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Fungal Mycelia as Bioadhesives

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Abstract
Mycelium-bonded bio-composites are an emerging class of novel materials developed over the past ten years. These have attracted significant research and commercialization interest with a steadily increasing number of papers, patents, and products. The basic idea behind these novel composites is to use fungal mycelia to bond substrates, mostly lignocellulosic biomass, together. Therefore, fungal mycelia in the system actually work as adhesives. This kind of adhesive is different from traditionally recognized resin-based adhesives in characteristics, processing, application field, and evaluation criteria. Adhesive performance evaluation depends on the form of the final product. Data on the performance of fungal mycelia adhesives is limited and inconsistent and requires more fundamental research and analysis. It appears that usual technical specifications will be achieved preferably by low-density products such as for packaging and insulation. Achieving all the required properties of standard panels, such as M2 grade particleboards, remains elusive.

***Keywords*:** Mycelium, fungi, lignocellulosic biomass, adhesion

16.1 Introduction

Fungal mycelia are networks of interconnected fungal cells. They spread on interior and exterior surfaces of lignocellulosic substrates for colonization and obtaining nutrition [1, 2]. This unique feature was exploited successfully to produce mycelium-bonded bio-composites [3, 4]. Fungal mycelia are a very low percentage of the product mass but use their entangled structure to hold wood substrate particles together. This feature of mycelia fits into the general definition of "adhesive," a substance that bonds two or more objects together mechanically [5]. Meanwhile, they are also distinct from common adhesive resins. Rather than being applied as a liquid and cured, they are grown on the substrate and modify the substrate chemistry in the process.

Specific products were developed based on their unique characteristics. The first developed product was a low-density packaging and insulation material [6, 7]. Lately, hot-pressed panel products with higher mechanical properties have received more attention [8, 9]. Most recently, "engineered living and regenerative" products have been demonstrated,

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where mycelia in the final product are alive and thus can grow across gaps, joining pieces together after assembly [10]. Using lignocellulosic-mycelial composites in additive manufacturing has also been explored [11].

This chapter reviews recent developments in using mycelia as adhesives in various composite systems. The functionality of fungal mycelia in different systems, their relationship to adhesion performance, and improvement strategies are summarized and discussed.

16.2 Basics of Fungal Mycelia

16.2.1 Fungal Species

Among millions of different fungal species [12], only very limited have been explored as adhesives for lignocellulosic bio-composites. According to a patent survey in 2019 [3], only 20 fungal organisms were found in granted patents, all saprotrophic members of the phylum *Basidiomycota*. As natural decomposers of lignocellulosic biomass, these fungi break down lignocellulosic materials, using them as nutrient sources to support their expansion [13, 14]. Hypha grow by tip extension, branching and penetrating all exterior and interior surfaces of their substrates seeking nutrition [2]. Hyphal branching and extension are key features of the adhesive function, while the ability to thrive on lignocellulose is key to the efficient production of lignocellulosic bio-composites.

Although wood decay fungi present three basic morphologies, almost all the species used for bonding composites belong to the white-rot fungi [3]. This is commonly attributed to their 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 [15, 16]. 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 [17, 18]. Note that the criteria and conditions for attaining selective degradation are sometimes contradictory in the literature. The selectivity of different fungi varies for different lignocellulosic biomasses and with the pretreatment time [19, 20].

16.2.2 Fungal Cell Wall

The fungal cell wall is a multi-lamellar structure [21]. 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 usually contains a proteinaceous layer, an alkali-soluble glucan layer and a glycoprotein layer. A water-soluble mucilage or capsule layer may cover the surface of the hyphae, particularly at the apical level [21–23]. This mucilaginous layer often forms the direct contact to the substrate surface and determines the interfacial interaction and compatibility of mycelia with the substrate.

