

MECHANICAL TESTING OF A DATE PALM FIBER- REINFORCED MYCELIUM-BASED COMPOSITE BIOMATERIAL

Amirreza Kaffash,¹ Javad Janpoor,² Parsa Safacian,¹ Seyed Abdolmajid Yousefsani,^{1*} and Masoud Tahani¹

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*The global bio-hazardous waste generation has prompted research towards developing advanced biocompatible and sustainable materials. Mycelium-based biocomposites have emerged as promising alternatives to conventional synthetic foam materials, offering biodegradability and recyclability with diverse applicability including thermal and acoustic insulation, packaging, and green construction. This study focuses on a novel biocomposite that forms by growth of a dense matrix of *Pleurotus eryngii* mycelium within a substrate made of shredded leaf sheaths of date palm as reinforcement fibers. A detailed fabrication protocol was developed, and the compressive and flexural characteristics of the biocomposite were measured. Three cylindrical specimens with identical composition but different pre-loads were fabricated to investigate the effects of pre-loading on the compressive properties. Results indicated a range of compressive strength from 0.34 to 4.55 MPa depending on the level of pre-loading, with higher pre-pressure leading to the improved compressive properties without significant increase of the apparent density. Moreover, two prismatic specimens featuring different fiber sizes were tested to assess the flexural properties. Results indicated that the specimen with small fibers exhibited superior flexural strength and modulus of 21.9 kPa and 2.98 MPa, respectively, in comparison with 11.7 and 1.29 MPa obtained for the large-fiber specimen. Depending on the fabrication conditions, the physical and mechanical characteristics of this biological composite foam can be adjusted for a particular application.*

¹Department of Mechanical Engineering, Faculty of Engineering, Ferdowsi University of Mashhad, Mashhad, Iran

²Industrial Fungi Biotechnology Department, Research Institute for Industrial Biotechnology, Academic Centre for Education, Culture, and Research (ACECR)-Khorasan Razavi Branch, Mashhad, Iran

*Corresponding author; tel.: +98 5138805448; e-mail: yousefsani@um.ac.ir

1. Introduction

Indiscriminate generation and disposal of packaging wastes has brought about an urgent environmental crisis since the 1970s. The accumulation of litter and solid wastes in the nature and the increasing water and air pollution are some of the consequences of plastic-based packaging [1], which has risen the global concerns about the future of non-biodegradable materials traditionally used for packaging.

Polymeric foams such as polystyrene (Styrofoam) and polyurethane are produced through the irreversible synthesis of oil-based materials. These foams possess low density, high specific strength, moisture and photolysis resistance, electromagnetic shielding, and excellent thermal and acoustic insulation properties, which make them suitable materials for various applications like packaging [2]. However, it is important to acknowledge that the synthetic foams are neither biodegradable nor completely recyclable. Additionally, their production and utilization entail complex manufacturing processes with high-embodied energy and waste generation.

In response to the issues stemming from the massive consumption of non-biodegradable materials, there has been an increasing demand for eco-friendly, biodegradable, and recyclable alternatives [3, 4]. Recent advancements in biomaterials science and engineering have led to introduction of new families of sustainable and fully degradable materials such as mycelium-based biocomposites (MBCs).

Over the past few years, the MBCs have emerged as a promising substitute to conventional materials, particularly the fossil-based products, due to their unique characteristics and sustainable production process [5, 6]. The unique composition of these randomly distributed, chopped fiber-reinforced composites, formed by spatiotemporal growth of a dense fungal mycelial network within a porous wooden substrate, offers a range of desirable properties such as biodegradability, lightweight, insulation, and fire resistance [7, 8].

The production of MBCs involves utilizing renewable biomass feedstocks and various fermentation techniques to achieve tunable physical and mechanical properties. In recent years, researchers have been exploring the innovative methods for production of the MBCs, such as cultivating the mycelium in nutrient-rich substrates from the agricultural wastes to address the environmental challenges [9, 10], and at the same time, to achieve the material properties comparable to their synthetic counterparts [6, 11].

MBCs have the potential to make a substantial contribution to the future of the environmentally-friendly building construction [7, 12]. In particular, MBCs offer a sustainable alternative to conventional building materials, helping to reduce the current dependency on the fossil fuels [6, 10]. Furthermore, the mechanical properties of MBCs can be tailored to meet specific packaging needs [13]. Consequently, MBCs serve as biodegradable materials, offering an eco-friendly substitute to polystyrene and other traditional synthetic polymer foams [8, 14]. Moreover, MBCs contribute to the waste management by utilizing agricultural and urban waste as substrates, promoting a circular economy [6, 15].

Reportedly, the mechanical properties of MBCs are influenced by several factors, including the type of feedstock used as the reinforcements as well as the mycelium species utilized to form the binding matrix phase. Different types of agricultural waste, such as hemp, rice straw, wheat straw, and corn stover, have been used as the feedstock for the MBC production [3, 9]. Selection of the proper feedstock type and processing plays a substantial role in tuning the desired physical and mechanical properties of the MBCs such as the strength, stiffness, and durability [10]. For instance, the size, shape, and composition of the feedstock particles can influence the packing density, inter-particle bonding, and overall structural integrity of the composite material [16, 17]. Substrates such as hemp and straw exhibit superior thermal insulation and water absorption [18]. Rice straw-based MBCs show high water absorption, while corn stover variants have enhanced flexural, impact, and tensile strength [14, 19]. The type of substrate may affect the thermal conductivity and mechanical properties, as the corn husk has been reported to enhance the bending strength and reducing the shrinkage [19, 20]. Although a proper facial adhesion is critical, mechanical performance of the substrate depends more on the fiber processing (e.g., the particle size and shape, pre-compression, etc.) rather than their chemical composition [18]. Furthermore, proper selection of the substrate material directly affects the mycelium colonization efficiency, as previous reports have shown that flax, hemp, and straw can foster robust structural networks because of enhanced fungal growth [18, 21].

