

Progress in Using Fungal Mycelia as Adhesive in Composites

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Abstract

Fungi grow dense webs of mycelia that naturally form a 3D matrix, binding together any particles they encounter during growth. This property has been successfully exploited to create 100% bio-based lignocellulosic composites bound with mycelia. In this chapter, we review recent research on production procedures and performance of these composites. We identify various roles of mycelia in the composite performance and mechanisms by which mycelia contribute to composite properties. In addition, we point out critical knowledge gaps and identify commercial challenges facing this technology.

Keywords: Fungal composite, fungal binder, fungal adhesive, bio-based composite, low density composite, lignocellulosic

11.1 Introduction

The growing demand for sustainable and environmentally-friendly materials drives innovation across various industries. One promising avenue involves using bio-based materials, replacing traditional petroleum-derived products. Serving as the binding agents in many composite materials, adhesives are crucial in this context. Traditional adhesives, such as

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formaldehyde and isocyanate-based synthetic resins, present significant environmental and health concerns [1–3]. This has led to concerted efforts to either replace these adhesives with bio-based alternatives or to develop entirely binderless, bio-based composites [4, 5].

Fungal mycelia, which naturally produce intricate networks of interconnected thread-like fungal cells, provide a remarkable alternative to adhesives for producing composites. The mycelia naturally spread and adhere to both the interior and exterior surfaces of substrates facilitating colonization and extraction of nutrients from the biomass substrate [6, 7]. This unique feature has been successfully exploited to produce composites where fungal mycelia serve an adhesive function without the addition of other binding agents [8]. In some applications, fungal mycelia are used much like conventional adhesive resins, being applied with a high moisture content, and then dried and cured to form a strong bond. In these circumstances, they are similar to other adhesives being used in particleboard and fiberboard, as they bind the particles at many discrete points, not continuously. We use bind, rather than bond, because one threadlike fungal connection allows movement between the two bound particles, similar to tying the particles with a string. Bonding one spot with an adhesive, by contrast, allows no relative motion between the particles. What truly distinguishes mycelia composites, however, is the utilization of the living feature of fungi. When given time to grow, they construct a complex interwoven framework, binding the substrate together with the 3D network of interconnected mycelia, while making up only a minimal part of the composite's overall weight. Furthermore, they not only adhere to the substrate; they also alter its chemistry, potentially improving the quality of adhesion in the composite.

Various products have been developed using fungal mycelia as the sole adhesive in composite structures. The very early applications focused on low-density foams for packaging and insulation [9, 10]. There have also been attempts toward hot-pressed panels with enhanced mechanical properties [11, 12]. Additionally, innovative processing methods like 3D and 4D printing have been explored to utilize the benefits of mycelial adhesion. Most recently, “engineered living and regenerative products” have been demonstrated, where mycelia in the final product are alive and thus can grow across gaps, joining pieces together after assembly [13]. Interest in this domain has surged notably. Figure 11.1 illustrates the evolving research trends in publications related to composites that employ mycelia as the adhesive, derived from a search in The Web of Science. This chapter reviews recent advancements in employing fungal mycelia as adhesives in different composite systems. We will examine the specific roles and

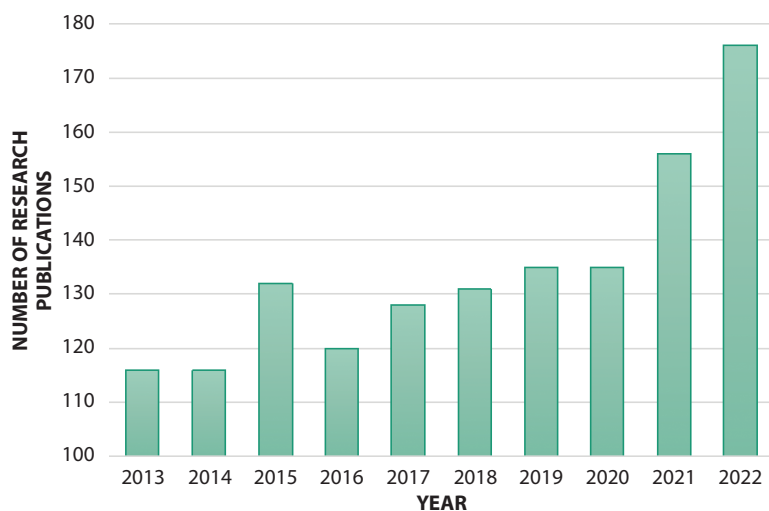


Figure 11.1 Research trends in relevant publications on composites using fungal mycelia as adhesive. Search string: mycelium (title) or mycelium-bound (title) or mycelium-based (Title) and materials (Title) and composites (title) and biocomposites (Title).

mechanisms through which fungal mycelia contribute to adhesive effectiveness, as well as avenues for further optimization and enhancement.