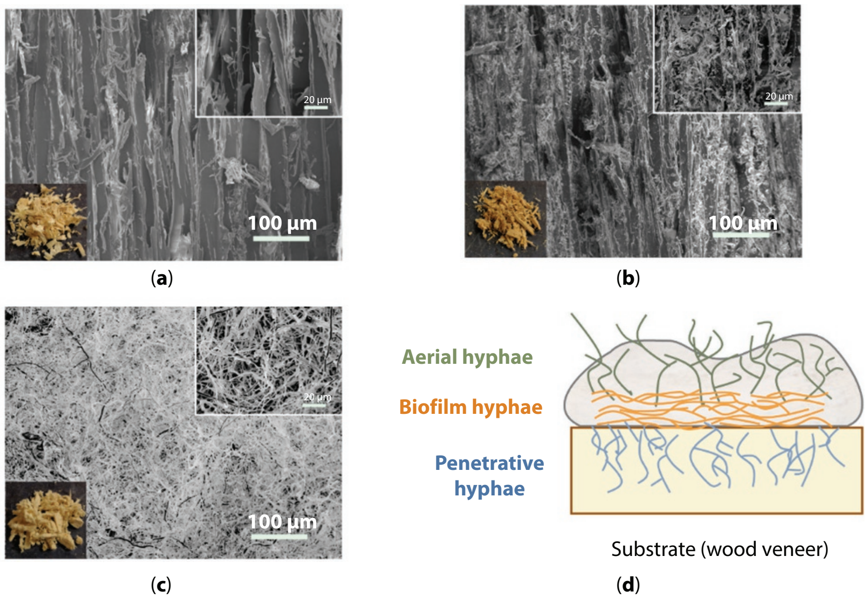

Figure 16.1 Morphology of wood particles before (a) and after fungal degradation (b); pure mycelium (c); schematic classification of hyphae (d). Adapted from [9] and [30].

16.2.3 Effects of Fungal Mycelia on Lignocellulosic Substrates

The cell walls of lignocellulosic substrates are aggregates of microfibrils surrounded by a matrix of hemicellulose and lignin. Over the first two weeks of fungal pretreatment, it is unlikely that the chemical composition of the cell wall is dramatically altered, as the 1-3% mass loss typically reported is more limited to hemicelluloses and extractives [24]. Therefore, the rougher surface of the degraded wood substrate seen in Figure 16.1b as compared to Figure 16.1a 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 [9, 25] (Figure 16.1c). The aerial mycelia themselves contain amphiphilic proteins called "hydrophobin", which mediate the escape of hyphae from the aqueous environment into the air and make the surface hydrophobic [26, 27] with water contact angles higher than 120° [28, 29]. The biofilm hyphae, which are located at the bottom surface of the mycelia mat, show stronger adhesion to lignocellulosic substrates than aerial hyphae [30] (Figure 16.1d). 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 [31].

16.3 Production Procedure

The basic production procedure for bio-composites using fungal mycelia as adhesives is illustrated in Figure 16.2 and includes several indispensable (boxed) and optional (circled) steps.

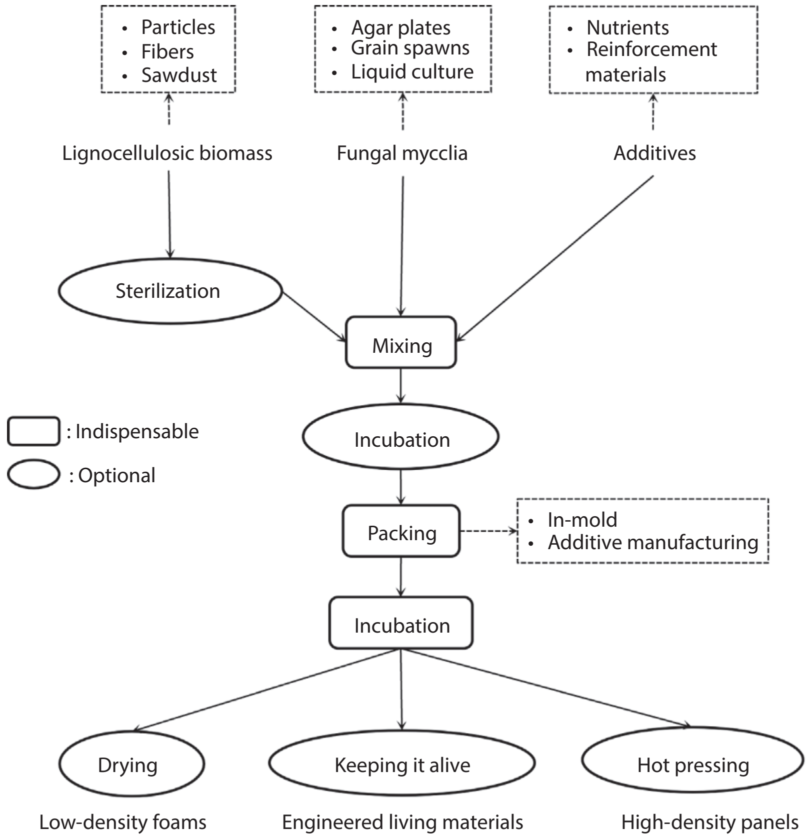

Figure 16.2 Production procedure for fungal mycelium-bonded bio-composites.

