Exploring the Feasibility of Mycelium-based Cups as a Sustainable Alternative to Single- Use Beverage Cups

By

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Abstract

Disposable paper (DP) cups, a staple in on-the-go beverage culture, contribute to environmental challenges due to their non-biodegradable polyethylene (PE) liners. This research presents an eco-friendly alternative by harnessing fungal mycelium combined with North American wetland biomass. The study evaluates three fungal species—*Ganoderma lucidum*, *Pleurotus ostreatus*, and *Polyporus squamosus*—cultivated on canola straw (*Brassica napus L.*) and cattail substrates (*Typha latifolia*) to produce mycelium composite materials. Among these, *Ganoderma lucidum* demonstrated superior mycelial growth and structural integrity when paired with cattail substrate over a 14-day period. Initial growth assessment was conducted using Potato Dextrose Agar (PDA), with further optimization through Yeast Extract Peptone Dextrose Broth (YEPDB) liquid media. The research explored the production of compostable coffee cups and mycelium sheets using a 3D poly-lactic acid (PLA) mold and aluminum foil, respectively. Mycelium composites were harvested at two growth intervals (2 weeks and 4 weeks) and analyzed for their morphological, chemical, thermo-mechanical, crystallinity, thermodynamic, and mechanical properties. The resulting composites exhibited notable thermal stability exceeding 267°C, low thermal conductivity (0.03-0.04 $Wm^{-1}K^{-1}$), and inherent hydrophobicity with a water contact angle surpassing at 100°. However, the mechanical properties, such as Young's modulus, were significantly lower than those of commercial coffee cup paper, with values of 11.27 MPa and 80.91 MPa for the 2-week and 4-week samples, respectively, compared to 1349.35 MPa for standard coffee cup paper. These findings demonstrate that cattail can serve as a viable substrate for producing mycelium-based bio-composites, suitable for applications requiring thermal stability and hydrophobicity.

Key words: Mycelium-bio composite, compostable coffee cup, Ganoderma Lucidum, Cattails, Hydrophobicity, Thermal stability.

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Abbreviations

ADF= Acid Detergent Fiber
ADL= Acid Detergent Lignin
ANOVA = Analysis of Variance
ASTM= American Society for Testing and Materials
CAGR = Compound Annual Growth Rate
DCM = Di-Chloro Methane
DP cups = Disposable Paper cups
DSC= Differential Scanning Calorimetry
F&H = Fools and Horses
GPGP = Great Pacific Garbage Patch
ISO= International Organisation of Standardisation
MBCs = Mycelium-Based Bio-Composites
NDF= Neutral Detergent Fiber
PDA= Potato Dextrose Agar
PDB = Potato Dextrose Broth
PDPB = Potato Dextrose Peptone Broth
PE= Polyethylene
PHA= Polyhydroxyalkanoates
PLA= Poly-Lactic Acid
REB = Rice Extract Broth
RO water= Reverse Osmosis water
SD = Standard Deviation
SDS = Sodium Lauryl Sulfate
SEM = Scanning Electron Microscopy
TAPPI = Technical Association of the Paper and Pulp Industry
TGA= Thermogravimetric Analysis
XRD= Xray Diffraction
YEPDB = Yeast Extract Peptone Dextrose Broth

Chapter 1

1.0 Introduction:

The surge in utilization of disposable paper (DP) cups is largely a consequence of the escalating consumption of coffee and other beverages ingested outside of domestic environments, a trend driven by the hectic pace of urban lifestyles. However, the convenience afforded by these cups denies the environmental cost exacted by the disposal of billions of them into landfills annually—a fate unrecognized to many consumers. Aggravating this issue is the non-biodegradability of the majority of these cups, which can be attributed to the integration of polyethylene liners, in combination with suboptimal sorting, collection, and recycling protocols. It is imperative to enhance consumer perception regarding the environmental consequences of single-use cups and to institute robust strategies that both encourage the adoption of eco-friendly substitutes and upgrade waste management systems.

1.1 Background

The global paper cup market has grown significantly in recent years, reaching USD 6.8 Billion in 2023, and is projected to increase to USD 9.0 Billion by 2032, with a Compound Annual Growth Rate (CAGR) of 3.1% during 2023-2032 (iMarc, n.d). The popularity of paper cups can be attributed to their ease of handling, low cost, and convenience, which have gained significant traction among the general population. The rise of dine-in services, takeaways, and online food delivery platforms has further bolstered the demand for disposable coffee cups. The United States has been identified as the largest consumer of disposable paper cups, with over 50 billion used and discarded annually. Other countries, such as China, the UK, and Taiwan, use 10 billion, 2.5 billion, and 1.5 billion disposable cups respectively each year (Ma, 2018). Unfortunately, the majority of these one-time use coffee cups end up in landfills, as they are not properly recycled or biodegraded.

In order to determine the ecological cost associated with paper-based cups, it is essential to examine their life cycle, including material extraction, processing, manufacturing, and disposal. To meet the growing demand for disposable paper cups, approximately 20 million trees are harvested annually in Brazil and Russia alone (Ma, 2018). The paper industry is known for its high energy consumption and resource usage, with significant amounts of energy required for raw material processing and paper manufacturing. Specifically, the production of one metric ton of paper requires 9000-12000 kg of steam, 960-1000 kW of electrical power, and 50 m^3 of cooling water (Connolly et al., 2012). Furthermore, the disposal of these paper-based cups is not environmentally friendly due to the polyethylene (PE) lining, which is highly resistant to natural biodegradation. Moreover, a lack of proper recycling infrastructure often results in these single-use paper cups being sent to landfills, contributing to land and ocean pollution issues.

Disposable paper cups are primarily composed of virgin cellulose fiber board (95 wt%) and a PE inner layer (5 wt%) to provide waterproofing and improve barrier performance. However, some single-use beverage cups are made from expanded polystyrene, which is not biodegradable (Triantafillopoulos et al., 2020). In response to the adverse environmental impact of the excessive use of paper cups, many coffee shops and companies have started exploring alternative options.

Currently, PolyLactic Acid (PLA) liners are gaining recognition as a replacement for PE liners (Vermeulen and Bam, 2018). PLA has emerged as a prominent biodegradable polymer, garnering attention for its eco-friendly characteristics and diverse industrial applications. Various factors influence PLA degradation, including environmental conditions such as temperature, humidity, and microbial presence (Mehrpouya et al., 2021). Hydrolytic degradation, facilitated by moisture, leads to the cleavage of ester bonds within the polymer chain, with degradation

rates contingent upon environmental parameters. Enzymatic degradation, catalyzed by lipases and proteases, further contributes to PLA breakdown (Mehrpouya et al., 2021). Moreover, thermal degradation occurs during recycling processes, particularly at elevated temperatures, where moisture accelerates the degradation pathway (Mehrpouya et al., 2021). Additionally, exposure to ultraviolet radiation induces photodegradation of PLA, resulting in the deterioration of mechanical properties (Scaffaro et al., 2019). The rate of PLA degradation may vary depending on various factors such as temperature, humidity, and the presence of microorganisms.

1.2 Problem Statement

Firstly, the processing and manufacturing of raw materials for paper cups is a highly energy- and resource-intensive industry. To meet the increasing demand for paper cups and other packaging materials, millions of trees are cut down each year, resulting in less absorption of atmospheric CO_2, leading to ecological imbalances and ultimately contributing to global warming. The loss of millions of trees also means the loss of habitats and food sources for thousands of animals. Secondly, many people assume that disposable paper cups are biodegradable like some other packaging materials. However, paper cups are not biodegradable due to the polyethylene layer inside the cup. Due to strong adhesion, it is challenging to separate the paper and plastic liner, making these cups not naturally biodegradable. Additionally, inefficient recycling and sorting facilities mean that paper cups often end up in landfills. In the US, only one paper cup out of 400 is sent for recycling, with the rest ending up in landfills, taking up to 20 years for a PE liner paper cup to decompose (Ma, 2018). When disposed of in landfills, these cups emit methane, a greenhouse gas that is 23 times more effective at trapping heat than carbon dioxide (Prashanthi et al., 2017).

Disposable cups are also contributing to the disruption of ocean and marine life. The "Great Pacific Garbage Patch (GPGP)" is the largest example of plastic pollution found in the

Pacific Ocean. According to estimates, more than 1.8 trillion pieces of plastic have accumulated in the GPGP, which has a size greater than twice the size of Texas and weighs 87,000 tons (Lebreton, 2018).

It is important to note that plastic or paper cups, when improperly disposed of, can end-up in the ocean and be broken down into micro- and nano-plastic particles due to exposure to heat, sun, waves, and partial degradation by microorganisms. Marine life can then consume these micro- and nanoparticles, resulting in indigestion, suffering, starvation, and even death (Coral magazine, 2015). Figure 1.1 shows the ingested microparticles (glowing) in a zooplankton. This phenomenon poses a serious threat to the entire ecosystem and can lead to ecological imbalances.

Figure 1.1 Ingested glowing microparticles in marine zooplanktons (Coral magazine, 2015) [Permission under processing].

1.3 Mycelium-based Cups

Significant research endeavors have been undertaken to cultivate eco-friendly and sustainable materials derived from natural sources, including plants, animals, and microorganisms. Nevertheless, the development of such materials often involves substantial costs, time commitments, and yields that may be constrained by sophisticated production and extraction methodologies (Haneef et al., 2016). In response to these challenges, the exploration of bio-composite materials derived from various microorganisms was initiated several decades

ago, aiming to diminish dependence on petroleum-derived materials. Research had been carried out to manufacture utensils, furniture's, artificial leathers, decorative piece, and packaging containers as shown in Figure 1.2 from fungal mycelium (Ghazvinian et al., 2019).

Figure 1.2 Versatile products range from mycelium-based bio-composite materials (Ghazvinian et al., 2019) [permission- under processing].

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Table 1.1 Brief rationale for analysing mycelium composite material as a sustainable alternative to coffee cup paper.

Criteria	Mycelium composite	Synthetic foam (PS)	Paper cup materials
Material cost (USD/kg)	0.07-0.17 *[b]	2.1-2.3 *[b]	1.1-1.23 *[a]
Thermal conductivity ($Wm^{-1}K^{-1}$)	0.04-0.18 (57-99 kg/m^3 density) *[b]	0.03-0.04 (11-50 kg/m^3 density) *[b]	0.09 (818 kg/m^3 sheet density) *[b]
Hydrophobicity	Hydrophobic with water contact angle higher than 120° *[c]	Hydrophobic with water contact angle 90°*[d]	Hydrophilic with water contact angle 47.89 ± 1.53° *[e]
Production time	Weeks-months *[b]	Minute-days *[b]	Minute-days *[b]
Biodegradability	Home compostable (45-60 days in contact with moisture) *[b]	Recycling, landfill *[b]	Commercial compost plant (for polymer), recycling and landfill *[b]

*PS = Polystyrene; *[a] = data retrieved on Oct 17, 2022 from Alibaba.com, *[b]= reference from Jones et al., 2020, *[c]= reference from Haneef et al., 2017, *[d]= reference from Evgeniye & Wlaton, 2008, *[e]= determined in the present study

The process of cultivating materials from living organisms to attain specific material characteristics and functionalities is referred to as "Living Materials". Such materials are particularly designed through controlled growth and formation conditions, leveraging microorganisms and various nutritional substrates (Karana et al., 2018). Among the diverse opportunities explored, mycelium-based bio-composites have emerged as particularly promising and bio-compatible materials. This material has gathered recognition across sectors like construction, agriculture, packaging, furniture, artificial leather, and even biomedicine (Yang et al., 2021). By harnessing local biomass, it exists potential to craft eco-conscious mycelium-based materials, presenting a viable remedy to the widespread issue known as "coffee cup pollution."

While mycelium-based biomaterials are being used in numerous packaging sectors, it remains crucial to assess their thermal and moisture barrier properties, particularly concerning their applicability in beverage cups. Existing research suggests that their thermal stability, hydrodynamic characteristics, and biodegradability align well with the requirements of paper-based coffee cups. For further insights, Table 1.1 provides a comprehensive overview.

1.4 Statement of Purpose and Objectives

Mycelium cups must possess physical, mechanical, and thermal properties that are compatible with existing paper-based coffee cups for them to replace their less eco-friendly counterparts. While mycelium-based biomaterials are well-recognized in various packaging industries, it is essential to evaluate the thermal and moisture barrier properties of these materials to ensure their suitability for use in beverage cups. Factors such as fungal species, growth conditions, feeding substrate, material density, size, and thickness have an impact on the final material's physical, mechanical, morphological, and thermal properties (Haneef et al., 2017; Karana et al., 2018; Yang et al., 2021; Appels et al., 2019). Mycelium-based composites

represent a promising advancement in material design due to the conversion of agricultural waste into a valuable and sustainable solution for numerous industries. As this material is derived from a living organism and agricultural waste, it can fully decompose and return to nature, making it a potential solution to the current "paper-based cup" pollution problem.

The Objectives of this research are to 1) identify the optimal selection of fungal species; 2) Optimize the growth conditions, material density, and thickness to maintain the required material properties for beverage cups; and 3) Determine the material's physical, mechanical, morphological, and thermal properties.

1.5 Literature Review

1.5.1 Overview of Mycelium-based Bio-composite Materials

Over the past few decades, mycelium bio-composites have drawn significant attention from researchers and commercial manufacturers due to their wide range of applications, low energy consumption, biodegradability, and low cost (Jones et al., 2020; Yang et al., 2021). Mycelium bio-composites can be produced from agricultural or industrial waste that has little or zero commercial value. A significant amount of research has been carried out on the application and performance of mycelium-based materials. Although the targeted application area of this research is limited to a few sectors, such as structural materials, packaging materials, insulation and acoustic materials, artificial leather, food packaging, furniture, composite biopolymer, and laminated bio-composites (Attias et al., 2020; Jones et al., 2020).

However, only a few companies have been commercially working with mycelium biomaterials over the last few decades. Among these companies, Ecovative (Ecovative, n.d.) is a pioneer in manufacturing and commercializing mycelium-based materials. Ecovative's main focus is on replacing traditional packaging and insulating polystyrene materials with mycelium bio-composites (Bliss, 2013). Designer, Phillip Ross, founded MycoWorks Inc. (MycoWorks,

n.d.) and was one of the early explorers of mycelium-based bricks. Currently, the company is focusing on fabricating suitable alternatives to leather with mycelium-based materials. Another company, formerly known as "MyCoplast" but later renamed "Mogu" (Mogu, n.d.) is working on creating sustainable alternatives for interior design applications such as floors and acoustic tiles (Attias et al., 2020).

1.5.2 Mycelia (plural; Mycelium, singular)

Mycelia, the vegetative part of fungi, are composed of thread-like hyphae (ranging from 2-10 μm in length) that extend and branch into the substrate (Karana et al., 2018; Appels et al., 2019). The fundamental structure of a mushroom, including its fruiting body and mycelia, are shown in Figure 1.3. Due to the vast size of its individual networks, such as the *Armillaria* genus occupying almost 10 km² in Oregon's Blue Mountains, some fungi are recognized as the "largest living organism" on Earth (Haneef et al.,2017).

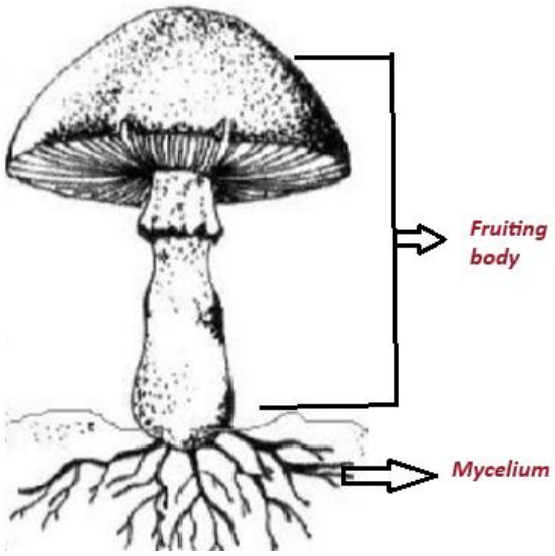


Figure 1.3 Structure of mushroom (Modified from Wille and Bento, 2021).

Mycelia are naturally occurring polymeric fibrous materials that are composed of chitin, cellulose, and proteins (Haneef et al., 2017). Chitin is a linear macromolecule composed of N-

acetylglucosamine units and is primarily found in the exoskeletons of insects and other organisms (Jones et al., 2020). The mycelial networks obtain their nutrients from substrate materials, typically derived from waste by products from plants or animals (Yang et al., 2021). Enzymes secreted from mycelia break down the substrate polymer and allow for its utilization as nutrients (Appels et al., 2019). Figure 1.4 provides a schematic representation of mycelium at different stages of development, including the elongated filamentous cells known as hyphae, the internal porous cross-section named septa, and the tubular cell wall composed of chitin, glucan, and an outer layer of proteins, such as mannoproteins and hydrophobins. The cell wall plays a significant role in providing mechanical strength and maintaining the morphological structure of mycelium (Haneef et al., 2017).

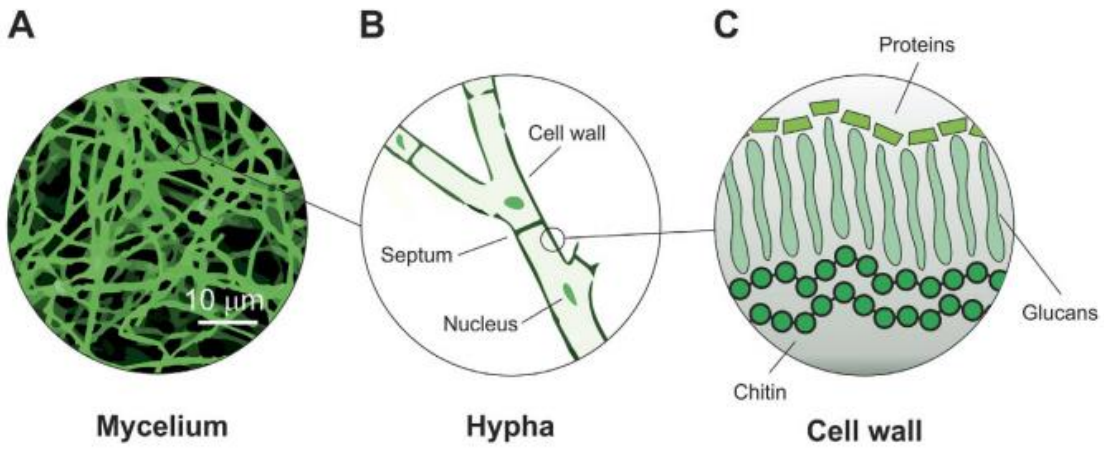


Figure 1.4 A schematic representation of mycelium at different stages of development (Haneef et al., 2017) [Permission not required].

To fabricate a mycelium-based material, a particular fungus is grown in a substrate, and its mycelium creates a network-like structure that binds the materials together, forming a composite-like material. The hyphal microfilaments of mycelium serve as a binder for

agricultural residues and can produce both low-value products, such as in the packaging industry, and high-value composite materials, such as in the construction industry (Jones et al., 2020)

1.5.3 Fungi Species for Growing Mycelia

Two subgroups of the fungal kingdom, Ascomycota and Basidiomycota, can produce mycelia. Ascomycota is the largest group in fungi and can be easily identified by a sac-like structure that produces ascospores. Basidiomycota covers almost one-third of all fungi and has a club-shaped structure known as basidium, where basidiospores are produced (Thomas et al., 2015). Hyphal anastomosis, or hyphal fusion, is a crucial phenomenon of Basidiomycota. Anastomosis is the property of a fungus to interconnect with adjacent hyphae, creating a cross-connected network and fusing together. This characteristic is considered crucial for creating mycelium networks because it facilitates the process of creating a fast-growing and strong network of mycelium (Chagnon, 2014). Mushrooms, smuts, and rusts are included in the Basidiomycota group, and they are major degraders of lignin, which is the main component of wood (Thomas et al., 2015). Most of the fungal species that have been used to fabricate mycelium materials belong to the Basidiomycota group (Attias et al., 2019).

Research has shown that several fungal species are suitable for growing mycelium-based materials, and the productivity, thickness, microstructure, and surface topography of mycelium fibers can vary depending on the species of fungus used (Yang et al., 2021; Haneef et al., 2017). The colonization of mycelium and the physio-mechanical properties of the mycelial material are also significantly dependent on the fungal species (Attias et al., 2019). Different fungal species used to fabricate mycelium-based bio-composite materials are listed in Figure 1.5, based on the literature. According to studies, the most commonly used fungal species are *Ganoderma lucidum* (25%) and *Pleurotus ostreatus* (12%), among other species (Yang et al., 2021).

The hyphal structures of Basidiomycetes can be divided into three types: generative, skeletal, and binding, based on cell wall thickness, internal structure, and branching characteristics (Jones et al., 2020; Yang et al., 2021). The key differences between these hyphal types are that generative hyphae are thin-walled, hollow, and branched, while skeleton hyphae are thick-walled, unbranched, and often solid. Binding hyphae are also thick-walled, solid, but highly branched. The three categories of mycelium network based on hyphal types are monomitic, dimitic, and trimitic. A monomitic structure only contains generative hyphae, while a dimitic structure consists of generative and skeleton types of hyphae. On the other hand, a trimitic structure contains generative, skeletal, and binding types of hyphae (Jones et al., 2020; Yang et al., 2021).

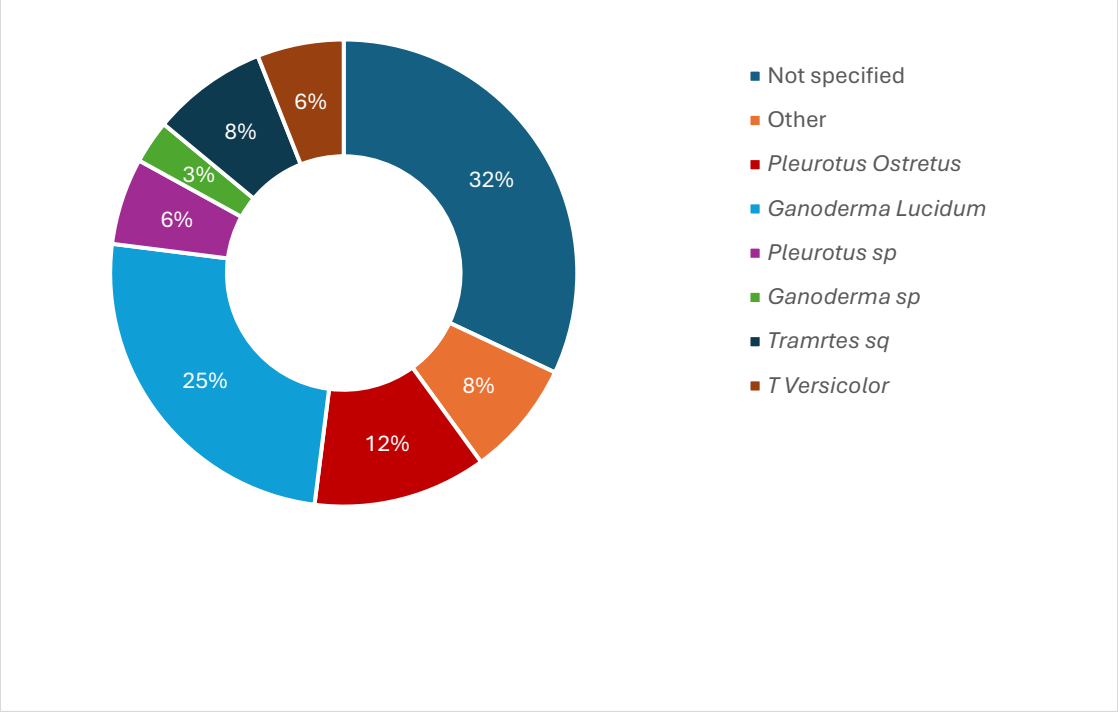


Figure 1.5 Different fungal species used in fabricating mycelium bio-composite material based on literatures (reproduced from from Yang et al,2021).