11.2 Basics of Fungal Mycelia

11.2.1 Fungal Species and Mycelia Characteristics

Among millions of different fungal species [14], only a limited few have been explored as adhesives for lignocellulosic bio-composites. According to a patent survey in 2019 [15], only 20 fungal organisms were found in granted patents, all saprotrophic members of the phylum *Basidiomycota*. The literature contains an additional 50 species used in mycelial composites [16]. As natural decomposers of lignocellulosic biomass, these fungi break down lignocellulosic materials, using them as nutrient sources to support their growth and metabolism [17, 18]. Hyphae grow by tip extension, branching and penetrating all exterior and interior surfaces of their substrates seeking nutrition [7]. Hyphal branching and extension are essential features of the adhesive function, while the ability to thrive on lignocellulose is key to the efficient production of lignocellulosic bio-composites. In a recent review, the following 11 fungal characteristics were deemed

effective in producing composites: rapid hyphae growth, ability to colonize quickly and exclude other microbes, dimitic or trimitic hyphal morphology, white rot decay type, high versatility in nutrition, high tolerance to substrate variability and environmental parameters, responsiveness to readily controlled factors, easy to deactivate, saprophytic, non-mycotoxic, and ability to biosynthesize natural active substances [16].

Although wood decay fungi present three basic morphologies, almost all the species used for bonding composites belong to the white-rot morphology [15]. This is commonly attributed to the white-rot's ability to decompose all major wood constituents, though we believe it is because the other two types, i.e., brown-rot and soft-rot fungi, are inherently unsuitable. In contrast to brown-rot, white-rot does little damage to the mechanical integrity of the wood substrate during early decay [19, 20]. In contrast to the soft-rot fungi, white-rots often grow quickly and are easily cultivated.

Selective white-rot fungi have received relatively more attention as they preferably degrade lignin, leaving the loadbearing cellulose relatively unmodified, as opposed to non-selective white-rots, which degrade all major wood constituents at similar rates [21, 22]. Note that the criteria and conditions for attaining selective degradation are sometimes contradictory in the literature. The selectivity of different fungi varies for different ligno-cellulosic biomasses, culture conditions and with the pretreatment time [23, 24].

The fungal cell wall is a multi-lamellar structure [25] (Figure 11.2). In the core, highly branched and stranded chitin is linked with alkali-insoluble β -glucans. This core layer forms the framework of the cell wall and provides the mechanical strength for fungal mycelia. The outer layer is more complex and more varied, usually containing a proteinaceous layer, an alkali-soluble glucan layer and may also contain glycoprotein layer, a variety of polysaccharides, a melanin layer, or hydrophobins. A water-soluble mucilage or capsule layer may cover the surface of the hyphae, particularly at the apical level [25–27]. This mucilaginous layer often forms direct contact to the substrate surface and determines the interfacial interaction and compatibility of mycelia with the substrate.

Like conventional adhesives, modifying fungal mycelia can have a profound impact on the properties of the composites they bind. This modification can be achieved through various approaches, including altering the fungal species, manipulating growth conditions such as time, temperature, nutrition type, and availability, or even modifying the genetics of the same fungal species [28, 29]. It is important to note that changing one factor may trigger a cascade of effects on others. For example, altering the genetics of a fungus may result in enhanced adhesion but could also impact growth rate,

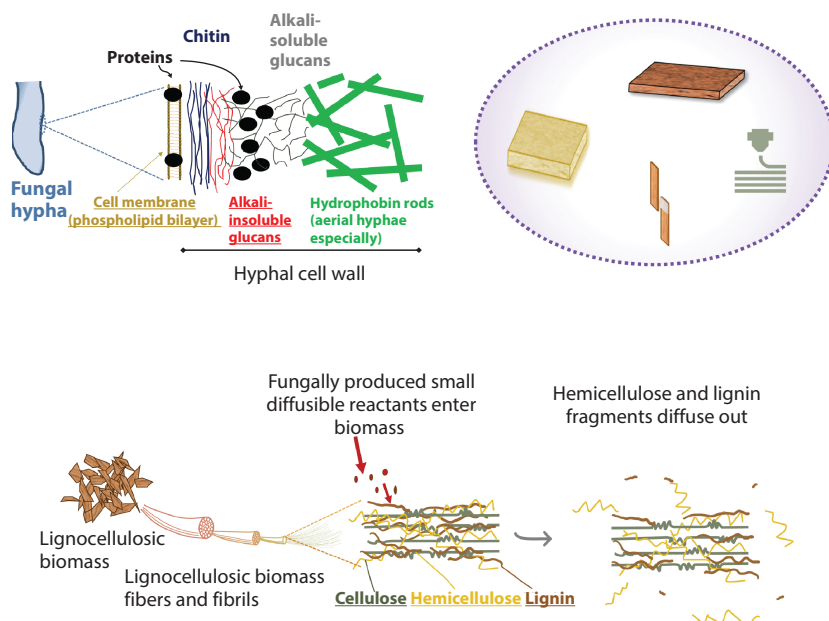


Figure 11.2 Fungal cell wall structure, types of fungal composites (as-grown foams, hot-pressed panel, 4-D printed, and fungal adhesive), and chemical modifications to lignocellulose during early decay. Cell wall diagram adapted from [25].

cell wall structure (strength), or other traits [25]. Therefore, establishing a definitive correlation between modification methods and the resulting properties of mycelium-bound composites presents a complex challenge, reflecting the complex regulation of fungal growth. While several attempts have been made to unravel these connections in the literature, the conclusions drawn thus far remain inconclusive.