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 fibers, or in particulate forms, such as sawdust and wood particles. To accelerate the colonization of fungi, liquid or solid nutrients may also be added to the substrate. To provide suitable humidity condition for the growth of fungi, the substrates are also hydrated to a desired water content level (optimum moisture content is between 40% and 80% [33]).

The prepared substrate typically undergoes a sterilization step. Substrate sterilization ensures that the fungus of interest (rather than a contaminating microbe) completely colonizes the substrate; this procedure is typical in research. In industrial application, incomplete sterilization (decontamination) such as exposing the substrate to steam for two minutes is much less costly and has been shown to give the white-rot fungus inoculum sufficient competitive advantage to reliably colonize the substrate [34]. Simply adding colonized wood chips to unsterilized raw chips has also been effective [35]. Most recently, *Ganoderma* sp. has been demonstrated to colonize non-sterilized substrates, which was attributed to genetic flexibility, but this effect can be described more accurately as fast growing and aggressive colonizing [10]. Thus, the sterilization step is optional.

Inoculation medium can be placed on any substrate, typically agar plates, grains or 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. The entire mixture is then 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 [36], gums [37] kaolin clay [38], and chitosan [39]. The desired structure is fabricated layer-by-layer through 3D printing.

Once the mixture is packed or printed, it is incubated in a controlled environment (20 – 30 °C, dark, humid, forced ventilation for larger volumes) for varying times, usually several days. For the in-mold-growing method, 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 them to obtain a continuous and uniform outer skin.

After mycelia colonization and bonding the substrates into an integral structure, heat treatment is usually applied to dehydrate and inactivate the organism. When being dried in an oven, the mycelium-bonded structure would maintain this highly porous structure and relatively low density, which are desirable characteristics for packaging and insulation materials [40–42]. When applying hot-pressing rather than oven drying, the bio-composites are densified, and the structure is similar to traditional particleboard or fiberboard [8, 9, 43]. 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 [44]. When applying this concept in mycelium-bonded bio-composites, it was found that the living structure could bond itself together and had sensing-reporting capabilities [10]. This opens up new possibilities for bio-composite applications.

16.4 Adhesive Performance

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 modify the substrates while growing and their function is not only limited as binders in some systems.

16.4.1 As-Grown Foams

Fungal mycelium-bonded as-grown foams are candidates to replace packaging and insulation petroleum-based foams such as polystyrene foams. Therefore, compressive strength is the most often tested mechanical property. In this system, the mycelia are believed to behave as a supportive matrix (Figure 16.3a), and the substrates act as reinforcing components in the mycelia network [45]. Thus, the mycelia matrix defines the elastic response

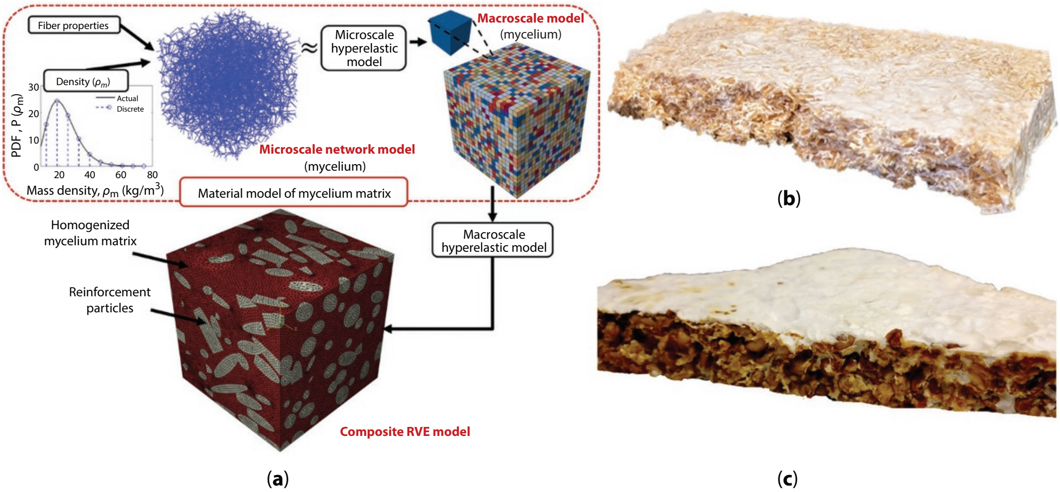

Figure 16.3 Schematic representation of multiscale modeling procedure developed for mycelium- bonded bio-composites (a). Adapted from [45]. Pictures show two different mycelium-bonded bio-composite structures: uniformly distributed mycelium (b) and core-shell structure (c) [31] and [46]. RVE: representative volume element.

under compression at initial small strains, while the stiffening at large strains is caused by the densification of the hard substrate materials [45].

