

In Situ Growth of Mycelium in a Lignocellulosic Scaffold Enabled by Cellulose Nanofibrils for Lightweight Insulation

Maryam El Hajam^{1*}, Wenjing Sun², Islam Hafez³, Caitlin Howell^{4,5}, Mehdi Tajvidi¹

¹ School of Forest Resources and Advanced Structures and Composites Center, University of Maine, Orono, ME, 04469-5755, USA

² Magan Centre of Applied Mycology, Cranfield University, Cranfield MK43 0AL, UK

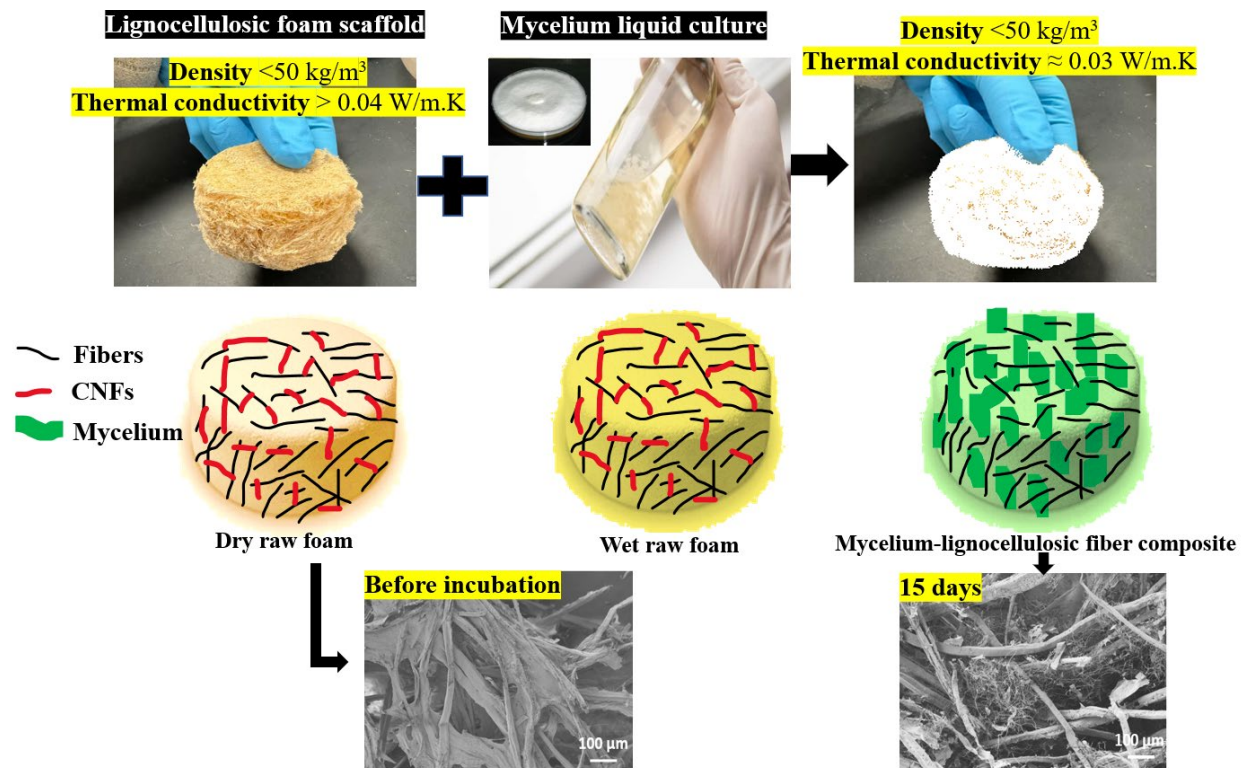
³ Department of Wood Science and Engineering, Oregon State University, Corvallis, OR, 97331, USA

⁴ Department of Chemical and Biomedical Engineering, University of Maine, Orono, ME, 04469-5755, USA

⁵ Graduate School of Biomedical Science and Engineering, University of Maine, Orono, ME, 04469-5755, USA

*Corresponding author: Maryam.el1@maine.edu

Graphical abstract



Abstract

Mycelium-lignocellulosic fiber composites (MLFCs) offer a promising sustainable alternative to lightweight, petroleum-based materials and are gaining significant interest across various sectors. However, creating low-density MLFCs that provide superior thermal and sound insulation properties along with good mechanical strength remains a substantial challenge. In-mold packing is the primary fabrication process explored so far for MLFCs, limiting the

ability to design complex shapes, and expanding potential applications. Growing mycelium on pre-produced low-density lignocellulosic foam scaffold substrates is an innovative process that represents a promising, yet underexplored, approach for MLFCs. This approach offers the potential for design flexibility and enhanced structural properties. In this study, hybrid low-density foams made from lignocellulosic fibers, cellulose nanofibrils (CNFs), and mycelium (*Trametes versicolor*) were developed and assessed as foam-like insulation materials. A foam-forming method enabled by a surfactant was used to prepare low-density foam scaffold substrates from hardwood and softwood fibers with CNFs as a binder. The raw scaffold substrates and the final MLFCs were characterized for thermal conductivity, sound absorption, water resistance, mechanical strength, and morphological properties. The composites demonstrated excellent thermal insulation, soundproofing, and mechanical strength, as well as thermal and sound properties comparable to or even better than expanded polystyrene (sound absorption coefficient > 0.9 at 1600 Hz). Overall, the results showed that MLFCs have strong potential for further exploration in semi-structural applications, such as lightweight insulating infill panels and protective packaging, offering environmentally friendly and resource-efficient solutions for the construction insulation and packaging markets.

Key words: Mycelium-lignocellulosic fiber composite, *Trametes versicolor*, scaffolding, thermal insulation.

1. Introduction

As the interdependence between human consumption habits and accelerating climate change becomes demonstrably clear, our planet faces an ongoing anthropogenic environmental crisis in the 21st century [1]. The use of traditional materials in the global construction industry substantially contributes to climate change by depleting over 45% of global resources and releasing up to 40% of energy-related carbon dioxide emissions into the atmosphere [2,3]. Additionally, building envelope systems in temperate and cold climates rely on insulation materials to improve indoor environmental control and reduce energy consumption. Insulation is particularly crucial in regions with high heating demand (latitude >40°), where it serves as a passive strategy to reduce heating loads and help achieve net-zero energy buildings. However, challenges in selecting suitable insulation materials arise due to inconsistent standards, discrepancies between manufacturers' specifications, and actual performance [4,5].

Lightweight thermal insulation materials play a pivotal role in improving building energy efficiency and facilitating the transport and storage of temperature-sensitive products [6,7]. However, the rapid increase in demand for thermal insulation materials has driven the development of petroleum-based polymers, such as extruded polystyrene (XPS)

and expanded polystyrene (EPS), that have been the standard materials for insulation in the construction industry due to their excellent thermal and acoustic performance along with light weight. While these foams are effective in enhancing energy efficiency and soundproofing, their production and disposal raise serious environmental concerns, as they are not biodegradable and pose health risks [8].

Over the past decade, architects and researchers have become progressively engaged in biobased materials that are made from renewable resources. These materials are designed to be biodegradable under appropriate conditions, reducing harmful substance release and creating sustainable approaches to address problems like fossil fuel dependence, greenhouse gas emissions, and solid waste generation, driving a successful transition from a linear economy model to a sustainable circular bioeconomy. Biocomposites, which can be derived from a variety of biomass sources such as plants, bacteria, algae, and fungi, play a key role in this transition [9]. These materials are driving new design methods, transforming designers into active creators and fostering collaboration among architects, biologists, and materials scientists to develop sustainable biomaterials [10].

The development of biocomposites produced by filamentous fungi colonizing lignocellulosic fibers has attracted considerable attention from both industry and academia as a sustainable alternative to traditionally used composites derived from or bound with petroleum-based materials. In particular, lightweight, foam-like mycelium-lignocellulosic fiber insulation composites (MLFCS) represent an emerging class of novel materials that have sparked significant research and commercialization interests.

Mycelium is the vegetative structure of filamentous fungi, consisting of a network of tubular filaments known as hyphae. These hyphae form an interconnected network that varies in morphology depending on the fungal species, development stage, and environmental conditions [11]. The cell walls of hyphae are primarily composed of chitin, glucans, proteins, and other biopolymers [12,13]. Mycelium develops on organic substrates through apical tip growth, where hyphae extend from germinating spores or inoculated fragments under ambient or controlled conditions. As growth progresses, hyphae can colonize the substrate and form aerial mycelia, which may appear fluffy, matted, or skin-like, depending on the fungal strain and growth conditions. Most studies on MLFCs have focused on white-rot fungi, as they tend to grow rapidly and form an intensive mycelium network and cause minimal damage to the mechanical integrity of the substrate at the initial stage [14]. These features make them particularly suitable for composite production. After mycelia colonization, heat treatment is usually applied to dehydrate and inactivate the

organism. The resulting as-grown MLFCS consist of a three-dimensional network interwoven hyphae and partially decomposed substrates, with densities that can be as low as 25 and as high as 462 kg/m³, depending on the production process, fungal species, and substrates [15]. The morphology of the resulting composites, often characterized by smaller pores and tortuous pathways, has been shown to enhance both thermal and acoustic insulation properties [16]. However, inconsistencies in mechanical properties reported across studies highlight the complexity and variability inherent in mycelium composite systems, pointing to a need for standardized testing protocols [17–19].

MLFCs have attracted growing interest as sustainable alternatives to conventional materials, due to their bio-based origin and perceived environmental benefits. They have demonstrated promising properties, including compressive strength and potential for thermal and acoustic insulation. The primary fabrication method explored in the literature is packing or molding, in which a mixture of lignocellulosic particles, nutrients, and fungal inoculum is packed into a mold. While this process is relatively simple and scalable, it can pose challenges in designing complex shapes, and expanding potential applications. For example, it is particularly difficult to pack fibers tightly into the edges of complex geometries. Recent innovations, such as additive manufacturing (3D printing), have emerged as promising techniques for overcoming these design limitations and expanding the potential applications of MLFCs [20].

