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Evaluation of different wood by-products for sustainable building biomaterial production using fungal mycelium

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ABSTRACT

As human population increases, the demand for new innovative, sustainable, and low impact construction materials also grows. Mycelium-based composites have shown to be an excellent alternative for traditional products ranging from low-density objects to semi-structural applications. They also present the advantage of using the waste streams from other productive processes as feedstock, enabling the upcycling of materials that can help us transition into a circular economy. In this study three different experiments were carried out: first the selection of the fastest growing fungal strains and the process' temperature; secondly, three different grain spawn media were evaluated for inoculum production and the last one was a qualitative screening of mycelium growth in different wood by-products. *G. lucidum, T. versicolor* and *P. ostreatus* grown at 25 °C were chosen due to their fast-developing rate and mycelium density in comparison to *P. eryngii* and *F. pinicola*. For grain spawn production of these strains, a 1:1 mix of wheat and millet was found to be the best option to accelerate the mycelium growth rather than using the grains separately. Different 9x12x4 cm samples were produced using a variety of wood by-product substrates and the shortest production time and more visibly homogeneous material was obtained when growing *G. lucidum* on beechwood. However, other preliminary test demonstrated the great potential of mixed substrates for production times reduction. The next steps for this research include substrate optimization using mixed wood substrates and further characterization of the bio-composites including thermal conductivity and humidity resistance tests.

Keywords: Mycelium based composites, fungal mycelium, lignocellulosic materials, wood by-products, material bio-fabrication.

1. INTRODUCTION

Over the past decades the construction sector has been under pressure due to the availability of raw materials and the continuous growth of population (Pheng and Hou 2019, Yang *et al.* 2021). This has caused an increasing demand for building materials and, more specifically in recent years, *sustainable* materials and production processes with lower environmental impact (Elsacker *et al.* 2020).

Mycelium-based composite materials have become an interesting solution in this matter (Elsacker *et al.* 2020, Jones *et al.* 2020). Mycelium is the vegetative growth of filamentous fungi and is composed of filaments of white hyphae (Chan *et al.* 2021, Jones *et al.* 2020). As the mycelium colonizes and grows on solid organic matter, it works as a natural biological binder (Jones *et al.* 2017, Tacer-Caba *et al.* 2020). This opens the possibility for upcycling lignocellulosic materials and by-products from other industrial and agricultural processes for the production of new bio-composites (Jones *et al.* 2020).

These new materials are versatile and can be used in different applications from low-density and planar objects to semi-structural materials for panelling and flooring (Yang *et al.* 2021, Jones *et al.* 2020). Additionally, depending on the production process, they have proven to meet functional requirements including thermal and acoustic insulation and fire resistance (Attias *et al.* 2020, Jones *et al.* 2020, Elsacker *et al.* 2021). Some of the additional advantages of this type of materials over their traditional synthetic alternatives are their production process' low energy and water requirements, the low cost of raw materials (by upcycling other processes' residues and by-products) (Tacer-Caba *et al.* 2020, Jones *et al.* 2020) and the final product biodegradability (Van Wylick *et al.* 2022).

The objective of this research is to evaluate different fungal strains, wood-byproducts and conditions in order to elucidate a feasible and fast process for mycelium composite production for semi-structural applications using wood waste materials with no commercial value as feedstock.

2. EXPERIMENTAL METHODS

2.1 Fungal strains selection

Five different fungal strains (*Ganoderma lucidum, Trametes versicolor, Pleurotus ostreatus, Fomitopsis pinicola* and *Pleurotus eryngii*) were grown in Petri plates with potato-dextrose-agar (PDA) at 22 °C, 25 °C and 28 °C. Radial growth of the mycelium was measured every 24 hours for 7 days.

2.2 Grain spawn selection

Millet, wheat and a mixture of 50 % millet and 50 % wheat were inoculated with the selected fungal strains from the previous step for mycelium propagation. The grains were soaked in water for 18 hours, drained for 1 hour and placed in 212 mL containers filled to ¾ of their volume. They were sterilized for 50 minutes by autoclave at 121 °C. After cooling, 5 mycelial plugs (~1 cm diameter) were placed on the grain's surface and the container was incubated at 25 °C. Mycelium growth was monitored linearly until the whole container was colonized by the fungi.

