

CSCE-Moncton-2023_FinalPaper-WORD_3983_eng

Potential of using the mycelium of *Pleurotus ostreatus* as a thermal insulator

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Abstract. The construction industry is one of the largest generators of waste. The majority of widely used insulation panels are made of synthetic materials that end up in landfill. The mycelium of the mushroom *Pleurotus ostreatus*, 100% compostable, has interesting properties that give it the potential to be the next trendy bio-based material. The literature reports its low thermal conductivity comparable to that of currently used insulation materials. The fire resistance of mycelium is also better than that of extruded polystyrene insulation materials. Mycelium has a lower heat production rate, it generates less smoke and CO₂ in addition to having a longer ignition time (300 s vs 50 s) limiting the spread of fires. Mycelium also has interesting acoustic properties. However, there is still much to do for the technology of growing and transforming mycelium into construction materials to be developed in Canada. The overall objective of this study is to produce an insulator that can be 100% composted after use. The specific objectives are to define the mycelium culture protocol, to define the optimal growth temperature of *Pleurotus ostreatus* on ash chips and to measure the thermal conductivity by the modified transient plane source method (MTPS) in order to compare them to those measured for hemp and glass wool. Different combinations of mushroom species and substrates can be used to manufacture mycelium-based products like that of *Pleurotus ostreatus* which is an edible mushroom safe for human health. The final properties of the material obtained will depend on the chosen mycelium-substrate composition. *Pleurotus ostreatus* grows on ash chips free of insecticide. The different experimental tests allow to define the optimal substrate composition (10% rye; 90% ash) for an optimized growth time at the best incubation temperature (25°C, 30°C, 35°C) determined by isothermal calorimetry tests with ash as the reference substrate. Preliminary tests allow to consider the use of mycelium as a thermal insulator following more in-depth studies.

Keywords: Mycelium, *Pleurotus ostreatus*, Thermal Insulation, Bio-based Material, Thermal Conductivity

1. Introduction

In this era of climate change where global climate conferences are happening at a rapid pace and where the UN defines its 13th sustainable development goal as taking "measures to combat climate change", a reassessment of our current practices and methods is necessary [1]. This includes, among other things, increasing the energy efficiency of buildings, but also questioning the practices of the construction industry which generates a significant amount of waste [2]. Indeed, the majority of materials used end up in the landfill at the end of their useful life where many years are needed to degrade them. The development of recyclable or compostable materials with a low carbon footprint is therefore the approach to advocate.

This is precisely why the potential use of mushroom mycelium as a binding agent for plant fibers from the circular economy is being studied in order to make it a potential thermal insulator for buildings. Characterization tests will allow the comparison of the mycelium of *Pleurotus ostreatus* used as a binder for plant fibers to proven insulators such as glass wool as well as to another biosourced insulator which is hemp wool. The objectives of the first phase of this study described in this article are to develop a mycelium culture protocol while determining its optimal growth temperature by isothermal calorimetry as well as to establish the thermal conductivity of the material thus obtained.

2. Literature Review

1. What is mycelium?

Mycelium is the lesser-known part of mushrooms that is equivalent to roots in plants. This highly interconnected underground network can extend for miles, allowing the organism to seek out organic matter to degrade into nutrients. Popularly, the term "mushroom" refers to the macroscopic fruiting visible on the ground that appears a few days per year when the conditions conducive to its growth are met. However, this fruiting is only the reproductive organ of the organism through which it will disperse its spores in the wind. Each spore has the potential to form a separate individual. These will remain dormant until the appropriate conditions are met for germination. The mycelium, on the other hand, also allows the symbiotic association of mushrooms with plant roots in the form of endomycorrhizae or ectomycorrhizae. This symbiosis provides water and nutrients (nitrogen) to the plant in exchange for carbohydrates provided by the plant to the fungus, which the plant produces in large quantities during the photosynthesis process. More than 90% of plant species on earth have a symbiotic association between their roots and fungi [3].

The growth of mycelium takes place continuously and indefinitely as long as space and nutrients are available. As time allows it to colonize all available space, the mycelium can take the desired shape with the help of a mold. Its ability to create a vast interconnected network allows it to serve as a binder between various fibers present in order to form a material.