To further reduce the bulk density of the MLFCs and improve thermal and acoustic insulation properties, novel fabrication processes need to be developed and evaluated. In this work, we present a technique to grow mycelium within a pre-fabricated lignocellulosic scaffold to produce ultra-low-density foams. Growing mycelium on low-density lignocellulosic foam scaffold substrates has been explored previously [21], but their reported material exhibited densities above 58 kg/m³. Our approach aims to achieve densities below 50 kg/m³ while maintaining thermal conductivities under 0.04 W/m.K for indoor insulation applications to compete with EPS and XPS foams, which currently dominate the building insulation market due to their low densities. To achieve this, we developed ultra-low-density lignocellulosic foam scaffolds (with an initially thermal conductivity (TC) higher than 0.04 W/m.K) to serve as substrates for mycelium growth, rather than using the traditionally packed, individually dispersed particles reported in most studies. In this regard, a foam forming method was used to prepare hybrid lignocellulosic foam scaffold substrates using thermomechanical pulp (TMP), yellow birch (YB), bleached pulp hardwood (BHP), and CNFs as binder. Three formulations were prepared by mixing 90% TMP/10% CNFs, 45% TMP/45% YB/10% CNFs, and 45% TMP/45% HBP/10% CNFs, with 1 g/L sodium dodecyl sulfate (SDS), following the same foam-forming protocol described in our previous work [22]. These specific ratios were selected based on preliminary trials aimed at

optimizing mycelial colonization using softwood and hardwood substrates. SDS was used as a surfactant essential to the foam-forming process, as it facilitates the creation of stable, low-density structures necessary for effective insulation performance. After washing the foam scaffolds to remove the excess of SDS and redrying them, the scaffolds were soaked in a liquid culture made by mixing two plates of pure mycelium from white rot fungus *Trametes versicolor* (*T. versicolor*) with 300 mL of nutrient solution (4% glucose (Gl.)/2% corn steep liquor (CSL) 1:1 v/v). The foam scaffolds were placed into autoclavable polypropylene boxes and then transferred to a dark incubator at 28 °C and 80% RH for up to 15 days. As the mycelium colonizes on the scaffold, the size of the pores decreases, leading to a reduction in TC, while maintaining the low-density of the composite, since pure mycelium is inherently lightweight. To prepare the MLFCs for characterization and application, inactivation and drying of the mycelium are crucial. In the present study, freeze drying was selected as the primary drying technique because it maintains the porous microstructure that is essential for low density and excellent thermal and acoustic properties. Although freeze drying is energy and time intensive compared to other methods such as air, oven and microwave drying, preliminary experiments with the growing conditions in this study concluded that freeze drying was more suitable for optimal performance structurally and functionally. A direct comprehensive comparison of drying techniques is still ongoing in detail and will be reported separately. In addition to the process development presented here, the physical, chemical, and morphological properties of the obtained MLFCs were analyzed and compared with the raw scaffolds and other conventional and synthetic composite materials.

Materials and Methods

1.1. Materials

Three different lignocellulosic substrates were utilized in this study: thermomechanical pulp (TMP), from a blend of softwoods (spruce, fir, and pine), with an average fiber diameter of $30 \pm 14 \mu\text{m}$ and length of $667 \pm 705 \mu\text{m}$ [22], supplied by TimberHP (Madison, ME); yellow birch (YB) veneers supplied by Columbia Forest Products LLC (Presque Isle, ME), were mechanically ground and manually sieved to particle sizes of 0.5–2 mm; and bleached hardwood pulp (BHP), derived from a blend of northern hardwoods (maple, birch, beech, and aspen), with an average fiber length $> 0.7 \text{ mm}$, was supplied by the University of Maine Process Development Center (Orono, ME) [23]. These materials were used as substrates to prepare the lignocellulosic foam scaffolds, using cellulose nanofibrils (CNFs) (3

wt% solid content and 90% fines, a product of the PDC [24]) as a binder and sodium dodecyl sulfate (SDS $\geq 98.5\%$) from Sigma-Aldrich as a foaming agent.

Trametes versicolor (*T. versicolor*) a white-rot fungus was supplied by North spore (Portland, ME) and chosen for this work due to its hyphae characteristics, high abundance, and strong growth potential. The pure *T.versicolor* plates grown on malt extract agar (MEA) were stored in a refrigerator at 4 °C. Three nutrient solutions, obtained from Sigma-Aldrich, were used in this study: corn steep liquor (CSL), glucose (Glu. >99.5%) and MEA.

1.2. Methods

1.2.1. Composite preparation

Mycelium-lignocellulosic fiber composites were prepared in four steps (A, B, C and D) (Figure 1):

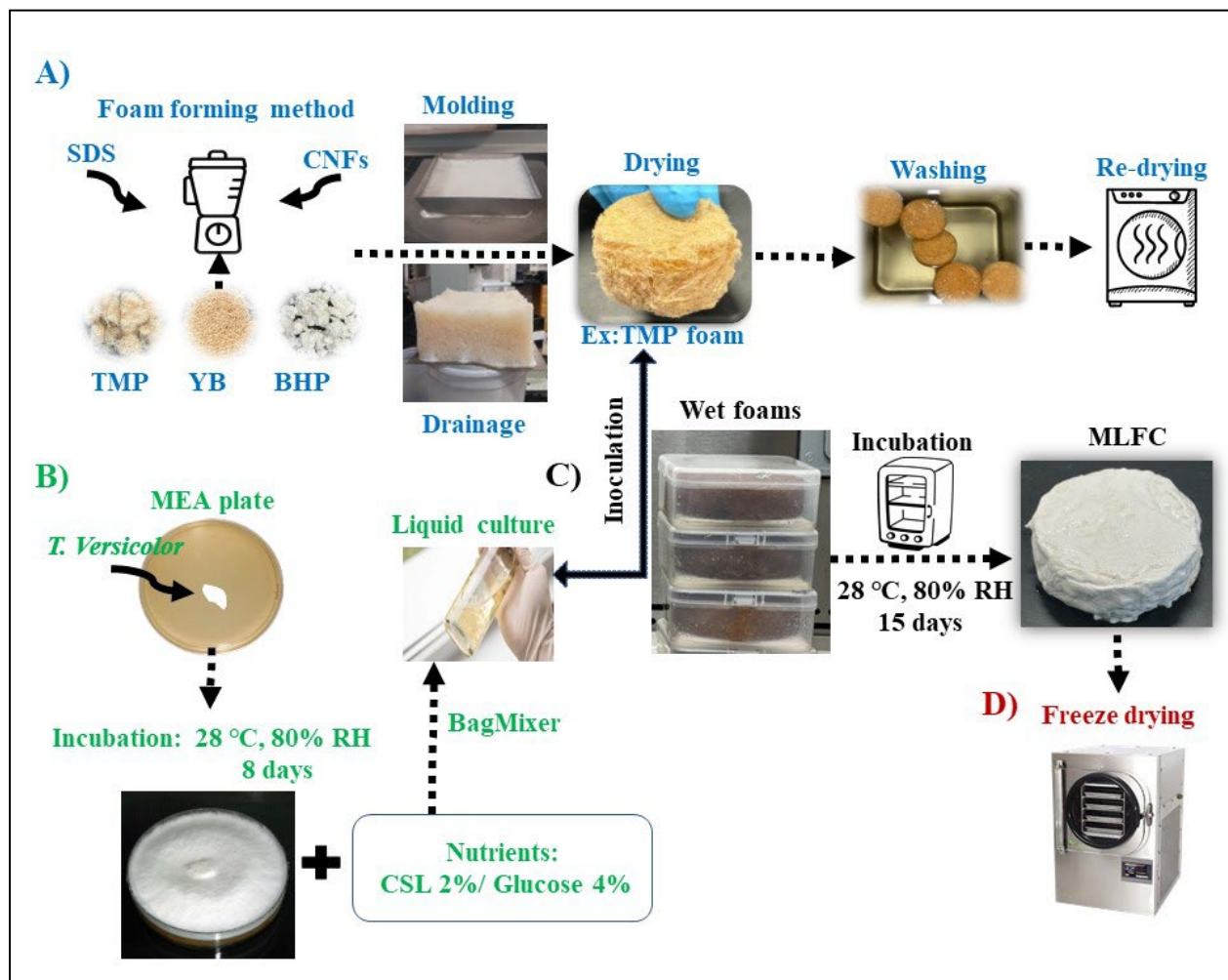


Figure 1: Schematic of the whole preparation process for mycelium–lignocellulosic fiber composites (MLFC)

A. Preparation of low-density lignocellulosic foam scaffolds

The first stage consisted of preparing the low-density lignocellulosic foam scaffold substrates using the foam forming method and 1g/l SDS as described in our previous studies [22,25,26]. Three hybrid formulations (solid content of 6%) were prepared for this study using: TMP 90%/CNFs 10%, YB 45%/TMP 45%/CNFs 10%, and BHP 45%/TMP 45%/CNFs 10%. The wet foams were first dried at 70 °C for 12 h followed by an additional drying step at 105-120 °C for 12 h to obtain foams that would be stable in water. This was necessary as a washing step was required to remove any residual SDS from the scaffolds that would inhibit fungal growth. An SDS concentration as low as 0.5 g/l had been previously found to have antifungal activity against *T. versicolor* [22].

B. Preparation of fresh *T. versicolor* mycelium plates and liquid culture

T. versicolor was used for the development of the mycelium. While the lignocellulosic foam scaffolds were prepared, the *T. versicolor* strain was incubated on MEA. A full-grown plate of the *T. versicolor* that was stored at 4 °C was sliced into 5 mm by 5 mm pieces using sterile forceps and each inoculum was transferred to the MEA plate (9 cm plastic petri dish) and kept for incubation at 28 °C and 80% relative humidity (RH) for one week or until the surface of the agar plate was fully covered by fungal hyphae [22]. The fungal plates were stored at 4 °C until the day of inoculation on the substrate.

The liquid inoculum was prepared by mixing two MEA plates overgrown by the fungus in 300 ml of 2% (w/v) sterile CSL and 4% (w/v) sterile Glu. (1:1 v/v) in a BagMixer (Interscience, St Nom, France) for 2 min. After that, the materials were transferred to a sterile mesh strainer (1mm x 1 mm) to remove the larger parts of agar that did not mix well. The liquid inoculum was prepared on the same day of the inoculation.

C. Inoculation of foam substrates with the fungus and incubation

The same day of inoculation, the redried washed foam scaffolds were steam-sterilized at 121 °C for 1 h. After cooling down to 40 °C, the foam scaffolds were inoculated with the *T. versicolor*, by soaking them in the liquid inoculum that was prepared on the same day for about 10 min, till the final water content value is around 80%. The CSL and Glu. in the liquid culture provide more carbon and nitrogen for the cultivation process. The foams were placed into autoclaved polypropylene boxes and then transferred to a dark incubator at 28 °C and 80% RH for up to 15 days. At this stage, the mycelium hyphae effectively bond the fiber core and fully cover the surface of the foam scaffold creating a white or off-white velvety layer.

D. Drying of the lignocellulosic/mycelium foam composites

After 15 days of incubation and before the product was ready for characterization, the composites were dehydrated by freeze drying for 48h, with the following temperature steps: 8 h at -30 °F, 15 h at 20 °F, 10 h at 40 °F, 10 h at 60 °F and 5 h at 90 °F.

