DEVELOPMENT OF MYCELIUM BIOCOMPOSIT FROM MYCELIUM FUNGI AND AGRICULTURE WASTE TO PROVIDE AS AN ALTERNATIVE TO CONVENTIONAL PLASTIC

FINAL YEAR PROJECT UG-12 (GROUP-9)

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This is to clarify that the

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APPROVAL SHEET

It is certified that contents and form of thesis entitled "Development of mycelium biocomposite from mycelium fungi and agriculture waste to provide as an alternative to conventional plastic" submitted by Hamza Arshad, Jawaria, and Muhammad Ammar have been found satisfactory for the requirement of the degree.

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DEDICATION

We dedicate this thesis to our family and friends for their unconditional love, support and guidance that helps us move mountains and cross oceans with ease.

CHAPTER 1: INTRODUCTION

1.0 Introduction

The use of mycelium materials as a sustainable alternative for packaging has gained significant attention in recent years. Mycelium refers to the vegetative part of fungi, consisting of a network of interconnected filaments called hyphae. This material can be grown using agricultural waste or other organic substrates, making it an environmentally friendly option.

One of the key advantages of mycelium-based packaging is its ability to biodegrade naturally. Unlike polystyrene, which takes hundreds of years to break down, mycelium packaging can decompose within a matter of weeks or months, depending on the specific conditions. This significantly reduces its impact on the environment and contributes to the development of circular economy systems.

Mycelium materials also offer highly tunable properties, allowing for customization based on specific packaging requirements. They can be shaped into various forms and sizes, providing versatility in design. Additionally, mycelium packaging exhibits excellent insulation properties, making it suitable for protecting fragile or temperature-sensitive products.

The potential of mycelium-based materials for sustainable packaging has been recognized by innovators and researchers, as indicated by the increasing number of patent applications in this field. It presents a promising solution for reducing the use of plastics and polystyrene, which have detrimental effects on global warming and contribute to waste accumulation.

In line with the focus on sustainable packaging, the European Union has prioritized the adoption of environmentally friendly alternatives. By promoting the use of sustainable materials like mycelium, the EU aims to work towards carbon neutrality and reduce the negative impact of packaging on the environment.

Overall, the utilization of mycelium materials in packaging offers a viable and eco-friendly solution that aligns with the goals of sustainability and circular economy systems. Continued research and development in this area hold the potential for further advancements and the creation of new natural products with diverse applications.

1.1 Mycelium – Opportunistic Material Of The Future

The field of fungal materials in material sciences has seen significant advancements in recent years, with a focus on developing green and biodegradable alternatives to unsustainable materials derived from crude oil. A survey based on patents published between 2009 and 2018 revealed 47 patents related to fungal materials (Cerimi, et.al., 2019). Among these patents, 26 were specifically related to the use of fungal mycelium materials, while 21 patents were still under consideration for future development.

The analysis of the patent situation indicates that the material-oriented fungal applications sector is experiencing substantial growth. Initially, the applications of fungal materials were mainly in the field of arts. However, more recently, there has been a shift towards industrial manufacturing and utilization of mushroom-based materials (Cerimi, et al., 2019). This shift highlights the expanding scope of fungal materials and their potential for broader applications.

The sustainable manufacturing processes and reusability of fungal-based materials, coupled with their innovative and unique properties, make them promising candidates for replacing existing materials in various industries. However, the extent to which fungal materials can replace traditional materials and the specific industries they will impact will become clearer in the future. The growth and adoption of fungal materials in practical applications require comprehensive transdisciplinary studies to address the challenges and demands of this emerging field (Elsacker, et al., 2020).

Overall, the advancements in fungal materials present exciting prospects for developing sustainable alternatives and reducing reliance on unsustainable materials. Continued research, development, and practical studies are needed to fully explore the potential of fungus-based materials and their applications across different industries.

1.1.1 What is Mycelium?

A filamentous fungus typically begins its life cycle as a small spore, which is only a few microns in diameter. In a favorable environment with sufficient humidity and nutrients, the spore germinates and develops a germ tube. This germ tube elongates and becomes a thread-like cell called a hypha. As the hypha continues to grow and elongate, it forms a network of interconnected hyphal threads known as a mycelium. The mycelium serves as the vegetative body of the fungus and plays a crucial role in nutrient acquisition and colonization.

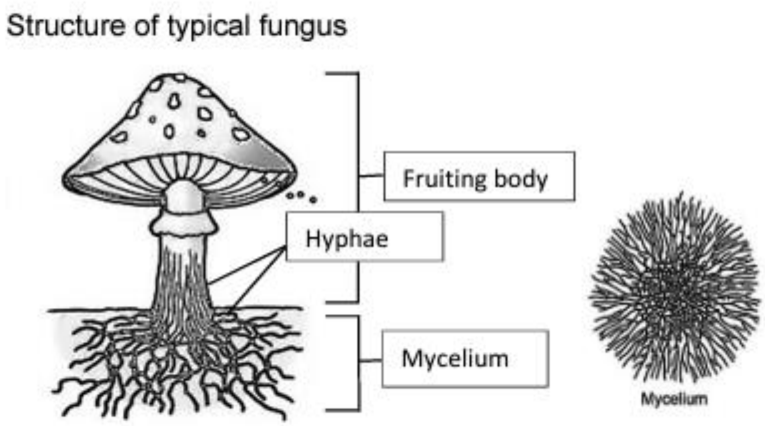


Figure 0.1 life cycle of mycelium

The mycelium of mushroom-forming fungi can also be cultivated on various forestry and agricultural by-products and waste materials. The composition and physical characteristics of the substrate, as well as the environmental conditions such as temperature, humidity, and pH, along with the genetic makeup of the fungus, all influence the efficiency of colonization and biomass generation (Meyer, et al., 2020).

Mycelium, particularly in its pure form or as composite foams, offers various advantages such as shape-fitting, recycling capabilities, and high-performance properties. This opens up a wide range of opportunities for its applications. The lightweight and foamy nature of mycelium-based products can be tailored by adjusting their density, providing versatility for different applications. Additionally, mycelium-based products have low manufacturing costs, require minimal energy input, have reduced environmental consequences, and leave a smaller carbon footprint. These characteristics make them attractive for a variety of uses (Girometta et. al., 2019).

In summary, fungal life cycles begin with spores that germinate and develop into hyphae, which further grow and form interconnected mycelial networks. Mushroom-forming fungi can cultivate mycelium on various organic waste materials. Pure mycelium and composite foam materials derived from it offer shape-fitting capabilities, recycling potential, and high performance. These lightweight products have low manufacturing costs, energy requirements, and environmental impact, making them versatile and environmentally friendly options for different applications.

1.1.2 Laboratory Treatment Of Mycelium

Fungal mycelia offer great potential as diverse and productive sources of bio composites that can replace conventional plastics. However, the utilization of fungal strains for this purpose is still limited, as many strains remain unexplored as alternatives to non-biodegradable materials (Tacer-Caba, 2020).

