



An experimental study on production opportunities of biocomposite by using fungal mycelium

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Abstract

Due to the adaptability, durability, and affordability of synthetic polymers, their usage has been increasing in the global industry. These petroleum-based polymers remain intact in nature for many years after they expire and cannot be included in the natural recycling network in any way. Producing polymers using fossil resources increasingly day by day threatens existing resources and affects the circular economy negatively. Considering the various negative effects of polymers on the environment, biopolymers could be seen as a strong alternative; which is a polymer group formed by living organisms such as plants, animals, and microorganisms. Ecological, low-emission, and recyclable biopolymers open up new and a broad range of topics in the field. Composite materials created with these biopolymer materials that act as natural adhesives; have different developing areas of applications such as packaging industry, textile, furniture, and industrial design sectors, architectural designs, and structural insulation materials. Fungal mycelium, a biopolymer, consists of fibrous filaments called hyphae, which can be defined as elongated cells, mainly composed of chitin, glucan, and proteins. The ability of fungal mycelium to digest and grow through organic matter makes it possible to produce biocomposites from mycelium. Mycelium-based composites are mixed with fungal mycelium, forming an interpenetrating three-dimensional filamentous network that binds the raw material to the material, and after completing the growth period, the mycelium growth is stopped by heat, thus offering an alternating fabrication paradigm based on the growth of materials. In this study, firstly, it was tried to find the most efficient ratio among different mixing ratios by using the mycelium of the genus *Pleurotus Ostreatus* and the same raw materials. Afterward, it was aimed to investigate the mechanical and physical properties through experimental studies, especially the production process, of mycelium-based composites formed by mixing different raw materials in determining proportions.

Keywords: bio-composite building material, biopolymer, mycelium, mycelium composite, sustainable materials.

1. Introduction

With the increasing population and urbanization in the world, the annual waste production and the consumption of available resources are gradually increasing. The waste generated mainly originates from trade centers, the construction sector, houses, agriculture, and various industries. Inappropriate recycling of the wastes produced causes pollution of water bodies, air, landfills, and fertile soils, causing serious damage to the environment. In addition, the rapid and unconscious consumption of natural resources necessitates the search for renewable and recyclable materials

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and the search for alternative ways to use existing resources (Abdel-Shafy & Mansour, 2018; Alemu et al., 2022; Joshi et al., 2020).

As a result of the current linear economy "produce, use and dispose of" model; It is seen that the construction sector has a significant share in global greenhouse gas emissions, depredation of natural habitats and industrial waste production. The increasing use of non-renewable materials such as concrete and steel in the sector creates environmental pressure on limited natural resources and is thought to lead to permanent depletion of resources in the near future. As a result of resource scarcity and public awareness of the building industry's increasing consumption of materials and energy, there has been a growing interest and demand for bio-based building materials and components. In this context, the emergence of biopolymers is considered promising for the future. Biopolymers are a powerful alternative to synthetic polymers. The production of biopolymers is based on living organisms such as plants, animals, and microorganisms. The increasing demand for biopolymer raw materials in industrial use leads to competition with existing stocks for food supply and complex socio-economic troubles. Therefore, the discovery of new alternative materials that are not only naturally grown and harvested, but also produced through developable processes that can be reused in waste streams and have reusability and recyclability at the end of the life cycle becomes important. (Bitting et al.,2022; Heisel & Rau-Oberhuber, 2020).

With its favorable material properties and rapid growth, fungal mycelium has become a popular research topic in recent years. Recent advances in mycelium-based renewable composites show significant potential in converting industrial waste streams into a suitable source to produce more sustainable and cyclical materials. Mycelium is the vegetative part of the fungus, which comprises of a dense network of microfilaments and is called hyphae. Myceliums are chitin-based biopolymers that can bind food, agricultural and industrial wastes with little or no commercial value and transform them into high-value composite materials with a wide variety of applications. (Bitting et al.,2022).

The quality of the composite formed with fungal mycelium as a natural adhesive material based on both the type of fungus and the type of raw material to compound. It also has unique properties low cost, low emission, and recyclable. In addition to using low amounts of energy during production and having a high biodegradability profile, mycelium-based materials are considered excessively customizable throughout the cultivation and manufacturing processes. This makes it possible to produce mycelium-based materials with several properties that can provide varying criteria from different disciplines and they are suitable for several applications. Structural applications of low-carbon materials such as mycelium in the construction industry have the potential to dramatically improve a building's environmental performance. When the structural applications of mycelium-based composites are examined, their application as a carrier structural element is restricted due to their low mechanical properties. However, with its insulating properties and moderate durability, this composite material is seen as an ideal bio-based substitute for conventional insulation elements. Today, the inadequacy of mycelium composites in large-scale architectural applications is due to the monopoly of mycelium-related patents on the market. This situation hampers the distribution of information for the mass production of mycelium composites. In addition, publications on new research and applications of mycelium-based materials prone to store data on the types of fungi used, incubation parameters, feedstock compositions, and detailed production procedures. The scarcity of generalized knowledge; makes it difficult for users to be aware of the existence of these materials and to trust large-scale applications (Attias et al., 2020; Bitting et al.,2022; Jones et al., 2020).

Within the scope of this study, mycelium-based composite material samples were produced based on the production methods and rates in the existing literature and physical and mechanical property tests of the samples produced and the results were evaluated. It is thought that this study will contribute to the development of environmentally friendly, sustainable alternative materials.

2. The Production Processes, Usage Areas and Current Studies of Mycelium Based Composites

This section describes the structure and production processes of the mycelium material. Afterward, an overview of the usage areas and the existing examples are presented.

2.1. Mycelium

Mycelium is a complex network of interlocked, microscopic, tubular fibrous cell chains, containing the vegetative part or roots of saprophytic fungi. It represents the structure that provides the necessary nutrition for the growth and development of fungi. Mycelium consists of fibrous filaments called hyphae, which are mainly composed of chitin, glucan, and proteins, which can be described as elongated cells. (Attias et al., 2020; Etinosa, 2019; Haneef et al., 2017). Schematic picture of mushroom mycelium is shown in Figure 1.

