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Advancing mycelium-based composites: Integrating strength optimization and porosity control for alternative construction materials

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ABSTRACT: Mycelium-based composites (MBCs) provide a sustainable alternative to fossil-based materials, combining lignocellulosic substrates and fungal mycelium to form lightweight structures. While their thermal properties are well-studied, mechanical performance remains underexplored. This study aims to enhance MBC compressive strength (σC) and elasticity (E) by minimizing porosity (ϕ) and refining fabrication techniques.

A novel methodology integrates gas pycnometry for non-destructive porosity (ϕ) analysis and uniaxial compression tests to assess mechanical properties. Using *Ganoderma lucidum* and various wood substrates, the study explores incubation periods, particle sizes, and wood-to-wheat bran ratios. Results show denser composites improve σC and E, with beech-based MBCs performing best. Surprisingly, poplar-based composites maintain integrity due to a mycelial "jacketing" effect. Sawdust substrates outperform wood chips, emphasizing substrate refinement's role.

This research advances MBC optimization, offering eco-friendly construction alternatives. Future studies should explore scalability and refine fungal strains for improved mechanical properties.

1 INTRODUCTION

Mycelium-based composites offer a sustainable alternative to fossil-based construction materials (Manan et al., 2021, p. 0; Özdemir et al., 2022; Rossi et al., 2024) and provide benefits like sound absorption and thermal insulation. They are produced using lignocellulosic substrates and filamentous fungi growth (Carlström et al., 2024; Haneef et al., 2017; Islam et al., 2017; Lelivelt et al., 2015) with densities ranging from 80 to 600 kg/m³ that resemble foam-like cellular materials such as cork (Jones et al., 2020, 2018); their mechanical properties are largely influenced by density and porosity, where porosity (Φ) represents the pore space fraction expressed as a percentage (0–100%) or as a fraction (0–1) (Ghazvinian and Gürsoy, 2022; Gibson and Ashby, 1997) and skeletal density (ρskeletal) is defined as the material's mass divided by its pore-free volume, with denser materials generally exhibiting lower porosity and improved mechanical performance advantageous for load-bearing applications; this study explores the correlation between porosity and mechanical performance in mycelium composites, initially focusing on developing a non-destructive method to measure skeletal density and porosity and examining growth conditions to minimize porosity using various lignocellulosic substrates and fungal strains, while previous research involving *Ganoderma lucidum* (GL) with

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beech wood—a high-quality hardwood rarely available as construction waste—suggests replacing beech with fast-growing softwoods like Spruce and Douglas fir, which are abundant as waste, in order to correlate porosity with key mechanical properties, particularly compressive strength (σC) and elasticity (E).

2 MATERIALS AND METHODS

2.1 *Wood particle characterization*

During the research phase, we used two hardwood and two softwood substrates in two particle sizes for growth tests. Particle size analyses were conducted using a sieve tower ("DIN 66165-1," n.d.) and static image analyses, and the results were compared. The wood types included beech, Douglas fir, poplar, and spruce, with beech divided into fine (1–2.5 mm) and coarse (2–4 mm) particles, Douglas fir into fine (2–8 mm) and coarse (8 mm) particles, poplar into fine (0.2–0.3 mm) particles, and spruce into fine (2–8 mm) and coarse (8–16 mm) particles. We employed a substrate preparation and cultivation protocol established in previous work and combined the wood-based substrates with nine fungal strains: *Fomes fomentarius* (FF), *Fomitopsis pinicola* (FP), *Ganoderma applanatum* (GA), *Ganoderma lucidum* (GL), *Hericium erinaceus* (HE), *Marasmius ssp.* (MS), *Phanerochaete chrysosporium* (PC), *Pleurotus ostreatus* (PO), and *Trametes hirsuta* (TH). Radial growth tests were performed to identify rigid network-forming combinations, using the standard GL-beech combination as a reference. Five Petri dishes of sterilized substrate for each wood type were inoculated centrally with liquid fungal pre-culture and grown at 24°C for fifteen days, with the reference GL-beech combination using a 10:1 wood to wheat bran ratio. To optimize growth conditions for different substrates and fungal strains, we conducted an agar growth test with two wood-wheat bran (WB) ratios (10:1 and 2:1) replacing potato dextrose agar (PDA) (Sisti et al., 2021). The mixtures were combined with deionized H_2O for the Petri-dish pre-cultures, with five tests per substrate combination, incubated at 24°C for fifteen days. For cylindrical growth tests, the liquid pre-culture replaced potato dextrose broth (PDB) with finely ground wood and WB in a 2:1 ratio mixed with deionized H_2O, and the cultures were incubated in a shaker at 24°C for twelve days.