All samples were conditioned at 23 °C and 50 (±2) % relative humidity for at least 72 hours prior to testing. Some foam samples were also processed into fine powders using a coffee grinder for chemical analysis. Depending on the characterization type, three to six replicates were tested for each formulation.

1.2.2. Composite characterization

a. Foams' morphology: Scanning Electron Microscopy (SEM)

An AmScope™ optical microscope (Model ME520TA, Irvine, CA) was used to analyze the surface and the internal morphologies of the foam before and after inoculation and incubation. Scanning electron microscopy (SEM) (Zeiss, Oberkochen, Germany) was used at an accelerating voltage of 6 kV to investigate the interior morphology of the foams before and after mycelium growth for 15 days at different magnifications. A 3–5 mm thick slice, which included the full cross-section of the specimen, was cut from the middle of the foams using a razor blade. These samples were sputter coated with a 6-nm-thick palladium layer to allow imaging of the samples.

b. Density and porosity

The density of the mycelium foams was determined after drying the samples at 70 °C for 24-72 h, following ISO 9427:2003 [27] by calculating the ratio of the oven-dry mass over the volume. Six replications were performed on each formulation.

The porosity of foam scaffolds and mycelium-lignocellulosic fiber composites was evaluated using weight and volume measurements, assuming a solid density of 1500 kg/m³ for both the lignocellulosic fibers and the fungal skeleton [28], using Equation (1).

$$\text{Porosity (\%)} = \left(1 - \frac{\text{bulk density}}{\text{solid density}}\right) \times 100 \quad (1)$$

c. Thermal conductivity

The thermal conductivity of the preconditioned foams before and after mycelium growth was evaluated using a TA Fox50 heat flow meter (TA Instruments, New Castle, DE) according to ASTM C518[29]. Circular samples of 5 cm in diameter with different thicknesses were used for the tests. The tests were performed at temperature gradients of 10-30 °C and 20-40 °C, and the reported values represent the averages.

d. Compressive properties

Compressive strength and compressive moduli of preconditioned foams before and after mycelium growth were measured according to ASTM C165 [30], using an Instron 5942 universal testing machine (Instron, Norwood, MA) with a 500 N capacity load cell. The samples had a circular shape with a 50 mm diameter, and their thickness varied depending on the tested formulation. Measurements were performed at a cross-head speed of 0.89 mm/min, with compressive strength reported at two strain levels: 10% and 25%. It is important to note that the initial contact surfaces between the load cell and the samples were not entirely flat, as the MLFCs naturally have rough surfaces.

e. Water absorption

Water uptake over time for mycelium foams was carried out after drying the samples at 70 °C and cooling them down in a desiccator for 24 h until their mass was stabilized, following the ASTM C272/C272M-18 method [31]. After measuring the initial mass of the samples (m_i), they were completely immersed in water at room temperature using a metal plate placed on the samples to prevent them from floating for a total duration of 240 h. The samples were then removed and wiped with paper towels to remove the excess water and weighed again to obtain the final mass (m_f) after 12, 24, 36, 48, 60, 72, 84, and 96 h. Water uptake was calculated by applying Equation (2):

$$\text{Water uptake} = \left(\frac{m_i - m_f}{m_f} \right) \times 100 \quad (2)$$

f. Sound absorption

An impedance tube BSWA-III-C021-03-0027-IMP (BSWA Technology, Beijing, China) was used to investigate the acoustic properties of the foams before and after the mycelium growth, following ASTM standard E1050 [32]. The samples had a diameter of 30 mm, with different known thicknesses, which were precisely measured. The tests were conducted across a frequency range spanning from 1000 to 6000 Hz.

g. Statistical analysis

All experimental data were subjected to a one-way ANOVA using SPSS 28.0 for Windows program. The difference in means was then tested using Duncan's multiple range test, at a probability level $p < 0.05$ to separate means.

2. Results and discussions

2.1. Preliminary study: choice of lignocellulosic substrate and the process conditions

Based on our previous study [22], by the application of wet-to-dry foam-forming process, we were able to obtain very low-density foams (11 to 45 kg/m³) using 90% TMP fibers, 10% CNFs and 1g/l SDS. However, these foams showed strong resistance to *T. versicolor* growth because of the residual SDS present in the foam structure, and also the source of the substrate (softwood or hardwood). Upon the identification of all barriers to mycelium growth on the foamed substrates, an experimental design was followed to overcome these problems and improve mycelium growth. As a conclusion, TMP, YB and BHP were selected as substrates in the screening process due to their availability and bio-compatibility with the foaming process. In this paper, we report the growth of the white-rot fungus *T. versicolor* on substrates formulated as follows: 45% TMP/45% YB/10% CNFs, 45% TMP/45% BHP/10% CNFs, and 90% TMP/10% CNFs. Washing and soaking the foam scaffolds in a nutrient solution of 2% CSL and 4% Glu (1:1. v:v) was beneficial for enhancing mycelium growth and colonization, despite the presence of potential inhibitory components, such as wood extractives. These conditions lead to the production of composites with excellent quality and quantity of mycelium production. More details about this preliminary study are presented in the supplementary information (Figures S1, S2 and S3).

After 15 days of incubation, the fully colonized samples were dried using freeze-drying, which was beneficial as it reduced distortion of the foams caused by surface tension during drying. Subsequently, mycelium growth and its impact on the structural, physical, and chemical properties of the foams were evaluated through different characterization methods outlined in the Methods section.

2.2. Growth assessment and behavior of mycelium on the foams' structure

Figure 2 (A and B) shows the mycelium growth progress on the pure TMP, YB/TMP, and BHP/TMP foams, respectively, after 15 days of incubation at 28 °C and 80% RH. Inoculating the prepared foams with *T. versicolor* culture resulted in successful colonization and the formation of a complex mycelium network through tip extension and continuous branching leading to a maximized surface-to-volume ratio, thus enhancing nutrient absorption during

substrate colonization [33]. We observed that hybrid formulations (YB/TMP and BHP/TMP) showed better growth compared to neat TMP foams in terms of the growth rate. TMP derived from softwoods retains a high amount of extractives even after processing (6.0% extractives and 41.3% lignin were reported in TMP fines [34], while our previous work showed that the yellow birch particles used here contained only 0.6% extractives and 26% lignin [35]. These compositional differences likely contribute to the observed growth differences. Other factors, such as fiber size, surface area, and interactions between residual nutrients and substrate, may also play important roles.

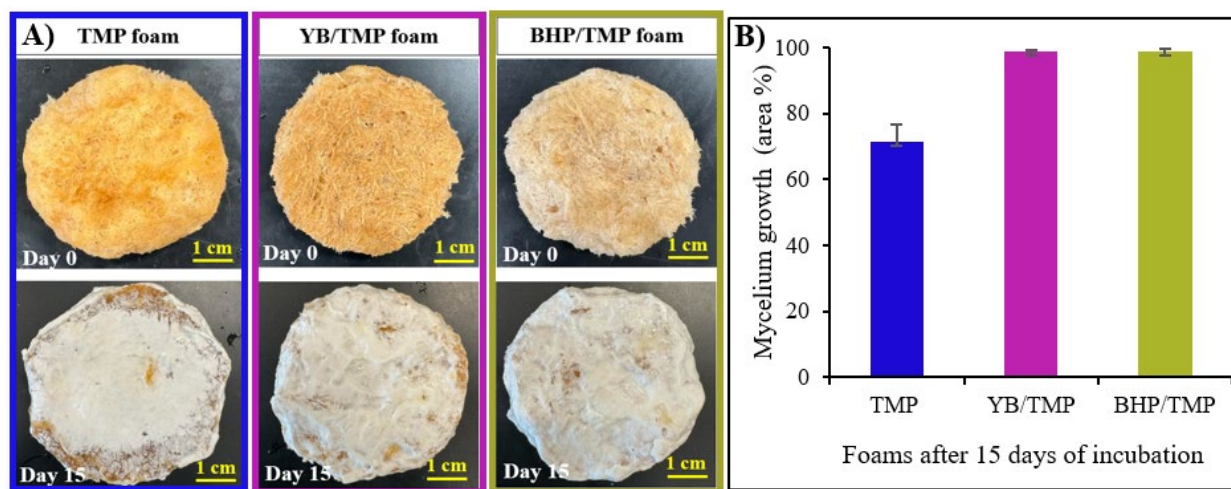


Figure 2: Development of surface mycelium on different foams before and after 15 days of incubation (A) and mycelium growth on the surface, expressed as the percentage of the sample surface area covered by mycelium (area %) (B).

Figure 3 shows the microscopic images of the external (A, C) and internal (B, D) surfaces of 45% TMP/ 45% YB/ 10% CNFs foam prior to incubation (A, B) and after 15 days of incubation (C, D) after drying. It was found that the external surface of this foam was fully covered with fungal mycelia (Figure 3-C). The internal structure of the foam (Figure 3-D) showed that the mycelium grew well inside the foam, resulting in a highly dense white fungal biomass inside and outside the foam composite. In addition to growth on the surface of wood fibers, patches of almost pure mycelium can be found in the larger pores of the scaffold indicating that the average pore size has been significantly reduced. These images indicate that there is successful mycelial growth both within the foam composites and on the external surface, confirming the fungus' ability to grow well under these experimental conditions. Based on a visual assessment of optical photographs of various samples, foams made from BHP/TMP had a high degree of mycelium density, followed by YB/TMP, however in TMP samples mycelium growth within the scaffold was limited.