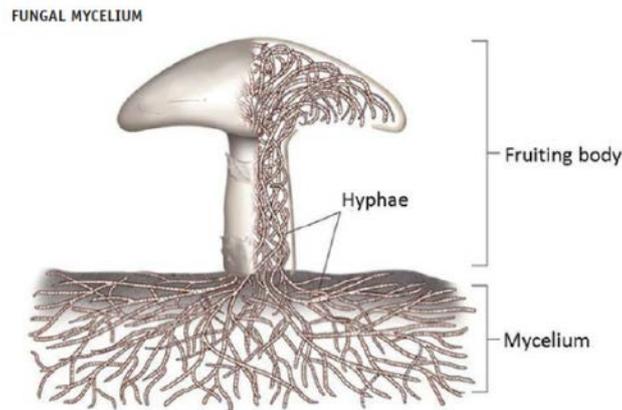


Figure 1 Fungal Composition Divided into Underground Mycelium and Fruiting Body (Url-1).

The ability of fungal mycelium to digest and grow through organic substances allows composites to be produced from mycelium. In short, mycelium acts as a natural binder that holds organic fibers or particles together, making it possible to create a natural, lightweight biocomposite. (Attias et al., 2020; Etinosa, 2019; Haneef et al., 2017). In Figure 2, mycelium morphology, hypha structure and cell wall are schematized.

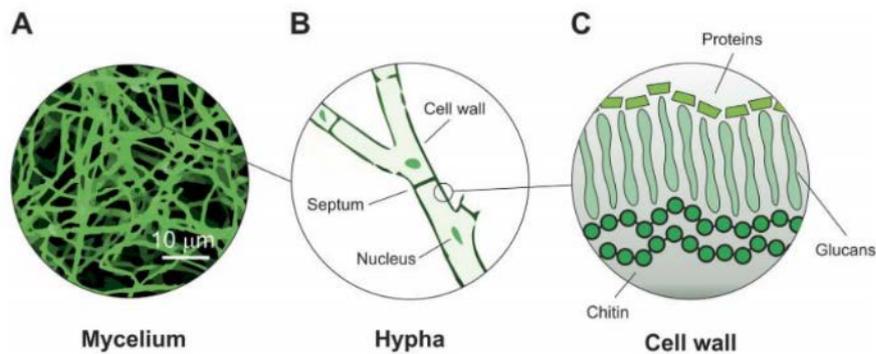


Figure 2 (A) Optical Morphology of Mycelium Fiber (B) Schematic Representation of Hyphae (C) Schematic Representation of The Cell Wall (Haneef Et Al., 2017).

2.2. General Overview of Production and Growth Methods of Mycelium Composites

The fungal myceliums represents the structure that provides the necessary nutrition for the growth and development of fungi. The ability of the fungal mycelium to be digested and grown through organic substances makes it possible to produce composites from mycelium. (Attias et al., 2020; Etinosa, 2019; Haneef et al., 2017).

The production processes of mycelium-based composite materials consist of several steps. First, the mycelium needs to be mixed with the nutritious feedstock to grow. The fact that these nutrients consist of materials with cellulose-rich content (straw, sawdust, hemp, cotton, etc.) is substantial for the growth of the fungus. Because fungi, unlike other organisms, have the ability to break down cellulose and convert it into glucose. Its means that fungi can grow swiftly in cellulose-rich environments. It is necessary to purify the selected feedstocks from other organisms that may infect the fungus before mixing it with mycelium, to prevent the formation of mold. For this reason, first of all, the feedstocks must be sterilized in the autoclave device. The raw materials that have been sterilized should be kept to cool before mixing with the mycelium, then mixed with the selected mycelium type using tools such as pre-sterilized containers. Then the molded mixture is left to grow. (Elsacker et al., 2020; Etinosa, 2019; Lelivelt, 2015).

It is substantial that the environment is dark during the growth phase. Because some species of mushrooms try to produce fruit in the presence of light, thinking that it has reached a free surface. In addition to darkness, the growth environment should contain high humidity (~80-90%), suitable temperature (~25-30°C) and oxygen. It is known that in optimized conditions, fungal growth lasts about 14-16 days. After the growth stage reaches the desired level, the drying process should be done. Otherwise, the mushroom micelles will begin to produce fruit after consuming all the feedstock. High temperature drying is required to terminate this growth. (Elsacker et al., 2020; Etinosa, 2019; Lelivelt, 2015).

Production variables are influential in the yield of fungal growth. In addition, it can be said that the fertile varies according to the structural properties of the components that make up the composite. Table.1 shows the factors affecting fungal growth.

Table 1 Factors Affecting Fungus Growth (adopted. Elsacker et al., 2020).

FACTORS AFFECTING FUNGUS GROWTH	
STRUCTURAL FACTORS	PRODUCTION VARIABLES
<ul style="list-style-type: none"> FUNGUS TYPE SELECTION 	<ul style="list-style-type: none"> SUBSTRATE STERILIZATION METHOD
Phylogenetic diversity Hyphal growth, branching and fusion Cell wall composition Lifestyle and capacity to degrade lignocellulose	<ul style="list-style-type: none"> INOCULATION METHOD PACKING METHOD GROWTH CONDITIONS
<ul style="list-style-type: none"> SELECTION OF RAW MATERIAL TYPE 	<ul style="list-style-type: none"> GROWTH TIME
Nature of lignocellulosic feedstock Direct impact of the feedstock type on the material properties Impact of feedstock on fungal biology	<ul style="list-style-type: none"> DRYING METHOD
	<ul style="list-style-type: none"> POST-PROCESSING

As it is known, since mycelium-based materials are naturally degradable, they can dissolve and rejoin the life cycle when they reach the end-user when they reach the end of their useful life. The diagram in Figure 3 shows the production stages of mycelium-based materials (Etinosa, 2019; Elsacker et al., 2020; Lelivelt, 2015).

