TUNING OF SELF-GROWING NATURAL COMPOSITE MATERIALS USING NON-PATHOGENIC FUNGAL STRAINS-TOWARDS 3D CONSTRUCTS

Doctoral Thesis by:
Kumba Bintunia Bonga

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Kumba Bintunia Bonga

M.Eng in Environmental Science and Engineering

S4783604
PhD Program in Bioengineering and Robotics (DIBRIS)

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Supervisors:

Dr. Athanassia Athanassiou

Dr. Despina Fragouli

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Dedication

I would like to dedicate this thesis to God Almighty and my family.

Author's Declaration

I hereby declare that except where specific reference is made to the work of others, the contents of this thesis are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This thesis is my work and contains nothing which is the outcome of work done in collaboration with others, except as specified in the Co-Authorship section. This dissertation contains fewer than 30,000 words including appendices, bibliography, figures, tables, and equations, and has fewer than 30 figures.

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Abstract

This PhD thesis investigates the utilization of non-pathogenic fungal strains for the development of self-growing natural composite materials, with a focus on their utilization as components of specific applications. The research explores the fundamental principles of fungal biology, materials science, and fabrication techniques to achieve precise control over the properties and shapes of these materials.

In particular, it explores a novel approach for creating self-growing natural biocomposite materials through the combination of the *Pleurotus ostreatus* mycelium with coffee silverskin grains. The growth conditions and parameters to tune the properties of the final products are optimized, and the physicochemical characteristics of the resulting biocomposites are evaluated, assessing also the feasibility of scaling up their production for practical applications.

To achieve these objectives, we employed key methodologies capturing the isolation and cultivation of mycelium, the definition of growth conditions in the presence of the coffee silverskin that promotes faster self-assembly and structural integrity, and the characterization of resulting materials using advanced analytical techniques such as microscopy, spectroscopy, and mechanical testing. We also conducted scalability studies to explore the potential for industrial applications. This research yielded several significant results, including the successful cultivation of the fungal strain capable of self-assembling into intricate 3D structures embedding the coffee silverskin grains, as well as, the potential of the resulting structures for use in specific applications such as sustainable construction, and biodegradable packaging.

The findings of this research have far-reaching implications. The development of self-growing biocomposites using a non-pathogenic fungal strain offers a sustainable and environmentally friendly alternative to conventional materials for defined applications. By harnessing the self-

assembly capabilities of fungi, we can reduce the environmental footprint associated with traditional manufacturing processes and contribute to a more sustainable future. This work opens the door to innovative applications and translational fields that prioritize eco-consciousness and biocompatibility, aligning with the growing demand for greener and more sustainable solutions in various industries.

Key-words: Mycelium, Agro Waste, Porous Biocomposites, Sustainability, Circular Economy, Green Materials.

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List of Abbreviations

BPA : Bisphenol A

CSF/P : Coffee Silverskin/*Pleurotus ostreatus*

FTIR : Fourier Transform Infrared Spectroscopy

ATR : Attenuated Total Reflection

WCA : Water Contact Angle

PHB : Poly-3-hydroxybutyrate

PHA : Polyhydroxyalkanote

ESOME : Epoxidized Soya Bean Oil Methyl Ester

MycB : Mycelium Biomass

MFI : Melt Flow Index

XRD : X-Ray Diffraction

1 Chapter One: Introduction

1.1 Synthetic Plastics - A Threat to Environmental Health and Safety

While plastics offer various benefits, such as durability and versatility, their widespread production, use, and disposal have led to numerous environmental challenges[1]. This is because the majority of synthetic plastics are non-biodegradable, meaning they do not break down easily over time.[2, 3] For example, the decomposition of some plastic bags may last for up to 1,000 years.[1, 4-7] Despite efforts to recycle or used them in waste-to-energy plants, only a small percentage of plastic waste is effectively managed.[8] Inadequate waste management infrastructure in many regions exacerbates plastic litter, and improper disposal can result in environmental contamination.[9-11] As a result, plastic waste accumulates in land, oceans, and other ecosystems, taking centuries to break down, releasing hazardous materials into the ground and water thus causing long-term environmental pollution,[12-15] and by this means affecting land-dwelling plants and animals and potentially harming humans.[16, 17]

Specifically, one serious and expanding global issue is plastic pollution in ocean waters.[18-20] Oceans are particularly affected by plastic pollution, impacting marine life. Over time, large plastic items break down into smaller particles known as micro and nano plastics. These tiny particles are found in soil, water, and air, threatening wildlife and human health as they can easily pass to the food chain.[3, 14] In fact, marine animals often mistake plastic debris for food, leading to ingestion which obstructs their digestive tracts, causing entanglement, and drowning.[7, 17, 21-25] In addition,

the environmental problem caused by trapped plastic along coastlines presents several issues that negatively impact tourism and impede economic growth.[7, 26-28] Agricultural land and atmosphere are also at risk due to plastic pollution,[29, 30] in addition to the marine environment,[6, 31, 32] causing harm to individual organisms and disrupting entire ecosystems [27, 31, 33-35]. Some plastics contain harmful chemicals that can leach into the environment, especially under certain conditions such as exposure to sunlight or heat[36, 37], posing risks to both aquatic and terrestrial ecosystems.[38-41] Moreover, the potential health risks associated with chemicals in plastics such as bisphenol A (BPA) and phthalates, raise concerns as they are known to interfere with the endocrine system and have been linked to various health issues[41], emphasizing the need for comprehensive efforts to address these challenges.[42, 43] Additionally, the production and use of synthetic plastics intensify resource depletion, as it relies heavily on fossil fuels.[1, 44] The extraction and processing of fossil fuels for plastic production contribute to air and water pollution.[45]

1.2 Mitigating the threats by Synthetic Plastic to Environmental Health and Safety

Addressing the need to invest in research and development of alternatives by fostering innovation in sustainable materials, bio-based plastics, materials that degrade through natural processes into simpler compounds when exposed to the environment, are promising in mitigating the threats posed by synthetic plastics to environmental health and safety.[46-50]

Many alternative materials are designed to be biodegradable, minimizing environmental impact throughout their lifecycle from production to disposal, by using fewer resources, generating less waste, and emitting fewer greenhouse gases compared to traditional plastics.[51] Biodegradable plastics, derived from renewable sources such as plant-based materials (i.e. agricultural waste, corn starch, sugarcane, or cellulose), or microbial fermentation, offer a valuable alternative.[52] In fact,

the field of biodegradable and natural-based plastics has seen significant advancements and innovations. Unlike traditional plastics, faster degradable plastic composites can help mitigate the long-term impact of plastic pollution boosting their environmental benefits and reducing the persistence of plastic waste.[50, 53-57] This facilitates the adoption of circular economy principles, [58, 59] where materials are designed for durability, reuse, [27, 60] recyclability,[61] biodegradability.[59, 62] This approach supports the efficient use of resources and reduces the reliance on single-use plastics.[49, 63-66] While the biodegradable and renewable natural based materials offer promising solutions to plastic pollution, their performance in the same, or even better, way for specific applications is a great challenge.

Some examples of the most promising natural based polymers for large scale applications are the here presented. Polyhydroxybutyrate (PHB) is a polyhydroxyalkanoate (PHA), a polymer belonging to the polyesters that represents a class of polymers that is synthesized by a variety of bacteria through fermentation of sugars or lipids, with a great variety of special features.[67-69] Other alternative natural-based and biodegradable materials include the following: Algae-based plastics that utilize algae biomass as a feedstock, chitosan derived from various crustaceans and cellulose derived from plants.[70-72 73-78]. Natural fibers such as hemp, bamboo, kenaf, and flax that can be combined with biodegradable resins to create composites with enhanced mechanical properties[79]. Also, starch that can be blended with other biodegradable polymers to improve the mechanical properties and biodegradability of the resulting materials[80, 81]. [82, 83]

Natural based polymers can be used in various applications. Specifically, alginate, derived from seaweed, is used in food packaging, wound dressings, and biomedical scaffolds.[84, 85] Chitosan, derived from the shells of crustaceans, has antimicrobial properties and is used in applications such as food packaging, biomedical devices, and agricultural films.[86, 87] Materials such as bamboo,

hemp, and cotton can be used to produce fibers for textiles and packaging.[88] Sustainable fabrics made from natural fibers like organic cotton, hemp, bamboo, and recycled materials are attaining acceptance as alternatives to synthetic textiles.[89, 90] Automotive manufacturers incorporate alternative materials for interior components, furnishings, and exterior parts to reduce weight, improve fuel efficiency, and enhance sustainability.[91, 92] Biodegradable and bio-compatible plastic materials are used in medical applications such as implants, surgical tools to improve patient safety.[93] Furthermore they are utilized in various consumer goods such as toys, household products, personal care items, and gardening supplies to offer more environmentally friendly options to consumers. Biodegradable mulch films, compostable plant pots, and other agricultural products made from alternative materials effectively reduce plastic pollution in farming and horticulture while promoting sustainable practices.[94-98]

Another promising avenue for overcoming the challenges of synthetic plastics is the creation of value from agricultural waste streams in combination with mycelium.[56, 99-104] Agrowaste, such as agricultural residues, byproducts, and biomass, often goes underutilized and can contribute to environmental pollution if not managed properly. However, it has been proved that the mycelium-agrowaste bound biomaterials can effectively create biodegradable products with properties similar to plastic[105-107] that can be used in packaging and construction due to their unique properties and sustainability. They are currently being adopted by retailers for shopping bags, packaging materials, and product displays to minimize plastic usage and demonstrate environmental responsibility. [108-115] On the top such an approach contributes to waste utilization and upcycling efforts, transforming low-value waste materials into value-added products with commercial potential.

Nonetheless, while the alternatives to synthetic plastics show great promise, challenges include scaling up production, improving mechanical properties and shelf life, addressing cost competitiveness compared to traditional plastics, and ensuring proper disposal and recycling infrastructure for biodegradable materials. Some alternative materials may be more expensive to produce compared to traditional plastics, primarily due to the cost of sourcing renewable resources and developing new manufacturing processes. Some may not possess the same level of durability, flexibility, or other desirable properties as traditional plastics, limiting their applicability in certain applications. Despite advancements, alternative materials may face resistance from industries and consumers accustomed to traditional plastics, hindering widespread adoption and market penetration. Utilizing renewable resources may compete with food production or other essential uses, raising concerns about land use, deforestation, and impacts on food security. Likewise, while alternative materials aim to reduce environmental harm, their long-term environmental impacts are not always well understood, and unintended consequences could emerge as they become more widely used. Addressing these challenges is essential for their widespread applications and to further enhance their sustainability[116-123]

The research and development of the composite materials in this thesis drive toward presenting an opportunity to mitigate the impact of plastic waste on our ecosystems,[124, 125] reduce reliance on non-renewable resources,[109] and address the challenges of agricultural waste management.[125, 126]

1.3 Research Objectives and Novelty

The need for research on mycelium-bound composites for insulation and other applications stems from the desire to develop sustainable, biodegradable, and effective alternatives to conventional materials, addressing environmental, health, and resource efficiency concerns in various industries

including construction. This thesis endeavors to comprehensively examine the manufacturing processes, material properties, and performance characteristics of mycelium agro-waste-bound biocomposites, shedding light on their potential as a sustainable alternative to conventional insulation materials.

This study aims to investigate and develop a novel approach for creating self-growing natural composite materials using non-pathogenic fungal strains with a specific focus on *Pleruotus ostreatus* species and explore their potential in the formation of 3D constructs, with a key objective the optimization of the growth conditions and parameters in order to enhance the material's properties. Furthermore, it explores the details of mycelium cultivation, the selection of appropriate filler materials including agro waste, and the fabrication process for creating the composites[112].. The mechanical, thermal, structural, and biodegradable characteristics of the resulting composite materials, assessing their biodegradability under controlled environmental conditions, and the feasibility of scaling up production for practical applications are also evaluated. By optimizing the formulation and processing parameters, this study seeks to achieve biocomposite materials with improved performance characteristics, making them suitable for a wider range of applications. It also fosters innovation in processing techniques and material design, driving advancements in sustainable materials technology. The outcomes of this study contribute to the broader goal of transitioning towards a more sustainable economy, where biomaterials play a pivotal role in reducing environmental impact and promoting resource efficiency. Through a synthesis of existing literature, experimental studies, and real-world applications, this research will contribute valuable insights to the growing body of knowledge on mycelium-bound biocomposites informing future research efforts of their design and development as innovative solutions for eco-

friendly composite materials applicable across various industries and fostering a deeper understanding of their capabilities and limitations. Ultimately, the findings of this study aspire to pave the way for a more sustainable and resilient future in building construction, packaging, and other applications where innovative materials play a pivotal role in mitigating environmental impact and promoting a healthier and greener environment.

2 Chapter Two: Self-growing (Mycelium-bound) Materials as a Potential Strategy for Environmental Sustainability

2.1 Background

The research background in mycelium-bound biocomposites reflects a convergence of technological innovation and sustainability imperatives. It represents a paradigm shift towards more nature-inspired, regenerative approaches to materials design and production, with the potential to transform industries and contribute to a more resilient and sustainable future. Self-growing materials, particularly those based on mycelium, hold significant promise as innovative and sustainable solutions for various environmental challenges in several ways.[127, 128] Mycelium is the root structure of fungi, consisting of a network of thread-like structures called hyphae [129-131] that possesses unique properties making it a versatile and environmentally friendly material (**Figure 2.1**).[116, 121]

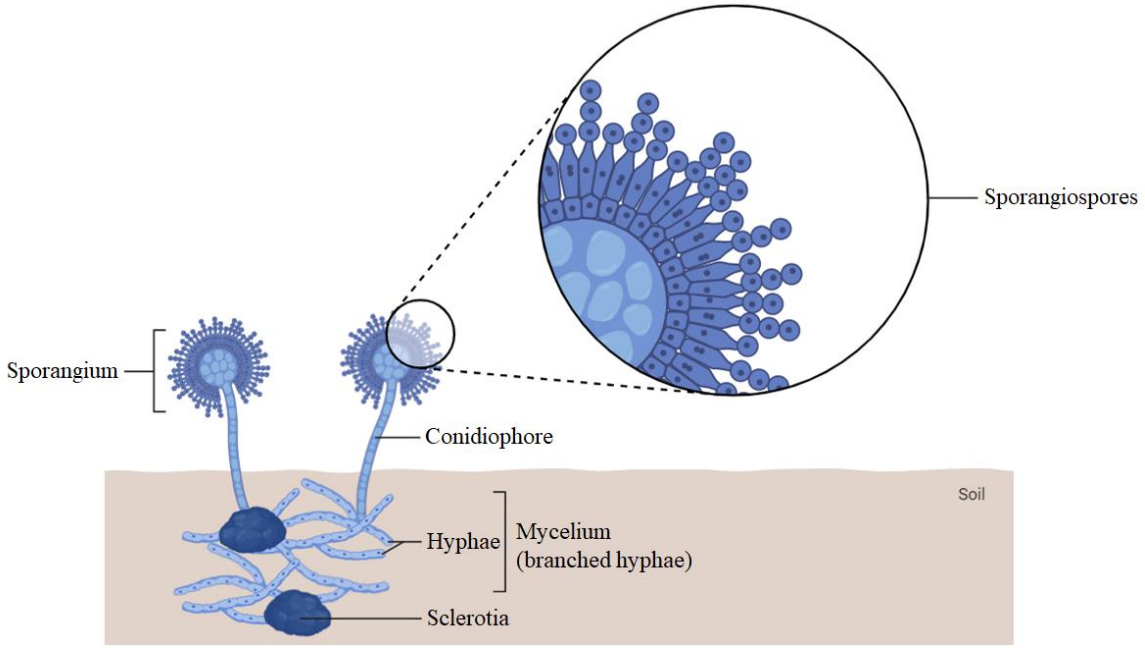


Figure 2.1 Morphological features of Mycelium

In particular, fungi's mycelium, which resembles roots, is made up of a dense network of hyphae, which are tiny, tubular filaments that are essential to the growth and survival of the organism. Hyphae, have the ability to form robust and wide-ranging networks. Their cell walls are rich in chitin (a polysaccharide also found in insect exoskeletons), glucans, proteins, and lipids. This composition gives mycelium both structural strength and resilience against environmental factors. In particular, chitin provides rigidity and glucans offers flexibility. Mycelium proteins facilitate structural formation and offer binding sites for other constituents. Smaller amounts of lipids help to make cell membranes flexible, which facilitates the absorption of nutrients. Numerous mycelium species generate enzymes and phenolic compounds that facilitate the decomposition of organic matter in the substrate. These enzymes are beneficial for composting and bioremediation processes and aid in the decomposition and nutrient cycling in ecosystems.

When creating materials like mycelium-based insulation and packaging, this interconnected structure acts as a natural "scaffolding," enabling it to grow into a variety of shapes or molds while maintaining structural integrity. The species, substrate, and growth conditions all affect the mycelium's porosity and density.

Mycelium is a useful material for a variety of applications, such as packaging, biofabrication, and sustainable building, due to its flexible, porous structure and chemically strong cell wall. With extra advantages like biodegradability and minimal environmental impact, its inherent qualities enable it to function as an environmentally beneficial substitute for synthetic materials.