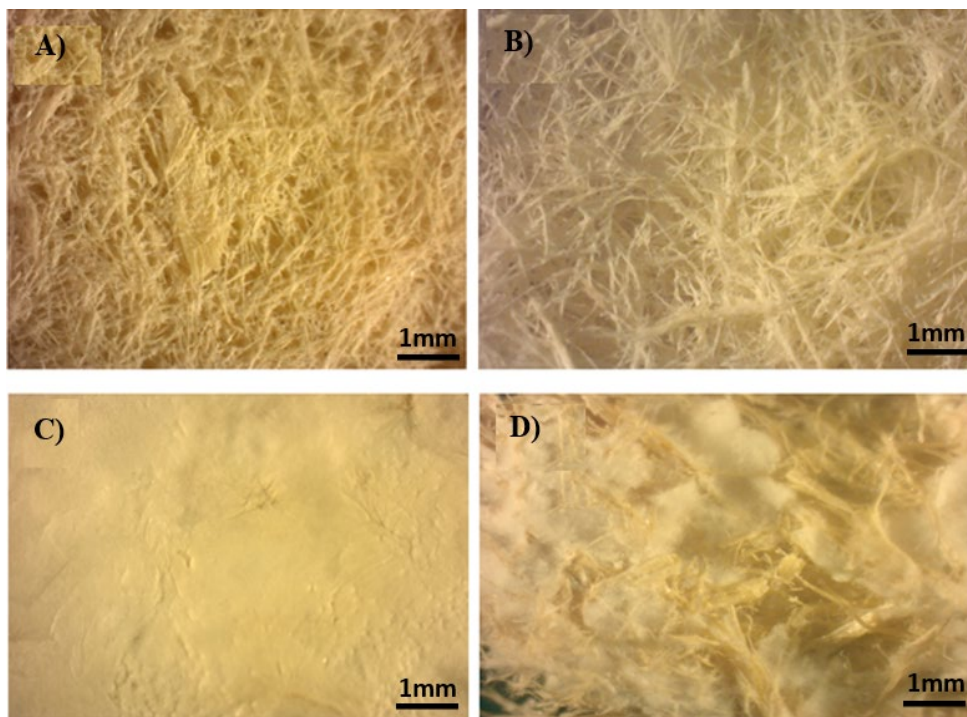


Figure 3: Optical microscopic images of the 45% TMP/ 45% YB/ 10% CNFs foam scaffold as an example before inoculation (A: external area and B: internal area) as well as the same surfaces after 15 days of incubation (C: external area and D: internal area).

As mentioned before, 10% (w:w) CNFs were used in the three formulations to enhance the stability of the foams and this was confirmed in our latest work [22]. The SEM micrographs of TMP, YB/TMP and BHP/TMP foams (Figure 4) clearly show the presence of CNFs as layers that bind the fibers together in the scaffold system. The SEM images after the first seven days of the growing process show that some fungal hyphae grew into the CNFs, partially consuming them as a source of nutrient. After 15 days of incubation, the growth of the mycelium on these structures (inside and outside) is clearly visible in the SEM images. Interestingly, almost all CNF content is consumed by the fungus. The growth of the mycelium involves the secretion of enzymes to both bind and degrade the substrate [36], and this is confirmed by the SEM images of the foams after 15 days of incubation, in which we noticed a total digestion of CNFs. Compared to YB/TMP and BHP/TMP foams, the 100% TMP foam showed less mycelium growth and less visible degradation or defibrillation in the SEM images. This may indicate a reduced extent of substrate breakdown, possibly due to the more intact fiber network, limited enzyme penetration, or simply the lower extent of mycelium colonization in the pure TMP structure.

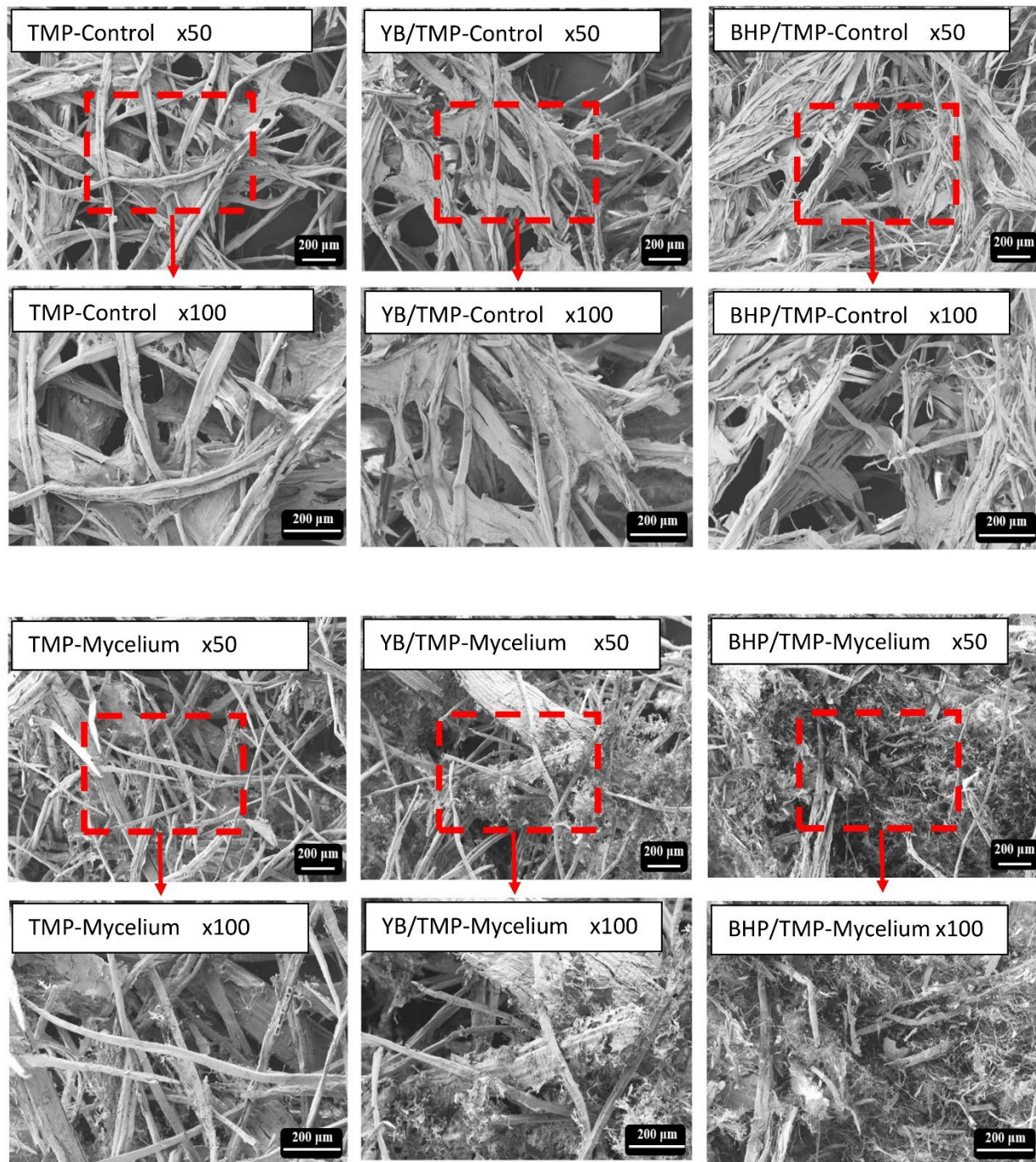


Figure 4: SEM micrographs of the internal surface of different foams before (control) and after 15 days of incubation (mycelium) at x50 and x100 magnifications.

2.3. Changes in the properties of as-grown foam composites

2.3.1. Density and thermal conductivity

Promising results were obtained when the ultra-low density foam composite samples were subjected to various characterization tests. Thermal insulation materials, like foams, are essential in the building and packaging industries. These materials are typically low-density and highly porous systems and enhancing their insulating properties can lead to significant energy savings. In our recent work, we developed sustainable ultra-low-density lignocellulosic foams with low thermal conductivity using TMP fibers [22]. In that work, we showed that the porous structure and pore size distribution of the foams affect the mechanisms of heat transfer through the material. For example, we found that the foams made from SDS were very porous with large pores and had very low densities (11-14 kg/m³). However, their thermal conductivities were around 0.045-0.055 W/m.K, which is relatively high for low-density materials. Thermal conductivity was further reduced with increasing density, reaching a minimum of 0.031 W/m.K at a density of 55 kg/m³ before increasing again once the density exceeded 55 kg/m³.

One main objective of growing the mycelium in these lignocellulosic foam scaffolds was to decrease the pore sizes of these foams in order to enhance their thermal insulation efficiency without affecting their densities. Effectively, Figure 5 (A) shows that the density of the foams did not change considerably after the mycelium growth; it increased by only 2 kg/m³ for the three formulations. The difference in density was not statistically significantly at 95% confidence level between the control group and the groups after growth for all three formulations. The densities remained under 50 kg/m³ and ranged between 35-45 kg/m³ before and after incubation.

Density values of MLFCs varies significantly based on the substrates utilized in their preparation and the initial packing density [37–39]. MLFCs made with denser scaffolds clearly exhibited higher density; for example, the 100% TMP foam scaffold, which was denser than the hybrid system, produced MLFCs with greater density. The density of the MLFCs produced in this work falls within the typical range of common polymeric insulation foams, such as expanded polystyrene (EPS), extruded polystyrene (XPS), and polyurethane (PUR), which generally range from 10 to 100 kg/m³ [40]. Moreover, while the density of the produced composites falls within the reported range for MLFCs (25-462 kg/m³) [15], it is at the lower end of that range, supporting their suitability for lightweight design needs.

It is evident in Figure 5 (B) that the thermal conductivity decreased significantly after the mycelium growth, especially for the hybrid systems (TMP/YB (0.035 W/m.K) and TMP/BHP (0.037 W/m.K)). This decrease was statistically

significant at 95% confidence level between the control and samples after growth for all three formulations. This trend can be logically linked to the rise in specific surface area by dividing the large pores into different smaller ones, as well as the reduction in average pore size. The obtained foams can match the performance of XPS (34 kg/m^3 , 0.03 W/m.K) [41] and meet/exceed characteristics of the conventional EPS foams ($0.031\text{-}0.038 \text{ W/m.K}$) [42]. Additionally, they are better than other natural insulators, including sheep wool (18 kg/m^3 , 0.05 W/m.K) and kenaf (105 kg/m^3 , 0.04 W/m.K) [42]. The effective thermal resistance and sustainable composition of these innovative low-density MLFCs allowed them to compete with traditional thermal insulation products as a promising alternative in the insulation market. This makes MLFCs a viable alternative to conventional commercial building insulation materials.

The reduction of thermal conductivity observed after the mycelium growth can be attributed to several interconnected factors. Firstly, the presence of numerous voids within the lignocellulosic fibers in low-density MLFCs plays a critical role in thermal insulation. These voids trap a considerable volume of air, which has an exceptionally low thermal conductivity of approximately 0.026 W/m.K [40]. This air-filled space significantly reduces the overall heat transfer through the material, as air is a poor conductor of heat. Secondly, the intricate arrangement of the lignocellulosic fibers contributes to the limitation of anisotropic heat flow. The irregularity in the fibers' arrangement creates a complex thermal pathway that impedes the direct transmission of heat, further enhancing the material's insulating properties. Additionally, the fungal activity involved in the degradation of wood cell walls leads to the formation of nanostructures, such as nanofibers and nanoscale holes. This microbial transformation not only increases the specific surface area of the composite but also alters its microstructure, introducing boreholes that disrupt thermal pathways [43]. Moreover, as the fungal degradation progresses, it reduces the spacing between the fibers and increases the number of smaller voids within the composite. This modification is crucial in further hindering heat transfer, as the smaller voids reduce radiative heat transfer leading to lower overall thermal conductivity. These factors collectively contribute to the remarkable thermal performance of MLFCs, making them suitable for various applications, including sustainable building materials and thermal insulation solutions.