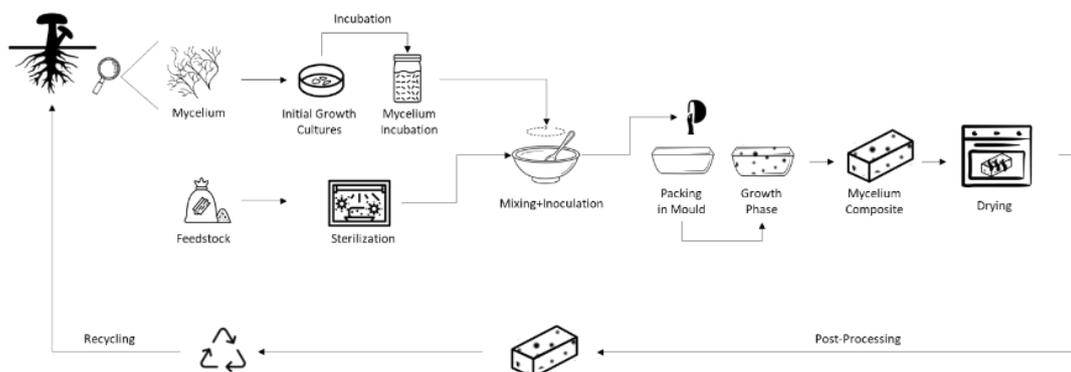


Figure 3 Diagram Showing the Production Stages of Mycelium-Based Materials (adopted Dias et al., 2021; Elsacker et al., 2020; Girometta et al., 2019; Lelivelt, 2015; Rafiee et al., 2021; Udayanga, D., & Miriyagalla, 2021).

There is no set standard for the production of mycelium-based materials. Although there are many studies as a result of the literature review, it is seen that information about mycelium composite production is deficient. It can be said that publications on new research and applications of mycelium-based materials prone to hide information and data on the types of fungi used, incubation parameters, feedstock compositions, or detailed production procedures. As the huge majority of authors are affiliated with commercial companies. Some data related to experimental studies on the production of mycelium-based materials, in which data on mycelium production stages are shared in the literature, are compiled in Table 2 below.

Table 2 Production Protocols Sorted by Years, Developed by Different Researchers (adopted. Elsacker et al., 2020).

Fungal Species	Feedstock	Sterilization Method	Growing Conditions	Growth Time	Drying Method	Application Field	Year	References
Coriolus Versicolor Pleurotus Ostreatus	Hemp hurd, wood chips, hemp mat, hemp fibres, non-woven mats	Pasteurization	25 °C 90-100% RH	30 Days	125 °C 120 min	Foam	2015	Leivelt et al.
Ganoderma Lucidum	Sawdust	Not Specified	25-30 °C	14 Days	70 °C	-	2016	Travaglini et al.
Pleurotus Djamor	Sawdust	Not Specified	20-25 °C, 80% RH	5-25 Days	55 °C 20 min	Foam	2016	Ahmadi
Ganoderma Lucidum Pleurotus Ostreatus	Cellulose Cellulosis- PDB	120 °C 15 min Autoclaving	25-30 °C, 70-80% RH	20 Days	60 °C 120 min	Fibrous Film	2017	Haneef et al.
Irpex Lacteus	Macerated Sawdust Millet Grain Natural Fibre Calcium Sulfate Wheat Bran	Pasteurization	Not Specified	14-42 Days	60 °C 24 h	Foam	2017	Yang et al.
Pleurotus Pulmonarius	Sawdust	121 °C 60 min Autoclaving	25 °C	28-35 Days	105 °C 48 h	Building Material	2017	Attias et al.
Ecovative Design	Biotex Jute, Biotex Flax, Biotex cellulose plain weave	Sterilized by 10% hydrogen peroxide solution	24 °C	5 Days	82 °C 12 h 93 °C 8 h	The core of sandwich structures.	2017	Jiang et al.
Pleurotus Ostreatus Fomes Fomentarius	Beech European oak pear wood chips sand gravel aggregates	Autoclaving	25 °C-28 °C	14-28 Days	95 °C	Building Material	2017	Moser et al.
Trametes Ochracea Pleurotus Ostreatus	Beech Sawdust Straw Cotton-fibre	Not Specified	25 °C 55-70% RH	24 Days	150 °C 20 min	Foam	2018	Appels et al.
Pleurotus Ostreatus Pleurotus Citrinopileatus Pleurotus Eryngii Ganoderma Lucidum	Jute Cotton Starch	80-90 °C sterilized by heating in the oven	25 °C	7 Days	90 °C 120 min	Textile Material	2018	Silverman
Trametes Virscicolor	Pirinç Kabuğu, Cam Kırıkları, Buğday Taneleri	121 °C 40 min Autoclaving	25 °C 50% RH	12 Day	50 °C 48 h	Building Material	2018	Jones et al.
Trametes Versicolor	Flax, flax dust, flax longtreated fibres, flax longuntreated fibres, flaxwaste, wheatstraw dust, wheat straw, hemp fibres and pinesoftwood shavings	121 °C 20 min Autoclaving	28 °C	8+8 Days	70 °C 5-10 h	Thermal Insulation	2019	Elsacker et al.
Pleurotus Ostreatus	Sawdust Coir Pith	121 °C 15-20 min Autoclaving	27 °C 80% RH	14 Days	140 °C 20 min	Building Material	2021	Sivaprasad et al.
Trametes Versicolor Ganoderma Resinaceum	Hemp Hurd Beechwood Sawdust	121 °C 20 min Autoclaving	26 °C 60% RH	9-11 Days	125 °C 10 h	Building Material	2021	Elsacker et al.
Pleurotus Ostreatus	Hemp, Rice Straw, Lacquer Wood Chips, And Oak Wood Chips	121 °C 90 min Autoclaving	25 °C 65% RH	21-25 Days	65 °C 24 h	Building Material	2021	Lee and Choi
Pleurotus Ostreatus Trametes Hirsuta	Oat Bran Coarse Wheat Flour	121 °C 30 min Autoclaving	27 °C	27 Days	120 °C 3 h	Building Material	2022	Kuribayashi et al.
Ganoderma Lucidum	Cellulose Fibre RPS	121 °C 40 min Autoclaving	30 °C 58% RH	7+14 Days	Not Specified	Building Material	2022	Gauvin et al.
Ganoderma Lucidum	Sawdust	60 min sterilized by heating in the oven	23-25 °C	7+7 Days	100 °C 45 min	Industrial Object	?	Url-2