Regarding the properties of mycelium-bound composites, some studies have attributed the differences in the mechanical properties to the type of hyphae within the fungal species, asserting that the species characterized by a combination of generative, skeletal, and binding types of hyphae tend to exhibit superior composite binding capability [30]. However, conflicting studies have found no significant distinctions [31]. Many of these studies use the same growth conditions and times for many different species, neglecting the fact that different fungi may have different optimal growth conditions that might result in specific property outcomes for one species but not for another.

Fungal metabolic diversity provides numerous genetic modification opportunities to alter the properties of mycelia through gene overexpression

or suppression [32]. It is worth noting that the integration of fungal genetic modification into the field of materials science remains relatively underexplored. As an example, the deletion of hydrophobin genes in *Schizophyllum commune* (*S. commune*) enhances mechanical properties of the resulting pure mycelia by affecting the density of the mycelia [28]. Additionally, the introduction of engineered bacteria *Pantoea agglomerans* has opened new avenues for the development of mycelial-bound living composites with novel biosynthetic and sensing-reporting capabilities [13].

11.2.2 Effects of Fungal Mycelia on Lignocellulosic Substrates

The cell walls of lignocellulosic substrates are aggregates of cellulose microfibrils surrounded by a matrix of hemicellulose and lignin. Fungi are typically chosen so that during first few days to ~two weeks of fungal treatment the cell wall properties and chemical composition changes are minor and performance is not negatively affected. Typical mass losses of the lignocellulose are 1-3%, primarily hemicelluloses and extractives [33]. Therefore, the rougher surface of the degraded wood substrate is most probably caused by the aggregation of smaller fungal mycelia at the microscale. The aerial hyphae on the surface create a microscale network on the original biomass surface [12, 34]. The aerial mycelia themselves contain amphiphilic proteins called hydrophobins, which make the surface hydrophobic with water contact angles higher than 120° [35, 36], mediating the escape of hyphae from the aqueous environment into the air [28, 37]. The biofilm hyphae, which are located at the bottom surface of the mycelia mat, show stronger adhesion to lignocellulosic substrates than aerial hyphae [38]. However, this bond is not water-resistant, which indicates that the major bonding mechanism is hydrogen bonds and van der Waals interactions due to the redistribution of surface components [39].

11.3 Production Procedures

The basic production procedure for bio-composites using fungal mycelia as adhesives is illustrated in Figure 11.3 and includes several indispensable (boxed) and optional (circled) steps.

Commonly used substrate materials are lignocellulosic biomass from agricultural and forestry by-products and wastes [32]. They could be in fibrous, such as hemp, straw, or cotton, or in particulate forms, such as sawdust and wood particles. Additionally, materials like food waste e.g.,

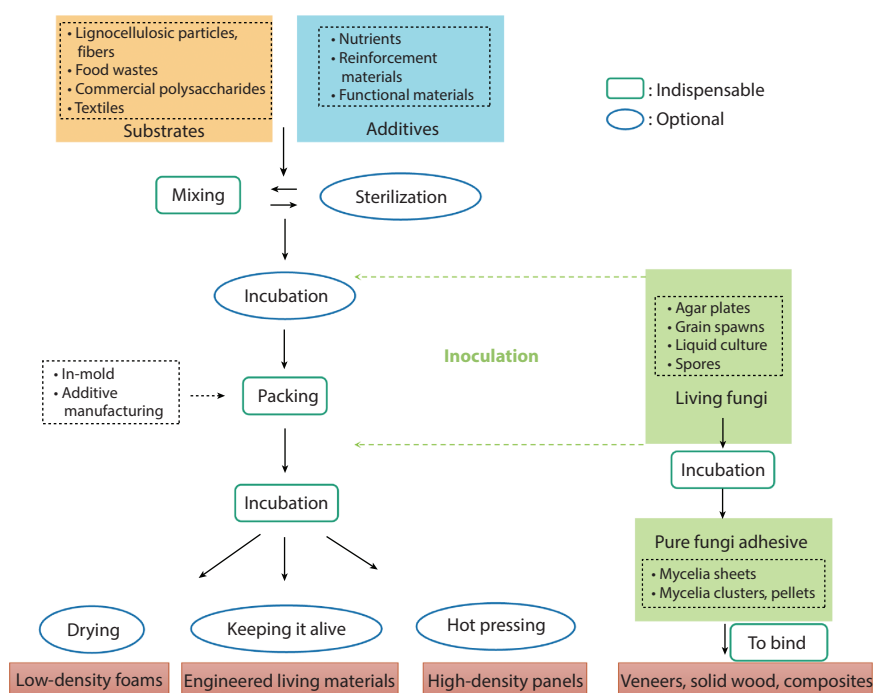


Figure 11.3 Production procedure for mycelium-bound composites.

wheat bran [40], coffee ground [41], coconut husk [42], and commercial polysaccharides such as agar [43] and textiles [44] have also been utilized as primary substrates to achieve the desired final product properties. To accelerate the colonization of fungi and introduce specific functional properties, liquid or solid nutrients and other additives may also be added to the substrate. To provide suitable moisture conditions for the growth of fungi, the substrates are also hydrated. The optimum moisture content is between 40% and 80% [45].