In addition to the feedstock, the mycelium species inoculated to form the filling matrix phase have direct influences on the mechanical characteristics of the biocomposite [11, 17]. Mycelium, which is the root structure of the fungi, can be constantly grown within the porous substrate to form a dense network of the fungal filamentous structure, say the hyphae, during its vegetative growth [22]. In fact, mycelial fungi serve as the binding agent for various natural substrates, ultimately leading to the formation of a strong and versatile MBC [5, 8]. The selection of fungal species in MBCs is pivotal, as their biological and mechanical characteristics are directly influenced by the growing fungus [23]. *Pleurotus ostreatus* (oyster mushroom) is prominent for packaging applications due to rapid substrate colonization, feedstock adaptability, and density ranges akin to the low-density polyurethane foam [24]. Similarly, *Pleurotus pulmonarius* and *Ganoderma lucidum* enable customizable sustainable packaging and structural components through substrate-dependent mechanical and morphological variations [17].

Species such as *Trametes versicolor*, *Ganoderma fornicatum*, *Lentinus sajor-caju*, and *Schizophyllum commune* offer distinct advantages, including enhanced water resistance, compressive strength, and flexural resilience in corn husk-based MBCs used for load-bearing applications [14]. Notably, *Pleurotus eryngii* (king oyster mushroom) stalk-reinforced composites exhibit exceptional mechanical properties (ultimate strength of 12.99 MPa; Young's modulus of 3.66 GPa), which rank them as potential candidates for high-performance engineering [25].

Mycelium has emerged as a sustainable biomaterial for environmental filtration, demonstrating efficacy in removing contaminants such as heavy metals from water and particulates from air. Research by Olorunfemi et al. [26] reveals that mycelium-colonized substrates eliminate 55-100% of iron, manganese, and lead from contaminated water, while Parasnis et al. [27] report 90% lead ion removal using dried mycelium membranes at specific flux rates. Chlebnikovas et al. [28] have extended their usability to air filtration by trapping the airborne particles.

Production of MBCs can adapt to regionally available materials. Agricultural residues like wheat straw, rice straw, hemp, and cotton byproducts offer viable substrate alternatives, maintaining comparable mechanical properties. Similarly, fungal species such as *Ganoderma lucidum* and *Trametes versicolor* provide flexibility in binding efficiency and material performance. These substitutes enable localized productions, while enhancing global scalability. By leveraging regionally abundant biomass and adaptable fungi, MBC fabrication can well align with the circular economy principles, while addressing geographic and industrial diversity.

The mechanical characteristics of MBCs are influenced not only by the type of mycelium and natural fibers (lignocellulosic substrate) but also by the method of fabrication, processing parameters, and growing conditions [17, 18]. Previous studies suggest that modifying the fabrication methods can indeed result in variations in the mechanical behavior of the MBCs [29, 30]. For instance, heat-and cold-pressing can enhance the mechanical properties of the MBCs by increasing the density, reducing the porosity, and improving the strength and structural integrity [31, 32].

Reportedly, incorporating additives like the paper waste or bacterial cellulose can improve the internal bonding and overall mechanical strength [33, 34]. Moreover, using small particles like chopped fibers as well as pre-compressing the fibers improve the compressive properties [18].

In MBCs, the mechanical properties such as compressive strength, flexural strength, density, and mycelium growth rates, are the mostly influenced characteristics by the choice of substrates with different nutrient content and fiber structure [31]. Commonly explored substrates include: (1) hemp, which provides excellent insulation and low water absorption, but may release toxins that impede fungal growth; (2) straw, which accelerates the colonization and boosts the production efficiency yet compromises the durability due to high water absorption; and (3) flax, which delivers high compressive stiffness ideal for structural uses [18, 31]. In previous research, date palm fiber (DPF) waste has largely remained overlooked. This lignocellulosic bi-product has the highest cellulosic content, nearly 50% among coir, sisal, and hemp, and its lower density makes it suitable for fabricating natural fiber biocomposites. It also costs only \$0.02 per kg, which is significantly lower than coir, hemp, and sisal [35]. Being abundant in date palm-rich regions, cost-effective, and lightweight, DPF offers competitive mechanical performance, positioning it as a regionally advantageous and sustainable reinforcement material for eco-friendly biocomposites. However, challenges such as the needle-like and non-uniform shape of the fibers may affect the mycelial adhesion.

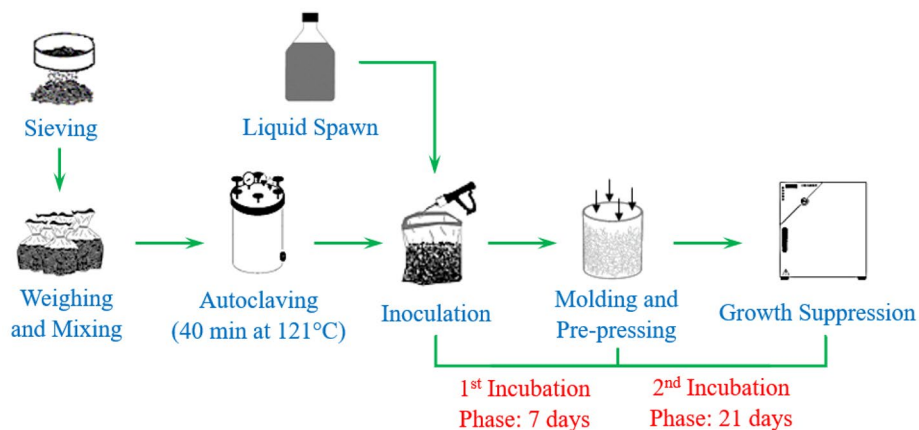


Fig. 1. Schematic of the fabrication process started from sieving and finished by demolding and growth suppression.

