
| CB | Styrofoam | 32.400 | 144 | 0.225 |

Table 4. 1: Compressive strength results for each composites

| Sample | Matric Composition | Maximum Load (N) | Area ($mm^2$) | Flexural stress (MPa) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| CI | Hemp + Dryad's Saddle mushroom | 18.050 | 450 | 0.393 |
| C2 | Hemp + Oyster mushroom | 25.000 | 450 | 0.397 |
| C3 | Hemp + Grey Dove mushroom | 15.255 | 450 | 0.347 |
| CA | Ecovative tile | 15.320 | 450 | 0.359 |
| CB | Styrofoam | 18.520 | 450 | 0.390 |

Table 4. 2: Flexural strength results for each composites

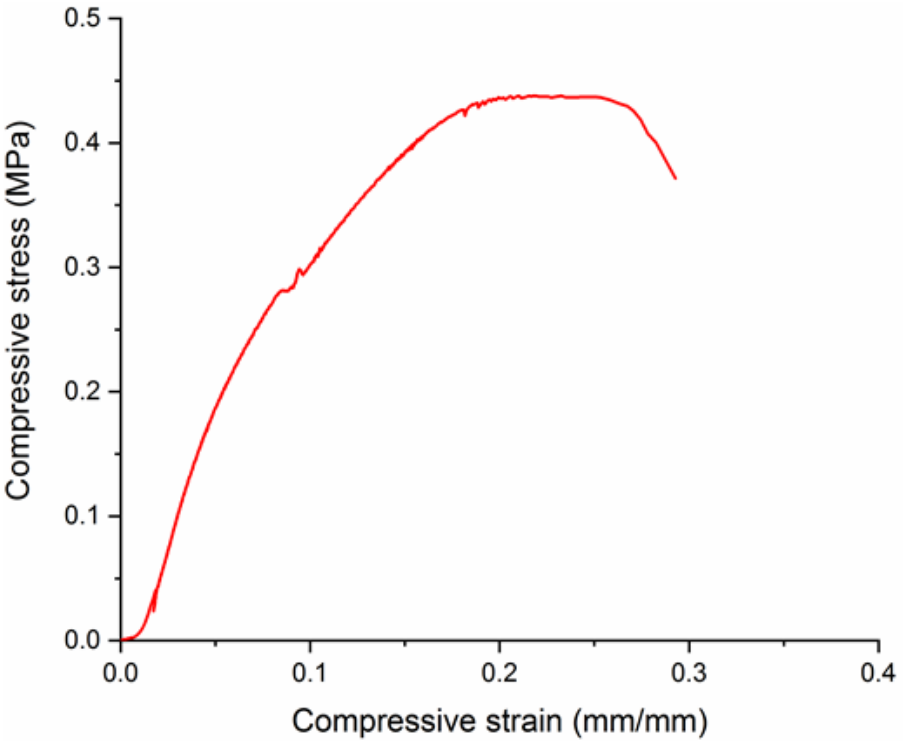



Figure 4. 9: compressive stress-strain curve for composite with Dryad's saddle specie