Research has shown that the mechanical properties of mycelium materials can vary based on the hyphal structure. Monomitic species (*P. ostretus*) have been found to exhibit poorer

mechanical strength compared to dimitic and trimitic species (*T. versicolor)*. For example, Apple et al. (2020) observed that *T. versicolor* grown on rapeseed straws had a tensile strength of 0.04 MPa, which is higher than the 0.01 MPa tensile strength of *P. ostreatus* (Jones et al., 2020; Yang et al., 2021)

1.5.4 Substrate Types

Mycelium-based bio-composites use a wide-range of substrates for their production. These substrates are selected based on research objectives and intended product usage, and often include agricultural by-products, industrial waste, and post-consumer waste (Sydor et al., 2022). Examples of such substrates include wood chips, sawdust, straw, corncobs, recycled paper, nut and seed hulls or meal, coffee pulp or grounds, and brewer's grain. The ideal substrates for mycelium-based bio-composites contain nitrogen and carbohydrates to facilitate the rapid growth of fungal mycelia (Sydor et al., 2022). The amount of required nitrogen and carbohydrate will vary based on fungi species, substrate types and other nutrients (Sydor et al., 2022). Figure 1.6 shows the most commonly used substrates in mycelium-based bio-composite material production across different research areas.

Jiang (2015) suggests that in substrate mixtures, carbohydrates and calcium are commonly added to promote mycelium development and bonding. However, the specific types of carbohydrates and calcium used are not typically specified in the studies. The raw materials used for mycelium-based bio-composites are lignocellulosic in nature, consisting of cellulose (30-50%), lignin (15-30%), hemicelluloses (25-35%), and other non-structural substances such as pectins, waxes, pigments, tannins, lipids, and minerals. The composition of these materials varies depending on their origin and species (Sydor et al., 2022).

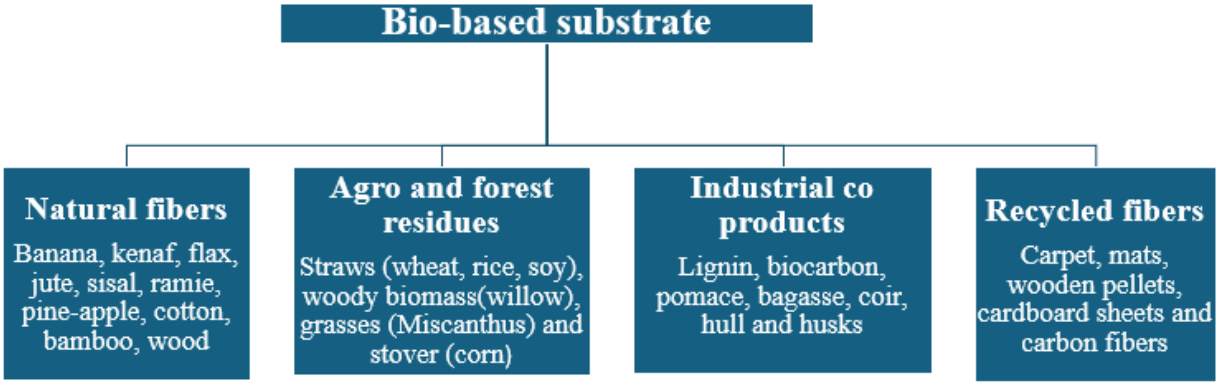


Figure 1.6 Substrates used in mycelium-based bio-composite material production (modified from Alaneme et al., 2023).

As suggested by Haneef et al. (2017), a substrate made of a mixture of pure cellulose and potato dextrose broth (PDB) is a simple and advantageous medium for mycelium growth. Cellulose, being abundant in hardwoods and crop wastes, serves as the material for mycelium growth, while PDB, rich in easily digestible simple sugars (dextrose), provides energy for mycelium growth. The two components are uniformly ground and mixed in a 1:1 weight ratio to create a substrate that ensures consistent growth of mycelium on a stable platform, resulting in a uniform material.

Cellulose, a natural polymer, is the primary structural component found in plant fibers and provides strength, stiffness, and stability to the materials. Hemicelluloses are polysaccharides that form short, branching chains and are closely associated with cellulose microfibrils, creating a matrix around cellulose. Lignin, a complex aromatic hydrocarbon polymer, confers rigidity to plants and enables them to attain considerable height. Lignin also facilitates water and solute transport through the plant's vascular system, while providing a physical barrier against pathogens and environmental stresses (Sydor et al., 2022).

Certain fungal mycelia extend from the substrate and create a dense layer referred to as the "fungal skin," which contains chitin and glucan, natural polymers (Aiduang et al., 2022). Lignin, characterized by an amorphous structure and high molecular weight, acts as a chemical adhesive within and between fibers due to its lower polarity (due to heterogenous structure and various aromatic rings) compared to cellulose (due to abundance of hydroxyl (–OH) groups). It enhances the structural integrity and rigidity of cell walls, and the composition of lignin varies depending on the source of the lignocellulosic material. The proportion of different monolignols and chemical bonds present in the lignin structure also varies based on the type of lignocellulosic biomass, such as hardwood, softwood, or grass (Sydor et al., 2022). The cell wall of hardwood plants is characterized by higher toughness and strength compared to annual plants (the plants complete its life cycle in one year), attributed to the presence of a thick layer of lignin that binds cellulose fibrils in a cross-orientation. White rot fungi possess a unique ability to break down the complex molecular structure of lignin. Typically, short sugars and starches are degraded first, as they are more readily available compared to complex polysaccharides (Xu et al., 2013).

For instance, in softwoods, lignin is predominantly composed of guaiacyl-units linked by ether and carbon-carbon bonds, while in hardwoods, there are equal amounts of guaiacyl- and syringyl-units in lignin. Grass lignin, on the other hand, contains guaiacyl-, syringyl-, and hydroxyphenyl-units (Sydor et al., 2022).

The degradation trends of these structural components depend on the type of fungi and the carbohydrate composition of the plant material used as substrate (Hori et al., 2013). Moreover, the type, size, and processing method of substrate particles have a direct impact on various properties of the final bio-composite (Elsacker et al., 2019). Most studies use annual plants or softwood as substrate materials, while only a small number utilize hardwood-based

substrate, often combined with annual plant residues and carbohydrates as a nutritional supplement (Attias et al, 2020).

Grains used as inoculum or nutritional supplements can significantly increase the overall mycelium density, two- or three-fold, respectively, compared to wood particles (Girometta et al., 2019). Therefore, providing a detailed description of the substrate composition and processing method is crucial for a better understanding of mycelium-colonization trends and their effect on final material properties. Shivaprasad et al. (2021), developed a mycelium-based bio-composite material for a sustainable replacement of polystyrene foam. In this study, mycelia from *P. ostretus* were grown in a sawdust-coir pit substrate (ratio: 3:2) with promising results that suggested it could be a successful replacement for polystyrene materials (Sivaprasad et al., 2021).

1.5.5 Mycelium-based Bio-composite Materials

Mycelium-based materials are derived from the Polyporales fungi, which utilize lignocellulosic material as their substrate. These materials have several advantages, including carbon neutrality, sustainable production processes, and biodegradability. One of the key advantages of mycelium composite materials is their use of natural fibers as reinforcements, which reduces reliance on synthetic fibers and their negative environmental impact. These natural fibers include flax, hemp, jute, sisal, kenaf, pineapple, abaca, coir, and banana. The use of natural fibers in mycelium composite materials offers numerous benefits. First, natural fiber reinforced mycelium composites are lightweight, making them ideal for applications where weight is a critical factor, such as aerospace and automotive industries (Summerscales et al., 2010). Second, natural fibers have excellent biodegradability properties, allowing for the disposal of mycelium composite materials without causing harm to the environment. Furthermore, natural fibers exhibit good mechanical properties, including high strength and

stiffness, which make them suitable for structural applications. Additionally, the use of natural fibers in mycelium composites can help address concerns related to the high densities and low modulus of elasticity typically associated with traditional mycelium composites.

To improve the mechanical properties of natural fiber reinforced mycelium composites, various techniques can be employed. These include chemical modifications to the matrix and fiber, as well as the use of adhesion promoters to enhance interfacial interactions between the filler particles and the mycelium matrix. The global trends in the marketplace show a shift towards natural fiber use in composites due to societal and environmental concerns (Karana et al., 2018). Nevertheless, the main concerns with traditional composite materials (made from fibre and fossil-based resin such as epoxy resin) are their lack of environmental soundness, high densities, and low modulus of elasticity (Yang et al., 2021). To address these concerns, researchers and fossil-based have invested in the development of natural fiber-reinforced composites as a viable alternative. Utilizing natural fibers in mycelium composite materials not only addresses these concerns but also offers additional benefits such as cost-effectiveness, availability of renewable resources, and improved overall sustainability. use of natural fibers in mycelium composite materials offers a promising solution to address the environmental concerns associated with traditional synthetic composites. Moreover, natural fiber-reinforced mycelium composites have demonstrated their potential in various industries including aerospace, automotive, and civil engineering.

1.5.5.1 Growing Methods and Conditions for Fungal Mycelia

Researchers use various methods to cultivate fungal mycelia, including liquid shaken cultures, solid culture media, and substrate-based growth. Each method offers unique advantages and challenges, influencing the resulting material properties and applications. Liquid shaken cultures involve suspending fungal spores or mycelium fragments in a liquid medium, promoting

rapid growth and homogeneous distribution (Apple et al., 2020). Solid culture media use agar-based substrates, providing a supportive matrix for mycelium growth (Rathinamoorthy et al., 2023). Substrate-based growth involves inoculating organic substrates, such as agricultural waste or sawdust, with fungal spores to foster mycelium colonization and material formation (Rathinamoorthy et al., 2023).

Growth conditions significantly impact mycelium development and material properties Factors such as temperature, humidity, carbon dioxide concentration, and substrate composition influence mycelium growth rates, density, and structural characteristics (Apple et al., 2020). Optimal conditions vary depending on the fungal species and intended application, necessitating careful control and monitoring throughout the cultivation process (Rathinamoorthy et al., 2023). Closed growth systems, such as plastic bags with filtered air exchange, allow mycelium cultivation without fruiting body development, which is often desirable for creating a dense and homogeneous mycelial network (Lelivelt, 2015). Additional details on growing conditions, including humidity of 55%, pH of 5.5, incubation times, supplementation (such as urea and superphosphate percentages), and substrate turning, are discussed by Lelivelt (2015).

To enhance mycelium production and material quality, researchers employ various optimization strategies (Rathinamoorthy et al., 2023). Sterilization and pasteurization techniques are used to eliminate contaminants and promote uniform mycelium growth (Attias et al., 2020). Chemical treatments may modify substrate properties or enhance fungal colonization (Attias et al., 2020). Additionally, precise control of environmental parameters such as temperature, humidity, and airflow are essential for optimizing growth conditions and maximizing material yield (Apple et al., 2020)

Innovative approaches are explored to enhance mycelium production efficiency and material properties (Soh & Ferrand, 2023). Specific molds and inoculation techniques are employed to control mycelium growth and achieve desired shapes and structures (Escaleira et al., 2020). Porous structures may be constructed to facilitate dense mycelium formation and improve mechanical properties (Soh & Ferrand, 2023). Additionally, advancements in 3D printing technology enable precise deposition of mycelium-containing substrates, offering new possibilities for customized material fabrication (Escaleira et al., 2020)

1.5.6 Areas of Application

In recent years, there has been a growing emphasis on sustainability and environmental consciousness across various sectors. Consequently, industries are increasingly seeking alternative materials that minimize environmental impact while maintaining performance and functionality. Mycelium composite materials have emerged as a promising solution to meet these demands, offering a renewable and biodegradable alternative to conventional materials as listed in Table 1.2.

Packaging Material: Mycelium-based bio-composites (MBCs) serve as an excellent alternative to synthetic foam and plastics in packaging applications due to their biodegradability and the use of agricultural by-products for production. Several research efforts have aimed to find suitable biodegradable alternatives to plastic packaging materials.

Construction Materials (Thermal and Acoustic Insulation): With their low thermal conductivity, MBCs provide an advantage as insulating materials in construction, offering better fire safety properties than some traditional materials. Additionally, their high acoustic absorption makes mycelium composites beneficial for sound insulation in construction and architectural acoustics.

Furniture and Design Elements: Mycelium-based composites' workability and aesthetic make them suitable for application in furniture, paneling, and other design elements within interiors.

Art: Mycelium composites can be molded into various forms, and their unique texture can be utilized in artistic and sculptural works.

Table 1.2 Relevant research of utilizing mycelium-based bio-composite materials in various applications.

Area of application	Product	Fungal species	Substrates	Results	Reference
Packaging	Packaging materials and insulation materials	Ganoderma Sp.	Cotton plant biomass	MBC meets the characteristics of poly styrene foam	Holt et al.,2012
	Insulated packaging materials	Not specified	Rice husk, wheat grains	Demonstrated potential for 100% natural cycle insulative packaging with minimal resource usage, slight variations in density were observed, while mycelium showed promise as a renewable alternative for biodegradable polystyrene foam, with specimen A exhibiting the highest apparent porosity at 66%	Arifin & Yusuf, 2013
	Insulating foam packaging	Coriolus Versicolor, Pleurotus Ostreatus	Wood chips, hemp hurd, loose hemp fiber and non-woven mats of hemp fiber	The combination of hemp mat with T. versicolor exhibits the greatest compressive strength	Lelivit et al., 2015
	Furniture packaging (particle board)	Not specified (white rot basidiomycota provided by Ecovative)	Wood particles (mixture of spruce, pine, particle board particles and cellulose nanofibrils	This innovative composite system demonstrated favorable physical and mechanical characteristics and holds promise as a substitute for formaldehyde-based composites.	Sun et al., 2019

Area of application	Product	Fungal species	Substrates	Results	Reference
Packaging	Packaging materials	Pleurotus Sp	Crop residues	The material didn’t full fill all the requirement of expandad polystyrene	Nava et al., 2012
	Packaging materials	Not specified	Outer layer: jute, flax, cellulose and core: agricultural waste	The flexural strength relies on how much the mycelium has spread into the outer layers and how well these outer layers are connected to the core. The stiffness, on the other hand, is determined by the core itself. Weakly attached outer layers don't contribute much to the bending strength	Jiang et al., 2015
	Packaging boards	Pleurotus ostreatus	Wood and coir pith	The mycelium bio composite showed satisfactory performance to replace expanded poly styrene	Sivaprasad et al., 2021
	Packaging boards	Ganoderma lucidum	Wheat straw (90%), polypropylene with bacterial spores (10%)	The fungal biocomposite showed comparable compressive strength and better thermal insulation ability than polystyrene	Raut et al., 2021

Area of application	Product	Fungal species	Substrates	Results	Reference
Construction materials	Insulation foam	Oxyporus latermarginatus, Megasporoporia minor, Ganoderma resinaceum	Wheat straw	The study emphasizes the diverse growth patterns of different fungal species within building materials. Choosing the right fungi is crucial for insulation materials. Factors such as rapid mycelial growth for binding are important, but excessive substrate decay is undesirable. Even growth throughout the blocks is preferred	Xing et al.,2018
	Thermal insulation board	Polyporus arcularis, Trametes suaveolens	Birch wood	The results of the initial physical screening test, especially regarding thermal conductivity, indicated that these panels performed similarly to conventional insulation materials	Wimmers et al., 2019
	Thermal insulation foam for water container	Colorius versicolor, Trametes ochracea, Ganoderma sessile	Vine and apple tree-pruning woodchips mixed with wheat straw	The experimental study successfully grew three fungal species on woodchip substrates sourced from agricultural waste, yielding different material densities and mechanical properties	Attias et al., 2019
	Acoustic absorption panels	Not specified	Switch-grass, rice straw, sorghum stalks, flax shive, kenaf and hemp	Mycelium-based composites exhibit their best performance at a noise frequency of 1000 Hz. They are on par with polyurethane foam board and surpass plywood in terms of performance	Pelletier et al., 2013
	Insulation material from mycelium-latex composite	Pleurotus ostreatus	Cotton seed hauls, Carboxylated styrene butadiene rubber latex, Silane coupling agent (aminopropyltriethoxysilane)	Adding less than 5% latex affects mycelium growth slightly, improving bonding between the culture medium and mycelium	Juan et al., 2014

Area of application	Product	Fungal species	Substrates	Results	Reference
Construction materials	Board	Trametes mu lticolor , Pleurotus ostreatus	Cotton fibre, Rapeseed straw, beech sawdust supplemented with bran	Mycelium composites derived from straw exhibit higher stiffness, but lower moisture resistance compared to those derived from cotton	Appels et al., 2019
	Thermal insulation	Trametes versicolor	Flax, flax dust, flax long treated fibres, flax long untreated fibres, flax waste, wheat straw dust and wheat straw	The thermal conductivity and water absorption rate of mycelium composite are similar to those of rock wool, glass wool, and extruded polystyrene. The mechanical properties of MBC are influenced more by the arrangement of fibers than by the chemical composition of the fibers	Elsacker et al., 2019
	Composite boards	Ganoderma lucidum	Palm sugar fiber and cassava bagasse	The composite board's physical and mechanical properties meet the standards set by the Japan Industrial Standard for particleboard	Agustina et al., 2019
	Composite block	Ganoderma lucidum	Cotton stalk	Properties showed notable enhancement as the hot-pressing temperature increased	Liu et al., 2019
	Building materials	Trametes versicolor	Hemp shives, wood chips	The comparison focused on the strength, water absorption, and biodegradability of five different combinations of fungi and substrates	Zimele et al., 2020
	Non structural building materials	Ganoderma lucidum	Bamboo fiber	The findings suggest that this board has potential applications in interior building use, particularly in high-rise buildings requiring lightweight insulation and partition boards	Ridzqo et al., 2020

Area of application	Product	Fungal species	Substrates	Results	Reference
Construction materials	Bioboard (plywood) without synthetic adhesive	Ganoderma lucidum,Pleurotus ostreatus,Auricularia polytricha	Food waste, diaper waste and sawdust	Ganoderma lucidum has the potential to serve as an excellent natural adhesive, offering a sustainable alternative to formaldehyde-based adhesives in the wood composite industry.	khoo et al., 2020
	Brick	Ganoderma lucidum and Pleurotus ostreatus	Clay mixed with sawdust, bleached and unbleached cellulose	Mycelium strengthens tensile strength along the extrusion axis and improves layer connections	Jauk et al., 2021
	Construction block	Ganoderma lucidum, Trametes hirsuta, Picnoporus sanguineus, Fomes fomentarius	Beech wood	Ganoderma lucidum shows promise as one of the most appropriate fungi for producing fungal mycelium and chipped wood composites, particularly for use in construction components	Saez et al., 2021
	Board	Ganoderma lucidum, Bacillus amyloliquefaciens	Wheat straw with bacterial spores	The fungal biocomposite demonstrated comparable compressive strength and enhanced thermal insulation capabilities when compared to polystyrene	Raut et al., 2021
	Wall insulation material	Ganoderma lucidum	Cellulose fibe, rapseed bagasse	The rapeseed bagasse substrate demonstrated superior thermal conductivity, low density, and excellent dimensional stability, comparable to conventional EPS polymer	Gauvin et al., 2022
	Bricks and beams	Ganoderma lucidum, pleurotous ostreatus	Beech sawdust, oak sawdust, bleached cellulose pulp, shredded cardboard, shredded newspaper, cotton fibers, soy silk	The hygroscopicity of MBC varies significantly depending on the type of substrate utilized	Vasatko et al., 2022

Area of application	Product	Fungal species	Substrates	Results	Reference
Miscellinous	Furniture	Pleurotus ostreatus	Hemp and wood	The primary challenge in manufacturing on an architectural scale is the need to halt the growth process	Nguyen et al., 2022
	Textile substitute	Penicillium camemberti	NA	Mycelium sheets, with a tensile strength showed lower properties than comparable nonwoven and knitted fabrics. However, their excellent permeability and biodegradability suggest potential for textile applications	Rathinamoorthy et al., 2023
	Paper	Pleurotus pulmonarius, Pleurotus osreatus, Penicillium camemberti	NA	The economics of mycelium paper present significant challenges, including feedstock scale, manufacturing complexity, and upgradation	Mazur, 2015
	Artificial leather	Polyporales	Oak sawdust and rice bran	Mushroom-derived leather has been proposed as a potential competitor to both animal and synthetic leather, providing a sustainable alternative for various leather products	Raman et al., 2022

1.6 Research Gap

The study of mycelium-based bio-composite materials is not a recent development, as research has been ongoing for years to explore environmentally friendly alternatives to fossil-based materials. Mycelium-based bio-composites have been investigated for various applications including construction, packaging, textiles, furniture, leather, and paper. However, there has been a notable absence of experiments focusing on the feasibility of using mycelium-based bio-composite materials to address plastic cup pollution. This study represents a novel approach aimed at determining the viability of producing compostable beverage cups from mycelium composite materials. Furthermore, agricultural waste such as rice, cotton, sawdust, wood, pine wood, wheat straw, flax, hemp, and bamboo have been widely utilized as substrates for mycelium composite materials. Notably, there has been limited exploration into the use of canola straw and cattail plant as substrates. The current study also seeks to adopt a novel approach by investigating the potential of these North American crops as substrates, thereby utilizing agricultural waste and discarded materials to create value-added products that are environmentally sustainable.

Chapter 2: Material Selection and Making Compostable Cup from Fungal Mycelium

2.0 Preamble

This project received partial funding from Fools and Horses (F&H), a coffee shop located in Winnipeg, MB, Canada. Consequently, the study utilized F&H coffee cups as a benchmark to assess and compare with the mycelium-based composite materials. The research was divided into two parts. In the first phase, compostable coffee cups were fabricated using fungal mycelium, conducting various trials and experiments based on prior research and the following hypotheses:

Mycelium-based biomaterials are inherently hydrophobic, and their low thermal conductivity, biodegradability, and lower material cost could be crucial properties for their use as an alternative to commercial coffee cups. In the second phase, a sheet-like material was prepared according to the chosen protocol to analyze the material characteristics, aiming to replicate the properties required to replace a commercial coffee cup.

2.1 Material and Methods

Several trials were conducted to determine the most suitable materials for fabricating mycelium composite materials. Selecting fungal species, identifying appropriate substrates, and choosing nutrient-rich growth media were crucial steps in this process. Each step was essential to cultivate the fungus and produce compostable beverage cups.

2.1.1 Selection of Fungal Species

Based on the literature review, the two most utilized fungus species, *Ganoderma lucidum* and *Pleurotus ostreatus*, were selected for this project. The mother cultures of these species were obtained from the Department of Microbiology at the University of Manitoba, grown on solid culture media in 90 mm petri dishes, and stored at 4°C until needed. Additionally, the research group possessed a DIY package from Ecovative Ltd. dating back to 2019. Initially, the goal was to employ the same fungal species utilized by Ecovative as a control, and to isolate fungal

species according to the procedure outlined in the Ecovative package. Following the retrieval of the fungal culture in a liquid medium, the spores were subsequently cultured on solid potato dextrose agar (PDA) plates. Two distinct microorganisms were observed, which were subsequently separated and cultured on separate PDA plates, as illustrated in Figure 2.1. Author tried to retrieve the genomes sequence of the fungus species obtained from Ecovative packages. The attempt to sequence the genomes of the fungi from Ecovative package was unsuccessful.

A) B)

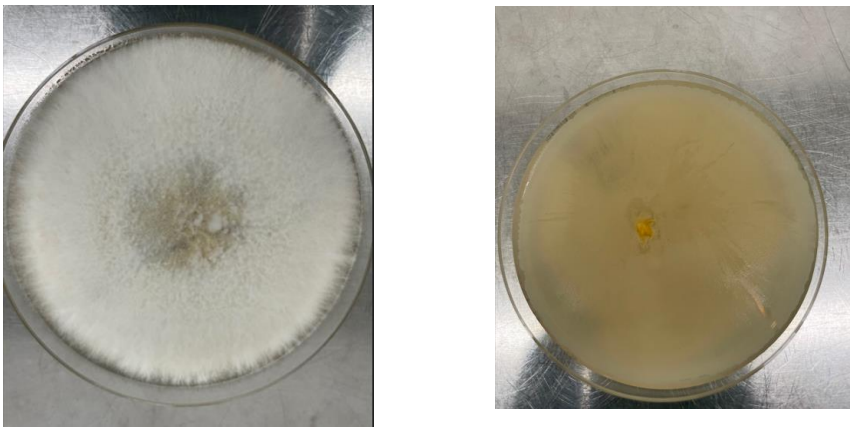


Figure 2.1 Microorganism retrieved from the DIY pack of Ecovative. A) Fungal isolates 1 from the Ecovative sample; B) Fungal isolates 2 from the Ecovative sample.