Specifically, new research has demonstrated that mycelium biocomposites are capable of acting as CO2 sinks[132-135]. Additionally, their production requires 1.5–6 times less energy than other systems[136-139], and they have a smaller effect on water use[140], particle emissions, and global climate

change than other systems.[99, 105, 111, 125, 141, 142] In light of this, mycelium has emerged as a versatile and sustainable building block for the creation of biocomposites.[122, 143]

2.2 Mycelium-Bound Composites: state of the art

A rapidly expanding field of study is the development of biocomposite materials based on fungal mycelium, as industry, society, and researchers actively seek new sustainable materials to address modern material challenges.[144] Fungi are one of the key biological resources that can be used to develop a wide range of sustainable products including biodegradable materials with promising applications, and with zero waste generation during the production process.[144, 145] Since ancient times, people have used fungi primarily to process food, such as baked goods, alcoholic beverages, fermented cheese, beans, and vegetables.[144] Modern times are seeing the emergence of new avenues for human-fungus interaction, such as the creation of structural materials based on the resilient network structure of fungal mycelium.[146] These materials can be entirely based on pure fungal mycelium,[147] or they can take the form of biocomposites made of a lignocellulose substrate bound into a cohesive structure by the hyphal mycelium network that forms the bulk surrounding matrix.[117, 122] The many variations available in the production phase lend to a versatile range of possible material outcomes and properties.[144] The development of composites with a variety of useful properties is the result of advancements in the selection of fungal strains,[110, 148, 149] substrate utilization,[149] and processing methods.[148] Currently, research is being done on the fascinating potential of fungal mycelium materials in relation to a number of applications including bio-composites, packaging materials,[150] textiles that resemble leather,[109, 151] food ("meatless" meat),[110, 152] fashion,[153] design,[154] and biomedicine[155] and even in architecture[154] and construction. [144] This

process is central to its biological utility, as it can recycle nutrients back into the ecosystem and decompose waste.[125]

The phyla Ascomycota and Basidiomycota contain the most frequently studied fungi[156] because t hey grow mycelially efficiently and yield durable materials.[157] Several important strains employed in studies are as follows: *Ganoderma lucidum,* which possess strong mycelial growth and well-known medicinal qualities (Table 2.1), [158-161] is a popular strain for the synthesis of biocomposites.[162] *Pleurotus ostreatus*, also known as oyster mushrooms, is one of the strains of mushrooms that has been studied the most[163] because of its quick growth and ability to adapt to different substrates (Table 2.1).[164, 165] *P. ostreatus* is frequently used to make mycelium-based packaging[166, 167] and insulation materials (Table 2.1).[168, 169] Medical applications for mycelium-based materials, such as drug delivery systems, tissue engineering,[170] and dressings for wounds,[161] are also being investigated. *Schizophyllum commune* (Table 2.1)*[162]* and *Ganoderma lucidum* (Table 2.1)*[155]* have been investigated for creating scaffolds and membranes that promote cell growth and tissue regeneration. *Trametes versicolor*, also known as Turkey Tail, is a strain of bacteria that has been studied for its potential to produce materials for biomedical applications[171] and to aid in environmental remediation by decomposing pollutants.[172] Brown mold (*Fomes fomentarius* (Table 2.1)), was traditionally used as a fire starter,[173] and is now being studied for potential uses in textiles and materials that resemble leather[153]. *Schizophyllum commune* (Table 2.1) is well-known for having a strong mycelial network, and its application in biodegradable packaging[174] has been studied. Due to their cellulose content, cardboard and recycled paper make great substrates for a variety of fungal strains, including *S. commune*.[109] This substrate produces strong, flexible materials that can be used for packaging or as an alternative to biodegradable plastic.[83, 175] Food leftovers like fruit skins and potato peels have also been investigated as possible

low-cost mycelium cultivation substrates.[176, 177] These waste streams can be utilized to cultivate fungi, such as *Pleurotus* species, which can produce materials for use in edible packaging and other non-food applications.[178-180] Mycelium has drawn a lot of interest as a biodegradable packaging substitute.[166, 167, 181] The use of *P. ostreatus* grown on agricultural waste to create mycelium-based packaging that can replace plastic foam and other non-biodegradable materials has been pioneered by companies like Ecovative Design.[116, 182-184] Mycelium composites have demonstrated promise as environmentally friendly building materials,[185-187] particularly those cultivated from strains of Ganoderma[162] and Trametes[162] on wood-based substrates.[188] These materials can be used to create insulation panels,[122] bricks,[185] and even whole architectural structures[105, 189] because they have qualities like thermal insulation,[169] soundproofing,[169] and fire resistance. In mycoremediation, fungi are utilized to degrade heavy metals and hydrocarbons[190] found in the environment. Two such species of fungi are *Pleurotus ostreatus*[191-193] and *Trametes versicolor*[194]. Mycelium-bound composites have also been investigated for their ability to convey electrical signals,[195] thereby enabling the transmission of frequency-modulated information.[195]

Table 2.1: Properties of different strains of fungal mycelium

Species	Chemical composition	Primary Applications
Ganoderma lucidum	Polysaccharides, triterpenoids	Supplements, biocomposites, biomedical
Pleurotus ostreatus	Beta-glucans, lignin-degrading enzymes	Biodegradation, bioremediation, insulation, packaging
Schizophyllum commune	Beta-glucans, schizophyllan	Biomedical, durable materials, water/fire-resistant materials
Fomes fomentarius	Polysaccharides, phenolic compounds	Eco-friendly products

Scientists and engineers have successfully developed mycelium-bound biocomposites by growing mycelium through a cultivation process on a variety of organic substrates (Figure 2.2), such as agricultural waste (e.g., rice hulls, straw, sawdust), wood by-products, hemp fibers, textile waste or other biomass in the form of powder, fibers or solid substrates.[122]

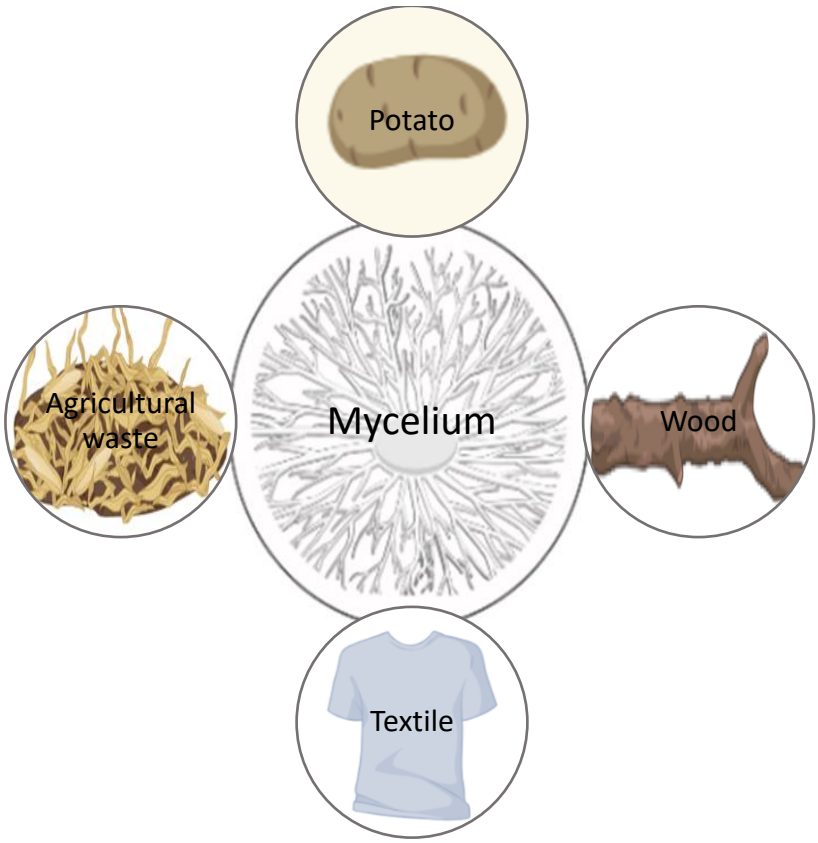


Figure 2.2: An illustration of mycelium with varieties of growth substrates used to develop biocomposites

This cultivation process involves mixing the mycelium with the organic substrate (Figure 2.2) in a controlled environment and allowing it to propagate through and bind the substrate particles together. As the mycelium grows, it colonizes the substrate forming a dense network of interconnected fibers, acting as a natural adhesive effectively binding the substrate particles into a cohesive material and improving its overall performance. This material presents a high degree of

customization and design flexibility,[121, 189] as it can then be molded into desired shapes, and it can be allowed to dry and harden, creating a solid product that exhibits enhanced strength, durability, and lightweight comparable to traditional materials. Different processing techniques have been applied to manufacture mycelium-bound biocomposites of complex shapes and structures, including compression molding, casting, and 3D printing. In fact, research and development on mycelium-bound biocomposites have made significant strides in exploring their potential as a replacement for conventional materials, such as polystyrene foam or particleboard within a wide range of applications in sustainable design and manufacturing like structural components in interior design elements,[124] industrial design and architecture,[143, 154] construction (e.g., insulation panels, acoustic tiles, flooring, roofing),[109, 114, 128, 154, 196] furniture and even fashion (e.g., biodegradable textiles and leather alternative).[108, 116, 117, 154, 155, 167, 197-201]

Understanding the material properties and growth behavior of mycelium enables scientists to develop innovative materials with unique properties and functionalities. Therefore various studies focus on characterizing the physicochemical properties of the mycelium-bound biocomposites. Several startups and companies have emerged to commercialize these mycelium-bound products for industrial-scale production. Collaborations between numerous startups, industries, research institutions, and established corporations are helping to advance the field and bring mycelium products to the market, by actively investing in research and development[202]. For instance, in recent years, industries have created protective and biodegradable mycelium-bound packaging materials that are gaining popularity as an alternative to traditional packaging like polystyrene foams.[109, 120, 123, 124, 147, 150] This collaboration within the scientific and business communities will play a crucial role in shaping the future of mycelium-based materials.[113, 203, 204] As technology advances and economies of scale are achieved, mycelium-bound materials may become more cost-

competitive with traditional materials, making them more attractive to industries and consumers.[105, 143, 205, 206] Researchers leverage these technologies to optimize production processes, improve material properties, and scale up manufacturing for commercial applications. Ongoing research is likely to uncover new and innovative uses for mycelium-bound products in the global market since continuous advancements in cultivation techniques and genetic engineering of mycelium strains may lead to faster growth rates and tailored material characteristics.[117, 147, 189, 207-212]

3 Chapter Three: *Pleurotus ostreatus* Coffee silverskin-bound Biocomposites as Thermal and Acoustic Insulation Materials in Building Construction

3.1 Abstract

The predominant use of synthetic materials, such as fiberglass and polymeric foams, for thermal and acoustic insulation in the construction sector contributes to the recalcitrant waste accumulation in the environment and is not economically sustainable in the long term. This is because they are developed with linear economy standards, they are neither reusable nor recyclable, and, at their end of the lifecycle, they are not compostable, with a great amount of them finishing in landfills. This work is focused on the development of natural, self-growing mycelium-biocomposites as sustainable alternatives to these conventional synthetic materials. Specifically, fungal mycelium derived from the non-pathogenic fungal strain *Pleurotus ostreatus* was fed by coffee silverskin flakes, a lignocellulosic agrowaste from roasted coffee seeds, forming three-dimensional biocomposites. The physicochemical properties of the obtained composite were thoroughly investigated, with a final focus on their thermal and acoustic insulation properties. As proved the natural agrowaste-mycelium composites possess high porosity and thus low density, good thermal properties, and satisfactory sound absorption capability. Such properties combined with the minimal energetic requirements for their growth and their fully compostable end-of-life nature make them valuable alternatives for thermal and acoustic insulation in building construction, among other applications, promoting environmental and economic sustainability.

Keywords**:** Coffee Silverskin, *Pleurotus ostreatus*, Porous Biocomposites, Self-Growing materials, Sustainability

3.2 Introduction

In recent years, the global construction industry has witnessed a paradigm shift towards sustainable and environmentally friendly practices, driven by the urgent need to address climate change and minimize the ecological footprint of human activities.[213-216] The construction sector is regarded as one of the main contributors to greenhouse gas emissions in the atmosphere, which have a substantial impact on climate change. It accounts for roughly 19% of global greenhouse gas emissions, making it a pollution hotspot that necessitates immediate mitigation measures.[216] In this context, the exploration and development of innovative materials have become imperative to meet the growing demand for energy-efficient and eco-friendly building solutions.[217]

In order to comply with environmental regulations and lessen its negative effects on the environment, the building industry has lately concentrated its efforts on developing bio-based materials to be used in the construction of sustainable building structures. Currently, traditional polymeric foams used for thermal insulation of buildings in temperate climate regions include fiberglass (Table 3.1), rock mineral wool (Table 3.1), polystyrene (Table 3.1), and polyurethane-based (Table 3.1) components. [218, 219] Furthermore, porous and fiber-based materials, such as metallic frames, fiberglass fabrics, and polyurethane foams, are commonly used for acoustic insulation (Table 3.1). These materials are typically applied as panels to walls, floors, and ceilings.[220, 221] While these materials can effectively insulate buildings from heat and sound, resulting in a safe and comfortable interior environment, the majority of them have various restrictions on their recycling and reuse,[222, 223] and they do not biodegrade (Table 3.1), with the exception, in some cases, of composting at the end of their lifespan (Table 3.1). Furthermore, a significant amount of energy is used in the complex manufacturing processes involved in their production (Table 3.1).[105, 224-228] Lastly, they may release dangerous compounds like carbon

monoxide, hydrogen cyanide, isocyanates, etc.[227, 229, 230] when exposed to severe environments like an unintentional fire, creating a well-known environmental health concern.[231, 232]

Biodegradable and bio-based thermal and acoustic insulation components are environmentally and economically sustainable substitutes for synthetic insulation,[233] since they typically require less energy to produce and transform than traditional systems. Using bio-based engineered building insulation reduces the risks of plastic pollution to the environment by acting as a counterbalance to the over-exploitation of finite natural resources and as a critical mitigation strategy to achieve environmentally safe solutions.[234-236]

Mycelium agro-waste-bound biocomposites offer a promising avenue for sustainable thermal and acoustic insulation in building construction (Table 3.1). As technology and research in this field advance, these materials may become more prevalent in the construction industry, contributing to a more sustainable and eco-friendly built environment. Paired with agrowaste materials, which are abundant byproducts of agricultural processes, mycelium-bound biocomposites present a unique opportunity to address both the environmental impact of waste disposal and the demand for effective insulation materials in construction. [237-239]

Mycelium-bound materials are lightweight yet durable, making them suitable for construction applications. The composites can be customized for specific structural and insulation requirements. In light of this, mycelium has emerged as a versatile and sustainable building block for the creation of biocomposites.[122, 143] Specifically, the network of branching, tube-like filaments known as hyphae that naturally grow beneath the earth known as the mycelium or vegetative portion of fungi,[240] can bind with various agricultural waste components, creating a strong and lightweight composite material.

Table 3.1: Comparative analysis of the properties of mycelium-based and conventional materials used for insulation applications

Material Property	Mycelium-Based Insulation	Fiberglass	Mineral Wool (Rock Wool)	Polyurethane Foam	Expanded Polystyrene (EPS)
Thermal Conductivity	(~0.03 - 0.10 W/m·K)[105, 241]	(0.034 - 0.040 W/m·K)[105]	(0.033 - 0.040 W/m·K)[105]	(0.020 - 0.030 W/m·K)[105]	(0.035 - 0.040 W/m·K)[105]
Acoustic Insulation	Excellent acoustic absorption)[105]	Excellent[105, 242]	Excellent[105]	Good[105]	Moderate[105]
Density	Low[105, 241]	High[105, 241]	High[105, 241]	High[105, 241]	High[105, 241]
Moisture uptake	Moderate[105, 241]	High[105]	High[105]	High[105]	Low[105]
Environmental Impact	Low, derived from natural, renewable resources[105, 241]	High, energy-intensive production, non-biodegradable[105]	Medium, requires mining, non-biodegradable[228]	High, derived from petrochemicals, non-biodegradable[227]	High, derived from petrochemicals, non-biodegradable[228]
Mechanical Properties	Compressive strength (0.17-1.1 MPa)[105];	Low compressive strength ; fragile to touch	Compressive strength; brittle when loaded	Compressive strength (0.002-48MPa)[105]	Compressive strength (0.03-0.69 MPa)[105]

	load-dependent				

Using natural substrates as feedstock, fibrous mycelium networks can grow economically and can form 2D fibrous mats,[243-247] or 3D porous structures depending on the environment.[248] Consequently, several mycelium strains, including *Ganoderma lucidum*, *Pleurotus ostreatus*, *Trametes versicolor*, *Schizophyllum commune*, and *Agaricus bisporus*, grown on wood by-products or agricultural waste such as wood chips, sawdust, straw, and other organic materials, molded into different shapes, have been proposed for components in furniture, [139] accessories,[139] fabrics,[201, 205] and packaging materials among others.[138, 139, 201]

Mycelium-bound biocomposites have natural insulating properties due to the entangled mycelial network, creating a porous structure. The mycelium itself acts as a thermal insulator, providing resistance to heat transfer. The porous structure of mycelium-bound biocomposites also contributes to effective sound absorption, making them suitable for acoustic insulation. The mycelial network can dampen sound waves, reducing noise transmission and improving indoor acoustic comfort.

Since its filamentous network forms a dense, three-dimensional structure with interconnected pores and the right porosity, density, pore structure, and filament dimensions for these kinds of applications, mycelium also naturally possesses insulating qualities.[105, 249] These properties act as a barrier against external stimuli like temperature changes[250] or the transmission of sound waves.[105, 221, 251-253] Mycelium in particular was discovered to be a remarkable mid-low frequency (< 1500 Hz) acoustic absorber. Different mycelia species have been mixed with different kinds of agricultural waste to produce panels and bricks for insulation applications. These products have

demonstrated good thermal and acoustic properties, meeting the requirements of the construction industry.[105, 168, 249, 250, 254] Furthermore, their acoustic insulation properties make them valuable components for creating soundproof environments, thereby addressing the acoustic challenges prevalent in urban and industrial settings.[105, 252] Examples that are representative of this type of cultivation include *Ganoderma resinaceum* mixed with Miscanthus giganteus fibers,[241] *Pleurotus ostreatus* grown on a combination of rice hulls, birch sawdust, and rye grain,[255] or on bagasse,[256] coconut husk,[257] rice husk,[121, 257, 258] juncao grass,[257] sawdust,[258] and cotton,[116, 259] *Trametes versicolor* grown on oak heartwood,[260] or on sawdust in combination with *Ganoderma lucidum*,[168] and *Trametes multicolor* cultivated on straw.[116, 259]

However, the choice of mycelium strain and agrowaste substrate affects not only the performance of the insulation but also the final product's overall characteristics and scalability.[105, 130, 259]

The main obstacles to their viability for large-scale construction projects are, in fact, the inappropriate mechanical characteristics of the final mycelium-agrowaste structure, the restricted supply of the selected agrowaste substrate, or the sluggish growth rate of some mycelium strains. Large-scale incubator investments and research on scaled-up controlled growth using readily available agricultural waste components are necessary for this material to become a competitive substitute for the insulating materials that are currently on the market.

We employed the *Pleurotus ostreatus (P. ostreatus)* strain in this study to address these problems because it grows quickly on lignocellulosic agrowastes and other natural substrates. The growing substrate was coffee silverskin flakes (CSF), a byproduct of the coffee industry. When green coffee beans are roasted, the outer layer known as CSF is released as the only-by product.[261] It makes up 4% (w/w) of the coffee bean, and since 10 million tons of coffee are produced annually, the coffee roasting industry produces about 400 thousand tons of waste annually.[262, 263] Its primary

ingredients are cellulose and lignin, and because of its availability and abundance, it's a desirable and renewable resource for making composites for a range of uses.[264]

Specifically, this study presents the production of 3D porous biocomposite materials with mechanical characteristics and thermal and acoustic insulation properties appropriate for the building construction industry, all while cost-effectively promoting environmental sustainability. This is achieved through the combination of CSF with *P. ostreatus* mycelium. The first weight ratio of (1:5) agrowaste: mycelium produces composites with the highest sound absorption capacity by encouraging the most effective mycelial growth colonization on CSF in less than a week. The produced biocomposites have low heat conductivity as well, on par with traditional insulating materials. This is a breakthrough in the state-of-the-art biocomposites for the construction industry that are made from inexpensive, readily available, renewable resources and require little energy to develop. In fact, the components shown here, along with the method of fabrication, are sustainable and environmentally friendly substitutes for the conventional materials used for thermal and acoustic insulation, such as fiberglass, expanded polystyrene, and polyurethane foams.