2.2 *Preparation and cultivation of specimens*

During specimen cultivation, substrates were prepared using 2:1 and 10:1 ratios of finely chipped wood to WB, along with a 5:2 ratio for the D-based substrate (Sisti et al., 2021). The different wood-to-WB ratios were used to assess WB's influence on the cultivation period before growth formation. The cylindrical forms were filled with 41.5–64 grams of substrate, depending on the wood type. Finally, the samples were incubated for four weeks (or as indicated) and then denatured after incubation at 70°C for 24 h.

2.3 *Porosity determination methodology*

Different cultivation periods were analyzed to develop a robust density measurement and porosity calculation methodology. Since this research is based in Europe, we adhere to European standard norms. Standard-sized cylindrical forms (h = 69 mm, d = 45 mm, according to DIN EN 826:2013 ("DIN EN 826," n.d.)) were grown using a beech (B) substrate and *Ganoderma lucidum* (GL) fungal strain. The specimens' density was measured with an *Anton Paar Ultrapyc 5000* Gaspycnometer following DIN 66137-2 ("DIN 66137-2," n.d., pp. 66137–2). This method replaces the classical liquid displacement technique with inert gas—Nitrogen (N) in this case—allowing the gas to penetrate the specimen's smallest pores for more accurate results. Ultrapyc's chambers ensure precision by selecting a cell size based on the sample, with the pycnometer automatically using the corresponding reference chamber volume. The sample chamber is pressurized with the inert gas until a stable pressure is reached, then a valve opens and the gas expands into an equal-volume reference chamber. The pore-free volume and

skeletal density are calculated from the pressure drop (Baumgartinger, 2023). A reproducible protocol for both calibration and measurement was established, as outlined in Figure 1, to measure the specimens' pore-free volume and skeletal density.

The Φ can be calculated from the ρ_{bulk}, and $\rho_{skeletal}$ with the following equation (1) (Baumgartinger, 2023):

$$\Phi = 1 - \left(\frac{\rho_{bulk}}{\rho_{skeletal}}\right) \tag{1}$$

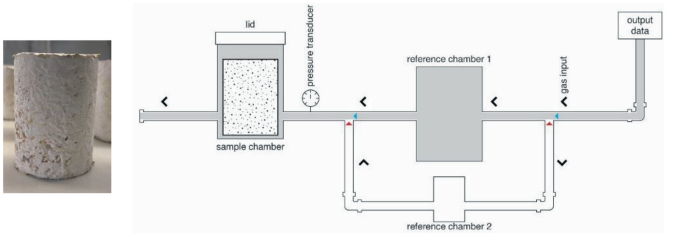

Figure 1. Outline of the density measurement process. Left: Cylindrical specimen [Z1]. Right: measurement process with the cylindrical specimen in the sample chamber.

2.4 *Mechanical tests*

This research phase assessed the mechanical properties under load, including the compressive strength and the elasticity of the selected specimens with various fungal-substrate combinations. Besides this, part of the investigation aimed to analyze the correlation between the material's compressive strength (σ_c), and the determined porosity according to the outcomes of the material tests. The compressive strength was tested for the cylindrical specimens cultivated with the GA+S, GL+B, GL+D, GL+P, and GL+S combinations according to DIN-EN 826:2013. The tests were carried out on a 50 kN universal testing machine as a uniaxial compression test, displacement-controlled at a speed of 10 mm per minute. For each test, the force-displacement curve was recorded, and the stress-strain curve was calculated from it.