2.3 Wood substrates evaluation

Four different wood by-products were tested as substrates for the selected fungal strains: pine sawdust, oak shavings, ailanthus chips and beechwood. The wood materials were supplemented with 10 wt.% of oat bran to enhance the mycelium's growth in the first stages of development in the substrate. Then, they were soaked in water for 18 hours and hand-pressed for excess water removal. Approximately 500 grams of moist substrate were placed in polypropylene bags and sterilized for 45 minutes by autoclave at 121 °C. After cooling, 10 wt% of the corresponding fungal spawn was mixed with each substrate in laminar flow hood. The bags were incubated at 25 °C until the mycelium colonized the material completely, point in which the substrate was kneaded and introduced into 9x12x4 cm moulds for 4 to 5 more days of incubation at 25 °C. After this time, the substrate had taken the shape of the rectangular mould, so they were removed from it and were oven dried at 60 °C for 48 hours. This step dehydrates the material and neutralizes the fungus,

stopping its growth. Bulk density measurements were made for each sample, as well as an x-ray (50 kV, 3 mA for 8 min) to complement the visual and qualitative analysis.

3. RESULTS AND DISCUSSION

Three different experiments were carried out. The first one was for selecting the fastest growing strains and the process' temperature; the second one was for grain spawn determination and the last one was a screening of mycelium growth in different wood by-products.

3.1 Fungal strains selection

The following fungal strains' growth were evaluated over time in PDA at three different temperatures: *G. lucidum, T. versicolor, P. ostreatus, F. pinicola* and *P. eryngii.* As shown in Fig. 1, the fastest growing strains were *G. lucidum, T. versicolor, P. ostreatus* and *F. pinicola.* In general, there was no significant difference in the growth rate observed at 25 °C and 28 °C, so a temperature of 25 °C was chosen for the subsequent experiments. *P. eryngii* had the slowest growth rate, reason why it was decided not to use it for further testing.

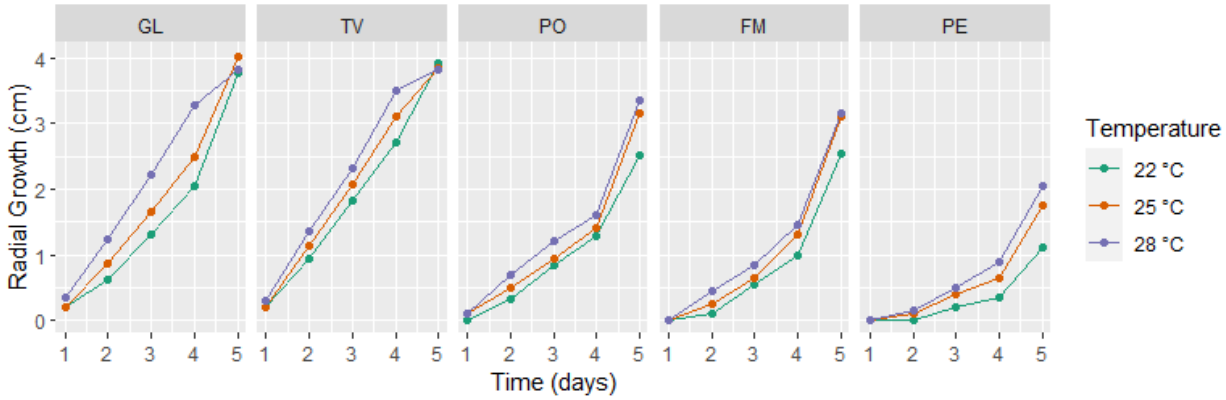

Figure 1: Radial mycelial growth on PDA plates at different temperatures for *G. lucidum* (GL), *T. versicolor* (TV), *P. ostreatus* (PO), *F. pinicola* (FM) and *P. eryngii* (PE).

Even though *P. ostreatus* and *F. pinicola* had similar growing tendencies, the mycelium of the last was less robust, as it can be seen from Fig. 2, so the following experiments were carried out with *G. lucidum, T. versicolor* and *P. ostreatus*.

Figure 2: (From left to right) *G. lucidum, T. versicolor, P. ostreatus, F. pinicola* and *P. eryngii* in PDA after 7 days of incubation at 25 °C.

