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Insight into mycelium-lignocellulosic bio-composites: Essential factors and properties

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ABSTRACT

Mycelium-bonded bio-composites are promising new materials for replacing non-sustainable products. In such composite systems, fungal mycelia work as an adhesive, bonding together lignocellulosic substrate particles. In this work, we focus on the two groups of mycelium-bonded bio-composites: as-grown foams and hot-pressed (densified) panels. We used *Trametes versicolor* mycelium and yellow birch wood particles as a substrate and incubated the mixture for up to 30 days. We investigated the relationship between mycelium growth and essential end-use properties. We revealed that in as-grown foams, mycelial colonization does not significantly alter physical and mechanical properties but can reduce sound absorption. In contrast, increased mycelial density in hot-pressed panel products resulted in an increasing modulus of rupture, modulus of elasticity, and internal bond strength. In hot pressed panels, mycelia appear to act as an adhesive to bond particles forced into contact during compaction.

1. Introduction

The growing demand for sustainable materials to reach a circular bioeconomy has led to the increasing interest in all-natural materials to replace non-renewable resources in many applications [1]. As an abundant and diverse natural resource, lignocellulosic biomass is a major component in composite systems for a wide variety of applications, such as construction, furniture, and packaging. A range of products including foams, panels, and films can be produced from lignocellulosic building blocks such as pure cellulose, partially delignified fibers, whole fibers, and particles through various processing methods [2–4] where typically a synthetic or natural binder is used to adhere these particles together. Fungal mycelium is a new and unique natural binder that can both grow on and be incorporated into lignocellulosic bio-composite systems [5]. Filamentous fungi are known for their ability to colonize lignocellulosic biomass in nature. They grow a network of continuous hyphae, bind the biomass substrate particles together, and create a three-dimensional matrix [6]. After drying, the as-grown composite has a foam-like structure with a density normally ranging between 0.06 and 0.30 g cm^{-3}, depending on the substrate and processing method [7]. These foams have been commercialized as packaging and insulation materials to replace petroleum-based polymeric foams such as expanded polystyrene and polyurethane [8,9]. Pressing the as-grown mycelium foams can turn these lightweight composites into a higher-density fiberboard or particleboard-type panels without the need for additional petroleum-based adhesives [10–12].

Multiple mycelium-lignocellulosic composites have been marketed and discussed in the scientific literature [13–16]. However, the complexity of the system makes it difficult to link product properties to the contributions of major components of the system. Therefore, there are multiple conflicting explanations of structure-property relations in the literature. As the main component of the hybrid system, the lignocellulosic biomass strongly influences many composite properties. However, the hyphae of filamentous fungi play a key role in the system as well. These fungal components bind the individual substrate elements together by bridging adjacent biomass particles. The hyphae together, known as mycelium, are distributed throughout the system and serve as an additional phase [17,18]. The process of fungal degradation additionally modifies the chemical and physical nature of the biomass.

White-rot fungi feed on and therefore chemically modify the biomass

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substrate, resulting in reduced substrate mass, mechanical properties, and density [19], as well as increased porosity and water absorption [20]. The presence of fungal mycelia in the relatively large gaps between biomass particles reduces pore sizes and increases water retention by capillary action. Fungal hyphae also retain high amounts of water, and some species can transport water into the substrate [21], which may increase thermal conductivity and the risk of deterioration.

The highly complicated interactions among the substrate, fungal species, incubation conditions, and extent of growth have caused multiple confusing, and at times contradictory, claims in the literature regarding several important properties. For as-grown foams, the whole system was modeled by Islam *et al.* as a matrix (mycelia) reinforced by biomass substrate particles. They found that the mycelium matrix controlled the primary compressive behavior under small strains (<20%) while substrate particle size had no effect in this region, and the lignocellulosic substrate was responsible for stiffening at larger strains [22,23]. In contrast, Bruscato *et al.* reported that the compressive strength relates to the characteristics of the fungal mycelia, with leathery mycelia corresponding to a higher compressive strength in the final composite compared to fragile mycelia [24]. Elsacker *et al.* found that the samples with a smaller fiber size resulted in a higher compressive modulus [25]. In some studies, a hydrophobic surface mycelium layer on a composite resulted in less water absorption for the composite [8,25] whereas other studies reported that water absorption in a pure mycelial layer was much higher (>300%) [26] compared with lignocellulosic biomass alone. In fact, the surface hydrophobicity of mycelium does not influence the water absorption of the material because the hydrophobin protein can adapt its structure and change the surface to hydrophilic when coming in contact with water [16]. These conflicting observations might have been arisen from the difference in testing time and methods in various studies.

Conflicting information on mycelial biocomposites in the literature may also be due to the fact that there are not yet enough reports on mycelium-containing compressed panels to establish the essential factors influencing the properties of the system. In some papers, the growth of mycelium has been seen as primarily an approach to modify the surface chemistry of the substrate to achieve better bonding [11,27]. In others, the mycelium matrix itself has been pointed to as the main bonding and strength provider for panels [28]. If the latter is truly the case, understanding the link between the composite properties and structure and the properties of the hyphae is critical.

Our review of available literature shows that despite efforts to understand the contribution of mycelia and lignocellulosic substrate materials to the structure and properties of both the foam and panel-like composite systems, a systematic evaluation to elucidate governing factors is currently missing. Thus, this work aimed to further understand the principles that determine the performance of both as-grown mycelium-based foams and hot-pressed panels. To evaluate this dynamically changing system in detail, we focused on one substrate and one fungal species. We monitored the development of the physical and chemical structure of the composites by incubating the fungi for different periods. By comparing the essential properties of these composites, we attempted to link the changing system to its changing properties.