3 RESULTS

3.1 *Screening of various fungal-substrate combinations to broaden composite fabrication*

This study investigated the potential for expanding fungal colonization and wood modification processes from the established GL-beech specimens developed in our previous research to include alternative wood types, particularly softwoods. The initial focus on GL-beech provided a robust model for fungal-wood interactions with desirable material properties. However, to diversify the application potential of MBC, we explored the compatibility and efficacy of softwood substrates. Our objective was to assess fungal adaptability and colonization efficiency across these wood types, aiming to establish protocols for broadening the range of fungi and lignocellulosic substrates for MBC.

The growth behavior of the different fungal-substrate combinations was observed through radial growth test (examples with two strains depicted in Figure 2), and rigid network formation have been identified manually after drying. From nine tested fungal strains GL, GA, FF, and TH strains exhibit sufficient mycelium growth and connectivity to the beech substrate. The GL and FF combinations show sufficient growth but weaker connectivity when combined with D and S substrates. These variations in growth and network formation can be attributed to the different compositions of cellulose, hemicellulose, and lignin in the substrates. Since hemicellulose, along with lignin, serves as a primary nutrient source for white-rot fungi, their higher content enhances enzymatic activity and promotes mycelial growth (Blanchette, 1991; Kirk and Farrell, 1987). To

optimize conditions on wood substrates other than beech towards sufficient growth, different wood-wheat bran (WB) ratios (Sisti et al., 2021) have been examined. Only the fine substrate geometries were utilized during this test because the first growth test results showed insufficient mycelium growth on the substrate with coarse particle size. The agar growth tests were analyzed with those four fungal strains (GL, GA, FF, and TH), which performed the fastest and strongest growth on the D, P, and S substrates during the radial growth tests.

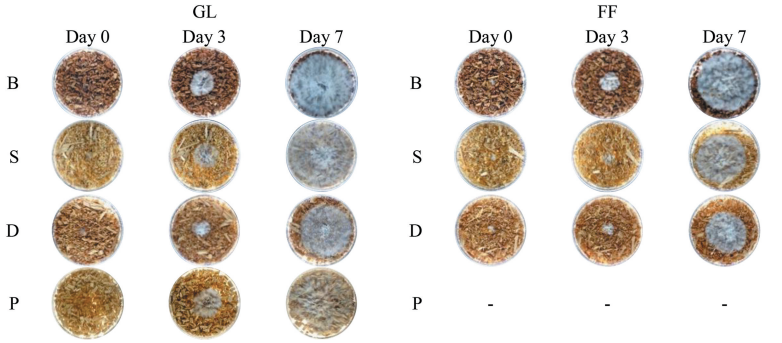

Figure 2. Examples of radial growth tests with various fungal-substrate combinations. (GL) Ganoderma lucidum; (FF) Fomes fomentarius; (B) beech; (S) spruce; (D) Douglas fir, (P) Poplar.

3.2 *Fabrication of specimens with different fungal-substrate combinations*

Based on the outcomes of this investigation, the substrate mixtures for the specimen cultivations were inoculated with FF, GA, and GL strains. The pre-cultivation of the different ratios of wood-WB combined with three fungal strains showed that the higher WB content usually results in a shorter incubation period. Figure 2 compare the pre-cultivation periods of the various fungal-substrate combinations.

The five fungal-substrate combinations shown in Table 1 resulted in cylindrical specimens suitable for conducting compression tests (see Section 3.3). None of the combinations with FF, and GA, strains led to appropriate specimens, while the GA strain exhibited sufficient growth only when combined with a S-based substrate. The cylindrical sample growth experiments indicated that the GL is the most suitable for material cultivation, as all substrate mixtures inoculated with this fungal strain yielded stable, suitable samples for further material tests.

3.3 *Assessment of skeletal density, skeletal volume, and porosity of tests conducted with different fungal-substrate combinations*

The cylindrical test specimens were initially used to measure the skeletal density, skeletal volume, and porosity of the fungal-strain combinations presented in Table 1. Since the method applied for these measurements was non-destructive, the same cylindrical specimens were subsequently employed for the compression tests outlined in Section 3.4.