Fungi have been used in industry for a very long time and we owe them a lot. Indeed, just in the food industry, they are used in the composition of cheeses (*Penecillium camemberti* and *Penecillium roqueforti*), yogurt and kombucha. In the case of yeasts which are part of the

same kingdom in the tree of life, they are used in the fermentation processes of beer and wine in addition to being useful to make bread rise (*Saccharomyces cerevisiae*). In the pharmaceutical industry, some antibiotics, including penicillin, have active ingredients that come from toxins secreted by certain species of fungi such as *Penicillium notatum* [3].

In the case of mycelium, the discovery of its interesting properties by the industry has only recently been made. One of the leaders in the field is the American company Ecovative which uses mycelium to create packaging that has the potential to replace styrofoam, as well as leather-like fabrics and even offers mycelium-based vegan bacon [4].

2. Material composition

First and foremost, it is important to use a harmless mushroom for humans, ideally edible, as a binder for plant fibers. Then, a market analysis was conducted to determine which plant material could be sourced locally and at a low cost while keeping the goal that it be present in large quantities and revalued according to the principle of the circular economy.

In the case of the city of Montreal in 2022, the main source of available plant material is ash wood. Indeed, since 2011, the emerald ash borer, *Agrilus planipennis* , has been wreaking havoc in the virgin ash populations of the island of Montreal where their proportion among the city's forest ecosystem is significant [5]. This beetle from South Asia is believed to have arrived in North America by boat via wooden packaging around 1990. When it becomes endemic in a region, the larva will infect and induce the mortality of up to 99% of the individuals present on the territory over a period of 10 years [6]. As municipal forestry organizations cannot keep up with this rapid pace of treatment, cutting and planting, many dead ashes wait too long before being cut down which affects the quality of the wood. The chip industry is then the only option. For the use of ash chips as a fungal growth substrate, it is important to use wood from trees not treated with the insecticide TreeAzin® to avoid undesired antifungal effects [7].

The species of fungi chosen for this study is *Pleurotus ostreatus* or by its common name oyster mushrooms. The choice is based on the fact that this edible saprotrophic species has a majority growth on hardwood [8]. However, as ash chips are not a rich enough substrate to start the growth of the mycelium, a richer intermediate substrate is used as a catalyst to achieve a better colonization time. This is the case with organic rye grains which will make up about 10% of the final material. Despite their use not taking into account the principle of circular economy and revaluation, in addition to using agricultural land for something other than human food, their small proportion in the context of the study is acceptable. However, an alternative would be important in the case of larger scale use.

3. An insulator?

Numerous studies have reported on the potential insulating properties of mycelium and agree that they are promising. Indeed, some thermal conductivity readings by Elsacker and al (2019) [9] are almost as low as for glass wool, which is one of the insulating materials used on a large scale by the industry with values of 0.0404 to 0.0578 W/mK for mycelium compared to 0.033 W/mK for glass wool. However, Xing et al (2018) [10] obtain thermal conductivities from 0.078 to 0.081 W/mK for high-density mycelium-based samples. In the

case of Pelletier and *al* (2017) [11], they have demonstrated the interesting acoustic properties of mycelium as a binder of agricultural waste fibers when formed into high-density panels. These testify to a sound absorption equivalent and even superior to commercial acoustic panels. Jones and *al* (2018) [12] report interesting fire resistance properties of mycelium-based samples compared to certain thermoplastic polymers. Indeed, the mycelium delays the spread of flames due to its longer ignition time and its lower heat release rate. These studies gather many properties sought in an insulating material that mycelium seems to possess, when associated with plant fibers.

3. Experimental method

1. Isothermal calorimetry

The TAM Air 8-channel device was used for isothermal calorimetry tests due to its high sensitivity which allows a detection limit as low as $2\mu\text{W}$ [13]. The controls used consist of an average of 2.04 g of ash chips, which is about half the volume of the ampoule, while the samples consist of the same mass of ash chips to which 100 μL of a liquid mycelium solution of *Pleurotus ostreatus* was added. The ampoules and the substrate were sterilized in the autoclave according to a 20-minute liquid cycle at 121°C and cooled before being seeded and sealed. The tests were carried out for 8 replications at 20°C , 25°C and 30°C for 14 days to determine the optimal growth temperature of the mycelium.