Based on the literature review, another fungal species and *Polyporus squamosus* was included in the study. *P. squamosus* was procured from the UAMH Centre at the Gage Research Institute in Toronto in the same size solid culture media (9mm petri dishes) and stored in 4°C as other species in PDA petri plates.

Ganoderma lucidum required 14 days to achieve full growth within the petri dishes, while the other species, *Pleurotus ostreatus* and *Polyporus squamosus,* exhibited half-plate growth within the same period, as illustrated in Figure 2.2. Among these three species, *Ganoderma lucidum* was chosen for further investigation owing to its rapid growth, dense hyphal

network, and compatibility with the selected substrate. The growth conditions were 25-28°C and 60-65% RH for all three fungal species.

A) B) C)

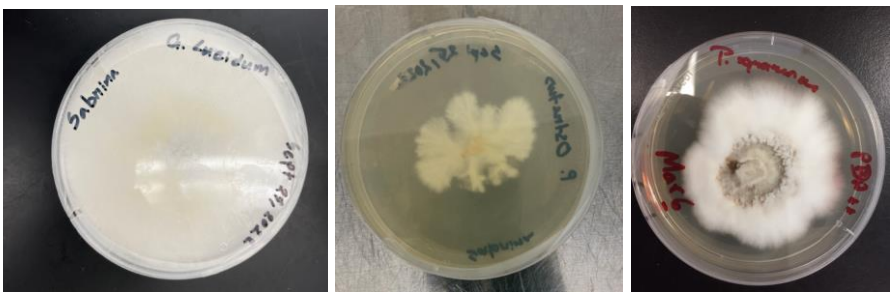


Figure 2.2 Growth Rates of A) *Ganoderma lucidum*, B) *Pleurotus ostreatus*, and C) *Polyporus squamosus* on Potato Dextrose Agar (PDA) Media 14 Days Post-Inoculation.

2.1.2 Selection of Fungal Growth Medium

Initially, *G. lucidum* was cultured on Potato Dextrose Agar (PDA) to isolate the strain from mother plates. As depicted in Figure 2.2, *G. lucidum* exhibited the most rapid growth rate on PDA culture 14 days post-inoculation. However, cultivating the fungus in a liquid medium proved to be the most viable method for fabricating a bio composite material. Various media, including Potato Dextrose Broth (PDB), Potato Dextrose Peptone Broth (PDPB), Yeast Extract Peptone Dextrose Broth (YEPDB), and Rice Extract Broth (REB), were assessed to determine the optimal growth conditions for *G. lucidum* within a 7-day period. The recipes for these growth media are provided in Appendix Table A1.

The yield of mycelium growth is presented in Table 2.1. YEPDB was subsequently selected for further cultivation of *G. lucidum*, yielding a maximum dry mass weight of mycelium of 9.1 ± 1.46 g/L. (batch wise data has been added in Appendix Table A2) The fungi were

cultured on an electrical shaker for 7 days at a temperature of 28°C, humidity (60-65%) and a speed of 200 rpm across all growth media.

Table 2.1 Dry Mass Weight of Mycelium 7 Days Post-Inoculation in Various Liquid Culture Media

Medium	Dry mycelium mass (g/L)
Potato dextrose broth	4.33 ± 1.45
Potato dextrose broth with peptone	3.77 ± 1.19
Yeast extract peptone dextrose	9.1 ± 1.46
Rice extract	1.85 ± 0.66

2.1.3 Selection of Substrate

The study aimed to explore the use of local biomass substrates for the production of value-added products. To this end, canola straw (*Brassica napus* L.) and cattail (*Typha lotifolia.)* were investigated as substrates for fungal growth in the current study. Both substrates were utilized in their raw state and subsequently ground into small particles using a food processor (Cuisinart) set to high-speed (23,000 rpm). Initially, multiple trials were conducted using different ratios of substrate, water, and fungal spores. Subsequently, a standardized and optimized recipe was determined, which will be outlined in the manufacturing steps of the mycelium cups.

G. lucidum was inoculated into both substrates for a duration of 14 days, and the growth of their mycelium networks was observed on a weekly basis. Among these two substrates, cattail demonstrated the most promising potential for fabricating a bio composite material with *G. lucidum* mycelia. This was attributed to its robust mycelial growth, shorter

cultivation period, and superior structural integrity. Figure 2.3 illustrates that, within a span of 14 days, *G. lucidum* developed a denser mycelial network compared to *P. ostreatus*.

Table 2.2 Composition analysis for cattail, canola, and hemp.

Fibers	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Wax (%)	Pectin (%)	Ash (%)	Moisture Content (%)	Reference
Cattail	22.4	21.8	20.6	11.5	0.18	4.5	8.6	Wu et al., 2021
Canola	44	-[b]	19.21	6	-[b]	13	-[b]	Kiaei et al., 2014
Hemp[a]	44.2	30.3	24.4	3.5	-[b]	1.4	-[b]	Stevulova et al., 2015

*a = mostly used substrate as per literature review, *b= not determined

Subsequent experiments involving substrate selection did not include the third fungal species (*P. squamosus*) due to its prolonged inoculation time during subculturing in petri plates from mother cultures. The reason for the suboptimal performance of the *P. ostretus* and P. *squamosus* fungal species with canola straw remained unclear, despite their similar cellulose and hemicellulose content (Table 2.2). Notably, this content is approximately half that of the holocellulose (cellulose + hemicellulose) content in hemp composition.

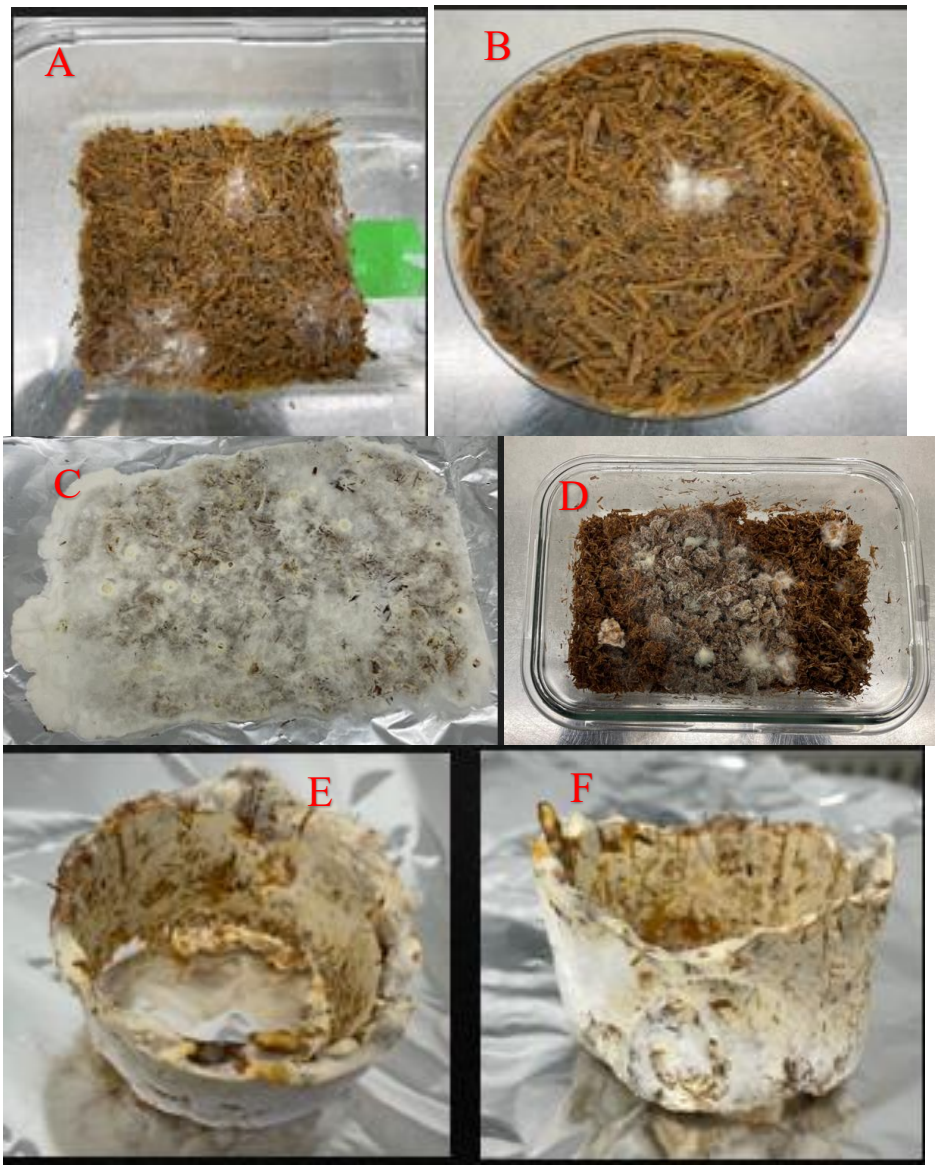


Figure 2.3 A) *G. lucidum* + canola after 14 days of inoculation; B) + Canola after 14 days of inoculation; C) *G. lucidum* + cattail after 14 days of inoculation; D) *P. ostreatus* + cattail after days of inoculation; E, F) First structure using DIY plastic cups from *G lucidum* and cattail substrate after 20-21 days of incubation.

2.2 Nutrients and Growth Conditions for Fungi

Several trials were conducted to determine the optimal nutrient source for fungal growth in conjunction with the cattail substrate, drawing from various sources in the literature.

2.2.1 Carbon source

Cattail biomass served as the primary carbon source to facilitate fungal growth. Additionally, PDB medium was utilized as a secondary carbon source to enhance mycelial growth and maintain adequate moisture levels. Yeast Extract Peptone Dextrose Broth (YEPDB) was explored as an alternative to PDB. However, the resulting material exhibited brittleness and flakiness (Figure 2.4A).

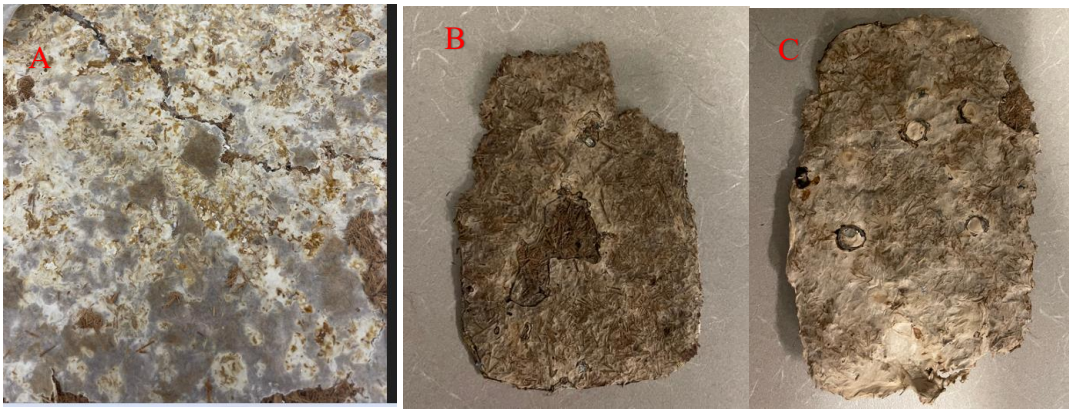


Figure 2.4 A) Cracked mycelium-cattail composite using YEPDB; B) Mycelium composite made with PDPB medium (very fragile); C) Mycelium composite made with $MgSO_4$ (no significant improvement)

2.2.2 Nitrogen source

Peptone was employed as a nitrogen source (at a ratio of 20:1 carbon to nitrogen), following a method similar to that of Zeid and Gimenez (2017). However, this trial proved unsuccessful as the material became excessively fragile after the use of peptone, as depicted in Figure 2.4B.

2.2.3 Inorganic salts

Previous studies (Zeid and Gimenez, 2017; Liu et al., 2020) have suggested that the addition of inorganic salts can enhance mycelial growth. However, the incorporation of $MgSO_4$ + KH_2PO_4 (100 -150 gm/L) did not yield any significant differences compared to conditions

without any inorganic salt (Figure 2.4 C). On the other hand, the addition of gypsum ($CaSO_4 \cdot 2H_2O$) resulted in enhanced growth, as observed in the study by Zeid and Gimenez (2017). Consistently, the current study also observed a similar result after incorporating gypsum into the material to promote hyphal growth and maintain a pH level between 4.5 and 5.

2.3 Fabricating Mycelium Cattail Biocomposite Cups

Raw cattails were ground using a food processor (Cuisinart brand) set to a high-speed (23,000 rpm) and then sterilized in an autoclave (Steris Amsco Lab 250) at 120°C for 45 minutes. Potato dextrose broth (PDB) medium was prepared by dissolving 24 grams of dehydrated PDB (Fisher Scientific brand) in 1 liter of distilled water. The final material mixture was prepared, comprising ground cattails, 200% volume of Potato Dextrose Broth (PDB) liquid nutrients, 50% flour as natural additives, and 1% inorganic salt ($CaSO_4$). The pH of the mixture was adjusted to between 4.5 and 5. Subsequently, 10-15% fungal spores were inoculated into the moist material. The inoculated material was then incubated in a static chamber at 28°C with a relative humidity 60-65% for a duration of 7 days.

After the completion of the incubation period, the mycelial colonies were manually homogenized. The resulting homogenized material was poured into a plastic compression mold to shape into cups. An external contractor prepared a mold using Poly-lactic acid (PLA), which was utilized for the fabrication of mycelium cups in the current study. The dimensions of the mold were as follows: lower cup - length: 115 mm, diameter: 88 mm, thickness: 10 mm; upper cup - length: 109 mm, diameter: 82 mm, thickness: 10 mm. The mold featured multiple small holes (0.75 mm) across the external part to ensure proper aeration for mycelium growth. Prior to use, the PLA mold was sterilized by washing with bleach liquid (H_2O_2) and then exposing them to UV-light (254 nm) in a biosafety cabinet for 45 minutes. After that the mold containing the homogenized material was placed in a static incubator for an additional 3 to 4 weeks at 28°C and

60-65% relative humidity. Following this period, the mold was carefully removed from the material, leaving behind the cup structure. Finally, the mycelium cup was sterilized in an autoclave at 120°C for 45 minutes to deactivate any remaining microorganisms. Figure 2.5 illustrates the procedural steps involved in creating the mycelium cup.

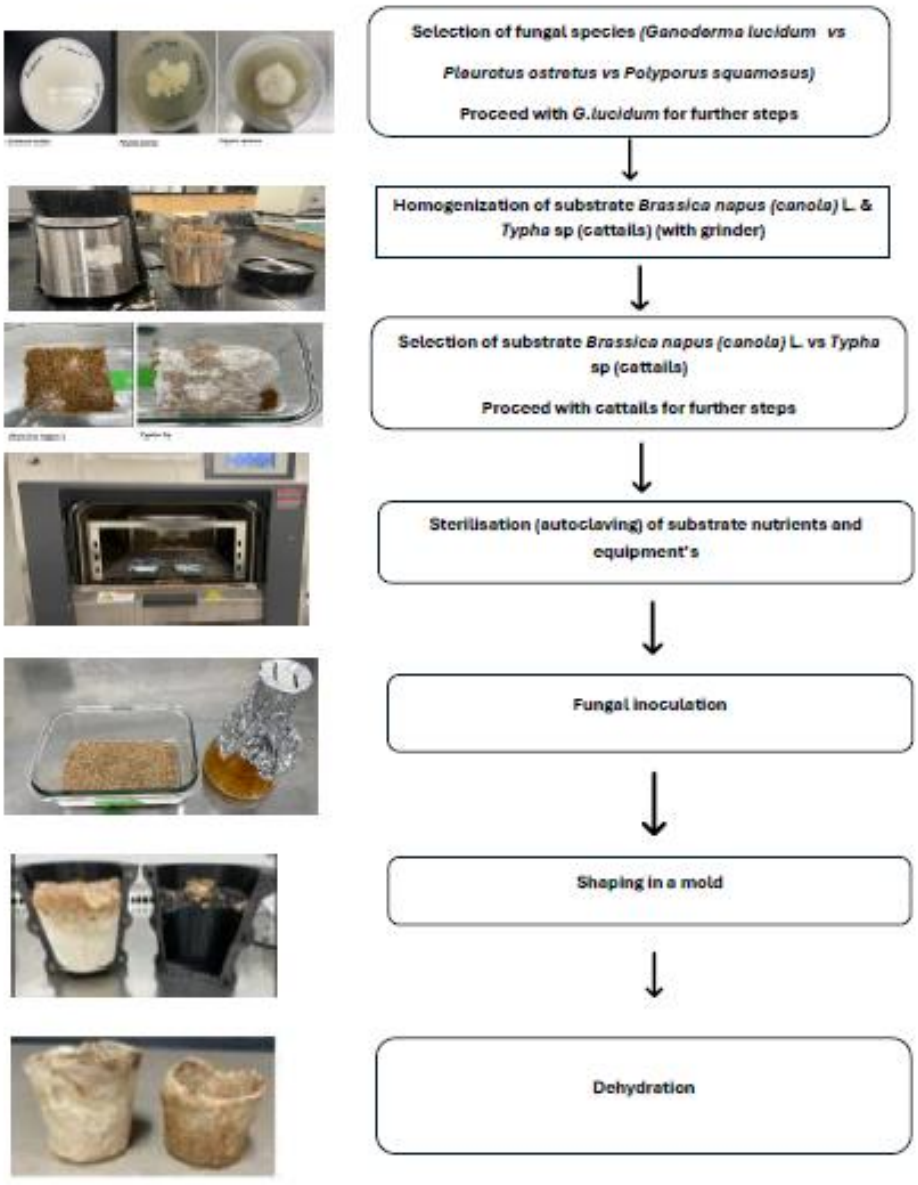


Figure 2.5 Flow chart of the process steps to make compostable coffee cups from fungal mycelium and cattail.

2.4 Mycelium-Cattail Composite Sheet

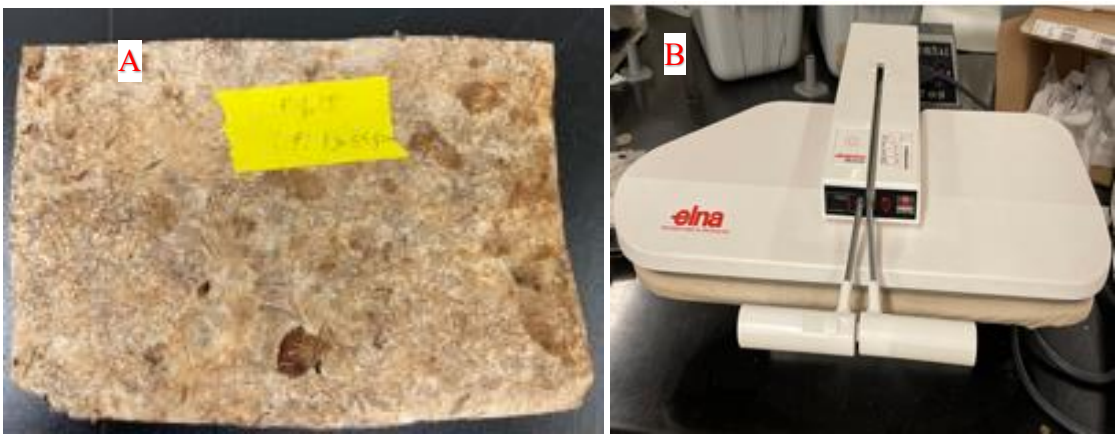


Figure 2.6 A) Mycelium sheet; B) clothing press machine

A mycelium composite sheet was prepared to assess the feasibility of traditional cup manufacturing processes, akin to those used in commercial coffee cup production from paper. Additionally, these mycelium sheets were utilized to characterize the materials for their morphological, thermal, hydrodynamic, and mechanical properties. The same process as followed for preparing both cups and mycelium sheets. The only distinction was that, after homogenization, the material was poured onto a foil sheet instead of a mold. The material was manually spread using a wooden bread roller and a metal frame measuring 12 inches by 12 inches (Figure 2.7). All instruments used, such as the wooden bread roller and metal frame, were cleaned with liquid H_2O_2 and then exposed to UV-light in a biosafety cabinet (254nm) for 45 minutes. All other processes were similar to cup production, including final autoclaving and dehydration. Figure 2.6A displays the final outcome of the mycelium sheet. Due to the manual distribution of living materials, the sheet's thickness varied frequently. Consequently, the author attempted to utilize a cloth press machine (Figure 2.6B) as a hot press to achieve uniform thickness and a smooth surface. However, the available press machine did not provide adequate pressure and temperature for this purpose. Subsequently, a dead weight (20 kg) placed on top of

the mycelium sheet for 24 hours after final autoclaving to improve uniformity in material thickness.

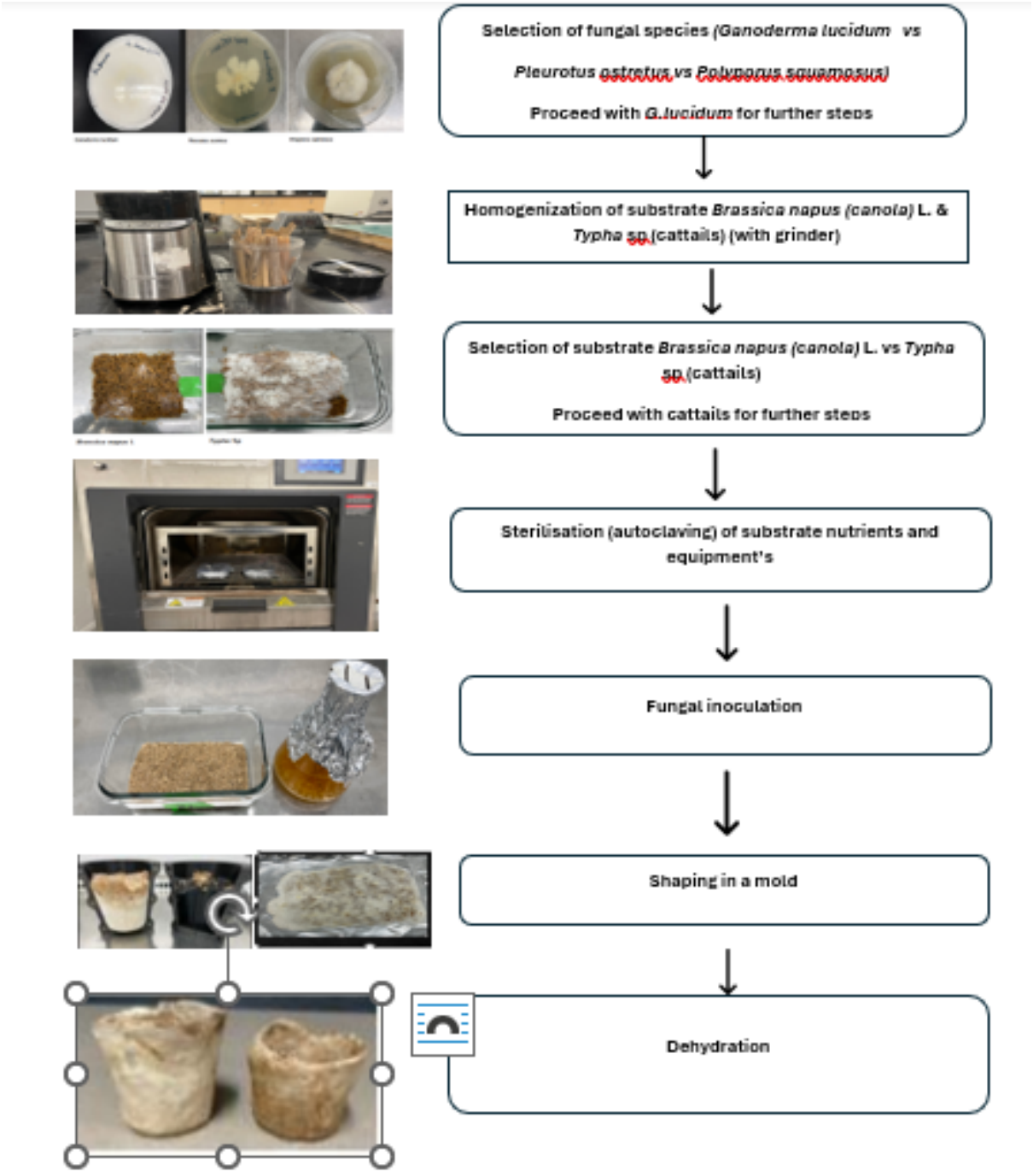


Figure 2.7 Flow chart of the process steps to make the mycelium-cattail composite sheet.