2. Material and methods

2.1. Materials

Yellow birch (*Betula alleghaniensis* Britt.) wood veneers with a thickness of 0.62 (±0.04) mm were kindly supplied by Columbia Forest Products LLC (Presque Isle, ME). *Trametes versicolor* was supplied by Ecovative Design LLC (Green Island, NY) and had been maintained on agar plates at 4 °C and was preincubated (28 °C, 80% relative humidity (RH)) on malt extract agar (MEA) plates before the incubation process.

2.2. Mycelium incubation

Wood veneers were ground into particles and sieved to a size between 0.5 and 2.0 mm. A sample of 225 g wood particles was poured into a filter patch bag and steam-sterilized at 121 °C for 60 min. One MEA plate of a 7-day preincubated fungal mycelium was mixed in 300 mL of 2% (w/v) sterile corn steep liquor (CSL) (Sigma-Aldrich, Saint Louis, MO) in a BagMixer (Interscience, St Nom, France) for 3 min after which the materials were transferred to the filter bag and were mixed with wood particles. The filter bags were incubated at 28 °C, 80% RH for 8 days, then the mixture was transferred to a stand mixer (KitchenAid, Benton Harbor, MI) with a paddle mixing blade and was mixed at speed 2 for 2 min. The mixture was then packed in square Petri dishes (80 mm × 80 mm × 14 mm) 50 g (dry weight: 19.8 (±1.3) g) per dish. The Petri dishes were incubated at 28 °C, 80% RH for up to 30 days.

2.3. Post-processing

After specific incubation periods, half of the samples were oven-dried for 48 h at 50 °C to produce as-grown foams. The other half of the samples were hot-pressed using a laboratory press (Carver, INC., Wabash, IN) to produce high-density panels. The hot-pressing process was conducted at 180 °C for 8 min with a thickness control of 4 mm using metal stops (pressure was not controlled during the process). The relatively high temperature and longer time were selected due to the high moisture content of the samples, which might be a limitation for scaled processing as most synthetic adhesives used in the industry for current commercial panels cure at lower temperatures (120 – 140 °C). The foams and panels were cut into various specimens using a laser cutter (Full Spectrum Laser LLC, NV). For foams, samples with dimensions of 25 mm × 25 mm × 13 mm were used for density, porosity, moisture content, water absorption analysis, and compressive strength analysis whereas samples with a diameter of 50 and 30 mm and thickness of 13 mm were used for thermal conductivity and sound absorption measurements, respectively. Incubation ranged from zero to 30 days. No Day 0 foam samples were tested as they were assembled right after mixing and there was not enough bonding between the particles to retain the shape. For panels, samples with dimensions of 25 mm × 25 mm × 4 mm were used for density, porosity, moisture content, water absorption, and thickness swelling analysis; samples measuring 25 mm × 80 mm × 4 mm were used for three-point bending and 20 mm diameter circular samples were used for internal bond strength. All samples were conditioned at 23 (±2) °C and 50 (±2) % RH for at least one week before testing. Parts of the foam samples were also milled into fine powders using a coffee grinder for chemical analysis.

2.4. Density and porosity

The bulk density (ρ_b) of the composites was determined by measuring the mass of the conditioned samples divided by their geometric volume. Six replicates were used in each group.

The true density (ρ_t) of the composites was measured with an AccuPyc II pycnometer (Micromeritics, GA) after drying the samples at 103 °C for 24 h. The porosity value was calculated using the following equation:

$$\text{Porosity} = \left(1 - \frac{\rho_b}{\rho_t}\right) \times 100 \qquad (1)$$

2.5. Light microscopy

Images were captured using a Nikon Ni-E (Nikon Instruments Inc., Melville, NY) upright microscope with Nikon Plan Fluor 4×/0.13 objective lens. The interior surfaces of both foams and panels were observed by the microscope. The foams were broken to expose the interior surface, and for the panels, failure areas were examined after the