This study presents a systematic production protocol for the fabrication of MBCs, utilizing the DPF as the fibrous reinforcing substrate and the *Pleurotus eryngii* mycelium to form the biological binding matrix. This research investigates how two key processing parameters, the fiber pre-pressing and the fiber size, can influence the compressive and flexural properties of these MBCs. The central research question is: Can DPF-based MBCs achieve comparable mechanical performance to the petroleum-derived polymer foams while offering superior environmental sustainability for packaging applications? Present work tries to answer this query through comprehensive mechanical characterization and comparative analysis with conventional synthetic foams.

2. Materials and Method

2.1. Natural components

The vegetative form of culinary-medicinal king oyster mushroom, *Pleurotus eryngii*, serves as a self-growing binding agent to cohere the natural fibers together forming an eco-friendly biocomposite. This fungus is capable of spatiotemporal growth in a symbiotic relationship within the natural wood substrates, resulting in the formation of a dense matrix that effectively binds the fibers together [5, 22].

Moreover, the DPF, as a substrate for fungal growth, are obtained by first shredding the leaf-sheath date palm agricultural wastes harvested from palm groves of Kerman province in Iran, and then washing the fibers with water. These natural fibers provide sufficient nutrients for mycelium network growth, given that the majority of the residue is made up of lignin-bound carbohydrates such as cellulose and hemicellulose [4].

2.2. Specimen preparation

Briefly, the fabrication procedure includes several steps: drying and sieving the DPF, cultivation of the liquid spawn, preparation of the substrate and sterilization, inoculation, primary incubation, molding and pre-pressing, secondary incubation, and finally, the mycelial growth suppression. Figure 1 depicts the fabrication protocol schematically. The entire in-lab incubation lasts for four weeks.

TABLE 1. The Size Specification of the Categorized Fibers Used as Substrates

	Unsorted fibers		S-fibers		L-fibers	
	Range	Mean	Range	Mean	Range	Mean
Fiber diameter, mm	[0.2, 8.7]	4.45	[0.2, 3]	1.6	[0.5, 4.7]	2.6
Fiber length, mm	[5.3, 55.5]	30.4	[5.3, 41.5]	23.4	[9, 55.5]	32.25

Initially, the fibers were air-dried at an average temperature of 25°C and ambient humidity of 40% and then classified using sieves of different mesh sizes. Two individual sets of substrates were required for conducting the compression and flexural tests. In the compression specimens, the fiber diameter ranges from 0.2 to 8.7 mm, with a mean value of 4.45 mm, while the fiber length varies between 5.3 and 55.5 mm, featuring an average value of 30.4 mm. In preparation of the flexural specimens, the fibers were classified into two statistically distinct size categories of large (L) and small (S) fibers in order to study the effect of fiber size on flexural characteristics of the MBC specimens. The diameter of the L-fibers varies between 0.5 and 4.7 mm, with a mean value of 2.6 mm, while their length varies from 9 to 55.5 mm, with an average of 32.25 mm. As for the S-fibers, the diameter ranges from 0.2 to 3 mm, with a mean value of 1.6 mm, and the length varies between 5.3 and 41.5 mm, with an average of 23.4 mm, as outlined in Table 1.

The liquid mycelium spawn used in this study was prepared following the established protocols for *Pleurotus eryngii*. In summary, to prepare two liters of liquid spawn of *Pleurotus eryngii*, a culture medium was formulated using potato extract broth (24 g/L) supplemented with yeast extract (1 g/L). The medium was inoculated with a mother culture of *Pleurotus eryngii* and incubated at 25°C under aseptic conditions with a filtered aeration system for seven days. On the seventh day, the mature liquid spawn was aseptically dispensed into individual replicates using 60 mL syringes to ensure uniform inoculation.

Three days post-inoculation of the substrate with *Pleurotus eryngii* liquid spawn, visible mycelial colonization manifested as fine, thread-like hyphal networks. Continuous monitoring of replicates was imperative to detect and mitigate potential microbial contamination. All inoculation procedures including the introduction of liquid spawn into the culture media and subsequent transfer of the inoculated substrates into the molds were conducted under a laminar airflow hood.

According to the protocol shown in Fig. 1, the mixture comprises 37 wt% (weight percentage) water, 33 wt% liquid mycelium spawn, and the remaining 30 wt% dry ingredients (containing 80% DPF and 20% wheat bran). Wheat bran facilitates the mycelial colonization on the DPF substrate, thereby decreasing the production time [36]. The mixtures were put into 3-litre autoclavable polypropylene bags (25×50 cm) plugged with nonabsorbent cotton plugs and autoclavable neck rings. Then, the bags were sterilized in the autoclave for 40 min at a temperature of 121°C and a pressure of 15 psi. The next step involves inoculating the 33 wt% of the liquid mycelium spawn shown in Fig. 2a to the sterilized bags. All the incubation-related steps were performed in a biosafety cabinet (JTLVC2X160, JALTAJHIZMEHRAN Company, Karaj, Iran). This process was conducted at room temperature (25 ± 2°C) and the ambient humidity of 40%. For the first 7 days of the incubation the bags were placed in a growth chamber at 27°C and 60% relative humidity (RH) as displayed in Fig. 2b. During this time, unlike the conventional composites, the growing mycelial networks, which form a primary matrix phase, consume the lignocellulosic substrates, resulting in a variable weight percentage of the fibers and matrix.

After the initial 7-day growth phase, when the mycelial networks formed an initial matrix, the contents of the bags were gently shaken and stirred so that all the primary mycelial networks were deliberately torn. This increases the homogeneity and results in more tips of segments, which in turn provides further nucleation for additional mycelial networks formation in the secondary incubation phase.

Prior to begin the secondary 21-day incubation phase, the contents of the incubation bags were molded to form the standard cylindrical compression test specimens according to the ASTM C165-07 [37] and the standard prismatic flexural test specimens according to the ASTM D790 and ASTM C203-05a [38, 39]. This process was conducted at room temperature (25 ± 2°C) and the ambient humidity of 40%. To attain sufficiently compact compression specimens, the mixture was compressed in the PVC tubes to form cylindrical specimens with identical diameter and height of 55 ± 5 mm.