Two sets of samples were prepared, corresponding to growth durations of 2 weeks and 4 weeks, to assess and compare the material properties. Subsequently, both the mycelium cup and mycelium sheet underwent autoclaving in a waste cycle at 120°C for 1.5 hours to sterilize them. This was followed by a drying phase at 60°C for 24 hours in an isotherm oven to remove residual moisture.

2.5 Coating the Mycelium Material with Polymer

Initially, it was decided to coat the cup with a biodegradable polymer to enhance its barrier properties. Considering factors such as biodegradability, availability, and feasibility for commercial use, Poly-Lactic Acid (PLA) was chosen for the coating instead of other non-degradable fossil-based polymers. PLA granules measuring 3-5 mm in size, with a melt flow rate of 8 and a molecular weight of 193.3 kg/mol, sourced from Goodfellow, were utilized in the current study.

2.5.1 Selection of Solvents

After conducting a comprehensive literature review to identify potential solvents for PLA, nine mono-solvents and three binary solvents were selected for solubility testing. The chosen mono-solvents were Hexane, Acetone, Dimethylformamide, Chloroform, Ethyl acetate, Aniline, Tetrahydrofuran, and Acetonitrile. The selected binary solvents were: DCM:Acetonitrile (1:1), Tetrahydrofuran:Sodium sulfide (1:1), and Tetrahydrofuran:Dimethyl sulfoxide (1:1). Table 2.3 outlines the treatment temperature, duration, and the solubility status of PLA in each solvent.

PLA was soluble in Dichloromethane, Chloroform, Aniline, and DCM:Acetonitrile (1:1) at room temperature and at elevated temperature (80°C). However, due to the associated health hazards with Aniline and Chloroform, they were excluded from further consideration. DCM and the binary solvent DCM:Acetonitrile (1:1) were chosen for trials in polymer film preparation

using the spin casting method. The polymer films produced from both solvents displayed similar properties except for color. Films made from DCM alone were more transparent, while those from the binary solvent had a slight whitish cast and were prone to tearing when removed from the spin casting surface. Considering commercial application and cost-effectiveness, the mono solvent DCM was selected for use as a solvent for PLA in the current study

Table 2.3 Solvent comparison chart for PLA solubility

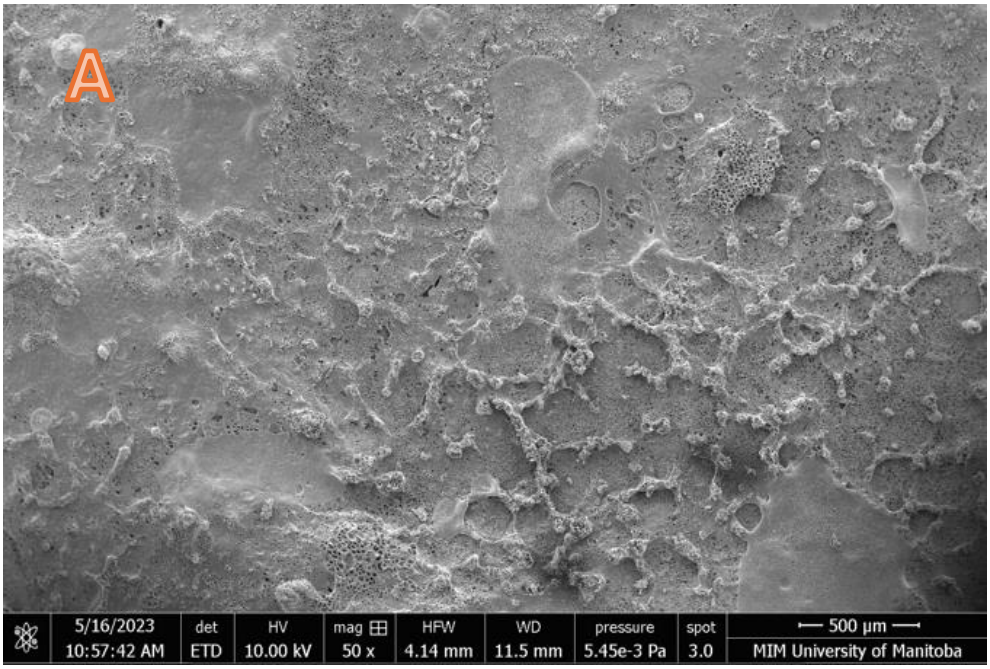
Types	Solvent	Boiling point (°C)	Temperature (°C)	Duration of magnetic stirring (HR)	PLA solubility
Single solvent	Hexane	69	80	24	insoluble
	Acetone	56.2	80	24	insoluble
	Di-methyl formaldehyde (DMF)	153	80	24	insoluble
	Di-chloro methane (DCM)	39.6	Room temperature (23-25)	2	**highly soluble**
	Chloroform	61.2	Room temperature (23-25)	2	**highly soluble**
	Ethyl acetate	77.1	80	24	insoluble
	Aniline	184	80	24	**highly soluble**
	Tetrahydrofuran	66	80	24	soluble (condensed in room temp)
	Acetonitrile	81	80	24	insoluble
Binary solvent	**DCM: Acetonitrile (1:1)**	-	80	24	**highly soluble**
	Tetrahydrofuran: sodium sulfide (1:1)	-	80	24	soluble (condensed in room temp)
	Tetrahydrofuran: dimethyl sulfoxide (DMSO) (1:1)	-	80	24	soluble (condensed in room temp)

2.5.2 Polymer Coating Method

Various techniques are employed for polymer coating, including spraying, spin casting, and electrospinning, among others. In consideration of availability and cost-effectiveness, a paint spray gun was chosen for coating the interior of the mycelium cup with PLA polymer.

Figure 2.8 Spray gun that was used to coat the mycelium materials.

A "Pindex 1000 cc Aluminum Cup with Gauge Air Spray Gun, HVLP Gravity Feed Air Spray Gun" was procured from Amazon for this research, as depicted in Figure 2.8. A nozzle size of 2.55 mm was utilized in this study.



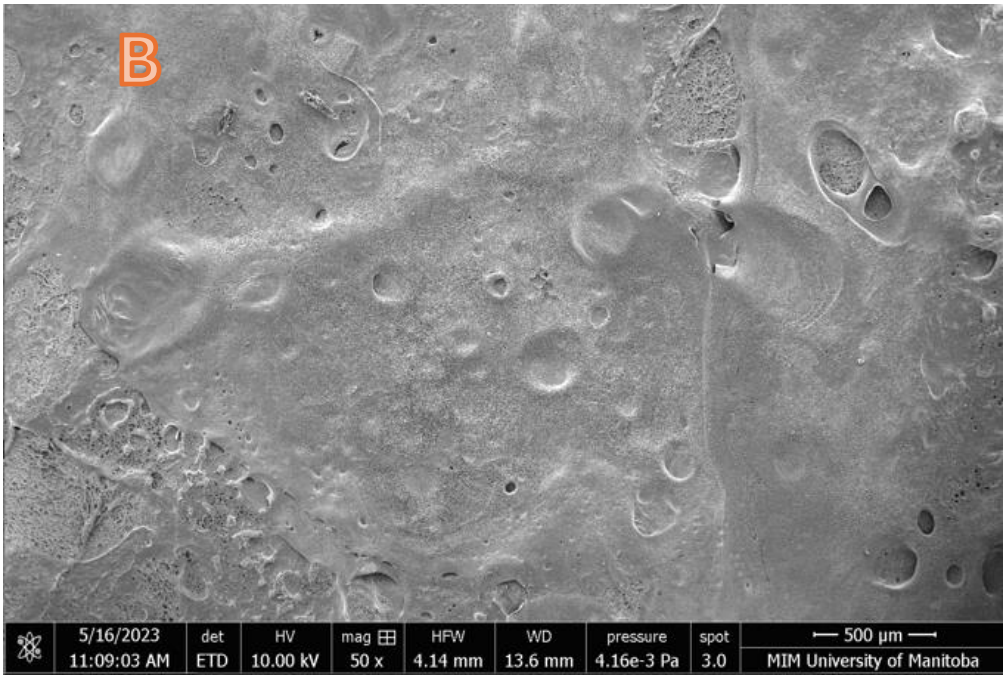


Figure 2.9 SEM images of coated mycelium materials (A) mycelium composite coated with 2% PLA, (B) Mycelium composite coated with 5% PLA.

Three different PLA concentration solutions (2%, 5%, and 6% w/v) were prepared using DCM as the solvent. These solutions were prepared at room temperature using a magnetic stirrer for 2 hours. However, the 6% solution could not be utilized due to frequent nozzle clogging issues. As hand spray- painting was employed for the coating process, assessing the uniformity of the coating was crucial. Scanning electron microscopy (SEM) was used to image the mycelium sheets coated with 2% and 5% PLA solutions (Figure 2.9). In both cases 4 strokes were used while doing the coating. From the SEM images, it was evident that the PLA polymer coating exhibited unevenness in both concentration solutions. However, the coating appeared to be less uneven and more uniform in the 5% PLA solution compared to the 2% PLA solution.

Later, the experimental protocol was modified to omit the polymer coating step in the process of making compostable coffee cups. This decision was based on findings from the current study, which revealed that the mycelium material is inherently hydrophobic and

possesses sufficient thermal barrier properties. Additionally, since the polymer coating would have provided additional barrier properties, its omission was deemed appropriate.

2.6 Use of Commercial Plasticizer to Improve Bending Properties

A commercial food-grade plasticizer, Triethyl citrate (Sigma Aldrich), was utilized to enhance the plasticity of the mycelium materials. A solution was prepared by mixing 20% Triethyl citrate with water, and then the mycelium sheet was submerged in the solution for 48 hours. Subsequently, the mycelium sheet was dried in an isothermal oven for 24 hours at a temperature of 60°C. The plasticizer-treated material exhibited improved bending properties. However, it also became highly hydrophilic. An attempt to measure the water contact angle of the treated sheet failed, because it rapidly absorbed water (within milliseconds), making it impossible to obtain accurate measurements. Given that the intended application of this bio-composite was in beverage cups, hydrophobicity was considered a crucial requirement for the material. Consequently, the treatment with plasticizer was deemed an unsuccessful attempt.

2.6.1 Improved Bending Properties after Rehydrating the Mycelium Sheet

After observing that the mycelium composite made from *G. lucidum* and cotton stalk exhibited maximum mechanical properties after a 30% water uptake (Liu et al., 2020), the mycelium sheet was rehydrated using a wet paper towel. It was found that in its wet condition, the mycelium sheet behaved more like a plastic material. Figure 2.10 depicts the appearance of the mycelium sheet after rehydration. If the mycelium composite were to be utilized in commercial industries, their unique properties in wet conditions could potentially be leveraged in cup production processes, similar to traditional paper cup manufacturing techniques.

Figure 2.10 Different bending attempts of mycelium sheet after rehydration.

2.7 Challenges with Working with a Microorganism

The author maintained all safety protocols established in the microbiological laboratory, including sanitizing equipment, utilizing a biosafety cabinet, and employing autoclaved equipment and materials. Despite the high level of precautions taken while working with fungus, the research group encountered occasional issues with contamination from other airborne fungi and bacteria. Additionally, microorganisms tended to proliferate on autoclaved final mycelium sheets if they were not adequately dried beforehand. Figure 2.11 illustrates some of the issues the author encountered while working with mycelium composite materials. As microorganisms require a considerable amount of time to grow, encountering contamination after a certain period often leads to the material being compromised and ultimately discarded. This situation can be disheartening, especially after investing a significant amount of time in the growth process.

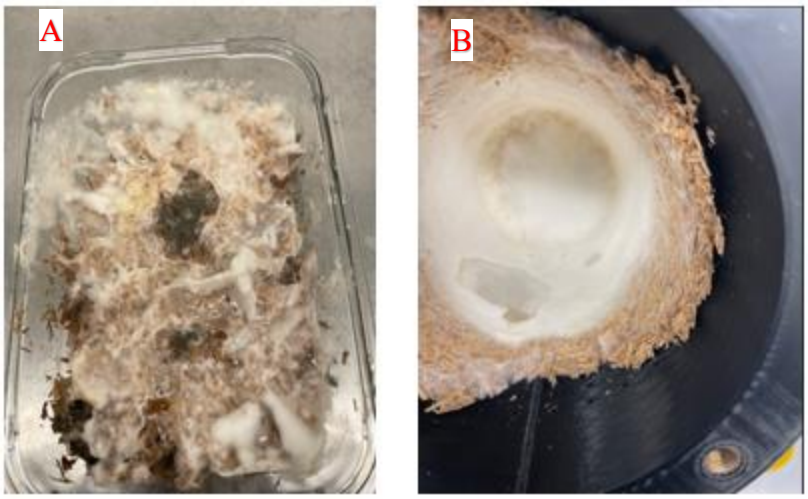


Figure 2.11 Contamination of mycelium material A) contamination with airborne fungus while incubation period B) contamination with unknown microorganism while molding the mycelium inoculated material in a cup mold.

Chapter 3: Characterization of Mycelium-Cattail Composite Materials

3.0 Preamble

This study not only involved the production of a compostable cup using fungal mycelium but also included an analysis of the characterization of the novel bio-composite. This analysis aimed to assess the feasibility of employing them as beverage packaging materials and to provide insights for future researchers to refine certain characteristics through material engineering. Various properties, including morphological, chemical, thermomechanical, hydrodynamic, and mechanical properties, were evaluated in the current study.

3.1 Characterization of Mycelium-Cattail Composite Materials:

The characterization of mycelium-cattail composite materials involves a multidimensional analysis aimed at understanding their physical, mechanical, and environmental properties. Through various techniques such as microscopy, spectroscopy, and mechanical testing, researchers assess the structural integrity, porosity, and thermal stability of these sustainable bio composites. Morphological studies reveal the interplay between mycelium and cattail fibers, elucidating their bonding mechanisms and overall architecture. Furthermore, chemical analysis provides insights into the material's composition, highlighting its potential applications in biodegradable packaging, insulation, and construction. This comprehensive characterization not only enhances our fundamental understanding of bio-based materials but also paves the way for their innovative use in environmentally friendly technologies.

3.2 Methods

3.2.1 Morphological Characterization

The morphological characteristics of the mycelium composite were meticulously examined utilizing a Scanning Electron Microscope (SEM) using a FEI Quanta 650 FEG (Manufacturer: Thermo Fisher Company, USA) located at the Materials Institute of Manitoba

(MIM) at the University of Manitoba, at an accelerating voltage 10.0 kV. This analysis aimed to scrutinize various parameters, including hyphal growth density, hyphal diameter, and cross-sectional characteristics, across samples harvested at 2-week and 4-week intervals. SEM enabled detailed analyses of the intricate structure of the mycelium composite, providing valuable insights into its microscale features and development over time. This detailed examination serves as a foundational step in understanding the structural properties of the composite, crucial for its application in beverage packaging materials.

Two different mycelium samples, based on their respective incubation periods of 2 weeks and 4 weeks, were analyzed in the current study. At the same time, raw cattails and pure mycelium images were also analysed to understand the influence of raw materials in the composite structure.

Initially, a small piece of each sample (5mm x 5mm) was cut and affixed to the base using double-sided carbon tape. The size of the sample was adjusted to fit the dimensions of the base. Subsequently, the samples underwent sputter coating with a gold/palladium (60/40 alloy) coating (20nm film) using a Desk II Cold Sputter Unit under the chamber pressure of 30 mTorr to render them conductive. Finally, the prepared samples were placed inside the Quanta FEG 650 chamber, as illustrated in Figure 3.1.

Images were taken in different magnitudes ranges from 500µm, 400 µm, 100µm, 50µm, 40µm, 20µm, 10µm to get clear images from the samples. 10 µm magnitudes images were used to determine the hyphal diameter. Cross sectional images of 2 weeks sample and 4 weeks sample were also taken and analysed further to see the impact of incubation period on composite structure. To determine the hyphal diameter, a total of four images were captured from random

regions of each sample, hyphal diameter of each sample was analysed from over 100 measurements randomly captured from different places.

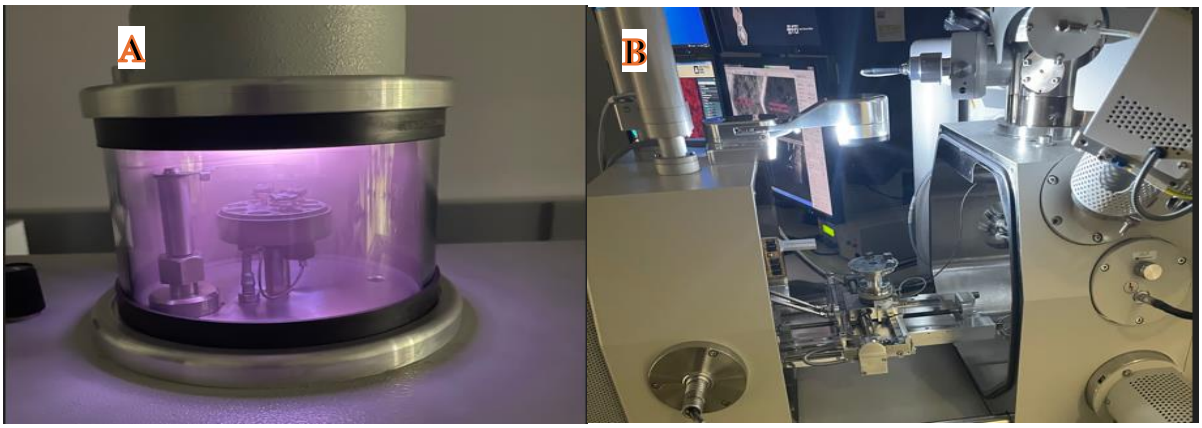


Figure 3.1 A) Desk II Cold Sputter coating chamber; B) Sample chamber inside the FEI Quanta FEG 650 Environmental SEM

3.2.2 Chemical Characterization

3.2.2.1 ANKOM Fibre Analyzer

To comprehensively understand and engineer the mechanical properties of a material, it is essential to analyze its chemical composition. The ANKOM 200 fiber analyzer (Manufacturer: ANKOM Technology, USA) located at department of Animal science, University of Manitoba was utilized to determine the cellulose, hemicellulose, and lignin content of the samples. These samples were prepared by grinding and sieving to maintain a particle size of 1 mm. 0.50gm samples were used in a ANKOM F57 bag to conduct the analysis. The composition percentages of cellulose, hemicellulose, and lignin were then determined using an ANKOM Delta Fiber Analyzer. Four samples with triple replication - raw cattails, pure mycelium, mycelium composite at 2 weeks, and mycelium composite at 4 weeks - were analyzed to assess the compositional changes resulting from mycelial growth. Figure 3.2 shows the ANKOM unit used for this study.

Figure: 3.2 ANKOM fibre analyser used for chemical composition analysis (cellulose, hemicellulose and lignin).

According to ANKOM 200 fiber analyzer protocol (ANKOM Technology, n.d.), the samples were prepared by grinding or chopping, and then the sample bags (F57) were sealed and weighed properly. The first step involved determining the Neutral Detergent Fiber (NDF) by preparing a 20 L NDF solution composed of Sodium lauryl sulfate (SDS), Ethylene glycol, and reverse osmosis (RO) water. The sample bags were subsequently placed in the digestion vessel and submerged in the NDF solution with agitation for 60 minutes. Following this, the bags were neutralized by immersion in an 100% acetone solution and air-dried at room temperature until all acetones had evaporated. Finally, the bags were dried in an oven at 100°C for 4 hours.

NDF% were determined by Eq1:

$$\%\text{NDF} = \frac{(Final\ weight - bag\ weight - bag\ correction*)}{Sample\ weight\ on\ dry\ mass\ basis} \times 100 \quad \ldots\ldots\ldots \text{Eq 1}$$

Where "Bag correction*" = Initial blank bag weight - the final blank bag weight

After NDF analysis, the study adhered to the protocol for determining Acid Detergent Fiber (ADF) using the filter bag technique. Initially, a 20 L solution of ADF was prepared by diluting 558 g of Sulfuric acid with RO water in a carboy until the volume reached 20 L. The sample bags were then placed in the digestion vessel and submerged in the ADF solution with agitation for 75 minutes. Subsequently, the bags were neutralized by immersion in an 100% acetone solution and then air-dried at room temperature until all acetones had evaporated. Finally, the bags were dried in an oven at 100°C for 4 hours.

The detailed formula for calculating the ADF percentage of a sample is represented by Eq2.

$$\%ADF= \frac{(Final\ weight - bag\ weight - bag\ correction*)}{Sample\ weight\ on\ dry\ mass\ basis} \times 100 \ldots\ldots\ldots \text{Eq 2}$$

Where, "Bag correction*" = Initial blank bag weight - the final blank bag weight

The final stage involved determining the Acid Detergent Lignin content (ADL) in the sample. Following the ADF determination, the ADL process was conducted in a beaker by submerging the sample bag in 72% Sulfuric acid for 3 hours. Subsequently, the bags were rinsed with tap water followed by an 100% acetone solution rinse until neutralized. Finally, the bags were dried in an oven at 105°C for 2-4 hours. Additionally, the sample bags underwent an ash procedure.

The ADL percentage was calculated using the Eq 3:

$$\text{ADL\% (Lignin content)} = \frac{(Weight\ loss\ after\ ashing - (bag\ weight * blank\ bag\ correction*)) \times 100}{sample\ weight\ on\ dry\ mass\ basis}$$

…Eq 3

Where "Bag correction*" = Initial blank bag weight - the final blank bag weight.

The hemicellulose, cellulose, and lignin content were determined by using Eq 4, Eq 5 & Eq 6 respectively:

Hemicellulose % = NDF% - ADF% ……Eq 4

Cellulose % = ADF% - ADL% ……Eq 5

Lignin % = ADL%" ……. Eq 6

3.2.2.2 Protein Analysis

The Leco Truspec-N (FP828) (Manufacturer: Leco corporation, USA) situated at Animal Science department, University of Manitoba, was utilized to quantify the protein content in both pure mycelium and mycelium composites at 2 weeks and 4 weeks. These experiments aimed to assess the variations in protein content resulting from fungal growth. The LECO FP828 is a combustion nitrogen/protein determinator that operates within a pure oxygen environment in a vertical quartz furnace. A thermoelectric cooler is employed to remove moisture from the combustion gases before they are collected in a ballast. Subsequently, the gases undergo equilibration and mixing within the ballast before a representative aliquot (3 cm or 10 cm volume) of the combustion gas is extracted and introduced into a flowing stream of inert gas (Helium or Argon) for analysis. The aliquot gas is then conveyed to a thermal conductivity cell (TC) for the detection of nitrogen (N). The AOAC 990.03 - Protein (Crude) in Animal Feed, Combustion Method, was employed to determine the protein content. One (1) gram of finely chopped or grinded material were used to determine the Protein analysis. Total 3 different samples were analysed along with triple replications: Pure mycelium, 2 weeks mycelium, 4 weeks mycelium composite samples. Raw cattail was excluded from determination of protein analysis.

3.2.3 Thermomechanical Characterisation

3.2.3.1 Thermogravimetric Analysis (TGA)

The thermal stability of mycelium-composite materials was investigated using a thermogravimetric analyzer, Manufacturer: TA Instrument, model: Q500, USA (Figure 3.3),

located at the Mechanical Department, University of Manitoba, MB, Canada. The configuration of this equipment includes a temperature range of ambient to 1000°C, with atmospheres of nitrogen and oxygen available. Sample size ranged from 08 to 30 mg.

This study aimed to determine the thermal decomposition behavior of the mycelium composites harvested at different growth stages. The Q500 model allowed for the assessment of thermal decomposition temperature and associated weight loss in the composite samples obtained after 2 weeks and 4 weeks of growth. Moreover, raw cattails and pure mycelium samples were analysed for determining thermal stability. Total 4 samples (raw cattails, pure mycelium, mycelium composite 2 weeks sample and mycelium composite 4 weeks sample) were analysed in this study.

Analytical procedures involved subjecting 08-15 mg of powdered sample placed in a Tzero aluminum pan to a temperature range of 25°C to 500°C, with a ramp rate of 5°C/min under a controlled nitrogen atmosphere to prevent oxidation. No isotherm was applied during the analysis. TA "Universal analyser" software was utilized to interpret the results in this study.