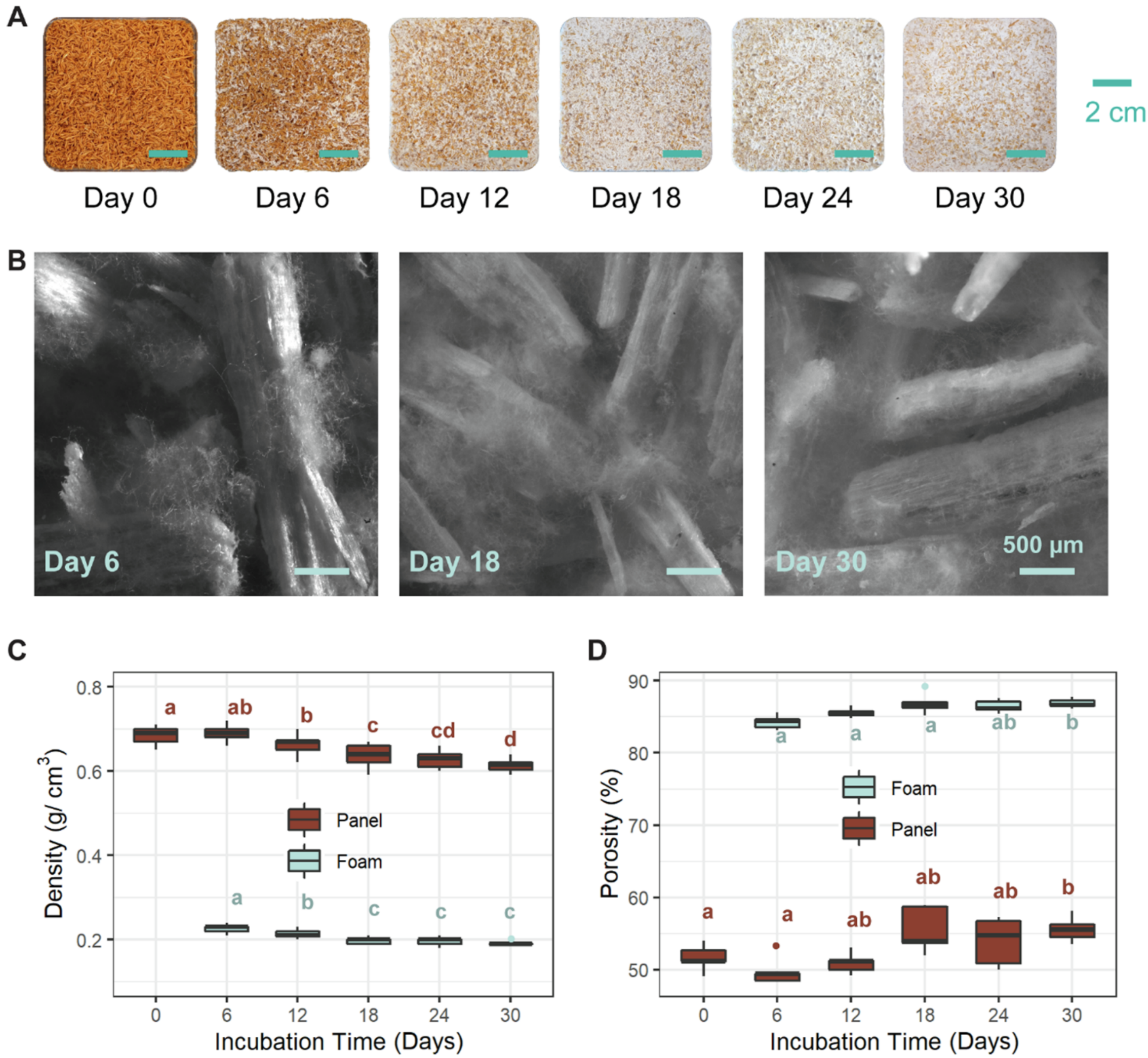

Fig. 1. Changes in the appearance and properties of the incubation system after different incubation times: (A) development of fungal mycelium in the substrate; (B) EDF images of the internal surfaces of as-grown foam; (C) density changes of as-grown foams and compressed panels; (D) porosity change of as-grown foams and compressed panels. Within each group, common letters indicate no significant difference at 95% confidence level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

internal bonding strength tests. Z-stack images were acquired at 30 µm intervals for 1.5 mm and processed using the extended depth of field (EDF) plugin in the NIS-Elements software.

2.6. Moisture content, water absorption and thickness swelling

The moisture content, water absorption and thickness swelling were measured according to ASTM D1037-12 with modifications of the sample size and duration of soak. For water absorption and thickness swelling analysis, the samples were immersed in distilled water in room temperature (23 ± 2 °C) and the weights and thicknesses were measured after 2, 24, 72, 144, and 288 h. The water absorption and thickness swelling values were determined from the weight or thickness difference in relation to initial weight or thickness. Twelve replicates were used in each group.

2.7. Mechanical properties

For foams, compressive strength was measured according to ASTM C165-17 [29]. For panels, modulus of rupture (MOR), modulus of elasticity (MOE) and internal bond strength (IB) were determined according to ASTM D1037-12 [30]. All mechanical tests were performed with an Instron 5942 universal testing machine (Instron, Norwood, MA) with a 500 N capacity load cell. For the compression testing, each piece was compressed up to 40% deformation at a rate of 1 mm min^{-1}. For shape-recovery measurements, after the samples were compressed to 40%, the load was released, and the thickness recovery was measured immediately and after 24 h. For three-point bending, samples were tested using a span of 70 mm and a cross-head speed of 3 mm min^{-1}. For internal bond, circular specimens were tested at a cross-head speed of 0.4 mm min^{-1}. Between 12 and 18 replicates were tested in each group.