To this end, the values of heat flow as well as total heat over time will be compared for each of the samples. In the case of heat flow, it represents the energy that is transferred from the growing mycelium sample to the environment which is here the sealed ampoule. The power of this transfer is measured in Watts (W) or rather in mW in the present case. Regarding total heat, it represents the amount of heat released by the growth of the mycelium and is measured in Joules (J) [14].

2. Material growth

The NSP1 strain of *Pleurotus ostreatus* is preserved and propagated on Sabouraud-chloramphenicol agar until complete colonization of it. The incubation temperature for optimal growth time is 25°C . About 5 pieces of mycelium are then seeded by 240 mL of dry organic rye grains previously rehydrated for 24h and sterilized for 40 min at 121°C . When the rye grains are fully colonized by the mycelium, they are diluted 1:10 in the ash chips previously hydrated and sterilized under the same conditions as the rye grains. Once complete colonization is obtained, flour is added and everything is homogenized before being put into molds with manual compaction. After 14 days of incubation, everything is demolded and returned to the incubator for 6 to 8 days to allow uniform colonization of the outer sides. Then, the bricks obtained are put in the oven at 80°C for 24 hours in order to inactivate the mycelium and to allow the moisture contained in the material to evaporate [15].

3. Thermal Conductivity

The C-Therm Trident device was used to perform the thermal conductivity measurements of the materials compared at an ambient temperature oscillating around 24°C. These were taken according to the modified transient plane source (MTPS) mode for which the accuracy of the measurements is estimated to be within 1% for conductivity values ranging from 0 to 100 W/mK [16]. The measurements were taken at least in triplicate for each sample at each analyzed orientation of glass wool, hemp wool, mycelium bricks with medium chips, and mycelium bricks with fine chips. The control used is the LAF6720C, a reference material provided by Trident, due to its thermal conductivity at 25°C which is similar to those expected for the materials to be analyzed according to the chart present in the appendix of the C-Therm user manual [16]. In the case of hemp wool, due to good maintenance of the material's shape and similar fiber orientation, the thermal conductivity was analyzed perpendicularly to the hemp fibers (horizontal) as well as parallel to the hemp fibers (vertical). In the case of glass wool, due to the malleability of the material, vertical measurements were not possible, but were instead replaced by a comparison of the thermal conductivity between a thickness of 5 cm and 10 cm. Finally, the same principle was applied to the mycelium samples due to a variable fiber orientation for thicknesses of 7 cm and 10 cm.

4. Results and Discussion

1. Isothermal Calorimetry

The TAM Air 8-channel device records every 30 seconds the variation of the heat flow as well as the total heat in each of the test ampoules compared to the baseline given by the control ampoules. Figures 1 and 2 below respectively represent the evolution of the heat flow and the total heat during 12 days of tests at each of the analyzed temperatures.