In the first step to developing a robust methodology for the density measurement and porosity calculation with Anton Paar Gas pycnometer, we assessed the standard combination of beech substrate and GL fungal strain. Subsequently, the skeletal density measurements and porosity determination of the different fungal-substrate combinations were conducted according to the established protocol. In addition to the combinations with D, S, and P with different WB proportions, the combination of GL+B, and GL+S with PDB were also plotted. The measurement data shows that the growth conditions, especially the amount of the utilized nutrient sources (PDB, wheat bran), significantly influence the substrate's pre-cultivation, skeletal density, and porosity of MBC. The cultivation of material with reduced easily accessible sugars led to higher skeletal density and lower porosity values. Beyond this, the type of wood used as a substrate also affects the resulting material's properties. While beech is a hardwood, poplar is a lightweight wood with

considerably lower density and characteristic strengths, which could influence the final material properties. Their modulus of rupture differs significantly, with beech at 103 MPa and poplar at 63 MPa.

Table 1. Mycelial strain-substrate combinations in different testing phases My1, My2, und My3.

Test phase	Strain	Substrate	Geometry	Filling level	Incubation time	WB	Designation
My1	GL	Beech	chips	100%	3 weeks	10:1	My-1-D-Z1-1-7
	GL	Beech	chips	120%	3 weeks	10:1	My-1-D-Z1-8-13
	GL	Beech	chips	100%	4 weeks	10:1	My-1-D-Z1-14-20
	GL	Beech	chips	100%	5 weeks	10:1	My-1-D-Z1-21-27
	GL	Beech	chips	100%	6 weeks	10:1	My-1-D-Z1-28-34
	GL	Beech	chips	100%	7 weeks	10:1	My-1-D-Z1-35-40
My2	GL	Beech	chips	100%	2 weeks	10:1	My-2-D-Z1-1-7
	GL	Beech	chips	100%	3 weeks	10:1	My-2-D-Z1-8-13
	GL	Beech	chips	100%	4 weeks	10:1	My-2-D-Z1-14-18
	GL	Spruce	sawdust	100%	4 weeks	10:1	My-2-D-Z1-26-31
	GL	Spruce	chips	100%	4 weeks	10:1	My-2-D-Z1-20-25
	GL	Spruce	sawdust	100%	4 weeks	2:1	My-2-D-Z1-32-37
My3	GL	Spruce	sawdust	100%	4 weeks	2:1	My-3-D-Z1-1-4
	GL	Spruce	sawdust	100%	4 weeks	10:1	My-3-D-Z1-5-7
	GL	Poplar	sawdust	100%	4 weeks	10:1	My-3-D-Z1-9-13
	GL	Poplar	sawdust	100%	4 weeks	2:1	My-3-D-Z1-14-18
	GL	Douglas fir	sawdust	100%	4 weeks	2:1	My-3-D-Z1-19-23
	GL	Douglas fir	sawdust	100%	4 weeks	5:2	My-3-D-Z1-24-27

3.4 *Assessment of the compressive tests with different fungal-substrate combinations*

The compressive tests on fungal composites are presented via stress-strain diagrams (Figures 3, 4), which evaluate mechanical performance under varying conditions such as substrate type, incubation period, and density. Samples are labeled systematically—"My-X-D-Z1" indicates test phase (X: My1, My2, or My3), test type (D: compression), and specimen geometry (Z1: cylindrical)—with additional labels for density (e.g., 100% for 0.55 g/cm³, 120% for 0.63 g/cm³) and incubation periods (e.g., 3Wo for three weeks), ensuring clear comparisons.

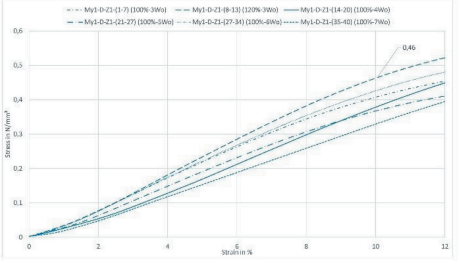

Figure 3. Stress-strain diagram of My1.