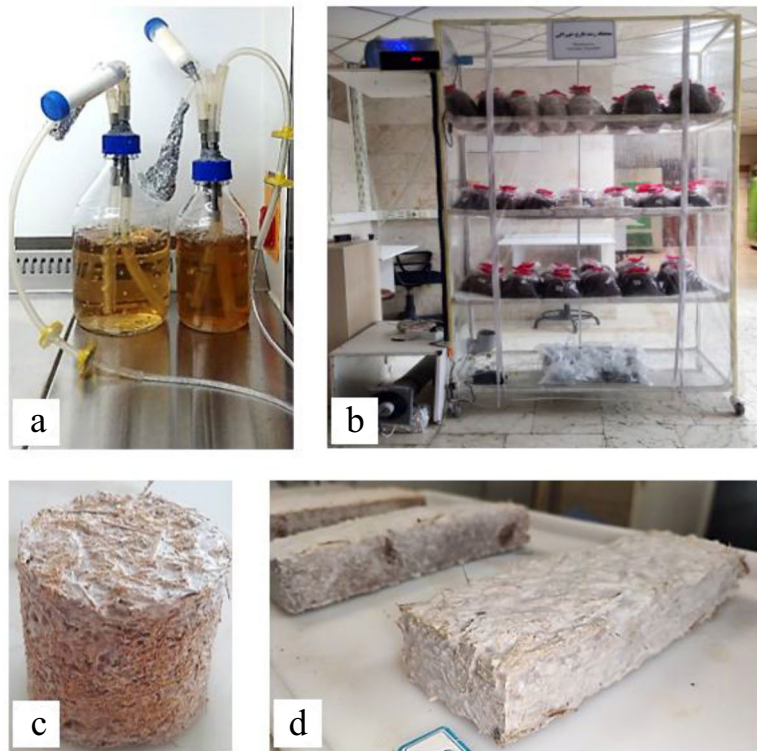


Fig. 2. Liquid mycelium spawn in the cultivation culture (a), the growth chamber (b), the 6-kg pre-pressed MBC compression specimen (c), and the prismatic flexural specimens made of small and large fiber sizes (d).

The compression specimens with similar composition were molded under different compressive axial pre-loads of 6, 10, 13, and 15 kg to investigate the effect of pre-pressure on the compressive properties. Axial pre-pressure was applied to the specimens within the molds by using different calibrated weights of 6, 10, 13, and 15 kg placed for 1 min on a light rigid plate. In a similar manner, the $(20 \pm 5) \times (60 \pm 5) \times (180 \pm 5) \text{ mm}^3$ flexural specimens were molded under a consistent pre-load of 18 kg imposed on the largest side of the prismatic molds.

Following the fabrication protocol shown in Fig. 1, after an additional 21 days of secondary incubation, which led to substantial branching of the hyphae, the cylindrical specimens of the compression tests as well as the prismatic specimens of the flexion tests were demolded, as shown in Figs. 2c and 2d. Subsequently, the fungal growth in the specimens was stopped by heating them for 24 h in a chamber (Binder Model ED 23, binder-world company, Tuttlingen, Germany) with natural convection and uniform drying at constant temperature of 70°C.

During the secondary incubation phase, one compression sample subjected to a 10 kg pre-load was found infected and subsequently rejected. Infected sample was immediately removed from the growth chamber and disposed in compliance with the biosafety protocols to prevent cross-contamination.

2.3. Test conditions

The physical and mechanical testing of the MBCs has not yet been standardized, and hence, researchers should follow the instructions developed for testing of the conventional composite materials [15, 40]. More comprehensive research and explorations are required to address the practical limitations and considerations for testing of the MBCs.

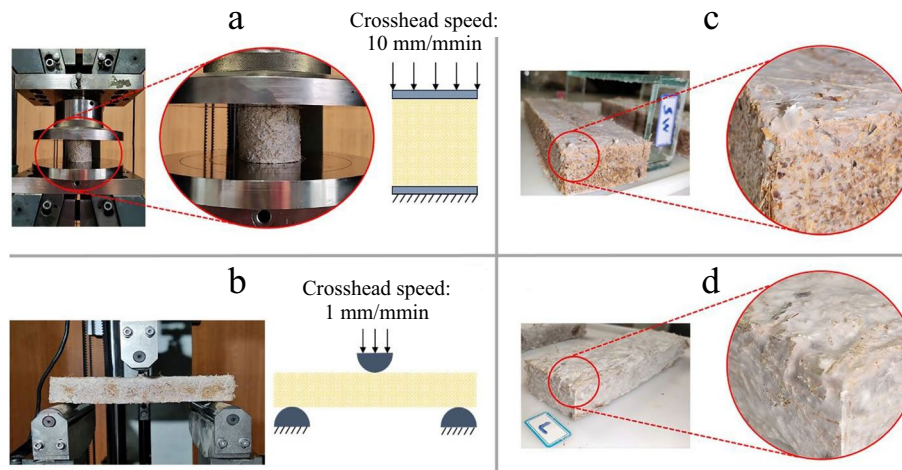


Fig. 3. Universal testing machine used for axial compression (a) and three-point bending (b) tests and small (c) and large (d) fiber MBC flexural specimens after demolding.

2.3.1. Compressive test. The cylindrical specimen shown in Fig. 2c was fabricated and tested in accordance with the ASTM C165-07 material testing standard [37]. The MBC specimens were tested under quasi-static displacement-controlled axial compression using the Zwick Z 250 universal testing machine, as illustrated in Fig. 3a, with a constant crosshead rate of 10 mm/min and a pre-load of 5 N to remove the clearance. It is noteworthy that the 1:1 diameter-to-height ratio prevents buckling. The compression test was terminated at approximately 75% axial strain, and the force-displacement data was continuously recorded. Using this data, the compressive stress and strain values were subsequently calculated.