3.3 Materials and Methods

3.3.1 Materials

Materials. Potato dextrose broth (PDB) (P6685) was purchased from Merck and used as a growth medium. The *P. ostreatus* active culture was purchased from DSMZ, Germany, and maintained in a 100 mm Petri dish with PDB, transferring the culture to a fresh medium every 30 days. The coffee silverskin flakes (CSF) 0.3-1 cm in size were gently offered from Covim S.p.A., Genova, Italy. MilliQ water was used in all experimental activities.

3.3.2 Biofabrication Techniques

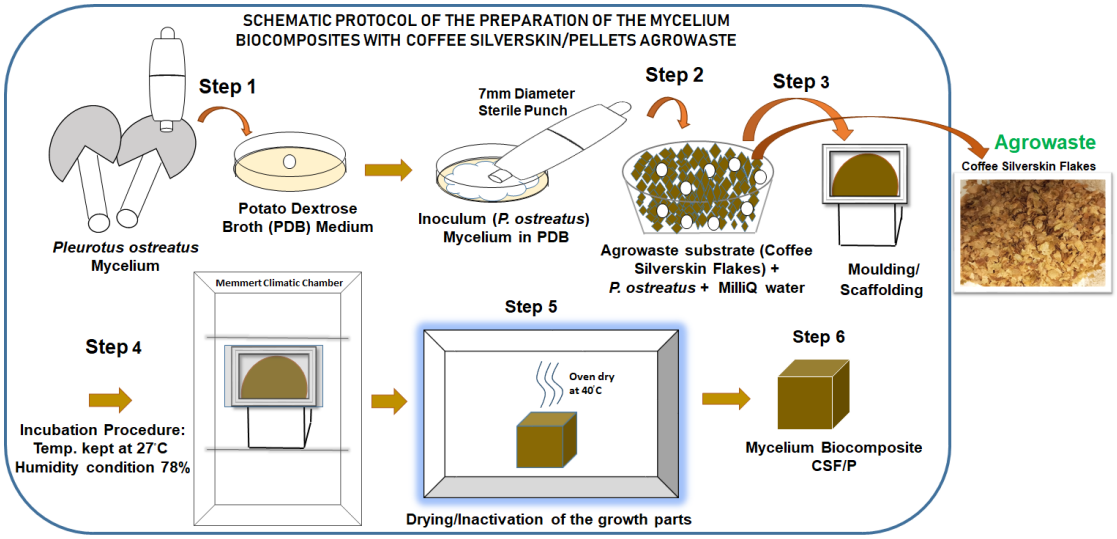


Figure 3.1 Schematic protocol for the fabrication of the 3D mycelium-bound biocomposite materials.

3.3.2.1 Fabrication of the Biocomposites

The 3D mycelium-CSF biocomposites were fabricated following the scheme shown in **Figure 3.1**. Firstly, the mycelium (*P. ostreatus*) was cultured in a 2D form for 28 days in a PDB medium. The CSF flakes were grinded to decrease their sizes and filtered by sieving in order to obtain flakes with sizes ranging between 3 and a few tens of micrometers. A 10g of CSF powder was mixed with 10 mL of MilliQ water, and then sterilized in an autoclave (Systec VX-40, SN: 6050, Germany) at 121°C for 1hr 30 min. Subsequently, 7 mm diameter punches of the mycelial inoculum were mixed with the CSF dispersion using a sterilized spatula under the biohazard hood

(Angelantoni Life Science Srl, VBH 48 equipped with a 30W germicidal ultra-violet lamp, wavelength 253.7 nm (UV-C)). The different initial weight ratios of CSF/mycelia are listed in **Table 3.1**. The mixtures were then placed in sterilized cubic-shaped (5x5x5cm^3) silicone molds. The silicone molds were covered with sterile aluminum foils and placed inside a climatic chamber (Memmert HPP 260) to be incubated at controlled environmental conditions under dark for a period of one week at 27°C and 78±2% relative humidity (RH), to ensure stable growth of the mycelium biocomposite. During this period the growing mycelium was fed on the CSF and colonized the organic substrate through the formation of interwoven three-dimensional filamentous networks, into the shaped molds resulting in the formation of lightweight block (4x4x3cm^3 in length, width, and height respectively) of biocomposites in silicone mold. The biocomposites developed were then removed from the silicone mold, oven-dried at 40°C for 10h to deactivate the mycelium growth, and kept at room temperature for further characterization.

Table 3.2: Labels and initial Agrowaste/Mycelium composition ratios (wt. %), before the incubation process, of the different biocomposite samples.

LABEL	AGROWASTE/MYCELIUM COMPOSITION RATIO	CSF/P Wt %	MYCELIUM Wt %
CSF/P 1:1	CSF 10g/P 10g	50%	50%
CSF/P 1:2	CSF 10g/P 20g	33.33%	66.67%
CSF/P 1:5	CSF 10g/P 50g	16.67%	83.33%

3.4 Characterizations

3.4.1 Morphological Characterization

To determine the morphology of the developed samples, digital photos were captured, using a Canon digital camera (Canon EOS 5D Mark II, DS126201, Japan). Scanning electron microscopy (SEM) analysis was conducted using a JEOL JSM 6490LA microscope at 10kV accelerating voltage. For the observation, samples were sputter coated with a 10nm thin gold layer (Cressington 208HR, Cressington Scientific Instrument Ltd., UK) and mounted on aluminum stubs, with double-stick carbon tape.

3.4.2 Chemical Composition Analysis

For the FTIR analysis, the biocomposite materials were pressed in a carver press (Model 3853CE Carver Inc. USA) for 5 minutes at 35°C under a clamping force of 2 metric tons. The test was performed at 50% Relative humidity (RH). The chemical composition and the possible chemical interactions of the components of the developed samples were studied using a single-reflection attenuated total reflection (ATR) accessory (MIRacle ATR, PIKE Technologies) coupled to a Fourier Transform Infrared (FTIR) spectrometer (Equinox 70 FT-IR, Bruker). All spectra shown were averaged from 128 repetitive scans recorded from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1}.

3.5 Density and Porosity

Skeletal density, i.e. the ratio of the mass of the solid to its volume excluding open and closed pores, was measured by helium pycnometry (Thermo Scientific Pycnomatic ATC). To do so, the dry samples (~0.129g of each sample) were placed in a 4 cm^3 cuvette and suspended in the pycnometer at 20.0 ± 0.01 °C, using helium as a measuring gas. The measurements were repeated ten times for each sample at (20°C and 51%RH), and the accuracy was set to be ± 0.001%.

The obtained skeletal density was then used to calculate the effective porosity and pore size distribution using a Pascal 140 Evo and a Pascal 240 Evo mercury intrusion porosimetry (MIP; Thermo Fisher Scientific) with mercury intrusion pressure ranges of 0.001-0.400 MPa and 0.1-200.0 MPa, respectively. The data obtained from both porosimeters were combined and correlated to an equivalent pore size range of 0.01-100.0 μm using the S.OL.I.D. Evo Software by the Washburn equation assuming a cylindrical and plate model, and a mercury contact angle and mercury surface tension of 140° and 0.48 N m^{-1}, respectively. For the measurement, the sample was rolled up in special support for membrane analysis and inserted in the dilatometer.

3.5.1 Water Interaction

An OCA 20 contact angle goniometer (DataPhysics, Instruments GmbH, Filderstadt, Germany) was used to define the wettability of the samples at room temperature. To ensure a flat and uniform surface, the samples were pressed in a carver press (3853CE Carver Inc. USA) for 5 minutes at 35°C under a clamping force of 2 metric tons at 22°C and 60%RH. 5 μL droplets of deionized water were deposited on the samples' surface and the contact angle was calculated from the side view with the help of the instrument's software. For each sample, the measurements were carried out at five random locations of their surface, and their average values were presented. To determine the humidity adsorption efficiency, the samples were dried first at 105°C for 4h and then conditioned in a desiccator to remove any adsorbed humidity. The dried samples were then weighed on an electronic balance and then placed in a humidity chamber (RH 100%). After remaining in the humidity chamber for 9 days each sample was removed and weighed and the amount of adsorbed humidity was calculated as presented in Equation 1.

$$Humidity\ Adsorption\ (\%) = \frac{M2-M1}{M1} X100\% \quad \text{.....(1)}$$

Where M_1 is the initial dry mass and M_2 is the mass of the samples after the conditioning in the humidity chamber. During the 9 days of conditioning in the chamber the humidity was monitored using a Tinytag Ultra2 635509 Hygrometer device.

3.5.2 Mechanical Characterization

The mechanical properties of the samples were determined by a uniaxial test (compression test) on a dual-column universal testing machine Instron 3365 (Instron, Norwood, Massachusetts, USA). The compression tests were carried out on the composite sample cubes (4x4x3 cm^3) using a 2 KN load cell. The compression strain rate was set to 10%min^{-1}. The compression modulus was determined from the slope of the initial linear region of the stress-strain curve. The test was performed at ambient conditions (25°C and 50%RH).[198] For each growth condition (CSF/P 1:1, 1:2, and 1:5), five samples were tested, and the results were averaged to obtain a mean value.

3.5.3 Thermal Characterization

The thermal conductivity of the biocomposites was measured using a modified transient-plane source technique on a thermal conductivity analyzer (C-Therm Technologies, TCi) following the ASTM D7984 test method. For the analysis, up to five measurements were performed for each sample at (24°C and 50%RH). Thermogravimetric (TGA) analysis on the samples was performed using a Q500 analyzer (TA Instruments, USA) at 20°C and 53%RH. 5-10mg of the biocomposites, were placed into 100µL platinum pans. Each one of the samples was heated at a rate of 10°C min^{-1} from 30°C to 800°C under a nitrogen atmosphere with a flow rate of 50mL min^{-1}.

3.5.4 Acoustic Test

The acoustic measurements were performed by a portable sound level meter (SLM) (type 01 dB Fusion, Acoem, France) at (25°C and 46%RH). The test container (a cubic PVC box) had external dimensions of 20x20x21cm^3 and an opening of 5x5cm^2 to which the biocomposite and reference test samples were fixed before the acoustic measurements. The biocomposite samples prepared for this test, had dimensions 10x10 cm^2, with a thickness of 2.4 cm. A flexible, open cell, polyurethane foam with an eggshell structure (skeletal density 1.17±0.03 gr/cm^3, highly interconnected pores, and mean pore size 148±99 μm with dimensions 12x12 cm^2, with a thickness of 2.5 cm, used for sound absorption applications, was also tested as a reference sample. The test procedure was performed in several steps

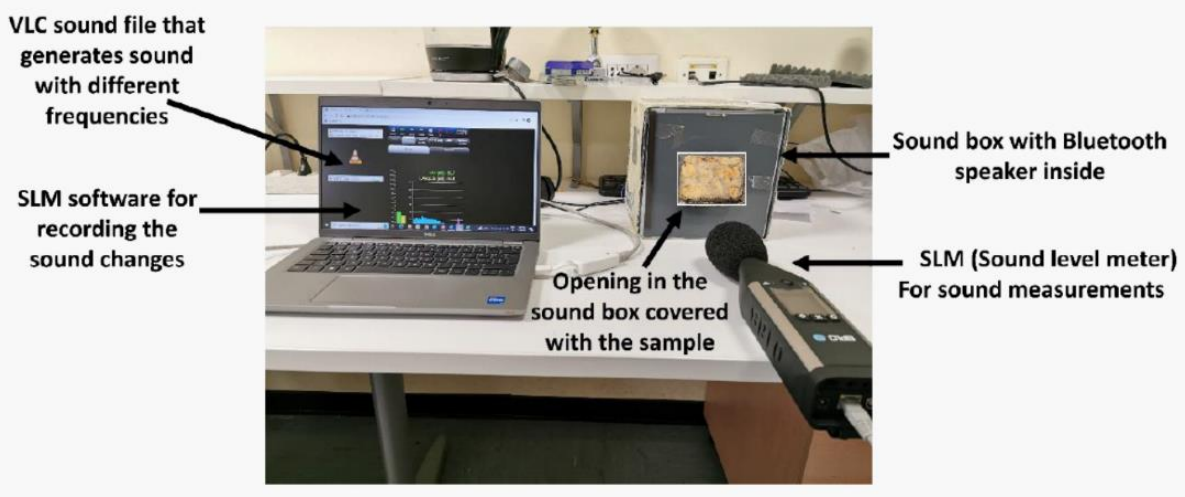


Figure 3.2 Test setup for the evaluation of the sound absorption capabilities of the composite CSF/P samples.

Figure 3.2 gives a demonstration of the set up for the acoustic test: Firstly, the speaker was switched on and placed inside the test container, then the testing sample was fixed in order to cover completely the opening of the container, and the cubic box was sealed with black magnetic tape. The SLM was then placed 12 cm away from the sample and pointed towards the source (0° incidence). Subsequently, audio playbacks of stored audio recording files at frequencies between 5-10000Hz, used as the sound source, were transmitted by the speaker. The sound test

measurements involved measurements of the background noise level while the sound source is not operating, and the transmitted, though the polyurethane foam or composite samples, sound when the sound source is operating. All steps of sound transmission and recording have been performed through the control laptop and the FUSION wireless network.

3.6 Results and Discussion

3.6.1 Morphological characterization

The photographs of the biocomposites derived from the various CSF to mycelia weight ratios are shown in **Figure 3.3 (A-C).** The images indicate a successful growth of the *P. ostreatus* mycelium within and around the CSF since the whitish mycelial colonization can be observed throughout the samples. Notably, the mycelium component in the final composite samples increases, as the mycelium inoculum in the initial composition increases (from 1:1 to 1:5 CSF: mycelia weight ratio, CSF: P). This observation is further confirmed by the SEM analysis where the microstructure of the fibrous mycelium within the biocomposites was evaluated. Indeed, as shown in the SEM images of the composite samples, in **Figure 3.4 (A-F)**, the non-directional growth of the mycelium filaments (of a few mm diameter), forms an interconnected fibrous network in between and around the CSF particles. Such a network results in a 3D porous structure comprising air pockets formation. This filamentous network becomes denser as the amount of the mycelium inoculum in the initial composition increases, resulting in a better-defined fibrous 3D network.

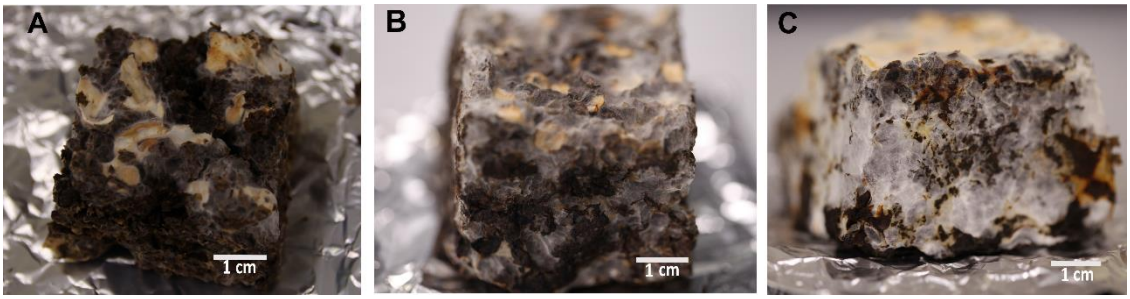


Figure 3.3 Photographs of the 3D biocomposite samples with initial weight ratios CSF/P 1:1 (3x4x2cm³) (A), 1:2 (2x5x3cm³) (B) and 1:5 (2x5x4cm³) (C). The initial ratio between the mycelium and the biomass used for its growth clearly affects the morphology of the final samples after one week.

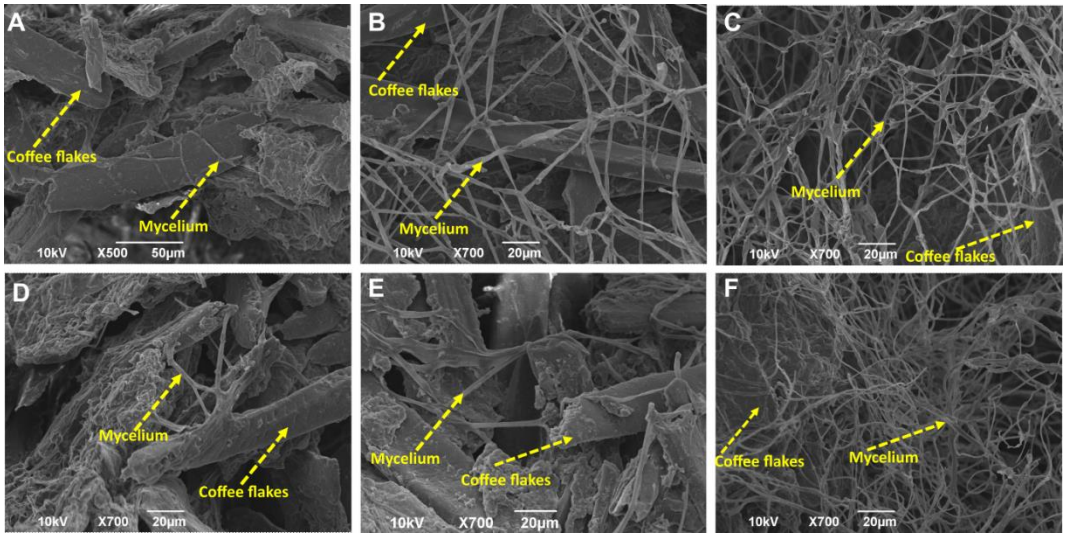


Figure 3.4 Morphological characterizations. (A-C) Surface and (D-F) Cross-sectional SEM images of the CSF/P 1:1 (A,D), 1:2 (B,E) and 1:5 (C,F) samples, showing the morphological differences between them.

3.6.2 Porosity Analysis

The MIP analysis was performed, in order to determine the porosity and the pore size distribution of the developed 3D biocomposites, focusing on those with the highest amount of mycelium fibers (CSF/P 1:5). As shown in **Figure 3.5**, the biocomposites pores' size distribution is broad, but around 80% of the cumulative pore volume has pores with diameter between 3.6 to 100.0 µm, while the overall porosity is 53.46%.