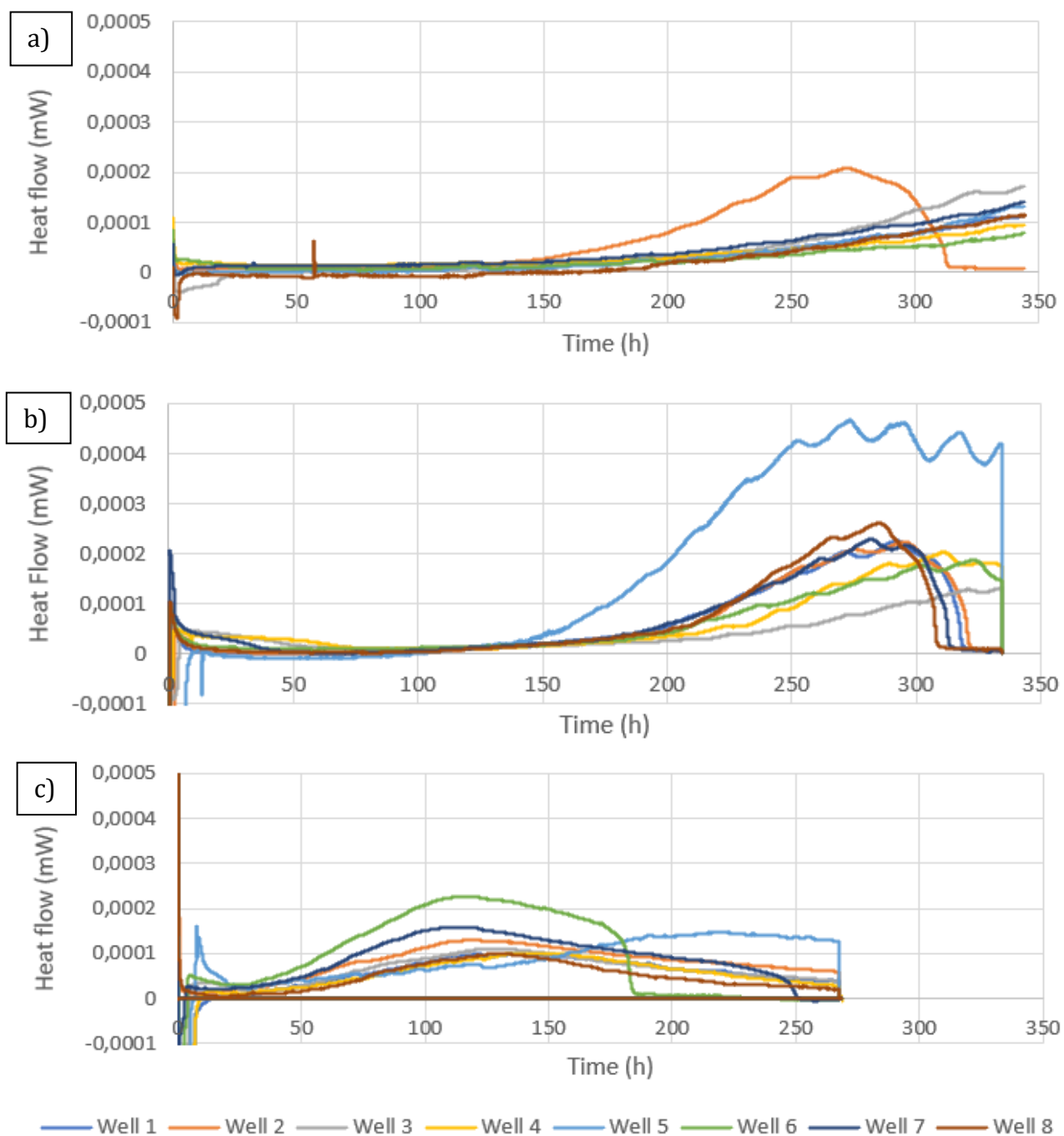


Fig. 1. Evolution of the heat flow released by the growth of *Pleurotus ostreatus* on an ash chip substrate over time for temperatures of a) 20, b) 25 and c) 30°C

