

The production process and compressive strength of Mycelium-based materials

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THE PRODUCTION PROCESS AND COMPRESSIVE STRENGTH OF MYCELIUM-BASED MATERIALS

R. Lelivelt^{1*}, G. Lindner¹, P. Teuffel¹, H. Lamers¹

¹ Eindhoven university of technology, unit Structural Design, Eindhoven, The Netherlands

*Corresponding author; e-mail: RobertLelivelt@gmail.com

Abstract

In the transition towards a world in dynamic equilibrium with its natural resources, oil and all oil based products must be replaced by more renewable alternatives.

Mycelium-based materials are renewable and can replace fossil-based plastics. A mycelium-based material is a composite consisting of a natural reinforcement or filler, such as hemp fibers, and the mycelium of a fungus, see *Fig. 1*. A mycelium is a dense network of thin strands, called hyphae that grow and fuse together into a continuous material. The mycelium acts as a matrix that binds the natural substrate into a lightweight material. This paper aims to provide an insight into the production methods of mycelium-based materials and an indication of the structural performance of such materials. The stress at 10% deformation of the best mycelium-composite was found to be between 24 and 93 kPa.

Keywords:

Bio-based, Mycelium, Composites, Renewable, Sustainable, Natural fiber, Hemp, Coriolus Versicolor

1 INTRODUCTION

Within the range of green materials a new subdomain is emerging: bio-based materials. The term bio-based material is here defined as: "a material of which one or more of its components are sustainably grown and are fully renewable". Such materials offer a solution to the global waste problem and provide an essential component for a cleaner, more sustainable future.

Within the domain of bio-based materials, composites consisting of a ductile matrix and high-strength reinforcement offer high performance at low cost and great freedom in designing a material for a specific application (Mallick 2008). Though many composites boast a natural reinforcement, the matrix is often based on petrochemicals (Faruk, et al. 2012) (Koronis, Silva and Fontul 2013) (La Mantia and Morreale 2011). A renewable alternative for these matrices is the vegetative state of a fungus, the mycelium. Such a matrix is fully bio-based and can be produced at low cost. Therefore research into the mechanical properties of mycelium-based materials is useful.

This paper will start with the process of making mycelium-based materials. Then the results of visual and compressive tests on several samples will be discussed.

2 PROCESS

As mycelium-based materials are an entirely new category of materials no standard yet exists that prescribes the production method for such materials. Therefore a method was established using input from experts with experience in mycelium-based materials (Montalti 2014) (van Belle 2009) (Wösten 2014) and adaptations from the methods used in existing agricultural mushroom cultivation (van der Horst 2014).

The process to create mycelium-based materials consists of six steps. Four steps are needed to cultivate a mycelium and two more are required to make it a usable material. (Montalti 2014). The process is shown in *Fig. 2*. The first step involves the creation of a habitat for the fungus; the substrate. The substrate can be any cellulose-rich material such as straw, wood and hemp.

The substrate needs to have high cellulose content for two reasons. Firstly, a fundamental difference between fungi and other organisms is that fungi can break cellulose down into glucose. This means that in cellulose-rich environments fungi can grow rapidly, whilst other organisms cannot. Therefore it is practical to use cellulose-rich materials when growing fungi to prevent contamination by other organisms (Wösten 2014). The second advantage is that at the molecular



Fig. 1; A mycelium-based material is a composite consisting of a natural reinforcement or filler, such as hemp fibers, and the mycelium of a fungus.

level, many natural fibers and wood-like materials are a composite of rigid-high strength cellulose embedded in a lignin matrix. Therefore, high cellulose content predicts high tensile strength (Faruk, et al. 2012) (Satyanarayana, Arizaga and Wypych 2009). As the substrate will act as the reinforcement of the material, a high tensile strength predicts a high mechanical performance of the composite overall (Mallick 2008).

Once the substrate has been selected and mixed, the substrate needs to be sterilized to prevent other organisms from infecting the fungus during growth. This can be done by boiling the substrate in water or by treating it with hydrogen-peroxide.

After sterilization the substrate can be inoculated with the spawn of the desired fungus. Preferably, pre-grown spawn is used that is cultivated by specialist companies that work under specific conditions to create very pure and reliable spawn (Montalti 2014). The group of fungi to use preferably is the basidiomycota as this group has the ability to fuse its mycelium into a dense mass (Carlile and Watkinson 1995).