The production of mycelium can be achieved through various methods and using different materials, but a common procedure involves a substrate, a specific fungal strain, and an incubation process. For instance, in one experiment, plant cellulose from a wastewater treatment plant was used as a notable case of open-loop recycling, utilizing a resource that would otherwise become waste. The selected fungal strain for this experiment was Ganoderma Resinaceum, a type of bracket fungus commonly found in northern and central Europe (Andrew L. Loyd, 2016).

In the laboratory, a stock colony of the fungi is maintained to inoculate a sterilized substrate, which is then allowed to grow in an autoclave bag within a 30°C incubator for a week. After a week of growth, the substrate is manually broken into small pieces, and the mold is filled with the infected substrate. It is crucial to maintain sterility during this process to prevent the growth of bacteria and other contaminants on the substrate, which can weaken the bonding of the mycelium and affect the properties of the final products (Appels, 2018).

The filled mold is then incubated at 30°C for another week before the mycelium is removed from the mold (PICTURE 2) and dried overnight at 65°C. During drying, it is important to place a weight on top of the mycelium to prevent curling due to water evaporation. Once dried, the mycelium is considered finished, but further processes can be implemented to enhance its quality. For example, the mycelium can be compressed to increase density and strength, or it can be colored to enhance its visual appeal (Jones, et al., 2020).

In summary, the production of mycelium-based materials typically involves a substrate, a specific fungal strain, and an incubation process. Maintaining sterility throughout the procedure is crucial to ensure optimal growth and bonding of the mycelium. After drying, additional processes such as compression and coloring can be applied to further improve the quality of the mycelium-based products

1.1.3 Uses Of Mycelium

Mycelium-based materials possess a wide range of properties that make them suitable for various industries, including building materials, packaging, textiles, medical applications, furniture, automobile manufacturing, and the food and cosmetics sectors (Manan, et.al., 2021). As the qualities and capabilities of mycelium materials expand, an increasing number of consumer goods are being produced by companies, including high-performance foams for garments and skincare products (Mycoflex), leather-like textiles (Mylo, Reishi, Mylea, Forager), and meat substitutes (Atlast; Vandelook, et al., 2021).

Foams constitute a significant portion of mycelium production for building and packaging purposes, and the production methods are largely similar. A sterilized substrate is inoculated with the chosen mushroom strain and incubated until the desired density is achieved. Once the design is complete, the material is dried at a high temperature of around 60 degrees Celsius to kill the fungus (Cerimi, et. al., 2019). These composites are moldable, do not require specialized equipment, and exhibit minimal water and energy absorption. They can be used as heat or acoustic insulation, flooring, paneling, cushioning, in cattle feed processing, and as packaging materials (Van Der Hoeven, 2020; Freek Appels & Wosten, 2020).

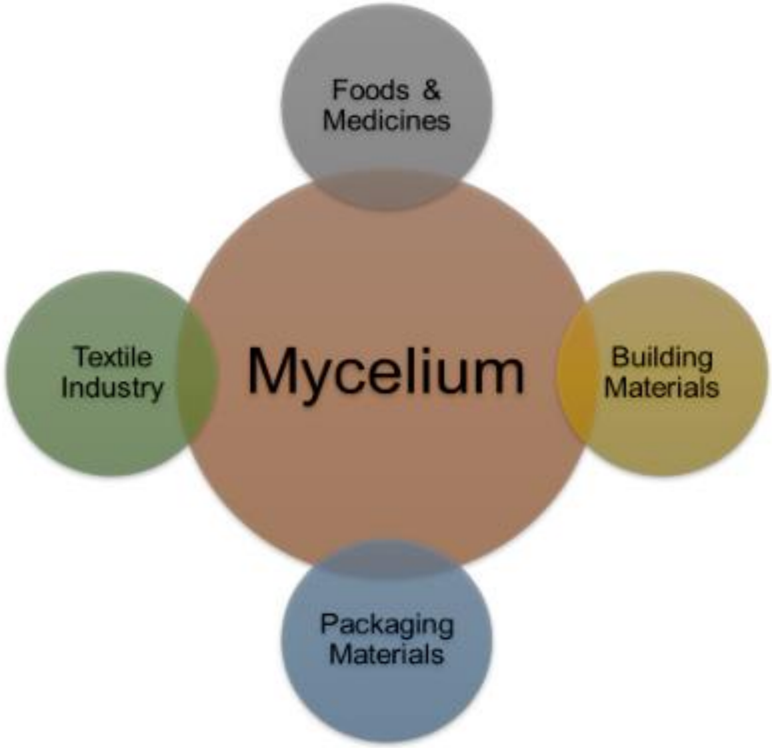


Figure 0.2 sectors where mycelium can be used

When aiming to produce fungal products, two main strategies are explored: growing on a solid medium or growing in a liquid medium. Both methods have their limitations. Steady growth on solid or liquid substrates is possible in controlled environments. Solid medium production allows for the harvest of fungal "skin," while solid substrates only allow for the collection of a portion of the mycelium. Growing as a liquid fermentation, such as in bioreactors, can be labor-intensive and challenging to scale up due to the static condition required. Additionally, it requires significant financial investments. Despite these challenges, mycelium materials hold great potential for replacing leather, paper, and even serving as bacon substitutes after appropriate processing (Atlast, 2020; Butu, 2020; Gandia, 2021).

In conclusion, mycelium-based materials find applications across various industries, and their properties make them suitable for replacing conventional materials in sectors such as building, packaging, textiles, medical, furniture, automobiles, and food and cosmetics. Foams produced from mycelium are commonly used for building and packaging, while different growth methods are explored to create fungal products. The versatility and potential of mycelium materials make them a promising avenue for sustainable and innovative alternatives in various sectors.

1.2 Mushrooms Used For Mycelium Production

Mushrooms have long been recognized for their use as a food source, medicinal remedies, and active ingredients in cosmetics (Rathore, et.al., 2019). While the qualities and applications vary, research suggests that including mycelium extracts from specific mushrooms in the diet can provide nutritional and medicinal benefits to the human body (Ramos, et al., 2019). Fungi have the potential to contribute to all aspects of human activity, promoting healthier and more ecologically friendly lifestyles.

Mycelium, the filamentous network of fungi, is a natural polymeric composite fiber composed primarily of natural polymers like chitin, cellulose, and proteins. The unique structure and composition of mycelium make it a promising material for the generation of a wide range of mycelium-based products. Currently, a US-based company utilizes unprocessed biomass bonded together by mycelium to create foamy structures, but there is still significant potential for further development and research in this field (Haneef, 2017).