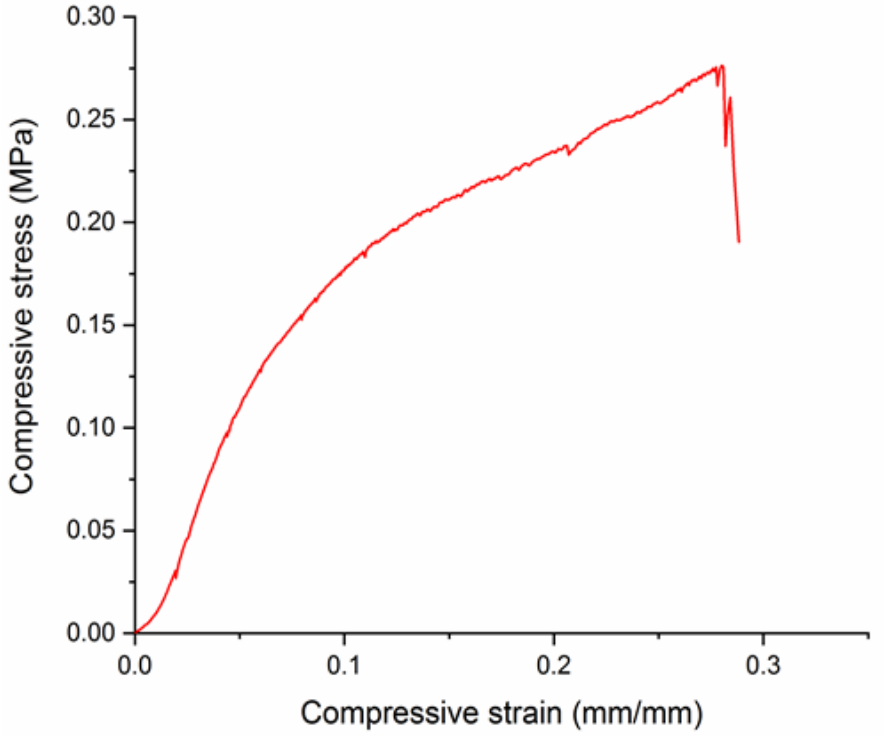



Figure 4. 10: compressive stress-strain curve for composite with Oyster specie

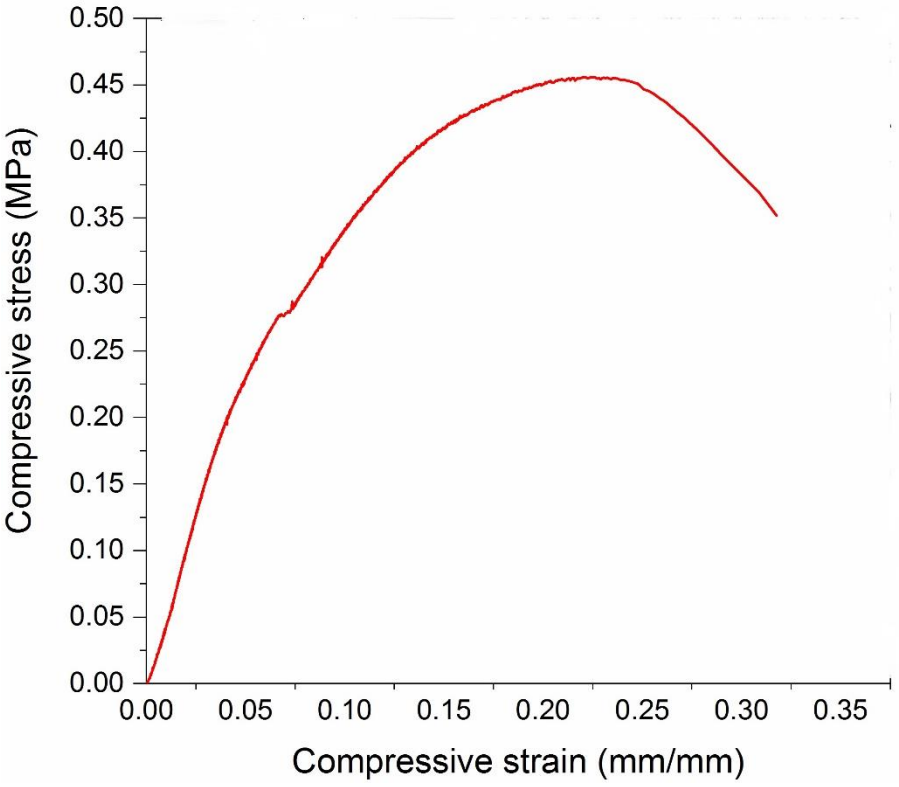



Figure 4. 11: compressive stress-strain curve for composite with Grey dove specie

