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AN EVALUATION OF THE FUNDAMENTAL FACTORS INFLUENCING THE CHARACTERISTICS OF MYCELIUM-BASED MATERIALS: A REVIEW

PROCENA FUNDAMENTALNIH FAKTORA KOJI UTIČU NA KARAKTERISTIKE MATERIJALA NA BAZI MICELIJUMA: PREGLED

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ABSTRACT

The mycelium-based materials (MBMs) are produced by growing the vegetative part of the mushroom-forming fungi - from Dikarya group: phylum Basidiomycota and Ascomycota, on different organic substrates, mostly due to containing important mycelium characteristics: septa and anastomosis. Moreover, function of these composites can be further tuned by controlling the species of fungus, the growing conditions, and the processing methods to meet a specific mechanical requirement in their further applications. The material formed after full colonization of the substrate, needs to be exposed to dry heating in order to remove the moisture content and to inactivate the mycelium, giving us the lightweight, and biodegradable material with great potential to replace fossil-based and synthetic materials such as polyurethane and polystyrene.

Their low carbon footprint, low energy and processing cost, biodegradability, low heat conductivity, high acoustic absorption, and fire safety qualities were some of the main characteristics that encouraged the use of mycelium based composites (MBCs) in the construction and building sector, especially as paneling, insulation, and furniture materials.

Since mycelium products are quite new and there is limited industry peer-reviewed testing data available, there is a need for standardized mechanical properties, universal testing requirements and published standards (ISO, ASTM) to ensure that qualification and testing programs can be developed to support the manufacture and use of MBCs.

***Keywords**: mycelium based composites; biomaterials; fungi.*

REZIME

Materijali na bazi micelijuma (MBM) nastaju gajenjem vegetativnog dela gljiva koje formiraju gljive – grupa Dikarya iz razdela Basidiomycota i Ascomycota, na različitim organskim supstratima, uglavnom zbog posedovanja važnih karakteristika micelijuma: prisustvo septi i anastomoza. Funkcija ovih kompozita može se dodatno podesiti kontrolom sledećih faktora: vrsta gljive, uslovi rasta i metode obrade finalnog proizvoda kako bi se ispunili specifični mehanički zahtevi u njihovoj kasnijoj primeni. Materijal nastao nakon pune kolonizacije supstrata, treba da se izloži suvom zagrevanju kako bi se uklonio sadržaj vlage i deaktivirao micelijum, dajući nam lagani i biorazgradivi material sa velikim potencijalom da zameni fosilne i sintetičke materijale npr. poliuretan i polistiren. Njihova niska emisija ugljendioksida, niska energija i troškovi obrade, ali i biorazgradivost, niska toplotna provodljivost, visoka akustična apsorpcija i kvalitet i zaštite od požara jesu neke od glavnih karakteristika koje su podstakle upotrebu kompozita na bazi micelijuma u građevinarstvu i građevinskom sektoru, sa posebnom primenom u oblogama, izolacijama i materijalima za nameštaj. Međutim, micelijumski proizvodi su prilično novi i podaci o njihoviom testiranju su veoma oskudni. Zbog toga postoji potreba za standardizovanim svojstvima materijala: mehaničkim, univerzalnim zahtevima za ispitivanje i objavljenim standardima (ISO, ASTM) kako bi se osiguralo da se program kvalifikacije i testiranja mogu razviti za podršku proizvodnji i upotrebi kompozita na bazi micelijuma (MBC).

***Ključne reči**: kompoziti na bazi micelijuma; biomaterijali; gljive.*

INTRODUCTION

The current unsustainable production of polymers, carbon emissions, the generation of non-biodegradable waste and the increasing generation of agricultural residues has been recognized as the most serious environmental issue. Products such as cement, concrete, metals, and polymers which are conventionally used, often have high energy consumption during production, high processing costs, complex infrastructure for design and production, and cause pollution problems (Haneef et al., 2017; Yang et al., 2021). Additionally, some reports state that many of these materials don’t have good biodegradability and can last for hundred years until decomposed completely (Vandelook et al., 2021).