The production of fungal bio composites involves the colonization of a substrate, which can be shaped simultaneously or after mycelial development. After colonization, the bio composite material is pressed and dried using various pressure and temperature procedures (Haneef, 2017). In a particular study, researchers investigated the use of mycelium in conjunction with polysaccharide-based substrates of various compositions to create precisely tailored fibrous films with customizable characteristics. They employed edible and medicinal fungal species, Ganoderma lucidum (G. lucidum) and Pleurotus ostreatus (P. ostreatus), which are white rot fungi capable of releasing a range of enzymes, including those involved in the degradation of complex plant components like lignin. These species were chosen for their ability to degrade the same substrates and form interwoven filamentous structures (Girometta, 2019).

In summary, mushrooms and mycelium offer a wide range of applications, including as food sources, medicinal remedies, and cosmetic ingredients. Mycelium-based materials hold promise due to their natural polymeric composition and unique structure. Current research focuses on the production of fungal bio composites, with various colonization and drying techniques employed. The selection of specific fungal species depends on their enzyme-releasing capabilities and the desired characteristics of the final product. Continued research and development in the field of mycelium-based products will likely lead to further advancements and expanded applications in the future

1.2.1 Pleurotus Ostreatus Mushroom

Pleurotus ostreatus, commonly known as oyster mushroom, is widely cultivated worldwide due to its ability to grow on a variety of agricultural and forestry residues with minimal infrastructure requirements (Zervakis & Venturella, 2002). In the production of P. ostreatus, cellulose fiber rejects from industrial-scale wastepaper recycling or pulping processes have been utilized as components in substrates (Grimm, et al., 2021). While wheat straw is often used for commercial production, the availability of cheaper and locally accessible plant residues highlights the need for more productive and cost-effective bio-conversion systems, as demonstrated in subtropical regions (Salmones, et al., 2005).

Figure 0.3 oystreatus mushroom grown on the dead wood

Pleurotus ostreatus is a white-rot fungus (PICTURE 4.) that has garnered significant attention for its remarkable lignin-degrading capabilities (Bellettini et al., 2019). It exhibits efficient cleavage of cellulose, hemicellulose, and lignin from wood, in contrast to brown-rot fungi that can only degrade cellulose and hemicellulose (Machado et al., 2016; Haneef, et al., 2017).

In terms of mechanical properties, P. ostreatus species was found to have lower compressive strength values compared to Ganoderma lucidum species, likely due to the softer mycelial characteristics when wheat straw is used as a substrate. However, P. ostreatus was stiffer than G. lucidum, which could be attributed to its higher polysaccharide content. The composition of mycelium, including polysaccharides, lipids, and chitin, as well as overall mechanical and morphological qualities, significantly influenced the substrate and resulting material properties (Haneef et al., 2017).

Overall, P. ostreatus has gained popularity in cultivation due to its ability to utilize a wide range of agricultural and forestry residues. Its ligninolytic characteristics and mechanical properties make it suitable for various applications in the field of mycelium-based materials. Further research and development in cultivation techniques and substrate utilization can contribute to more productive and cost-effective bio-conversion systems using P. ostreatus.

1.2.2 Ganoderma Mushrooms

The most often used fungal strain for mycelium composites is G. lucidum (PICTURE 5.) (Holt, et.al., 2012; López Nava, et.al., 2016; Jones, et.al., 2017). In mycelium composites, intrinsic and extrinsic variables dictate hyphal architecture, fungal cell wall composition, composite ingredients, and growth kinetics, which vary between species (Jones, et.al., 2017). Among the many varieties, lignocellulose degrading basidiomycetes notably P. ostreatus and G. lucidum may colonize and develop fast on cellulose, hemicelluloses, and lignin-containing substrates (Corrêa, et.al., 2015). P. ostreatus and G. lucidum, have been investigated in mycelium composites. With over 5.1 million known fungal species (Blackwell, 2011), novel fungal species may offer untapped potential if described in composite materials.

Figure 0.4 Ganoderma specie

PICTURE 5. Ganoderma, G. lucidum at Paussac, France (Eric Steinert, CC BY 3.0.) 14

Compressive strength is one of the most important material properties, especially in packaging applications designed to protect the internal contents from mechanical harm (Jones, et.al., 2017). Previous research has linked increased mycelial density to increased hyphae branching, resulting in a more compact structure with fewer micropores. More precisely, hyphae branching in G. lucidum has been proposed as the reason of their density disparities in film application, rather than longitudinal hyphae growth in P. ostreatus (Haneef, et.al., 2017). This conclusion contrasts with the current findings, which reveal that P. ostreatus mycelia cultivated on both substrates had larger densities than G. lucidum. It has been found that replacing the substrate with fungal material reduces the density of mycelium composites (Islam, 2017). The current findings complement prior findings that the compressive strength of mycelium composites is significantly reliant on substrates and mycelia strains, both of which alter the morphology of the mycelium composites (Holt, et.al., 2012).

1.3 Scope Of Project

The purpose of this thesis is to provide a comprehensive overview of mycelium packaging composite materials, including their applications, functions, market importance, and potential benefits for the future. The research aims to explore current trends and future perspectives of fungal mycelium materials across various fields of application.

The thesis will delve into the characteristics and properties of mycelium-based packaging composites, highlighting their potential as sustainable alternatives to conventional packaging materials. It will examine the unique features of mycelium, such as its natural polymeric composition and ability to biodegrade, which make it an attractive option for environmentally friendly packaging solutions.

The research will also emphasize the market importance of mycelium materials, analyzing their current market presence and growth potential. It will identify key players in the industry and examine the factors driving the adoption of mycelium-based products in various sectors. Additionally, the thesis will discuss the economic and environmental benefits associated with mycelium materials, including their potential for circular economy practices and reduced carbon footprint.

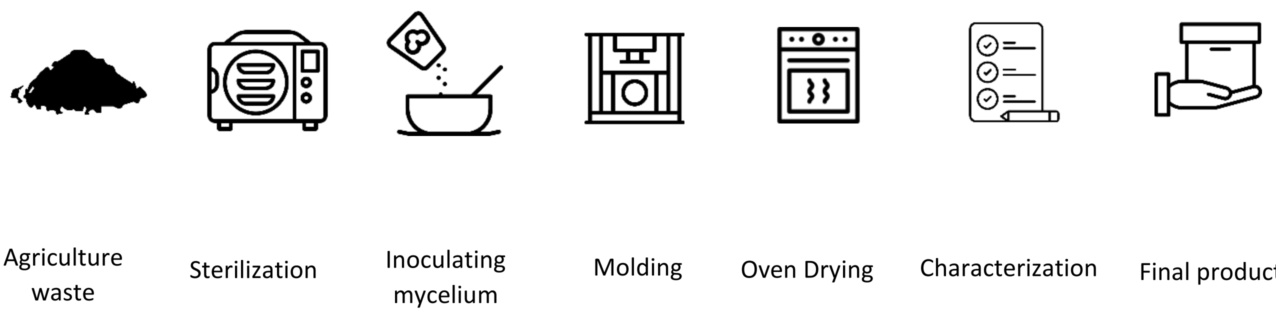
Furthermore, the study will assess current trends in the development and utilization of mycelium materials, considering advancements in manufacturing techniques, substrate selection, and product customization. It will also explore the challenges and limitations faced in the widespread adoption of mycelium-based materials, highlighting the need for further research and development in this field.