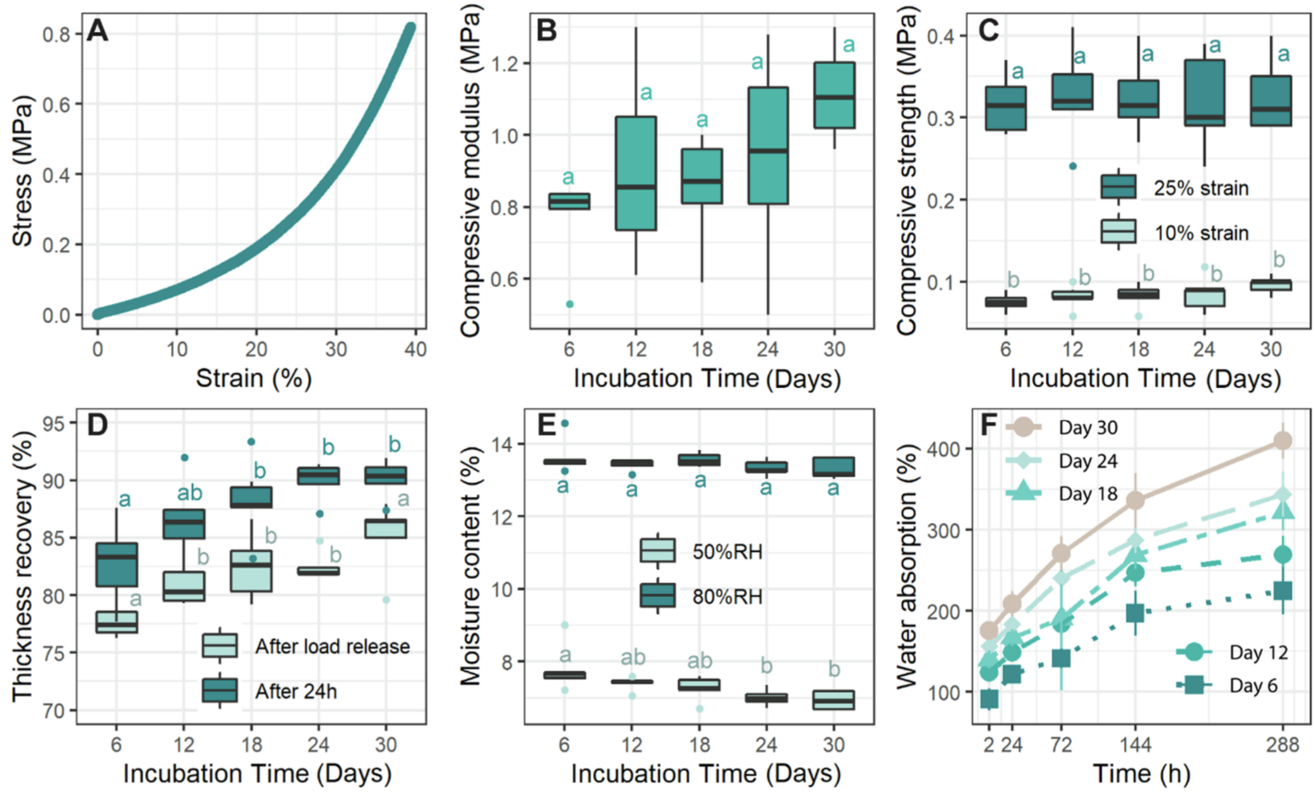

Fig. 2. Essential properties of as-grown composite foams: (A) representative stress-strain curve; (B) compressive modulus (elastic region, < 5% strain); (C) compressive strength at 10% and 25% strain (D) thickness recovery after 40% compression, immediately and after 24 h; (E) moisture content at 50% RH and 80% RH; (F) water absorption. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.8. *Thermal conductivity*

Thermal conductivity values of foams were measured using a TA Fox50 heat flow meter (TA Instruments, New Castle, DE) according to ASTM C518-17 [31]. Temperature gradients of 10 °C to 30 °C and 20 °C to 40 °C were used, and the average of the two gradients was reported. Six replicates were tested for each group.

2.9. *Acoustic properties*

Sound absorption measurements were conducted using an impedance tube BSWA-III-C021-03-0027-IMP (BSWA Technology, Beijing, China) according to ASTM E1050-19 [32]. Foam samples were tested in triplicate. Sound absorption coefficients were reported for the frequency range from 1000 to 6000 Hz.

2.10. *Statistical analysis*

Data were analyzed using one-way analysis of variance (ANOVA) to determine statistical differences between the means. A Tukey's honestly significant difference (HSD) multiple comparison test was then performed to further assess the significance level of the mean values for each treatment level. All comparisons were made at 95% confidence level. All the analyses were performed using RStudio (RStudio.com, Version 1.2.5033).

3. Results and discussion

3.1. *Growth of mycelia on the substrate*

The development of fungal mycelia in between the substrate particles is shown in the series of photos in Fig. 1A. The white mycelial tissue is easy to distinguish from the brownish wood particles. The growth of fungal hyphae gradually expanded within the wood particles and by Day 12 had covered most of the particle on the surface. From Day 12 to Day 18, the aerial mycelia grew thicker and denser and further whitened the surface. There was no apparent visual difference between Day 18, Day 24, and Day 30. The EDF images taken from the inner surface of the foam (Fig. 1B) show a similar trend, with some mycelium clusters attached to the particles on Day 6, to those fully occupying the space between the particles on Day 18 and Day 30 (Fig. 1B). Unlike the significant difference in hyphal density between the core and the surface of the final composite reported by other research groups [28,33], in our growing system, the substrate was fully packed and attached to the Petri dish, and the size of the particles was relatively large to ensure minimal oxygen availability difference between the surface and the core. The cross-section picture shown in Figure S1 reveals that the growth of mycelium was uniform across the whole composite.

Fig. 1C and 1D show the changes in density and porosity of the directly oven-dried foam and hot-pressed panels. The density of both foams and panels generally decreased with colonization time. However, if there were density differences among Days 18, 24, and 30, they were not statistically significant, which corresponded well with the visual changes in Fig. 1A. While colonizing the substrate, fungi utilize the substrate as a food source and gain energy from the respiration of organic compounds [34]. Therefore, the unchanged density after Day 18 directly relates to the unchanged weight, as the difference among the volume shrinkage of samples incubated for different days (thickness and volume difference among different groups shown in Figure S2A and B) was negligible. Three factors influence the weight change of the composite: the loss of the substrate, increase of fungal mycelia body, and metabolic conversion of biomass to CO_2 and H_2O. If the total dry mass does not change, the fungus must have stopped growing. By continuing to incubate some samples for longer times, we confirmed that the fungus did not stop growing (the density of the Day 42 foam sample decreased to 0.17 ± 0.01 g cm^{-3}). We further confirmed this by comparing the