Fungi are organisms that are using absorptive heterotrophy for living both in wild (Karaman et al., 2012, 2022a) and under laboratory conditions during submerged cultivation (Mišković et al., 2021; Žižić et al, 2024). Their biological role in the natural (urban) ecosystems is to decompose organic matter and to recycle it in the general biogeocycle of energy and organic matter. Their absorptive abilities are influenced exactly by the same factors that impact on their nutrient composition, since mushrooms are generally used in humans diet as food or food supplements, and varies greatly depending on the following: 1) taxonomic position – genera/species/strain, environmental factors including 2) substrate/microhabitat: wild or cultivated (Krsmanović et al, 2023) the geographic location/climate factors, 4) biological factors: maturity/growth stages: fruiting bodies/mycelia/sclerotia and 5) morphological factors of the fruiting bodies: the cap/stalk/hymenophore.

Biocomposites have emerged as an attractive alternative to match current needs for new materials using the available agricultural waste to produce greener materials (Alabi et al., 2019) contributing at the same time to the UN agenda for Sustainable Development Goals 2030. There are various applications of agricultural waste. The agricultural waste can be used for biogas generation (Al Afif et al., 2023), production of material called biochar wich has similar properties as coal (Pavkov et al., 2022), production of particles suspended in biofluids (Bikić et al., 2022; Radojčin et al., 2022) etc.

Research has led to the discovery of numerous species' genome sequences, which has made it possible to genetically modify them. Research on composite biomaterials based on mycelium is still in its early stages, but it is a hot area for future study. The development of composite biomaterials is moving from simple mixing to synthesis for gene editing, i.e., synthetic biology is used to transform mycelium-based composites and use chassis cells in biological systems to endow them (Wang et al., 2022).

Agricultural production has expanded by more than threefold in the last 50 years in response to increased food demand from ever growing global population and generated a large quantity of lignocellulose biowaste each year (Acevedo et al., 2020). In the Republic of Serbia agricultural residues originate from agriculture, forestry, food and wood industries. Residues from agriculture can be classified into three main groups: 1) residues produced in the process of growing field crops, 2) residues originating from fruit and 3) residues resulting from livestock farming. These residues have multiple and significant applications in agricultural holdings, and thus a certain economic value (Ministry of Environmental Protection, 2022). In general, residues burned in fields increase emissions of carbon monoxide (CO), carbon dioxide (CO_2), and particulate matter (PM_{10} and $PM_{2.5}$) causing a range of health risks for humans and environmental problems (Arunrat et al., 2018). Recycling of such wastes into sustainable, energy efficient biomaterials is a viable solution for the problem of pollution and natural resource conservation for future generations (Donner et al., 2020, Karaman et al, 2022b)

Recent research has demonstrated the potential of fungal mycelium to produce biocomposites as substitutes for traditional non-renewable polymers (Jones et al., 2020, Karaman et al, 2022b), Due to their low density, impact resistance, low thermal conductivity, and low cost (Jones et al.,2020), these materials are starting to impact several diverse fields such as construction industry (materials for packaging, insulation panels) engineering, architecture (building blocks, environmental restoration materials), interior design (acoustic and floor tiles, paneling, furniture, decking) and even the Arts (leather and textiles) (Holkar et al., 2016; Elsacker et al., 2020; Vandelook et al., 2021; Sydor et al., 2022) (Fig. 1).

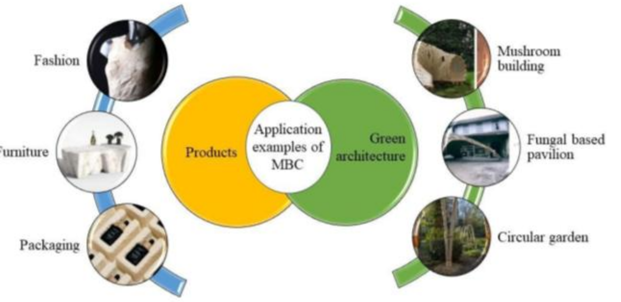

Fig. 1. Application of mycelium-based composites (MBC)

Mycelium-based composites derive their name from mycelium, the filamentous part of a fungi. When these fibers grow on organic materials such as straw, sawdust, woodchips, cotton, and rice husk, among others, and colonize the substrate, the hyphae of the fungus create an intertwined network through the cellulose, hemicellulose, and lignin-rich substrate, bonding with it to form mycelium-based materials (MBMs) (Attias et al., 2020).