Following data acquisition, interpretation, and analysis were conducted using TA Instrument Explorer software, facilitating a comprehensive understanding of the thermal behavior of the mycelium material. The findings contribute valuable insights into the thermal stability of mycelium composite materials, which holds significance for various potential applications in industry and research.

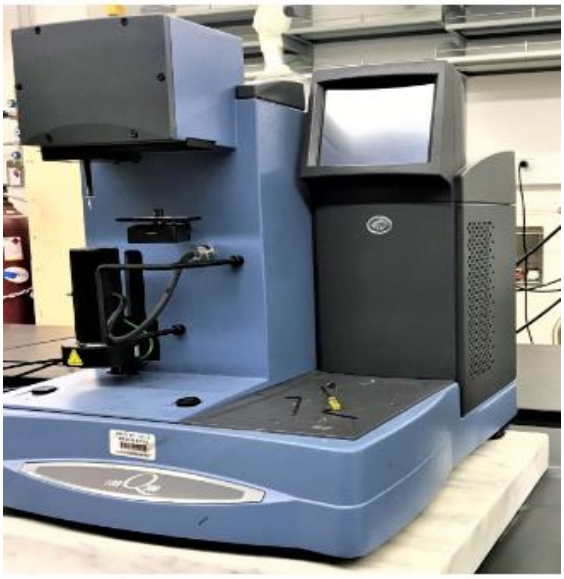


Figure: 3.3 Thermogravimetric analyzer Q500 used for decomposition temperature analysis.

3.3.2.2 Differential Scanning Calorimetry (DSC)

The critical temperature and thermal properties of mycelium composite materials were investigated using a Differential Scanning Calorimeter (DSC) Manufacturer: TA Instrument model: Q200, USA (Figure 3.4), located at Food and Human Nutritional Science department, University of Manitoba, MB, CA. The configuration of this equipment includes a temperature range of -90° to 725°C, with atmospheres of nitrogen available. Sample size ranged from 05 to 50 mg.

This analytical technique is invaluable for studying the thermal behavior of materials by measuring the heat flow associated with thermal transitions. The primary objectives of this investigation were to mycelium-composites, raw cattails, and pure mycelium. By subjecting approximately 10 mg of powdered sample contained in a Tzero aluminum pan to the specified temperature conditions, the DSC Q200 instrument facilitated the detection of thermal transitions within the materials. Figure 3.4 shows the equipment set used for this analysis.

Total four samples were analysed to determine the critical temperature. These samples are raw cattails, pure mycelium and mycelium composite samples for 2 weeks and 4 weeks.

Through the heating-cooling-reheating cycle, the glass transition and melting temperature of the mycelium composite were identified. However, despite thorough analysis, no crystallization point was observed within the tested temperature range. The detailed protocol for the DSC analysis included the application of heating, cooling, and reheating cycles, with temperature parameters ranging from -90°C to 300°C, a ramp rate of 5°C/min, and a 5-minute isotherm to ensure consistent temperature conditions during analysis. TA Universal analyser software was utilized to interpret the results in this study. Overall, this study provides valuable insights into the thermal properties of mycelium- composite materials, shedding light on their potential applications in various fields including biotechnology, materials science, and sustainable engineering.

Figure: 3.4 DSC Q200 equipment used for critical temperature analysis.

3.2.4 Crystallinity Analysis by X-ray Diffraction (XRD)

The crystallinity of mycelium-composites was evaluated through X-ray diffraction (XRD) analyses conducted using a diffractometer, Manufacturer: Siemens (Bruker) model: D5000, USA, located at Department of Geology, University of Manitoba (Figure 3.5).

The XRD analyses facilitated the identification and characterization of crystalline phases present in the mycelium-composites, offering crucial information regarding the material's structural properties. These findings contribute to the understanding of mycelium-based materials and their potential applications in various fields, including biotechnology, sustainable engineering, and material science.

Siemens (Bruker) D5000 diffractometer was equipped with a Cu X-ray tube operating at 40 kV and 35 mA, ensuring optimal conditions for diffraction measurements. A pre scan was done from 3-60° revealed diffraction inside 5-50°. As a result, the scan range was set from 5° to 50° 2θ, allowing for comprehensive exploration of diffraction patterns. A step interval of 0.02° and a dwell time of 1 second were employed to ensure precise data acquisition. OriginPro 2023 data analysis software was used in study to analysis XRD data. Crystallinity was measured using Origin pro software following Eq 7.

$$\text{Crystallinity Index} = \frac{Area\ of\ crystaline\ peaks}{Total\ area\ (crystaline+amorphous)} \quad \ldots\ldots \text{ Eq 7}$$

The area of crystalline peaks and total area of crystalline and amorphous were analysed and determined by using OriginPro 2023 software.

Total 4 types of samples were analysed to get X-Ray diffraction data. These are pure mycelium, raw cattails and mycelium composite sample in both 2 weeks and 4 weeks. Initially, grinded powdered sample was prepared to use for these experiments. Later, the samples were pulverized with mortar & pestle, but this wasn't very effective- so instead did some rapid chopping with razor blade on glass plate; some success in particle reduction. Sample was dry packed into a zero-background specimen holder.

This allowed for the comparison of crystalline structures between samples harvested at different growth durations, providing valuable insights into the influence of fungal growth periods on the material's crystallinity.

Figure 3.5 Siemens (Bruker) D5000 diffractometer used for XRD analysis.

3.2.5 Hydrodynamic characterization

3.2.5.1 Water Contact Angle

The hydrophobicity of mycelium composites was investigated through a water contact angle test conducted using an instrument from Ramé-Hart Inc., Model 200-00-115 (Figure 3.6) located at Manitoba Institute of Materials, University of Manitoba. This experimental technique allows for the measurement of the angle formed between a water droplet and the surface of the material, providing insights into its hydrophobic or hydrophilic properties.

Samples from both the 2-week and 4-week mycelium composites were carefully prepared and analyzed using the water contact angle test. This comparative approach enabled the

assessment of hydrophobicity variations between samples harvested at different growth durations. The study also attempted to measure the water contact angle for pure cattails. However, the approach was unsuccessful because the super hydrophilic nature of cattail plants made it unsuitable to measure the water contact angle with the Ramé-Hart Inc., Model 200-00-115. Moreover, conducting water contact angle analysis with the same equipment on pure mycelium was not possible due to the sample being in powder form rather than sheet form.

2 µl of volume of distilled water droplet was used to measure the water contact angle and each droplet was observed approximately 10 seconds to take the measurement of contact angle and images. Two types of samples (mycelium composite for 2 weeks and 4 weeks) with 6 replications were investigated for determination of water contact angle.

The water contact angle measurements provide valuable information regarding the interaction between the mycelium composite surface and water molecules. A higher contact angle indicates greater hydrophobicity, suggesting that the material repels water effectively. Conversely, a lower contact angle signifies higher hydrophilicity, indicating that the material has a greater affinity for water.

By conducting water contact angle tests on mycelium composites aged for different durations, this study aimed to elucidate the influence of fungal growth periods on the hydrophobic properties of the material. The findings contribute to a better understanding of the surface characteristics of mycelium-based materials, which is crucial for their applications in fields such as biotechnology, environmental engineering, and sustainable construction.

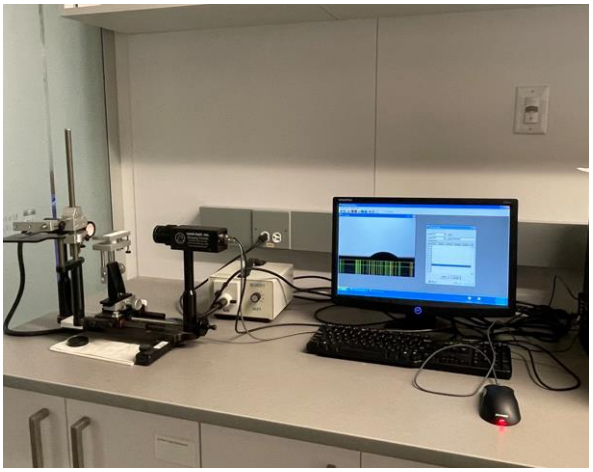


Figure 3.6 Ramé-Hart Inc., Model 200-00-115 used for water contact angle measurement.

A valuable method for understanding these interactions between a substrate surface and water through measuring the contact angle. Imagine a drop of liquid on a flat surface. The angle where the liquid meets the air, and the solid is the contact angle. It's where all three phases - the liquid, the solid, and the air - meet, called the "three-phase contact line" (Figure 3.7. This angle's size depends on the balance of forces between the solid, liquid, and air. Ideally, the contact angle should stay the same for a surface under specific conditions. This idea was first developed by Thomas Young and helps us understand how these three phases reach equilibrium (Hebbar et al., 2017).

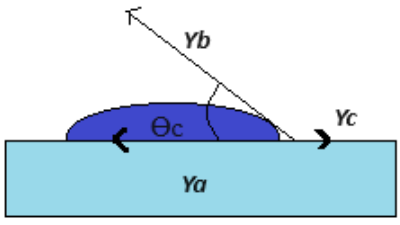


Figure 3.7 Liquid spread formed contact angle over a surface (modified from Hebbar et al., 2017)

The calculation is as below:

$\cos\Theta = \frac{Yc-Ya}{Yb} \ldots\ldots\ldots Eq\ 8,$

Where, Θ represents the contact angle, Yb, Yc, and Ya symbolize the liquid-vapor, solid-vapor, and solid-liquid interfacial tensions, respectively (Hebbar et al., 2017).

In the realm of surface science, specific thresholds are often used to categorize materials based on their hydrophobic or hydrophilic properties (Table 3.1). When the contact angle exceeds 150°, the surface is typically classified as superhydrophobic. Contact angles over 90° are indicative of hydrophobic surfaces, while angles below 90° suggest hydrophilic behavior. Finally, contact angles below 10° are associated with super hydrophilic surfaces (IGL Coatings, n.d.)

Table 3.1 Rating of hydrophobicity based on water contact angle (modified from IGL Coatings, n.d.)

Water contact angle	Rating of hydrophobicity	Wetting properties
> 150˚	Super hydrophobic	Non- wetting
> 90˚	Hydrophobic	Poor wetting
< 90˚	Hydrophilic	High wetting
< 10˚	Super hydrophilic	Super wetting

3.2.6 Mechanical Characterisation

3.2.6.1 Tensile Strength Analysis

The tensile strength of the samples was evaluated using an Instron tensile tester (Model: 5965, Sl: VS02075661, Noorwood, USA) located at Textile lab, University of Manitoba (Figure 3.8), a widely recognized instrument for material testing, following the standardized procedures outlined in the TAPPI T 494 standard (TAPPI/ANSI T 494, 2022). Total 4 different samples were tested to determine the tensile strength. They are mycelium composite sample for 2 weeks, mycelium composite sample for 4 weeks, Fools and Horses (F&H) coffee cups paper (both

coated and uncoated). The uncoated sample was prepared by removing the laminated sheet with hand and needle. Sample size was kept 9 in * 1 in, so that 7 in sample could be clamped between the jaws as per TAPPI T 494 standard (TAPPI/ANSI T 494, 2022). Total 6 samples were examined for mycelium composite samples (2 weeks and 4 weeks sample). 3 samples were examined for F&H coffee cup samples (coated and uncoated).

This standard ensures consistent and accurate assessment of tensile properties in various materials. According to TAPPI T 402 standard (TAPPI/ANSI T 402, 2021) both the mycelium composite sample and the coffee cup sample were conditioned for 24 hours at 50.0% ± 2.0% RH and 23.0 ± 1.0°C. All samples were tested under dry conditions. Tensile testing was done at a crosshead speed of 25 mm/min, using a 5-kN load cell. Sample width and thickness in millimetre (mm) were inserted to obtain the tensile strength in MPa.

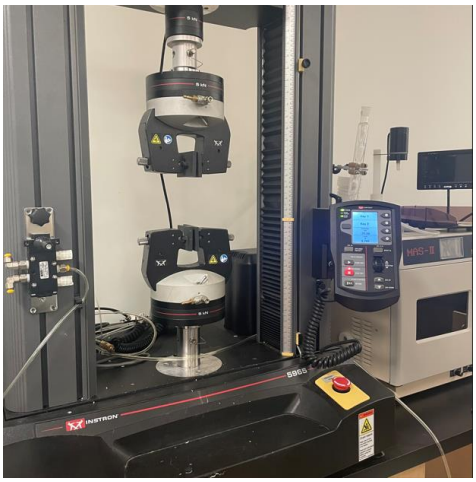


Figure: 3.8 Instron instrument used in measuring tensile strength.

To provide a comparative perspective, a paper cup sample sourced from Fools and Horses was included in the analysis. This allowed for an assessment of the tensile strength of the mycelium composites in relation to a commercially available paper cup. By conducting these tests, we aimed to gain insights into the mechanical properties of the mycelium-based materials and their potential suitability for applications such as packaging and disposable products.

3.2.6.2 Compression Strength

Compression strength assessments were conducted using the Instron tensile tester (Model: 5965, Sl: VS02075661, Noorwood, USA) located at Textile lab, University of Manitoba, adhering to the standardized protocol outlined in the ASTM D6797-24 standard (ASTM International, 2024). This method ensures consistent and accurate measurement of compression strength across various materials. Similar to the tensile tests, samples collected at both the 2-week and 4-week intervals were subjected to scrutiny to evaluate their compression strength characteristics. According to TAPPI T 402 standard (TAPPI/ANSI T 402, 2021), all samples were conditioned for 24 hours at 50.0% ± 2.0% RH and 23.0 ± 1.0°C. The compression strength were conducted with a 5-kN load cell and speed of 305mm/min. Sample width and thickness were inserted in mm in the software.

To provide a benchmark for comparison, a paper cup sample sourced from Fools and Horses was included in the analysis and tested under the same standard conditions. This allowed for a comparative assessment of the compression strength of the mycelium samples against a commercially available paper cup. A total of 7 replicates were conducted for 4 weeks mycelium composite while 2 replication was conducted for 2 weeks mycelium sample. It is north worthy to mention that the author tried to do replicates of the 2 weeks mycelium- composite sample several times, but due to contamination the samples were discarded. For F&H coffee cups (both PLA coated and uncoated) total 3 replication was conducted for determining the tensile strength. All sample size was maintained 2.5 in* 2.5 in as per ASTM D6797-24 standard. (ASTM International, 2024).

By conducting these assessments, we aimed to gain insights into the ability of mycelium-based materials to withstand compression forces, which is crucial for applications such as

packaging and container manufacturing. The Instron set up used for this study is showing in Figure 3.9.

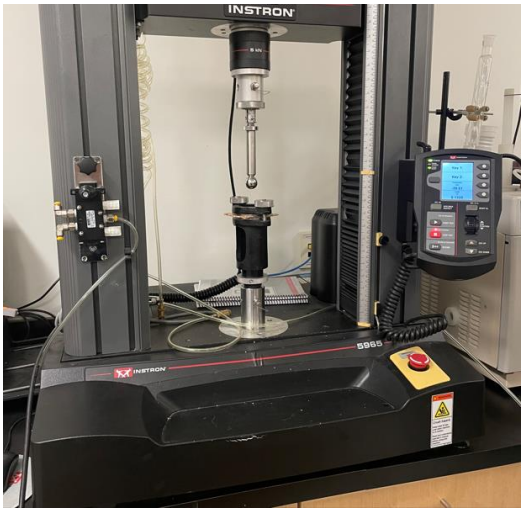


Figure 3.9 Instron instrument used for measuring compression strength.

3.2.7 Thermal Conductivity Analysis

Both the 2-week and 4-week mycelium samples were analyzed to determine their thermal conductivity. Thermal conductivity analyses were conducted using a Fox 314 Heat Flow Meter (Brand: Ta Instrument, USA) and Fox TA analysis software in accordance with ASTM C518-21 standard (ASTM International, 2021). A 12-inch by 12-inch sample was positioned between two plates within the test stack, and a temperature gradient was established across the material's thickness. The plates could be adjusted to a user-defined thickness or using the auto thickness feature, where the instrument automatically adjusted to establish contact with the sample. The *in-situ* sample thickness was measured using four optical encoders, one at each corner of the plate, providing stable measurements to within 0.025 mm.

Mycelium composite samples from both 2 weeks and 4 weeks were analyzed to determine the thermal conductivity of the material. Two samples of each type were analyzed in the current study. However, the study was unable to conduct the thermal conductivity test on the

F&H coffee cup due to the inability to achieve the required sample size (12 inches by 12 inches) with the largest cup available from F&H.

During testing, the default parameters were utilized, with the upper plate temperature set at 10°C and the lower plate temperature at 35°C, resulting in a temperature difference of 25°C between the two plates. Upon completion of the test, the results were automatically calculated using the TA analysis software according to Eq 8:

Thermal conductivity $\gamma = \frac{Calibration\ factor \times heat\ flow\ of\ transducer \times thickness\ of\ the\ sample}{temperature\ difference\ between\ 2\ plates}$

$Wm^{-1}K^{-1}$------Eq8

3.3 Statistical analysis:

Outlier from the database was removed with Interquartile (IQR) method in excel. The IQR method used here following the 1.5 scale as per Gaussian distribution. The IQR method follows the process as below:

Finding the first quartile and third quartile using excel function. Finding Interquartile range by Eq 9 and finally set the upper and lower boundary with the Eq 10 and 11 respectively.

Interquartile range (IQR) = Third quartile – First quartile -----Eq 9

Upper bound= Third quartile + (1.5*IQR) ------ Eq 10

Lower bound = First quartile- (1.5*IQR) ------Eq 11

Analysis of Variance (ANOVA) through Excel was done for applicable experiments to verify the significancy of the variance. The variance was considered significant if the p-value was less than 0.05 and F value is bigger than the critical F value (F > F crit). In case of significant ANOVA result, for more that two variables, a post hoc analysis was conducted through IBM SPSS software with one-way Tukey test. The variance was considered significant if the significance value was less than 0.05.

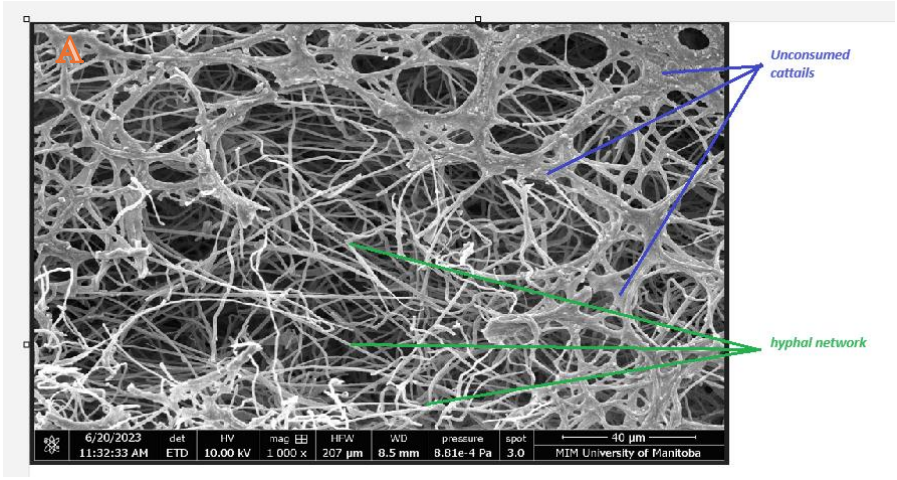
Chapter 4: Results and Discussion

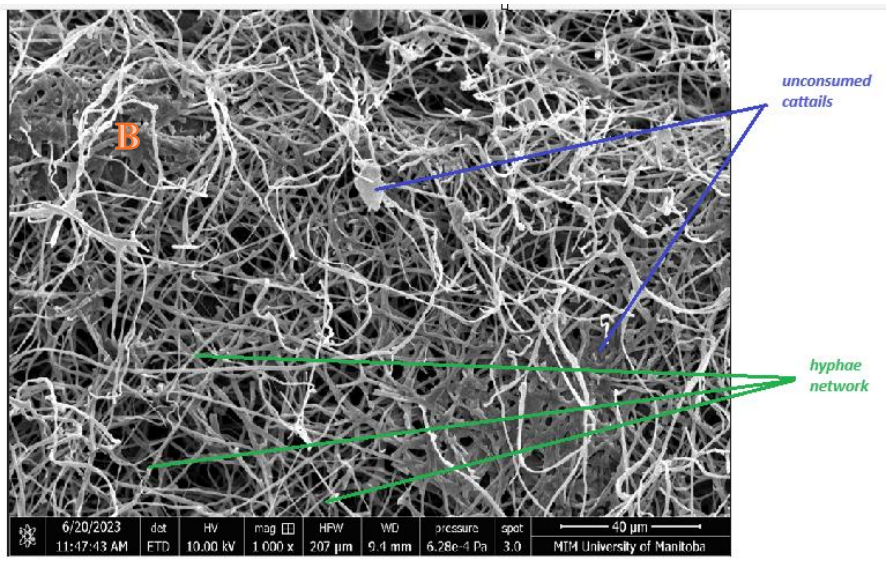
The results presented in this chapter encompass both ready-to-use (RTU) cups and mycelium sheets, as the materials, incubation period, and growing conditions were identical for both, except for the molds used for growth. Experiments such as SEM, DSC, TGA, XRD, and composition analysis required ground/powdered samples to represent both RTU cups and composite sheets.

However, for the determination of mechanical properties (tensile and compression) and thermal conductivity, it was not feasible to conduct tests on the 3D cups due to the large sample size requirements and machine limitations. Consequently, these experiments were performed on mycelium sheets, ensuring a thickness comparable to that of the mycelium 3D cups (5-6 mm)

4.1 Morphological Analysis

SEM analysis facilitated the interpretation of the mycelial structure within the cattail-mycelium-composite material, including the determination of hyphal diameter, which is an essential parameter for understanding biomaterial characteristics. Previous studies by Haneef et al. (2017) and Antonori et al. (2021) noted the random orientation of hyphal networks. Figure 4.1 illustrates predominantly tube-like hyphae in both the 2-week and 4-week samples. Analysis of Figure 4.1 reveals a notably lower hyphal network in the 2-week sample (Figure 4.1A) compared with the 4-week sample (Figure 4.1B). Additionally, a higher presence of unconsumed cattail material is visible in the 2-week sample. The hyphae network of pure mycelium sample is shown in Figure 4.1C, where both hyphal network and aggregated cell wall is visible.