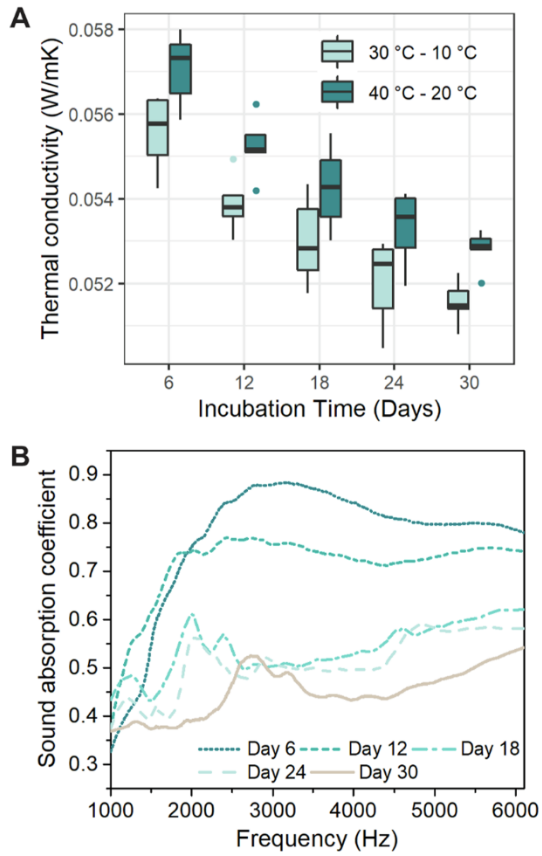

Fig. 3. Thermal conductivity (A) and sound absorption coefficient (B) of as-grown foams. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

chemical features of the composites using ATR-FTIR and TGA analysis (Figure S3). The FTIR curves and peak ratios also show that the fungus preferably decayed the hemicellulose fraction over cellulose [35,36] (details in Supporting information). The results from TGA analysis (Figure S3 B and C) revealed a trend similar to the observations made in Fig. 1. It appears that the physical and chemical features of composites fell into two major groups: Day 0 and Day 6 and Day 18, 24, and 30. Day 12 appeared to be a transition state between the two groups.

3.2. *Essential properties of as-grown foams*

As described earlier, as-grown mycelium-lignocellulosic foams were specifically developed for packaging [37], thermal insulation [38], and acoustic absorption [39] applications. Our data for mechanical and physical properties related to these applications are presented in Figs. 2 and 3.

The typical stress-strain response (Fig. 2A) of the as-grown composite foams under uniaxial compression revealed that it was linear below about 10% strain (compression). Similar to what other researchers have reported for low density mycelium-lignocellulosic biomass composites [22,40], stiffness gradually increased with compaction. We expect this is a result of increasing particle-particle contact with densification. Even though the mycelium concentration increased significantly between Day 6 and Day 30 (Fig. 1A and 1B), the trend toward increasing initial stiffness over this period was not statistically significant. This suggests that the initial compressive modulus of mycelium foams are largely independent of mycelium matrix density.

According to Islam *et al.* [22], both experimental and modeling data concluded that the linear region is primarily controlled by the mycelium matrix. Although wood particles still form the bulk of the system, there are likely few in direct contact with each other leading to a lag before a stiffening is observed. As shown in Figure S4A, the specific compressive modulus did show a slight but significant increase over incubation time, suggesting that the hyphae contribute to stiffness. Similarly, while there was no significant difference in compressive strength at either 10% or 25% strain (Fig. 2C), the specific compressive strength shown in Figure S4B revealed an increasing trend at 10% strain, before particle-particle interactions overwhelm the contribution from hypha. Although the density of the composites decreased with longer incubation, the compressive strength remained unchanged, indicating an improved composite structure with more mycelia matrix. The upward trend in compressive modulus, though not statistically significant, also suggests that hypha contribute to stiffness at low deformations.

Thickness recovery after 40% compression (important for packaging) increased significantly over the course of incubation, reaching as high as 90% after 24 h for samples incubated more than 12 days (Fig. 2D). This is likely a result of the interconnected network of mycelia pulling the composite back to its original shape, but the role of substrate chemical modification cannot be ruled out.

Substrate degradation by the fungi may also cause changes in the equilibrium moisture content, which may cause changes in other properties. The moisture contents of the foams were generally small or not significantly changed with incubation time (Fig. 2E). Considering that the mycelia continued to develop throughout the growing period, their influence on moisture content is interesting. As listed in Table S3, the moisture content of pure mycelium was 11.5 ± 1.3 % at 50% RH and 19.5 ± 2.3% at 80% RH, which was higher than corresponding values for the as-grown foams. This may have been compensated by the selective removal of hydrophilic wood components during the early stages of decay. In contrast, after immersion, more water was retained in samples incubated longer (Fig. 2F). One reason for the increase in water absorption (i.e. water retention upon removal from a bath) is that the large gaps between wood particles were divided into smaller ones by the hyphal network, which was much better at retaining water through capillary action. Pure mycelium can retain 1579 ± 390% of their dry weight as water after full immersion (Table S3), which is more than three times higher than Day 30 as-grown foams [41]. Also, fungal colonization is known to increase the bulk flow of water through wood, potentially resulting in more water being taken up inside the wood particles [19].

Fig. 3A shows that the thermal conductivity of the foam composites decreased with longer incubation. This is reasonable, as thermal conductivity correlates strongly with the density in porous materials. The lower the density, the higher the quantity of air, which has an extremely low thermal conductivity and corresponds to the lower thermal conductivity of the foam. The 30 days of degradation only caused a density reduction of about 10% and a thermal conductivity reduction of less than 10%, indicating that fungal degradation is not an efficient approach to achieve composites with low thermal conductivity. It would therefore be more efficient to choose a substrate with naturally low thermal conductivity or modify the construction method to lower the density of the foam structure.