Standard materials testing machines control the cross-head displacement, and the force applied is measured using load cells. Here, the quasi-static displacement-controlled compression method was selected because of the strain-rate-dependency of the MBCs' mechanical response, which is in accordance with the ASTM C165-07 standard [37], to ensure the compatibility with conventional testing protocols for foams and composite materials.

2.3.2. Flexural test. According to the ASTM D790 and ASTM C203-05a [38, 39] material testing standards, the dimensions of the prismatic specimens shown in Fig. 2d were as follows: thickness of 20 ± 5 mm, width of 60 ± 5 mm, and length of 180 ± 5 mm (Figs. 3c and 3d). The quasi-static displacement-controlled three-point bending test was conducted using the same testing machine but different fixtures suggested by the corresponding testing standard, as displayed in Fig. 3b. A pre-load of 2 N was also applied to remove the loading clearance, and the crosshead rate was maintained at 1 mm/min. The flexural test was terminated at almost 5% strain, when visible signs of fracture appeared on the outer surface of the test specimens. Subsequently, the flexural stress and strain values were calculated based on the recorded force-displacement data.

As recommended by the ASTM standards D790 and C203-05a for material testing [38, 39], three-point bending was selected over other flexural tests. Unlike four-point bending, which requires larger specimens and is more sensitive to material inhomogeneity, three-point bending is less susceptible to stress concentration artifacts in MBCs with variable fiber distribution. Moreover, it can be simply conducted and is suitable for small-scale, laboratory-prepared specimens.

3. Results and Discussion

3.1. Compressive behavior

The stress-strain curves of the compression specimens presented in Fig. 4 show the compressive behavior of the MBC specimens subjected to different pre-loads of 6, 13, and 15 kg during the fabrication process. The specimen subjected

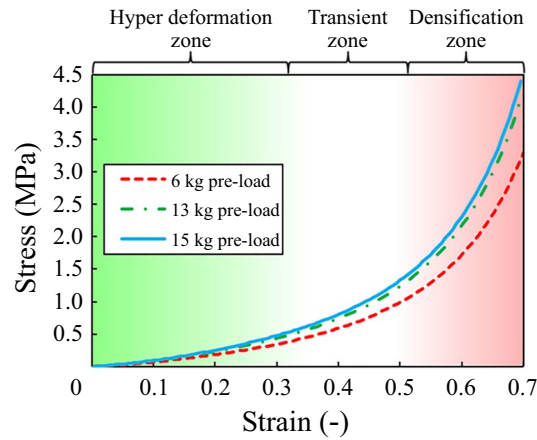


Fig. 4. Stress-strain response of the MBC specimens with different pre-loads under axial compression.

TABLE 2. Summary of the Compressive Properties of the MBC Specimens with Different Pre-loads

Pre-load, kg	Apparent density, kg/m ³	Compressive modulus, kPa	Compressive strengths, kPa at different strains			Mass-specific compressive strength, kPa/g at different strains		
			30%	50%	70%	30%	50%	70%
6	207.98	509.2	343	990	3300	13.5	38.7	129.2
13	210.21	711.4	431	1239	4269	16	45.9	158.3
15	213.09	744.5	475	1328	4549	17.4	48.6	166.4

to 10 kg pre-load was found infected, and subsequently, was rejected. Results show a direct correlation between the increase of the pre-load and enhancement of the compressive response of the MBCs. Compressive strength of the MBCs is directly affected by the level of pre-loading during fabrication [32]. Increase of the pre-loading results in the compaction of mycelium filaments, which strengthens the interconnections within the composite, and subsequently, enhances the load-bearing capacity of the material. This indicates that higher pre-loads contribute to a more robust composite structure, capable of resisting greater compressive forces. Moreover, the specimens show continual increase of the compressive strengths by increasing the strain, which is followed by a gradual densification induced by overly compressed and piled up fibers.

As it can be observed in Fig. 4, the MBC specimens have stress-strain responses different from the engineering materials without an identifiable yield point. Upon increasing the compression, all specimens showed apparent strain-stiffening behavior leading to continuous densification as the strain increases [5]. This is in agreement with previous observations reported in [41].

The compressive characteristics of the MBC specimens subjected to different pre-loads are listed in Table 2. These characteristics include the compressive modulus, the compressive strengths, and the mass-specific compressive strengths at different strain levels. It is noticeable that the specimens have different apparent densities because of different levels of compaction during pre-loading.

Table 2 illustrates a slight density change, including 1.07% increase observed, when the pre-load increases from 6 kg to 13 kg, followed by an increase of 1.37%, when it increases to 15 kg. Table 2 shows also the compressive characteristics of the specimens at three strain levels of 30, 50, and 70%. In this table, the mass-specific compressive strength is defined as the compressive strength divided by the mass of the specimen. As it can be observed, more compacted specimens exhibit higher compressive strength and mass-specific compressive strength. Indeed, by increasing the pre-loads from 6 kg to 15 kg, the empty spaces between the fibers get smaller resulting in higher apparent density as indicated in Table 2, and hence, preloading improves the interfacial bonding strength of the mycelium network to the substrate fibers leading to enhanced load-bearing capacity [42]. By increasing the density without a proportional increase in mass, pre-loading can enhance

the mass-specific compressive strength and improve the strength-to-weight ratio [14]. This is pivotal for applications like packaging, where a lightweight but strong material is required. Nevertheless, it is worth to note that excessive pre-pressure impedes the growth in overloaded specimens due to insufficient oxygen supply.