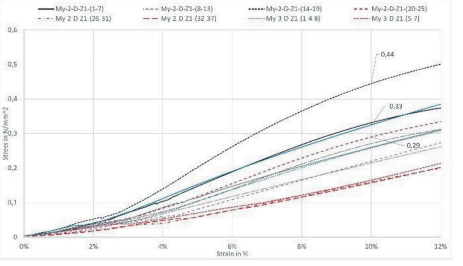

Figure 4. Stress-strain diagram of My2 And My-3.

Figure 3 shows stress-strain curves for MycoPor 1 (My1) samples made from beech wood chips and GL substrates. These samples were incubated for 3 to 7 weeks and had densities of 100% (0.55 g/cm³) or 120% (0.63 g/cm³). The curves reveal that stress increases with strain, indicating resistance to deformation; compressive strength is evaluated at 10% strain. At this point, the 120%-4Wo sample reached the highest stress of 0.46 N/mm² (SD 0.26 N/mm²), while samples incubated five or more weeks (100%-5Wo, 100%-6Wo, and 100%-7Wo) showed

lower stress, suggesting that prolonged incubation increases mycelial biomass and ductility but weakens structural integrity.

Figure 4 compares compressive performance in MycoPor 2 (My2) and MycoPor 3 (My3) samples, focusing on substrate type and incubation period. Stress-strain curves are categorized by substrate: black curves represent beech, while red curves represent spruce, including sawdust-based samples. At 10% strain, beech samples with a four-week incubation (My-2-D-Z1-(14-19)) reached 0.44 N/mm². Samples incubated for two and three weeks (My-2-D-Z1-(1-7) and My-2-D-Z1-(8-13)) had intermediate stress, while spruce sawdust samples (My-2-D-Z1-(20-25) and My-2-D-Z1-(32-37)) outperformed spruce chips but remained below beech performance. In MycoPor 3, with a uniform four-week incubation, color-coded curves show that poplar (blue, My-3-D-Z1-(9-13)) achieved the highest stress of 0.33 N/mm², beech (red, My-3-D-Z1-(1-4 and 18-7)) had moderate performance at 0.22 N/mm², and Douglas fir (black, My-3-D-Z1-(19-23) and My-3-D-Z1-(24-27)) displayed the lowest stress, indicating weaker compressive strength.

Overall, the tests reveal that a beech wood substrate with a four-week incubation consistently delivers the highest compressive strength (0.44 N/mm²). Poplar also performs well (0.33 N/mm²) but not as high as beech. Additionally, using sawdust rather than wood chips generally enhances compressive performance, while longer incubation times beyond four weeks increase ductility at the expense of compressive strength.

3.5 *Comparison of porosity and compressive tests' results*

In the My1 phase, comparing porosity (Φ) and compressive strength (N/mm²) across different cultivation periods for MBC using beech as a substrate reveals clear trends. In these charts, cyan values represent beech, while blue and red mark the minimum and maximum average measurements.

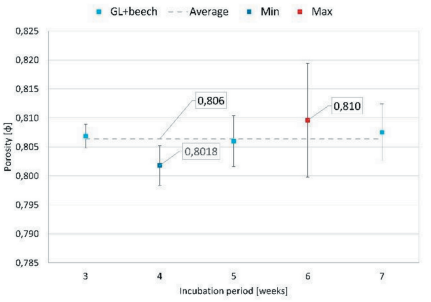

Figure 5. Porosity of My1.

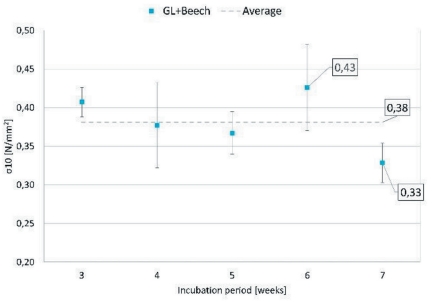

Figure 6. Compressive strength My1.