Inoculation must occur under semi-sterile conditions to prevent contamination. Inoculation can be performed by mixing the spawn with the substrate.

After inoculation the fungus must colonize the substrate by growing through it (Step 4 in Fig. 2). In this step it is important to provide the correct growing conditions. Such conditions differ per species of fungus but some conditions are universal for all basidiomycota.

First of all, the growing must occur in dark conditions. For basidiomycota the presence of light is an indication that a free surface is reached. At such a surface, basidiomycota start producing fruiting bodies, i.e. mushrooms. When cultivating fungi for their mycelia it is important to prevent the forming of fruiting bodies as

they will slow the growing process and create heterogeneities in the mycelium.

Secondly moisture content needs to be very high. The relative moisture content in the air needs to be 90 to 100% (Yadav and Tripathi 1991). However it is also sufficient to ensure that the substrate contains enough moisture for the growth phase. Though the exact moisture content differs per substrate and species, a substrate that is 'wet to the touch' is deemed to be sufficient (Wösten 2014).

Table 1 ; growing conditions of basidiomycota (Montalti 2014) (Wösten 2014) (Yadav and Tripathi 1991).

Growing conditions	
Humidity	90-100% (moist to the touch)
CO ₂	High
Light	None
O ₂	Necessary for growth
Temperature	< 40 °C (heat is produced during growth)

Also airflow is required. Fresh air is needed as the mycelium requires oxygen and produces CO₂ during growth. However, fresh air also increases the chance for contamination so a controlled airflow is recommended. Furthermore a low CO₂-content is also an indication for a basidiomycete that a free surface is reached. Such a stimulus results in the production of fruiting bodies, which is not favorable for mycelium cultivation. Therefore the CO₂ content should be kept high.

The optimum temperature during growth differs per species but in general basidiomycota do not grow above 40 °C. For most purposes room temperature is sufficient. However as fungi also produce heat during

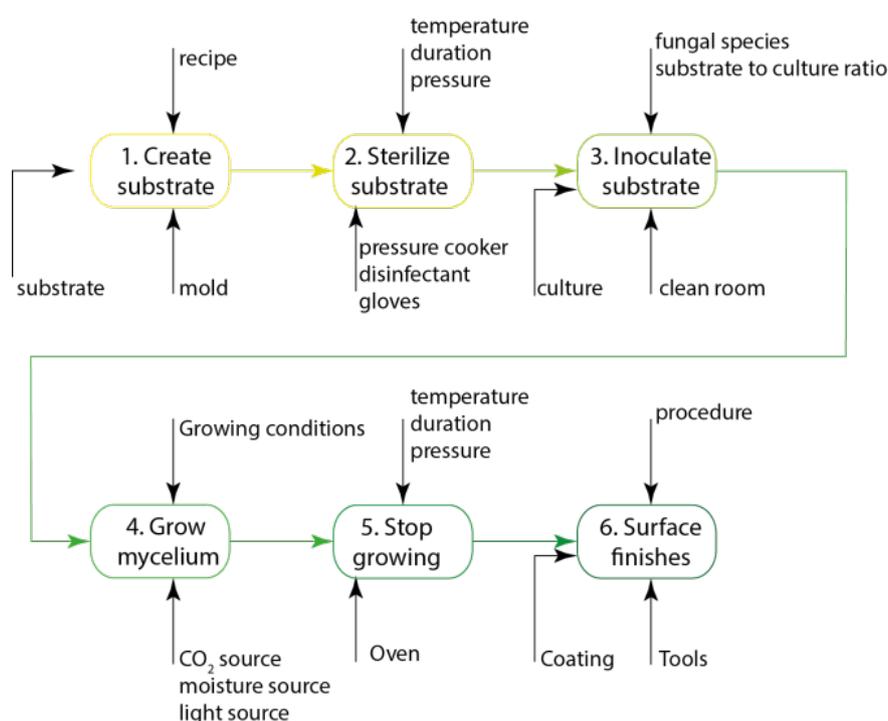


Fig. 2: SADT-Scheme of mycelium-based material production process.



Fig. 3 ; from top left, going clockwise: top, bottom and side view of a sample of *C. Versicolor* and non-woven hemp mats.

growth, it is possible that the fungi overheats in closed conditions.