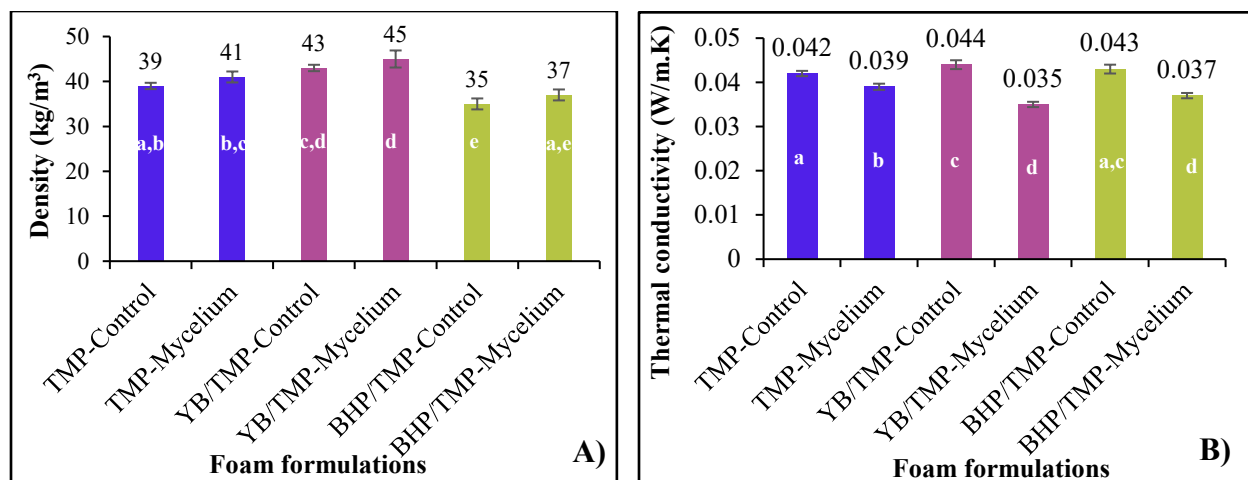


Figure 5: Density (A) and thermal conductivity (B) of low density foams before and after mycelium growth. Columns not sharing a letter are significantly different ($p < 0.05$).

2.3.2. Mechanical properties

Figure 6A and B show the evolution of the compressive strength and modulus after the mycelium growth on TMP alone, YB/TMP and BHP/TMP foam formulations. As mentioned before and explained in the introduction, the substrate significantly impacts all of the foam's properties. Even before mycelium growth, the hybrid foams were much better than the foams made with 100% TMP with regards to compressive strength (almost 2 times higher) especially at 25% compressive strain while being lighter in the case of BHP/TMP. After mycelium growth, the compressive strength increased significantly for all three formulations, however, this increase was more pronounced in the hybrid system, in which the growth rate was much higher inside and outside the substrate scaffolds compared with the pure TMP foams. At 10% compressive strain, the compressive strength of the pure TMP foams increased by three times while the YB/TMP and BHP/TMP hybrid foams increased by four to five times. At 25% compressive strain, this decreased to a 2x increase in compressive strength for both the pure and hybrid foams. The compressive moduli of TMP, YB/TMP and BHP/TMP foams also increased after mycelium growth, rising from almost zero to 1 kPa, 0.11 to 23.3 kPa and 0.12 to 40 kPa, respectively. Additionally, stiffness (modulus) also increased with compaction, which is a result of increasing fiber-fiber contact with densification.

In this study we found that the compressive strength and modulus did show a significant increase after the mycelium growth especially in the hybrid systems despite the total degradation of CNFs that were initially used in the low-density scaffolds' preparation as a binder. This suggests a compensatory process stimulated by fungal colonization

and structural remodeling. Figure S4 shows that fungal hyphae formed extensive interfacial networks within the scaffold, with visible attachment and entanglement. These interactions prove that the mycelium effectively replaces the structural role of CNFs after its enzymatic degradation. The developing hyphal mesh enhances fiber-fiber contact and acts as a natural biopolymeric binder. This system of replacement is also supplemented by comparative structural analysis of scaffold forms. In TMP-based scaffolds, with limited internal mycelial development, post-CNF mechanical strength was enhanced but was significantly lower than that in the hybrid formulations, which promoted more extensive internal colonization, and thus higher compressive strength. This emphasizes the impact of internal mycelial network formation on reinforcing the scaffold when CNF is lost. In addition to internal colonization, we observed the formation of a homogeneous outer mycelial shell on the scaffold surface during subsequent growth stages that had a woody and leathery surface morphology. This shell binds the structure together, giving the composite its cohesive, unified form, and may also act as a secondary reinforcement layer to further enhance mechanical performance by distributing external loads and stabilizing the inner fiber network. This effect is particularly pronounced in the hybrid system when a mix of TMP (softwood) and YB or BHP (hardwood) is used. Overall, the internal hyphal bridging and external mycelial encapsulation would likely be responsible for maintaining the mechanical stability of the scaffold upon CNF degradation.

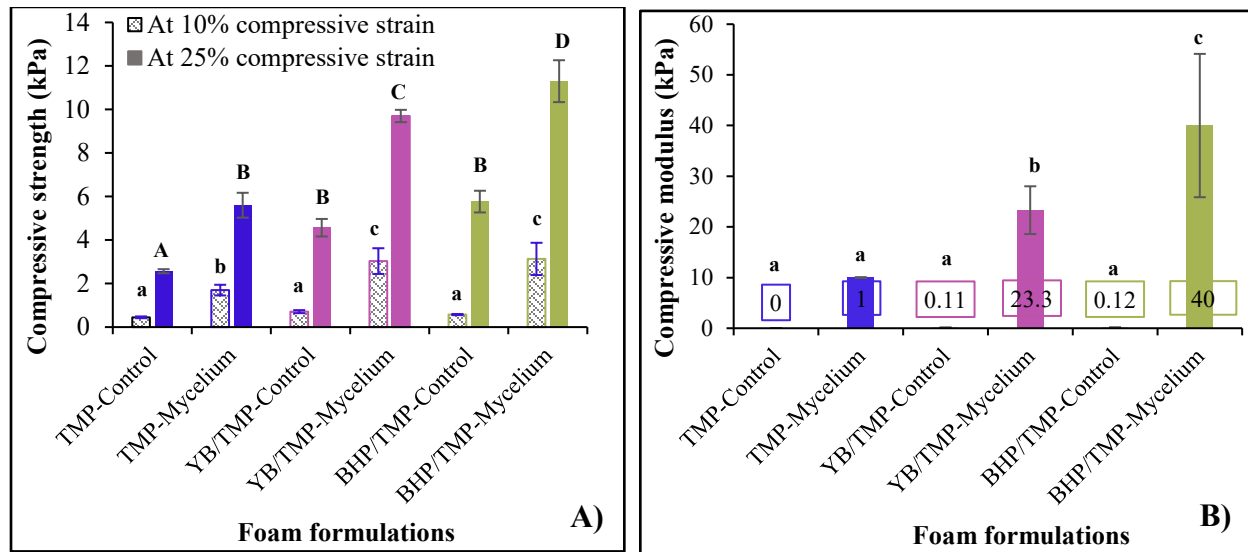


Figure 6: Compressive strength (A) and compressive modulus (B) of low-density foams before and after mycelium growth. Solid bars represent values at 25% compressive strain, while patterned bars represent values at 10% compressive strain.

2.3.3. *Water absorption and acoustic properties*

The water absorption capacity of the obtained low-density MLFCs was determined by submerging the foams in water over a period of up to 240 h. All samples with and without mycelium, maintained their shape throughout the entire soaking period/full immersion without any deformation, decomposition, or warping attributed to the improvements in water resistance by the applied heat treatment. When water was applied to the surface of mycelium foams, they initially exhibit very good water resistance (Figure S5) due to the presence of the hydrophobin proteins in the structure of aerial mycelium [44]. Although surface wettability is normally tested through contact angle measurements, in our case, they were not feasible due to irregular and non-planar (i.e., non-flat) sample surfaces; we were unable to obtain these measurements, since surface irregularities and curvature made it impractical to create stable drops on the materials for measuring the contact angle. However, after complete immersion, a large amount of water was retained in the samples. This is due to the development of the hyphal network dividing the large gaps between substrate particles into smaller ones, enhancing water retention through capillary action [45]. Pure mycelium can retain $1579 \pm 390\%$ of its dry weight in water after full immersion [45]. Additionally, fungal colonization is known to enhance the bulk flow of water through wood, potentially leading to greater water uptake within the wood particles [45]. Our results revealed that foams before the mycelium growth exhibited lower water absorption capacity regardless of the substrate types, while after the mycelium growth, the foams exhibited a significantly high degree of water absorption in all substrate types.

As can be seen in Figure 7A, the water absorption of the raw foams was about two times lower than those after the mycelium growth, which confirmed the effect of smaller pore sizes. It was found that the water absorption capacity of low-density foams before mycelium growth increased over a 24 h period and then slowly stabilized between 24 and 144 h before increasing again after 240 h. However, in the case of MLFCs, water absorption remained unchanged from 2 to 24 hours for the hybrid systems (BHP/TMP and YB/TMP), then increased gradually over 72 hours before re-stabilizing between 72 and 240 hours. For TMP foams, water absorption slightly increased from 2 to 24 hours, then stabilized, with no further absorption occurring after 24 hours. In addition, MLFCs obtained from BHP/TMP displayed the highest degree of water absorption capacity, followed by foams obtained from TMP and then YB/TMP. This means that the water absorption capacity is directly related to the substrate nature (size of particles and their nature: raw or treated) [46].