After full colonization of the substrate, heat is used to remove the moisture content and kill the organism. The product formed is an inert, lightweight, and biodegradable material known as mycelium-based composite (MBC) (Jones et al., 2020). These biomaterials are clean, safe, strong, and biodegradable with huge potential to substitute fossil-based and synthetic materials such as polyurethane and polystyrene (Haneef et al., 2017; Attias et al., 2020). Given the amazing potential and multiple advantages of such a material, we are reviewing the key determinants of characteristics of MBCs.

MATERIAL

According to many experimental results with MBCs (see reference list), biocomposite characteristics used to determine their final purpose and application depend on 4 major factors:

1) *Selection of fungal species mycelium (strain) used to bind the agricultural waste*

The mechanical properties of the mycelium bio-composite are affected mostly by fungal species, which can be introduced either by using spores or mushroom spawn in the first stage of the mycelium incubation (Yang et al., 2021)

The selection of appropriate fungal species, the most appropriate fungal strain, according to the substrate, growth conditions, incubation time, growth rate, characteristic density, etc. is one of the most important steps (Vandelook et al., 2021). Based on the species of fungus, its productivity and the thickness of mycelium fiber, the surface topography will differ as well (Haneef et al., 2017; Jiang et al., 2017; Appels et al., 2019; Girometta et al., 2019).

Additionally, the genetic traits of the organism (fungal species) have been identified as one of the factors that determine the properties of the MBCs (Haneef et al., 2017) and different results have been obtained in terms of material properties with the use of different fungal species for the production of MBCs (Islam 2017). For example, *Trametes multicolor* on rapeseed straw results in a smooth and foam-like structure while *P. ostreatus* produced MBMs with rough structure on the same substrate (Apples et al., 2019)

The most commonly used fungi in the production of MBCs are from lignicolous fungal species belonging to genera Ganoderma, Pleurotus, Pycnoporus, and Trametes (Attias et al., 2019).

2) *Selection of substrate (the specific combination of different agricultural and forestry organic waste)*

The substrate for mycelium growth is usually a mixture consisting of different lignocellulosic agricultural crop waste such as cotton, corn, wheat, and hemp, flax residues (Jiang et al., 2017; Appels et al., 2019; Girometta et al., 2019) and fibers, sawdust, and wood chips as organic materials (Jones et al., 2020). The substrate for the mycelium-based foams always uses lignocellulosic waste since fungi can decompose cellulose or lignin in plant biomass (Girometta et al., 2017). Haneef et al. (2017) stated that a mixed substrate made of pure cellulose and potato dextrose broth (PDB) has advantages in growing mycelium. Teixeira et al. (2018) used coconut powder with five fungal isolates (*Pleurotus ostreatus*: POS-W, POS-SP1; POS98/38; *P. eryngii* and *Pycnoporus sanguineus*). Attias et al. (2019) produced MBCs by growing three different fungi species

(*Colorius sp*., *Trametes sp*., *Ganoderma sp*.) on woodchips prepared by mixing ground residues of apple and vine crops with 1% flour, 3% wheat straw, and 62% distilled water. Elsacker et al. (2019) used lignocellulosic substrates made from flax, wheat straw and hemp fibers, with mycelium spawn from *T. versicolor*.

3) *The interaction between the mycelium, substrate and environmental factors such as temperature, humidity, CO_2, etc.*

The temperature and humidity are important factors that can affect mycelium growth. The best temperature for growing mycelium is room temperature (24–25° C) (Hoa and Wang, 2015). Considering that mold with inoculated substrate should stay in a relatively high humidity environment, humidifiers or sprinkler systems can be used to increase humidity. Another way to create a high humidity and sterile environment for mycelium growth is to use a semi permeable polypropylene bag (provides up to 98% relative humidity) (Jiang et al., 2017).