Overall, the thesis aims to provide a comprehensive understanding of mycelium packaging composite materials and their potential future impact. By examining their applications, market importance, and future prospects in various industries, it seeks to contribute to the knowledge and awareness of this emerging field and its potential for sustainable and innovative material solutions.

CHAPTER 2: METHODOLOGY

2. METHODOLOGY:

The fundamental methodology applied in this research includes the inoculation of mycelium spawns, which serves as a binding material, with substrate such as agriculture waste. They are mixed and cultivated together until they make foam like composite material and are subjected to test their characterization. The core procedure is represented as:



2.1 Substrate Selection:

The initial step constitute the identification and collection of most suitable substrate. Pakistan's agricultural industry generates a lot of agricultural waste, including sawdust, husk, wheat straw, and rice straw. Farmers burn this waste to cleanup land for new crop which poses serious

Figure 0.5 Wheat straw

Rice straw

environmental concern. Agriculture waste was collected as a primary raw material and utilized as a substrate for this project. Mostly wheat straw and saw dust was used and the results were compared.

2.2 Binding Material

Next step involves the identification of binding material. Mycelium spawns was selected as a binding material. Various types of mycelium species were identified. Among all oyster specie was found to be most suitable due to its local availability and compatibility to grow with selected substrate.

2.3 Sterilization of Agriculture Waste

Sterilization of agriculture waste was the crucial step to extract dirt and pathogen from the waste that could hinder mycelium growth. For that, the waste was first soaked in water for **two hours** and was washed properly to remove all the dirt and pathogens entrapped within the straws. After

Figure 0.6 Washing of agricultural waste

washing, it was dried under direct sunlight for **two days**.

For the sterilization, two methods were performed to ensure the complete sterilization of waste material.

2.3.1 Hot plate boiling method:

Heat sterilization or hot plate method is the most widely used method for sterilizing various products. 1000ml of beaker containing water and wheat straw was laid on hot plates to boil for 30 minutes at 50 ° c ensuring that the heat source was reliable and continuously heated the process.

The next step after sterilizing the material was cooling and managing the trash. The material was taken off the hot plate and allowed to cool until it was safe to handle for processing purposes.

Figure 0.7 Sterilization using boiler method

2.3.2 Autoclave method:

Autoclaving is the most important step in eliminating all pathogens, microorganisms, and materials that can stop the substrate mycelium from growing. The following steps were carried out during autoclaving:

First the autoclave was prepared and attention was paid to cleanliness. Then add the appropriate amount of water to the autoclave tank. Pour the washed agricultural waste into the autoclave bags, available from local market, to avoid contamination. Arrange these bags in the autoclave, making sure that the same amount of heat was circulated through each bag. Close the autoclave to prevent steam from escaping.

Autoclave was set to 121°C for 2 hours. After this period, the substrate was removed from the autoclave bags and allowed to cool for later use.

Figure 0.8 Sterilization Using Autoclave

2.4 Grinding of wheat straw

Wheat straw categories among the agriculture waste to use as substrate for mycelium bio composites. Due to larger particle size of straw, they were shred into different particle size to compare the strength of mycelium growth. Grinder was used in SCME to grind the wheat straw. Different sieves were used to separate the particles of different sizes.

Figure 0.9 Grinding Of wheat Straw

2.5 Preparation Of Composites And Molding

Next step includes the inoculation of mycelium spawns with prepared agriculture waste. Use 8 % of water content. Additional nutrient was added to enhance the growth of mycelium spawns. The mixture was filled into the plastic bags. Perforation was made into the bags to ensure proper air contact with mycelium spawns facilitation their optimal growth. The mixture was placed in dark and humid area at room temperature.

After 1 week, mycelium cultivation was observed within the selected substrates. Cultivated mycelium was shifted from bags into different molding shapes to initiate second phase of growth. Before transferring the matter the shapes were sterilized with 70% ethanol and was done in sterile environment. The shapes containing mycelium mixture was wrapped with plastic paper and same holes were made to ensure air contact with mycelium spawns.

After 20^{th} days they were taken out of molding shapes and left undisturbed for self-growth of mycelium at room temperature for 4 to 5 days.

2.6 Oven Drying and Final Product

After self-growth, final product was obtained by oven drying the samples at 100 ^oC for around 1 hour to stop further mycelium growth and to evaporates the water content with in the samples until 1/3 of the weight is lost.(file:///C:/Users/cWW/Downloads/Documents/ecaadesigradi2019_465.pdf) The samples were subjected to dry till further characterization.

Figure 0.10 Oven Drying

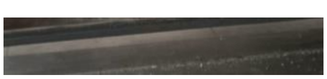


Figure 0.11 Final Product After heat treatment

CHAPTER 3: CHARACTERIZATION

3.0 Characterization

Characterization of mycelium bio composites involves assessing various properties and characteristics to understand their performance at various applications. Different tests were performed including mechanical test, physical test and biological test to check their properties.

3.1 Compressive Strength Test

Compressive test of mycelium bio composites were conducted using Compressive test machine (CTM) in SCME (school of chemical and materials engineering).

Measure the dimensions of the sample using vernier caliper. Place the sample over one plate of the machine. Align the specimen to ensure uniform load distribution on the sample and prevent any slippage or any misalignment during the test. Maximum force of **50KN** was applied to the sample with the speed of **1mm per second** to test the properties of sample under the applied force.

Continuously monitor the applied force and deformation of the sample under applied stress. The CTM was connected with the software to measure the applied stress and strain for that sample.

Data such as maximum load applied to the sample, stress-strain curve and modulus calculations were recorded for comprehensive characterization of sample.

3.2 Flexural Test 3 Point

Flexural test also known as the 3 point test is used to measure the stiffness and strength of materials. To check the strength of these mycelium biocomposites, flexural test was performed in SCME. Dimensions of samples were measured using vernire caliper and was placed symmetrically on a support of the machine. The pressure was forces on the sample with the speed of 2mm per second. The point at which cracks begin to appear testing machine automatically stops further application of pressure. Data such as maximum force applied, stress-strain, displacement and stroke was recorded for comprehensive characterization of sample.