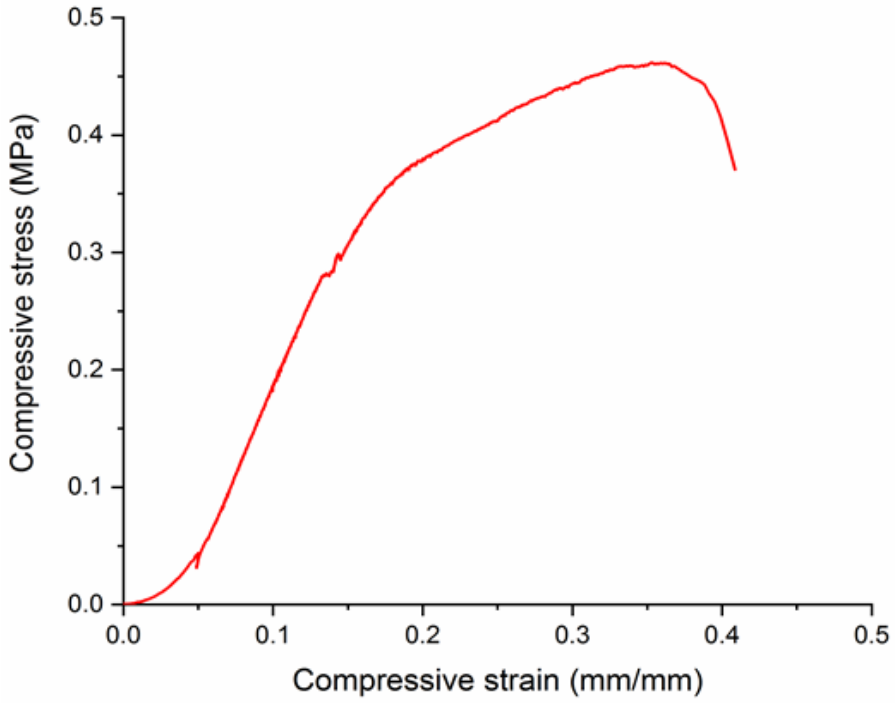



Figure 4. 12: compressive stress-strain curve for Ecovative grown composite

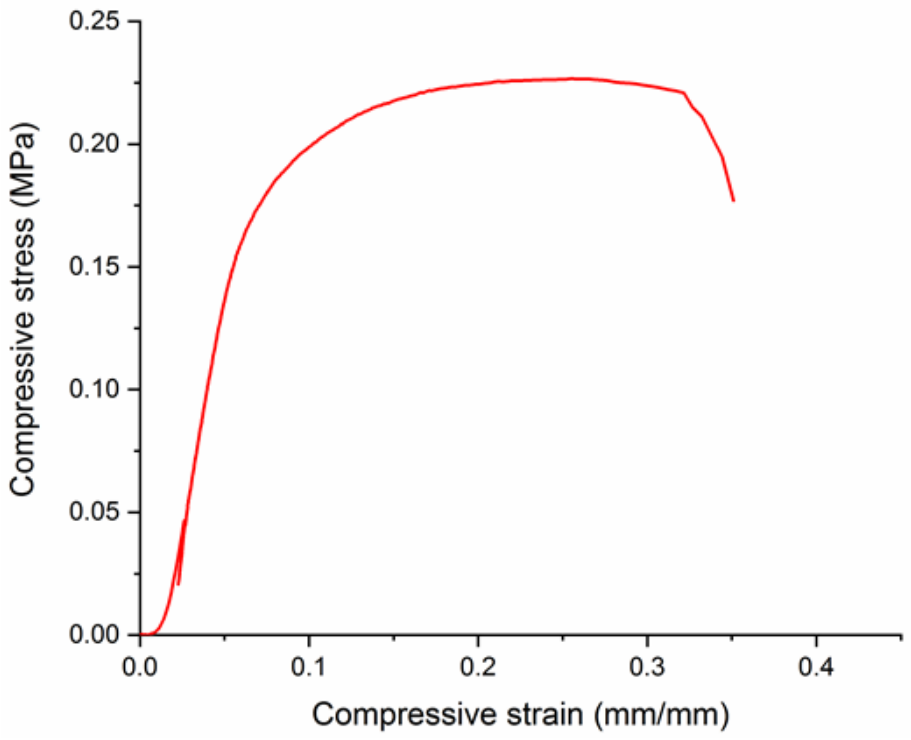



Figure 4. 13: Compressive stress-strain curve for Styrofoam

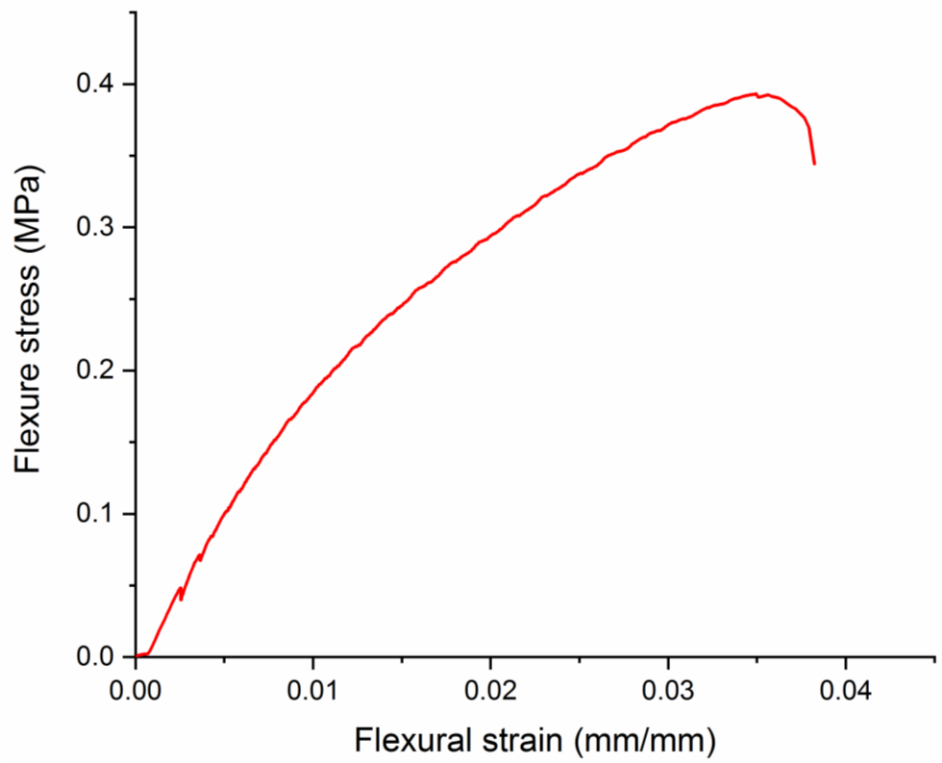



Figure 4. 14: Flexural stress-strain curve for composite with Dryad's saddle specie