The studies examined show that the production conditions are generally similar to each other. In some cases, it can be said that differences are also observed according to the type of mushroom and feedstock chosen.

Usage Areas of Mycelium Composites and Current Studies

Today, there is a growing interest in mycelium-based materials. In the studies on this promising new material, it is known that the companies are in partnership with the academy. Therefore, most academic studies do not include information about materials and data due to commercial concerns. (Attias et al., 2020).

One of the leading companies in the mycelium sector is Ecovative Design LLC. The company focuses on developing mycelium in the areas of protective packaging products and the production of insulating material to replace conventional polystyrene-based materials. Grown.bio is a company focused on creating ecologically based alternatives to fossil fuel-based plastics such as polystyrene (EPS) and expanded polypropene (EPP) in the packaging industry, industrial products, and construction industry using mycelium technology pioneered by Ecovative Design LLC. Mogu, another company conducting mycelium composite studies, aims to develop sustainable alternatives in interior and product design applications. It produces acoustic insulation and floor tiles made of mycelium. Another company that is active in the industrial potential of mycelium-based composites is MycoWorks Inc. The company, which started its activities with studies on mycelium bricks, focuses on mycelium-based leather production today. (Url-3, Url-4, Url-5, Url-6).

The application scale of mycelium-based materials; is divided into two categories: the basis of the product described as small and the basis of the architectural project described as large. Some examples of applications at the product scale include interior architectural furniture, industrial design products, and consumer products such as clothing, insulation materials, and building materials. At the architectural scale, it includes large assembly applications with the gathered individual modules. (Bitting et al., 2022).

Product Scale Application Areas of Mycelium Composites

In this section, examples of Product Scale Application Areas of Mycelium Composites are discussed.

Furniture Area

The chairs designed by Eric Klarenbeek, who developed products produced by three-dimensional printing where the materials used were bioplastics and mycelium instead of fossil-based materials, constitute unique examples of the use of mycelium in the furniture sector. Products produce oxygen throughout life by using mycelium material. In addition to minimum energy use in the production process, the products are also considered very substantial in terms of being fully recyclable after completing their useful life (Attias et al., 2020; Bitting et al. 2022; Elbasdi, 2016). The picture of the example is shown in Figure 4.



Figure 3 Eric Klarenbeek's Mycelium Studies (Url-7).

Packaging Area

The first examples of the use of mycelium in the packaging industry are produced by Ecovative Design LLC. with a mixture of hemp husk and mycelium. Packages that have completed their useful life can be separated naturally and reintegrated into the life cycle without any additional processing. (Url-3). The picture of the examples are shown in Figure 5.



Figure 4 Ecovative Design LLC. Packaging Products (Url-3).

Textile Area

It is seen that the use of mycelium material is directed toward productions that can be an alternative to leather, especially in the field of textiles. MycoWorks Inc., one of the leading companies in mycelium production, focuses its work on the production of mycelium-based leather. In addition, as a result of designer Aniela Hoitink's work by combining mycelium with textile elements, a flexible composite product called MycoTex was developed. Again, as a result of the fashion world's search for alternatives to plastic and leather, the MYCL company offered a series of products made of mycelium for sale. (Elbasdi, 2016; Url-6; Url-8; Url-9). The picture of the examples are shown in Figure 6.



Figure 5 a) Mycelium Leather (Url-6) b) Mycelium Dress (Url-8) c) Mycelium Shoes (Url-9).

Electronic Area

As an alternative to the plastic parts used in the electronics sector, mycelium blocks are used, which are enlarged in molds and then shaped with a laser for the necessary equipment. The properties of mycelium such as deficiency of electrical conductivity and high fire resistance provide advantages in such applications (Bitting, 2022; Vasquez& Vega; 2019). The picture of the example is shown in Figure 7.



Figure 6 Applications of Mycelium Material in Electronics Industry (Url-10).

Building Materials Area

When compared to other conventional composites Mycelium-based composites have many advantages, such as lower energy consumption, low carbon footprint, recyclability, low density, and cost. Mycelium composites are composed entirely of a combination of organic materials. Thanks to this, fully compostable materials support the transition to the cyclical economy as they preserve their economic value and prevent waste generation. Although mycelium composites, which are advantageous in many respects, produce advanced solutions in various fields such as conventional plastic films, and synthetic foams, they constitute a new research area developing for the building industry (Javadian et al., 2020).

The thermal and fire resistance properties of mycelium composite materials pave the way for their use as a layable material in structural applications. The company Biohm produces mycelium insulation materials with a thermal conductivity coefficient of 0.024 W/m.K. These materials are capable of competing with conventional materials in terms of both performance characteristics and production costs. (Url-11).

Another feature of mycelium composites is that they are suitable for acoustic panels. In addition to its insulating performance, there are also areas of use such as floor coverings and tiles produced by Mogu company. (Bitting et al. 2022; Url-5). The picture of the examples are shown in Figure 8.



Figure 7 a) Mycelium Thermal Insulation Panel (Url- 11) b) Mycelium Acoustic Panel (Url-5) c) Mycelium Floor Tiles (Url-5).