The trade-off between the pre-loading and fungal growth arises from competing demands: higher pre-load enhances the compressive strength (by improving the fiber-mycelium integration). As shown in Table 2, increasing pre-loads (from 6 to 15 kg) results in marginal density increases (1.07-2.4%) but significant improvement in compressive strength (34.1-38.5%) and modulus (39.7-46.2%). However, excessive pre-pressure may restrict the oxygen availability and nutrient depletion, hindering mycelial colonization. Excessive pre-loading reduces the porosity and restricts the oxygen diffusion, which is critical for hyphal extension. Additionally, denser substrates may impede mycelial access to the lignocellulosic nutrients in DPF. Finding an optimum trade-off between these factors requires further in-depth investigations using predictive or statistics-based algorithms.

During the initial stages of the compression test (i.e., less than 5% strain), when all specimens exhibit a nearly linear behavior [41], the compressive modulus represented by the slope of the stress-strain curve ranges from 509.2 to 744.5 kPa, as indicated in Table 2. The specimens subjected to higher pre-loads exhibit higher initial elastic response. Results obtained for the MBCs fabricated in this study suggests that the compressive modulus of the specimens surpasses those reported by Elsacker et al. [18] ranging from 770 to 1320 kPa. Additionally, considering the densities and compressive moduli reported in Table 2, these MBCs can be categorized as foams according to the Ashby material selection chart [43].

Table 2 reveals more interesting results. Upon increase of the pre-load from 6 to 13 and 15 kg, the apparent density increases only 1.1 and 2.4%, respectively; while the compressive strength at different strain levels increases 25.2-29.4% and 34.1-38.5%, respectively. Comparable improvement is observed for the mass-specific compressive strength under different strain levels with an increase of 18.5-22.5% at 13 kg pre-load and 25.6-28.9% at 15 kg pre-load. The compressive modulus also follows a similar trend, as it increases by 39.7 and 46.2% under 13 and 15 kg pre-loads, respectively. This enhancement in the compressive properties without significant increase of the weight is very beneficial, particularly in the applications such as packaging where the lightness, load-bearing capacity, and the structural integrity are important [30].

In the design of packaging components used to protect the interior contents from mechanical damages, a critical characteristic of the material is the compressive strength [14]. According to the results of compression tests, pre-loading can play a key role in adjustment of the physical and mechanical properties of the MBCs [32]. For instance, higher density and compressive modulus is desirable for structural components in building construction, whereas lower density may be preferred for thermal or acoustic insulation. Considering the commercial viability, these MBCs can serve as a promising alternative to synthetic foams such as polystyrene (PS). Specifically, the MBC specimens in this study exhibit compressive strength in range of 0.34 MPa (at 30% strain) and 4.55 MPa (at 70% strain) as reported in Table 2, which is comparable to compressive strength of PS (0.03-0.69 MPa) [29] and higher than the compressive strength of MBC specimens presented by Lingam et al. [44] (7.7-78.34 kPa), Etinosa et al. [41] (0.34-0.74 MPa), and Rigobello et al. [30] (89-306.38 kPa) but quite comparable to those reported by Chan [45] (0.71-4.44 MPa). Moreover, Yang et al. [22] reported the compressive strength between 350 and 570 kPa at a strain of 15%, while Ziegler et al. [16] showed that the compressive strength varies between 0.67 and 1.18 MPa at 60% height deformation.

3.2. Flexural behavior

In this Section, the flexural behavior of the prismatic MBC specimens with different fiber sizes was studied under three-point bending test. Figure 5 shows the stress-strain curves of the MBC specimens fabricated with small and large fiber size categories as indicated in Table 1. Each specimen features two curves, the original and smoothed data. As illustrated in Fig. 5, the smoothed data curves are running the averages of the original data for both specimens. Moreover, the details about the physical and flexural properties of the specimens are shown in Table 3.

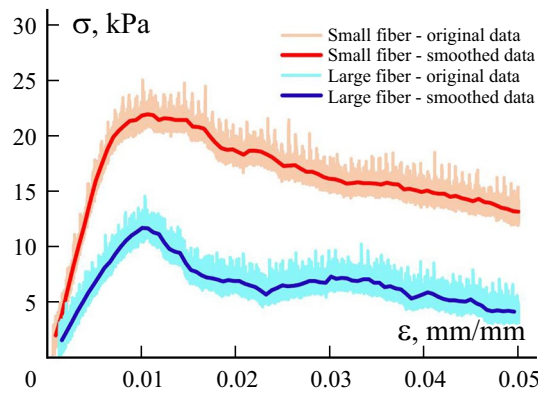


Fig. 5. Flexural behavior of the MBC specimens with different fiber sizes.

TABLE 3. Summary of the Physical and Flexural Properties for the MBC Specimens with Different Fiber Sizes

Fiber size	Apparent density, kg/m ³	Flexural modulus, MPa	Flexural strength, kPa	Specific flexural strength, kPa/kg
Small	153.22	2.98	21.9	491.2
Large	153.83	1.29	11.7	283.4

As indicated in Fig. 5, the stress-strain curves of the both specimens show two distinct regions with different behavior. Under three-point bending, the specimens initially exhibited a monotonic increase in the stress as the strain rises with an almost linear trend suggesting a constant flexural (elastic) modulus, which is typically defined as the slope of the stress-strain curve in the initial linear region. Despite both specimens have almost the same apparent density, examining the slope of the linear portion of the stress-strain curves reveals that higher flexural modulus is observed for the specimen containing small fibers. Moreover, as it can be observed in Fig. 5, at a similar strain level of ~ 0.01 , associated with the peak stresses in both curves, the small-fiber specimen shows a flexural strength almost 87% higher than that of large-fiber specimen, indicating a better load-bearing capacity prior to begin to fail under bending. This might be attributed to the more uniform and tightly bonded network of small fibers enhancing the stress distribution and structural integrity.