In the optimized conditions of large-scale agricultural mycelium production, a growth phase takes 16 days (van der Horst 2014). The general growing conditions for basidiomycota are listed in *Table 1*.

After the growing phase, the growing needs to be stopped. This is imperative as otherwise the fungus would ultimately consume the entire substrate or start to produce fruiting bodies. The termination of the growing phase of the mycelium can be done by heating the mycelium. When the growing has been stopped, the sample can be removed from the mold. To improve the properties of the material a coating might be added as a last step.

3 EXPERIMENTAL

Three sets of samples were made. The first set was made with different fungi, substrates and sterilization methods. The combination from the first set that yielded the highest compressive strength was made in larger numbers for the second and third set.

3.1 Materials

Tests were performed on samples consisting of mycelium of *Coriolus Versicolor* and *Pleurotus Ostreatus*. Substrates were used consisting of wood chips, hemp hurd, loose hemp fiber and non-woven mats of hemp fiber. The mycelium was grown using pre-grown spawn cultivated on rye that was bought at

Table 2 ; overview of substrates, fungal species and sterilization methods in the first group of tests

Number	Substrate	Spawn	Sterilization method
1	hemp hurd	<i>C. versicolor</i>	boiling
2	wood chips	<i>C. versicolor</i>	boiling
3	hemp hurd	<i>P. ostreatus</i>	boiling
4	wood chips	<i>P. ostreatus</i>	boiling
5	hemp mat	<i>C. versicolor</i>	boiling
6	hemp fibers	<i>C. versicolor</i>	boiling
7	hemp mat	<i>P. ostreatus</i>	boiling
8	hemp fibers	<i>P. ostreatus</i>	boiling
9	hemp fibers	<i>C. versicolor</i>	H2O2
10	wood chips	<i>C. versicolor</i>	H2O2

Mycobois. The non-woven hemp mats were kindly provided by HempFlax b.v. The wood chips and hemp hurd were bought at a local pet store.

For the first group a spawn to total weight ratio of 20% was used. For the second and third group a ratio of 10% was used. The first group was given a higher ratio to ensure a faster growth.

3.2 Method

The worktop, gloves and all other equipment used in this procedure was cleaned with a 95% alcohol solution to prevent contamination of the samples.

First group

The composition of the first group of samples can be found in *Table 2*. The substrate was sterilized by placing it in boiling water for 100 minutes. Three samples were treated with 0,3 % hydrogen-peroxide solution instead of boiling. After the treatment the substrate was squeezed by hand to drain excess moisture. The substrate was then placed into transparent plastic molds. The substrate was mixed with particles of the spawn.

The molds were closed and then placed in larger boxes that could be closed off. The samples were allowed to grow in dark conditions at room temperature. To ensure a completion of the growth process, a long growth period of 30 days was used.

After the growth period the samples were placed inside an oven at 125 °C and dried for 2 hours. Afterwards the samples with a sufficiently dense and coherent mass were cut into rectangles.

Second and third group

The combination of non-woven hemp mats and spawn of *C. versicolor* was selected for the second and third test series. For these series the non-woven hemp mats were sterilized by placing them in boiling water for 100 minutes. After the treatment the mats were squeezed by hand. The dry mats weighed 21,7 (1,6) grams. The squeezed wet mats weighed 49,2 (1,8) grams. The values in brackets are standard deviations. For the drying process, the samples were placed inside an oven at 125 °C and dried for 2 hours.

After drying the samples were cut into cylinders with a height of 32,1 (1,8) mm and a diameter of 27,0 (1,1) mm.

3.3 Compressive tests

The first group of samples was tested using a Zwick Z020 machine with a 1 mm/min load speed. A 5 kN cell was used. The rectangles were tested standing on their smallest surface.

For the second group the cylinders were placed inside an Instron testing machine and tested in compression with a 3 mm/min load speed. Again a 5 kN load cell was used. Strain was calculated from machine displacement and was also visually recorded using video equipment.

The cylinders of the third group were tested using a Zwick Z020 testing machine with a 1 mm/min load speed and a 5 kN load cell. The cylinders were first loaded up to 100 N and subsequently fully unloaded. The cylinders were then loaded until 200 N.