Despite the ecological advantages of mycelium materials, they have several limitations for their use in structural applications. These limitations are due to the low water resistance capacity compared to synthetic alternatives due to the low strength properties of the material and its organic origin. However, studies show that these limitations can be improved by production methods, the type of mycelium, and the feedstock used. (Appels et al. 2019; Javadian et al. 2020).

Architectural Scale Applications of Mycelium Composites

In this section, examples of architectural scale applications of mycelium composites are discussed.

Mycotecture

Designed by artist Philip Ross, this work represents the shape of an arch created by combining materials in a prefabricated manner. All of the bricks used in the project were grown from mycelium. (Url-12). The picture of the examples are shown in Figure 9.



Figure 8 Mycotecture-2009 (Url-12).

Hi-Fi Tower

The Hi-Fi Tower project, designed by Living, is a tower of 10,000 bricks made from fibrous mushrooms connected to agricultural waste. After the structure was on display for three months, it was fully composted and distributed to local gardens, returning it to its life cycle. (Url-13; Uri; 14). The picture of the examples are shown in Figure 10.



Figure 9 Hi-Fi Tower-2014 (Url-13).

Shell Mycelium Pavilion

It is a study that aims to introduce mycelium as an alternative material suitable for constructing temporary structures owing to the environmentally friendly properties of mycelium, which consists of the root network of fungi. (Url-15). The picture of the example is shown in Figure 11.



Figure 10 Shell Mycelium Pavilion-2016 (Url-15).

MycoTree

The MycoTree project uses improved mycelium composites to create a load-bearing structure. The strength and rigidity of the structure is derived from its geometry rather than its material (Heisel et al., 2017). The picture of the example is shown in Figure 12.



Figure 11 MycoTree-2017 (Heisel et al., 2017).

Monolithic Mycelium Experiments

The monolithic mycelium experiments, led by Jonathan Dessi-Olive, consist of a series of studies to adapt mycelium. While the belt created in the studies can carry a person's weight, it shows both the significance of geometry and the possibility of growing in place in an unsterile conditions (Url-16). The picture of the example is shown in Figure 13.



Figure 12 Mycelium Experiments of Jonathan Dessi-Olive-2017 (Url-16).

The Circular Garden

Scope of the project, the myceliums were grown over two months to create a series of arches. This project brought a new approach to mycelium composites by combining monolithic mycelium structures with prefabrication technique (Url-17). The picture of the example is shown in Figure 14.

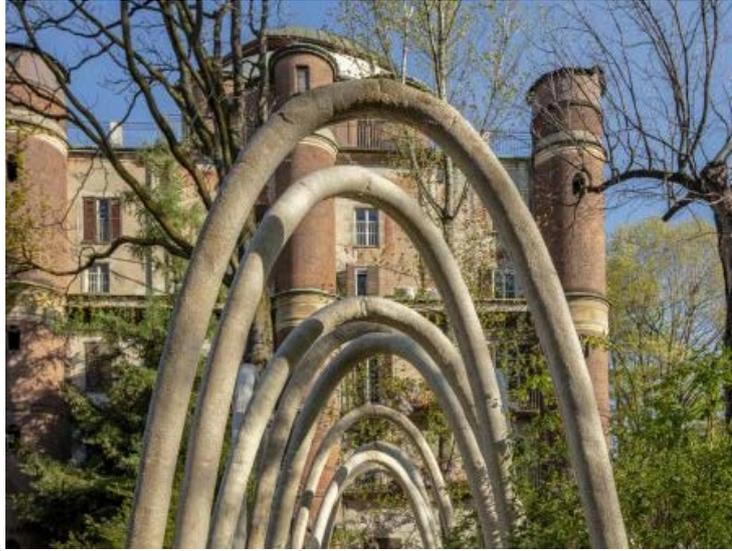


Figure 13 The Circular Garden-2019 (Url-17).

The Growing Pavilion

The pavilion displays a combination of 88 façade panels made of mycelium, combining various bio-based materials such as wood, hemp, mycelium, long tail, and cotton. (Url-18). The picture of the example is shown in Figure 15.



Figure 14 The Growing Pavilion-2019 (Url-18).

MY-CO-X

The project is a prototype for temporary housing and is used as a sleeping and learning station, as well as an exhibition room. Its morphology is based on an uninterrupted functional diagram. The species *Fomes Fomentarius* was grown on pieces of hemp with mycelium (Url-19; Url-20). The picture of the example is shown in Figure 16.



Figure 15 MY-CO-X-2021 (Url-19).

MycoCreate 2.0

MycoCreate 2.0, offers a computational form-finding strategy for compression-only, component-based spatial structures produced with mycelium-based composites (Url-21). The picture of the example is shown in Figure 17.



Figure 16 MycoCreate 2.0.-2022 (Url-21).

La Parete Fungina

La Parete Fungina is a wall inspired by historic spiral brick walls on UVA's campus but created with fully biodegradable mycelium bricks (Url-22). The picture of the example is shown in Figure 18.



Figure 17 La Parete Fungina-2022 (Url-22).

Talinn Architecture Biennale

The project prints the wooden structure according to a generative algorithm using an industrial robot, demonstrating the potential of combining new technologies with natural organisms using mycelium. (Url-23). The picture of the example is shown in Figure 19.



Figure 18 Talinn Architecture Biennale-2022 (Url-23).

Many of the project applications of mycelium demonstrate tendency to use mycelium-based composites grown in molds. In most cases, the structure is divided into smaller components that will be prefabricated and assembled in place in a controlled environment. In these projects, the mycelium-based composite is used, then components that require an exoskeleton or an auxiliary structure to ensure the main stability. Due to the low mechanical properties of the mycelium-based composite, the height of the structure appears to be a substantial limiting factor. In projects where mycelium is grown on-site, the size of the projects is quite small due to logistical difficulties and the long time conjunction with the growth of mycelium. In addition, inadequate material strength is not able to adapt to the increased self-weight as the size of the structure increases. As a result, it is seen that the most successful mycelium-based material applications in reaching the construction sector are heat or acoustic insulation panels (Bitting et al. 2022).