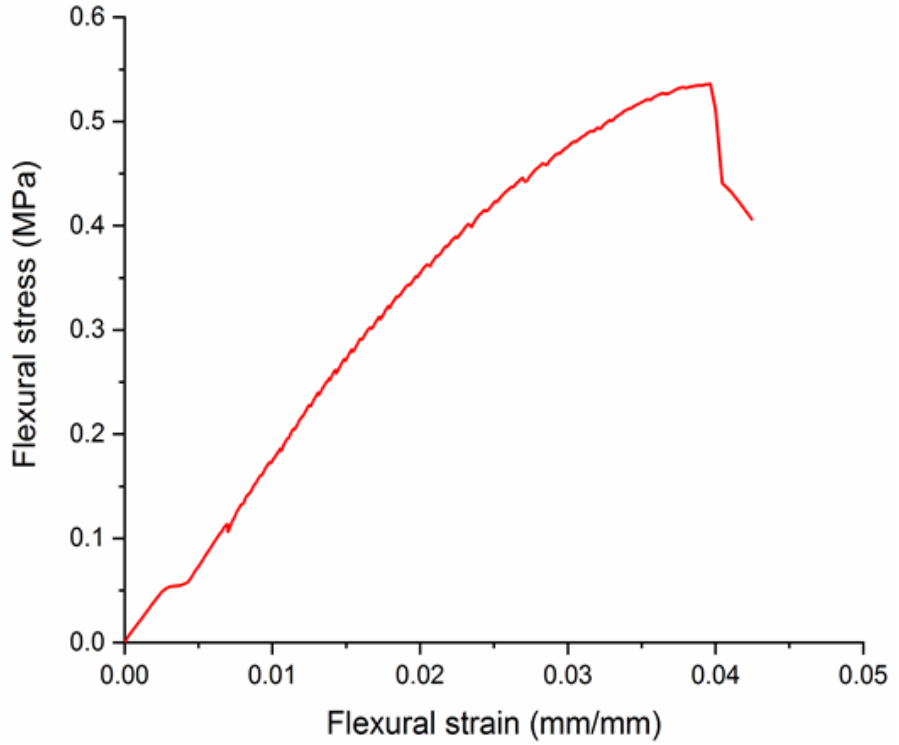



Figure 4. 15: Flexural stress-strain curve for composite with Oyster specie

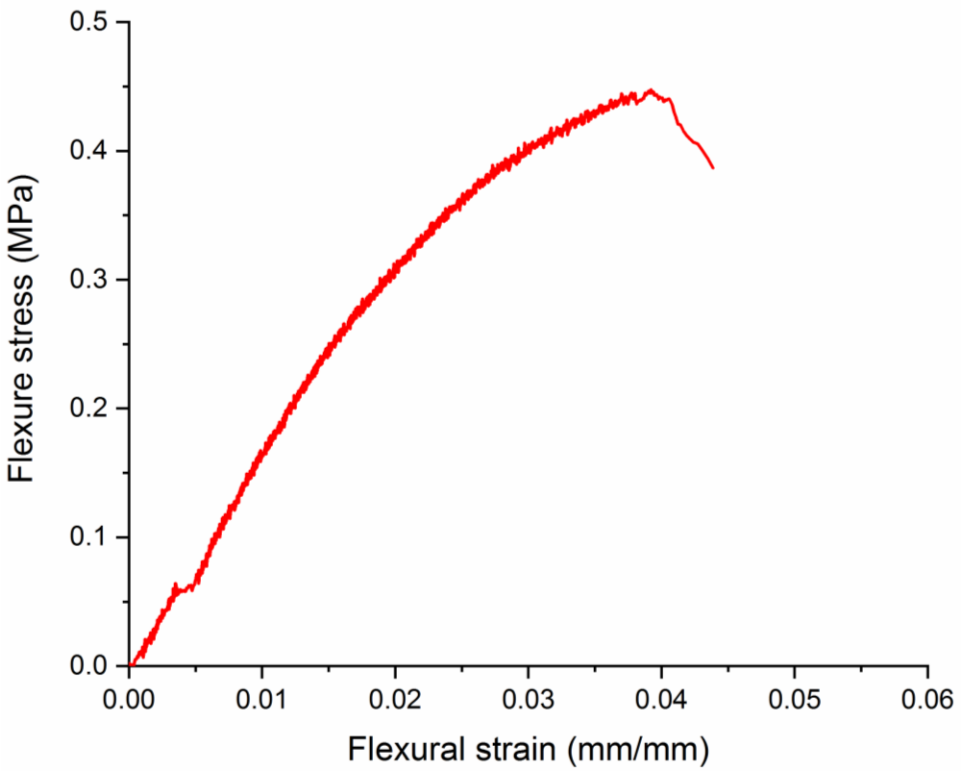



Figure 4. 16: Flexural stress-strain curve for composite with Grey dove specie

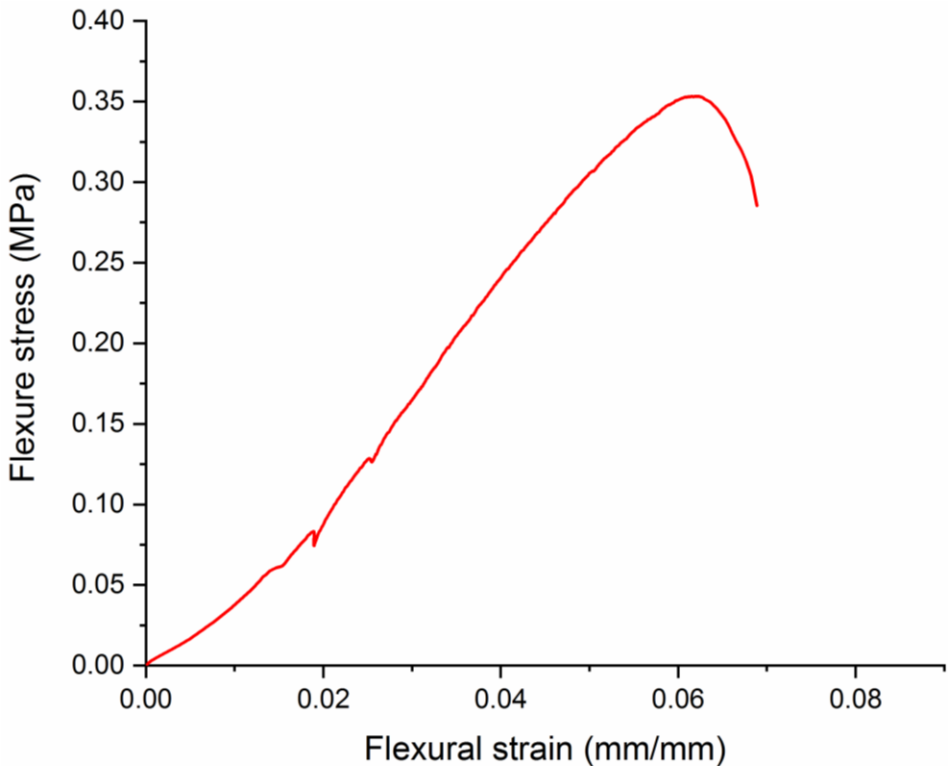



Figure 4. 17: Flexural stress-strain curve for Ecovative grown composite