However, the results obtained in different studies do not always follow this pattern and are sometimes contradictory. Elsacker *et al.* found that the samples with a smaller fiber size resulted in a higher compressive modulus [41]. In contrast, Islam *et al.* found that the compressive properties of mycelium-bonded bio-composites are particle size-independent [45]. Elsacker *et al.* also claimed that denser mycelia colonization resulted in a higher compressive modulus [41], whereas Sun found that the different densities of the mycelia matrices in the bio-composite did not significantly affect either the compressive modulus or strength [31].

Some studies have found a relationship between fungal species and product strengths. 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 [42]. Teixeira and coworkers tested the compressive strength on different 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 15 days of incubation used as minimum time span [47].

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 [32, 48]. However, in another study no notable difference was found between *Pleurotus ostreatus* and *Trametres hirsute* (also having trimitric hyphae system) [49].

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 16.3b 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 less mycelial density inside (Figure 16.3c). One reason is that the core of the bio-composite has less oxygen supply for metabolic reactions compared to the shell. Some mycelium growth also continues out of the mold to create a continuous white layer of pure mycelia on the surface. Secondly, many substrate particles are in direct contact with each other rather than not contacting at all as modeled in Figure 16.3a, providing 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 [50]. Substrate surface morphology and chemistry also change during the incubation.

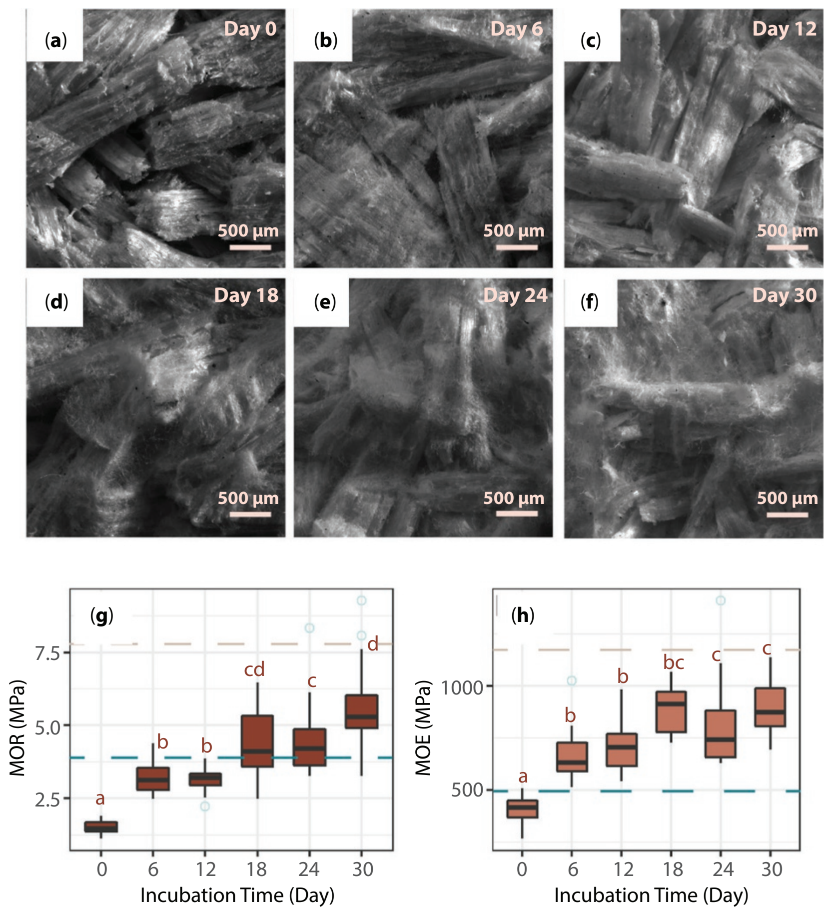

Figure 16.4 Extended depth of field (EDF) images of the internal surface of hot-pressed panels after internal bond (IB) strength test at day 0 (a); day 6 (b); day 12 (c); day 18 (d); day 24 (e); day 30 (f). MOR (g) and MOE (h) of hot-pressed bio-composite panels. Adapted from [31].