In academic settings, the prepared substrate formulations typically undergo a sterilization step. Substrate sterilization ensures that the fungus of interest (rather than a contaminating microbe) completely colonizes the substrate. In industrial applications, incomplete sterilization (decontamination) such as exposing the substrate to two minutes of steam is much less costly and has been shown to give the white-rot fungus inoculum sufficient competitive advantage to reliably colonize the substrate [46]. Simply adding colonized wood chips to unsterilized raw chips has also been effective [47]. Most recently, *Ganoderma* sp. has been demonstrated to colonize

non-sterilized substrates, which was attributed to its genetic flexibility, but can be described more accurately as fast growing and aggressive colonizing [13]. Thus, the sterilization step is optional if the right fungi and process are in place.

In most research, the procedure involves initially mixing the substrate and additives, followed by inoculation with an inoculation medium. The inoculation medium can use many different substrates, typically agar plates, grain spawns, lignocellulosic spawns, or liquid culture. The inoculum, and sometimes extra nutrients such as corn steep liquor, wheat flour, or minerals are then thoroughly mixed with the biomass. Inoculation with spores typically affords the best distribution of fungi through the product, but spores have the disadvantage of requiring more nutrients than the vegetative state, and also there is normally an induction period (delay) while spores transition to active growth. The entire mixture is then loosely packed in containers or molds and incubated in a controlled environment, as explained below. Depending on conditions, growth of the mixture for some time before packing it into molds may allow faster or more uniform growth. Besides using molds and containers as the shape-forming tools, additive manufacturing has also incorporated mycelia as a bonding agent. In this case, substrate-nutrient-fungi mixtures are processed into an extrudable paste by incorporating other additives such as flours [48], gums [49] kaolin clay [50], and chitosan [51]. The desired structure is fabricated layer-by-layer through 3D printing. Recent innovations have introduced an alternative approach where the final structure or its elements, created through methods like additive manufacturing [41], are initially created. Subsequently, fungal organisms are introduced through indirect inoculation by bringing the materials into contact with a bed of mycelium-bound composite and letting the mycelia grow through the pre-structured material.

Incubation is usually conducted in a controlled environment (20–30 °C, dark, humid, forced ventilation for larger volumes) for varying times, usually a few days to two weeks. Once mycelia grow through the substrate, an additional out-of-mold growing process (removing the substrate from the mold and letting the surface be exposed to the air) may be applied to obtain a continuous and uniform outer skin. Fungi typically grow a thick mat on the outer surface of the substrate. Poor to no growth from lack of oxygen has been reported for biomass that is too wet or too thick. There was extensive research in the 1990s on a very similar process called biopulping, where wood chips were inoculated with white rot fungi for ~14 days before papermaking. Much of the findings of that research are summarized in a

1998 book by Young and Akhtar. Topics relevant to mycelial composites include research on decontamination/competing fungi/inoculation methods, mass loss vs growth period, tracking biomass generation, engineering considerations upon scale-up, large-scale production of inoculum, etc. [52].

After mycelia colonize and bond the substrates into an integral structure, heat treatment is usually applied to dehydrate and kill the organism. When being dried in an oven, the mycelium-bound structure would maintain its highly porous structure and relatively low density, which are desirable characteristics for packaging and insulation materials [40, 53, 54]. When applying hot-pressing rather than oven drying, the bio-composites are densified, and the structure is similar to traditional particleboard or fiberboard, depending on the form of the particles used [11, 12, 55]. Typical temperatures and times used for oven drying (50–125 °C, 2–48 h) and hot-pressing (130–250 °C, 5–20 min) are enough to cause the mortality of the fungi. Engineered living materials with capacity to self-repair and self-replication are believed to be the next generation of materials [56]. When applying this concept in mycelium-bound composites, it was found that the living structure could bond itself together and had sensing-reporting capability [13]. This opens up new possibilities for bio-composite applications.

11.4 Adhesive Performance of Fungal Mycelia in Different Composite Systems

The adhesive performance of fungal mycelia in bonding lignocellulosic substrates is often indirectly expressed by the mechanical properties of the final bio-composite product. Specific mechanical properties are usually selected based on the application requirements of the product, which may not fully reveal the performance of the adhesive. Expressing the performance of the “adhesive” itself is challenging as mycelia are small and come in varied forms. They can also modify the substrates while growing and therefore their function is not only as binders in some systems.