3. Material and Method

The production of mycelium-based composite material is a new research topic. There is no determined standard for its production. In this study, first of all, samples were created with the mixture ratios of the two referenced studies in order to determine the yield according to the mixture ratios of the produced composites. Then, based on the determined ratio, samples were created in which different raw materials were mixed and studies were carried out to determine the mixture rate to be referenced and the raw material to be used. In addition, in order to compare samples at different incubation periods, each mixture was kept in a light- and the airtight environment under standard room conditions for up to 28 days with 7-day periods.

3.1. Materials

In this study, fungal myceliums of the genus 'Pleurotus Ostreatus' pre-grafted with oat bran and stored in the freezer were used. Myceliums, which contain a large number of hyphae, vegetative tissues, and spores, were purchased from a specialized company. In this preliminary study, the feedstocks required for the nutrition of myceliums were selected as hay, rice shell, beech shavings, walnut shell, and rice shell powder in line with the studies in the literature and the fastest supply conditions. (Gauvin et al., 2022; Jones et al., 2018; Url-2; Url-24).

The materials used as raw materials differ in terms of dimensions and the cellulose, hemicellulose, and lignin ratios they contain. In Figure 20, the images of the raw material types and the graph of the composition ratios are given.

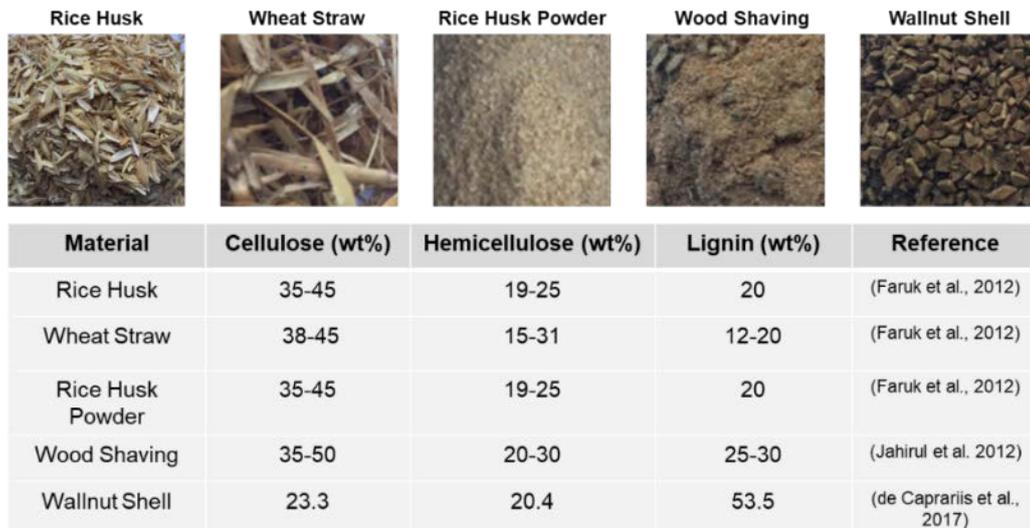


Figure 19 Compositions of Feedstocks Used

To create a mycelium composite, fungal mycelium of the genus *Pleurotus Ostreatus* and straw were mixed with the mixing ratios of two different studies, and production was made based on the production stages shown in Figure 3. Information on reference mixing ratios is shown in Table 3.

Table 3 Mycelium Composite Mixing Ratios of Reference Studies

Mycelium	Feedstocks	Reference
50 g	200 g	(Url-2)
100 g	200 g	(Gauvin et al., 2022)

According to the mixture ratios in the reference studies, the samples produced were evaluated and a mixture ratio was determined. To observe the effect of feedstocks using the determined ratio, 5 different mixtures with rice shell, straw, rice shell powder, sawdust, and walnut shell were produced based on the stages in Figure 3.

The mixing ratios of the experimental studies are given in Table 4.

Table 4 Mycelium Composite Mixing Ratios of Experimental Studies

Experiment No	Ingredients	Mixing Ratio
1	Mycelium+Rice Husk	1:2
2	Mycelium+Wheat Straw	1:2
3	Mycelium+Rice Husk Powder	1:2
4	Mycelium+Wood Shaving	1:2
5	Mycelium+Walnut Shell	1:2

3.2. Mixture Preparation and Growing Conditions

In this study, the solid grafting method was preferred instead of a liquid method with ovulation. While the mycelium is growing, each surface to be used to prevent bacteria-induced mold is sterilized with 70% ethanol. The myceliums, previously inoculated with oat bran, were cut into pieces by hand in a container and mixed with sterilized feedstocks. The materials mixed until homogeneous are left to grow in bags for 7 days to create a more comfortable growth environment

before molding. The incubation medium created for mycelium composite samples; is completely dark, with a relative humidity of ~75-80% and set at 24°C. Steps of the preparation of the mixture are shown in Figure 21.



Figure 20 a) Preparing the Mixture According to The Proportions b) Mixing The Mixture c) Samples Kept In The Bag For 7 Days d) Molded Samples And The Prepared Sterile Mold e) Drilling Holes In The Samples After Molding

At the end of the first period of 7 days, the mixture in the bag was poured into the sterile container, crushed and mixed until the whiteness dissipated. Then the mixture is sterilized and allowed to grow in cling film-coated molds with dimensions of 4x4x16cm. After the molding process, the top of the mold is covered with stretch film and holes are drilled on it for the sample to breathe. The samples left to grow in the mold were removed from the molds at the end of 7 days to ensure equal growth on each surface. The specimens, which were planned to have a growth period of 7 days, were dried at 100°C for 45 minutes to stop the growth of mycelium. The remaining samples were taken from the growth medium according to the planned periods of 14, 21, and 28 days and dried at 100°C for 45 minutes. Visuals of the post-growth stages of composite samples are shown in Figure 22.