Holt et al. [13] have reported a maximum flexural strength of 20.8 kPa for an MBC made of cotton plant byproducts. A maximum flexural strength of 220 kPa at 1.5% rupture strain for a cold-pressed MBC specimen was reported by Appels et al. [31]. Moreover, using a heat-pressed fabrication method, Elsacker et al. [34] found a maximum flexural strength of 1460 kPa for a pure MBC specimen and 1910 kPa for a bacterial cellulosic MBC specimen. In a more recent study, Lingam et al. [44] developed a juncao grass (JG) MBC specimen with a recorded maximum flexural strength of 400 kPa. In the present research, the maximum recorded flexural strength for the S- and L-specimens were 21.9 and 11.7 kPa, respectively, which are comparable to the results reported by Holt et al. [13] but significantly lower than those reported by Appels [31], Elsacker [34], and Lingam [44].

DPF-reinforced MBCs exhibit lower flexural strength (11.7-21.9 kPa) compared to the synthetic foams like polystyrene, PS, (0.07-0.70 MPa) [29] and polyurethane, PU, (0.21-57 MPa) [29]. The inhomogeneous microstructure of the MBCs, characterized as a discontinuous lignocellulosic fiber network embedded within a mycelial matrix, results in local stress concentrations at the fiber-matrix interfaces promoting matrix rupture and interfacial debonding. Unlike the strong chemical bonds in PS and PU, weak interfacial adhesion between the growing matrix and the needle-like DPF further reduces the effective load transfer. It is worth to note that despite the MBC specimens tested in this study showed flexural properties weaker than their synthetic counterpart, PS, this study focused on the sole effect of the fiber size on the flexural properties. Various factors such as the type and composition of the ingredients including the fungal type and nutrients, the growth period, pre-loading, and the ambient conditions should be further studied to obtain the optimal physical and mechanical characteristics of this MBC.

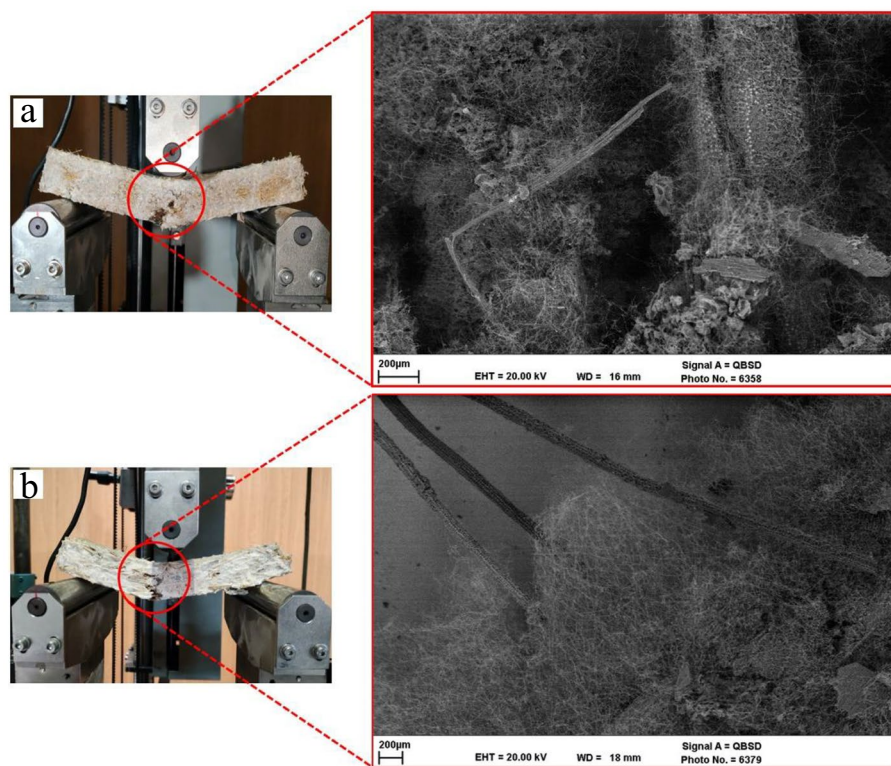


Fig. 6. The flexural specimens, containing small (a) and large (b) fibers, failed after three point-bending standard test along with the scanning electron micrographs.

As also reflected in Table 3, similar result was expected for the mass-specific flexural strengths, considering that both specimens have similar weights. Higher specific flexural strength of the small-fiber specimen suggests that it can better serve in applications requiring lightweight components with high resistance to bending forces such as packaging and lightweight building construction [4]. Additionally, according to the densities and flexural moduli of the specimens reported in Table 3, the present MBC specimens can be classified within the range of foam materials, particularly the flexible polymer foams, in the Ashby material selection chart [43].

As shown in Fig. 5, followed by a peak stress, when the specimens began to fail under bending, the next region of the stress-strain curves was observed with a descending linear trend. At this stage, the connections between the fibers and the mycelial matrix were destroyed, resulting in an abrupt drop of the measured force and visible signs of specimen failure. Furthermore, Fig. 5 suggests that the final fracture stress is higher for the specimen containing small fibers.

Identification of the failure modes in MBCs is inherently complicated. A closer look at the scanning electron micrographs of the flexural specimens shown in Fig. 6 reveals different failure mechanisms, including the matrix rupture, the fiber-matrix debonding followed by fiber pull-out and partial fiber breakage. These observations highlight the weakness of matrix and its interfacial bonding with the fibers.

As illustrated in Figs. 6a and 6b, debonding initiated at the mycelium-fiber interface due to weak adhesion. This inherent weakness is mainly related to the physical entanglement rather than the chemical bonding [17, 31]. These observations align with prior studies on lignocellulosic-MBC systems, where insufficient interfacial bonding compromises load transfer [21, 46]. Subsequent fiber pull-out, which is dominant in the large-fiber specimens (Fig. 6b), reflects the poor stress transfer at the interfacial discontinuities [34]. Moreover, thin fibers were subjected to fracture as shown in Fig. 6a. These findings emphasize the important role of the fiber size and the fiber-matrix adhesion in mechanical failure of the MBCs. Further study is required to enhance the mechanical properties of the mycelial matrix and its bonding to the fibers.