Mycelium is rich in water (approx. 60%) (Velasco et al., 2014; Elsacker et al., 2019) or 70–80% (Deacon, 2005), but the percentage of water in the finalized samples is approximately 10–15% (Deacon, 2005). Therefore, the water content of the final mycelium-based bio-composites is the primary consideration for the mechanics of the mycelium samples and most of the water must be removed because it needs to be provide a high and reliable mechanical performance and be dry enough to terminate the fungal growth (Girometta et al., 2019). The substrate and the species of fungi affect the final mycelium water content. For instance, a substrate made of hemp pulp absorbs more water than that made of cotton wool (Ziegler et al., 2016).

Mycelium requires oxygen for growth and to produce carbon dioxide. Low carbon dioxide content initiates the formation of a fruiting body therefore to prevent the formation of the fruiting body and ensure efficient mycelium growth a high CO_2 level should be maintained according to Lelivelt et al. (2015).

4) *Processing and Post-processing methods*

The material formed after full colonization of the substrate, needs to be exposed to heat in order to remove the moisture content and to inactivate the mycelium, giving us the lightweight, and biodegradable material for multiple purposes (Elsacker et al., 2020). These MBCs are using less energy in the production process, they are safe for the environment, and represent strong materials with great potential to replace fossil-based and synthetic materials such as polyurethane and polystyrene (Haneef et al., 2017; Elsacker et al., 2019)

The shape of the MBCs products depends on the shape of the moldthat the substrate is placed in (Vandelook et al., 2021).

There are several methods that use a broad range of temperatures and different time intervals to remove the moisture content. According to some authors infrared oven heating, microwave heating, and oven baking should be at a temperature range of 60 to 125 ◦C for 2 h (Haneef et al., 2017), oven-drying should be for 48 h at 60 ◦C (Attias et al., 2019); while convection oven drying should be at a temperature of 70 ◦C for 5 to 10 h (Elsacker et al., 2019).

Additionally, greener method, using solar dryers is carried out for 8 h at 60 °C or for 2 h at 220 °C (Jiang et al., 2017) and convection heating using solar dryers at 82 ◦C for 12 h and thermal pressing at 250 ◦C for 20 min. (Jiang et al., 2019);

Using thermal press gave good results with thermal pressing at 250 ◦C for 20 min. (Jiang et al., 2019); and hot-pressing with a force less than 30kN at 150 ◦C for 20 min. (Appels et al., 2019);

Liu et al., (2019) observed that high pressing temperatures of 200 °C develop hydrogen bonding between the *G. lucidum*-produced mycelium and cotton stalk particles, thus greatly contributing to the enhanced physico-mechanical properties of the MBCs.

Depending on the final application of MBC, different types of coatings such as oils (natural oil, linseed, coconut oil) or wax, (carnauba, beeswax) can be used to improve the surface properties of MBCs (Jiang et al., 2019; Vandelook et al., 2021).

DISCUSSION

Species selection

The fungus species with different growth density, used as a binder for agricultural waste appears to impact the material's mechanical characteristics (Jones et al., 2018). Because most MBCs are cultivated on lignocellulosic agro-based by-products and waste materials, poor in simple sugars (glucose, sucrose, and fructose), white rot fungi from Genus Ganoderma, Pleurotus and Trametes are the most commonly used (Jones et al 2018; Jones et al., 2019).

Haneef et al. (2017) grew *G. lucidum* and *P. ostreatus* on cellulose-potato dextrose broth (PDB) and pure cellulose. They found that while *G. lucidum* had a higher percentage of critical strain on both substrates, *P. ostreatus* fibers are stiffer and have a higher Young's modulus (MPa). Furthermore, it is mentioned that the presence of PDB can reduce the Young's modulus and increase the critical strain of the mycelium fiber film, making it softer but more flexible. Nonetheless, the mycelium species has very little effect on critical stress (Haneef et al., 2017).

The biosynthesis of plasticizers (lipids, proteins) is stimulated by the addition of potato dextrose broth to the pure cellulose substrate, increasing the flexibility of the MBCs. The shape and elasticity of the structure of the composites made from the *G. lucidum* fungal strain were responsible for the increased ultimate strength of these composites as compared to *P. ostreatus* composites. This finding implies that the tensile qualities of MBCs are influenced by the fungal species chosen for their biofabrication.