3.2 Grain spawn selection

Fig. 3 presents the growth tendency of the three previously selected fungal strains in millet, wheat, and a mixture of 50 % millet and 50 % wheat. For the three strains, the fastest growth rate was

achieved when using the mixture of grains, while wheat had the slowest results. This may be due to the grain's complementing mineral composition and carbohydrate availability, as both wheat and millet have similar protein (around 10 to 15 %) and starch (between 60 to 70 %) contents (Jocelyne *et al.* 2020, Hassan *et al.* 2021, Shewry and Hey 2015).

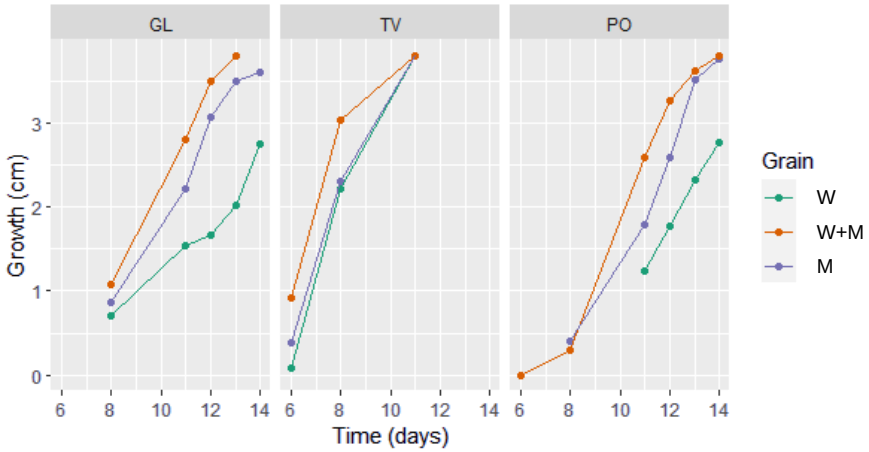

Figure 3: Mycelial growth trend on wheat (W), millet (M) and a 50 % millet and 50 % wheat grain mix (W+M) over time.

3.3 Wood substrates evaluation

Different samples were developed using the previously selected fungal strains and the mixed grains as inoculum to determine the compatibility of the fungi with the selected substrates. Table 1 presents the results for the best specimens obtained for each fungus. In general, ailanthus chips performed poorly regardless of the strain used, resulting in brittle materials that crumbled easily. This can be attributed to the physical state of this substrate, since the chips had dimensions of ~4 mm and are not a fibrous material that facilitates and favors the union of the substrate with the fungal hyphae.

Table 1: Results of wood substrate's evaluation for bio-composite production using fungal mycelium.

Fungal strain	Best substrates	Mean bulk density [kg/m^3]	Material's production time[a] [days]
G. lucidum	Pine sawdust	243,2 ± 10,6	22
	Beechwood	376,5 ± 21,4	16
T. versicolor	Oak shavings	256,1 ± 33,8	21
	Pine sawdust	205,0 ± 13,3	21
P. ostreatus	Oak shavings	251,4 ± 25,0	21

[a]*Corresponds to the sum of the days of mycelium development in bag, in mould and drying time.*

Overall, the bulk densities obtained in this study range between the expected values for as-grown mycelium composites containing forestry by-product substrates (87-300 kg/m^3) (Jones *et al.* 2020). Also, production times were similar regardless of the fungal strain, even though *G. lucidum* grew faster in the beechwood substrate.

Additional tests were carried out using substrate mixes (oak shavings mixed with pine sawdust and beechwood mixed with the last two) and *G. lucidum*. This samples were produced in 16 days and had similar properties as the pure-substrate ones, demonstrating the potential for substrate optimization using wood by-product mixes.

4. CONCLUSIONS AND FUTURE WORK

G. lucidum, *T. versicolor* and *P. ostreatus* grown at 25 °C were chosen to produce bio-composites using wood by-products due to their fast-developing rate and mycelium density in comparison to *P. eryngii* and *F. pinicola*. A 1:1 mix of wheat and millet was found to be the best option for fungal spawn production for these strains, instead of using the grains separately. Several samples were produced using different wood by-product substrates and the fastest and more visibly homogeneous material was obtained when growing *G. lucidum* on beechwood. Nonetheless, mixed substrates demonstrated great potential for substituting pure substrates. Consequently, next steps for this research include substrate optimization using mixes and further characterization of the bio-composites including thermal conductivity and humidity resistance tests.

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