3.3 Thermal Conductivity Test

Thermal conductivity is the mechanical property of mycelium is performed to determine the thermal conductivity value of mycelium bio composites to check how well they conduct heat. Thermal conductivity test of mycelium bio composites was performed in SMME (School of mechanical and manufacturing engineering) Lab. KD-2 pro thermal analyzer was used to check thermal conductivity. Using this instrument was beneficial because of it high accuracy, short measurement time consuming and easy to use and maintain data. Heat was applied to heating needle for set period of time. Temperature was measured in the monitoring needle at the distance of 6 mm during the heating and cooling period. The readings was then processed by subtracting the ambient temperature at time 0, multiplying it with 4π and dividing by the heat per unit length, q. The resulting data are were fitted to the following equations (Yangang Xing et al., 2018) :

$$T = b_o t + b_1 E_i \left(\frac{b_2}{t}\right)$$

$$T = b_o t + b_1 \{E_i \left(\frac{b_2}{t}\right) - E_i (\frac{b_2}{t - t_h})\}$$

$$T = 4\pi \frac{T - T_o}{q}$$

E_i = exponential integral

T_o = temperature at start of measurement

b_1 and b_2 = constant

q = heat to be applied

First equation was used when the heat was being applied and the second equation was used when heat was off. To determine the conductivity of sample following equation was used in which K represents thermal conductivity value:

$$K = \frac{1}{b_1}$$

3.4 Water Absorption Test

Water absorption test is conducted to check the absorption capacity of the samples and to check their resistance to mycelium. Different samples of equal dimensions were used for water absorption test. The initial dry weight was measured using mass balance. Samples were soaked in 1000ml flask containing water. Allow the sample to soak in water. And measure the wet weight of the sample after every 2 hours.

After immersion period (which was 72 hours) sample was removed form the water and blot the surface to remove excess water from the sample. Don't not squeeze the sample.

Then calculate the final wet weight after 72 hours. And calculate the water absorption test using the formula:

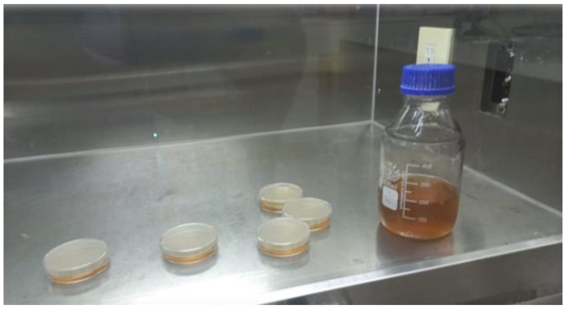
$$Water\ Absorption = [(Wf - Wi) / Wi]\ x\ 100$$

Wf = final wet weight of the sample

Wi = Initial weight/dry weight of the sample

3.5 Mycelium Viability Test

Viability of mycelium plays an important role to ensure biological safety during usage. To verify the inactivation of mycelium fungi growth after oven drying mycelium viability test was performed. A portion from the sample was taken and was placed on sterilized petri dish containing 2% MEA(Malt extract agar) spread on the plate for 13 days at 25 ºC. All steps were performed in laminar flow hood to avoid any contamination. After incubation, the inoculated petri dish were analyzed under microscope to observe mycelium viability.



CHAPTER 4: RESULTS

4.0 Pre-Screening

4.1 Mushroom Species

There are many kinds of mushrooms, each with an own set of qualities and attributes. Agaricus bisporus (white button mushroom), Pleurotus ostreatus (oyster mushroom), Lentinula edodes (shiitake mushroom), and Hericium erinaceus (lion's mane mushroom) are a few of the most well-known edible mushroom species. Mushrooms are utilized in traditional medicine all over the world and offer a variety of medical benefits in addition to their culinary usage. Some types of mushrooms are also employed in the manufacturing of enzymes and bioremediation processes, among other industrial uses. Overall, the group of creatures known as mushrooms is intriguing and significant, and they are used for a variety of purposes.

In this case, we've decided to work with oyster mushrooms, or Pleurotus ostreatus. This species is a great choice for our product because it offers several benefits. Compared to certain other species, oyster mushrooms are comparatively simple to produce and mature fast. They are a sustainable alternative since they are also extremely versatile and can be grown on a range of substrates, including agricultural waste materials. Oyster mushrooms are also well-known for their high quantities of protein, fiber, and vitamins, which contribute to their nutritional value. They have long been a part of conventional medicine and has therapeutic qualities as well. Overall, oyster mushrooms are an intriguing and potential study topic because of their unusual combination of gastronomic, nutritional, and therapeutic benefits. And moreover, this is readily available in Pakistan and easy to purchase.

4.2 Substrate:

Substrate selection is one of the most important parameters that should be examined under critical observation. There are several substrates available, but we must choose one with a higher organic content that is capable of binding with mycelium and forming a proper structure. So, for that reason, we work on multiple substrates. First, we worked on wheat straw, and we achieved a proper product in this case. After this, we worked on sawdust, but unfortunately, we could not succeed in this case due to certain parameters like temperature and humidity.

4.3 Growing Conditions:

Depending on the individual species of mushroom being used and the required characteristics of the finished product, different mycelium-based composite materials might require different growth conditions. The substrate type, temperature, humidity, and nutrient availability are among variables that might impact the development and characteristics of the mycelium-based composite. To maximize mycelium development and create composite materials of the highest caliber, it is crucial to manage these factors. So, for that reason with the help of literature review and studies we set some parameters for our work. We kept the sample in the dak room where there is certain availability if oxygen and moisture content. Humidity level and Temperature should be maintained at a certain limit. Humidity level should be around 70-80% and temperature should be around 23-28 $^{\circ}$C. For some substrate there is the requirement of nutrients while for some there is no requirements.

4.4. Final Experiment

4.4.1 Visual Observation:

Mycelium-based composite materials can be observed visually before and after heat treatment to study their physical and structural changes.

In our case, the mycelium-based composite material has a foam-like texture before heat treatment and seems soft, malleable, and spongy. Depending on the type of agricultural waste utilized, the color varies, although in our case it is light brown and white. Mycelium strands that have developed and connected the substrate together can be seen on the surface.

After the heating treatment, the mycelium-based composite material altered dramatically. The strength and durability of the material are improved by heat treatment, improving its suitability for use in packaging. The color is also altered after heat treatment, becoming uniformly brown. The material looks smoother and less pliable due to the heat-induced densification of the substance.

4.4.2 Density and Compressive Strength Test Data:

4.4.2.1 Density Comparison of Wet And Dry Sample:

To check their strength and compare them with standard EPS, we conducted several tests on our product. The first is a density comparison of products based on moisture content and size of substrate. So, we obtained several results based on this test, which are as follows:

It was discovered that mycelium-based composite materials with higher moisture contents and smaller particle sizes had higher densities when comparing their densities to those of materials with lower moisture contents and larger particle sizes. This is because wet mycelium-based composite materials have a greater density because the particles pack closer together. But it is also important to keep in mind that the strength and durability of wet mycelium-based composites cannot be accurately predicted by the material's density. Wet materials are not suited for use in many applications because, while observing them, it was concluded that they are weaker and more prone to harm. The material must be dried as a last step to further reinforce it. The process of drying eliminates excess moisture from the material, making it stiffer and more durable.