11.4.1 As-Grown Foams

Mycelial composites are categorized as unhybridized (pure fungi and substrate also known as as-grown mycelium-bound foams), hybridized (blended with additives), reinforced (incorporating woven or rigid

materials), or in a sandwich structure (with materials adhered to both sides). Ongoing research aims to improve these composites beyond the current method [57].

As-grown mycelium-bound foams were perhaps the first group of mycelial composites produced and reported in the literature. The production process of as-grown foams starts by preparing the substrate that can be any type of lignocellulosic material, from wood particles to agricultural by-products to waste materials. The particles are reduced in size to usable fractions normally through a grinding process and then sieved to separate desired dimensions. They then follow the process outlined above: decontamination/sterilization if needed, inoculation, molding, and oven drying.

Fungal mycelium-bound as-grown foams are candidates to replace petroleum-based packaging and insulation materials such as polystyrene foams. Therefore, compressive strength is the most often tested mechanical property. In these systems, the mycelia are believed to behave as a supportive matrix (Figure 11.4a), and the substrates act as reinforcing components contributing to the mycelia network [58]. Thus, at initial small strains the mycelia matrix determines the elastic response under compression, while the stiffening is observed at large strains is attributed to the hard substrate particles coming into direct contact with each other [58].

One of the interesting features of mycelial composites is the morphology of the fungal “adhesive”, which creates threadlike structures throughout the void spaces of the product. The smaller pores and more tortuous flow paths

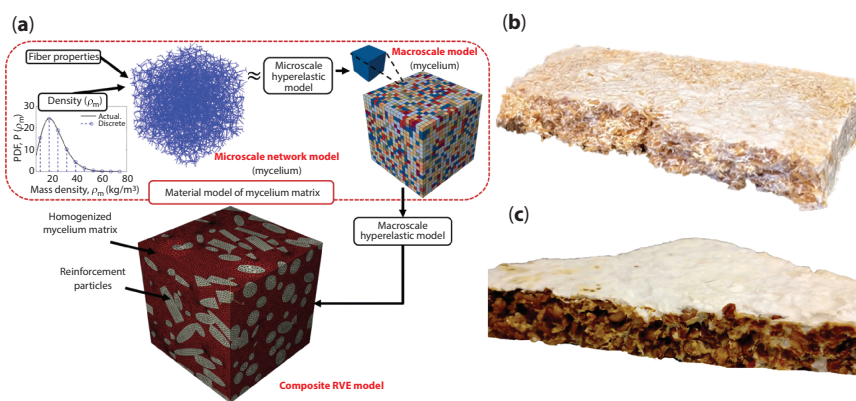


Figure 11.4 Schematic representation of multiscale modeling procedure developed for mycelium-bound composites (a). Adapted from [58]. Pictures show two different mycelium-bound composite structures: uniformly distributed mycelia [39] (b) and core-shell structure [59] (c). RVE: representative volume element.

that result from fungal colonization in voids could potentially improve sound and thermal insulation properties, though the experimental data is inconclusive. The mechanical reinforcement of this network holds the product together and they are ideally suited for low-density applications such as packaging and insulation.

Some studies have found a relationship between fungal species and product strengths. For example, Bruscato *et al.* compared different fungal species colonized on the same substrates and found that leathery mycelia corresponded to higher compressive strength compared to fragile (easily broken) mycelia [54]. Teixeira and coworkers tested the compressive strength of various fungi-colonized substrates and found that the bio-composites colonized by *Pycnoporus sanguineus* had much higher strength than those colonized by *Pleurotus spp.* However, there was no change in the elastic behavior with further increase of the culture period and therefore hyphal density, beyond the initial 15 days of incubation [60].

Tensile and flexural moduli and strengths were also reported in the literature, again with contradictory results. In one study, *Trametes versicolor* and *Trametes multicolor* exhibited higher tensile and flexural strengths and moduli than *Pleurotus ostreatus*. This was explained as corresponding to the trimitic hyphae system, which produces generative, binding, and skeletal hyphae, compared to the monomeric hyphae system of *Pleurotus ostreatus* comprising only of generative hyphae [30]. However, in another study, no notable difference was found between monomeric *Pleurotus ostreatus* and trimeric *Trametes hirsute* [31].

The complexity of the bio-composite system makes it difficult to reliably determine properties. Firstly, in most of the bio-composite systems, fungal mycelia are not uniformly distributed (Figure 11.4b shows an example of relatively uniformly distributed mycelia on the substrate). These bio-composites usually contain a core-shell structure, with a continuous mycelial shell on the surface but with lower mycelial density inside, attributed to lower oxygen concentration (Figure 11.4c). Secondly, many substrate particles are in direct contact with each other, as opposed to the model in Figure 11.4a where there is no particle-particle contact. Particle contact should provide compression resistance and some flexural stiffness. These facts might explain the contradictory compressive test results in the literature. Thirdly, the period of fungal growth is not a simple process of filling the gaps among the substrate particles or fibers. In the meantime, the mycelia are degrading the substrates and thus altering their mechanical properties, though not substantially [61]. Substrate surface morphology and chemistry also change during the incubation.