A
Unconsumed cattails
hyphal network
6/20/2023 11:32:33 AM
det ETD
HV 10.00 kV
mag 1 000 x
HFW 207 µm
WD 8.5 mm
pressure 8.81e-4 Pa
spot 3.0
40 µm
MIM University of Manitoba


B
unconsumed cattails
hyphae network
6/20/2023 11:47:43 AM
det ETD
HV 10.00 kV
mag 1 000 x
HFW 207 µm
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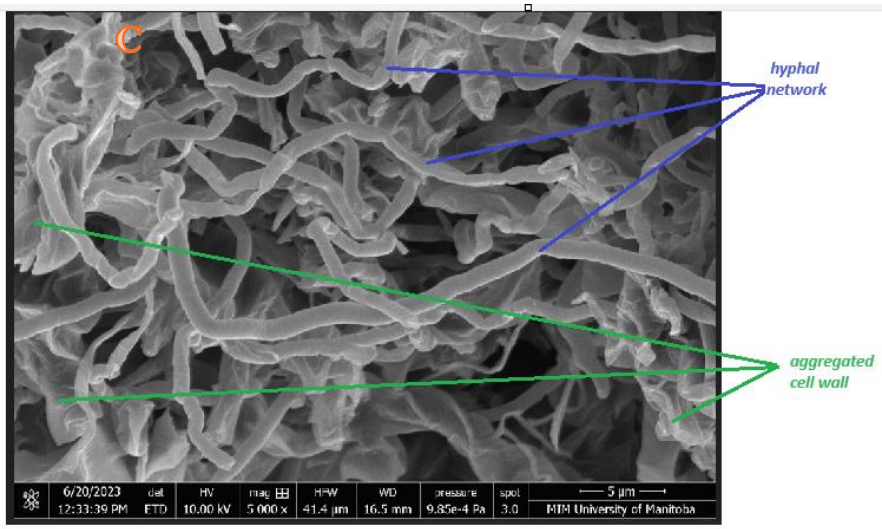
pressure 6.28e-4 Pa
spot 3.0
40 µm
MIM University of Manitoba


C
hyphal network
aggregated cell wall
6/20/2023 12:33:39 PM
det ETD
HV 10.00 kV
mag 5 000 x
HFW 41.4 µm
WD 16.5 mm
pressure 9.85e-4 Pa
spot 3.0
5 µm
MIM University of Manitoba

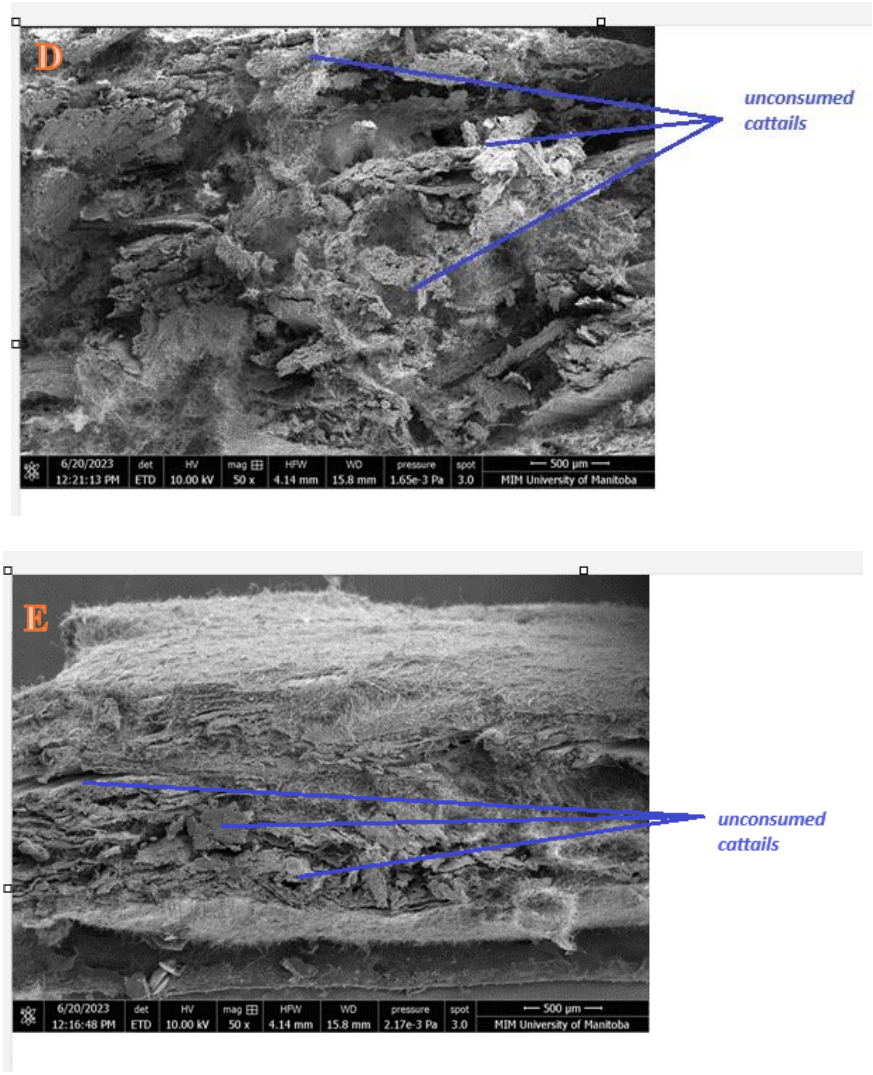


Figure 4.1 Morphological features of the mycelium composite A) 2-week surface view; B) 4-week surface view; C) Pure mycelium D) 2-week cross-sectional view; E) 4-week cross-sectional view.

The cross-sectional images confirm that the 2-week sample (Figure 4.1 D) is less dense that the 4-week sample (Figure 4.1E), which has fewer remnants of unconsumed cattail biomass. This structural inconsistency underscores the binding efficacy of mycelium in the fabrication of self-assembled bio composites. The cross-section images indicates that after 4 weeks incubation period is favourable for forming a densely composite material.

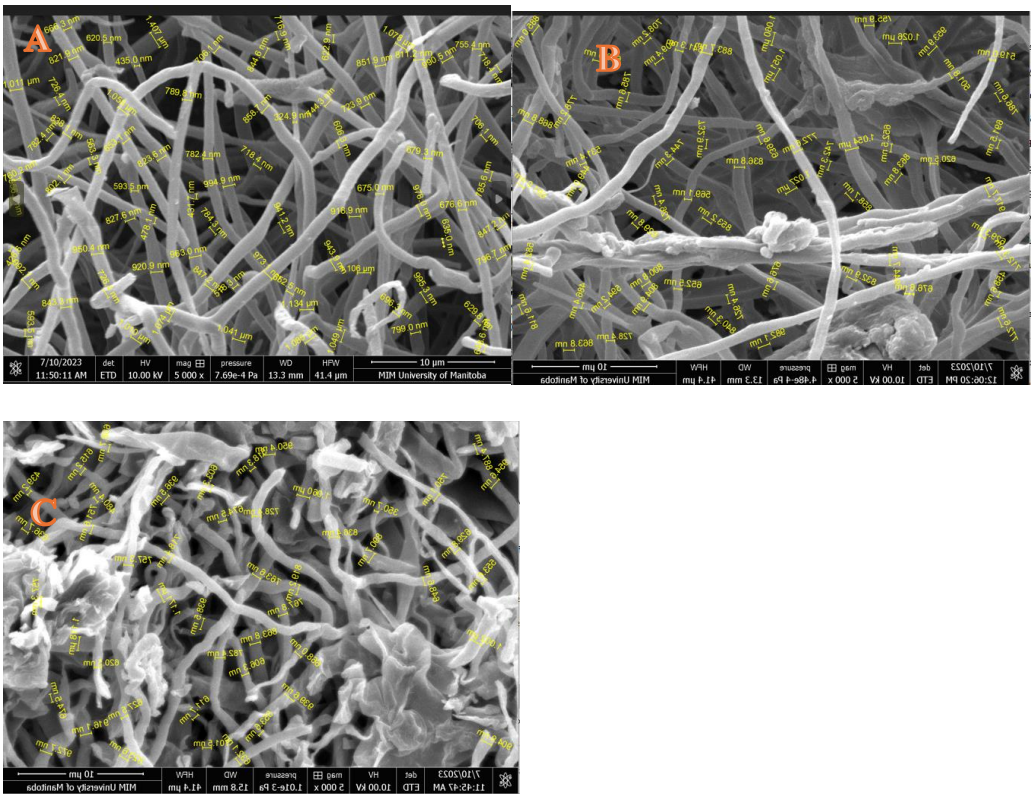


Figure 4.2 A) 2 weeks aged mycelium composite sample indicating hyphae diameter B) 4 weeks aged mycelium composite sample indicating hyphae diameter C) Pure mycelium indicating hyphal diameter

The current study also examined the hyphae diameter of 2 weeks, 4 weeks and pure mycelium samples. The outlier had been removed and raw data were presented in the Appendix Table A3-A5. The study observed that the hyphal diameter of the mycelium composite remained consistent for both the 2-week and 4-week samples, measuring between 0.77 ± 0.19 µm and 0.85 ±0.28 µm (Figure 4.3). Figure 4.2 A, B & C shows the hyphal diameter of 2 weeks, 4 weeks mycelium composite and pure mycelium sample respectively. In contrast, pure mycelium cultivated in YEPDB media exhibited a greater hyphal diameter of 1.00 ± 0.20 µm (Figure 4.3). Haneef et al. (2017) reported similar hyphal diameters (0.8 µm) for *G. lucidum* cultivated on

both cellulose and PDB-cellulose medium. In another study, Antonori et al. (2021) documented a hyphal diameter of 0.7 ± 0.2 µm for *G. lucidum* grown on PDB media.

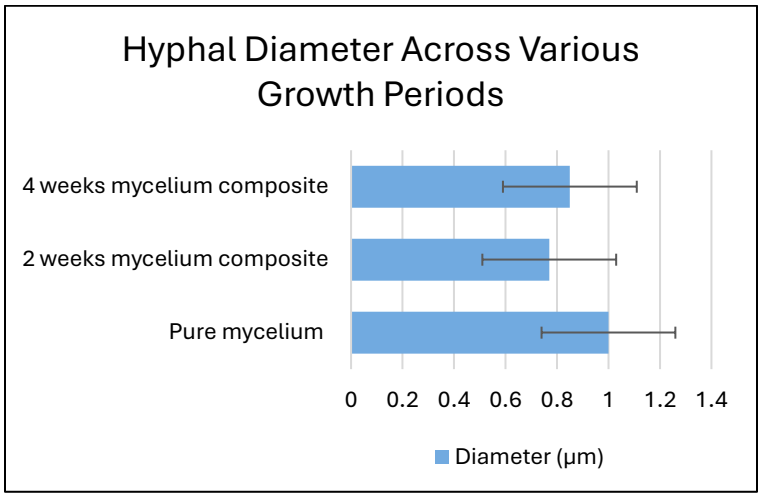


Figure 4.3 Analyses of hyphal diameter from 2-week and 4-week samples compared with mycelium from fungi cultured with YEPDB medium.

Analysis of variance (ANOVA) for both 2 weeks, 4 weeks mycelium composite and pure mycelium sample are showing in Table 4.1 There were statistically significant differences between the groups means by One-way ANOVA ($F(2,289) = 26.32$, $p = 3.1E\text{-}11$)), where critical F value is 3.02 ($P<0.05$, $F>F$ crit).

Table 4.1 ANOVA analysis for hyphae diameter for 2 weeks vs 4 weeks sample vs pure mycelium.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.778987	2	1.389493	26.32216	3.15E-11	3.027001
Within Groups	15.25572	289	0.052788			
Total	18.03471	291				

As a result, a post hoc (Tukey test) were conducted and the results are represented in the Table 4.2. Tukey test analysis tables were added in the Appendix (Table A6). From Table 4.2, it is evident that the hyphae diameters are not statistically significant for the 2-week and 4-week samples. However, they are statistically significant when comparing the diameter of pure mycelium to the 2-week and 4-week samples.

Table 4.2 Significant value from Tukey-test analysis for 2 weeks, 4 weeks mycelium composite and pure mycelium sample.

Sample Group	Sample Group	Sig	comments
2 weeks sample	4 weeks sample	0.133	Statistically not significant
	pure mycelium	<0.001	statistically significant
4 weeks sample	2 weeks sample	0.133	Statistically not significant
	pure mycelium	<0.001	statistically significant
pure mycelium	2 weeks sample	<0.001	statistically significant
	4 weeks sample	<0.001	statistically significant

4.2 Chemical Analyses

Ganoderma lucidum, along with certain other white rot fungi, has the capability to degrade lignin in lignocellulosic materials without significantly affecting the holocellulose content, which comprises cellulose and hemicellulose (Sydor et al., 2022; Haneef et al., 2017). Lignin serves as a fundamental structural component in lignocellulosic materials; however, it can also contribute to the degradation of mechanical properties (Liu et al., 2020). Therefore, optimizing lignin removal is essential to preserve the desired mechanical attributes.

Total four types of samples: raw cattails, pure mycelium, mycelium composite harvested over 2 weeks and 4 weeks, were to assess compositional changes in the cattail-mycelium composite over 2-week and 4-week periods. This investigation encompassed the evaluation of hemicellulose, cellulose, lignin, and protein content as illustrated in Table 4.3. The details data is added in Appendix (Table A10).

Results indicated a noticeable reduction in lignin content after 2 weeks (9.25%) of fungal growth, with a further decrease observed after 4 weeks (4.85%), (Figure 4.4) raw cattails were excluded from the protein analysis due to the absence of inherent protein content in their structure (Wu et al., 2021). Additionally, no replication was conducted for the composition analysis of pure mycelium due to the lengthy sample preparation process and its associated low yield. Table 4.3 showed the data on lignin content in cattail, 2-weeks sample and 4-weeks sample.

Table 4.3 Lignin% for cattail and mycelium composite samples.

Sample ID	Lignin (%)
Raw cattail	11.27
2-weeks sample	9.25
4-weeks	4.85

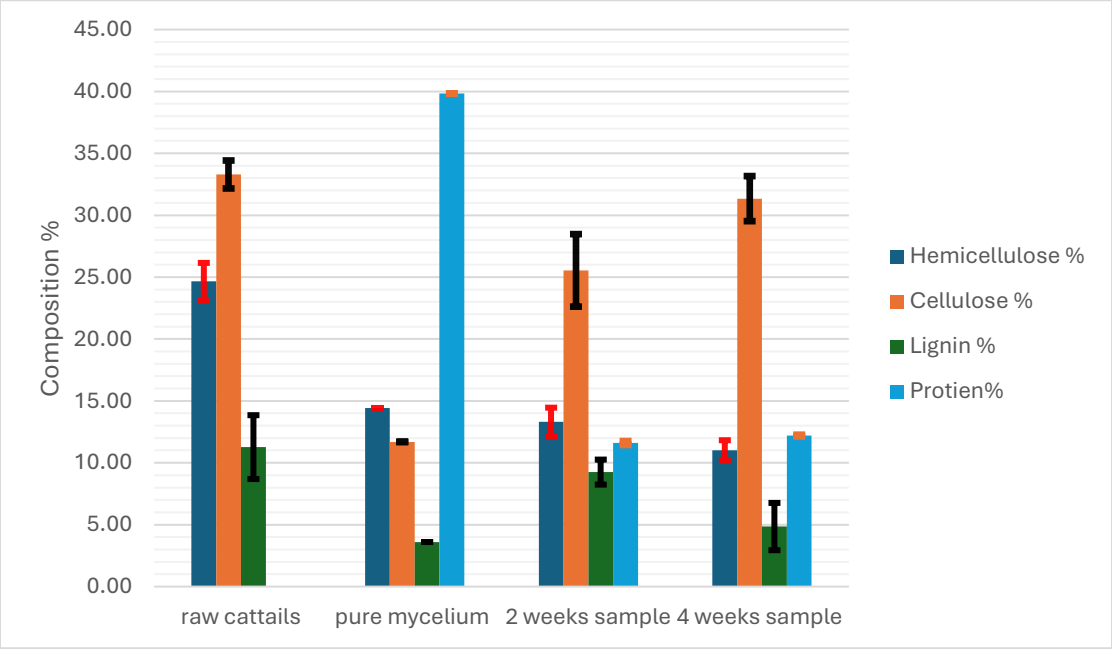


Figure 4.4 Percent composition of cellulose, hemicellulose, lignin, and protein in raw cattail, pure mycelium, and mycelium-composites.

4.3 Thermomechanical Characterisation

4.3.1 Thermal Stability Analysis of Mycelium Composite

The assessment of thermal stability is paramount when contemplating the utilization of bio composites in applications such as hot beverage cups. Through the utilization of a thermogravimetric analyzer, this study aimed to evaluate the thermal decomposition temperature and weight loss characteristics of the materials under consideration. Specifically, the investigation focused on two mycelium-composite samples inoculated for durations of 2 weeks and 4 weeks. The objective was to discern the influence of fungal growth periods on the thermal stability of the materials.

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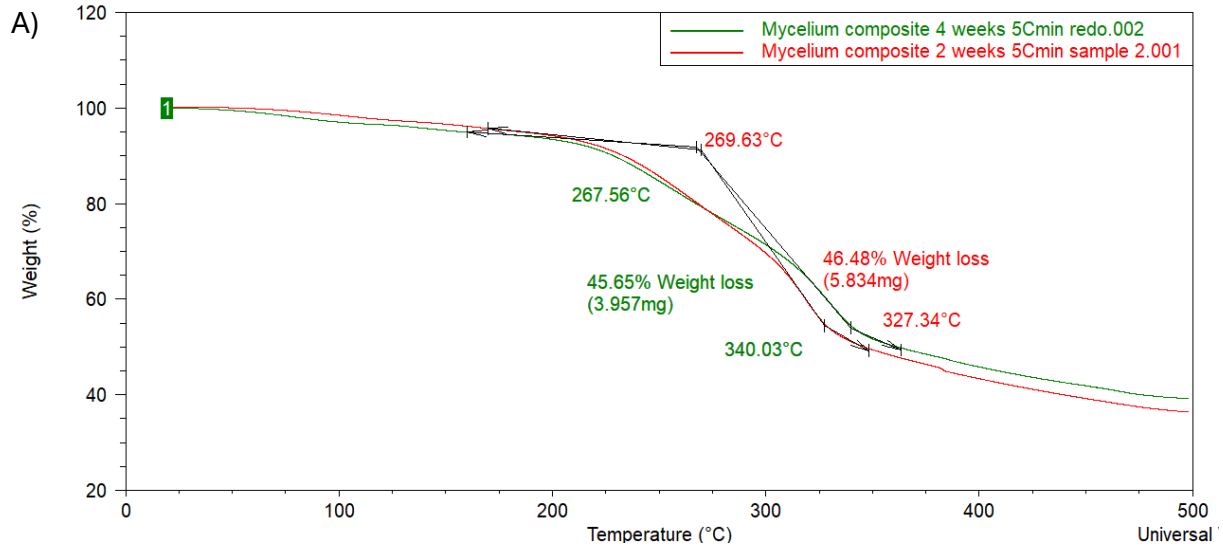


B)

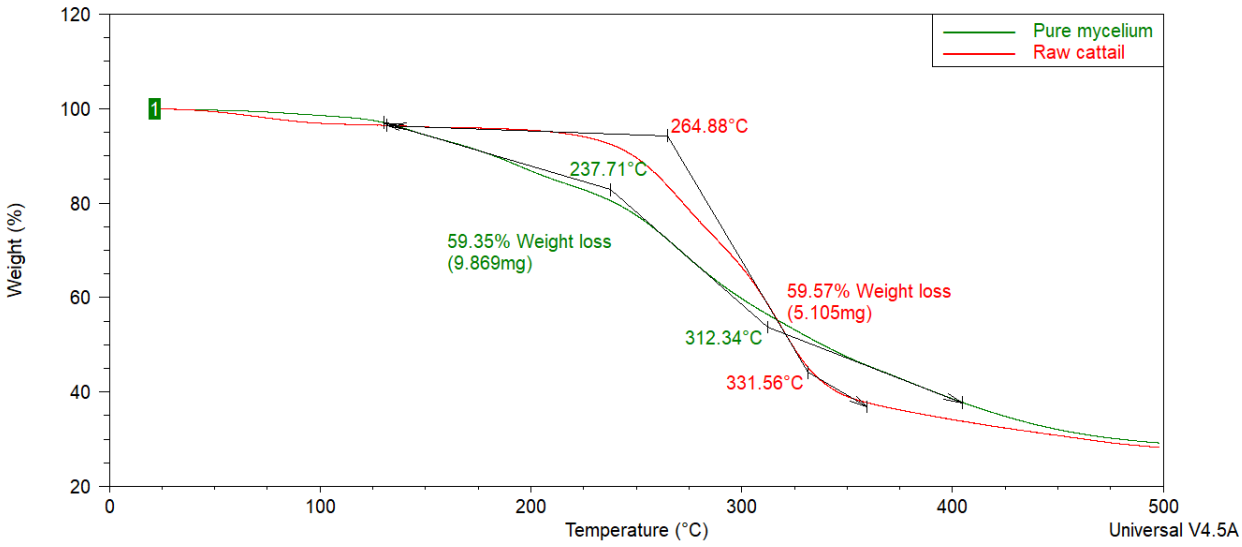


Figure 4.5 A) Thermogravimetric analyses of 2 weeks and 4 weeks mycelium composite sample. B) Thermogravimetric analyses of raw cattails and pure mycelium

In Figure 4.5 A presents the degradation curves, revealing two distinct stages of degradation. Initially, a minor decomposition occurred at temperatures below 100°C, primarily attributed to moisture evaporation from the samples, resulting in a weight loss ranging between 2-5%. Subsequently, a more pronounced degradation phase occurred, indicating material degradation or combustion reactions. Similar initial degradation patterns have been observed in previous studies by Escaleria et al. (2021) and Sakir et al. (2020).

Upon analysis, it was observed that for 2 weeks aged mycelium composite sample a larger second stage mass loss happened between 269.63°C to 327.34°C with a weight loss of 46.48% (5.83mg) while the original sample weight was 11.5mg. This major degradation probably indicated the decomposition of most of the organic constituents (example: amino acid, chitin, polysaccharide). Similarly, 4 weeks aged mycelium composite sample a larger second

stage mass loss happened between 267.56°C to 340.03°C with a weight loss of 45.65% (3.97mg) while the original sample weight was 9 mg (Figure 4.5A)

Figure, 4.5 B is showing the thermal degradation of pure mycelium sample and raw cattails sample. From the graph, it is obvious that for raw cattails the most degradation happened between 264.88°C to 331.56°C with a weight loss of 59.57% (5.11mg), whereas original sample weight was 9 mg. However, for pure mycelium the degradation temperature is slightly lower than the raw cattails. The major degradation happened from 237.71°C to 312.34°C with a weight loss of 59.35% (10 mg), where the original sample weight was 16 mg

ANOVA was performed to assess the significance of the thermal decomposition start temperature during the second major mass loss of mycelium composites, comparing the 2-week and 4-week samples (Table 4.4).There were not statistically significant differences between the groups means by One-way ANOVA ($F(1,2) = 0.63$, $p = 0.51$)), where critical F value is 18.54 ($P>0.05$, $F<F\ crit$). The elevated degradation temperature suggests that the mycelium-composite material possesses thermal stability, rendering it suitable for use in production and applications requiring resistance to high temperatures. The raw data had been added in Appendix Table A7.

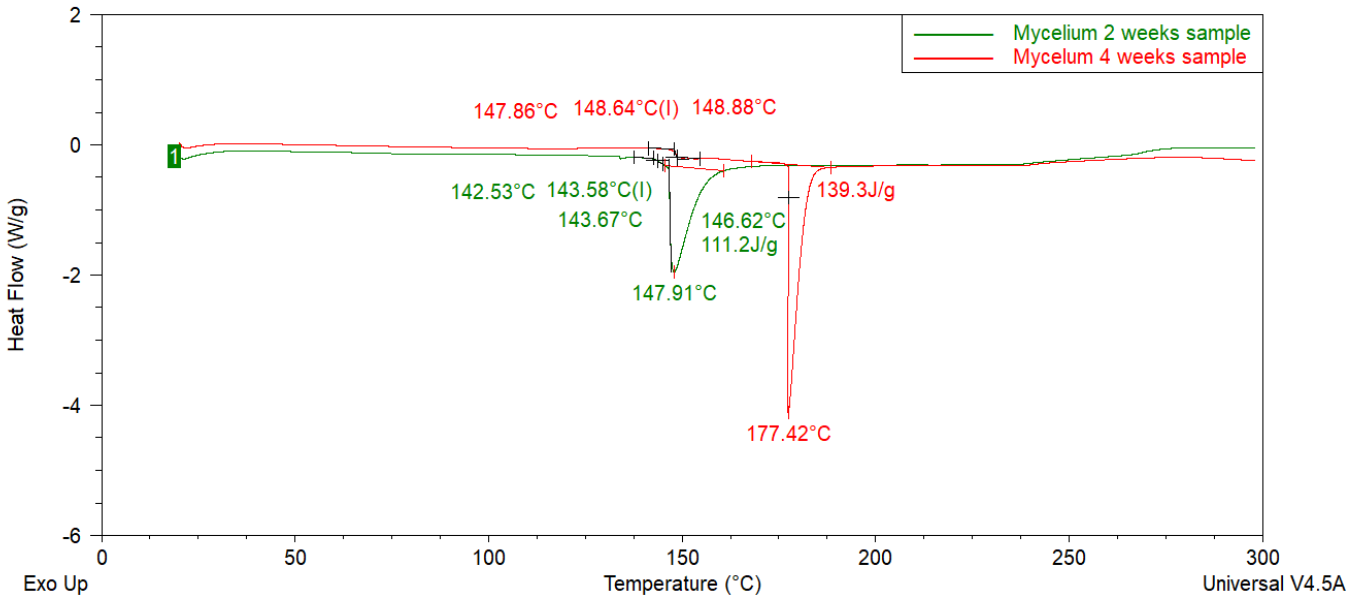
Table 4.4 ANOVA analysis starting of 2nd stage degradation temperature for 2 weeks and 4 weeks mycelium composite samples.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14.0625	1	14.0625	0.631096	0.510244	18.51282
Within Groups	44.5653	2	22.28265			
Total	58.6278	3				

4.3.2 Critical Temperature Analysis

Thermal transitions in the composite samples were analyzed using a differential scanning calorimeter (DSC) as depicted in Figure 4.6. The DSC revealed variations in the fungal mycelium across various inoculation days. While the mycelia may share similar chemical compositions, their quantities differ, influencing the density of the mycelium. The increased density of the mycelium correlates with the observed thermal transitions at elevated temperature ranges, as noted by Sakir et al., 2020.