16.4.2 Hot-Pressed Panels

The adhesive performance of hot-pressed fungal mycelium-bonded panels is evaluated in most of the studies as modulus of rupture (MOR) and modulus of elasticity (MOE) using three-point bending tests. 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 16.4a-f). MOR and MOE increased with the density of mycelia, corresponding well with the microscopy images of the interfaces (Figure 16.4g, h).

Besides the density of the fungal mycelia, higher temperature during the hot-pressing process usually brings about higher MOR, which is attributed to the formation of more hydrogen and chemical bonds. 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 [8]. 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.

16.4.3 Engineered Living Materials

In engineered living mycelium-bonded structures, the fungi are alive, hydrated, and have the ability to respond to chemical, optical, tactile and other environmental stimuli [10, 51]. Because of the existence of a large amount of water, the mechanical properties of living bio-composites are much lower than the dried samples [49, 52]. Unlike dried mycelium-bonded foams, where mycelia are much more fragile than lignocellulosic substrates, the hydrated living mycelia improve shape-retention and flexibility in the wet state bio-composite. It seems that the presence of a dense and continuous exterior shell layer is essential. While these materials need to be kept moist while consolidating or healing, keeping them moist indefinitely will likely lead to colonization by other microbes. Therefore, they must either have relatively short lifespans or eventually be dried.

The safety profile of mycelium-bonded bio-composites is expected to be excellent, especially after a heat treatment to kill the fungus and evaporate any potential fungi-generated volatiles in the bio-composite. It must be considered that still living fungi, metabolites, and spore generation have the potential to create air quality issues beyond the production environment [53, 54].

16.5 Improvement Strategies

The exploration of bio-composites bonded by fungal mycelia is still in an early stage. Very few improvement strategies have been explored and they are all common strategies used in other lignocellulosic bio-composites.

16.5.1 Incorporating Natural Fibers

Adding natural fibers into the incubation mixture was effective at increasing the elastic stiffness of as-grown foams and prevented or reduced the occurrence of surface cracks [52]. Incorporating cellulose nanofibrils (CNFs) at 2.5% by weight in mycelium-bonded particleboards more than doubled the dry MOR and MOE [9].

16.5.2 Infusing Bio-Resin

Infusing the spaces between mycelia with bio-resin has been shown to greatly improve strength and stiffness [55]. It is still unclear how much of the improvement was due to additional adhesion from the resin vs. simply from filling voids.

16.5.3 Incorporating Natural Reinforcement Particles

Adding carbonate sand as natural reinforcement particles into the substrates obviously improved the compressive strength of the mycelium-bonded bio-composites. However, the density also increased significantly [56].

16.6 Prospects

Bonding with fungal mycelia is a unique, untraditional way of fabricating bio-composites. The resulting material is typically 100% Biobased and easily recyclable. The research on using fungal mycelia as an adhesive in bio-composites is still at a very early stage. The complexity of the system requires more investigations in multidisciplinary fields. The structure and distribution of the mycelia in the product could be better predicted and controlled by monitoring the fungal culture by species and strain selection, and gene modification. The substrate materials could be specially selected for specific applications. For example, insulation applications would favor lightweight and more porous substrates. Different fungi seem to prefer different substrates, complicating further development. Meanwhile, standardized testing procedures and requirements are needed to support future development of this material.

16.7 Summary

In this chapter, we reviewed the functionality and behavior of fungal mycelia as adhesives in various bio-composite systems. Fungal mycelia as adhesives are well positioned to contribute to the circular economy and the desire for higher Biobased content in materials. Because they are living, they are self-replicating and 100% Biobased. On the other hand, they also require much longer time to create the bonding, are more difficult to control, and their strength, especially wet strength, appears to be limited. Their use is complicated by the many fungal species, and their interactions with substrates, growth conditions, and time of harvest. Fungi also alter the substrate materials during colonization. Fundamental and systematic research is needed for better understanding and development of this unique material as well as its recyclability and potential health concerns.

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List of Abbreviations

MOR	Modulus of rupture
MOE	Modulus of elasticity
EDF	Extended depth of field
IB	Internal bond
FTIR	Fourier transform infrared
CNF	Cellulose nanofibril

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