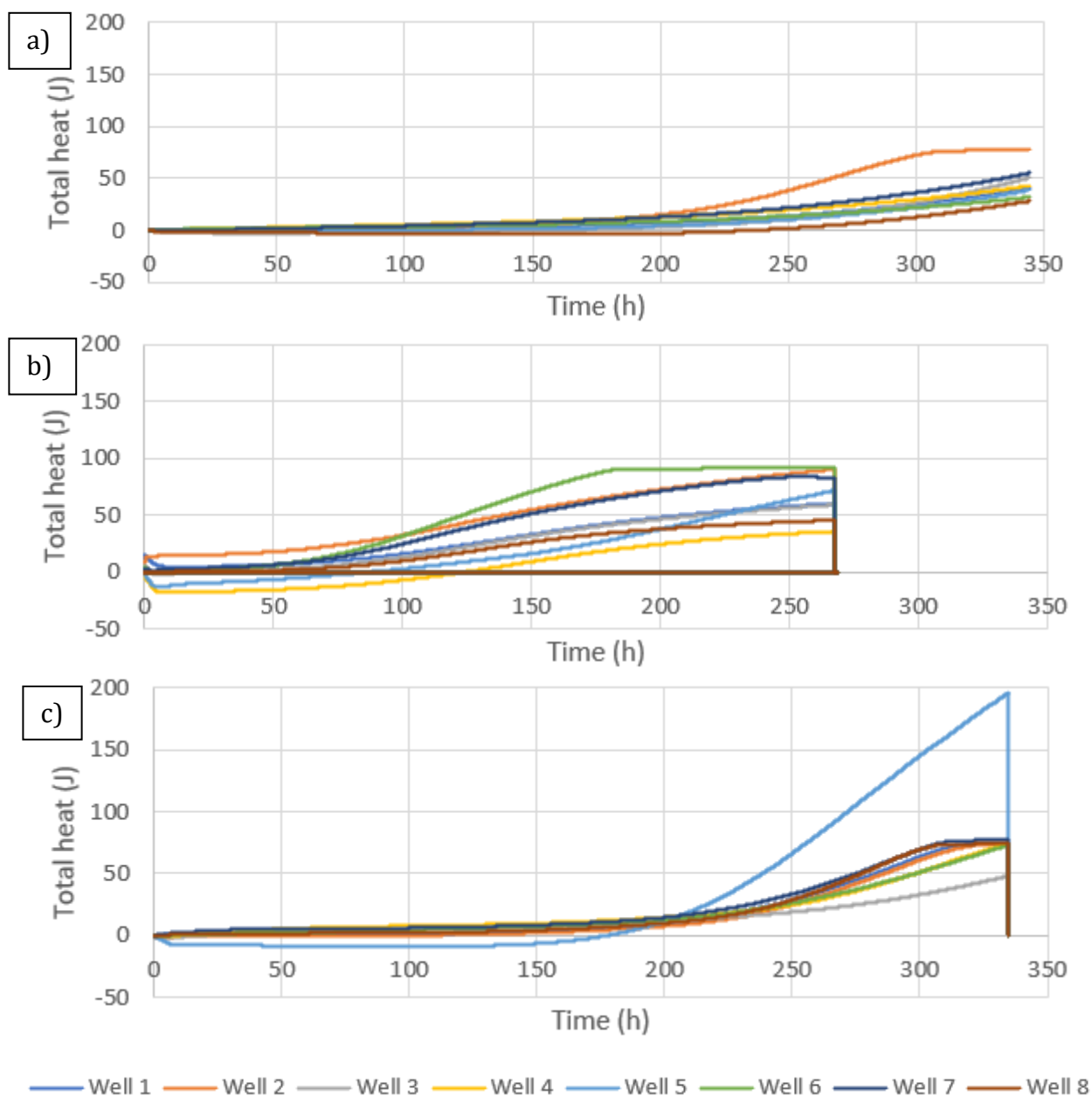


Fig. 2. Evolution of the total heat released by the growth of *Pleurotus ostreatus* on an ash chip substrate over time for temperatures of a) 20, b) 25 and c) 30°C

It is possible to note that the peak for heat flow and total heat occurs earlier at 25°C than at the other analyzed temperatures. However, higher values are reached for the heat flow at 30°C, but more time is required.

Regarding the isothermal calorimetry results obtained with the TAM Air 8-channel device, the analysis at 25°C was performed first and was stopped when the total heat curves began to stabilize and the heat flow curves were in regression, which occurred after about 250 hours (10 days). Indeed, the stabilization of the total heat curve indicates that there is no

more reaction taking place in the medium, which is explained by a stop in the growth of the mycelium due to a lack of nutrients, moisture, or oxygen. In the case of heat flow, a regression of the curve corresponds to a decrease in the power of heat transferred when the growth of the mycelium decreases until it stops when the curve returns to zero.

It is possible to observe a more significant growth of the sample in well 6 (green curve) reached its peak more quickly and then decreased. This may have been caused by a mycelium sample whose incorporation was deficient within the liquid inoculum mixture, resulting in a higher concentration of mycelium for the same volume of mother solution inoculated by ampoule. In the case of sample 5, in turquoise, growth seems to have been slower to start, but it eventually reached values similar to other samples before decreasing. This may be due to initial conditions in the ampoule slightly different from other replicates (more time exposed to air therefore lack of humidity) which may have hindered the initiation of mycelium growth. Finally, in the case of sample 8, in burgundy, there seems to be little or no growth. This may be due to an inoculum that was not viable or had a very low concentration of mycelium at the base. However, for the majority of the replicates analyzed at 25°C, the heat flow seems to reach its peak around 120 hours at about 0.0015mW on average and the total heat tends to stabilize after 250 hours around 50 Joules.