The variations in water absorption capacities among different composites were found to be linked to differences in their chemical components. Various research studies have concluded that MLFCs are typically hydroscopic materials, increasing in weight by ~40–580 % when in contact with water for 48–192 h [47,48]. Variations in water uptake findings can be attributed to several factors, including differences in research methodologies, testing standards, and sample preparation protocols, which complicate direct comparisons between studies. The high-water absorption affinity of MLFCs more influenced by the interconnected pores (open cell) structure of the foams which allows the absorption and retention of water due to capillary action. Other factors involved are the lignocellulosic filler components, which contain a large number of accessible hydroxyl groups [49], and the hydrophilic, porous nature of the mycelium binder itself, together promoting water absorption through wicking [50,51]. The non-linear water absorption pattern can be attributed to the interplay between the hydrophobicity of mycelium, the hydrophilic nature of the cellulosic materials, and the bonding interactions between the surface mycelium layer and the plant fiber core. While the high water uptake of MLFCs may pose challenges in certain construction applications, many uses such as acoustic or thermal insulation are typically for indoor or dry environments, where exposure to weather is minimal, thus mitigating this potential issue. Water absorption can be effectively reduced by either increasing the material's density or by applying a hydrophobic coating to its outer surface, which helps minimize moisture uptake [38]. To reduce the hydrophilicity of lignocellulose fibers and minimize water absorption in MLFCs, further research is required to achieve fully developed mycelium that effectively envelops substrate particles. Specifically, the development of a continuous and dense/compact aerial mycelium layer on the foam scaffold surface may enhance short term water resistance, as the aerial hyphae are known to exhibit greater hydrophobicity [44].

Beyond their thermal insulating properties, low-density MLFCs have shown great promise in acoustic insulation applications. Figure 7B shows the sound absorption coefficients of the foams in the range of 1000-6000 Hz. For neat foam scaffolds, the minimum sound absorption coefficients were at 1000 Hz (0.25 for TMP, 0.2 for BHP/TMP and 0.15 for YB/TMP) while the maximum values were at 3700 Hz for TMP (0.75), BHP/TMP (0.7) and at 6000 for the YB/TMP (0.6). However, after the mycelium growth, the as-produced foams showed interesting enhancement in the sound absorption coefficients at low range of frequencies. The BHP/TMP/Mycelium foam showed the highest sound absorption coefficient of about 0.98 at 1800 Hz, followed by the YB/TMP/ Mycelium sample with a value of 0.96 at 2500 Hz and then TMP/Mycelium with 0.94 at 1600 Hz. Contrary to Sun et al. [45] who reported that the sound absorption coefficients generally increased with sound frequency, in this study the sound absorption coefficients of

the mycelium foams decreased when the frequency increased to 4000 Hz and reached 0.72 for BHP/TMP/Mycelium, 0.82 for TMP/Mycelium. At around 4800 Hz, the sound absorption coefficient of YB/TMP/Mycelium foam decreased to 0.78 and reached 0.72 at 6000 Hz. However, in the case of BHP/TMP/Mycelium and TMP/Mycelium foams, the sound absorption values increased again to 0.84 and 0.88 respectively when the frequency was around 5500 Hz. Before mycelium growth, the sound absorption pattern vs frequency was following a similar trend for all formulations.

The changes in sound absorption coefficient after the mycelium growth may have been caused by the changes at the microscopic level such as tortuosity, airflow resistivity, and thermal characteristic lengths [52]. While MLFCs made from agricultural residues have shown strong absorption around 1000 Hz [14], their performance at lower frequencies is more limited. The exceptional acoustic absorption of MLFCs is largely due to their porous, fibrous structure, which facilitates sound wave dissipation. The acoustic properties of materials, such as impedance and propagation constants, are strongly influenced by air flow resistance. Materials with higher airflow resistance tend to exhibit greater sound absorption, as they better impede the movement of air, thereby converting more sound energy into heat [53]. The hyphae in MLFCs serve as frictional barriers that resist the motion of acoustic waves, causing the sound waves to lose energy as they navigate through the material's complex, tortuous structure. This energy is then converted into heat [54]. Thin fibers are particularly effective at improving acoustic absorption, as they are more flexible and allow for greater movement. Additionally, a higher density of fibers per unit volume creates more convoluted pathways, further increasing airflow resistance and enhancing the material's ability to absorb sound [55–57].

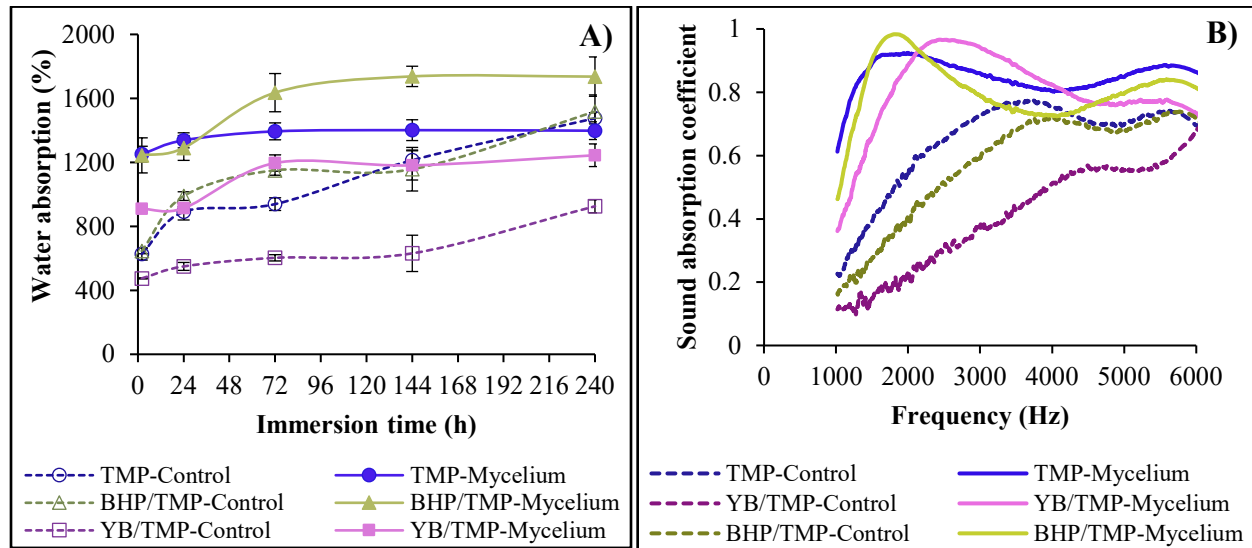


Figure 7: Water absorption of the lignocellulosic fiber composites before and after the mycelium growth (A) and their sound absorption coefficient (B).

2.4. Critical assessment of MLFCs and scalability considerations

The development of mycelium-lignocellulosic fiber composites (MLFCs) is a promising step towards more sustainable solutions to synthetic thermal insulation materials.

Since 2018, the laboratory of Renewable Nanomaterials at the University of Maine has been actively working on the development of novel hybrid systems of all-biobased materials for insulation applications. These systems are made from lignocellulosic particles bonded with renewable natural materials, including CNFs and mycelium. Working with industrial collaborators our team at UMaine developed fungal mycelium-based panels and objects with good thermal insulation. Moreover, it has shown that hybridizing the mycelium technology with CNFs as binder can greatly benefit the produced composites especially with respect to compressive strength at lower densities.

The current study presents a method for producing low-density MLFCs with enhanced physical and functional properties, specifically for thermal and acoustic insulation. Compared to conventional MLFCs produced by the packing process where density control is an issue, our system allows for more reproducible material properties by using lignocellulosic scaffolds inoculated with *Trametes versicolor*.

The morphological, physical, mechanical, and chemical properties of our MLFCs made from lignocellulosic scaffolds and *T. versicolor* fungi can guide us in the evaluation of their potential to be applied in various practical applications, especially insulation. The comparison of some properties of our MLFCs with other MLFCs made using the packing method with the same fungus and different substrates, other MLFCs made from other fungi species, as well as synthetic foams is shown in Table 1. The prepared MLFCs are competitive in terms of density (35-45 kg/m³), with other conventional MLFCs prepared using the packing method and also with synthetic insulation foams according to ASTM C578-23, such as EPS type XIV, XV, and VII (35-48 kg/m³) and XPS type V (48 kg/m³) [58]. Our production technique offers a major benefit in terms of thermal insulation as it results in MLFCs with lower thermal conductivities (0.035–0.039 W/m.K) than the conventional MLFCs, creating better thermal insulators that can compete with EPS (0.029-0.035 W/m.K) and XPS (0.029 W/m.K) [58].

The produced MLFCs are weaker than all other types of conventional mycelium or synthetic foams in terms of compressive strength. Therefore, these produced composites are not suitable for load-bearing applications, but they would very likely be better suited for use in other applications such as lightweight filling materials in the construction

of door panel cores. However, for load-bearing applications or components in construction, their strength remains insufficient and would require further reinforcement, as also noted by Ghazvinian et al. [59]. For non-structural uses such as insulation panels within wall assemblies, reinforcement may not be necessary.

Regarding acoustic insulation properties, there are limited data on the sound absorption coefficients of conventional MLFCs or synthetic insulator foams across a wide range of frequencies; for this reason, we did not include them in table 1. But interestingly, our produced MLFCs offer more than 95% sound absorption at 1600 Hz. Additionally, the produced low-density MLFCs display a higher tendency for water absorption compared to both synthetic foams and conventional MLFCs. However, this challenge can be mitigated through hydrophobic treatments or additives. However, all these challenges may be outweighed by the environmental benefits of MLFCs. They upcycle lignocellulosic fiber into higher-value fully biodegradable materials through natural fungal growth and can be potentially composted in a field within a few months at the end of their life [60]. Realistically, MLFCs show promising potential for use in thermal and acoustic insulation due to their combination of biodegradable content, low density, and low thermal conductivity. In particular, our materials exhibited good acoustic properties at specific frequencies, and they can be as good as, or even superior to, conventional alternatives, though a more comprehensive comparison is limited by the lack of standardized data in the same frequency range. While these functional characteristics show the potential of MLFCs, further investigation of the cost of production, along with practical considerations of processing and scalability, are needed to support their competitiveness and commercial adoption.

One major limitation to the scalability of MLFCs is the reliance on freeze-drying, a time and energy-consuming process, not practical for the large scale. More critical, though, is the 15-day incubation period of the fungus necessary for total scaffold colonization, which will be a limitation in time-critical or industrial applications. While this incubation time was sufficient in our experimental conditions, future efforts are needed to reduce fungal growth time to increase feasibility. Moving forward, efforts need to be made to reduce incubation time by modulating CNFs and nutrients optimally, such as incorporating nutrients that can be quickly accessed by fungi and materials that allow them to grow faster. To counterbalance the trade-off between scaffold porosity and mechanical strength, highlighted by relatively lower compressive strengths reported here when compared with denser MLFCs, density-gradient scaffolds can be recommended. For example, this could involve the production of a low-density, lightweight foam core covered or wrapped with a denser outer layer, balancing high porosity and lowest weight with improved mechanical performance. Such designs would expand the range of possible

applications of these materials to semi-structural applications. Together, these strategies would support a path forward to creating an energy-efficient, high-throughput manufacturing process for MLFCs of improved versatility and performance.