Fig. 3B shows the sound absorption coefficient of the foams in the range of 1000–6000 Hz. For all samples, the peak values of the sound absorption coefficient curves were all above 0.5. Interestingly, the sample with the shortest incubation showed the best sound absorption. The sound absorption coefficient generally increased with sound frequency. For the Day 6 foam, the maximum absorption coefficient was 0.87 at 2800 Hz, but they were all above 0.8 at higher frequencies. With the increase of incubation time, the sound absorption decreased. The Day 12 sample showed a similar trend as Day 6 sample with a lower peak (0.76). Day 18, 24, and 30 samples showed a much lower coefficient in the majority of the frequencies above 1500 Hz. The highest values were 0.61, 0.56, and 0.53, respectively. Multiple small peaks also appeared at different regions that were different from Day 6 and Day 12, which only had one major peak. As shown in Fig. 1C and 1D, the density of Day 18,

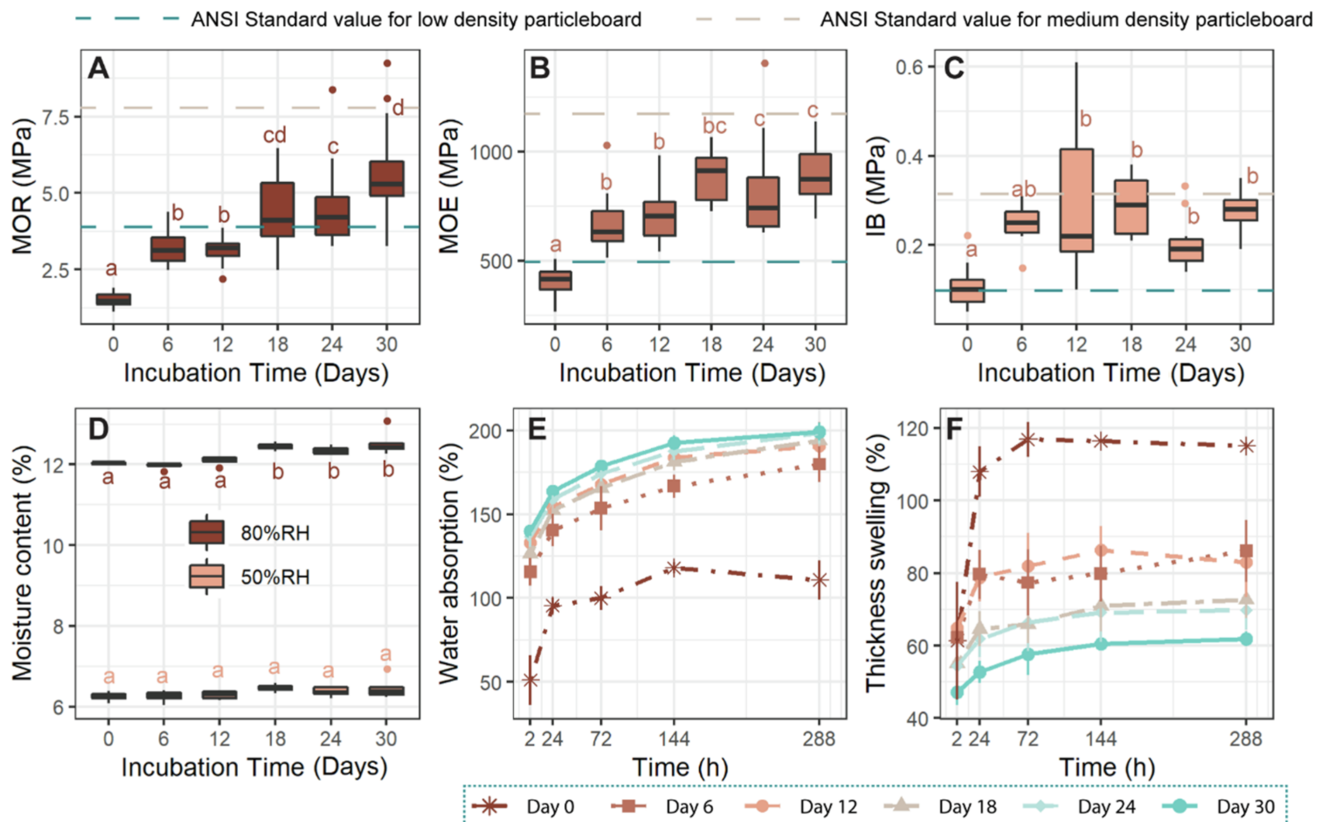

Fig. 4. Essential properties of hot-pressed composite panels: (A) MOR; (B) MOE; (C) IB; (D) moisture content after conditioning at 50% RH and 80% RH; (E) water absorption; (F) thickness swelling. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

24, and 30 samples were lower than Day 0 and Day 6 samples, and the porosity was higher as expected.

The changes in sound absorption coefficient during incubation may have been caused by the structural changes at the microscopic level such as tortuosity, airflow resistivity, viscous, and thermal characteristic lengths [42]. At the beginning of the incubation, when the mycelium did not fully colonize the large spaces in between wood particles, the larger pores permitted more air vibration within the sample. The subsequent growth of mycelium narrowed down the air channels at the inter-particle scale, consequently reducing the pore diameter gradient and mean pore size and thus causing a lower air viscosity ramp during the movement of sound energy [43]. At the same time, it has been previously found that both mycelium-bonded composites and pure mycelium foams showed improvement of sound absorption at lower frequency range [39,44]. We also found a similar trend in Day 12, 18, and 24 samples, but it was combined with the substantial reduction of sound absorption at a higher frequency range. To achieve a higher sound absorption coverage at all frequencies, one approach could be a hybrid system composed of different materials.