The data from the analysis reveals consistent transition points in both the 2-week and 4-week samples. Specifically, the glass transition temperature for the 2-week sample was observed at 143.58°C indicated with (I) with onset and end set temperature 142.53°C and 143.67°C respectively. The melting temperature of 2 weeks sample was recorded at 147.91°C with the enthalpy of 111.2 J/g. In comparison, the 4-week samples exhibited a glass transition temperature 148.64 °C indicated with (I) with onset and end set temperature 147.86 °C and 148.88°C respectively. The melting point of 4 weeks sample registered at 177.49°C with the enthalpy 139.3 J/g (Figure 4.6 A).



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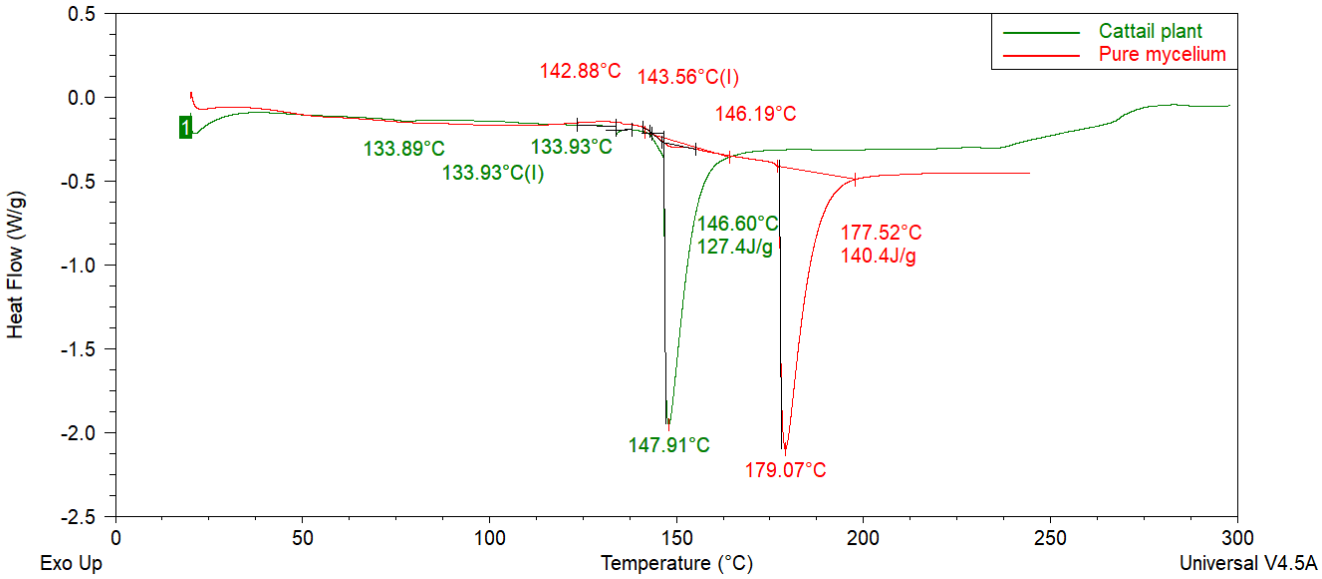


Figure 4.6 A) Glass transition temperature and melting temperature analysis of mycelium-composites harvested at 2 weeks and 4 weeks. B) Glass transition temperature and melting temperature analysis of pure mycelium and raw cattails.

ANOVA analysis of glass transition temperature for both 2 weeks and 4 weeks mycelium composite samples are listed in Table 4.5. There were statistically significant differences between the groups means by One-way ANOVA ($F(1,4) = 118.23$, $p = 0.000406$), where critical F value is 7.71 ($P<0.05$, $F>F$ crit). ANOVA analysis dataset is added in Appendix A8.

Table 4.5 ANOVA analysis of glass transition temperature for 2 weeks and 4 weeks mycelium composite samples.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	40.56	1	40.56	118.2335	0.000406	7.708647
Within Groups	1.3722	4	0.34305			
Total	41.9322	5				

From the figure, 4.6 B, it is evident that the glass transition temperature for the raw cattails was observed at 133.93°C indicated with (I) with onset and end set temperature 133.89°C and 133.93°C respectively. The melting temperature of raw cattails was recorded at 147.91°C with the enthalpy of 127.4 J/g. In comparison, pure mycelium exhibited a glass transition temperature 143.56 °C indicated with (I) with onset and end set temperature 142.88 °C and 146.19°C respectively. The melting point of pure mycelium sample registered at 179.07°C with the enthalpy 140.4 J/g. The elevated glass transition and melting temperatures observed in the composite suggest that this material possesses notable thermal stability. Such characteristics position it favorably for applications in biomaterials where enhanced thermal resilience is essential.

4.4 Crystallinity Analysis by X-ray Diffraction (XRD)

XRD analysis was conducted to assess the crystallinity of mycelium composites harvested at two distinct durations: 2 weeks and 4 weeks. A total of four samples were examined: pure mycelium, untreated cattails, and mycelium-cattail composites harvested at 2-weeks and 4-weeks. The crystallinity of these biomaterials was determined as shown in Figure 4.7. The XRD spectrum indicated crystallinities of 76% for the 2 weeks sample considering peaks at 2θ values of 5.30°, 14.26°, 14.91°. 20.04°, 20.04°, 21.61°, 22.69°, 24.37, 25.45°, 28.09°, 30.04°, 31.28°, 32.20°, 38.25°, 40.30°,42.68°,46.36°,47.98. For 4 weeks sample crystallinity found 73% considering peaks at 2θ values of 6°, 9.02°, 10.31°, 14.20°,14.75° 16.15°, 18.01°, 22.20°, 24.37°, 28.88°, 32.09°, 34.58°,38.25°,39.71°. The marginally higher crystallinity observed in the 2 weeks sample may be attributed to residual cattails, given that raw cattails inherently exhibit greater crystallinity than the other samples. Conversely, the spectrum for pure mycelium exhibited the least pronounced crystalline peaks. Similar crystallinity (71.89%) was found in the mycelium produced from *Penicillium camemberti* (Rathinamoorthy et al., 2023).

Crystallinity %:

2 weeks mycelium composite: 76%
4 weeks mycelium composite: 73%
Pure mycelium: 70%
Raw cattails: 80%

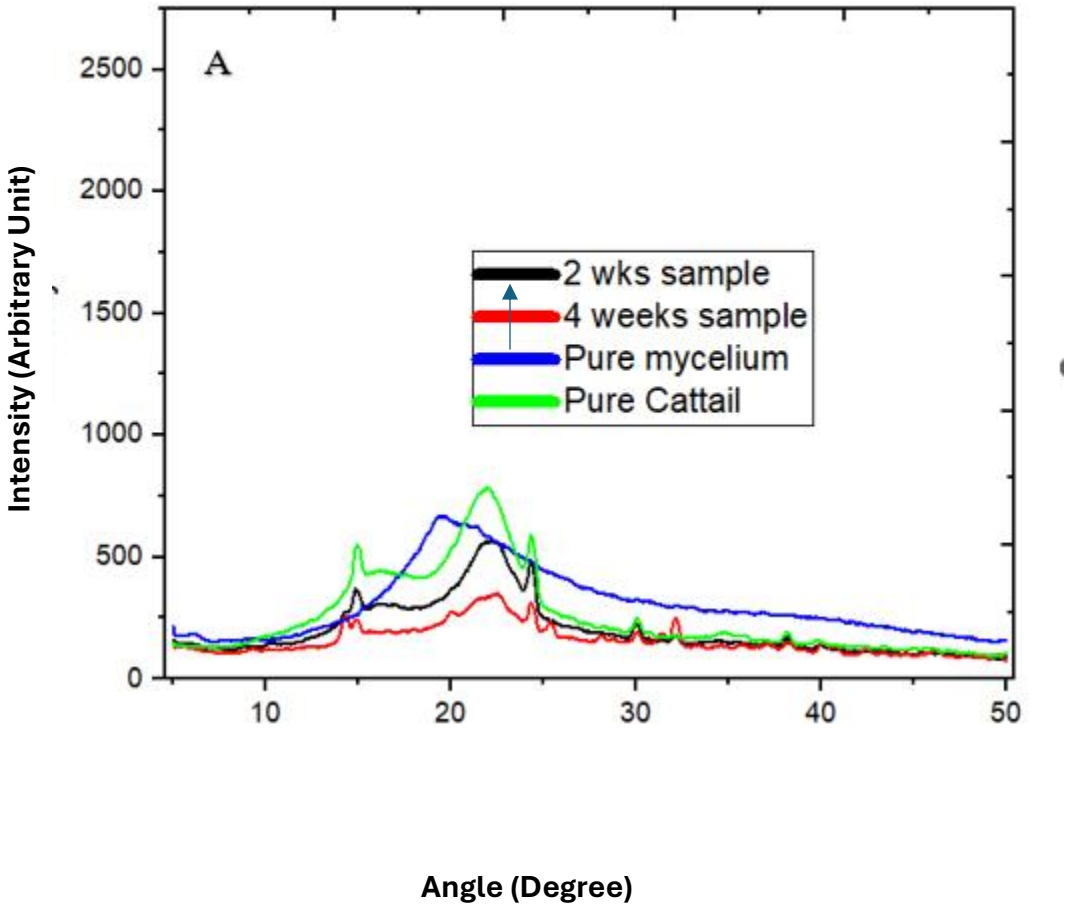


Figure 4.7 X-Ray Diffraction graphs of mycelium-composites, untreated cattail and pure mycelium.

The XRD spectrum indicated crystallinities of pure mycelium was found 70% considering peaks at 2θ values of 6.10°, 7.83°, 19.50°, 20.74°, 21.34°, 29.88°, while the crystallinity of raw cattails was found 80% considering peaks at 2θ values of 15.02°, 16.26°, 18.10°, 24.42°, 29.98°,34.74°, 35.77°, 38.14°, 39.76°, 43.49°, 45.92°.

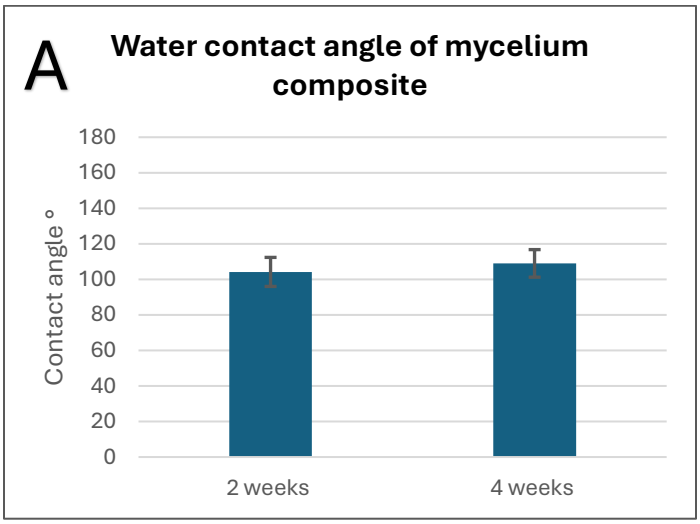
Previous research on chitin extracted from mycelium of *Penicillium camemberti* identified characteristic peaks at 2θ values of 5.48°, 9.13°, and 19.03°, indicative of α-chitin and its inherently rigid crystalline structure (Rathinamoorthy et al., 2023). In our investigation, peaks were observed at 2θ values of 14°, 22°, 24°, 30°, and 32°. Upon examining the distinct chitin types—α, β, and γ—Jang et al. (2004) delineated that α-chitin is characterized by peaks at 2θ values of 9.6°, 19.6°, 21.1°, and 23.7°, β-chitin by 2θ values of 9.1° and 20.3°, and γ-chitin by 2θ values of 9.6° and 19.8°. Consequently, our findings suggest that the chitin present in the developed mycelium sheet aligns with α-chitin, showcasing its rigid crystalline nature.

4.5 Water Contact Angle

The contact angle assessment with water was executed on the cattail-mycelium bio composite. The results revealed a hydrophobic characteristic in the biomaterial, with all samples exhibiting a contact angle with water exceeding 90°. The elevated water contact angles can be ascribed to the fibrous architecture of the mycelium, where the hyphae construct a microporous dense layer. Additionally, the hyphal cell walls incorporate proteins, including mannoproteins and hydrophobins, which possess hydrophobic attributes, thereby augmenting the observed high water contact angle (Yuan and Randall, 2013).

Figure 4.8 represents the water contact angle data and images of mycelium-composite harvested for 2 weeks and 4 weeks. The detailed data has been added in appendix (Table A8). The data indicated that both samples exhibited water contact angles exceeding 100°, with the 4-week sample registering a marginally higher angle than 2 weeks sample. This observation could be attributed to the extended culture duration, which resulted in denser mycelium growth and reduced residual hydrophilic lignocellulosic materials. The chemical composition analysis in the

present study showed degradation in lignin and an increase in protein content over the incubation period, from the 2-week sample to the 4-week sample.



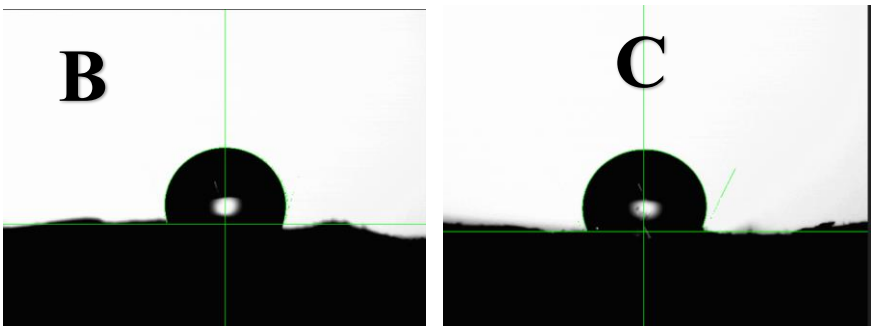


Figure 4.8 A) Graphical representation of water contact angle of mycelium composites. B) image of water drops on 2 weeks sample C) image of water drop on 4 weeks sample.

The study encountered difficulties in obtaining water contact angle data using the Ramé-Hart Inc., Model 200-00-115 equipment due to the highly hydrophilic nature of cattail plants. This characteristic caused rapid absorption of the water droplet, rendering accurate measurements unfeasible. Similarly, assessing the water contact angle on pure mycelium was impeded by the sample being in powder form instead of sheet form. Table 4.6 showed the

ANOVA analysis for both 2 weeks sample and 4 weeks sample. They were not statistically significant between the groups means by One-way ANOVA ($F(1,10) = 0.51$, $p = 0.49$)), where critical F value is 4.96 ($P>0.05$, $F<F$ crit). Dataset is added in Appendix A9.

Table 4.6 ANOVA analysis of 2 weeks and 4 weeks mycelium composite samples for water contact angle

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	75	1	75	0.509626	0.491626	4.964603
Within Groups	1471.667	10	147.1667			
Total	1546.667	11				

4.6 Tensile Strength

Tensile stress (MPa), tensile strain (mm), and Young's modulus (MPa) were determined, as detailed in Table 4.7. For reference, coffee cup paper from ‘Fools and Horses' was utilized in this study. Notably, the harvested mycelium composite was devoid of any finish and had not undergone either hot or cold pressing processes. In contrast, the coffee cup paper was finished on both sides and had clearly undergone a compressing process.

Mycelium, when dry, exhibits brittleness, a trait that necessitates its incorporation into composites for applications in construction, thermal/acoustic insulation, and design manufacturing (Antinori et al., 2021). Current study observed that under dry conditions, the biomaterial becomes brittle and is not conducive to bending due to its inherent properties. In contrast, the mycelium composite sheet demonstrates greater flexibility and ductility when subjected to wet conditions. Literature suggests that the existence of voids and defects within composites can compromise their mechanical and thermal properties (Sakir et at., 2020).

Table 4.7 Mechanical properties of mycelium composite materials and Fools and horses' coffee cup paper (with and without coating).

Sample name	Sample ID	Tensile strain at max tensile stress (mm/mm)	Tensile stress (MPa)	Young's modulus (MPa)
Mycelium composite sample 2 weeks	S1	0.0052	0.0223	12.47
	S2	0.0076	0.0802	13.70
	S3	0.0107	0.0970	13.07
	S4	0.0096	0.0700	7.98
	S5	0.0109	0.0692	9.11
	Average	0.0088	0.07	11.27
	SD*	0.0020	0.02	2.28
Mycelium composite sample 4 weeks	S1	0.0075	0.2802	59.41
	S2	0.0049	0.2439	63.29
	S3	0.0068	0.4249	94.62
	S4	0.0057	0.3305	79.91
	S5	0.0068	0.5038	107.34
	Average	0.0064	0.36	80.91
	SD*	0.0008	0.10	18.22
Standard (FH paper cup without coating)	S1	0.0279	14.2499	1188.50
	S2	0.0265	14.4917	1059.15
	S3	0.0340	17.5428	1249.29
	Average	0.0295	15.43	1165.65
	SD*	0.0033	1.50	79.29
Standard (FH paper cup with PLA coating)	S1	0.0424	20.3641	1596.22
	S2	0.0408	19.2971	1156.39
	S3	0.0441	18.5753	1295.45
	Average	0.0424	58.24	1349.35
	SD*	0.0013	0.73	183.56

* SD= Standard deviation, FH= Fools and Horses

Analysis of Table 4.6 reveals a noticeable difference in the Young's modulus between the 4-week sample (80.91 MPa ± 18.22) and the 2-week sample (11.27 MPa ± 2.28), indicating superior mechanical properties in the former. However, both mycelium samples exhibit significantly lower Young's modulus values compared to the uncoated F&H coffee cup paper (1165.65 MPa ± 79.29). Moreover, the coated F&H paper demonstrates further improvement in Young's modulus (1349.35 MPa ± 183.56), highlighting the mechanical superiority of the coated paper over both mycelium samples.

4.7 Compression Strength

To assess the compressive strength of the bio composite, compression stress was determined for two distinct types of mycelium composite samples. For comparative purposes, 'Fools and Horses' coffee cup paper served as the benchmark. It is pertinent to note that the harvested mycelium composite was unaltered, lacking any finish, and had not been subjected to either hot or cold pressing. Conversely, the coffee cup paper had been treated with finishes on both surfaces and had undergone distinct compressive procedures.

Table 4.8 Compression stress and extension of mycelium composite materials and Fools and Horses coffee cup paper

Sample type	Sample ID	Compression stress (N)	Compression extension (mm)
Mycelium composite	2 weeks sample- S1	3.52	62.02
	2 weeks sample- S2	4.47	95.06
	Average	4.00	78.54
	SD	0.48	16.52
	4 weeks sample-S1	4.15	77.78
	4 weeks sample-S2	5.21	41.69
	4 weeks sample-S3	12.03	36.1
	4 weeks sample-S4	5.14	89.47
	4 weeks sample-S5	3.2	65.07
	4 weeks sample-S6	6.32	48.3
	4 weeks sample-S7	4.12	63.55
	Average	5.74	60.28
	SD	2.73	17.99
Uncoated FH paper	Sample 1	75.87	74.45
	Sample 2	70.98	46.67
	Sample 3	86.48	53.54
	Average	77.78	58.22
	SD	6.47	11.82
PLA Coated FH paper	Sample 1	125.88	59.33
	Sample 2	126.63	71.37
	Sample 3	114.98	52.30
	Average	122.49	61.00
	SD	5.32	7.87

*SD= Standard deviation, FH= Fools and Horses.

The data displayed in Table 4.8 clearly indicate that the compressive strength of the mycelium composite is notably inferior to that of standard coffee cup paper. The uncoated paper showed a compressive strength of 77.78 ± 6.47 N, while the PLA coated paper showed 122.49 ± 5.32 N. However, the mycelium composite sample cultivated for 4 weeks exhibited a marginally elevated compressive stress of 5.74 ± 2.73 N compared to the 2-week sample, which recorded a compressive stress of 4 ± 0.48 N.

4.8 Thermal Conductivity Analysis:

Thermal conductivity plays a crucial role in engineering biomaterials for beverage cups. It directly impacts the cup's ability to retain or dissipate heat, which is essential for maintaining the desired temperature of beverages. For instance, a biomaterial with low thermal conductivity would help to insulate hot beverages, keeping them warmer for longer periods without transferring excessive heat to the user's hand. Conversely, for cold beverages, a biomaterial with higher thermal conductivity could assist in dissipating heat from the cup's surface, preventing condensation and ensuring a comfortable grip. Therefore, understanding and optimizing the thermal conductivity of biomaterials is vital for designing beverage cups that provide optimal insulation or cooling properties, enhancing the overall user experience.

Table 4.9 Summary of thermal conductivity results of 2-week and 4-week samples.

Sample type	Sample ID	Sample thickness (mm)	Thermal conductivity ($Wm^{-1}K^{-1}$)	Literature value ($Wm^{-1}K^{-1}$)
2 weeks sample	S1	5.3	0.0356	Copier paper: 0.1321[a]
	S2	3.35	0.0365	
	Average	0.325	0.0360	
	*SD		0.0004	
4 weeks sample	S1	4.76	0.0344	
	S2	4.98	0.0349	
	Average	4.87	0.0346	
	*SD		0.0002	

*SD= Standard deviation; *a= Lavrykov & Ramarao, 2011

From Table 4.9, it is evident that the thermal conductivity of the 4-week sample (0.0346 to 0.0360 $Wm^{-1}K^{-1}$) is slightly lower than that of the 2-week sample (0.0360 $Wm^{-1}K^{-1}$), indicating that the 4-week sample possesses marginally better heat insulating properties. It's important to note that thermal conductivity can vary depending on the thickness of the materials. Lavrykov & Ramarao (2011) reported a conductivity range of 0.0740 $Wm^{-1}K^{-1}$ to 0.1816 $Wm^{-1}K^{-1}$ for thicknesses ranging from 0.085 mm to 0.22 mm.

Moreover, the author conducted a quick test during her experiment by placing hot water (100°C) in the produced mycelium cup for 2 hours. The test revealed excellent thermal stability, with no noticeable heat transfer to the touch and no leakage of the liquid. It is noteworthy that the thickness of the cup was 5-6 mm. ANOVA analysis showed the thermal conductivity difference between 2 weeks sample and 4 weeks sample in table 4.10. They were not statistically significant between the groups means by One-way ANOVA ($F(1,2) = 7.61$, $p = 0.11$)), where critical F value is 18.51 ($P>0.05$, $F<F$ crit).

Table 4.10 ANOVA analysis of thermal conductivity of 2 weeks and 4 weeks mycelium composites

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	1.88E-06	1	1.88E-06	7.614199	0.110070596	18.51282
Within Groups	4.93E-07	2	2.47E-07			
Total	2.37E-06	3				

4.9 Discussion

The analyses conducted on the mycelium and cattail-based bio composite materials offers valuable insights into its suitability for applications, particularly in comparison to conventional coffee cup materials. A summary of the characteristics of the Mycelium-composite materials harvested at 2-weeks and 4-weeks is presented in Table 4.11.

Morphological examination through SEM imaging revealed the bio composite's ability to form a cohesive structure, potentially suitable for use in coffee cup production. The observed increase in mycelium density over four weeks suggests a maturation process that could enhance the structural integrity of the material. Denser hyphal network would provide superior mechanical properties as because of better adhesion. As a result, denser network is preferable for using mycelium bio-composite in coffee cup production.

Chemical analysis indicates a decrease in lignin content and a marginal increase in protein content, which could contribute to the bio composite's mechanical strength and durability. This is crucial for end uses such as coffee cups, where the material must withstand handling and heat exposure without compromising on performance.

Thermo-mechanical analyses revealed stable thermal properties, with consistent glass transition and melting temperatures regardless of the composite's maturation stage. This is essential for coffee cup materials, ensuring they can withstand the temperature variations associated with hot beverages without deforming or losing their structural integrity. Crystallinity analyses suggested ongoing biodegradation processes within the bio composite, which could enhance its flexibility and adaptability. This is advantageous for coffee cup materials, as they need to be able to accommodate different cup shapes and sizes while maintaining their functionality. Additionally, the mycelium-composite materials exhibited hydrophobic properties, making it resistant to moisture ingress.