Table 1: Comparison of the properties of the produced mycelium-lignocellulosic fiber composites with synthetic foams.

Foam type	Manufacturing process	Density kg/m ³	Thermal conductivity W/m.K	Compressive strength at 10% deformation (kPa)	References
Mycelium-lignocellulosic fiber composites using different substrates and processes					
<i>T. versicolor</i> - hemp, flax, flax waste, pine wood, wheat straw	Packing	94–135	0.04–0.058	–	[39]
<i>T. versicolor</i> on YB particules	Packing	200	0.053	60-100	[45]
<i>T. versicolor</i> on Beech sawdust	Packing	200	0.067	17.2	[61]
<i>T. versicolor</i> on Beech wood sawdust	Packing	270	0.057	–	[62]
Other fungi with other substrates	Packing	70-280	0.05–0.18	–	[63,64]
<i>T. versicolor</i> on cellulose fiber foam	Growth on scaffold substrate made using foam forming method	58–77	0.032–0.06	–	[21]
<i>T. versicolor</i> on lignocellulosic scaffolds	Growth on scaffold substrate made using foam forming method	37–45	0.035–0.039	3.15	This study
Synthetic foams as specified in ASTM C578–23					
Type XIV	EPS	Expansion, aging and molding	38	0.034	276
Type XV			48	0.033	414
Type VII			35	0.029	414
Type V	XPS	Extrusion and expansion	48	0.029	690

3. Conclusions

This study reports the successful development of novel sustainable lightweight thermal insulation mycelium-lignocellulosic fiber composites (MLFCs) was through the cultivation of the white rot fungus *Trametes versicolor* on lignocellulosic foam scaffold substrates. These substrates were made from different hybrid systems (TMP/CNFs,

YB/TMP/CNFs, and BHP/TMP/CNFs) using a surfactant-assisted foam-forming method, followed by a washing step to remove residual surfactant. The visual appearance, morphology, density, porosity, thermal insulation, sound absorption, chemical composition, thermal and mechanical properties, and water-absorbing behavior of both raw foam scaffolds and the resulting MLFCs were evaluated. It was found that the properties of MLFCs as well as the mycelium growth rate were directly affected by the type of substrate, and the incubation conditions (moisture content, nutrients, and air availability).

Overall, the results demonstrated that the presence of surfactant on the substrate fibers completely inhibited mycelial growth. For this reason, a washing step after preparing the scaffolds and before inoculating them with the mycelium was mandatory. *Trametes versicolor* demonstrated optimal growth on hybrid scaffolds containing hardwood fibers (YB/TMP and BHP/TMP) in terms of growth rate, but the growth was significantly less on scaffolds composed only of TMP fibers. The morphology of the MLFCs indicates that the entanglement within the structure contributes to the material's lightweight characteristic as it resists collapse during water removal and drying. In addition to the enzymatic digestion processes associated with mycelial growth and the development of the fungus itself, the chemical changes observed in the FTIR spectra confirm that the chemicals present in the substrate are responsible for the distinct alterations in the material's composition. Therefore, the growth conditions of the mycelium should be optimized according to the type of the used substrate and the desired properties of the final material.

The mycelium materials exhibited densities ranging from 35 to 38 kg/m³, which are within the same range as those of many synthetic foams. The thermal conductivity and sound absorption coefficient of the produced MLFCs have shown an overall good insulation behavior (thermal conductivity of 0.035-0.039 W/m.K and sound absorption coefficient >0.9 %) compared to conventional insulation materials. Based on these results, we conclude that the mycelium growth enhances the thermal/sound insulation efficiency and the mechanical strength of the foam scaffolds without affecting their density, despite the digestion of all the CNFs used as binder. The role of the CNFs in these composite formulations is to hold the structure together until enough mycelium tissue is produced to assume the binding role of CNFs.

This research has shown that MLFCs can meet the necessary requirements for thermal and sound insulation foams and could replace fossil-based composites in these applications. The proposed approach in this study can serve as a valuable resource for architects, scientists, and engineers in exploring the potential new applications of MLFCs.

Further optimization of mechanical properties and water absorption could enhance the final properties of the novel MLFCs.

Acknowledgement

This material is based upon work supported by the US Department of Energy (DOE), Office of Energy Efficiency and Renewable Energy, Advanced Materials and Manufacturing Office under CPS Agreement 35863, and Oak Ridge National Laboratory/University of Maine SM²ART program with research and resources used at the School of Forest Resources and the Chemical and Biomedical Engineering department at University of Maine.

References

- [1] Tonnessen M. Anticipating the societal transformation required to solve the environmental crisis in the 21st century. *Sign Syst Stud* 2021;49:12–62. <https://doi.org/10.12697/SSS.2021.49.1-2.02>.
- [2] Dahy H. Biocomposite materials based on annual natural fibres and biopolymers – Design, fabrication and customized applications in architecture. *Constr Build Mater* 2017;147:212–20. <https://doi.org/10.1016/j.conbuildmat.2017.04.079>.
- [3] Dahy H. Towards Sustainable Buildings with Free-Form Geometries: Development and Application of Flexible NFRP in Load-Bearing Structures. In *Biocomposite Materials: Design and Mechanical Properties Characterization*. 2021. https://doi.org/10.1007/978-981-33-4091-6_2.
- [4] Roberts BC, Webber ME, Ezekoye OA. Development of a multi-objective optimization tool for selecting thermal insulation materials in sustainable designs. *Energy Build* 2015;105:358–67. <https://doi.org/10.1016/j.enbuild.2015.07.063>.
- [5] Bomberg M. Glows and shadows of thermal insulation. *Front Archit Res* 2013;2:263–6. <https://doi.org/10.1016/j.foar.2013.06.001>.
- [6] Fan W, Zhang X, Zhang Y, Zhang Y, Liu T. Lightweight, strong, and super-thermal insulating polyimide composite aerogels under high temperature. *Compos Sci Technol* 2019;173:47–52. <https://doi.org/10.1016/j.compscitech.2019.01.025>.
- [7] Zhang X, Zhao X, Xue T, Yang F, Fan W, Liu T. Bidirectional anisotropic polyimide/bacterial cellulose aerogels by freeze-drying for super-thermal insulation. *Chem Eng J* 2020;385:123963. <https://doi.org/10.1016/j.cej.2019.123963>.
- [8] Xu Y, Yan C, Du C, Xu K, Li Y, Xu M, et al. High-strength, thermal-insulating, fire-safe bio-based organic lightweight aerogel based on 3D network construction of natural tubular fibers. *Compos Part B Eng* 2023;261:110809. <https://doi.org/10.1016/j.compositesb.2023.110809>.
- [9] Weiss M, Haufe J, Carus M, Brandão M, Bringezu S, Hermann B, et al. A Review of the Environmental Impacts of Biobased Materials. *J Ind Ecol* 2012;16. <https://doi.org/10.1111/j.1530-9290.2012.00468.x>.

- [10] Modanloo B, Ghazvinian A, Matini M, Andaroodi E. Tilted arch; implementation of additive manufacturing and bio-welding of mycelium-based composites. *Biomimetics* 2021;6. <https://doi.org/10.3390/biomimetics6040068>.
- [11] Lew RR. How does a hypha grow? the biophysics of pressurized growth in fungi. *Nat Rev Microbiol* 2011;9:509–18. <https://doi.org/10.1038/nrmicro2591>.
- [12] Ghanbari F, Costanzo F, Hughes DP, Peco C. Phase-field modeling of constrained interactive fungal networks. *J Mech Phys Solids* 2020;145:104160. <https://doi.org/10.1016/j.jmps.2020.104160>.
- [13] Shakir MA, Azahari B, Yusup Y, Yhaya MF, Salehabadi A, Ahmad MI. Preparation and characterization of mycelium as a bio-matrix in fabrication of bio-composite. *J Adv Res Fluid Mech Therm Sci* 2020;65:253–63.
- [14] Zabel RA, J. J. Morrell. Changes in the strength and physical properties of wood caused by decay fungi. *Wood Microbiol.*, ademic Press, San Diego (2020); 2020, p. 271–91.
- [15] Aiduang W, Chanthaluck A, Kumla J, Jatuwong K, Srinuanpan S, Waroonkun T, et al. Amazing Fungi for Eco-Friendly Composite Materials: A Comprehensive Review. *J Fungi* 2022;8. <https://doi.org/10.3390/jof8080842>.
- [16] Sun W. Understanding the Adhesion Mechanism in Mycelium-Assisted Wood Bonding. University of Maine, 2021.
- [17] Kuribayashi T, Lankinen P, Hietala S, Mikkonen KS. Dense and continuous networks of aerial hyphae improve flexibility and shape retention of mycelium composite in the wet state. *Compos Part A Appl Sci Manuf* 2022;152:106688. <https://doi.org/10.1016/j.compositesa.2021.106688>.
- [18] Sun W, Hunt CG, Tajvidi M. Progress in Using Fungal Mycelia as Adhesive in Composites. *Prog Adhes Adhes Vol 9* 2025;9:469–92. <https://doi.org/10.1002/9781394315086.ch11>.
- [19] Goodell B, Winandy JE, Morrell JJ. Fungal degradation of wood: Emerging data, new insights and changing perceptions. *Coatings* 2020;10:1–19. <https://doi.org/10.3390/coatings10121210>.
- [20] Shen SC, Lee NA, Lockett WJ, Acuil AD, Gazdus HB, Spitzer BN, et al. Robust myco-composites: a biocomposite platform for versatile hybrid-living materials. *Mater Horizons* 2024;11:1689–703. <https://doi.org/10.1039/d3mh01277h>.
- [21] Amstislavski P, Pöhler T, Valtonen A, Wikström L, Harlin A, Salo S, et al. Low-density, water-repellent, and thermally insulating cellulose-mycelium foams. *Cellulose* 2024;31:8769–85. <https://doi.org/10.1007/s10570-024-06067-5>.
- [22] El Hajam M, Sun W, Hossain R, Hafez I, Howell C, Tajvidi M. Surfactant-assisted foam-forming of high performance ultra-low density structures made from lignocellulosic materials and cellulose nanofibrils (CNFs). *Ind Crops Prod* 2024;221:119357. <https://doi.org/10.1016/j.indcrop.2024.119357>.
- [23] Woodland Pulp LLC. Northern Bleached Hardwood Kraft. 2023.
- [24] Raheem M, Tripp CP, Tajvidi M. Oil and grease-resistant molded fiber containers produced by partial substitution of wood pulp with wood particles enabled by cellulose nanofibrils. *Cellulose* 2024;32:1851–66. <https://doi.org/10.1007/s10570-024-06340-7>.
- [25] Alonso MP, Hossain R, Hajam M El, Tajvidi M. Enhancement of the Physical and Mechanical Properties of