3.3. Essential properties of hot-pressed panels

The most important physical and mechanical properties of hot-pressed panels made from wood particles and mycelium are shown in Fig. 4. Both MOR and MOE reveal a similar trend (Fig. 4A and B). The Day 0 sample showed very low values; Day 6 and Day 12 samples showed improved MOR (~3 MPa) and MOE (~700 MPa). Day 18 samples improved further to 5 MPa and 900 MPa, respectively. Further incubation did not cause any further change. However, this trend is not as obvious in the IB test result. The IB values are constant at ~0.3 MPa from Day 6 to Day 30. Half of our processed hot-pressed panels (Day 18, 24, and 30) are considered low-density (<0.64 g cm^{-3}), according to ANSI A208.1-2016 [45]. As shown in Fig. 4, their mechanical properties are notably higher than the required value for low-density panels, relatively close to the value for medium-density particleboard. With an increase of density or the inclusion of other components such as face and back layers, these composites have potential as medium-density panels for specific applications such as home and office furniture. It should be mentioned that even if longer incubation time leads to better bonding and higher mechanical properties, it also causes more loss of wood (Fig. 1C) which is not favorable. Therefore, a shorter time that would lead to sufficient properties is preferred.

Moisture content, water absorption, and thickness swelling properties of the panels are shown in Fig. 4 D, E, and F, respectively. Similar to the foams, the moisture content values remained relatively unchanged during incubation, but with more mycelium growth, more water was absorbed (retained) in the panels. The hot-pressing process compressed the panels and decreased the space between particles for water to reside, resulting in less water absorption for the panels than comparable foams. The thickness swelling values of the panels show an opposite trend: longer incubation produced lower swelling. This indicates that the fungi improve bonding during hot-pressing, as the longer-incubated samples were better able to resist moisture-induced swelling forces.

Mycelium incubation can reduce wood MOE, MOR, and density. At only 2% weight loss in hardwood, white-rot fungi may typically cause a decline of ~13% in MOR and ~4% in MOE [46]. Interestingly, the decreased wood quality and density did not appear to harm the mechanical properties of the panels. The improvement of inter-particle adhesion may compensate for the change in wood quality and the reduction of wood particle density and stiffness can improve bonding by providing a higher compaction ratio. The broken surface of the panels after IB test are shown in Fig. 5. On Day 0 (Fig. 5A), when there was almost no mycelial growth, the surface texture of the wood particles is clear and there were many unoccupied spaces between individual wood

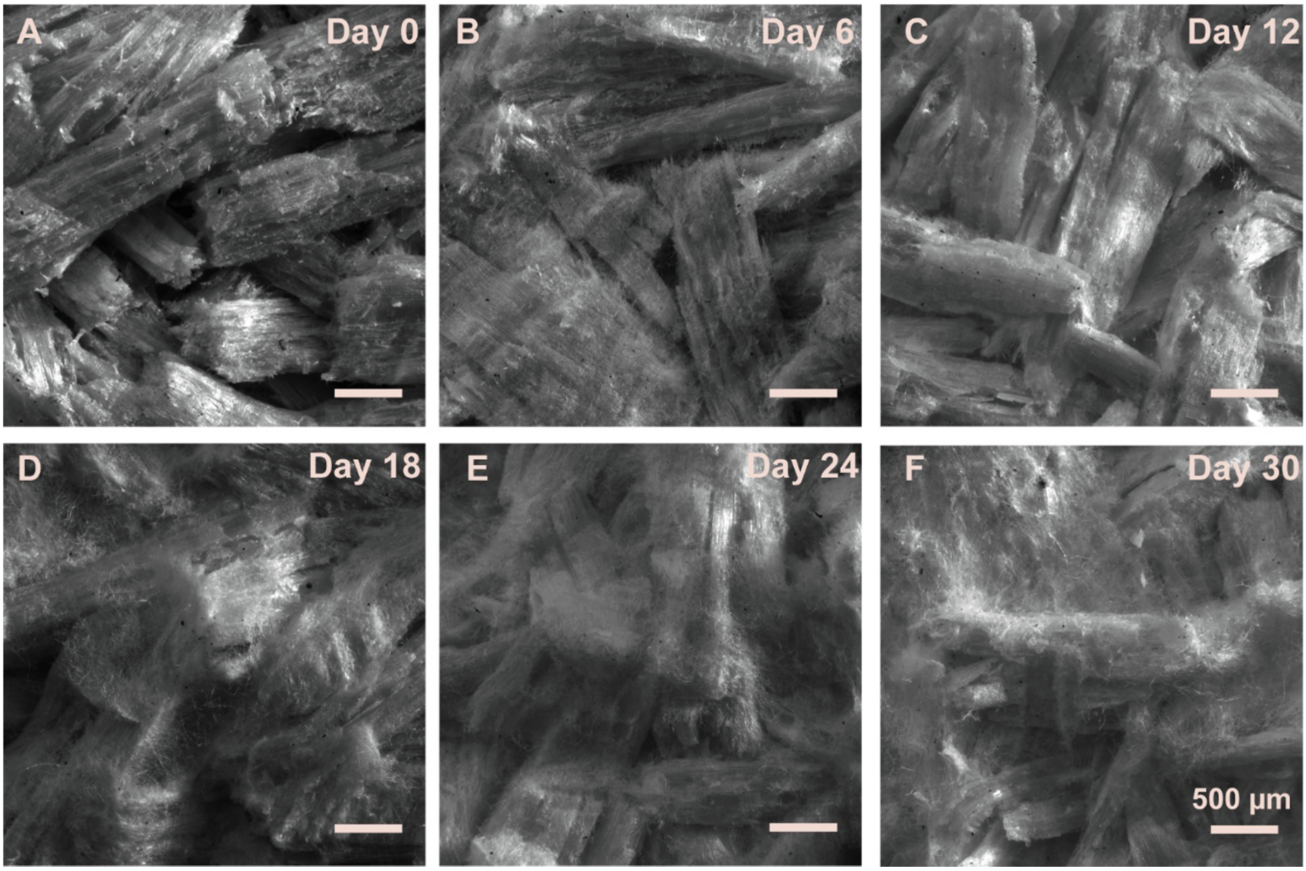

Fig. 5. EDF images of the internal surfaces of hot-pressed panels after IB test (A) Day 0; (B) Day 6; (C) Day 12; (D) Day 18; (E) Day 24; (F) Day 30.