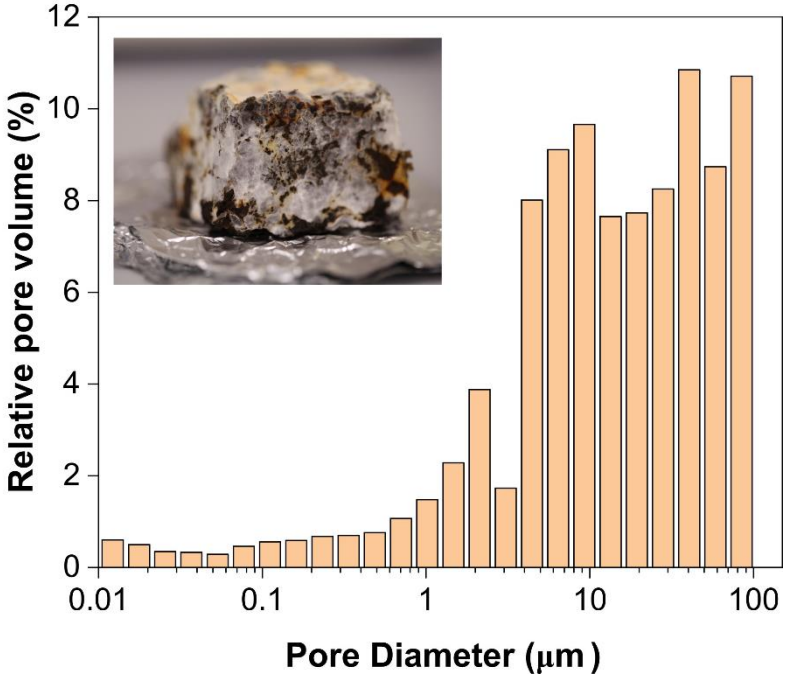


Figure 3.5 Pore size distribution measurement for the CSF/P biocomposites.

3.6.3 Chemical Composition Analysis

The *P. ostreatus* mycelium mats grown on PDB, the pristine CSF, and the produced biocomposites were analyzed by ATR-FTIR spectroscopy, and the results are reported in (**Figure 3.6)**. The spectrum of *P. ostreatus* (black line) presents the typical bands of the O-H and N-H stretching modes between 3500 and 3100 cm^{-1}, attributed to the protein and saccharide components and the adsorbed humidity; the asymmetric and symmetric CH_2 and CH_3 stretching modes at 2955, 2918, 2876, 2851 cm^{-1} which are due to the lipidic part of the mycelium; the amide I and amide II stretching modes centered at 1641 and 1541 cm^{-1} attributed to the proteins of the mycelium, while the C-O and C-O-C stretching modes between 1200 and 1000 cm^{-1} are typical of polysaccharides.[130, 243]

At the spectrum of CSF (red line) the O-H and N-H stretching modes between 3500 and 3100 cm^{-1} are also related to adsorbed humidity, protein, and saccharide components; the aromatic C-H stretching centered at 3060 cm^{-1} is attributed to small aromatic molecules such as caffeine, and phenolic acids such as chlorogenic acid, caffeic acid, and coumaric acid;[265] the asymmetric and symmetric CH_2 and CH_3 stretching modes at 2959, 2920, 2887, 2851 cm^{-1} are linked to the caffeine and the lipidic part of coffee; the C=O stretching at 1736 cm^{-1} is attributed to the fatty acids present in the coffee; the C=O and C=C stretching modes between 1640 and 1510 cm^{-1} are due to the lignin and phenolic acid structures; the peaks 1450 cm^{-1}, 1377 cm^{-1} and 893 cm^{-1} are assigned to the β-linkage of cellulose, and the C-O and C-O-C stretching modes between 1238 and 1028 cm^{-1} are due to the presence of the polysaccharides, monosaccharides, and phenolic compounds present in the coffee.[265-269]

In the CSF/P biocomposites, the spectra display overlapped peaks of the isolated components of the pure CSF and *P. ostreatus*. However, moving from the ratios 1:1 to 1:5, the intensity of the C=O stretching of the fatty acid present in the coffee decreases. As we move to the CSF/P 1:5 sample, the concentration of the mycelium component is higher, and probably more fatty acids are metabolized causing their reduced concentration in the final sample after growth. Moreover, the peak of the amide II, 1541 cm^{-1} typical of the mycelium protein increases, confirming a higher final concentration of the mycelium in these samples (**Figure 3.6).** Overall, we can conclude that the pure mycelium and the CSF are both present in the biocomposites and that probably in the sample of a higher concentration of mycelium, the coffee is metabolized at a higher degree by the mycelium during its growth.

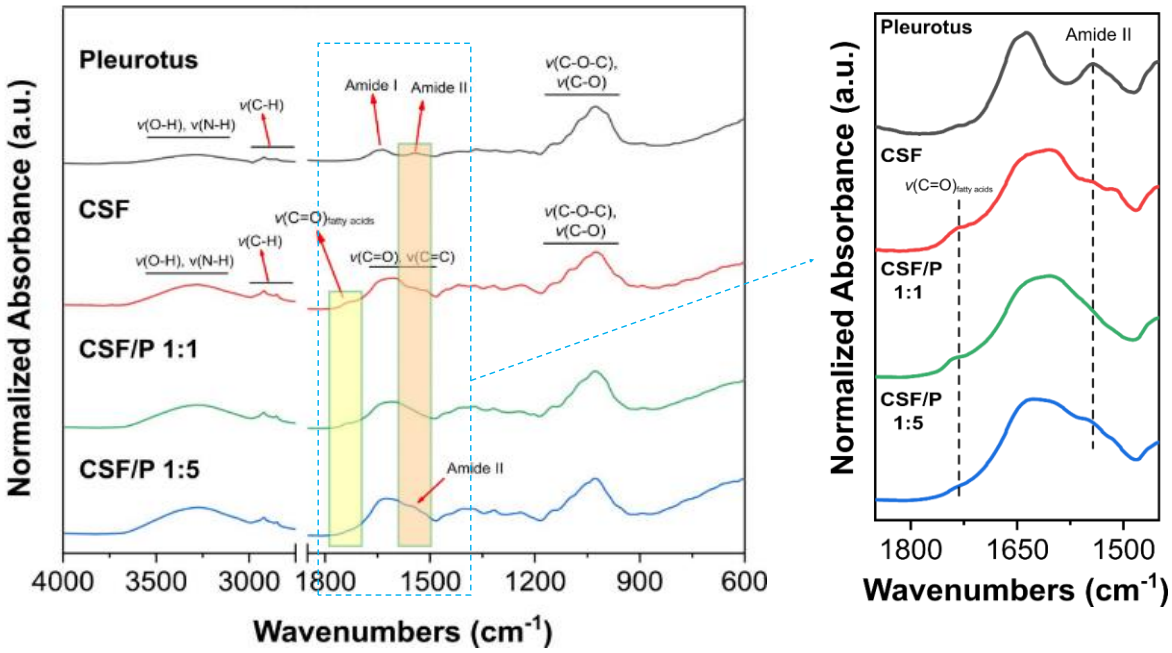


Figure 3.6 ATR-FTIR spectra for P. ostreatus, CSF, CSF/P 1:1, CSF/P 1:5 samples, respectively. The area of the C=O stretching of the fatty acids in the coffee and the Amide II stretching of the mushroom proteins are highlighted by the yellow and red bars.

3.6.4 Thermogravimetric Analysis

To evaluate the thermal degradation behavior of the CSF/P biocomposites in comparison with the individual components, TGA was performed as shown in **Figure 3.7 (A-B)**. For all samples tested, the initial degradation step below 100°C depicts the desorption of humidity. For the pure mycelium sample the small weight loss observed between 120 °C and 205 °C may be related to the degradation of polysaccharide side chains alone (such as α-glucans) or in glycoproteins, mainly branched glucomannan.[270] The main degradation step was observed between (200–325 °C) with a T_{max} at 304°C prevalently attributed to the breakdown and volatilization of the polysaccharides backbone and especially of the β-glucans. Finally, the last degradation step observed in the range between 325°C and 487°C is related to the degradation of chitin-glucans and pure chitin[270]. On the other hand, for the CSF, two main degradation steps with T_{max} 248°C and 300°C were observed, **Figure 3.7(B).** The step at T_{max} 248°C is mainly attributed to the degradation of hemicellulose,

while the second one, with T_{max} 300°C, is attributed to the degradation of cellulose.[271] Concerning the CSF/P biocomposites, there are not extra degradation steps apart from those of pure mycelium and CSF, indicating that their interaction did not produce components that degraded earlier.

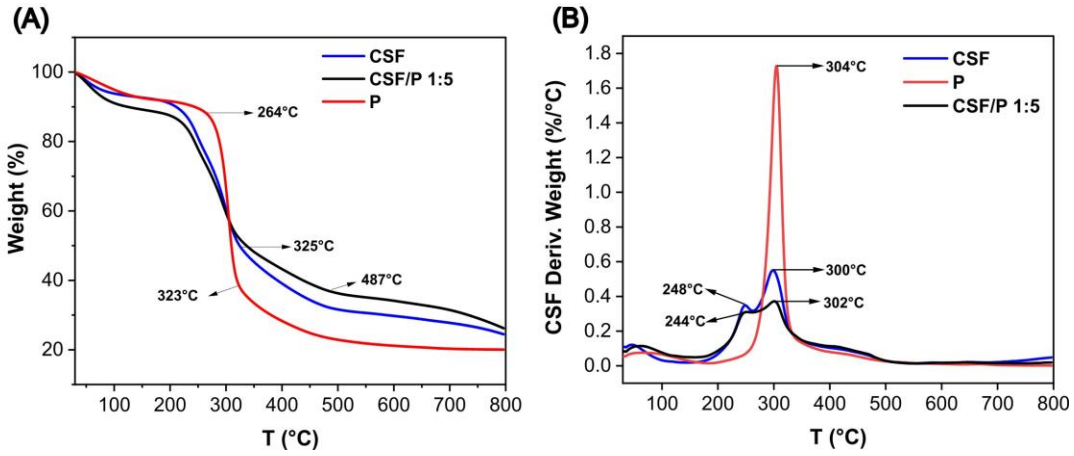


Figure 3.7: (A) TGA thermograms and (B) derivative thermogravimetric curves analysis of the CSF, P. ostreatus, and of the CSF/P 1:5 samples.

3.6.5 Wetting Properties

To determine the wetting properties of the developed mycelium biocomposites, water contact angle (WCA), and relative humidity measurements were performed. As shown in **Figure 3.8(A)** for all samples, the WCA values are around 114, 122, and 139°, with an increasing trend as the mycelium amount increases (i.e. the 1:5 biocomposite is the most hydrophobic), attributed to the hydrophobic nature of specific proteins (such as mannoproteins and hydrophobins) that can be found in the outermost layer of the fungal cell wall, and also to the micrometric roughness of the samples related to the fibrous nature of the developed systems.[130] This is further supported by the humidity adsorption study (**Figure 3.8(B)**). As shown, the values of the relative humidity adsorbed on the samples are very low (< 20 %) for all cases. However, the humidity adsorption decreases with increasing mycelium amount in the biocomposites, clearly indicating that upon its growth,

the mycelium metabolizes the hydrophilic CSF into hydrophobic mycelium cell wall components.

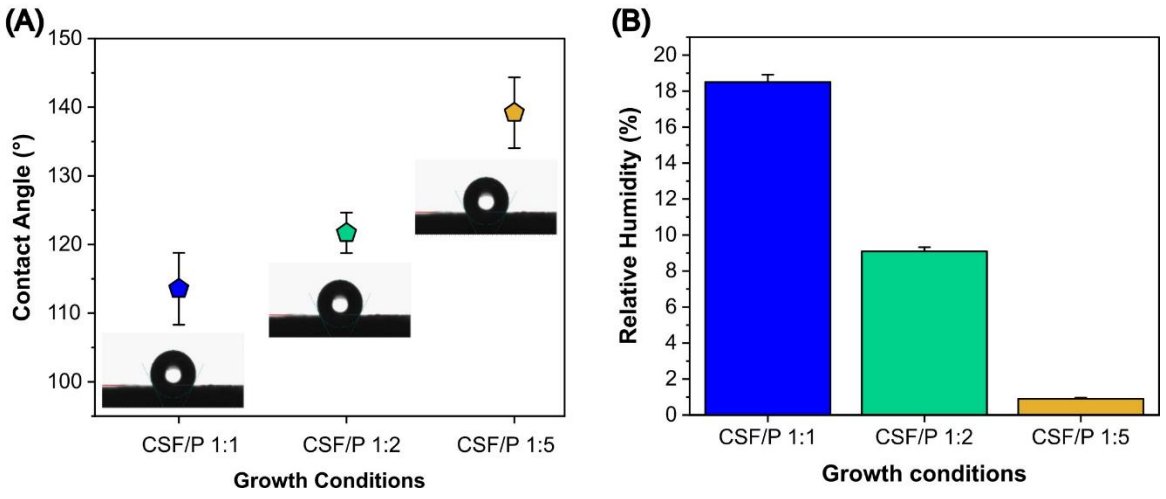


Figure 3.8: Water Interaction. (A) Static water contact angle measured on the CSF/P biocomposites. (B) Humidity adsorption of the CSF/P biocomposites in the various growth conditions (CSF/P 1:1, 1:2, and 1:5).

3.6.6 Mechanical characterization

To determine the mechanical strength of the developed biocomposites, compressive strength tests were conducted. The compressive strength of the mycelium-CSF cuboid samples depends on their porosity, pore size, and material characteristics including the interaction of the CSF to the *P. ostreatus*mycelium, and to their density (0.066gcm^{-3}, 0.071gcm^{-3} and 0.090gcm^{-3} for CSF/P 1:1, 1:2 and 1:5 respectively). **Figure 3.9(A-B)** shows that CSF/P 1:5 samples display a higher compressive strength and compressive modulus when compared with the other two types of biocomposites (CSF/P 1:1 and 1:2). In particular, Young's compressive modulus of the biocomposites is 0.06, 0.37 and 0.40 MPa, for the CSF/P 1:1, 1.2, and 1.5 samples, respectively (**Figure 3.9B**). The performance falls within the same range as other mycelium-bound composites of similar density and is comparable with traditional synthetic insulation polymers. In particular mycelium composites containing fibrous dispersed, flax and hemp hurd, with slightly higher

density (0.099 and 0.094 g cm^{-3}, respectively) have shown Young's compressive moduli of 0.73 and 0.64 MPa[198], respectively. Furthermore, conventional thermal and sound insulation products made from expanded polystyrene, with densities between c.a. 0.048 and 0.793 g cm^{-3} show a compressive strength between c.a. 0.02-2.50 MPa.

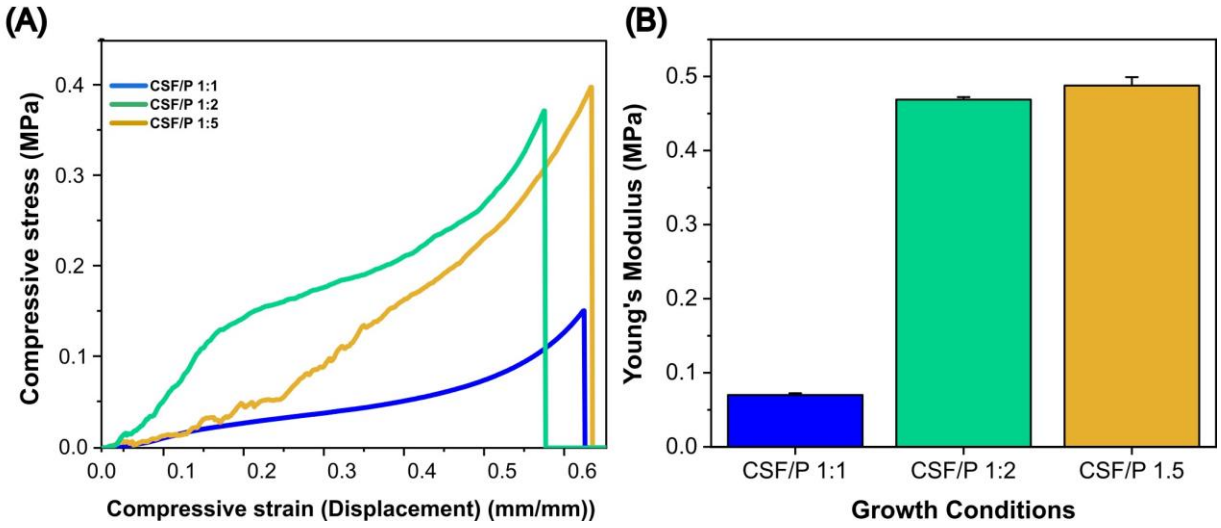


Figure 3.9: Mechanical characterizations: (A) Compression strength, (B) Young's modulus for the CSF/P biocomposites.

3.6.7 Insulating Thermal and Acoustic Properties

To evaluate the insulation capabilities of the developed samples, their thermal conductivity (TC) was measured. As can be seen in **Figure 3.10(A),** the mean TC values of the biocomposites fall within the range of (0.03-0.04) $Wm^{-1}K^{-1}$, values representative of most of the insulation materials of natural or synthetic origin reported so far,[241] indicating their suitability for thermal insulation applications. In particular, mycelium-bound composites containing high-performance natural insulators such as straw and hemp fibers present thermal conductivities values between 0.04–0.08 $Wm^{-1}K^{-1}$[1105]. Such performances are comparable with the ones of conventional thermal insulation

products, such as expanded polystyrene (EPS (0.03-0.04 $Wm^{-1}K^{-1}$))[105] and glass wool (0.04 $Wm^{-1}K^{-1}$).[105]

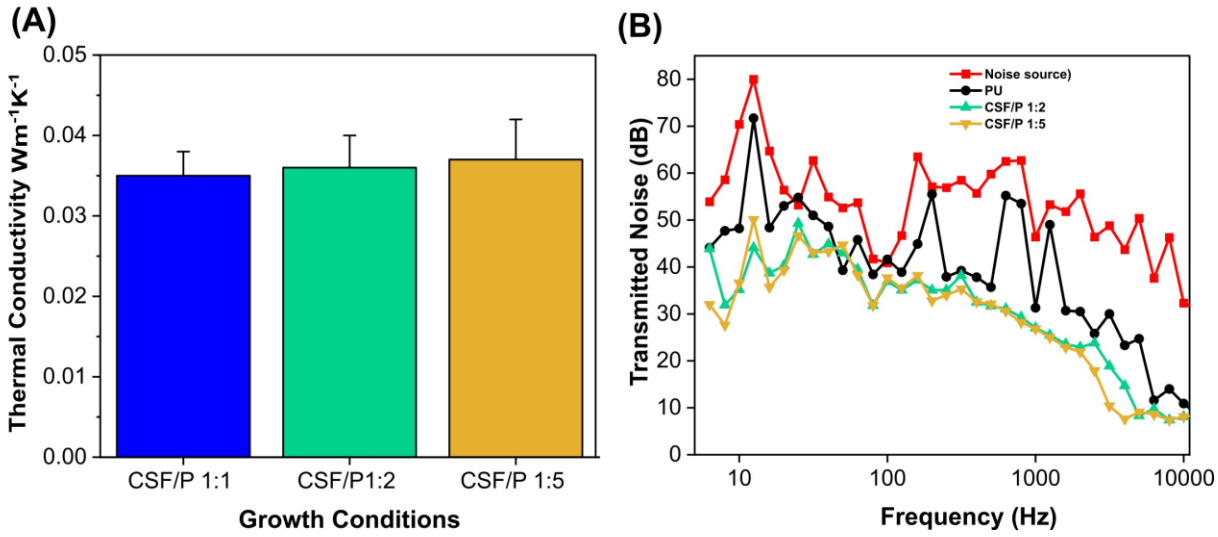


Figure 3.10: (A) Thermal Conductivity of CSF/P biocomposite samples. (B) Sound absorption coefficient of the biocomposites compared to that of Polyurethane foam. The data is based on an integrated speaker noise excitation (0-10000Hz).