Over the past several years, efforts have been made to improve the physical and mechanical properties of as-grown mycelial foams with hybridization. Hybridization with additives may include the use of inorganic materials (calcium carbonate [62], clay [63] and latex [64]). The inorganic additives mainly improve properties through increasing density, which could have been achieved by the densification of the foam as well. Organic additives such as bacterial cellulose [65] and processed cellulose [66] have also been reported in the literature. Other hybridization methods include fiber reinforcement either through woven fibers or rigid reinforcement [67].

11.4.2 Hot-Pressed or Densified Panels

The adhesive performance of hot-pressed fungal mycelium-bonded panels is evaluated in most studies in terms of modulus of rupture (MOR) and modulus of elasticity (MOE) using three-point bending tests [68]. In contrast to as-grown low-density foams described above, after applying heat and pressure, fungal mycelia play a more important role in the bio-composite system in these panels.

When fungal mycelia were absent from the hot-pressed panel system, many gaps were seen in between individual wood particles. With mycelial growth, voids were filled with entangled fungal mycelia and thus the mechanical properties increased with the density of the mycelia (Figure 11.5 a-f). MOR and MOE increased with the density of mycelia, corresponding well with the microscopy images of the interfaces (Figure 11.5 g, h) [69].

Besides the density of the fungal mycelia, higher temperature during the hot-pressing process usually brings about higher MOR, which is typical of any pressed lignocellulosic composite. The higher properties are attributed to the formation of more hydrogen and chemical bonds, as well as the plasticization and deformation of lignocellulosic materials at higher temperature which results in more contact area between particles and higher density. Liu *et al.* compared *Ganoderma lucidum* pretreated cotton stalk bio-composites made at different hot-press temperatures (160, 180, and 200 °C) with controlled density and thickness [11]. The interfacial bonding strength increased significantly with the increased temperature (230% increase in MOR from 160 °C to 200 °C). They also observed more repolymerization, esterification, and formation of hydrogen bonds from Fourier transform infrared (FTIR) spectra.

To enhance the properties of hot-pressed mycelium-bonded panels, several strategies have shown promise. Incorporating cellulose nanofibrils (CNFs) at 2.5% by weight in mycelium-bonded particleboards more than

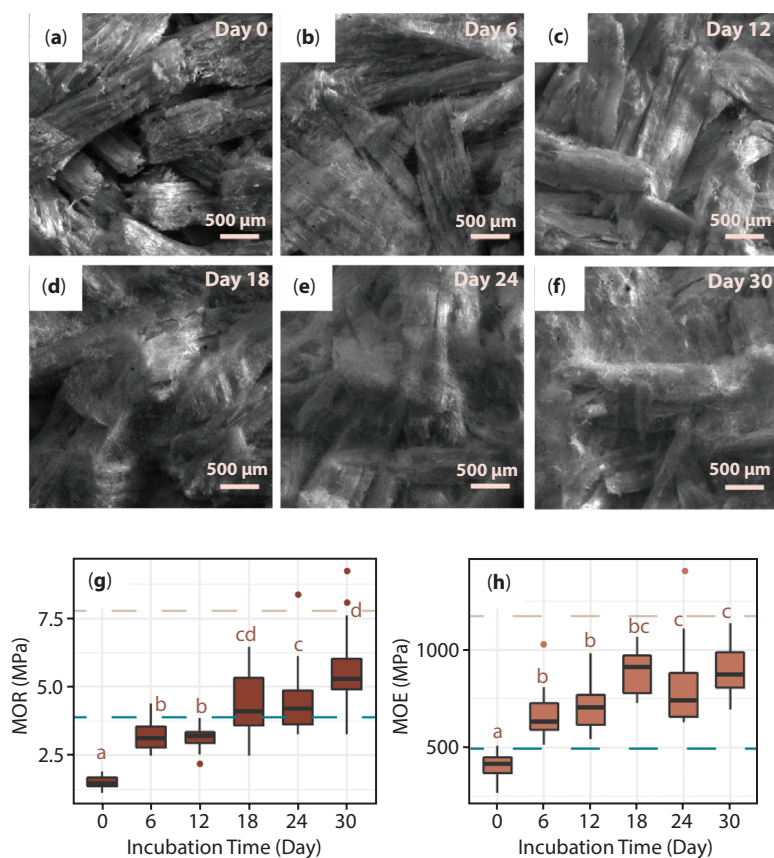


Figure 11.5 Extended depth of field (EDF) images of the internal surface of hot-pressed panels after internal bond (IB) strength test at Day 0 (a); Day 6 (b); Day 12 (c); Day 18 (d); Day 24 (e); Day 30 (f). MOR (g) and MOE (h) of hot-pressed mycelium-bonded panels. Adapted from [69].

doubled the dry MOR and MOE [12]. Additionally, blending in bacterial cellulose doubled MOE and substantially improved tensile strength and internal bonding [70]. Another notable approach involved introducing processed cellulose from wastewater treatments into the composite system, where the resulting hybridized composite exhibited twice the strength of the control [66]. Yang and Qin made dogbone specimens and looked for increases in specific (property/density) strength, stiffness, and toughness. They found that long pressing times (4+ hours) were universally beneficial but optimal pressures (6.8–20.3 MPa) and temperatures (80–100°C) were found throughout the range tested [71].