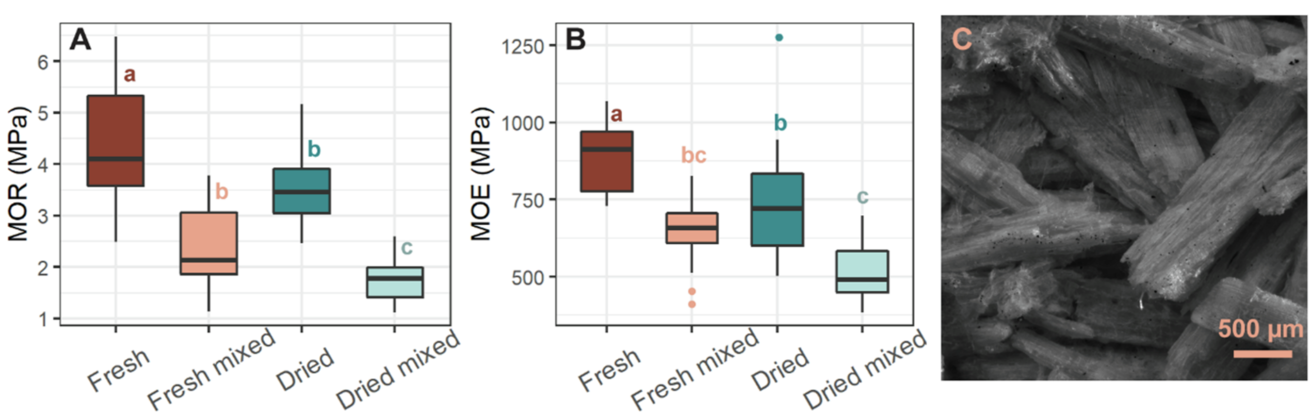

Fig. 6. Essential properties of Day 18 hot-pressed panels with several pretreatments before hot-pressing: (A) MOR; (B) MOE; (C) EDF image of the internal surfaces of hot-pressed panels after IB test: Day 18 "Fresh mixed" group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

particles. With mycelium growth, voids were filled with entangled mycelium (Fig. 5B, C, D). The mycelium fully covered the interface on Day 18 (Fig. 5E) so that the texture of the wood surface can be barely seen. The microscopy images correspond well with the mechanical property results, showing how fungal hyphae worked to bind the particles together. Our previous work on the functionality of surface mycelium on wood bonding has shown that the chemical components (fungal biofilm and degraded small molecules of wood components) located at the mycelium and wood substrate interface play a role in bonding [47]. This mechanism may also be a factor in the composite system and provide inter-particle bonding.

To confirm the reinforcing functionality of the mycelium network and the bonding chemistry at the mycelium-wood interface, we conducted the following tests on Day 18 samples before hot pressing them: (1) break up the mycelium network with a mixer, (2) oven dry to "pre-cure" the potential interface adhesives, then add the water back into the mixture before pressing, (3) both mix and dry. Fig. 6A and B show the MOR and MOE values of the control and three test groups. The mechanical properties decreased both after mixing and after pre-drying. The mixing step destroyed the connections between the wood particles caused by the mycelium so that the voids in between wood particles were again present (Fig. 6C). Seen another way, the web of hyphal strings connecting particles was broken. The drying process can reorient and aggregate the proteins and polysaccharides located at the interface between wood and mycelium and create hydrogen bonding. Although the dried group maintained the mycelium connections, its MOR and MOE still decreased to a level similar to the "fresh mixed" group (Fig. 6A, B). The group ("dried mixed"), subjected to both mixing and drying, had the lowest mechanical properties. These observations confirm the contribution of the two essential components connecting wood particles: mycelium and mycelium surface active components. It is also worth mentioning that although the mechanical properties of "fresh mixed" and "dried" groups were significantly lower than the "fresh" group, they are still much higher than the ANSI standard value for low density particleboard [45]. Even the weakest "dried mixed" group has been shown to reach the standard ANSI value by incorporating 2.5% cellulose nanofibrils (CNF) [11]. These findings offer flexibility while producing hot-pressed panels. Multiple starting materials can be processed in specific ways to reach different application requirements.

4. Conclusions

Fungal mycelia behave differently in different forms of bio-composites. For the low-density as-grown foam, the primary effect of fungal mycelia seems to be to bind the particles together, with little impact on mechanical properties. The lignocellulosic substrate and gaps between particles played an essential role in the sound absorption and thermal insulation properties of the foam, as a denser mycelium structure negatively affected these properties. In the higher-density hot-pressed panel system, the fungal mycelia contributed to bonding both through the network of hyphae directly connecting individual particles and as an adhesive, with bonds formed during the hot press. ANSI minimums for MOE, MOR, and IB were attained by low-density hot-pressed panels and in some cases came close to the standard for medium density panels. Future mycelium-lignocellulosic based bio-composite development should include developing much higher strength and water resistance by tailoring the substrate, growing time, and processing method to the required applications in both initial design and production. Overall, our findings help to understand the development of a wide variety of mycelium-based bio-composite properties and can facilitate current and future commercial developments.

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CRediT authorship contribution statement

Wenjing Sun: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Mehdi Tajvidi:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Caitlin Howell:** Resources, Writing – original draft, Writing – review & editing. **Christopher G. Hunt:** Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.compositesa.2022.107125.

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