Figure 3.10(B) shows the transmitted sound energy derived from the sound tests conducted on the CSF/P samples**.** The results indicate that the biocomposite materials developed in this study exhibit a better acoustic insulation performance compared to a conventionally used acoustic insulation material (polyurethane foam) at all the frequency levels tested. In particular, from the results obtained, it can be deduced that both of the biocomposite samples tested (CSF/P 1:2, CSF/P 1:5) are promising for improved performance over the standard sound absorption system, especially in the region of 1000 Hz which is the critical frequency range for absorption of road noise. The CSF/P 1:1 composite was excluded from the sound test, due to its above presented poor mechanical properties. The performance of the other two biocomposite samples is similar to what is reported in the literature for some other mycelium-agrowaste biocomposites that have been developed with different mycelium strains and agrowaste substrates.[105, 251] In particular, acoustic perception of

road noise through mycelium-bound composites with fillers such as rice straw-sorghum fiber, rice straw-cotton bur fiber, and sorghum fiber-switchgrass was reported in the range 45.5-47.0 dB[105, 251] whereas the perception through the CSF/P samples produced in this work was found to be lower, between 25-30 dB. Such performance is attributed to their porous and fibrous nature. In fact, the thin fibers in the mycelium biocomposites provide good acoustic absorption, acting as frictional elements since they can move easily resisting the acoustic wave motion and decreasing its amplitude. The sound waves that attempt to move through the undulating passages of the material are converted to heat in the process.[251]

3.7 Conclusion

The successful development of novel agrowaste-based mycelium biocomposite materials has been achieved in this study. The biocomposites produced using CSF agrowaste substrate and *P. ostreatus* mycelium possess characteristic properties such as low thermal conductivity between 0.03 to 0.04 $Wm^{-1}K^{-1}$, and good acoustic isolation, which together with the thermal stability and hydrophobicity make them valuable candidates for biodegradable, thermal, and acoustic insulation materials in building and construction. The herein proposed systems can replace the conventional insulation and acoustic materials such as glass or rock mineral wool, polyurethane extruded polystyrene foams, etc. with natural systems of low environmental impact. In fact, due to its self-growing nature and its natural components, the system requires minimum energy consumption for its fabrication, while at the end of its lifespan, it can be disposed of in compost and treated as common organic waste.[141] This work paves the way for innovative solutions that prioritize eco-

consciousness, aligning with the growing demand for greener and more sustainable solutions in the construction industry.

4 Chapter Four: A Green-Processing Route for Mycelium Biomass/PHB Biocomposites

4.1 Abstract

This research investigates a green-processing route for the development of biocomposites utilizing mycelium biomass and polyhydroxybutyrate (PHB). The methodology involves cultivating mycelium on an organic substrate (Coffee silverskin agrowaste) to form a dense network of hyphal threads, which serve as a natural binder within the biocomposite structure. Subsequently, the mycelium biomass/PHB biocomposites are fabricated using environmentally friendly processing techniques, including extrusion, injection, and compression molding, to achieve desired shapes and mechanical properties. This research investigates the effects of mycelium content, processing parameters, and sample composition on the performance of the biocomposites, including mechanical strength, thermal stability, biodegradability, oxygen permeability, food migration, and antioxidant properties. The results demonstrate the feasibility of utilizing mycelium biomass as a sustainable reinforcement in PHB-based biocomposites, offering potential applications in the packaging and consumer goods industries. The biocomposites are presented as a unique avenue for sustainable materials fostering innovation in biomaterials research.

Key words: Sustainable materials, Extrusion, bio-based polymers, Biodegradable composites, PHB, Mycelium

4.2 Introduction

Owing to the pressing need to address environmental issues and lessen the ecological footprint of human activity, the search for sustainable and environmentally friendly materials has gained attention in several industries in recent years.[272] In this regard, mycelium-based composite materials have come to light as a promising solution that is consistent with the sustainable development and circular economy.[124, 125, 154] In fact, the utilization of mycelium for the development of composites, has the potential to completely transform material science and industrial processes due to its extraordinary ability to bind organic substrates into cohesive structures.[273, 274]

Issues regarding the industrial applicability of mycelium-based composites are an important topic that this thesis seeks to unearth, addressing the major obstacles and offering insights into scalable production processes. Actually, the increasing need for sustainable substitutes for current plastic components makes it critical to comprehend how mycelium-based materials can be effectively incorporated into current industrial structures in order to ensure their widespread adoption and potential impact[154]. This research adds to the body of knowledge that directs the development of sustainable materials suitable for mass production by exploring the complexities of fungal growth, and manufacturing process optimization. Furthermore, it clarifies whether integrating mycelium-based composites into standard industrial processes is both feasible and affordable. This is explored by the incorporation of the mycelium-bound composite into bio-based polymer materials, particularly PHB combined with a plasticizer, (Epoxidized soyabean oil methyl ester (ESOME)) to develop biodegradable composite materials by melt extrusion, injection, and compression molding processes. ESOME was added into the biocomposites to improve the interfacial adhesion

between the fibers and polymer matrix, enhance the processing stability, and impart specific properties such as the reduction of the surface roughness.

Extrusion was employed to mix the natural fibers or fillers with the polymer matrix for efficient manufacturing. It is a commonly used process in the manufacturing of biocomposites due to its ability to efficiently blend materials, control the composition, and produce various shapes. Biocomposites developed with extrusion involve the use of advanced manufacturing techniques to produce composite materials that combine natural fillers with a polymer matrix. The process involves melting the polymer, mixing it with the fillers, and forming the composite into the desirable structure. Twin-screw extrusion was used to optimize the dispersion of fibers and achieve the desired properties. To this end, various biodegradable or bio-based polymers are used as the matrix material. Examples include polylactic acid (PLA), PHA, polybutylene succinate (PBS), and polyethylene derived from renewable resources. In this research PHB is used as the polymer matrix where the mycelium biomass is introduced following the abovementioned processes, complementing each other for the formation of a sustainable biocomposite system. Since the use of ESOME as a plasticizer in PHB, MycB melt extruded composites have not been explored by existing literature; the results would be helpful in the development of novel PHB/ESOME/MycB composite.

The developed biocomposites through extrusion, injection, and compression molding can be used in a wide range of applications including biodegradable food packaging,[275] disposable cutlery,[276, 277] agricultural films,[94] interior automotive components, decking and fencing, furniture,[115, 120, 278] 3D printing filaments,[279] construction to textiles and beyond.[122, 184] As will be proved in this study, the incorporation of mycelium based fillers into the PHB matrices, expands the range of sustainable biomaterials available for composite manufacturing and enhances the mechanical

strength, thermal stability, biodegradability, and customizable material properties of the PHB-based biocomposites. Additionally, the introduction of the mycelium biomass into the polymer matrix offers advantages such as lowering the composites overall cost, and lowering their density. This study builds upon and contributes to current research by exploring the utilization of mycelium biomass as a natural reinforcement in biocomposites, which is relatively novel compared to conventional reinforcements such as plant fibers or nanoparticles. It advances the current understanding and capabilities in the field of sustainable biomaterials and biocomposite manufacturing and hence represents a promising approach for creating sustainable, high-performance materials compared to conventional plastics or even traditional biocomposites[124, 128] This study focuses on green processing techniques avoiding the use of toxic chemicals typically associated with traditional manufacturing processes, contributing, with this low-impact production, to a more sustainable industrial ecosystem.[128, 280, 281] The comprehensive characterization and thorough evaluation of the mechanical, thermal, and biodegradability properties of the mycelium/PHB biocomposites through scanning electron microscopy (SEM), mechanical testing, thermal analysis, and degradation studies gives clarity about the structure-property relationship of these materials to ascertain their suitability for packaging and other applications. Overall, this study leads to the discovery of new formulations, processing techniques, and additives that enhance the performance and sustainability of bioplastic composites, driving the industry toward more environmentally friendly solutions.[281]

4.2.1 State of the art

Research on materials is moving toward the creation of polymeric materials made from renewable resources as worries about the effects of synthetic plastics on the environment grow.[201] Combining biopolymers like Polyhydroxybutyrate (PHB) with mycelium represents a potentially fruitful field of research.[282] Microorganisms naturally produce PHB, a biodegradable and biocompatible polymer that is usually synthesized in environments with limited nutrients.[283] Mycelium, on the other hand, provides an inexpensive, renewable platform for making sustainable composites.[284] The potential for PHB and mycelium to work together to create new, eco-friendly materials that could take the place of synthetic polymers and conventional plastics in a range of applications has drawn attention to this synergy.[282] Unlike conventional plastics, both mycelium and PHB are biodegradable, meaning that they can decompose naturally without leaving behind hazardous residues.[282] Using mycelium in conjunction with PHB presents a viable path toward the creation of biodegradable and sustainable materials. Because of its physical similarities to polypropylene and its biodegradability, PHB is considered a promising substitute for conventional plastics.[283, 285] The mixing of mycelium together with polymers have been the subject of recent research aimed at creating novel biocomposites with improved mechanical strength, flexibility, and biodegradability.[130, 286] PHB can be made more mechanically effective while maintaining its biodegradable properties by blending it with materials such as mycelium.[282] PHB's strength is increased by mycelium, which acts as a reinforcing matrix, enabling the composite to be used in structural and other suitable applications.[282, 287-289]

The main focus of research efforts has been on abundant and low-cost substrates, particularly agricultural by-products which offer a stable foundation for mycelial growth (Lignocellulosic materials).[157, 290] The mycelium-based composites having tailored structural, physical, chemical,

mechanical, and biological properties rely on the strain, feeding substrate, and the manufacturing process.[201] Biodegradable composites that provide strength and structural support are commonly made from materials such as straw,[291] rice husks,[292] and corn stalks (Agricultural residues).[293-295] As sustainable substrates for mycelium growth, materials like coffee grounds, spent grains, and fruit peels have also been investigated;[296] this addresses the issues of material development and food waste reduction.[296] The mechanical, thermal, and biodegradability properties of the final material are all directly impacted by the substrate choice.

So far, mycelium-based materials have been produced mainly from varying mycelial strains such as *Pleurotus ostreatus*[201, 297, 298] and *Trametes multicolor*[201, 286, 299] and also from *Ganoderma lucidum*[298] because of their strong and resilient mycelial structures and excellent bonding properties that produce composites with good mechanical strength and flexibility. Biodegradable packaging materials are commonly made from *Pleurotus ostreatus* (oyster mushroom), which is valued for its quick growth and capacity to colonize a variety of substrates, including agricultural byproducts.[300] *Trametes versicolor* is employed in the production of mycelium composites that possesses flexibility to environmental stimuli due to its remarkable capacity to decompose lignocellulosic materials.[301] The potential of other fungi, like *Schizophyllum commune*[207, 302] and *Fomes fomentarius*,[303] in composite formation has also been investigated. Manufactured mycelium composites have been used in a variety of industries, including furniture,[304] and construction.[201] For packaging in particular, they provide a sustainable substitute for synthetic materials like polystyrene.[111]

4.3 Materials and Methods

4.3.1 Materials

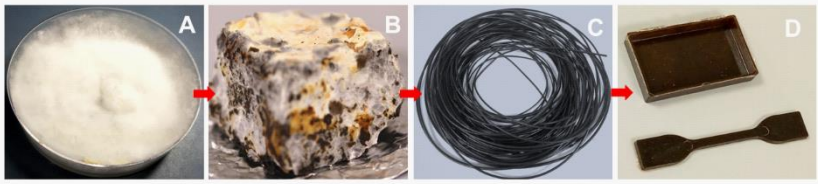
The commercial polyester PHB was purchased from NaturePlast (France). The coffee silverskin flakes with a particle size of around 3mm, were kindly offered by Covim S.p.A., Genova, Italy. Epoxidized soybean oil methyl ester (ESOME) was obtained from ATP R&D Srl (Camisano, Italy). The *Pleurotus ostreatus* mycelium (P) active culture was purchased from DSMZ, Germany, and maintained in a 100 mm Petri dish with PDB. The culture is transferred to a fresh medium every 30 days.

4.3.2 Preparation of the Mycelium Biomass

The protocol for the preparation of the mycelium biomass is described in detail in the previous chapter. In brief, the first processing was the fabrication of the mycelium-bound biomass (MycB) with the utilization of 20g of P mycelium as a natural binder for 10g of coffee silverskin agrowaste under controlled environmental conditions, i.e. under dark for a period of one week at 27°C and 78±2% RH, **Figure 4.1:(I (A-B))**.

4.3.3 Preparation of Biocomposites

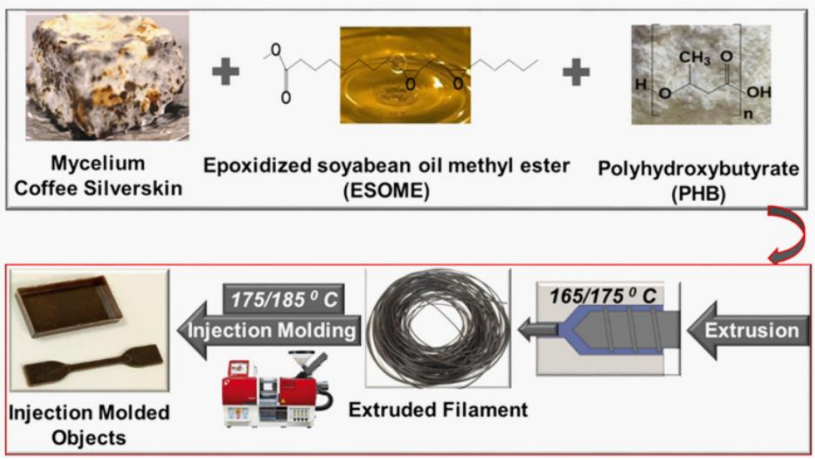
(I)



A: Mycelium fungus grown in the laboratory PDB medium
B: Mycelium grown in coffee silverskin (Biomass)
C: Extruded filaments of PHB/mycelium/coffee silverskin
D: Injection molded objects of PHB/mycelium/coffee silverskin

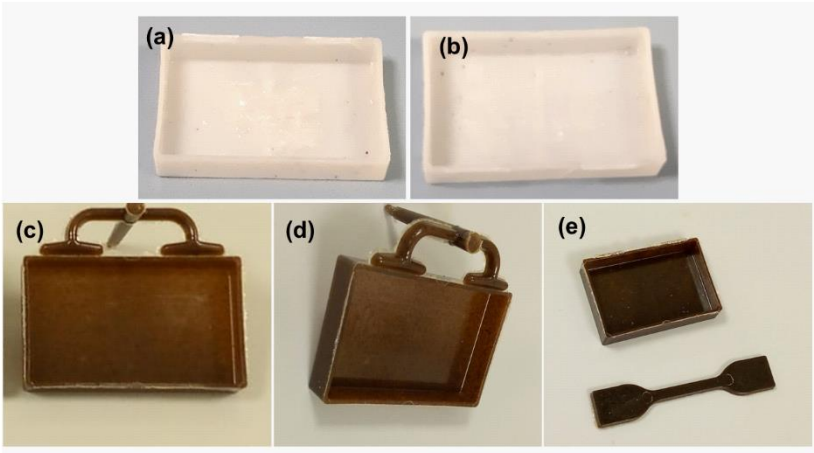
(A)- (D) Steps involving the preparation of the Biocomposites

(II)



Preparation of the Biocomposites through industrial applicable approach

(III)



(a) Pure PHB molded object (b) PHB/ESOME molded object
(c)PHB/ESOME/5 MycB loaded injection-molded box with trimming,
(d)PHB/ESOME/7.5 MycB loaded injection-molded box with trimming, and
(e)PHB/ESOME/15 MycB loaded box and dog-bone injection-molded object after cutting trimming

Figure 4.1: (I) –(III): Preparation of the Biocomposites: (I) steps involving the preparation of the biocomposites (A) Mycelium fungal grown in the laboratory PDB medium, (B) Mycelium grown in coffee silverskin biomass, (C) melt extruded filaments of PHB/mycelium/coffee Silverskin biomass, (D) Injection molded objects of PHB/mycelium/coffee silverskin biomass. (II) Preparation of the biocomposites through Industrial applicable processes. (III) different biocomposite objects made with BabyPlast industrial machine (a) Only PHB molded object, (b) PHB/ESOME molded object, (c) PHB/ESOME/5

MycB loaded injection-molded object , (d) PHB/ESOME/7.5 MycB loaded injection-molded object, and (e) PHB/ESOME/15 MycB loaded injection-molded object

At first, the MycB sample was crushed into powder with an Oster Versa 1400 blender and sieved using a 300μm standard Endecotts Sieve Shaker Minor 200 (London, UK) at room temperature (RT) resulting in particles with dimensions lower than 300μm. Both MycB powder and PHB pellets were dried at 50 °C in an oven for 24 h. Before extrusion, the PHB, MycB, and ESOME were premixed for about 15 min in a polyethylene bag by shaking. For the preparation of the final biocomposites, the extrusion was performed using a tween-screw extruder (Luigi Bandera S.p.A., 2C15 Extruder with length to diameter ratio (L/D) 45), and the screw speed was at 140 RPM in all formulations, with a temperature profile of 130, 160, 170, 175, 175°C **Figure 4.1:(II)**, starting from the feeding section. The diameter of the extruded filaments was ∼1.85 mm. The extruded filaments after being cooled down at RT, were then pelletized in the Bandera pelletizer, in pellets of ∼4 mm diameter and dried at 50 °C overnight in an oven. The pure PHB and PHB-ESOME were also extruded and pelletized to be used as reference samples. The generated pellets were then injection molded at a temperature profile of 175/185°C to produce dog-bones (1.0 mm of thickness and an overall length of 75 mm, a gauge length of 25 mm and a width of 5 mm) and box-shaped (LxWxH: 50x30x7 mm^3) mycelium agro-waste-bound composite materials **(Figure 4.1:(II) and Figure 4.1:(III) (a)-(e))**. The resulting components were then stored for conditioning at 21 ± 2 °C and 50 ± 2% relative humidity (RH). The formulations of the prepared biocomposites and the sample names are listed in **Table 4.1**.