While various strategies have shown promise in enhancing hot-pressed mycelium-bonded panels, some challenges remain. Notably, these panels exhibit relatively high water absorption and thickness swelling, exceeding the standard requirements. This issue restricts their suitability for use in humid or wet conditions. Nevertheless, in some particleboard-related applications, which are typically designed for interior use, this is less a concern. Significant improvements are necessary for medium density fiberboard applications, however.

11.4.3 4-D Printed Composites

There has been significant academic interest in printing large scale 3D structures such as small buildings. Because of the added dimension of time in developing the material, these are sometimes referred to as 4-D materials. Mycelial composite 3D structures are not immune to the challenges typical of 3D printing: properties of the extruded material, properties of the finished ribbon, processability, interlaminar bonding, production rate, etc. We can borrow many lessons from 3D printing of plastics and apply them to printing biomass. A fundamental hurdle to 3D printing is the need for low viscosity in the printing mechanism quickly followed by solidification to prevent sagging of the lower layers from the weight of the upper layers. In plastics 3D printing, amorphous polymers are extruded above the glass transition temperature (T_g) and quickly cooled below T_g , increasing elastic modulus (stiffness) by several orders of magnitude. Crystalline polymers are not 3D printed largely because they do not have a T_g , and therefore no mechanism for speedy strength development (they also shrink considerably upon cooling leading to part warpage). Some groups have attempted to solve this stiffness problem by adding biomass with strong gelling characteristics, such as psyllium powder. Another approach has been to fill voids with sand or gravel while the extruded material solidifies [72].

It would seem that a prerequisite for a functional fungal composite is that the substrate can retain its shape during fungal growth. Therefore, the current lack of a mechanism for supporting the structure during printing is a key barrier to development in this area.

Another common problem with 3D printed structures is a weak boundary between layers, as no particles or fibers bridge the gap between subsequent extrusions. Here mycelia have the potential to provide some bridging between layers, also known as “biowelding” as demonstrated by Modanloo and coworkers [49]. The amount of this enhancement mechanism will be determined by the strength of the mycelial layer and its adhesion to the substrate, discussed in the next section. Another possibility is

to add the fungal inoculum after printing. The inoculum could be sprayed on the interlaminar surface during the printing process to maximize the interlayer bonding, or applied after printing is complete.

Mycelial composites may be able to assist in another significant challenge to 3D printed biomass, the drying process. Most biomass is printed wet, to provide flow in the printer and cohesion upon deposition. However biomass will support microorganisms if wet, and so the products need to be dried out for use. If the product has a colony of selected fungi on it, these fungi can prevent undesirable species from colonizing the product during passive, low energy intensity drying over the incubation period (weeks).

A common problem with 3D printing of animal or human cells for biomedical applications is that the high shear and acceleration conditions at the print head kill the cells. In the case of printing biomass, this has not been a problem because the print heads for biomass applications are typically centimeters, rather than micrometers, in width. Unlike mammalian cells, fungal hyphae have the benefit of tough cell walls to further increase their odds of surviving extrusion.

The main challenges of mycelial composites are in thick applications requiring supporting the weight of upper layers, drying of thick parts, flow in the printing apparatus, and recovering the cost of processing. Therefore the most promising applications of myco-extruded materials are in creative high value products such as artificial skin [43].

Technical articles on 3D printing of biomass focus on issues of printability with different recipes, wait times, and printing conditions [48, 51, 73–75]. When searching the literature, we found that a significant fraction of articles discussed the architectural or visionary aspects of the material rather than technical specifics.

11.4.4 Other Forms of Composites

The performance of mycelia as adhesives for solid wood has been lightly investigated, presumably because virtually all solid wood product standards require significant adhesive wet strength.

Sun and coworkers measured shear strength between veneers using ASTM D7998, and obtained dry strengths of ~ 1.5 MPa after pressing at 180°C for 5 minutes [38]. Spoelstra measured the fracture energy of the mycelial web growing between two veneers to be typically 2 J/m^2 , with a maximum value of 3.5 J/m^2 [76]. These are very low values compared to traditional adhesives which typically range from 200 – 800 J/m^2 [77]. Spoelstra also noted that failures were cohesive, indicating the hyphae were breaking before detaching from the wood [76].