It is noted that the dry samples have greater densities when comparing the densities of wet and dry mycelium-based composite materials, as you can see in the table below. This is due to the elimination of extra moisture during drying, which enables the particles to pack even more densely and produce a greater density. The dry mycelium-based composites are better suited for use in applications that need a high level of structural integrity since this increase in density is frequently accompanied by an improvement in strength and durability.

Substrate	M.C%	Density Dry kg/m^3	Wet Density kg/m^3
Wheat straw fine 1	70	482.66	273.01
Wheat straw fine 2	50	332.24	164.30
Wheat straw fine 3	50	377.93	181.39
Wheat straw fine 4	70	420.20	268.12
Wheat straw coarse 1	70	190.12	91.60
Wheat straw coarse 2	50	134.48	60.96
Wheat straw coarse 3	50	155.21	71.12
Wheat straw coarse 4	70	225.16	106.93

Table 0-1 Density Comparison between Moist and Dry samples

4.4.2.2 Density Comparison with EPS:

Though EPS and mycelium-based composites are both lightweight materials, their densities can differ greatly. Due to its low density, which normally ranges between 10 and 35 kg/m3 depending on the grade, EPS is well acknowledged. The density of mycelium-based composites, on the other hand, can vary from 100 to 500 kg/m3, depending on the mycelium's kind, moisture content, and particle size.

It is crucial to consider elements other than density when comparing the densities of mycelium-based composites with EPS, such as strength, durability, and environmental effect. Due to its affordability and light weight, EPS is a frequently used packaging material; however, it is also non-biodegradable and can have an adverse effect on the environment. In contrast, mycelium-based composites are more sustainable than EPS since they made from renewable materials and are biodegradable.

Substrate	Mean value Dry Density (kg/m^3)
Fine WS	164.30
Fine WS	181.39
Coarse WS	60.96
Coarse WS	71.12

Sample	Foam Density (kg/m^3)
EPS M1	52.3
EPS M2	72.6
EPS M3	77.8
EPS M4	69.1

4.4.3 Compressive Strength Test Data:

Compressive strength testing is a mechanical procedure used to determine the maximum compressive load that a material can withstand before breaking. A compression testing machine is a type of universal testing machine (UTM) designed specifically to assess the strength and deformation behavior of a material under compressive (pressing) pressure.

The results of a compressive strength test on materials made from mycelium composites may provide important details regarding the durability and stability of the substance. The greatest compressive load that the specimen can sustain before failing, the accompanying displacement of the specimen, and the estimated compressive strength are normally included in the compressive strength test data. The type of mycelium employed, the substrate materials, and the testing circumstances can all have a significant impact on the compressive strength of mycelium composite-based structures. Mycelium composite-based materials typically have lower compressive strengths than more conventional building materials like steel or concrete, but they can nevertheless demonstrate enough strength and durability for a variety of applications.

In our case we concluded that composite becomes more thick and solid as it is squeezed because the void spaces inside it are removed. This is due to the possibility that void spaces inside a material might serve as weak spots or regions where the material could flex or deform more readily. The material gets tougher and more rigid by eliminating these void spaces through compression, which also increases the material's resistance to bending and deformation.

Additionally, because composites are composed of many materials, they may possess special qualities like greater strength, durability, or flexibility. So, that’s why there was no breaking point for composite materials due to these qualities (You can see in the table shown below), which make composite more resistant to breaking or fracturing under stress. We observed breaking point, there might be the reasons that wheat straw has a characteristic for being a strong, durable material that can endure a lot of pressure (which is 50 KN in our case) before breaking. This is because of its structure, which comprises of lengths of fibrous tissue that are tightly entangled and interlocking, making them challenging to separate.

Table below shown the data that we obtained during Compression test.

Name	Parameters	Units	Values
Max Force	At entire area	N	50018.3
Max Stress	At entire area	N/mm^2	3.989
Max Area	At entire area	mm^2	37071.29
Max Strain	At entire area	%	97.5

Max Time	At entire area	Sec	1560.99
Break Force	At entire area	N	0
Max Stroke	At entire area	Mm	26.01
Speed	At entire area	mm/s	1

Table 0-2 data obtain from compressive strength test

4.4.4 Hypothesis Testing:

A statistical technique known as hypothesis testing is used to infer or draw conclusions about a population from a sample of data. It entails establishing two competing hypotheses, the alternative hypothesis (H1) and the null hypothesis (H0), before gathering and examining data to ascertain which is more likely to be accurate.

The null hypothesis (H0) states that there is no difference or effect. It is presumptively true that any observed difference or impact in the data results from random variation or chance. On the other hand, the alternative hypothesis (H1) stands in opposition to the null hypothesis. It implies that the population exhibits a particular impact, difference, or link. Mostly significance is 0.05 and the null hypothesis is rejected, and the alternative hypothesis is accepted if the p-value is less than the preset significance level, which is normally 0.05. This indicates that there is a substantial difference between the mycelium composite material and conventional composite materials. Too little information exists to support a significant difference if the p-value is higher than the significance level, failing to reject the null hypothesis.

When we performed hypothesis test, and we got the result that P value for 2 tail is 0.049 which is less than 0.05 which shows that null hypothesis is significant, and our product has potential for use as compared to EPS.

4.5 STRESS VS STRAIN GRAPHS

4.5.1 Mycelium Composite and Standard EPS

4.5.1.1 Mycelium Composite:

It is anticipated that the stress vs. strain graph of the mycelium composite will have a somewhat ductile behavior. The mycelium composite material initially experiences elastic deformation as stress is applied, meaning it will revert to its original shape once the load is removed. The mycelium composite material may exhibit plastic deformation as the strain (deformation) increases, meaning it experiences permanent deformation while maintaining structural integrity. Mycelium composite material's stress vs. strain curve could have a progressively inclining slope, signifying a progressive rise in stress with strain until it reaches its maximum strength. After the material reaches its maximum strength, stress may start to decline because of material failure. (You can see in graph).