In the case of the test at 20°C, sample 2, in orange, seems to have an aberrant behavior compared to the 7 other replicates whose behavior seems quite stable and similar. As in the case of sample 6 (green curve) at 25°C, this may be due to poor homogenization of the mother solution which resulted in a more concentrated inoculum for the same volume of seeding. It is possible to see that the heat flow and the total heat have not yet reached their peak after an incubation of 350 hours (14 days). This reflects a much slower growth rate than at 25°C.

In the case of the test at 30°C, replicate 5 (in turquoise) seems to have had much more pronounced and longer-lasting growth compared to the other replicates whose behavior seems to be similar to each other for a reason probably similar to that explained above. It is possible to see a maximum heat flow at 300 hours $\pm$ 20 at about 0.0002 mW on average and a total heat that stabilizes around 320 hours at about 75 Joules.

The growth rate of the mycelium of *Pleurotus ostreatus* on ash chips therefore seems to start more quickly at 25°C. However, once the latency phase is over, growth seems to be stronger at 30°C. In the case of large-scale growth in the laboratory, as the ash chips will be inoculated from previously colonized rye grains which are a richer growth medium, the latency phase will be shorter than that observed in isothermal calorimetry where the growth substrate is only ash. As the speed of obtaining the material is a limiting factor, the growth of the mycelium of *Pleurotus ostreatus* at 25°C was chosen.

2. Mycelium growth

During the step of inactivating the mycelium by oven drying, the weight of the bricks was measured at their exit from the incubator just before being put in the oven at 80°C. The weight was measured 24 hours later in order to quantify the loss of mass due to the evaporation of the water contained in the material. The *Table 1* below shows the average percentage of humidity contained in each of the bricks produced. Some of these are made

up of a substrate of coarse ash chips while the ash substrate has been sieved for the second part whose particles are finer. It is possible to see that the average percentage of humidity contained in the mycelium bricks made up of finer ash chips is slightly higher than for the bricks whose substrate is medium-sized ash chips.

Table 1. Average percentage of moisture contained in each of the mycelium growth trials

Trial no. ¹	3	4	5	6
Particle size	Average		Fine	
Number of replicas	6	5	6	5
Average humidity (%)	39	38	41	44
Standard deviation of humidity %	2.6			

As far as mycelium growth is concerned, patience is a virtue. Indeed, the different stages of colonization of the various substrates by the mycelium take time and can be slowed down if the growth conditions are not optimal. This is often the case with rye grains coming out of the autoclave whose humidity level seems to be good, but it evaporates when they return to room temperature. It is therefore important to add an adequate amount of sterile tempered water to allow the inoculated mycelium to start colonizing the substrate.

Wanting to skip steps by starting the next colonization stage too early simply has the effect of slowing down the overall process, as the volume of inoculum is then less significant. However, this does not compromise the viability of the mycelium, but rather affects its colonization vigor. This was observed during an unsuccessful demolding trial after 8 days of incubation of mycelium bricks with fine ash chips as a substrate. Indeed, the material did not hold in place during demolding. A 2nd trial 6 days later allowed to observe the benefits of an adequate incubation time on the cohesion of the substrate through the mycelium which acts as a binder of plant fibers.

As for the moisture content in the produced bricks, *Table 1* provides a good summary. Indeed, it is possible to observe a very similar moisture rate between the bricks coming from mixes 3 and 4 made up of medium-sized chips with respective values of 38 and 39% of the initial mass of the bricks. In the case of mixes 5 and 6, made up of finer chips, the average moisture content in the bricks is slightly higher with respectively 41 and 44% of the initial mass of the bricks attributable to water. This is explained by the larger contact surface available on the material due to the smaller size of the particles that make it up which allows a greater absorption of water by the substrate. On the other hand, some of the weight loss of the bricks can be attributed to their handling during the monitoring of their weight. Indeed, the mycelium bricks obtained can be quite fragile if they undergo localized shocks or if certain chips were not in a good position to begin with.

3. Thermal conductivity

During the thermal conductivity tests, the value of the amount of heat transmitted by the material per unit of surface and time λ obtained was compared according to the orientation of the fibers or the thickness of the material for glass wool, hemp wool and mycelium

samples made up of fine and medium chips. The *Table 2* below summarizes the values obtained. It is possible to note that the standard deviations obtained between comparables are low and testify to the precision as well as the reliability of the C-Therm device. Also, the thermal conductivity values are similar between mycelium, glass wool and hemp wool.