Interestingly, we were only able to find one article measuring the mechanical properties of individual hypha. The elastic modulus of wild type *Aspergillus nidulans* was 110 MPa but different growth conditions resulted in lower modulus, which they attributed to changes in cell wall architecture [78]. The authors did not measure tensile strength. Porter and Naleway [79] estimated the modulus, strength, and other properties of the three hyphal types by performing compression tests on mushrooms, imaging their structure, and modeling the performance. Not surprisingly, skeletal hyphae, whose purpose is to provide mechanical support, had the highest estimated stiffness (78MPa) and strength (89MPa). Estimates of bending stiffness, tensile stiffness, and failure load of hypha, if known, could be used to model the bonding behavior of fungal composites. Note that these studies have already shown that mechanical properties vary depending on moisture content, hyphal morphology, growth conditions, and species.

11.5 Knowledge Gaps and Commercial Challenges

Only a tiny fraction of the fungal diversity has been explored for use in composites. While just one culture collection at the Center for Forest Mycology Research (Madison, WI) has 24,000 isolates of 2,000 wood decay basidiomycetes, only 70 fungal species have been reported in fungal composites. Diverse growth morphologies (generative, skeletal, and binding, hyphal density, etc.) are produced by different species and strains, as well as by varying environmental conditions. This suggests that some benchmarking of the most promising fungi for industrially relevant properties such as growth rate, tolerance to the mechanical disruption of the inoculation process, and ability to exclude common contaminant organisms during incubation would be useful. In addition, there is extremely limited information on the mechanical properties of hyphae, which could be used to model structures and estimate mechanical performance. Finding hyphal structures with higher strength or elasticity could be broadly beneficial to the mycelial composite field.

3D printed mycelial composites are currently limited to only a few layers because we lack a means to switch the extruded material from fluid to solid upon printing. Without such a mechanism, 3D printing of biomass will continue to be limited to relatively flat profiles, and therefore developing a mechanism for fast solidification is a prerequisite for development of 3D printed mycelial composites.

To our knowledge, modern commercial implementation of mycelium-bound biomass products began in 2007 with the founding of Ecovative Design, which is still producing mycelial packaging materials and more [80]. Some of the largest hurdles to commercial implementation of fungal mycelia products are long production times (up to 2 weeks) and risk of contamination. For comparison, the average output of a medium density fiberboard plant in North America in 2022 was 144,000 m³/yr [81]. 14-day production times at this scale would require almost 5,700 m³ of material continuously in incubation. In addition, culture failures (contamination, failure to grow) are often not detectable for a day or more, resulting in large losses, and therefore risk in the eyes of investors. Mushroom growers have found a way to succeed despite these challenges, but their products command much higher prices than conventional lignocellulosic products. Therefore, simply replacing current low cost products such as packaging, insulation, and panels with an equal is not economically viable. Mycelial products have to deliver significant value to support the production costs.

Living materials have the advantage of self-healing, but if the healing is done by living fungi, then the healing can only occur when the fungi are metabolically active, meaning they must be consuming the substrate to maintain themselves. Clearly this can only work in situations where the product has a short lifetime. In addition, good growth conditions for the fungi so that they can repair the product requires a moist environment of moderate temperature, and therefore the conditions are right for colonization by other microbes such as mold.

Even if the fungi are dead and the product dried upon delivery, the fact that the fungi have colonized the substrate means that the substrate degradation process has begun, the material is hospitable to microbiological colonization as it lacks a broad spectrum toxins, and it contains food in the form of dead fungal or lignocellulosic substrates. Even when dry, fungal mycelia are an attractive food supply for silverfish (*Lepisma saccharinum* and relatives), and some fungus beetles. If the mycelia become wet, their biomass will support a variety of insects, molds, and other fungi.

11.6 Summary and Perspectives

Fungal mycelia are a 100% bio-based, compostable, renewable raw material attracting significant research interest. Because fungal hyphae naturally form low-density 3-D networks while growing on biomass, they have been investigated and commercially utilized to bind biomass particles into low-density materials. The largest interest has been in low-density

insulation and packaging applications, taking advantage of the practicality of simply composting the packaging material upon receipt. Pure mycelia products are also possible but outside the scope of this review. Fungi used to date have been fast-growing white rot basidiomycetes, whose abundant hyphae bind together biomass particles to make a product.

While multiple methods exist for making mycelial composites, they all involve providing moist biomass for fungi to colonize and feed on, distributing live fungi throughout or on the surface, and giving the fungi time to grow the mycelial network. Fungi take the shape of the biomass they feed on, allowing the use of molds for producing complex shapes. After mycelial growth, the product can be oven dried to make a low-density foam, fungus left alive for a self-healing product, or hot pressed to make higher density/higher strength products. The pure mycelia can also be harvested as a film adhesive or used as stand-alone foam.

Fungally-bound products have the advantage of being 100% bio-based and easily compostable. These and other advantages will need to be leveraged to compensate for the requirement of having a long period of fungal growth, limiting production rates, and the necessity of having water present during the growth period, while finished products typically need to be dry. Those who can develop commercially viable solutions to these challenges will be well rewarded.

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