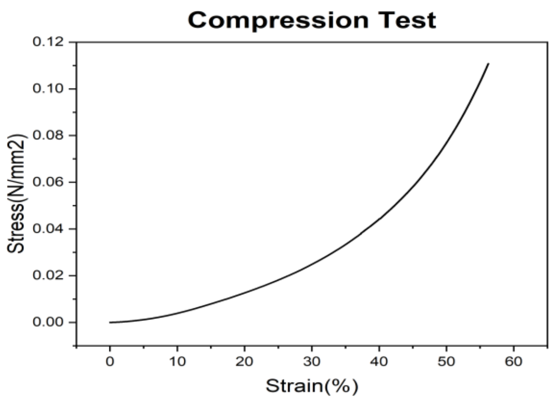


Figure 0.12 Stress Vs Strain Graph

4.5.1.2 Standard EPS:

Brittle behavior is frequently seen in the stress vs. strain graph of EPS.EPS will first deform elastically, just like mycelium composite material does. Before achieving its ultimate strength, EPS is anticipated to have a finite plastic deformation range. EPS will have no further deformation when the strain increases past the plastic region and is likely to fail suddenly with a considerable decrease in stress. (You can see in graph)

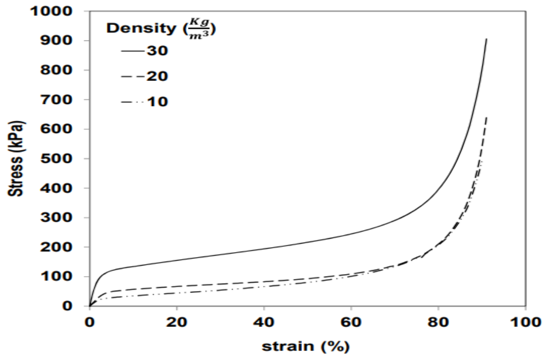


Figure 0.13 Stress Vs Strain graph

4.5.1.3 Conclusion:

We concluded that mycelium composite has a more ductile behavior with a gradual increase in stress with strain, while EPS display a more brittle behavior with a rapid increase in stress until failure, according to the stress vs. strain graphs of the two materials.

4.6 Flexural Test:

A mechanical test used to ascertain a material's stiffness and flexural strength is called a flexural test, occasionally referred to as a bending test. A test specimen is bent to determine how resistant it is to deformation and fracture under bending circumstances. Metals, polymers, composites, and ceramics are among the materials on which the test is frequently carried out.

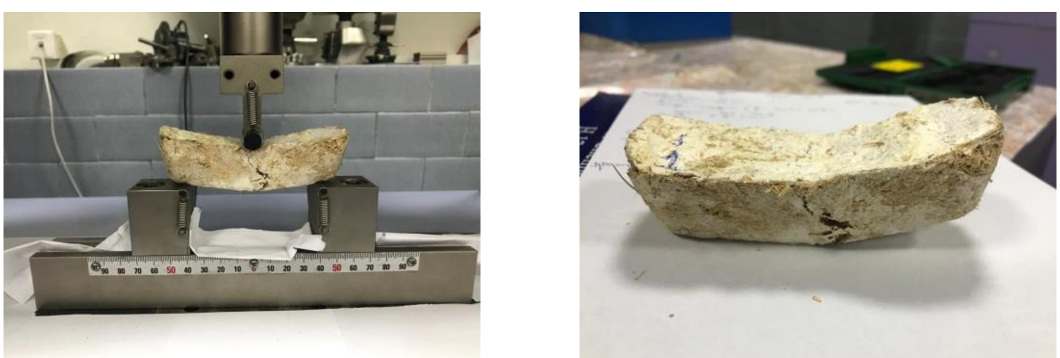


Figure 0.14 Product before flexural test Vs after flexural Test

Sample	Mycelium Bio-Composite		Expanded Polystyrene	
	Flexure Modulus (EH) MPa	Flexural Strength (SM) MPa	Flexure Modulus (EH) MPa	Flexural Strength (SM) MPa
1	2.78	0.167	0.305	0.029
2	2.17	0.18	0.126	0.0263
3	3.85	0.208	0.351	0.0283
4	3.09	0.174	0.854	0.0325

Table 0-3 Data obtained During Flexural Test

Table shows above is the comparison between Mycelium Composite and Expanded polystyrene and from table you can clearly see the value difference between both. These values show that mycelium composite has better elastic behavior than expanded polystyrene.

4.7 Hypothesis Testing for Flexural Test:

Based on the hypothesis test, if the two-tailed p-value obtained is 0.39%, which is less than the typical significance level of 5% (0.05), it indicates that the observed difference in flexural strength between the mycelium composite and the traditional composite material is statistically significant. This result validates the soft and elastic behavior of the mycelium composite in comparison to EPS (Expanded Polystyrene), which is the traditional composite material being used as a reference. The lower flexural strength observed in the mycelium composite suggests that it is softer and more elastic, meaning it can deform more under applied bending forces before fracturing.

The significance of this finding lies in the potential applications of the mycelium composite as a sustainable alternative to EPS. The soft and elastic behavior of the mycelium composite may make it suitable for specific purposes where flexibility and impact resistance are desired. It could also indicate that the mycelium composite possesses unique material properties that can be further explored and leveraged in various engineering and construction applications.

4.8 Thermal Conductivity Test:

During the test conducted at 40 degrees Celsius, we found that the mycelium composite material has higher thermal conductivity than EPS, it would be an interesting observation. Here are some possible remarks regarding this finding:

Enhanced Thermal Conductivity: The higher thermal conductivity of the mycelium composite compared to EPS suggests that the mycelium composite has a better ability to conduct heat. This characteristic could make it suitable for applications where efficient heat transfer is desired, such as thermal insulation or heat dissipation in certain industries.

Potential for Heat Management: The higher thermal conductivity of the mycelium composite material could indicate its potential for use in thermal management systems. It may offer advantages in dissipating heat and maintaining temperature stability in various applications, including electronics, construction materials, or packaging.

Structural Considerations: It's important to consider the overall structure and composition of the mycelium composite and EPS. The higher thermal conductivity of mycelium composite might be due to its unique composition or the presence of additives that enhance heat transfer. Understanding the underlying factors contributing to the thermal conductivity difference can provide insights into the material's structure and performance.

Further Research: The observed difference in thermal conductivity between mycelium composite and EPS could open avenues for further research and development. It may be valuable to investigate the specific properties and composition of the mycelium composite material to better understand how it achieves higher thermal conductivity. This knowledge can aid in optimizing the material for specific applications or in exploring new applications altogether.

4.9 Water Absorption Test:

The results of the water absorption test on the mycelium-based composite material indicate that initially, there was a significant increase in volume as the material absorbed water. However, over time, the rate of volume increases decreased. This finding suggests that the mycelium composite material has an initial high water absorption capacity, which is likely due to the porous nature of the material. The water is absorbed into the pores, causing the volume of the material to expand. As the material continues to absorb water, it reaches a point where most of the available pore space becomes filled, resulting in a reduced rate of volume increase. This could indicate that the material has reached a state of saturation or equilibrium, where further water absorption becomes limited.

The decrease in the rate of volume increase over time is a common behavior observed in many porous materials undergoing water absorption. It is influenced by factors such as the porosity and pore structure of the material, as well as the diffusion properties of water within the composite.