Table 4.1 Formulations of prepared composites and their names. The wt. % is with respect to the final composite

PHB (wt. %)	ESOME (wt. %)	Mycelium biomass (wt. %)	Final Product name
100.0	--	--	PHB
90.0	10.0	--	PHB/ESOME
85.0	10.0	5.0	PHB/ESOME/5 MycB
82.5	10.0	7.5	PHB/ESOME/7.5 MycB
75.0	10.0	15.0	PHB/ESOME/15 MycB

To prepare the biocomposite films that were used for the antioxidant test, the pellets were compression molded using a CARVER hot press (Model 4122) at 175 °C for 5 min at contact pressure and then another 5 min at 5 MPa. Subsequently, a cooling step with running tap water through the platens of the hot press was performed. The resulting films were then stored for conditioning at 21 ± 2 °C and 50 ± 2% RH. The thickness of the films was determined by an electronic digital micrometer (Mitutoyo, 543–470B, sensitivity: 1 μm), measuring different regions of each sample, and an average of at least five measurements was calculated.

4.4 Characterization

4.4.1 Morphological Analysis

The morphology of the biocomposites was investigated by an SEM microscope (Jeol JSM-6490LA, JEOL, Tokyo, Japan). The microscope was equipped with a tungsten (W) thermionic electron source working in a high vacuum, and the acceleration voltage was 10 kV. For the analysis, the samples were fixed on aluminum stubs using carbon tape, and a gold coating (thickness: 10 nm) was sputter-coated on each sample using a high-resolution sputter coater (Cressington 208 HR). For the cross-sectional analysis, the specimens utilized for the mechanical properties analysis, after their fracture during the tensile testing, were used.

4.4.2 Chemical and Structural Analysis

The chemical composition of all samples was investigated by FTIR-ATR (Vertex 70v equipped with an ATR unit: diamond crystal, Bruker Analytik GmbH). For each spectrum, 64 repetitive scans were averaged within the wavenumber range from 600 to 3600 cm^{-1}, with a resolution of 4 cm^{-1}.

X-ray diffraction patterns were collected on all the biocomposite samples from 5° to 60° by using a parallel beam geometry with Cu-Kα radiation (λ = 1.541874 Å) in an Empyrean diffractometer, working at 45 kV and 40 mA and equipped with a PIXcel 3D 2x2 area detector at ambient temperature.

The wetting characteristics of all samples were determined by water contact angle (WCA) measurements using a contact angle goniometer, (DataPhysics OCAH 200, Kruss). For each measurement, a three-microliter water droplet was placed on the surface of the films, and the WCA measurements were performed within 30 seconds. At least 10 measurements were performed for each sample in different areas, and the average values are reported with the standard deviation.

The tensile testing measurements were conducted with an Instron dual column tabletop universal testing system (T.A. Instruments, Instron, Model 3365L4052, Norwood, MA) with a 2 kN load cell, following the ASTM D882 standard test methods for tensile properties of thin plastic sheeting at 25 °C. Specimens of about 550 μm thickness were first conditioned for 48 h at standard laboratory conditions (21 ± 2 °C and 50 ± 2% RH). Then, they were cut with a dog bone press (as discussed in the previous sections) and they were tested at a rate of 5 mm/min. From the obtained stress-strain curves, Young's modulus, ultimate tensile strength, elongation at break, and toughness (area under the stress-strain curve) were calculated. For each biocomposite, five specimens were tested, and for each parameter, the mean value with the standard deviation was

reported. To analyze the data, analyses of variance (ANOVA), using OriginPro 2018 software (Northampton, MA) were performed. The multiple-range Tukey's test was used to compare the differences between the mean values of the measured properties (significance level: 0.05).

The thermal stability of the biocomposites was characterized utilizing thermogravimetric analysis (TGA) using a TA Q500 instrument (TA Instruments, Newcastle, EEUU). Each sample (15–25 mg) was placed in a platinum pan and heated from 30 to 600°C under an inert N_2 atmosphere, with a flow rate of 50 mL/min and a heating rate of 10°C/min.

4.4.3 Soil Biodegradation

The biodegradability of the composite materials and of their respective controls was analyzed over a 3 month-experiment following the methodology reported by Merino et al. 2019.[305] The samples were cut into 2 cm x 2 cm dimensions, put into a PE-mesh bag and buried in the biodegradation media. For that, a pot of 20 cm x 20 cm x 8 cm filled with soil for aromatic plants and vegetable garden (VIGORPLANT ITALIA S.R.L., Fombio, Italy) was used. The main physicochemical properties of the soil were a pH of 6.5, electrical conductivity of 0.4 dS/m, dry apparent density of 250 Kg/m^3, and total porosity of 87% v/v. According to the supplier it was composed of: acidic sphagnum peat, green composted soil improver (produced from mixtures of composted and not chemically treated plant materials), and simple non-composted vegetable soil improver all allowed in organic farming not chemically treated. At the beginning of the experiment, the soil was irrigated to half of its holding capacity, and its moisture was maintained during the experiment, with the evaporated water being compensated by regular additions of water. The experiment was conducted in an indoor environment. The assay was conducted at 18 °C ± 2°C and 60% ± 5% RH. Samples were initially dried for 24 h at 40 °C in an oven and weighed ($\boldsymbol{W_0}$). Then they were placed in hand-made PE-mesh bags and buried in the soil. The samples were removed at specific times

(the 26th day of each month) and the soil attached to the samples was carefully removed with a brush. After being dried overnight in a vacuum oven at 40 °C the samples were re-weighted ($\boldsymbol{W_t}$). Finally, the weight loss (%) of each sample was determined as shown in equation (3.1) and was represented as a function of time (months):

$$\boldsymbol{Weight\ loss}\ (\%) = (\boldsymbol{W_0} - \boldsymbol{W_t})/\boldsymbol{W_0} \times \mathbf{100} \ldots\ldots\ldots\ldots\ldots\ldots \quad \textbf{(3.4)}$$

Samples were analyzed in duplicates and results were expressed as average ± SD. Pictures of the dried films were also captured.

4.4.4 Oxygen Barrier, Overall Migration and Antioxidant Properties

The oxygen permeability of the samples was analyzed according to ASTM test method F 3136-15 (ASTM, 1989), using an Oxysense 5250i device (Oxysense) equipped with a film permeation chamber. The test was performed under standard laboratory conditions, (i.e., T = 21 ± 2 °C and RH = 50 ± 2%). The permeation chamber consists of a cylinder divided into 2 parts, (sensing well and driving well). At first, the sample was placed over the sensing well and the chamber was properly sealed by the locking bolts. The sensing well is equipped with a fluorescence sensor (oxidot) mounted on the nitrogen-purged side of the chamber while the driving well is kept open to ambient air. The oxygen gas transmission rate (OTR) of the biocomposites was measured at specific time intervals, using the Oxysense permeability analyzer (OxySense fiber-optic pen) with a fluorescence sensor. When oxygen passes from the driving well through the biocomposite films to the sensing well, the fluorescence is quenched, decreasing its lifetime proportionally to the oxygen concentration. The oxygen volumetric flow rate per unit area of the biocomposite samples and per time unit (in mL m^{-2} day^{-1}) was continuously monitored until a steady state was achieved. The Oxysense software translated these data to determine the OTR of the biocomposite samples. For each sample, at least five recorded values were taken and the average values are reported with

the standard deviation. The oxygen permeability (OP) of the biocomposites was then calculated according to the following equation[306]:

$$OP = \frac{OTR.\ t}{\Delta P} \quad \text{..... (3.5)}$$

Where OTR is the oxygen transmission rate, *t* is the thickness of the biocomposite films, and ΔP is the oxygen partial pressure difference between the sides of the films.

The migration of components from the different biocomposite samples in the food was tested by Commission Regulation (EU) 10/2011 using Tenax® as a simulant for dry food following the method reported in previous works.[306-308] For all the biocomposites, a round sample of 2.5 cm in diameter was put in a clean glass Petri dish and 80 mg of Tenax® was placed on each side. The petri dish was sealed and exposed to 70 °C for 2 hours in a vacuum oven. Finally, the samples were removed and cooled to laboratory temperature. Values for the overall migration (M) were obtained by calculating the mass difference of Tenax® before and after the treatment, applying the following formula[306]:

$$Overall\ Migration, M\ (\text{mg}\ dm^{-2}) = \frac{(m_0 - m_f)}{S} \qquad (3.6)$$

Where m_0 and m_f represent the weight of Tenax at the beginning and end of the test and *S* denotes the surface area of the test specimen intended to come into contact with the given foodstuff, in dm^2. The accuracy of the measurements was ensured by performing the test in triplicates.

The antioxidant activity of the samples was calculated by measuring the free-radical scavenging activity (RSA, also called antioxidant activity) of the antioxidant molecules released from the

samples. For that, the standard DPPH radical (DPPH•) assay was used during the experiments[309]. Briefly, 0.1 g of each film was immersed in 3 mL of a 0.1 mM DPPH• solution in ethanol. The antioxidants that migrate from the different samples react with the radical, leading to a detectable color change with a UV–vis spectrophotometer at 517 nm. The antioxidant activity of the samples was analyzed after 24 h in contact with the radical solution. The long-term antioxidant activity of the films was studied by measuring the RSA at different time intervals for 2 weeks. The RSA was estimated using eq 3.4.

$$RSA(\%) = \frac{A_1 - A_2}{A_1} \; X \; 100\% \qquad (3.7)$$

Where A_1 is the absorbance of the DPPH• radical solution at 517 nm, and A_2 is the absorbance of the radical solution with the sample immersed.

4.5 Results and Discussion

4.5.1 Morphological Analysis

In the SEM analysis **Figure 4.2 (a,b)**, it can be seen that the surface of the pure PHB sample is relatively smooth while it becomes smoother after the addition of ESOME. This can be seen also in the cross-section images where it is clear that the addition of ESOME gives a more compact structure (**Figure 4.2 (f,g)**). In fact, PHB is generally a highly crystalline polymer that starts to crystallize at a temperature very close to RT. However, when the 10 wt. % ESOME was added to the PHB, the surface of the biocomposites becomes more homogenous and smoother as the surface bumps almost disappear. When the MycB filler is introduced in the formulation, the formed composites (PHB/ESOME/5 MycB, PHB/ESOME/7.5 MycB, and PHB/ESOME/15 MycB also showed the smoother surface, **Figure 4.2 (c-e)**). In cross-sectional morphology there is also no

visible CS or MycB is observed, indicating that the mycelium biomass component is well introduced in the PHB matrix.

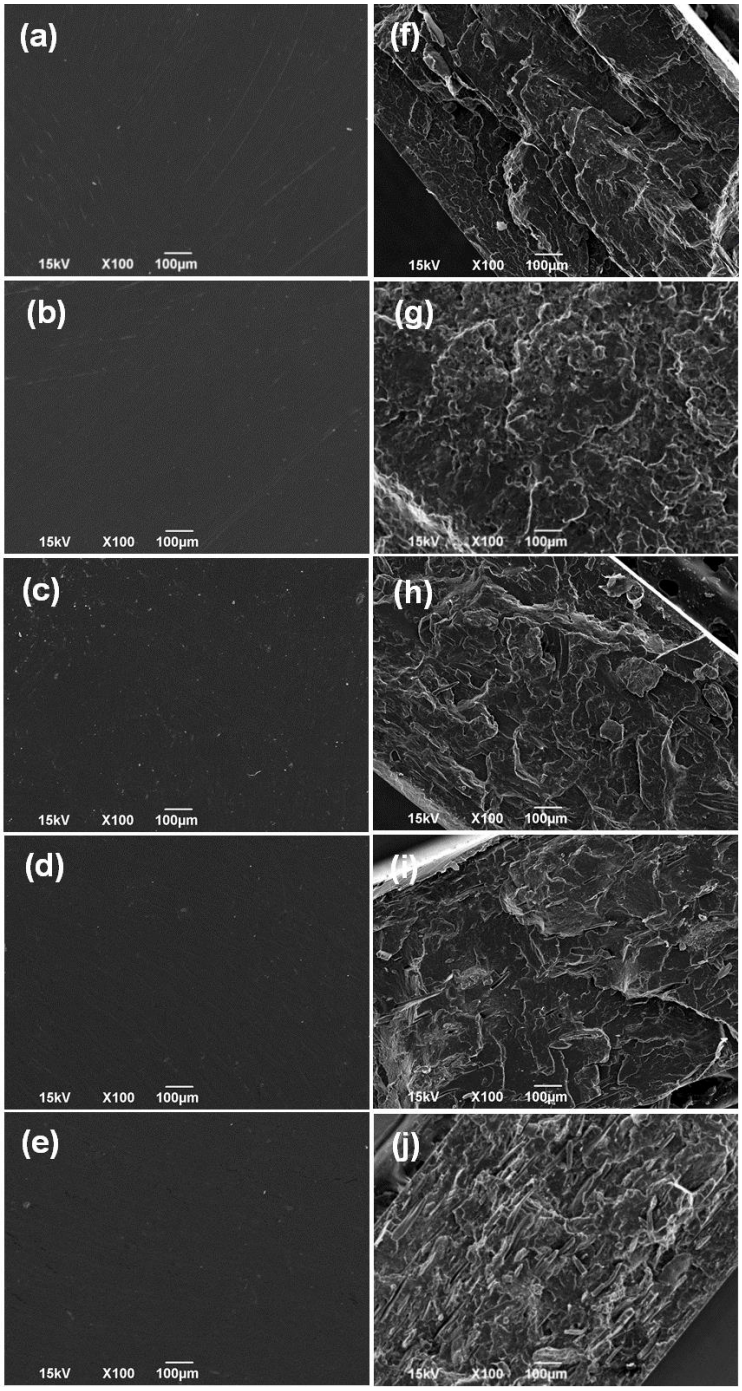


Figure 4.2: Scanning electron microscopy (SEM) analysis of the developed samples (a-e) surface and (f-j) cross section of: (a,f) Pure PHB, (b,g)PHB/ESOME, (c,h)PHB/ESOME/5 MycB, (d,i)PHB/ESOME/7.5 MycB, and (e,j)PHB/ESOME/15 MycB biocomposites.

The surface roughness of PHB/ESOME (4.4 µm) decreases as compared to neat PHB (5.1 µm), indicating that ESOME helps to plasticize the PHB, and decrease the surface roughness. The addition of MycB up to 7.5 wt.% in PHB/ESOME further decreases the roughness properties, implying that surface-level blending occurred for the PHB/ESOME/MycB composites compared to the PHB/ESOME sample (**Figure 4.3**). However, in the case of higher loading of MycB (15 wt.%), in PHB/ESOME, no significant decrease in roughness was observed possibly due to the higher amount of coarse MycB particles in the composites.

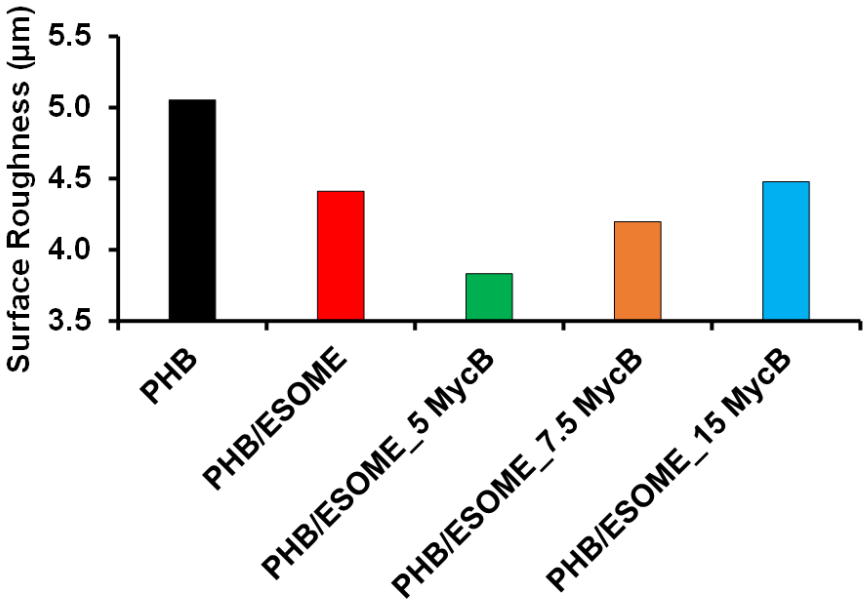


Figure 4.3: Surface Roughness of PHB, PHB/ESOME, and PHB/ESOME/MycB composites

4.5.2 Chemical and Structural Analysis

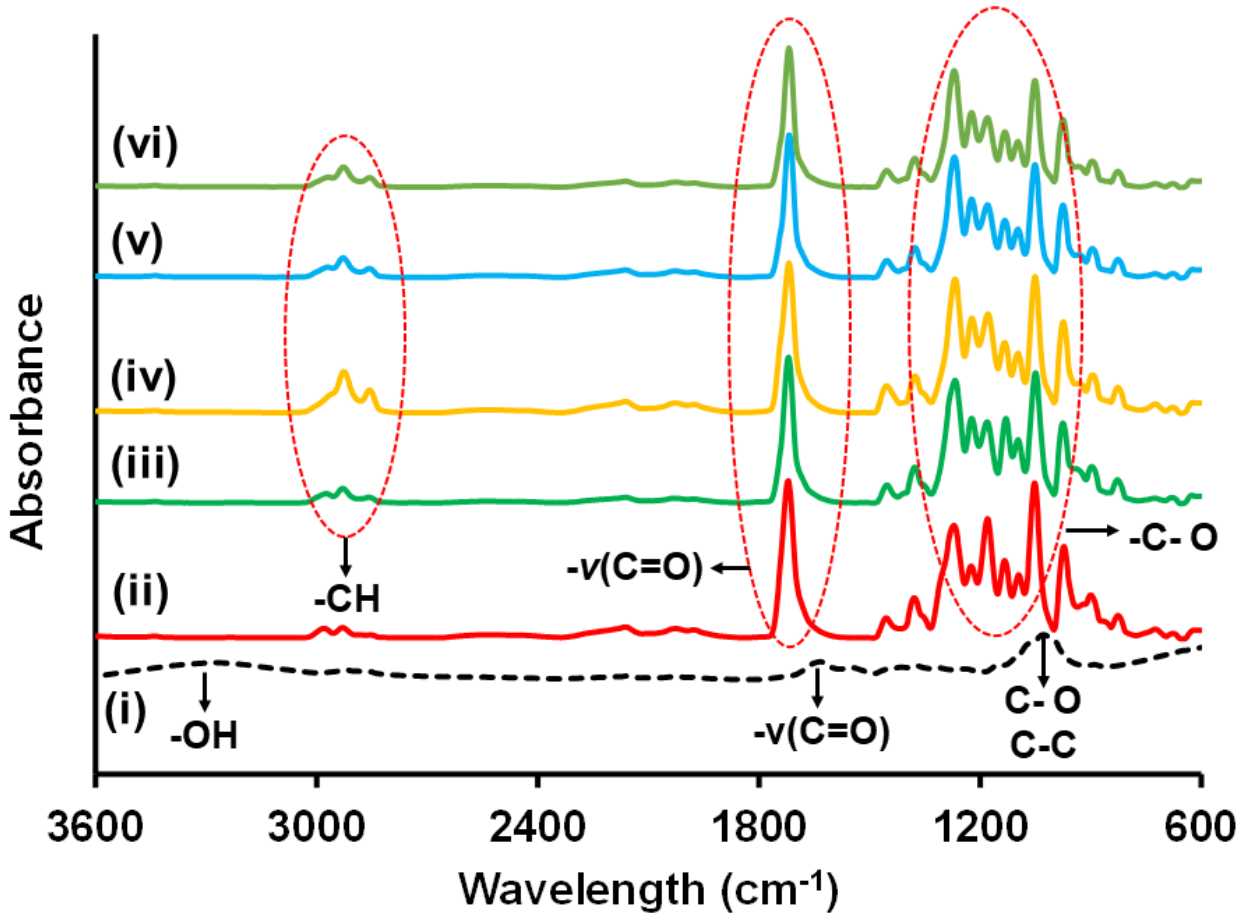


Figure 4.4: FTIR-ATR Analysis of (i) MycB, (ii) PHB, (iii) PHB/ESOME, (iv) PHB/ESOME/5 MycB, (v) PHB/ESOME/7.5 MycB, and (vi) PHB/ESOME/15 MycB biocomposites

Table 4.2 Main chemical functional groups of PHB, PHB/ESOME, and PHB/ESOME/MycB composites.