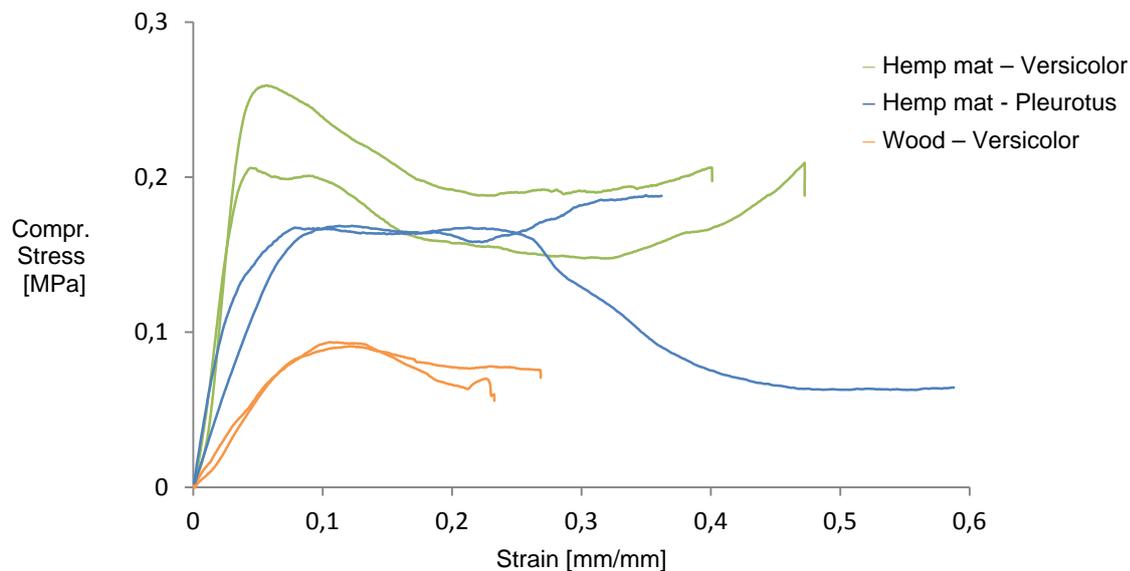


Fig. 4; Compressive stress - strain graph of the first group of samples

4 RESULTS AND DISCUSSION

4.1 Visual results

Infection rate

11% (2/18) of the samples with boiled substrate was infected. Of the samples treated with hydrogen-peroxide 33% (1/3) was infected. Because only a small amount of samples was prepared using the hydrogen-peroxide treatment, it is impossible to provide a definite conclusion on the effect of such a treatment. However, visual inspection also showed that mycelial growth in the hydrogen-peroxide treated sample was less dense than the samples with boiled substrates. A possible explanation is that after the treatment, the substrate remains protected. When boiling the substrate, the substrate will simply cool down and be susceptible to reentry of malicious organisms. With hydrogen-peroxide treatment, the chemicals remain in the substrate and provide ongoing protection against new organisms. The downside is that the hydrogen-

peroxide still damages the mycelium albeit not as severe as the other micro-organisms. Therefore growth of the mycelium will be slower compared to other methods.

Visual inspection of growth

All samples showed a strong gradient in mycelial density over the height, with stronger concentrations of mycelium at the interfaces. The concentration was also stronger at the top surface than at the bottom. Fig. 3 shows a top, bottom and side view of a sample in which it is clearly visible that the top and bottom have a much denser mycelium and that the top is denser than the bottom. Two explanations are possible for this effect. First of all air enters the mold trough the seams of the lid at the top. Therefore a gradient in oxygen concentration develops with most oxygen at the top and least at the bottom. Secondly, mycelium produces heat during its growth. The heat in the center of the mold will be less able to dissipate than the heat at the