Table 4.11 Summary of Mycelium-composite characteristics harvested at 2-weeks and 4-weeks.

Evaluated characterisation	Techniques	Results
Morphological analysis	SEM Imaging	- Hyphal networks were able to make bio composite from Mycelium and cattails. - Hyphae diameter was found 0.81-.82 with *G. lucidum* fungus and cattail. – 4-week sample had denser mycelium growth and less unconsumed cattails in the structure.
Chemical analysis	ANKOM Fibre Analyzer and Protein Analysis	- Lignin content decrease with the mycelium growth. 4 weeks sample showed least lignin content in the sample. - Protein content marginally increase in 4 weeks sample compared with 2 weeks sample
Thermo-mechanical analysis	Thermogravimetric Analysis	- Thermal decomposition temperature was higher in 2 weeks sample (269°C) while 4 weeks sample had slightly lower decomposition temperature (267°C)
	Differential Scanning Calorimetry	- Both the 2-week and 4-week samples showed similar glass transition temperature and melting temperature ranging from 140-148°C and 177 °C respectively
Crystallinity analysis	X-Ray Diffraction	1. Crystallinity of 2 weeks and 4 weeks sample was calculated 76% and 73% respectively.
Thermodynamic analysis	Water Contact Angle	1. all mycelium composite showed contact angle above 90°.
Mechanical analysis	Tensile Strength	1. Young modulus of mycelium composite is notably inferior (2 weeks sample: 11.27MPa, 4 weeks sample: 80.91 MPa, than coffee cup papers (1165.65 MPa)
	Compression Strength	1. Compression stress of mycelium composite is 5.74 N which is significantly lower than the coffee cup paper standard.
Thermal analysis	Thermal Conductivity	4 weeks sample had less thermal conductivity than 2 weeks sample

This is particularly important for coffee cups, as they need to prevent leakage and maintain the quality of the beverage contained within. While the bio composite may have lower mechanical strength compared to traditional coffee cup materials initially, the observed improvement in Young's modulus over time indicates potential for enhanced stiffness and structural integrity with prolonged maturation. This could be further optimized to meet the mechanical requirements of coffee cup applications through refining manufacturing processes or incorporating reinforcing agents. The mycelium and cattail-based bio composite show promise as a sustainable alternative for coffee cup materials. Its unique combination of morphological, chemical, thermo-mechanical, and hydrophobic properties makes it well-suited for this end use, offering potential advantages in terms of sustainability, performance, and functionality.

Chapter 5: Limitations of manufacturing RTU Cups and Sheets with Mycelium Composite Materials

The current study to create compostable coffee cups from fungal mycelium shows great promise as an innovative and sustainable alternative to conventional disposable cups. However, several challenges must be addressed to develop practical solutions for large-scale production. The researcher explored two approaches: creating complete cups using a 3D mold and producing paper-like sheet material that can be folded to form cups, similar to traditional paper cup manufacturing.

5.1 Challenges in Making RTU Cups with a 3D Mold

While this study successfully created mycelium coffee cups using a 3D PLA mold, there are several areas for improvement:

Compression Molding Issues: The use of compression molding for the cup mold presents difficulties in removing the cup after 3-4 weeks of mycelium growth, as the material tends to adhere to the mold's walls. This often results in cracks or breakage when attempting to remove the mold. Although the cups can naturally heal cracks if left for a few more days before autoclaving, the self-healing nature of the biological material can compromise the cup's shape.

Material Shrinkage: Cup shapes were compromised during the drying process due to high material shrinkage. Morphological analysis revealed a densely packed hyphal network in the mycelium composite, which can trap a significant amount of moisture. This results in high shrinkage after drying, altering the intended cup shape.

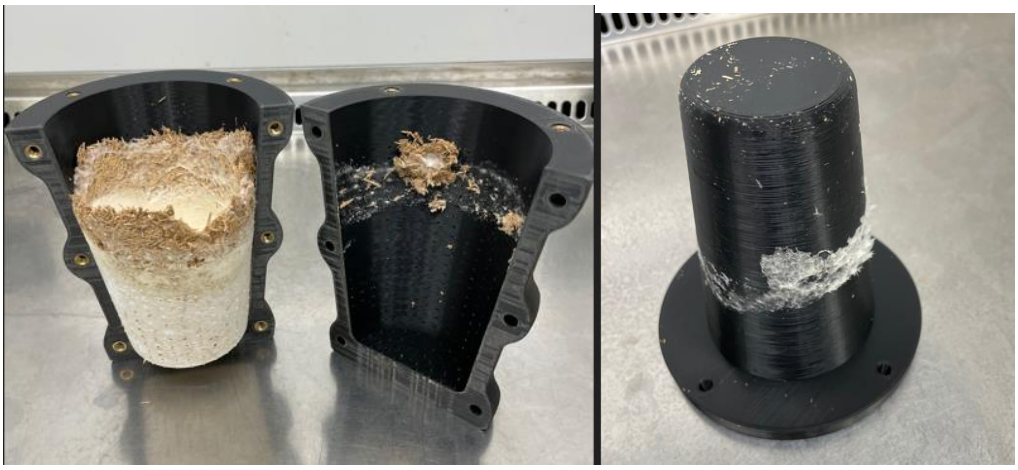


Figure 5.1 Mycelium cup after removing from the PLA mold.

Odor Issues: Despite the material's hydrophobic properties, allowing the cups to hold hot water (90-100°C) for 2-3 hours without leakage, there is a woody odor issue that must be addressed. Since the intention is to use these cups for coffee, they must be odor-free and not affect the taste of the beverage they hold.

Contamination Risk: As biological materials, the mycelium cups are highly prone to contamination with bacteria and other airborne fungi. Ensuring the sterility of the production environment and the final product is crucial for safe use.

Scalability Challenges: The mold cup-making procedure is time-consuming, labor-intensive, space-demanding, and costly to adapt to large-scale production as a replacement for conventional polymer-lined paper cups. Initially, they may be implemented on a small volume scale. This is why researchers are also exploring the possibility of making cups from mycelium composite sheets, which would be much more convenient to scale up for large-scale production.

5.2 Challenges in Paper-Like Sheet Materials

Brittleness: The primary challenge with mycelium composite sheets is their high brittleness, making them less flexible and unable to bend and roll like paper. This limits their usability in forming conventional cup shapes.

Mechanical Properties: Their tensile strength and Young's modulus are notably lower compared to conventional coffee cup paper. Enhancing the mechanical properties of the mycelium sheets is essential to meet the durability standards required for coffee cups.

Uniformity and Surface Quality: As biological materials, mycelium sheets do not grow uniformly, leading to an uneven and non-flat surface. This affects the aesthetic quality and functional integrity of the cups made from these sheets.

Contamination Risk: Similar to the molded cups, mycelium composite sheets are highly susceptible to contamination by bacteria and other airborne fungi. Maintaining sterility throughout the production process is vital.

Odor Issues: A woody odor in the material must be addressed to ensure that the cups do not affect the taste or aroma of the coffee they hold.

Chapter 6: Engineering Significance and Recommendations for Future Work

6.1 Engineering Significance

The *G. lucidum* mycelium and cattail bio composite materials display remarkable attributes that hold promise for various applications, particularly as a sustainable alternative to conventional paper cups. Initial evaluations have unveiled its distinct morphological characteristics, indicating a cohesive structure capable of withstanding the rigors of daily use. Moreover, its exceptional thermal stability and elevated critical temperature suggest resilience to high temperatures commonly encountered with hot beverages, reinforcing its suitability for beverage containers. Additionally, the inherent hydrophobicity of the bio composite further enhances its appeal, as it can effectively repel moisture and prevent leakage.

Despite these promising qualities, further research is warranted to optimize the mechanical properties of the mycelium-composite materials. Comprehensive studies focusing on the chemical composition and crystallinity of the material are essential. By delving deeper into these aspects, researchers can gain invaluable insights that may contribute to refining the material's structural integrity and enhancing its mechanical strength. Such enhancements are crucial for ensuring the bio composite meets the rigorous demands of industrial-grade applications, such as food and beverage packaging.

Overall, the *G. lucidum* mycelium-composite materials with cattail biomass demonstrates considerable potential as a sustainable solution for paper cup alternatives. Through continued research and development efforts aimed at improving its mechanical attributes and understanding its chemical composition, this bio composite could pave the way for environmentally friendly alternatives in various industries.

6.2 Recommendations for Future Work

To address the inherent lack of mechanical performance in the material, several recommendations for future research are proposed:

1) Utilization of Alkali-Treated Cattails: Alkali treatment of cattails is suggested as a method to effectively remove additional lignin from the raw material. This process can enhance the adherence properties of the cattails within the composite, potentially improving the overall strength and durability of the material. By reducing the lignin content, the compatibility between the cattails and mycelium can be enhanced, leading to a more cohesive and robust biocomposite structure.

2) Preparation of Homogenous Mycelium Composite Powder: To achieve a more uniform and controlled particle size distribution, it is recommended to prepare a homogenous mycelium composite powder through grinding and sieving. This step aims to standardize the particle size, ensuring consistent material properties and facilitating the subsequent mixing process with other additives or polymers.

3) Mixing with Biodegradable Polymer (PLA): The addition of a biodegradable polymer, preferably Polylactic Acid (PLA), is proposed to enhance the mechanical properties of the bio composite. Using a rotating screw extruder, similar to the method described by Patel et al. (2022) for mixing PLA with PHA and chitin, the mycelium composite powder can be blended with PLA under controlled conditions. Recommended extrusion parameters include temperatures ranging from 185-190°C, a screw speed of 300 rpm, and a duration of 30-40 seconds. These conditions aim to ensure thorough mixing of the components while minimizing material degradation during the extrusion process.

4) Optimization of Extrusion Temperature: Given the thermal degradation temperature of the mycelium-cattail composite exceeds 245°C, maintaining the extrusion temperature within a

range of 180°-200°C is considered favorable. This temperature range allows for efficient processing while minimizing the risk of material degradation. Controlling the extrusion temperature is crucial to preserving the integrity of the bio composite and maximizing its mechanical properties.

By implementing these proposed hypotheses, future research endeavors can potentially overcome the current limitations of the material and pave the way for the development of high-performance bio composite suitable for various industrial applications.

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8.0 Appendix

8.1 List of Appendix Tables:

Table A1 Recipes of fungal growth media.

Potato Dextrose Broth
Potato dextrose (Fisher scientific): 24 gm
Distilled water: 1000 mL
pH: 5.00

Potato Dextrose Peptone Broth:
Potato dextrose (Fisher scientific): 24 gm
Distilled water: 1000 mL
Peptone: 1:20 (w/v)
pH: 5.01

Rice Extract Broth:
Rice extract powder (Fisher scientific): 16gm
Distilled water: 1000 mL
pH: 6.53

Yeast Extract Peptone Dextrose Broth:
Potato dextrose: 20 gm (Fisher scientific)
Peptone: 4 gm (Fisher scientific)
Yeast: 10 gm (Fisher scientific)
Distilled water: 1000 mL
pH: 6.5

Table A2 Dry mass calculation of mycelium in 3 different batches

Batch	Medium	Dry mycelium mass (g/L)
S1	Potato dextrose broth	4.35
	Potato dextrose broth with peptone	2.90
	Yeast extract peptone dextrose	8.30
	Rice extract	1.20
S2	Potato dextrose broth	6.10
	Potato dextrose broth with peptone	5.45
	Yeast extract peptone dextrose	11.15
	Rice extract	2.75
S3	Potato dextrose broth	2.55
	Potato dextrose broth with peptone	2.95
	Yeast extract peptone dextrose	7.85
	Rice extract	1.6

Table A3 Hyphae diameter for 2 weeks mycelium-cattail composite (outliers in red letters).

2 weeks sample	
First quartile	0.602
Third quartile	0.84135
Interquartile range	0.23935
Upper limit	1.200375
Lower limit	0.242975

No of sample	Diameter (micro meter)	Outlier	No of sample	Diameter (micro meter)	Outlier	No of sample	Diameter (micro meter)	Outlier
1	1.189	FALSE	35	1.47	TRUE	69	0.8236	FALSE
2	1.192	FALSE	36	0.7602	FALSE	70	0.7824	FALSE
3	0.7339	FALSE	37	0.5562	FALSE	71	0.7184	FALSE
4	0.7339	FALSE	38	0.4266	FALSE	72	0.675	FALSE
5	0.7631	FALSE	39	0.9921	FALSE	73	0.978	FALSE
6	0.691	FALSE	40	0.5935	FALSE	74	0.6766	FALSE
7	0.7516	FALSE	41	0.8433	FALSE	75	0.7856	FALSE
8	0.6105	FALSE	42	0.8021	FALSE	76	0.8276	FALSE
9	1.689	TRUE	43	0.9504	FALSE	77	0.4781	FALSE
10	0.5736	FALSE	44	0.7261	FALSE	78	0.4317	FALSE
11	0.509	FALSE	45	1	FALSE	79	0.9949	FALSE
12	1.283	FALSE	46	0.7061	FALSE	80	0.7843	FALSE
13	0.691	FALSE	47	0.7898	FALSE	81	0.9412	FALSE
14	0.8219	FALSE	48	0.823	FALSE	82	0.9439	FALSE
15	0.7634	FALSE	49	0.5935	FALSE	83	0.106	TRUE
16	0.5935	FALSE	50	0.8276	FALSE	84	0.635	FALSE
17	0.5119	FALSE	51	0.5935	FALSE	85	0.8472	FALSE
18	0.7496	FALSE	52	0.8276	FALSE	86	0.7967	FALSE
19	0.6316	FALSE	53	0.4781	FALSE	87	0.9209	FALSE
20	0.8446	FALSE	54	0.9209	FALSE	88	0.963	FALSE
21	0.9905	FALSE	55	0.7061	FALSE	89	0.9731	FALSE
22	0.4975	FALSE	56	0.7169	FALSE	90	0.5183	FALSE
23	0.3652	FALSE	57	0.6229	FALSE	91	0.6525	FALSE
24	0.3777	FALSE	58	0.8519	FALSE	92	0.6963	FALSE
25	0.3815	FALSE	59	1.078	FALSE	93	0.9953	FALSE
26	1.01	FALSE	60	0.8112	FALSE	94	0.6298	FALSE
27	0.6663	FALSE	61	0.9905	FALSE	95	0.6486	FALSE
28	0.8219	FALSE	62	0.7554	FALSE	97	1.041	FALSE
29	0.6205	FALSE	63	0.7898	FALSE	98	1.085	FALSE
30	0.7264	FALSE	64	0.8587	FALSE	99	1.049	FALSE
31	0.435	FALSE	65	0.3249	FALSE	100	0.799	FALSE
32	1.058	FALSE	66	0.7443	FALSE	101	0.6531	FALSE
33	0.8381	FALSE	67	0.7239	FALSE	102	0.8809	FALSE
34	0.7824	FALSE	68	0.7061	FALSE			

Table A4 Hyphae diameter for 4 weeks mycelium-cattail composite (outliers in red letters).

4 weeks sample	
First quartile	0.5321
Third quartile	1.07763
Interquartile range	0.54553
Upper limit	1.89591
Lower limit	0.28619

4 weeks sample								
No of sample	Diameter (micro meter)	Outlier	No of sample	Diameter (micro meter)	Outlier	No of sample	Diameter (micro meter)	Outlier
1	0.7369	FALSE	35	1.292	FALSE	69	0.8098	FALSE
2	1.17	FALSE	36	0.6063	FALSE	70	0.675	FALSE
3	0.845	FALSE	37	0.7143	FALSE	71	0.7329	FALSE
4	0.8638	FALSE	38	0.6117	FALSE	72	0.8574	FALSE
5	1.322	FALSE	39	1.171	FALSE	73	0.7678	FALSE
6	1.19	FALSE	40	0.8608	FALSE	74	0.8784	FALSE
7	0.5838	FALSE	41	1.236	FALSE	75	1.159	FALSE
8	0.6878	FALSE	42	0.501	FALSE	76	1.157	FALSE
9	1.724	TRUE	43	1.034	FALSE	77	0.7264	FALSE
10	0.8688	FALSE	44	0.846	FALSE	78	0.8574	FALSE
11	0.712	FALSE	45	0.6298	FALSE	79	0.4618	FALSE
12	1.271	FALSE	46	0.5018	FALSE	80	1.271	FALSE
13	0.7364	FALSE	47	0.9045	FALSE	81	1.016	FALSE
14	0.7726	FALSE	48	0.8403	FALSE	82	0.9209	FALSE
15	0.7829	FALSE	49	0.6586	FALSE	83	1.033	FALSE
16	1.285	FALSE	50	0.7824	FALSE	84	0.7843	FALSE
17	0.3518	FALSE	51	0.7669	FALSE	85	1.017	FALSE
18	0.5456	FALSE	52	1.092	FALSE	86	0.7967	FALSE
19	1.304	FALSE	53	0.9045	FALSE	87	0.6936	FALSE
20	0.496	FALSE	54	0.84	FALSE	88	0.7259	FALSE
21	0.5342	FALSE	55	0.4266	FALSE	89	0.8932	FALSE
22	1.145	FALSE	56	0.5723	FALSE	90	1.16	FALSE
23	0.6936	FALSE	57	0.958	FALSE	91	1.1024	FALSE
24	0.2559	FALSE	58	0.5811	FALSE	92	0.8205	FALSE
25	0.8638	FALSE	59	1.557	FALSE	93	0.8532	FALSE
26	0.4359	FALSE	60	0.6164	FALSE	94	0.7462	FALSE
27	0.5314	FALSE	61	1.557	FALSE	95	0.7264	FALSE
28	0.3815	FALSE	62	0.7339	FALSE	96	0.7082	FALSE
29	0.4594	FALSE	63	0.9986	FALSE	97	1.133	FALSE
30	0.2868	FALSE	64	1.323	FALSE	98	0.8094	FALSE
31	0.8755	FALSE	65	1.408	FALSE	99	0.7726	FALSE
32	0.6663	FALSE	66	1.159	FALSE	100	1.026	FALSE
33	0.1088	TRUE	67	0.9746	FALSE	101	0.5972	FALSE
34	1.298	FALSE	68	0.691	FALSE	102	0.7061	FALSE

Table A5 Hyphae diameter for pure mycelium (outliers in red letters).

Pure mycelium	
First quartile	0.83745
Third quartile	1.11925
Interquartile range	0.2818
Upper limit	1.54195
Lower limit	0.69655

Pure mycelium								
No of sample	Diameter (micro meter)	Outlier	No of sample	Diameter (micro meter)	Outlier	No of sample	Diameter (micro meter)	Outlier
1	0.9504	FALSE	35	0.997	FALSE	69	0.8784	FALSE
2	0.6321	FALSE	36	0.6229	FALSE	70	1.089	FALSE
3	0.7967	FALSE	37	1.007	FALSE	71	1.048	FALSE
4	0.8368	FALSE	38	0.8165	FALSE	72	0.7726	FALSE
5	1.233	FALSE	39	1.428	FALSE	73	0.9177	FALSE
6	0.8638	FALSE	40	0.5691	FALSE	74	1.079	FALSE
7	0.922	FALSE	41	1.905	TRUE	75	1.051	FALSE
8	0.9228	FALSE	42	0.9806	FALSE	76	1.031	FALSE
9	0.6745	FALSE	43	1.027	FALSE	77	1.101	FALSE
10	1.067	FALSE	44	1.905	TRUE	78	1.147	FALSE
11	0.5633	FALSE	45	1.428	FALSE	79	0.8587	FALSE
12	0.7094	FALSE	46	1.031	FALSE	80	0.8784	FALSE
13	1.485	FALSE	47	0.9209	FALSE	81	1.085	FALSE
14	1.548	TRUE	48	1.016	FALSE	82	1.054	FALSE
15	1.402	FALSE	49	1.051	FALSE	83	1.593	TRUE
16	1.122	FALSE	50	0.9209	FALSE	84	0.9898	FALSE
17	0.8333	FALSE	51	1.267	FALSE	85	0.8364	FALSE
18	1.076	FALSE	52	1.026	FALSE	86	0.9712	FALSE
19	0.9986	FALSE	53	0.8467	FALSE	87	0.9165	FALSE
20	1.111	FALSE	54	1.195	FALSE	88	0.4325	TRUE
21	1.169	FALSE	55	1.081	FALSE	89	0.8633	FALSE
22	1.193	FALSE	56	1.347	FALSE	90	1.004	FALSE
23	0.9686	FALSE	57	0.8223	FALSE	91	1.13	FALSE
24	0.8467	FALSE	58	1.168	FALSE	92	1.64	TRUE
25	0.958	FALSE	59	1.051	FALSE	93	0.7782	FALSE
26	1.038	FALSE	60	1.43	FALSE	94	0.9303	FALSE
27	0.8907	FALSE	61	0.8837	FALSE	95	1.64	TRUE
28	1.091	FALSE	62	1.47	FALSE	96	1.191	FALSE
29	0.8394	FALSE	63	1.607	TRUE	97	1.085	FALSE
30	0.7462	FALSE	64	1.426	FALSE	98	1.275	FALSE
31	1.123	FALSE	65	1.075	FALSE	99	1.048	FALSE
32	0.567	FALSE	66	0.8784	FALSE	100	1.026	FALSE
33	1.209	FALSE	67	1.075	FALSE	101	1.699	TRUE
34	0.8467	FALSE	68	0.6418	FALSE	102	1.085	FALSE

Table A6 Tukey-test for hyphae diameter of 2 weeks, 4 weeks mycelium composite and pure mycelium samples.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: VAR00002

Tukey HSD

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
2 week sample	4 week sample	-.07333	.03805	.133	-.1629	.0163
	pure mycelium	-.27725*	.03805	<.001	-.3669	-.1876
4 week sample	2 week sample	.07333	.03805	.133	-.0163	.1629
	pure mycelium	-.20392*	.03805	<.001	-.2935	-.1143
pure mycelium	2 week sample	.27725*	.03805	<.001	.1876	.3669
	4 week sample	.20392*	.03805	<.001	.1143	.2935

*. The mean difference is significant at the 0.05 level.

Table A7 ANOVA analysis dataset for thermal decomposition temperature (due to second/major stage mass loss)

Sample ID	2nd stage degradation temperature	
2 weeks sample	269.63	260.54
4 weeks sample	267.56	270.11

Table A8 ANOVA analysis dataset for glass transition temperature for 2 weeks and 4 weeks sample

Samples	Glass transition temperature range		
2 weeks sample	142.53	143.58	143.67
4 weeks sample	147.86	148.64	148.88

Table A9 Water contact angle of 2 weeks and 4 weeks mycelium composite materials.

Sample Type	Sample ID	Water contact angle °
2 weeks	S1	126
	S2	110
	S3	112
	S4	100
	S5	91
	Average	104.2
	SD	7.76
4 weeks	S1	116
	S2	112
	S3	95
	S4	117
	S5	105
	Average	109
	SD	8.17

*SD = Standard deviation

Table A10 Chemical composition analysis of mycelium materials, raw cattails and pure mycelium

	Hemicellulose %			Cellulose %			Lignin %			Protein %		
Sample ID	**Hemi cellulose %**	**Average**	**SD**	**Cellulose %**	**Average**	**SD**	**Lignin %**	**Average**	**SD**	**Protein %**	**Average**	**SD**
raw cattails S0	23.95			31.48			15.20					
raw cattails S1	24.86	24.65	0.51	34.41	33.29	1.29	9.00	11.27	2.79			
raw cattails S2	25.14			33.99			9.60					
pure mycelium	14.43	14.43	0.00	11.69	11.69	0.00	3.60	3.60	0.00	39.83	39.83	0
2 weeks sample S0	16.58			30.22			12.30			11.7		
2 weeks sample S1	12.80			24.89			7.20			11.60		
2 weeks sample S2	12.81	13.31	2.02	23.08	25.55	2.77	10.00	9.25	2.07	11.50	11.6	0.07
2 weeks sample S3	11.03			23.99			7.50			11.60		
4 weeks sample S0	14.02			28.02			8.10			12.1		
4 weeks sample S1	9.89			33.30			2.80			12.40	12.2	0.16
4 weeks sample S2	9.74	11.01	1.76	32.05	31.34	1.99	4.30	4.85	1.97	12.00		
4 weeks sample S3	10.37			32.00			4.20			12.30		