	PHB	PHB/ESOME	PHB/MycB	PHB/ESOME/ 5 MycB	PHB/ESOME /7.5 MycB	PHB/ESOME /15 MycB	CS	MycB	Mycelium	ESOME	
O-H [cm^{-1}]	3736	--	3738	--	--	--	3289	3275	3287	-	
C=O [cm^{-1}]	1720	1720	1718	1718	1718	1718	1620	1634	1638	1709 / 1848	Carbonyl
$CH_{2\ assy}$ [cm^{-1}]	2980	2974	2980	2926	2928	2928	2918	2920	2922	2953	
$CH_{2\ sym}$ [cm^{-1}]	2930	2918	2926	2857	2857	2857	2851	2853	2847	--	

In order to investigate the interactions between PHB, ESOME, and MycB, chemical analysis was carried out through FTIR-ATR spectroscopy. **Figure 4.4** shows the infrared spectra of MycB, PHB plasticized with ESOME, and PHB/ESOME/MycB composites and **Table 4.2** shows their main characteristics peaks. The FTIR analysis of PHB/ESOME/MycB composites obtained by extrusion and injection molding revealed that all composites show similar characteristic peaks in the region of carbonyl and C-H stretching vibration, indicating there is no chemical interaction among the PHB, ESOME, and MycB. Concerning the MycB sample the region of 1634 cm^{-1} corresponds to the stretching vibration of C=O groups, mainly due to the amide (amide I) groups, while the absorption band at 1028 cm^{-1}, is characteristic for C-O and C–C vibration due to the presence of polysaccharides. The absorption band at around 1549 cm^{-1} for PHB/ESOME/MycB is ascribed to the -NH vibration of amide groups (amid II). Concerning PHB the characteristic peak around 1720 cm^{-1} corresponds to the carbonyl stretching vibration (νC=O) band. The carbonyl group of ESOME shows maximum absorption at around 1709 cm^{-1} (**Table 4.2**), while when ESOME is combined with the PHB it overlaps with the carbonyl absorption of PHB. All composites containing MycB show also a similar position of the νC=O as the one of PHB/ESOME (at 1720 cm^{-1}) (**Table 4.2**),

corresponding to the carbonyl stretching vibration band of PHB. The bands between 2980 and 2850 cm^{-1} in PHB/ESOME/MycB samples are belong to the -CH- asymmetric and symmetric stretching vibrations of CH3 groups in the side chains, whereas their bending vibration was observed at 1456 cm^{-1}. Overall, no new peaks were observed when ESOME was added to PHB or when MycB was added to the PHB/ESOME/MycB composites, confirming no chemical interaction between the components of the films.

4.5.3 X-Ray Diffraction (XRD) Analysis

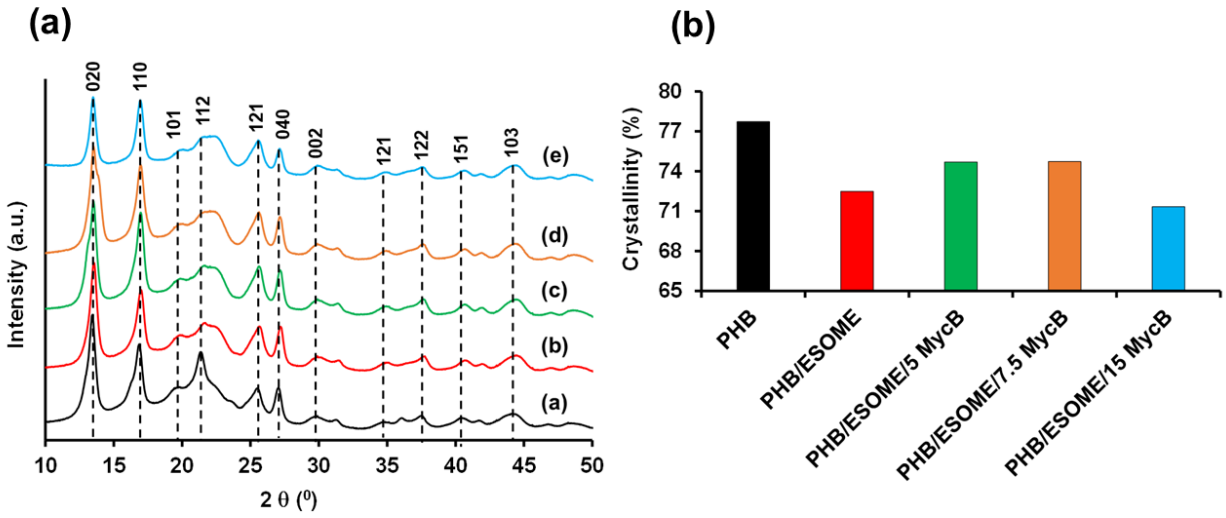


Figure 4.5: (a) X-Ray Diffraction patterns for (a) PHB, (b) PHB/ESOME,(c) PHB/ESOME/5 MycB, (d) PHB/ESOME/7.5 MycB, (e) PHB/ESOME/15 MycB, and (b) Crystallinity percentages of PHB, PHB/ESOME, PHB/ESOME/5 MycB, PHB/ESOME/7.5 MycB, PHB/ESOME 15 MycB.

The analysis of the crystalline structure of neat PHB and its plasticized blends was performed by X-ray diffraction analysis and a comparative plot of the crystalline profiles is shown in **Figure 4.5 (a)**. The spectra are comparable with the common X-ray diffractogram of PHB.[310] In particular, the crystalline structure of pure PHB presents the typical reflection peaks at 2θ = 13.4° and 2θ = 16.8°, as well as peaks at higher 2θ (21.4°, 25.5° and 27.0°), attributed to the orthorhombic crystal peaks of PHB. The crystalline peaks present in the spectra are not modified by the addition of

ESOME 2θ (13.6°, 17.0°, 21.7°, 25.7° and 27.2°). When the MycB is combined with the PHB/ESOME, the XRD pattern of the samples shows the same diffraction peaks at 2θ (13.5°, 17.0°, 21.6°, 25.6° and 27.2°). The crystallinity values obtained from the XRD spectra analysis are shown in **Figure 4.5 (b)**. The crystallinity of pristine PHB was 77.7%. The addition of ESOME to PHB slightly decreased the crystalline value (72.5%). Addition of MycB on PHB/ESOME slightly increased the crystalline values (74.8, 74.7, and 71.3%, respectively). However, these values are still lower than the pure PHB. This indicates that the addition of MycB to PHB/ESOME slightly affects the crystalline structure of the matrix.

4.5.4 Wetting Properties

The WCA analysis of the pure PHB composite films is around 109.1 ±2.4° (**Figure 4.6 (a))**, while the addition of ESOME slightly reduces its hydrophobic nature (92.5 ±3.1°), while the addition of the mycelium biomass (MycB), does not affect significantly this value. Specifically, in all cases, the WCA is around 85-90 ±1.5° (**Figure 4.6 (a))**.

This can be attributed to the smoother surface of the composite films, as already proved by the SEM and roughness study (**Figure 4.2 (a-e)** and **Figure 4.3** respectively**).** Smoother surface results in a lower number of air pockets trapped between the water droplet and the solid improving the hydrophilicity as described by the Cassie–Baxter law.[307, 311]

Furthermore, water absorption tests were performed as shown in **Figure 4.6 (b)**. To do so, the materials were soaked in water contained in glass vials for about 28 days, and subsequently their weight change was calculated. The results show that the final water absorption for pure PHB was around 0.5% which is significantly lower than the corresponding values for composites PHB/ESOME/5 MycB, PHB/ESOME/7.5 MycB and PHB/ESOME/15 MycB (1%, 1.9%, and 3.3%). The water absorption of PHB/ESOME gave slightly negative values, and this may be

attributed probably to the ESOME loss by migration from the composite to the water medium affecting significantly the measurement. Nonetheless, the trend of the water absorption for the MycB biocomposites, shows an increase for a higher concentration of the mycelium biomass in the biocomposites. This indicates that the increase in the water absorption in the biocomposites is solely attributed to the presence of the porous mycelium biomass content.

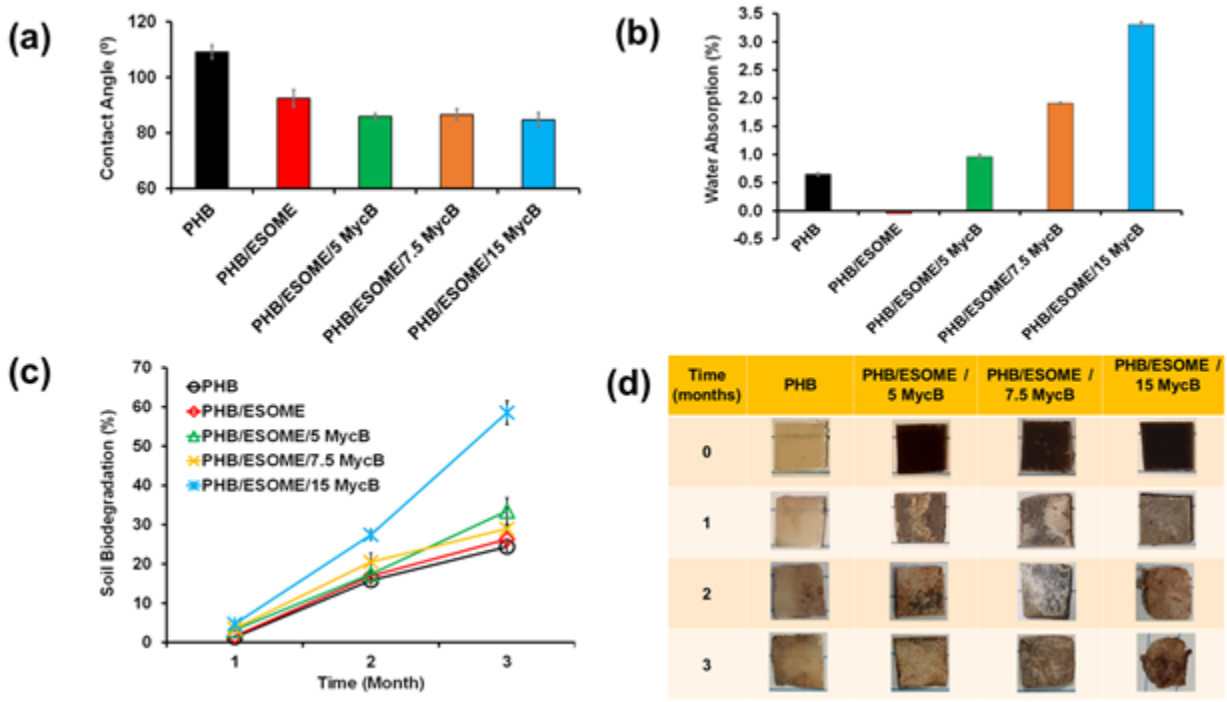


Figure 4.6: (a) Water Contact Angle (b) Water absorption (c-d) Soil Biodegradation of PHB, PHB/ESOME, and PHB/ESOME/MycB biocomposites

4.5.5 Soil Biodegradation

One of the most significant advantages of mycelium-bound composites is their biodegradability. These materials can be decomposed by microorganisms in natural environments, improving long-term environmental impact. **Figure 4.6 (c-d**) demonstrates results for the soil biodegradation test or the biodegradability assessment from 1-3 months. As shown, the rate of biodegradation for the

pure PHB is around 20-25% corresponding to the lowest degradation rate for all the tested samples. In the case of the composites with 5, 7.5, and 15% mycelium biomass (PHB/ESOME/5 MycB, PHB/ESOME/7.5 MycB), the weight loss increases to around 30-35% but with the addition of 15% MycB (PHB/ESOME/15 MycB), the loss rises to around 60%. Hence, as also shown in **Figure 4.6 (d)**, more mycelium incorporation in the PHB increased the biodegradation rate thus promoting faster biodegradability of the composite material.

4.5.6 Mechanical Properties

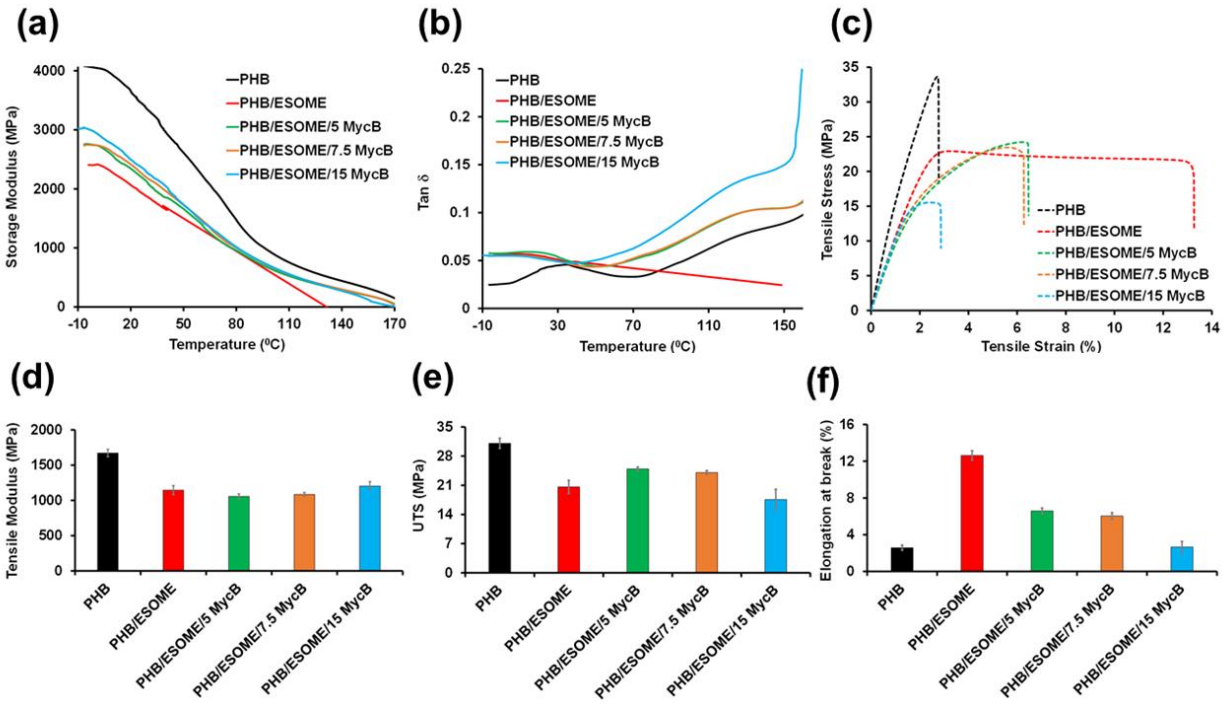


Figure 4.7: Dynamic mechanical and tensile properties of PHB, MycB, and its composites: (a) storage modulus vs temperature and (b) tan delta vs temperature, (c) stress–strain curves, (d) tensile modulus, (e) ultimate tensile strength (UTS), and (f) elongation at break.

The effect of ESOME and MycB content on the storage modulus (E′), and tan delta peak (tan δ) of neat PHB, PHB/ESOME, and PHB/ESOME/MycB -based composites is shown in **Figure 4.7 (a, b)** as a function of the temperature. It can be seen that the storage modulus as well as tan δ changes with the increasing temperature depend on the ESOME and MycB content in the samples.

A falloff in the storage modulus can be observed for all the samples with increasing temperature, which is attributed to the increased molecular mobility of the polymer chains. Furthermore, it can be seen that the storage modulus of the PHB/ESOME is lower compared to pure PHB indicating the plasticizing effect of ESOME. For the PHB/ESOME/MycB composites, the storage modulus is higher compared to PHB/ESOME owing to the reinforcing effect of MycB.

The tensile stress−strain curves of the PHB, PHB/ESOME, and PHB/ESOME/MycB composites shown in **Figure 4.7 (c)** indicate that the neat PHB behaves as a brittle material, whereas blending it with ESOME and later on with MycB results in a rather ductile behavior. The young's modulus of pure PHB also decreased by the loading of ESOME. Specifically, the tensile modulus of neat PHB was 1671.4 ± 53.2 MPa and showed a significant decrease (31.5%) for PHB mixed with ESOME (1144.8 ± 65.9 MPa). The addition of 5 and 7.5% MycB on PHB/ESOME slightly decreased the tensile modulus as compared to PHB/ESOME. However, the tensile modulus of composites containing 15% MycB was higher.