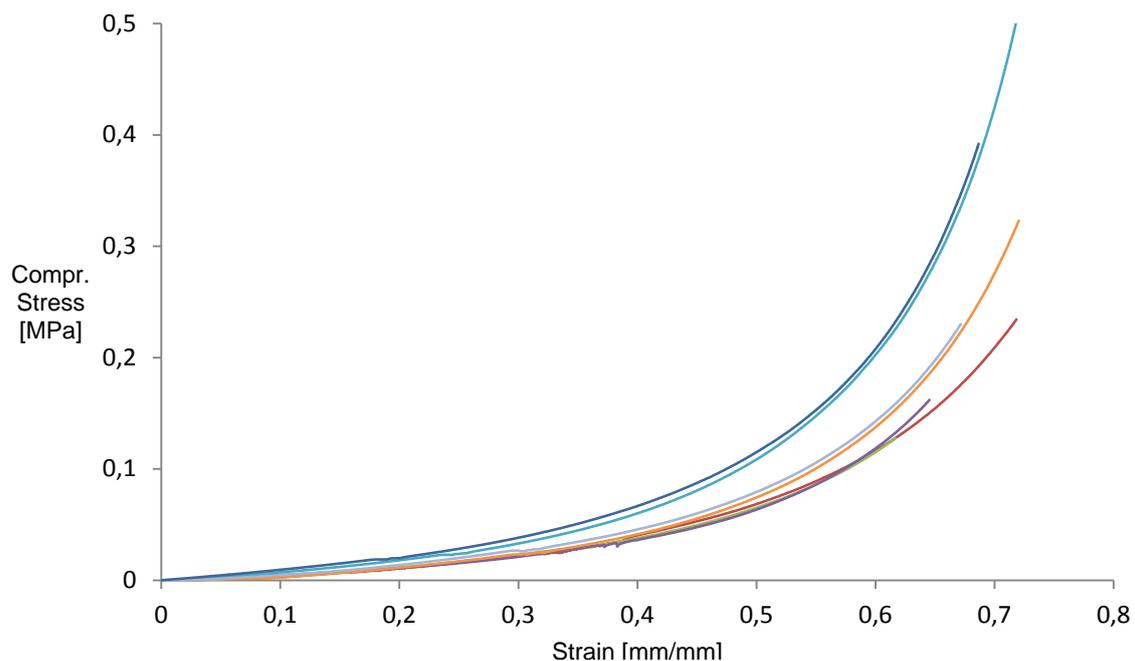


Fig. 5 ; Compressive stress - strain of the second series of samples. All samples of the second group consist of non-woven hemp mats and spawn of *C. versicolor*.

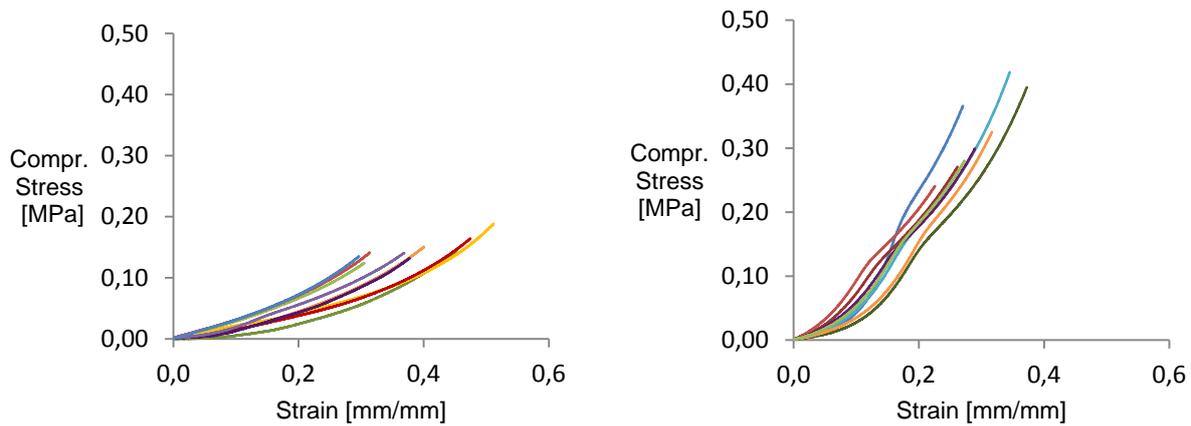


Fig. 7 ; Results of the third group. Samples were first loaded until a load of 100 N was reached (left). Then the load was removed and samples were loaded until 200 N (right).

interfaces. As oxygen stimulates growth and heat deters growth, this would explain why the mycelium is denser at the interfaces and denser at the top. If the thickness of the material is increased, there will be a point at which the center becomes too hot or too anaerobic to allow any growth at all. The implication is that mycelium-materials will have a maximum thickness unless measures are taken to create an even oxygen and temperature distribution.

Regarding the substrates the non-woven hemp mats showed the best compatibility with mycelia as growth could be observed to be much denser. The samples with loose hemp fibers also showed dense colonization but resulted in a mass too incoherent to be used for testing. The hemp hurd samples showed a comparable but slightly less dense growth. The samples with wood chips were markedly less dense and in some cases showed no growth at all.