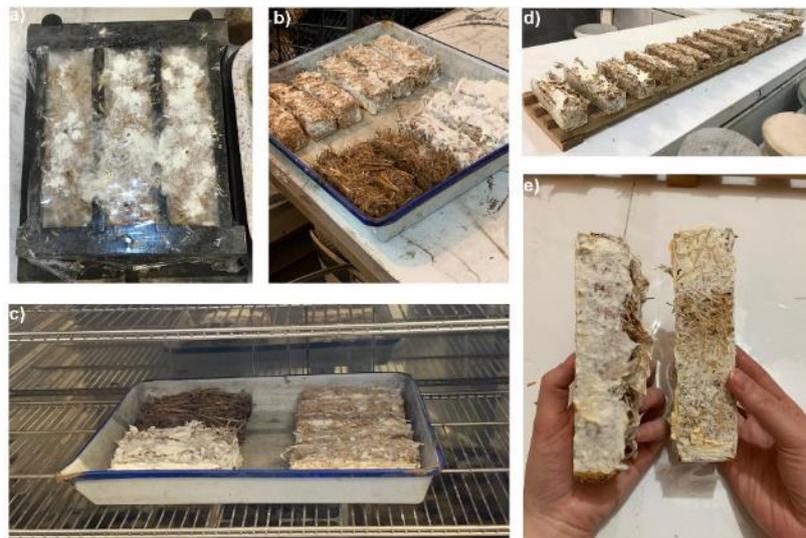


Figure 21 a) Sample for The Samples That Have Completed the Growth Period b) The State of The Different Samples Before Drying c) The Drying of The Samples In The Oven d) The State of The Samples After Drying e) The Sample Sample After The Drying Process

3.3. The Experimental Studies

Experimental studies are described in this section.

Determination of Physical Properties

Experiments were carried out to determine the dry density, moisture content and water absorption rates of the composite material.

Dry density was calculated by the ratio of oven-dry mass to volume based on ISO 9427:2003.

$$\rho = \frac{m}{b_1 b_2 t} \times 10^6$$

m : is the mass of the test piece, in grams (g),

b_1 and b_2 : are the width and the length of the test piece, in millimetres (mm), ($b_1 = b_2$),

t : is the thickness of the test piece, in millimetres (mm).

Moisture content Calculated based on ISO 16979:2003.

$$H = \frac{m_0 - m_1}{m_1} \times 100$$

m_0 : is the initial mass of the test piece, in grams (g),

m_1 : is the mass of the test piece after drying, in grams (g).

Based on BS EN 1097-6:2022 for water absorption rate measurements by weight.

$$WA_{24} = \frac{M_1 - M_4}{M_4} \times 100$$

M_1 is the mass of the saturated and surface-dried aggregate in the air, in grams (g),

M_4 is the mass of the oven-dried test portion in air, in grams (g).

In the test of water absorption rate by weight, a mechanism was created for the samples floating in water. The mechanism are shown in Figure 23.



Figure 22 Mechanism Formed for Water Absorption Measurement

Determination of Mechanical Properties

The compressive and bending loads of the samples were determined using the Instron load bench. It should be taken into account that the samples are not full-sized (4x4x16cm) due to mycelium growth and have rough surfaces. In addition, mycelium composites, which are ductile materials, were not divided into two separate parts after bending tests. In line with these reasons, the samples were divided into 3x4 pieces with a utility knife and compressive tests were performed. The test was stopped at the point where surface cracks began to form in the test samples. The visuals of the mechanical experiments are shown in Figure 24.



Figure 23 Mechanical Measurements

Flexural strength measurement is based on BS EN 196-1:2016.

$$R_f = \frac{1,5 \times F_f \times l}{b^3}$$

R_f is the flexural strength, in megapascals,

b is the side of the square section of the prism, in millimetres,

F_f is the load applied to the middle of the prism at fracture, in newtons,

l is the distance between the supports, in millimetres.

Compressive strength measurement is based on BS EN 196-1:2016.

$$R_c = \frac{F_c}{1600}$$

R_c is the compressive strength, in megapascals,

F_c is the maximum load at fracture, in newtons,

1600 is the area of the platens or auxiliary plates (40 mm × 40 mm), in square millimetres.

4. Results and Discussion

This section presents the results of the experimental study.

4.1. Sample Description and Growth Examination

In the production of mycelium composites, two different mixing ratios used in the referenced studies were tested by mixing mycelium and straw. According to the results of this production, mycelium growth was not observed for 28 days in the mixture with a mycelium-feedstock ratio of 1:4, while a gradually developing growth was observed in each of the 7-day periods in the mixture with a mycelium-feedstock ratio of 1:2. Growth observations according to the competition ratios are shown in Table 5.

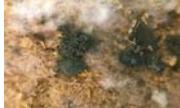
Table 5 Growth Observation of Samples of Different Mixing Ratios

Ratio	14 Day	21 Day	28 Day
114			
112			

Since no growth was observed in samples with a mycelium feedstock mixing ratio of 1:4, the ratio of 1:2 was taken as the basis for later experiments. Since the complete coating of the composite surface with mycelium was completed within 28 days, the samples were left to grow for 28 days in the next stage.

In the second stage, in order not to observe the effect of feedstocks on growth and the properties of the formed composite; Samples were formed by mixing five different feedstocks as rice husk, straw, rice husk powder, sawdust, and walnut shell separately with mycelium. Details of the samples produced are given in Table 6.