Porosity (Figure 5) remains stable across incubation periods (3 to 7 weeks), ranging between 0.8018 and 0.8100. This stability suggests beech-based composite porosity is unaffected by cultivation time, reflecting the predictable structure of the substrate combined with mycelium. Conversely, compressive strength (Figure 6) varies more dynamically. The average strength is 0.33 N/mm², peaking at 0.38 N/mm² in week four and 0.43 N/mm² in week six. However, the pattern isn't progressively stronger over time. Notably, porosity's minimum (blue) appears in week four and maximum (red) in week six, though values are too close to confirm a strong influence on material strength.

In the My2 and My3 tests, porosity (Φ) (Figure 7) and compressive strength (σC) (Figure 8) highlight substrate influence. The GL+beech combination (cyan) serves as a reference. Spruce and Douglas fir, common construction by-products in Germany, were tested but showed poor mycelium growth in coarse particle sizes (8-16 mm). Consequently, finer particle sizes were used in subsequent tests. The beech composite has a porosity of 0.8637 and an average strength of 0.38 N/mm², establishing a baseline. Generally, higher porosity correlates with reduced strength —a typical trade-off in material science (Xia et al., 2013). Most composites align with this

trend, clustering near beech's porosity level and showing declining strength with increased porosity.

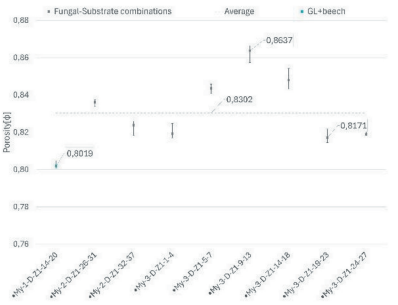

Figure 7. Porosity of My2 and My3.

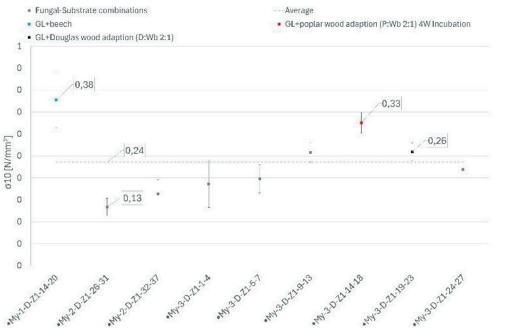

Figure 8. Compressive strength of My2 and My3.

However, the poplar composite (marked in red) defies this pattern. Despite its relatively high porosity (0.8637, just below beech), it shows an unusually high compressive strength of 0.33 N/mm². This suggests that poplar introduces unique structural traits that enhance strength independently of porosity, a phenomenon explored further in Chapter 4.

4 OUTLOOK AND DISCUSSION

4.1 *Impact of substrate type on mycelium growth and material properties*

The choice of substrate significantly influenced mycelial growth, density, porosity, and compressive strength. Beech emerged as a consistently reliable substrate, exhibiting stable porosity and the highest compressive strength among the tested species, especially following a four-week incubation period. However, spruce and Douglas fir also present improvement potential compared to previous experiments conducted by this research group (Saez et al., 2022, 2021). This potential is particularly notable when we consider that spruce and Douglas fir are softwoods primarily used in the construction industry and locally sourced within Germany, potentially appearing as a byproduct, a more sustainable and economically viable option for MBC production. Despite its higher porosity, poplar-based MBC exhibited considerable compressive strength, likely due to unique structural interactions between the mycelium and the poplar substrate.

As showed in Figure 9, the beech samples (a) show a fragmented and crushed appearance. The material appears to have less ductility, failing abruptly with a more extensive structure breakdown. The fractures are more widespread, and the specimens have lost much of their cylindrical shape, indicating a more brittle failure mode with less resistance to compressive forces than the poplar specimens. The first poplar specimens (b) appear to be a relatively intact cylinder with some visible deformation, suggesting some plastic behavior before fracturing. The material seems to be fracturing in a way consistent with brittle behavior. Still, it has some fibrous tearing, indicating that it resists compression initially but disintegrates as it reaches a higher compression point. The specimens maintain a fair degree of integrity at the edges, meaning that the failure is more internal with some minor external cracks, evidence of the jacketing effect. The second series of poplar tests (c) show slight deformation similar to the first poplar specimen but with more visible vertical cracking along the edges. There is an indication of some compression bulging, which may suggest a degree of plasticity before failure, though it remains more intact than the first poplar tests specimens. The cracks suggest a mixed fracture mode, with both compressive and shear forces contributing to failure.