Substrate selection

Because of their symbiotic relationship, the mycelium and substrate create a network structure that expands, increasing the contact area within the complex substrate. Many authors (Haneef et al., 2017; Jiang et al., 2017; Appels et al., 2019) pointed out that the properties of the MBCs surface, produced after full colonization of the substrate vary based on the organic substrate. Branched filaments and network structure govern the mechanical properties of mycelium (Islam et al., 2017). Therefore, one of the key factors influencing the mycelium-based composite's density is its substrate. Typically, higher density is achieved using the higher proportion of grain (fibers, husks, or wood pulp) in the substrate (Arifin and Yusuf, 2013). Javadian et al. (2020) reported that the compressive strength of MBCs can be increased by appropriate selection of the growth substrate. For instance, Ganoderma material grown on cotton plant biomass materials demonstrated a bending strength of 7–26 kPa (Ziegler et al., 2016). In another study the compressive strength of mycelium *P. ostreatus* composite grown on wheat straw substrate increased from 0.02 to 0.15 MPa when grown on white oak sawdust substrate (Ghazvinian et al., 2020). The tensile strength of the fungal-based mycelium among other properties are highly sensitive to the type of substrate. The composites grown on oak sawdust demonstrated high tensile strength as compared with those prepared from the beech sawdust (Faruk et al., 2012).

Haneef et al. (2017) explained that the microstructure of mycelium-based materials depends on their growth substrate which in turn influences their mechanical properties. In a study, *P. ostreatus* grown on potato dextrose had frequent hyphal collapse, low hyphal width, and less content of chitin as compared with *P. ostreatus* grown on cellulose. The bioproduced material with low chitin content had high water absorption, a high rate of elongation, and low Young's modulus (Haneef et al., 2017).

Because they can prevent noise from building up in a confined space by converting a significant amount of mobile air molecules traveling in sound waves into mild heat, MBCs are thought to have strong acoustic noise absorption properties. When compared to conventional absorbers like commercial ceiling tiles (61 dBa), urethane foam board (64 dBa), and plywood (65 dBa), certain MBCs have better acoustic absorption capacities, such as mycelium composites grown on rice straw (52 dBa), hemp pith (53 dBa), and flax (53.5 dBa) (Jones et al., 2019).

The excellent acoustic absorption properties exhibited by MBCs are the main reason why they have been recently utilized in the production of sound insulation of walls, doors, and ceilings of concrete halls, as well as broadcasting studios (Jones et al., 2019; Pelletier et al., 2019).

Environmental conditions

Favorable growth conditions vary from species to species on different substrates. For example, the incubation temperature varies between 21°C and 30 °C for different fungal species. Similarly, the average pH level for optimal growth of various species is in a range from 5 to 8, while the humidity levels are in range between 70% and 100% (Haneef et al., 2017; Appels et al., 2019).

The water content of the final mycelium-based bio-composites is the primary consideration for the mechanics of the mycelium samples and most of the water must be removed because it needs to be provide a high and reliable mechanical performance and be dry enough to terminate the fungal growth (Girometta et al., 2019). The substrate and the species of fungi affect the final mycelium water content. For instance, a substrate made of hemp pulp absorbs more water than that made of cotton wool (Ziegler et al., 2016).

High-density materials are produced in the dark with low carbon dioxide concentration and in light with high carbon dioxide concentration, showing a connection between light and CO_2 and their effect on fiber density (Appels et al., 2019).

Post processing method selection

Several authors (Elsacker et al., (2019) and Yang et al., (2017)) have observed that the substrate mold design affects the mechanical properties (compressive strength, stiffness, or Young's modulus) of the MBCs after production.

When the MBMs reach maturity, they are heated to a predetermined temperature and duration in order to kill the organism and remove moisture from the MBCs (Elsacker et al., 2020). The most common techniques used to dehydrate MBCs are hot pressing and oven drying, which frequently improves the material's mechanical qualities (Jones et al., 2019).

For the purpose of completely drying the bioproduced components, an oven with air circulation might be the best option. Materials that have been fully dried have lower thermal conductivity when compared to those that have retained moisture. Additionally, heat drying samples often have greater elastic moduli than living materials (Yang et al., 2017).