The samples with *C. Versicolor* showed a denser growth than *P. Ostreatus* in all cases. The combination *P. Ostreatus* with wood chips resulted in a mass that was too sparsely colonized to be used for testing.

4.2 Compressive results

The results from the compressive tests of the first group can be seen in Fig. 4. Both the stiffness and the top of the specimens with hemp mats are higher than the specimens with wood chips. Of the specimens with hemp mats, the specimens with *C. versicolor* show a higher strength and stiffness than the *P. ostreatus* samples. Some literature has reported on a specifically good compatibility between hemp fibers and mycelia (Pickering and Li 2009) (Li, Pickering and Farrell 2009). This concurs with the results shown here that samples with hemp mats show a greater strength and stiffness.

The results of the second group can be seen in Fig. 5.

Table 3 ; Comparison of lightweight structural materials. Strength is defined as stress at failure or 10% deformation.

Material	Strength	Density	Specific Strength	Source
	[kPa]	[kg/m ³]	[kPa m ³ /kg]	
Hempcrete	400	445	0,90	(Aigbomian and Fan 2013)
EPS	35 - 173	12 - 29	1,21 - 13,16	(Elragi 2006)
Cellular Concrete	2000-5000	380 - 720	2,78 - 13,16	(Cox, et al. 2007)
Hemp-mat - Versicolor	24 - 93	170 - 260	0,09 - 0,55	

The stiffness increases exponentially with stress. The stresses at 10% strain are 2,6 - 9,4 kPa. The behavior of the first group differs enormously from the second and third group. This can be explained by two reasons. First of all the specimens from the first test were of rectangular rather than cylindrical shape and also had larger dimensions. This might lead to a form of buckling which would explain the top in Fig. 4. The second reason for the difference might be that the rectangular specimens were tested with different orientation of the fibers in the hemp mats with respect to the load direction. This is schematically shown in Fig. 6. As composite strength is very dependent of fiber orientation (Mallick 2008) this might explain the relatively high stresses in Fig. 4.

Fig. 6 shows the results of the third group. The stiffness increases markedly when the samples are reloaded. The stress at 10% deformation during the

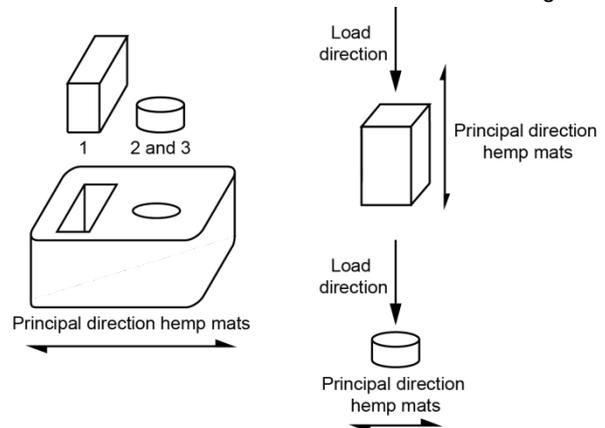


Fig. 6 ; Schematic drawing of the difference in fiber orientation between samples of group 1 and of groups 2 and 3.

first loading was 18,8 (7,0) kPa and the stress at the same deformation during the second loading was 46,5 (20,2) kPa.

5 CONCLUSIONS AND RECOMMENDATIONS

Samples of several substrates and strains of fungi were produced and tested in compression. Visual inspections showed that the combination of non-woven hemp mats and the strain *C. versicolor* showed the densest mycelial growth. This concurs with the results from the compressive tests which showed the highest strength and stiffness for these samples.

Reloading the material resulted in a marked increase in stiffness and greatly increased the stress at 10% deformation.

Table 3 compares the strengths of several lightweight structural materials with the results of the third group. Though the observed strengths are comparatively low it should be noted that Mycelium-based materials are fully bio-based and fully degradable whereas the other materials in *Table 3* are not. Furthermore, this paper presents only the first step in developing a production process for mycelium-based materials. There is room for many optimizations in the process, both in terms of composition and cultivation methods.

To better understand if mycelium-based materials can be of practical use the authors recommend further research into thermal properties of mycelium-based materials and experiments with other substrates.

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