4.10 Viability Check after Heat Treatment:

The viability of mycelium is essential to ensure the biological safety of the material during its usage. To verify the inactivation of fungus growth after treatment, a portion of the treated sample was inoculated into a sterilized petri dish and observed for 13 days. After the 13-day period, it was observed that no growth of fungus occurred in the petri dish. This result indicates that the treatment applied to the mycelium composite material was effective in inactivating or inhibiting the growth of fungi. The absence of fungal growth in the petri dish suggests that the treatment method employed successfully eliminated or rendered inactive any viable fungal spores or mycelium present in the treated sample. This outcome is crucial for ensuring the biological safety of the mycelium composite, as it confirms that the treatment was successful in preventing fungal growth, which could otherwise lead to potential health hazards or material degradation.

Chapter 5 COST ANALYSIS, ENVIROENMNTAL IMPACT AND RECOMMENDATION

5.0 Cost Analysis

5.1 Cost Analysis:

Estimation of cost of the product depends on various factors such as scale f project, cost of raw materials used, production cost, utilization cost and local market conditions. For the cost estimation of mycelium bio composites factors such as raw material cost, labor, production, equipment and finishing. For 1 square feet of mycelium bio composite with the thickness of 0.5 inch the cost was analyzed as:

5.1.1 Consumable Cost

1. Price of wheat straw = 50 Rs per 10 kg
2. Mycelium spawns cost (0.5 kg) = 400(one time cost)
   (For 1 kg of wheat straw 20 g of seed is being used = 15 Rs)
3. Molding shapes: 2-3 Rs per shape

5.1.2 Sterilization Cost

The cost during the sterilization of agriculture waste includes the cost during both process of sterilization (Hot plate and Autoclaving method). This cost was analyzed as:

1. By chemical Sterilization (by Carbon dazing) = for each 100 g = 450 Rs.

(for 1L of water 0.6 g carbon dazing is used = 3 Rs)

5.1.3 Heat Treatment Cost

1500-watt oven operated for one hour = 1.5KWhr.

total price= 50 Rs/hr.

Consumption by one composite = 2.5 Rs

5.1.4 Cost comparison of mycelium bio composite with conventional plastic:

According to the cost analysis of mycelium bio composites form experiment and cost analysis of conventional plastic form literature review estimated that mycelium being organic in nature is less expensive than conventional plastic.

Mycelium biocompoiste cost:

For 1 square feet composite with thickness of 0.5 inch = **22.5 Rs**

Polystyrene Cost:

Commercial cost for polystyrene 9 feet square/0.5 inch thickness= 300 Rs

For 1 feet square/o.5 inch thickness= **33 Rs**

5.2 Environmental impact

Mycelium bio composites poses several environmental benefits to environment when compared to conventional plastics. The points that possess environmental impact of mycelium bio composites includes(Kiana Rafiee, Guneet Kaur, Satinder K. Brar, Fungal biocomposites: How process engineering affects composition and properties?, Bioresource Technology Reports, Volume 14, 2021, 100692, ISSN 2589-014X,):

These products have the potential to reduce carbon emission by recycling the agriculture waste instead of burning. The growth of mycelium requires minimum energy input and this results in lower greenhouse gases emission during the process of production.

One of the major advantage of environment is their ability to naturally decompose. While other materials such as plastics or Styrofoam's persist the environment for centuries. Mycelium bio composites break down into organic matter contributing to soil health and the nutrient cycle. This characteristic of mycelium bio composites minimizes long term pollution and waste accumulation into the environment.

The production of mycelium bio composites requires less energy as compared to traditional methods. The cultivation occurs in controlled environment due to which less energy is required for this process. Mycelium composites requires minimal amount of water during production contributing to water conservation efforts.

These products offer a sustainable solution for waste management as well. These composites after used as a packaging materials can be composted, returning valuable nutrients to the soil and closing the material cycle while on the other hand, materials like conventional plastic often ends up in landfill or as a litter and this cause serious environmental harm and requires long term management practice.

While keeping these points under consideration mycelium biocomposites provide a valuable insights into their potential to mitigate waste reduction and promote sustainable resource use. They help in development of ecofriendly alternatives to conventional plastic materials

5.3 Recommendations:

The Final Year project was conducted in 2022 to produce energy briquettes from recycling agriculture waste. Chemical binder (waste engine oil) was used for this project. The project strongly addresses the issue of agriculture waste but the environmental drawback of this project was the use of chemical binder because of its non-biodegradability it produces toxic chemicals into the environment when burned. In our project we selected binder that is completely organic in nature. This choice strongly concern environment benefit and also represent the opportunity to utilize the products as energy briquettes after its usage as packaging material.

This innovation of this project has potential for sustainable development and resource conservation. To establish the efficiency of the product further studies are required focusing on the comparison of energy capacity of organic binder with that of conventional fuel. Considering the need for sustainable energy and strongly waste management practices, this thesis project offers valuable contribution to this field. This project has the potential to revolutionize agriculture waste and reduce the environmental impact associated with the chemical binder. We recommend the continuation and expansion of this project as it holds promise in addressing the major environmental and energy challenges being faced these days.

CHAPTER 5: CONCLUSION

CONCLUSION:

Mycelium composites, with their unique mechanical properties, have the potential to replace Expanded Polystyrene (EPS) in various applications. EPS is a commonly used material known for its lightweight and insulation properties, but mycelium composites offer additional advantages. One such advantage is the use of finer particles of wheat straw in the composite, which can enhance its strength. When using finer particles of wheat straw in the mycelium composite, the resulting material exhibits improved strength properties. The smaller particle size allows for better interlocking between the mycelium and the straw particles, resulting in enhanced bonding and structural integrity. This leads to a stronger composite material that can withstand higher loads and stresses. In terms of load-bearing capacity, compression tests on mycelium composites have shown promising results. The mycelium-based material exhibits high load-bearing capacity, meaning it can withstand substantial weight or pressure without significant deformation or failure. This characteristic is crucial for various applications that require structural stability and support, such as construction materials or furniture manufacturing. Moreover, manufacturing mycelium composites in reduced thickness can result in cost reduction. Due to the inherent strength and load-bearing capacity of the material, thinner layers can be used while still maintaining the desired performance. This leads to material savings and potentially lower production costs, making mycelium composites a cost-effective alternative to EPS and other traditional materials. However, to fully harness the potential of mycelium composites, further studies are needed, particularly focused on assessing different mycelium species in Pakistan. Mycelium species can vary in their growth characteristics, strength properties, and adaptability to local conditions. Conducting studies specific to Pakistan would provide valuable insights into the suitability of different mycelium species for composite manufacturing in the country. This information is crucial for optimizing the production process and selecting the most suitable mycelium species for specific applications. In conclusion, mycelium composites offer exciting opportunities as a replacement for EPS, primarily due to their unique mechanical properties. Utilizing finer particles of wheat straw enhances the strength of the composite, while compression tests have demonstrated the high load-bearing capacity of mycelium composites. Manufacturing these composites in reduced thickness can lead to cost reduction. However, further research focusing on assessing different mycelium species in Pakistan is necessary to fully explore and exploit the potential of mycelium composites in the country's context.