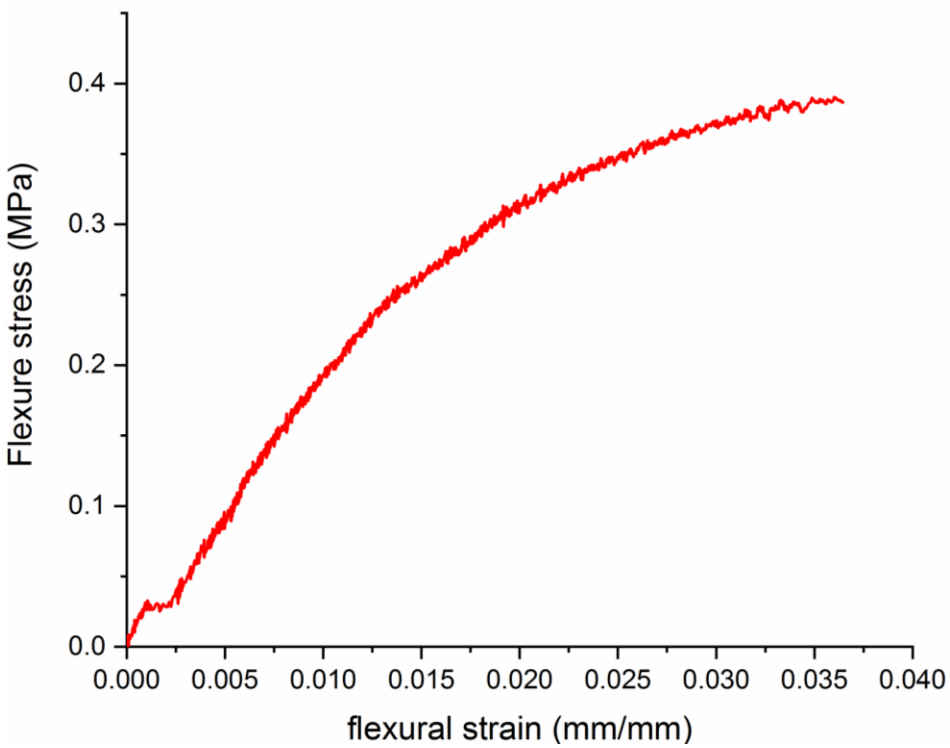



Figure 4. 18: Flexural stress-strain curve for styrofoam

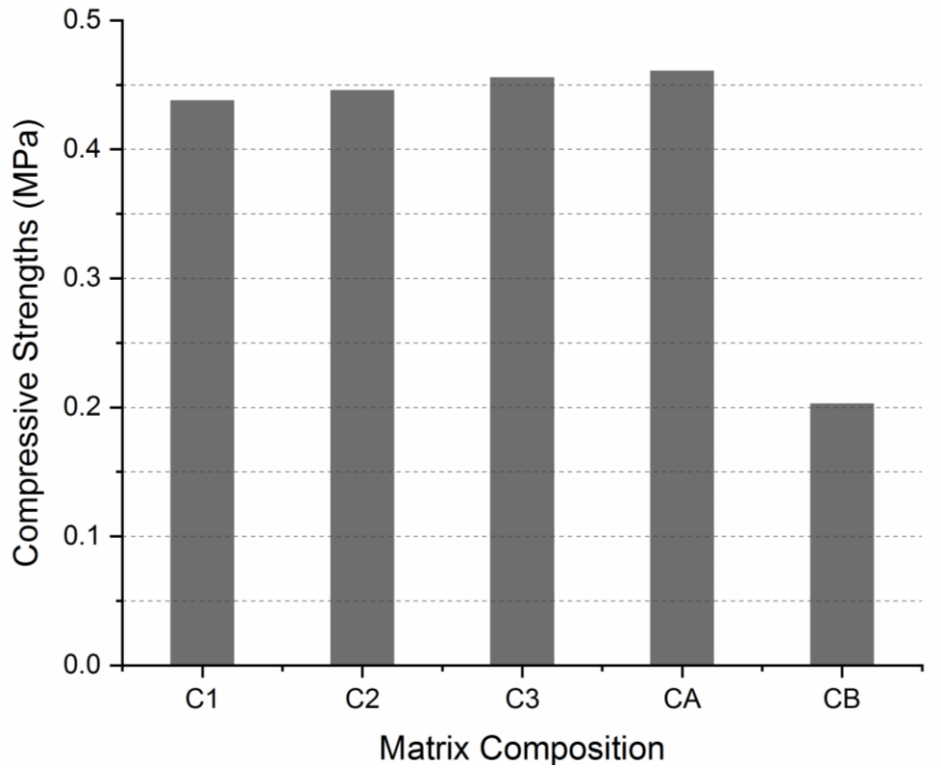



Figure 4. 19: Composite strengths at the bottom

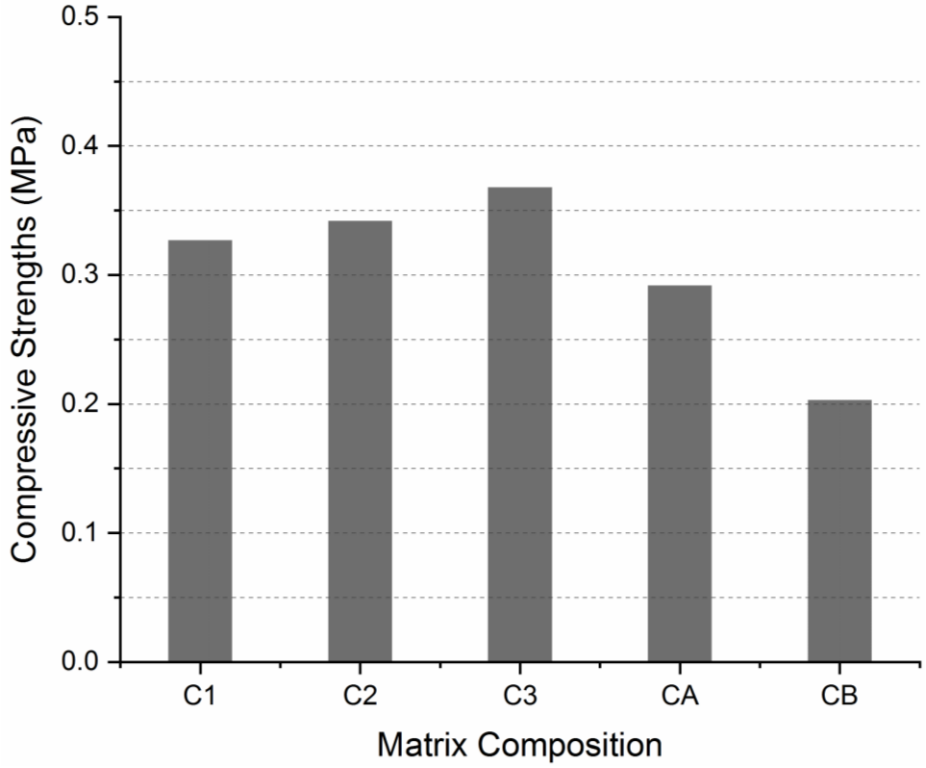


Figure 4. 20: Composite strengths at the top

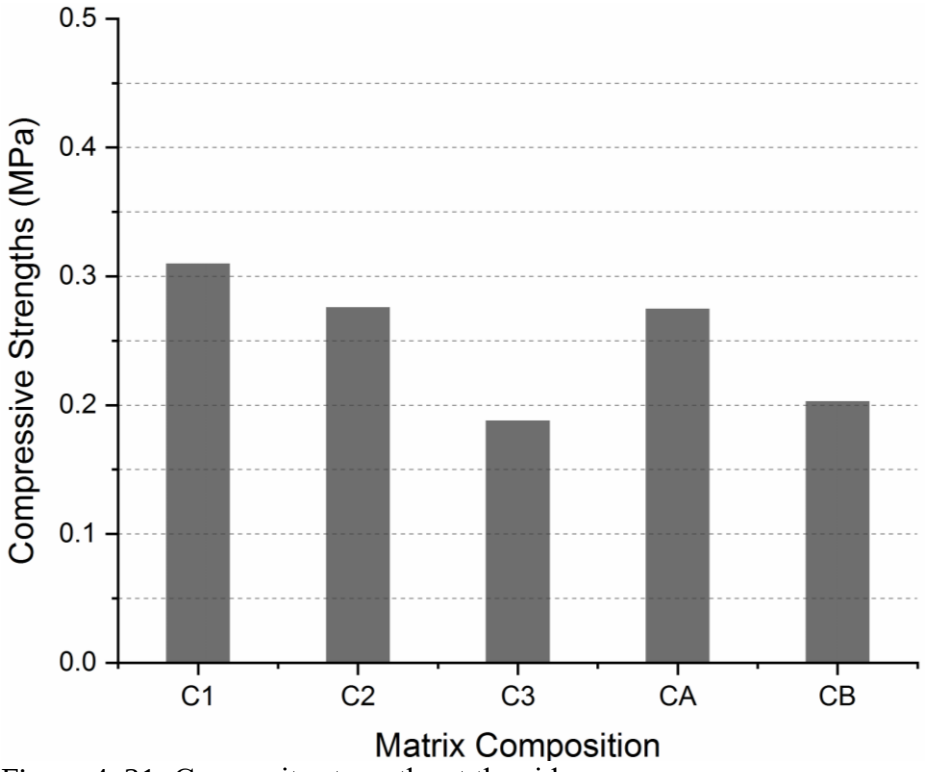


Figure 4. 21: Composite strengths at the side

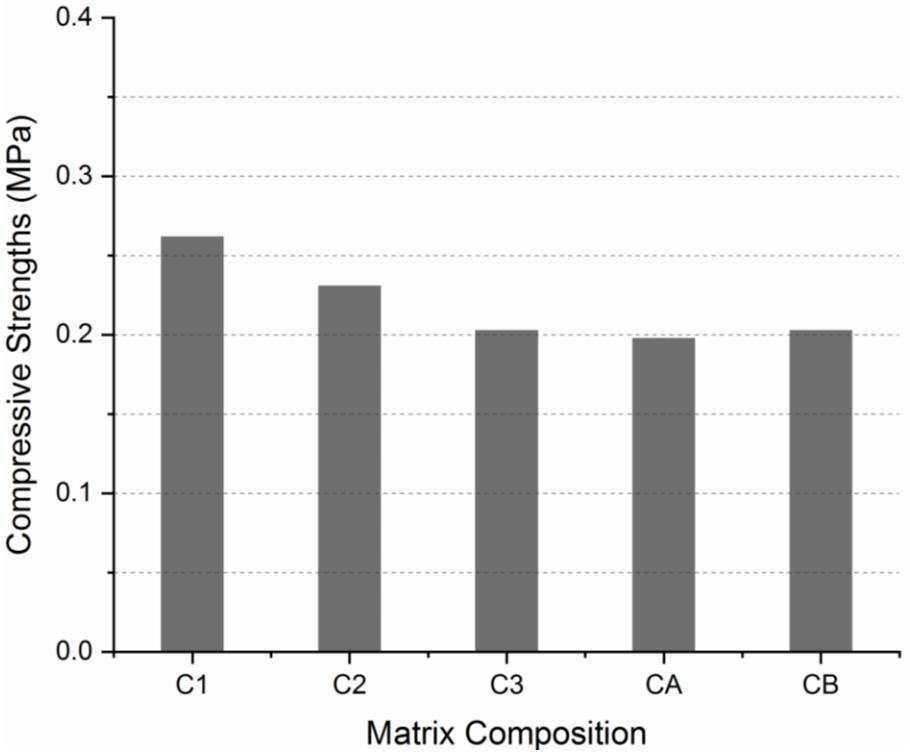


Figure 4. 22: Composite strengths at the middle

C1 = composite with Dryad's saddle mushroom

C2 = composite with Oyster mushroom

C3 = composite with Grey dove mushroom

CA = Ecovative grown composite

CB = styrofoam

## 4.5 Water Absorption and Dry density

Figure 31 and 32 illustrates the comparison between each composite of water absorption, dry and bulk density respectively. Composite with Grey Dove exhibit the highest degree of water absorption while samples with Dryad's Saddle shows the least water absorption. On the other hand, sample consisting of Dryad's Saddle and Oyster mushroom appear to possess a higher density than composite of Grey Dove. This trend indicate an inverse relation between water absorption and density.