The tensile strength of neat PHB is 31.1 ± 1.2 MPa. Plasticizing PHB with ESOME significantly decreased the tensile strength of the PHB by 33.7%, as shown in **Figure 4.7(e)**. However, the tensile strength of PHB/ESOME/5 and 7.5 MycB composites improves in a range of 17−21% when compared to those of PHB/ESOME. However, further increasing the amount of the MycB filler (15 wt.%) in the PHB/ESOME decreased the tensile strength value (17.6± 2.5 MPa). This may be due to the presence of a higher amount of MycB in the composites, which causes poorer interfacial interactions and cohesion with the polymer matrix. These interpretations are in accordance with the SEM results of the composites, which are described in **Figure 4.2**.

It can be also seen that the pure PHB is brittle with an elongation at break around 2.6%, whereas this value was found to be approximately 389% higher for PHB/ESOME. At the same time, both the tensile modulus and strength of PHB/ESOME decrease, indicating that ESOME effectively plasticizes the PHB, making it more flexible and ductile. The elongation at break of PHB/ESOME/MycB composites sensibly improves with an increase of 3−156% compared to PHB, suggesting that the ESOME effectively can plasticize the PHB even at relatively high MycB contents, which eases the penetration of its molecules into the interface between PHB and MycB, weakening the direct binding forces among the macromolecules, and in this way, the molecular chains can easily slide and move upon stress, resulting in an increase in the elongation at break. In conclusion, the biocomposites possibly due to the presence of ESOME experience decreases in their stiffness, which are highly desirable properties for molding and shaping processes.[312] The presented results clearly show that pure PHB modified with ESOME and MycB can overcome its intrinsic brittleness, resulting in a significant improvement in its ductility and toughness.

4.5.7 Thermal Properties

4.5.7.1 Thermogravimetric Analysis

The thermal degradation behavior of the biocomposites was investigated by TGA analysis as shown in **Figure 4.8 (a-b)**. The pure PHB starts to decompose to volatile compounds at around 250 °C with a maximum rate of 285 °C (**Figure 4.8 (b)**), while for PHB/ESOME the maximum decomposition rate occurs at 288°C. This improvement might be due to the synergistic effect of ESOME and PHB leading to an improved interfacial adhesion For the composite samples with MycB, the maximum decomposition rate is observed at 268 °C for PHB/ESOME/5 MycB while

the Tmax is reduced slightly to 262°C for (PHB/ESOME/7.5 MycB) and further reduced to 254°C for (PHB/ESOME/15 MycB). This gives an indication that the composites containing MycB degrade at lower temperatures compared to the pure PHB. In addition, the increase of the MycB content in the PHB/ESOME/MycB composites increased the residual mass at 600 °C.

4.5.7.2 Melt Rheological (Melt Flow Index/Capillary Rheometer), and Viscosity Properties

In **Figure 4.8 (c)** the melt flow index (MFI) of the developed materials were determined. From **Figure 4.8 (c)** MFI of pure PHB was around 15-16g/min followed by an increase to 25g/min due to the addition of the plasticizer. PHB/ESOME/5 MycB and PHB/ESOME/7.5 MycB composites show almost the same MFI value whereas the composite material containing the highest percentage of mycelium biomass (PHB/ESOME/15 MycB), shows the highest MFI reaching around 55g/min. This drive to the conclusion that the MFI of the composites can be tuned from low to high viscosity at a temperature of 190°C, by increasing the content of the mycelium biomass, indicating the possibility of developing a mycelium-bound composite material either with low or high viscosity based on the manufacturing requirements.

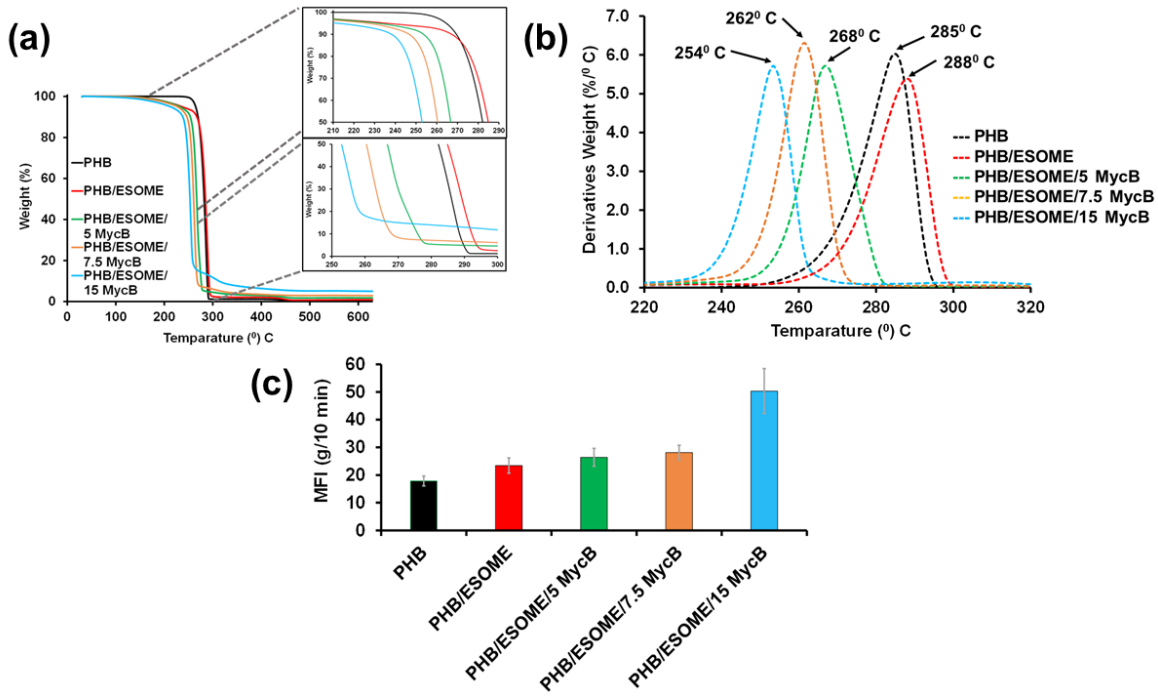


Figure 4.8: (a-b) TGA profile Curves and their derivatives, and (c) Melt flow index (MFI) Properties of the PHB, PHB/ESOME, and PHB/ESOME/MycB biocomposites

4.6 Oxygen Barrier Properties

The OP was calculated from the OTR for all of the prepared films to determine the oxygen barrier functionality of the developed films as shown in **Figure 4.9 (a)**. From previous studies conducted it has been confirmed that PHB is characterized by significant oxygen barrier properties, with OP of notably low values[313] and the results for the OP of pure PHB obtained in this study correlated with that assertion. The pure PHB sample exhibited very low oxygen barrier properties with OP reaching around 2500 mL.mm/m^2/day, one of the lowest values known for biopolymers and biocomposites**,** possibly due to the high crystallinity of the polymer which complicates the movement of the oxygen molecules, inevitably reducing the OP. After incorporating the ESOME plasticizer into the PHB biopolymer matrix, the OP of the PHB film increased greatly from 2500 to 70,000 mL·mm/m^2/day, and therefore the oxygen penetrates more easily. This is due to the

effect that ESOME has to the polymer chain interactions, permitting the easier penetration of oxygen. For (PHB/ESOME/ 5 wt. % MycB) and (PHB/ESOME/ 7.5 wt. % MycB) composite blends, higher values of 30,000 and 10,000 mL·mm/m^2/day were observed respectively. The highest OP value of 90,000 mL·mm/m^2/day was observed for (PHB/ESOME/ 15 wt. % MycB). Therefore, it is clear that the OP values were significantly affected by the presence of MycB (**Figure 4.9 (a)**). This could be due to the MycB particles, which enhance the possibility for the formation of voids/holes in the PHB structure. The oxygen can pass through this porous holes/voids zone and consequently increase the oxygen permeability value.

4.7 Overall Migration Analysis

Figure 4.9 (b) shows the overall migration test with Tenax (a dry food simulant) carried out to investigate the possible migration of molecules from the biocomposites toward dry food. The test simulates food contact, by using the food simulant according to the current EU legislation (EU Commission Regulation No. 10/2011 for plastic materials and articles) [307, 308, 314]. As presented the PHB, PHB/ESOME, PHB/ESOME/5 MycB, and PHB/ESOME/7.5 MycB samples had migration well below the 10 mg dm^{-2} that is the acceptable limit whereas, PHB/ESOME/15MycB show a migration of 18%, fair above the acceptable limit of 10 mg dm^{-2}. Excluding the latter sample, the rest of the collected data proved compliance with migration limits; thus, these biocomposites should not endanger human health.

4.8 Antioxidant Properties

Finally, the antioxidant properties of the PHB, PHB/ESOME, and the MycB composites were assessed by using DPPH radical scavenging agents. **Figure 4.9 (d)** shows the antioxidant behavior

of the films for 1, 7, and 28 days. A neat PHB blend was selected as reference material. The presence of MycB reduced the DPPH radicals, **Figure 4.9 (c)**, due to the reaction between the phenolic hydroxyl groups of mycelium and DPPH[315]. The incorporation of MycB (the mycelium biomass mixed with coffee silverskin) in the composites determined a decrease in the absorption values at 517 nm and, consequently, indicate an increase in the overall antioxidant activity of the samples (**Figure 4.9d**), highlighting how MycB could effectively act as an antioxidant agent. The PHB/ESOME composites with 5 and 7.5% MycB exhibited a delayed release of antioxidants, reaching antioxidant activity up to 16% after 28 days of incubation in the solution. However, the sample with PHB/ESOME/15MycB demonstrated the highest antioxidant activity after 28 days (antioxidant activity 59.5%). Nonetheless, the highest antioxidant activity was observed for only MycB (81%) after 28 days of incubation. The antioxidant performance of MycB is mainly due to the presence of phenolic compounds present in coffee silverskin. Therefore, biocomposites based on antioxidant-rich mycelium biomass could be very promising for food packaging.

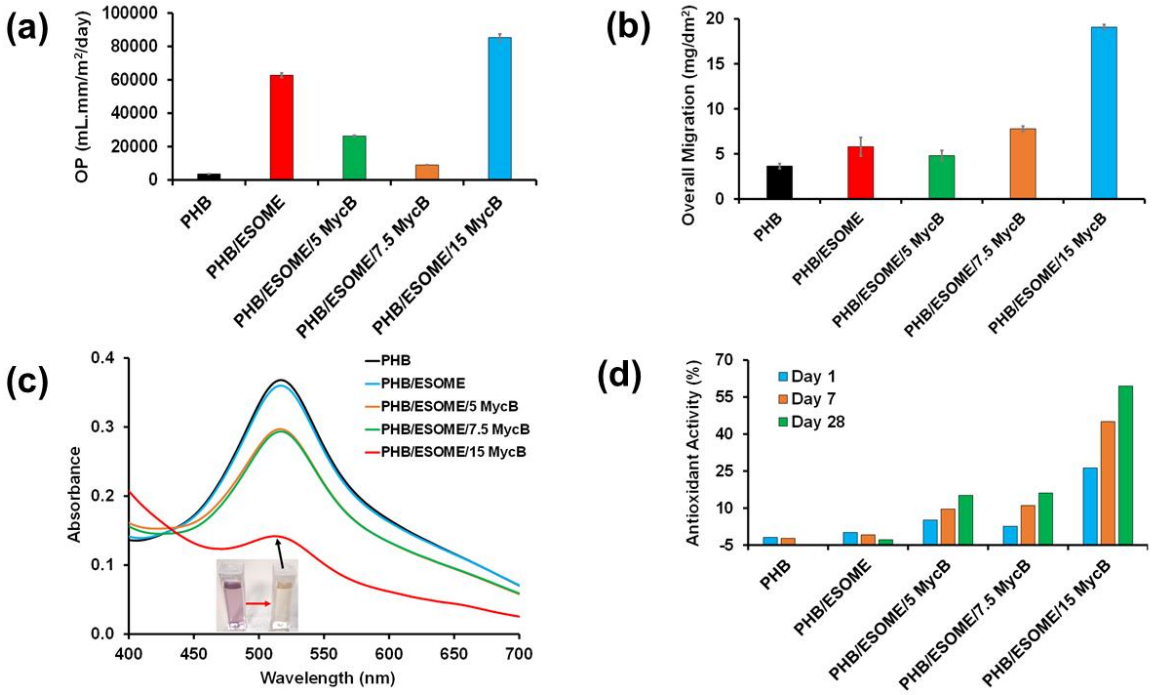


Figure 4.9: (a) Oxygen Permeability (b) Overall migration analysis of PHB, PHB/ESOME, PHB/ESOME/5 MycB, PHB/ESOME/7.5 MycB and PHB/ESOME/15 MycB biocomposites, (c) and (d) antioxidant activity of the PHB, PHB/ESOME, and PHB/ESOME/MycB biocomposites

4.9 Conclusions

In this study, PHB biocomposites containing up to 15 wt. % of mycelium biomass (MycB) were successfully produced by using a twin-screw extruder and an injection molding instrument. Fungal mycelium was inoculated with coffee silverskin and then used to prepare the mycelium-coffee silverskin biomass (MycB). The incorporation of ESOME with MycB improves its processability in terms of continuous feeding in the extruder, and injection molding. The present results clearly show that the addition of ESOME and MycB in PHB had a great influence on the thermal, mechanical, barrier, antioxidant, and biodegradation properties of the polymer. The PHB-based composites with ESOME and 5-7.5% MycB showed an increased flexibility and ductility and kept

the dry food simulant migration level of the PHB/ESOME/5 and 7.5% MycB composite below the EU acceptable level, which is 10 mg/dm^2. The composites also presented enhanced soil biodegradation level in comparison to the pristine PHB. Furthermore, it is demonstrated and enhanced antioxidant activity and acceptable gas barrier properties, while maintaining a balance between mechanical strength and flexibility.

5 Overall Conclusions

In this Thesis, the mycelium *Pleurotus ostreatus* commonly known as oyster mushroom, a non-pathogenic and an edible fungal strain, was used for the production of mycelium-bound composite materials in cooperation with coffee silverskin lignocellulosic agrowaste substrate. The characteristic properties of the developed composite materials earned them the possibility of serving as suitable alternatives to conventional synthetic materials in thermal and acoustic insulation, and food packaging.

In Chapter 1, it is stated that the growing interest in mycelium stems from its ability to function as a natural, bio-based material that can be manipulated into various forms. Mycelium-based research has become more popular in the past ten years as a means of creating sustainable, renewable, lightweight and biodegradable biomaterials and the substrate has a major influence on the strength, texture, and rate of growth.[162]. The opportunities for their use in numerous applications as in insulation and packaging is emphasized especially concerning their ability to promote environmental sustainability. Since mycelium is capable of decomposing a broad range of organic materials, a number of substrates including readily available and reasonably priced agricultural by-products like straw, husks, corn stalks and used coffee grounds containing a high nutrient content, have been investigated in an effort to maximize the growth conditions and material characteristics. The growth of the mycelium strain is aided by the substrate to produce mycelium-based biocomposites. Wood-based substrates are recommended for strains of fungi that naturally break down lignocellulosic materials, such as *Ganoderma lucidum*[110, 316] and *Trametes versicolor*.[122, 290] The challenges involved in the manufacturing of mycelium-bound materials and preferable solutions have also been highlighted.

In Chapter 2, the successful development of novel agro-waste-bound mycelium biocomposite materials has been achieved. The biocomposites produced using CSF agrowaste substrate and *P. ostreatus* mycelium blends possess low thermal conductivity and good acoustic isolation, which together with the thermal stability and hydrophobicity make them valuable candidates for biodegradable, thermal, and acoustic insulation materials in building and construction.

In Chapter 3, a green-processing route for mycelium biomass/PHB biocomposites was studied when varying the component mixtures of the biocomposite materials. In particular, the biocomposite materials were prepared through extrusion and injection molding. The incorporation of ESOME with MycB improves its processability in terms of continuous feeding in the extruder, and injection molding. The present results clearly show that the addition of ESOME and MycB had a great influence on the thermal, mechanical, barrier, antioxidant, and biodegradation properties of composites. It was found that the incorporation of MycB and ESOME into PHB increased the melt flow behavior and made the composites ductile. The PHB-based composites with 5-7.5% MycB showed an increase the flexibility and ductility and kept the food migration level below the EU acceptable level, which is 10 mg/dm^2. The composites also showed enhanced soil biodegradation rate compared to the pristine PHB. Furthermore, the developed samples demonstrated qood antioxidant activity and acceptable gas barrier properties.

In conclusion, the green approach used for the fabrication of the described materials and their many attractive properties paves the way to inspire further research and drive the integration of mycelium-based materials into manufacturing a more sustainable and industrially scalable production of MycB-based biocomposites with wide applicability in different applications such as packaging, or construction applications among others.

6 Appendix-I

6.1 Co-Authorship / List of publications

Chapter 2 includes published results in the form of an original journal article, and Chapter 3 reports materials which are to be submitted to a scientific journal for future publication. The large majority of the experimental work, analysis, and writing of the described chapters has been conducted by the author.

Chapters and manuscripts were reviewed by the thesis supervisors Dr. A. Athanassiou and Dr. Despina Fragouli.

The complete citations are provided below:

Bonga, K.B., Bertolacci, L., Contardi, M., Paul, U.C., Zafar, M.S., Mancini, G., Marini, L., Ceseracciu, L., Fragouli, D. and Athanassiou, A., 2024. Mycelium Agrowaste-Bound Biocomposites as Thermal and Acoustic Insulation Materials in Building Construction. Macromolecular Materials and Engineering, p.2300449.

Bonga, K.B., Chandra,P.U., Merino D., Fragouli, D. and Athanassiou, A., 2024. A Green-Processing Route for Mycelium Biomass/PHB Biocomposites. Macromolecular Materials and Engineering, *to be submitted for publication*.

7 Conferences/Presentations

Materials and Research Society (MRS) Spring Meeting and Exhibit

Symposium: SB01.06, Fundamentals and Applications of Engineered Living Materials.

San Francisco, California, U.S.A

April, 2023.

American Chemical Society (ACS) Fall Technical Meeting

Session: Innovative Materials for Environmental Sustainability, Moscone Center Room 3007, West Bldg.

San Francisco, California, U.S.A

August, 2023.

Career Paths for PhDs working as Climate Change Experts at the United Nations

Università degli Studi di Torino, Direzione Ricerca e Terza Missione.

Speaker: Dr. Andrea Camponagara PhD, Climate Change Expert,

Programme Officer UNFCCC Secretariat, ONU, Bonn.

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