Table 2. Comparison of thermal conductivity measured by MTPS with the C-Therm device for various materials

Materials	Orientation	Thickness (m)	Replica	Average λ (W/mK)	$\sigma \lambda$	R (W/m ² K)
Glass wool	Horizontal	0.10	6	0.0313	0.00010	3.245
	Horizontal	0.05	3	0.0312	0.00012	1.628
Hemp wool	Horizontal	0.076	12	0.0362	0.00174	2.104
	Vertical		9	0.0365	0.00047	2.089
Fine mycelium	Horizontal	0.07	6	0.0338	0.00209	2.069
	Vertical	0.10	3	0.0420	0.00026	2.418
Medium mycelium	Horizontal	0.07	9	0.0325	0.00041	2.149
	Vertical	0.10	9	0.0316	0.00062	3.216

Legend: λ = Thermal conductivity σ = Standard deviation R = Thermal resistance

Regarding the thermal conductivity of the 4 compared materials, it is important to qualify the analysis method used. Indeed, the measurement of thermal conductivity by MTPS requires a flat surface of the material to be in contact with the sensor. When this is not entirely possible, a contact agent in the form of paste, provided by Trident manufacturer, can be applied to the sensor so that this contact agent fills the interstices of the material. However, the device did not allow the use of a contact agent for the analysis of materials with a range of thermal conductivity characteristic of insulators. As the presence of the contact agent influences the thermal conductivity measurement obtained, it is important to specify its use and nature to the software so that it takes it into account as background noise and adjusts the λ values obtained. The analysis was therefore carried out without a contact agent, which can influence the data obtained due to the heterogeneity of the material and the contact surfaces with the MTPS sensor which were not exactly flat. The data obtained can therefore be considered as exploratory.

In the case of glass wool, which is a proven insulator, thermal conductivity measurements were taken for a single and double thickness of wool, therefore for 5 and 10 cm. The average values obtained are respectively 0.0312 and 0.313 W/mK with a very low standard deviation. The values are in line with the literature which establishes around 0.030 W/mK the thermal conductivity of glass wool, which confirms the method used [17]. In the case of hemp wool, which is a biosourced insulator, the average thermal conductivities obtained for a horizontal and vertical orientation of the fibers are respectively 0.0362 and 0.0365 W/mK. The values are therefore constant, regardless of the panel orientation, but the thermal conductivity of hemp wool is slightly less than that of glass wool. However, the

thermal conductivity values are slightly lower than the 0.040 W/mK indicated in the product sheet at the Nature Fibres company [18].

In the case of the mycelium samples, thermal conductivity measurements were taken for a thickness of 7 and 10 cm. In the case of fine chips, these are respectively 0.0338 and 0.0420 while they are 0.0325 and 0.0316 for medium-sized chips. As mentioned above, the literature reports values that vary between 0.0404 to 0.0578 W/mK which is slightly higher than the experimentally obtained values [9]. This can be explained by a different density of the samples as well as a homogeneity of the more variable mix. Therefore, samples with medium chips seem to have better thermal conductivity than those with fine chips and even slightly better than hemp wool. In all cases, the 4 materials analyzed have a thermal conductivity that falls into the "insulating" category according to the French standard RT 2012 which establishes it at 0.065 W/mK and less [19].

5. Conclusion

To conclude, this study has allowed to define an optimal growth temperature for the mycelium of *Pleurotus ostreatus* on ash chips at 25°C through isothermal calorimetry tests. Also, growth tests show a relative humidity contained in the mycelium bricks around 40%. Finally, preliminary thermal conductivity tests suggest a competitive thermal conductivity with glass wool. This opens the door to more in-depth studies on the possibility of using mycelium as a binder for plant residues in the development of thermal insulators usable on a large scale.

6. Acknowledgements

The authors thank the City of Montreal for providing a large quantity of ash chips, Biopterre for the strain of *Pleurotus ostreatus* and Nature Fibres for the hemp wool sample. The project was made possible thanks to the financial support of the NSERC throughout the project, through the master's excellence scholarship, as well as the École de technologie supérieure which allowed access to the laboratories.

7. References

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1. Tests 1 and 2 having suffered from contaminations, they were eliminated before reaching the molding stage. ↩