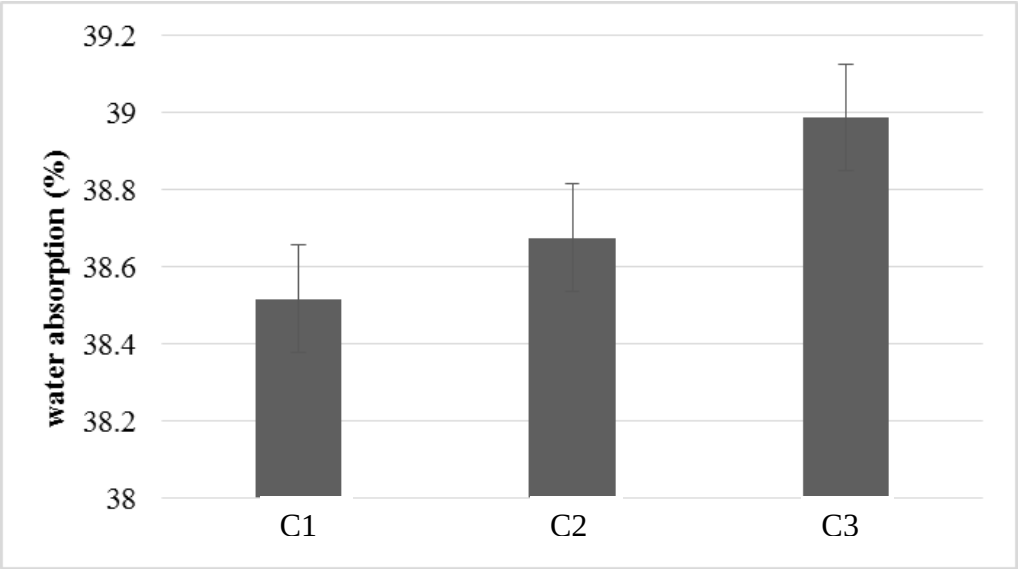



Figure 4. 23: Water absorption of each composite

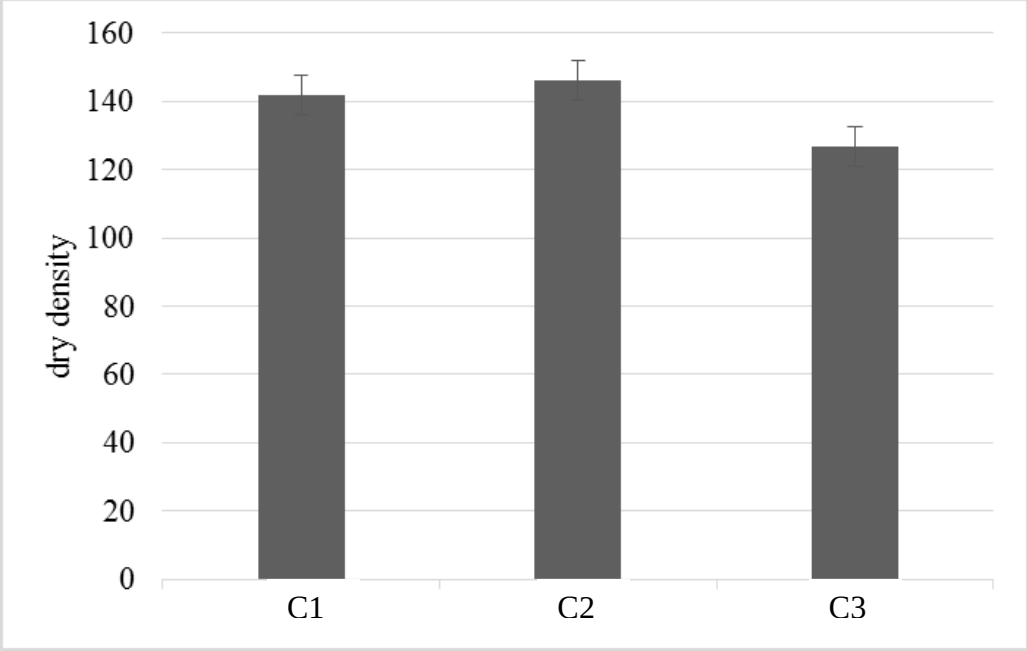



Figure 4. 24: Dry density of each composite

# CHAPTER FIVE

## 5.0 CONCLUSION AND RECOMMENDATION

## 5.1 CONCLUSION

Bio-composites consisting of natural fibers (loose hemp fibers) and mycelium matrices were prepared. The mechanical and physical properties of the various compositions were determined. The results in both cases were compared to verify the effects of reinforcement. The mechanical properties were determined using a universal testing machine. Scanning electron microscopy was used to characterize the surface morphology of the prepared composite.

Experimental incubation tests were performed on two groups of samples. The first group consisted of a mix of many different combinations of substrates and fungi. This group was used in an explorative fashion to study which combination provided the best results. It was found that sterilizing the substrate was an adequate method of pre-treatment. The hypothesis that hemp was very compatible with fungi proved to be true as it was found that the combination of *white oyster* and hemp fibers yielded the densest growth and the

highest flexural strength. The second group therefore consist of Grey dove, Oyster, Dryad's saddle and hemp fibers as substrates.

Higher density samples were observed to also have higher flexural strength, thus supporting the need for more densely packed composites and/or utilization of mushroom species with denser mycelium matrices. On the other hand, manufacturers seeking softer materials may pursue species with a lower flexural. Grey dove mushrooms displayed very good compressive strength comparable to Ecovative grown tile, providing opportunities for stiff but tough cork-like or sturdy materials for suitable applications. White Oyster mushroom displays good flexural strength even superior to Ecovative composite providing materials opportunity for flexibility.

## 5.2 RECOMMENDATION

Summarizing this project from the results obtained found that mycelium-based materials should not be equally compared with high strength materials such as polymer composites, wood or bamboo. Rather, they belong in the category of softer lightweight materials such as expanded polystyrene. This realization leads to the implication that some other properties than the ones studied in this research project are important for mycelium-based materials. In the group of soft lightweight materials, thermal and dynamic properties become far more important than mechanical properties such as strength and stiffness. It is for these reasons that the author recommends future research in the direction of properties important for lightweight materials.

To start, the thermal conductivity needs to be discovered and then the damping effect of mycelium materials should be studied. Especially in structures where vibrations are governing such as wooden floors, mycelium-based materials could be very useful.

Another application where mycelium-based materials can be interesting is in sandwich panels. The core materials are currently often EPS foams. Mycelium-based materials can offer a sustainable and cheap alternative. For core materials the behavior of the material in shear is crucial. Therefore the author recommends a study of mycelium-based materials loaded in shear.

There is also a need to investigate the hydrological characteristics and potential pretreatments that are capable of improving the hydrophilic properties. This, since the discovery of this novel material, have been a treat to its durability under exposed environment. Chemical modification of the natural fibers offers to be a good point to start.

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