Table 6 Test Samples

Experiment No	Feedstock	Sample	Detail
1	Rice Husk		
2	Wheat Straw		
3	Rice Husk Powder		
4	Wood Shaving		
5	Walnut Shell		

Mold growth occurred in the composites formed as a result of tests 1, 3, and 5 within a 28-day period. Mycelium growth has not reached a level that completely covers the surface. Fragmentation was observed because the binding of samples with low, mycelium growth was weak. In the composites formed as a result of experiments 2 and 4, mycelium growth surrounded the surface, and the materials adhered to each other. No mold was observed during the 28-day growth period. To analyze the internal growth of the samples, the non-dispersed samples were split in half from their midpoint. Although a homogeneous mixture is formed during composite mixture preparation, weak growth is observed inside the samples. It is thought that a possible explanation for the weak growth in the sample may be that the materials selected as feedstocks are large in size or that they cannot be easily digested by the mycelium due to growth conditions. As a result of this situation, it is seen that while the outer surface grows, the inner growth does not continue. The cross-section of the samples is shown in [Figure 25](#).



Figure 24 Cross-Sections of Samples from Experiments 2 and 4

The volume calculation was made taking into account the irregularity in the shape of the samples due to mycelium growth. The volume, starting weight, dry weight, dry density, and moisture content of the samples are given in Table 7. The measurements could not be made because the samples of experiment no 3 were scattered.

Table 6 Data of Obtained from Mycelium Composites

Experiment No	Volume (cm ³)	Weight Before Dry(g)	Weight After Dry (g)	Dry Density (g/mm)	Moisture Content %
1	234,68	50,77	34,76	148,13	46,18
2	217,8	61,47	28,29	131,19	117,03
3	Not Measured.				
4	287,4	114,81	71,95	383,57	59,71
5	212,15	134,66	104,32	495,15	30,69

4.2. The Results of Water Absorption Tests

The water absorption rate is an important factor in the application of mycelium composite as an indoor particle board or insulating element, as it will specified the durability of the material over time. Since composite components are organic products, high water absorption rates are expected. However, if we compare the composites with each other, it is seen that the samples consisting of mycelium + sawdust mixture absorb relatively less water than other mixtures. Data of the water absorption rate are shown in Table 8.

Table 7 Water Absorption Rate

Experiment No	Weight of Saturated Surface-Dried Sample(g)	Weight of Oven-Dried Sample (g)	Water Absorption %
1	103,55	50,77	206,81
2	139,49	61,47	411,13
3	Not Measured.		
4	125,23	114,81	74,51
5	242,33	134,66	135,22

4.3. The Results of Mechanical Property Tests

Since there is no standard test procedure for mycelium-based composites, it is settled when cracks begin to form in the sample for flexural strength. In compressive strength, the test was stopped when the compression value of 43% was reached based on the literature (Gauvin et al., 2022). The retention rates of the mixture components are very low because sufficient mycelium growth is not seen in samples containing rice husk, rice husk powder, and walnut shell. Therefore, the samples were scattered and mechanical tests could not be performed. The values are partially in line with previous growth observations. Sawdust-containing samples with dense white mycelium biomass resulted in higher values than those containing straw. In the reference study, samples containing rapeseed straw showed a compressive strength of 0.452 MPa, and samples containing cellulose fiber showed a compressive strength of 0.145 MPa (Gauvin et al., 2022). The findings obtained in our study were similar to the previous study. With the study where different feedstocks significant effect on the composite material. Data on the mechanical property tests are shown in Table 9.

Table 9 The Results of Mechanical Property Tests

Experiment No	Flexural Strenght (MPa)	Compressive Strenght (MPa)
1	Not Measured.	
2	0,37	0,21
3	Not Measured.	
4	0,39	0,32
5	Not Measured.	

5. Conclusion and Suggestions

The findings of this research contribute to the field of biological materials as they provide an overview of the production processes of mycelium-based composites, particularly mechanical and physical experiments. This study investigates the possibilities of manufacturing mycelium-based composites by combining Pleurotus Ostreatus type mycelium with different types of lignocellulosic supplements under the leadership of studies in the literature. The fundamental purpose of the study is to create the appropriate environment and conditions for the production of mycelium composites under existing laboratory conditions by conducting preliminary experiments and the dry density, moisture content, water absorption rate, bending, and compressive strength of the mycelium-based composites created were calculated.

This research is important in terms of eliminating the lack of information due to information not shared due to various concerns in the current literature by determining the mixing ratios to be used in creating mycelium composites. When the growth periods of the experimental mixtures prepared with the mixture ratios of the two referenced studies were observed, it was seen that the growth was at a minimum level in the mixture with a mycelium + feedstocks ratio of 1:4, while the expected yield was obtained from the mixture with a mycelium + feedstocks ratio of 1:2. Samples created with five different feedstocks at the determined mixing ratios then showed that mycelium composite production and mechanical properties depended on fiber types. The growth of samples containing wheat straw and sawdust resulted in more efficient results than samples containing rice husk, rice husk powder, and walnut shell. A possible explanation for these results is that there is a growth difference due to the difference in the ratio of cellulose, hemicellulose, and lignin contained in the feedstocks.

Although the mechanical properties are not yet optimal, this research shows that mycelium composites have the potential to replace fossil-based composites. However, the water absorption rate of the composite, which consists entirely of organic materials, must be reduced for structural applications. The study shows that the manufacturing process affects the desired properties of the composite. In addition, other properties related to insulation materials such as thermal

conductivity, fire resistance, aging, acoustics, and water vapor diffusion should be tested in further research.

The methodology used to assess the suitability and selection of organic waste streams, in general, has proven effective for mycelium-material manufacturing applications. The wide range of options for creating and growing mycelium composites complicates comparing the results with the available literature, as each parameter change affects the growth and mechanical behavior of the composite. More work needs to be done to improve growing conditions, optimize mechanical properties, and establish a standardized manufacturing protocol. Since it is thought that such studies will contribute to the solution of environmental and sustainability problems on a global basis, it is believed that they will shed light on other studies that can be done in this field.

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Resume

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