It is possible to efficiently improve the structural characteristics of mycelium-based composites by applying heat or cold pressing. Generally speaking, pressing decreases porosity and raises material density. Additionally, it thins the mycelium fibers and helps them reorient horizontally in a plane, increasing the contact between the fibers at sites where they overlap (Thoemen and Humphrey, 2005).

Once pressed, the air that once made the material light and airy is now lost and material becomes more dense with better structural qualities. Compared to non-pressed MBC, cold-pressed MBC has a higher tensile strength and elastic modulus and leads to a two-fold increase in density. In addition to having a density increase of more than three times, heat-pressed MBC also has an improved elastic modulus and tensile strength (Appels et al., 2019).

Due to the softening of the lignin which, after heat-pressing, reacts to form new cross-links that increase the material strength, properties are similar to those of panels, and compressive strength is on par with wood products like fiberboards and strand boards. In addition to the improved structural characteristics, heat-pressing produces panels with higher structural qualities as well as more uniform thickness and less density variation between panels (Bouajila et al. 2005).

Later, other authors (Appels et al. 2019; Yang et al., 2020; Manan et al. 2021), showed as well that the mechanical properties of MBMs are affected by the heat pressing which can substantially increase the tensile strength and stiffness of the MBCs.

The mechanical properties of mycelial-based materials are also greatly influenced by the pressing temperature. A study reported that the composites of *P. ostreatus* and rapeseed straw showed less stiffness and tensile stress upon cold pressing, as compared to high tensile strength, stiffness, bending properties, and low rupture strain when pressed under heat. Overall, the cold and hot pressings of *P. ostreatus* and rapeseed straw composites resulted in a two- and three-fold increase in density, respectively.

The *G. lucidum*-cotton stalk composite was heat-pressed at 200 °C to yield an internal bonding strength of 0.18 MPa, a modulus of elasticity of 680 MPa, and a rupture strength of 4.6 MPa. According to this study, the superior qualities of the composite are primarily due to esterification, repolymerization, and hydrogen bonding creation at high pressing temperatures (Liu et al., 2019).

Appels et al. (2019) observed that the *P. ostreatus* biocomposite grown on cotton seed hull substrate (processed by hot pressing at 150 ◦C, and force < 30 KN) had a higher tensile strength (0.13) than the 0.03 MPa obtained for the same composite when processed by cold pressing (at 20 ◦C for 20 min and dried afterwards).

We can conclude that the strength of MBCs is very dependent on the processing method as pressed mycelium samples were stronger than the mycelium products that have not been previously pressed.

Even though the MBCs show advantages for their mechanics, lightweight, and many environmentally friendly features, they have limitations and challenges for their large-scale applications. For instance, as a biomaterial, its production is far less standardized than conventional engineering materials such as steel, cement, and polymer, and it is not clear how to customize the types of substrates for the certain species of fungi to maximize the yield of mycelium and to optimize the composite mechanics. To have these standardized properties, there is a need for more R&D work. Moreover, unlike polymer foams, mycelium-based biocomposites cannot be mass produced within a short time by machines, as growing the mycelium takes 2 weeks or more. It is important to automatically control the growing factors, including temperature, humidity, supplied nutrition, and light within an incubating environment without direct usage of human labor during its growth.

CONCLUSION

The quest for green products and technologies for applications in the built environment has led to the birth of a new generation of sustainable materials, among which are mycelium-based composites. Using the unique capability to utilize agricultural crop waste, MBCs have been invented and widely applied to different areas, including construction, manufacturing, agriculture, and biomedical (chitin and chitosan production for wound healing). Their low-cost and environmentally friendly features attract interest in their research and commercialization. However, their low mechanical properties, high water absorption, and lack of standardized development methods limit their applications to semi-structural and non-structural materials such as paneling, furniture, and decking. Future research should aim at reconciling its varying mechanical properties based on fungal species, their substrate, related environments during the growth and processing method. An in-depth knowledge of the relative effect of each of these variables can considerably lead to the optimization of the production process, leading to the development of products with the appropriate spectrum of properties suitable for the building and construction industries.

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