Although our studies confirm that its porosity remains high, the combination of *Ganoderma lucidum* (GL) with poplar wood results in a superficial mycelial growth layer of approximately 1 mm, forming a jacketing-like structure (Figure 10). This effect can be attributed to the

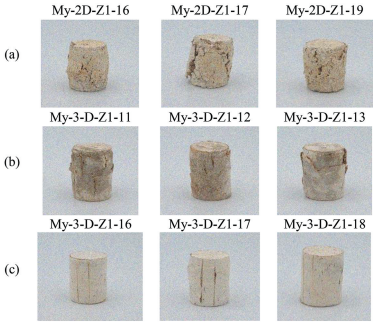

Figure 9. Different fracture behaviors for beech samples (a), poplar samples with a 10:1 ratio of wheat bran (b), and poplar samples with a 2:1 ratio of wheat bran (c).

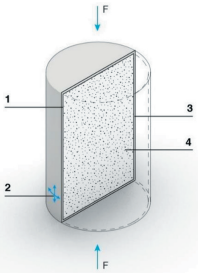

Figure 10. Illustration of the jacketing effect of the quasi-pure mycelium surface on the poplar samples where [$\rho_1 > \rho_2$]. (1) Binding are between myco-jacketing and MBC; (2) activation of the axial stresses alongside the myco-jacketing surface (3); Φ_1, ρ_1; (4) Φ_2, ρ_2.

substrate's lower lignin concentration and the higher starch content of wheat bran, which serves as an accessible carbon source, supporting fungal growth and mycelial development. This layer functions as a quasi-pure mycelial shell, potentially enhancing surface strength. Therefore, it is essential to consider the composite ratio between the substrate and the mycelial network and the bonding effect of this protective shell, which forms a cohesive jacket around the MBC itself.

4.2 *Influence of incubation periods on structural properties*

The experiments demonstrated that extended incubation generally leads to increased mycelial biomass but can also reduce compressive strength due to increased material ductility. Samples with shorter incubation periods generally maintained higher compressive strength in MBC with different substrate compositions, especially under standardized conditions at 10% strain. This observation suggests an optimal window for incubation, balancing adequate mycelial development with structural resilience. Further studies could refine this incubation period, particularly for industrial applications where uniformity and predictability are essential.

4.3 *Implications of material design and application potential*

The findings from this research contribute to the broader understanding of how biological substrates can be harnessed for composite materials with customized properties. The diverse responses of fungal strains to substrate variations indicate the possibility of tailoring composite characteristics through substrate manipulation and controlled incubation periods. For instance, selecting poplar as a substrate could provide lightweight yet resilient materials that are beneficial in applications requiring high strength-to-weight ratios.

Moreover, the comparison of sawdust and wood chips suggests that substrate refinement significantly enhances compressive strength. By offering a higher surface area for mycelium adherence, Sawdust contributed to denser and more robust composites, underscoring its potential as a preferable substrate form for load-bearing applications.

4.4 *Lessons learned and outlook*

Poplar-based MBC offers intriguing results, with a high porosity yet substantial surface strength due to a shell-like, quasi-pure mycelial layer formed by GL around the composite. Future studies should assess this "mycelial shell" effect to determine how it can be strategically applied in MBC development.

To fully realize the potential of spruce and Douglas fir, further studies should focus on optimizing their structural properties for mycelium growth. Research on particle size, surface area, and substrate preparation methods could help enhance the integration and bonding of mycelium with these softwoods. By refining these substrate characteristics, softwood-based

MBC could achieve improved mechanical properties, positioning spruce and Douglas fir as strong contenders for sustainable, high-performance mycelium-based materials.

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