In summary, the results of flexural test reveals that the fiber size can profoundly affect the physical and mechanical properties of the eco-friendly MBCs, and hence, the selection of the appropriate fiber size categories in the fabrication of MBCs has to be tailored to the intended application. Evidently, as indicated in Table 3, the flexural properties of the MBCs are influenced by variation of the fiber size. This can be justified considering that the L-specimen contains larger empty spaces in its fibrous network in comparison with the S-specimen, which prevents the growing matrix from sufficient consolidation. Moreover, the large empty spaces between the fibers in the L-specimen may cause the local formation of not-reinforced mycelium networks [8], which can result in fabrication of a soft and flexible specimen. Accordingly, the fungal structure can better grow in a substrate with shorter fibers and higher fiber density, primarily due to the smaller vacancies between the fibers as well as the larger surfaces available for the growth, which results in more rigid specimens [47]. However, it is important to note that not enough space required for sufficient oxygen supply there exists in the substrates made of very small fibers.

This study evaluates the feasibility of replacing conventional synthetic foams including polystyrene (PS) and polyurethane (PU) with sustainable MBCs through comparative analysis of material properties, environmental impact, and functional viability. The compressive strength obtained for the MBCs (0.34-4.55 MPa), which exceeds that of PS (0.03-0.69 MPa) [29], aligns with the requirements for protective packaging. Their competitive compressive modulus (509-744 kPa) and strength-to-weight ratio can make them suitable alternatives to synthetic foams. Moreover, due to their tunable density (153-213 kg/m³), MBCs are promising candidates for lightweight construction panels in modern buildings. However, MBCs exhibit weaker flexural properties (0.0117-0.0219 MPa) compared to PU (0.21-57 MPa) [29], and their use in applications with dynamic-loads are restricted. Developing the MBCs bring many environmental advantages, including but not limited to biodegradability, circular economy alignment by agricultural waste valorization, and reduced fossil fuel reliance. However, there are also some challenges like moisture and contamination sensitivity, biological variability which requires precise quality control, and prolonged production time.

3.3. Study limitations and future outlook

In this study the physical and mechanical characteristics of a new mycelium biocomposite made of shredded leaf sheaths of date palm, as a potential substitute for synthetic foam materials, were investigated. As earlier discussed in details, interesting results about the effects of pre-pressure and the fiber size on the mechanical characteristics of the MBC proposed were obtained; however, there remained some limitations to be addressed. From the industrial production point of view, several factors generally restrict the ease of production of the MBC, including the spawn scarcity, unpredictable cultivation, and time-consuming growth periods. Sterile conditions were crucial to prevent contamination, necessitating redoing steps [31, 44]. The variety of methods for MBC fabrication made it difficult to compare the results. Moreover, lack of specific standards for testing of the biocomposite materials forced us to use the available ASTM standards for testing of conventional composite materials [15, 40].

While the MBCs hold significant promise as sustainable alternatives to traditional foam materials, their fabrication is generally hindered by different challenges. Variability in mycelium growth rates can lead to inconsistent material properties. Factors such as the temperature, humidity, and substrate type significantly influence the growth quality, making it challenging to achieve uniform properties if not accurately controlled. Adjustment of the optimal moisture content and pH level is also crucial for an acceptable mycelial growth.

The laboratory-scale production of mycelium biocomposites has shown promise; however, scaling up to the industrial production requires logistics and economic considerations. Factors such as the availability of natural fiber substrates, production costs, and facility requirements need further investigation for a feasible large-scale production.

In this study, each testing condition (i.e., the pre-loads and the fiber sizes) was tested once per parameter set (i.e., three compression specimens and two flexural specimens). Further effort is required to fabricate more replicates to minimize the probable variability of the reported results. Moreover, a statistical analysis on mean and standard deviation has to be

performed to quantify the variability and reliability of results. Previous studies on lignocellulosic substrates conducted a minimum of three test replicates per individual experiment [18].

To minimize the inherent variability of *Pleurotus eryngii* mycelial growth conditions such as temperature, humidity, and substrate composition, developing standard protocols for fabrication and testing of MBCs is highly recommended. Future research should also focus on different fungal strains (e.g., *Pleurotus ostreatus*) to enhance the colonization speed and reduce the risk of contamination. Further studies can also be conducted on: adding DPFs with different shapes like wood flakes and chips, improvement of the substrate's chemo-mechanical properties and fiber-matrix adhesion through pre-treatments like alkali modification and using additives, optimization of environmental conditions, and development of scalable production methods. Finally, to broaden the applicability of the proposed MBC, additional performance tests for flammability and thermal and acoustic isolation should be conducted.

4. Conclusion

In this study, a new mycelium-based biocomposite material was developed by growing up specific fungal species on a natural wood substrate made of date palm waste. The study shared details of the fabrication protocol, and reported the effects of pre-loading and the fiber size on the compressive and flexural properties of the proposed MBC, respectively. Compression tests on specimens fabricated under different pre-loads showed that higher pre-pressures can improve the compressive properties; however, an excessive pre-pressure may compromise the fungal growth and disrupt the fabrication process. Moreover, the flexural tests on the MBC specimens with different fiber sizes revealed that the large fiber specimens show lower modulus and flexural strength. The new material exhibited physical and mechanical characteristics comparable to other MBCs as well as the synthetic polymer foams like the polystyrene, offering a potential alternative in various applications including the light and green construction and packaging. Although this study establishes foundational insights into DPF-reinforced MBCs as sustainable alternatives to synthetic polymer foams, there is plenty of room for improvement, including the fungal strain optimization, enhancement of the fiber-matrix adhesion by surface treatments, improving the colonization by using proper additives and nutrients, and designing protocols for scaling up to industrial production. These efforts aim to bridge the gap between the sustainable material innovation and the industrial feasibility, advancing the MBCs towards a scalable, eco-friendly, and cost-efficient level.

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