- Cellulose Nanofibril-Reinforced Lignocellulosic Foams for Packaging and Building Applications. *Nanomaterials* 2024;14:1837. <https://doi.org/10.3390/nano14221837>.
- [26] Hossain R, El M, Hafez I, Tajvidi M. Structure-property insights into foam-forming processing of lignocellulosic materials. *Constr Build Mater* 2025;489:142401. <https://doi.org/10.1016/j.conbuildmat.2025.142401>.
- [27] ISO 9427: Wood-based panels Determination of density. 2023.
- [28] Nawawi WMFW, Jones MP, Kontturi E, Mautner A, Bismarck A. Plastic to elastic: Fungi-derived composite nanopapers with tunable tensile properties. *Compos Sci Technol* 2020;198:108327. <https://doi.org/10.1016/j.compscitech.2020.108327>.
- [29] ASTM, C518: Standard Test Method for Steady-State Thermal Transmission Properties by Means of the Heat Flow Meter Apparatus, ASTM International. 2014.
- [30] ASTM. Standard Test Method for Measuring Compressive Properties of Thermal Insulations C165-07. 2012.
- [31] ASTM C272/C272M. Standard Test Method for Water Absorption of Core Materials for Sandwich. 2015.
- [32] ASTM E1050, Standard Test Method for Impedance and Absorption of Acoustical Materials Using a Tube, Two Microphones and a Digital Frequency Analysis System, ASTM International, West Conshohocken, PA, USA. 2024.
- [33] Heaton LLM, López E, Maini PK, Fricker MD, Jones NS. Growth-induced mass flows in fungal networks. *Proc R Soc B Biol Sci* 2010;277:3265–74. <https://doi.org/10.1098/rspb.2010.0735>.
- [34] Chen S. Thermomechanical pulp fines as additives for 3D printing filaments. The university of British Columbia, 2021.
- [35] Sun W, Hafez I, Cole BJW, Tajvidi M. A closer examination of white-rot fungal mycelia assisted wood bonding. *RSC Appl Interfaces* 2024;1:1036–44. <https://doi.org/10.1039/d4lf00061g>.
- [36] Goetten de Lima G, Schoenherr ZCP, Magalhães WLE, Tavares LBB, Helm CV. Enzymatic activities and analysis of a mycelium-based composite formation using peach palm (*Bactris gasipaes*) residues on *Lentinula edodes*. *Bioresour Bioprocess* 2020;7. <https://doi.org/10.1186/s40643-020-00346-2>.
- [37] Appels FVW, Camere S, Montalti M, Karana E, Jansen KMB, Dijksterhuis J, et al. Fabrication factors influencing mechanical, moisture- and water-related properties of mycelium-based composites. *Mater Des* 2019;161:64–71. <https://doi.org/10.1016/j.matdes.2018.11.027>.
- [38] Ziegler AR, Bajwa SG, Holt GA, McIntyre G, Bajwa DS. Evaluation of physico-mechanical properties of mycelium reinforced green biocomposites made from cellulosic fibers. *Appl Eng Agric* 2016;32:931–8. <https://doi.org/10.13031/aea.32.11830>.
- [39] Elsacker E, Vandeloock S, Brancart J, Peeters E, De Laet L. Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates. *PLoS One* 2019;14:1–20. <https://doi.org/10.1371/journal.pone.0213954>.
- [40] Hasanzadeh R, Azdast T, Lee PC, Park CB. A review of the state-of-the-art on thermal insulation performance of polymeric foams. *Therm Sci Eng Prog* 2023;41:101808. <https://doi.org/10.1016/j.tsep.2023.101808>.
- [41] Papadopoulos AM. State of the art in thermal insulation materials and aims for future developments. *Energy*

- Build 2005;37:77–86. <https://doi.org/10.1016/j.enbuild.2004.05.006>.
- [42] Asdrubali F, D'Alessandro F, Schiavoni S. A review of unconventional sustainable building insulation materials. *Sustain Mater Technol* 2015;4:1–17. <https://doi.org/10.1016/j.susmat.2015.05.002>.
- [43] Geoffrey Daniel. Fungal degradation of wood cell walls. Second. Xylem Biol. Orig. Funct. Appl., Academic Press (Elsevier); 2016, p. 131–69.
- [44] Wösten HAB, Schuren FHJ, Wessels JGH. Interfacial self-assembly of a hydrophobin into an amphipathic protein membrane mediates fungal attachment to hydrophobic surfaces. *EMBO J* 1994;13:5848–54. <https://doi.org/10.1002/j.1460-2075.1994.tb06929.x>.
- [45] Sun W, Tajvidi M, Howell C, Hunt CG. Insight into mycelium-lignocellulosic bio-composites: Essential factors and properties. *Compos Part A Appl Sci Manuf* 2022;161:107125. <https://doi.org/10.1016/j.compositesa.2022.107125>.
- [46] Aiduang W, Kumla J, Srinuanpan S, Thamjaree W, Lumyong S, Suwannarach N. Mechanical, Physical, and Chemical Properties of Mycelium-Based Composites Produced from Various Lignocellulosic Residues and Fungal Species. *J Fungi* 2022;8. <https://doi.org/10.3390/jof8111125>.
- [47] López Nava JA, Méndez González J, Ruelas Chacón X, Nájera Luna JA. Assessment of Edible Fungi and Films Bio-Based Material Simulating Expanded Polystyrene. *Mater Manuf Process* 2016;31:1085–90. <https://doi.org/10.1080/10426914.2015.1070420>.
- [48] Sun W, Tajvidi M, Hunt CG, McIntyre G, Gardner DJ. Fully Bio-Based Hybrid Composites Made of Wood, Fungal Mycelium and Cellulose Nanofibrils. *Sci Rep* 2019;9:1–12. <https://doi.org/10.1038/s41598-019-40442-8>.
- [49] Zabihzadeh SM. Water uptake and flexural properties of natural filler/HDPE composites. *BioResources* 2010;5:316–23. <https://doi.org/10.15376/biores.5.1.316-323>.
- [50] Dias PP, Jayasinghe LB, Waldmann D. Investigation of Mycelium-Miscanthus composites as building insulation material. *Results Mater* 2021;10:100189. <https://doi.org/10.1016/j.rinma.2021.100189>.
- [51] Lee T, Choi J. Mycelium-composite panels for atmospheric particulate matter adsorption. *Results Mater* 2021;11:100208. <https://doi.org/10.1016/j.rinma.2021.100208>.
- [52] Yang T, Hu L, Xiong X, Petru M, Noman MT, Mishra R, et al. Sound absorption properties of natural fibers: A review. *Sustain* 2020;12:1–25. <https://doi.org/10.3390/su12208477>.
- [53] Ren M, Jacobsen F. A method of measuring the dynamic flow resistance and reactance of porous materials. *Appl Acoust* 1993;39:265–76. [https://doi.org/10.1016/0003-682X\(93\)90010-4](https://doi.org/10.1016/0003-682X(93)90010-4).
- [54] Walter N, Gürsoy B. A Study on the Sound Absorption Properties of Mycelium-Based Composites Cultivated on Waste Paper-Based Substrates. *Biomimetics* 2022;7. <https://doi.org/10.3390/biomimetics7030100>.
- [55] Koizumi T, Tsujiuchi N, Adachi A. The development of sound absorbing materials using natural bamboo fibers. *High Perform Struct Mater* 2002;4:157–66.
- [56] Sun F, Banks-Lee P, Peng H. Sound absorption in an anisotropic periodically layered fluid-saturated porous medium. *Appl Acoust* 1993;39:65–76. [https://doi.org/10.1016/0003-682X\(93\)90030-A](https://doi.org/10.1016/0003-682X(93)90030-A).
- [57] Sivaprasad S, Byju SK, Prajith C, Shaju J, Rejeesh CR. Development of a novel mycelium bio-composite

- material to substitute for polystyrene in packaging applications. *Mater Today Proc* 2021;47:5038–44. <https://doi.org/10.1016/j.matpr.2021.04.622>.
- [58] ASTM 578. Standard Specification for Rigid , Cellular Polystyrene Thermal Insulation Boards. 2004.
 - [59] Ghazvinian A, Farrokhsiar P, Vieira F, Pecchia J, Gursoy B. Mycelium-Based Bio-Composites For Architecture: Assessing the Effects of Cultivation Factors on Compressive Strength. *Proc Int Conf Educ Res Comput Aided Archit Des Eur* 2019;2:505–14. https://doi.org/10.5151/proceedings-ecaadesigradi2019_465.
 - [60] Irbe I, Loris GD, Filipova I, Andze L, Skute M. Characterization of Self-Growing Biomaterials Made of Fungal Mycelium and Various Lignocellulose-Containing Ingredients. *Materials (Basel)* 2022;15. <https://doi.org/10.3390/ma15217608>.
 - [61] Schritt H, Vidi S, Pleissner D. Spent mushroom substrate and sawdust to produce mycelium-based thermal insulation composites. *J Clean Prod* 2021;313:1–8. <https://doi.org/10.1016/j.jclepro.2021.127910>.
 - [62] Nussbaumer M, Van Opdenbosch D, Engelhardt M, Briesen H, Benz JP, Karl T. Material characterization of pressed and unpressed wood–mycelium composites derived from two *Trametes* species. *Environ Technol Innov* 2023;30. <https://doi.org/10.1016/j.eti.2023.103063>.
 - [63] Girometta C, Picco AM, Baiguera RM, Dondi D, Babbini S, Cartabia M, et al. Physico-mechanical and thermodynamic properties of mycelium-based biocomposites: A review. *Sustain* 2019;11. <https://doi.org/10.3390/su11010281>.
 - [64] Holt GA, McIntyre G, Flagg D, Bayer E, Wanjura JD, Pelletier MG. Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: Evaluation study of select blends of cotton byproducts. *J Biobased Mater Bioenergy* 2012;6:431–9. <https://doi.org/10.1166/jbmb.2012.1241>.



DSPACE

<https://dspace.org/>

In situ growth of mycelium in a lignocellulosic scaffold enabled by cellulose nanofibrils for lightweight insulation

Hajam, Maryam El; Sun, Wenjing; Hafez, Islam; Howell, Caitlin; Tajvidi, Mehdi

2025-12-01

Elsevier

<https://doi.org/10.1016/j.compositesa.2025.109223>

Hajam ME, Sun W, Hafez I, et al., (2025) In situ growth of mycelium in a lignocellulosic scaffold enabled by cellulose nanofibrils for lightweight insulation. Composites Part A: Applied Science and Manufacturing, Volume 199, December 2025, Article number 109223