Final report

Research and Development Initiation Project

Exploring mycelium's development into earth bricks

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1. Introduction

The construction sector is currently addressing new issues. The need to adapt is crucial in order to meet the challenges of the 21st century. Indeed, this sector is essential for a society but today emits enormous quantities of carbon dioxide into the atmosphere. The Global Status Report for Buildings and Construction indicates that the construction sector consumes 32 % of global energy and contributes to 34 % of global CO2 emissions. The sector is dependent on materials like cement and steel that are responsible for 18% of global emissions and are a major source of construction waste [1]. In this context, the search for more virtuous materials is gaining momentum and techniques from the past (that are still use is some part of the word) are coming back. In developed countries we lost the skill of construction in earth material, but combining ancestral knowledge with new techniques could be one of the ways to reduce the impact of the construction on the environment.

Earth material has been used during centuries and is still used nowadays in some part of the world, but this material is subject to one main problem: the water. Earth bricks are sensible to humidity and need to be protected by plaster to have a durable construction. Very often the plaster is not an eco-friendly material and can't be recycled. Biopolymers—polymeric substances occurring in living organisms—have been used for centuries to improve the water resistance of earth materials. Different biopolymers are used worldwide, depending on local availability. However, biopolymer efficacy depends on their compatibility with local soil, and the physicochemical interactions between polymers and soils are still poorly understood and controlled. Moreover, the susceptibility of bio stabilized earth materials to microbial and mold proliferation is still a major concerned for the health. It can impact negatively air quality and causes health problems for the residents.

Instead of seeing the mold negatively, we could find some advantages from it if we succeed to control its development. From there, the use of mycelium in construction material could be one way to lower carbon emissions in construction sector and replace the usual plasters or cement. Combining mycelium and other material is something new but promising. It is essential to understand better the interaction between the mycelium and other material.

Fungus is the scientific word to describe any of a kingdom (Fungi) of saprophytic and parasitic spore-producing eukaryotic typically filamentous organisms formerly classified as plants that lack chlorophyll and include molds, rusts, mildews, smuts, mushrooms, and yeasts. The term mushroom is generally misused, in reality it only refers to the visible part which is called fruiting body. Mushroom is the reproductive part of the fungus, it generates spores that disperse via wind, water, or animals. However certain types of fungi do not have mushroom, some fungi reproduce using other methods like forming spores directly on hyphae or creating slime molds. The mycelium, meanwhile, is the vegetative part of a fungus, consisting of a network of fine, thread-like structures called **hyphae**. These hyphae branch extensively to form a dense web that infiltrates soil, wood, leaf litter, or other substrates where fungi grow. Mycelium absorbs nutrients by secreting enzymes that break down organic matter into simpler compounds, which it can then absorb. This mycelial network acts as a natural binder, similar to the role of cement in concrete. Unlike plants, fungi absorb oxygen and release carbon dioxide. They breathe like humans and do not participate in photosynthesis.

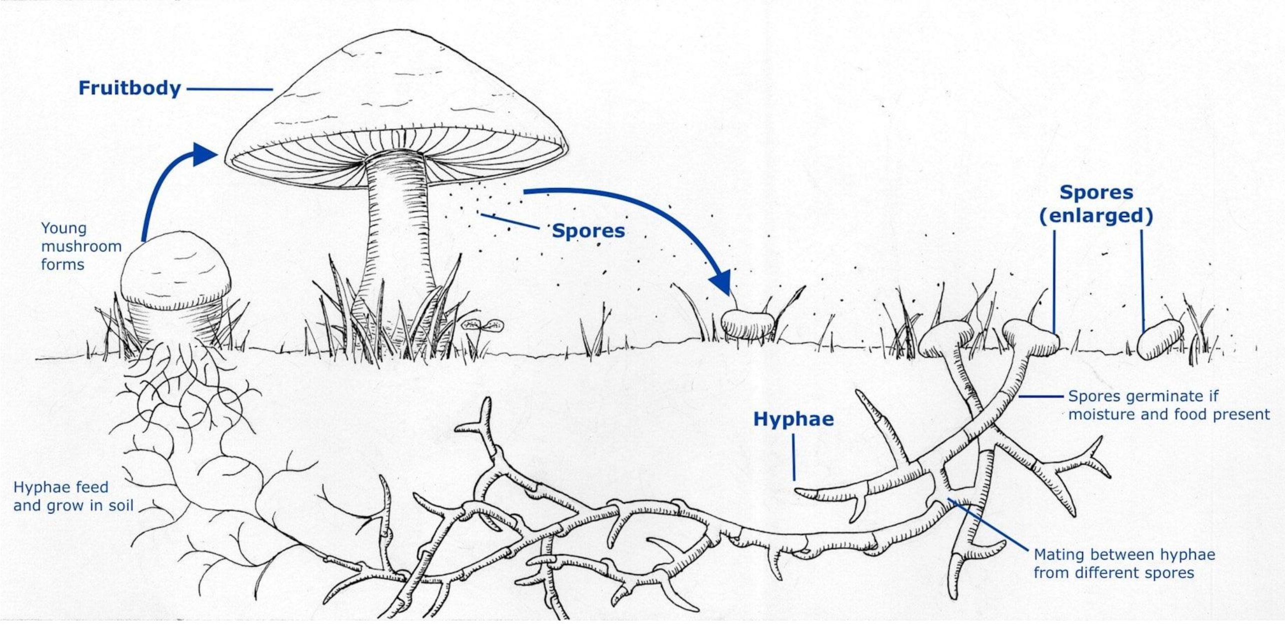

Figure 1: Mushroom life cycle simplified, Rights: Manaaki Whenua – Landcare Research

During the last decades, MBCs—Mycelium Based Composites—have emerged. Currently, the mycelium is mainly combined only with subtracts like hemp, sawdust, rice bran but without earth. These MBCs have various applications. Due to its high porosity, MBCs could be used in construction as a thermal and acoustic insulation material for buildings [2]. Even if, most of the studies that concern mycelium abord the subject of insulation, MBCs are also used in packaging [3] to propose an alternative eco-friendly to single-use plastic.

Most of the time, the mycelium is developed on a substrate and then is inactivated by heating it to stop mycelium growth. However, mycelium's development techniques differ slightly from one another because there is not yet a protocol that has been approved and adopted by all. Some techniques controlled the type of mycelium developed but others not.

For example, the process J.Maximino developed in his study [4] begins with the acquisition of the mycelium fungi from underneath a bamboo shoot. He used one kilogram of rice in a plastic container as the initial main substrate to cultivate the mycelium. He buried it below the soil near the bamboo shoot. The container was left underground for a total of five days. This was vital to allow the substrate to hold the fungal tissue. After the said period, the mycelium was segregated by the researchers from the rice substrate. After the segregation, the mycelium was mixed with 1 L of sugarcane molasses which was stored in a 1.5 L PET bottle. There is then an inoculation period of five days in constant ambient temperature, before it was used in the brick production as a mycelium serum. With this protocol, the essence of the developing mycelium is not controlled.

L.Walter and her team proceeds differently. They incorporated a fine subtract directly in the earth and then in the mold of the brick. They used 4 different substrates, leaf, cactus, grass and tannin but each time with a particle size of 250 µm. Formulations were prepared by mixing biopolymers with soil at a concentration of 2 % by mass of soil. Then, half of the specimens underwent a 21-day humid cure in a climatic chamber at 30 ◦C with 98 % relative humidity. The humid cure condition (30 ◦C/98 %RH) was selected to promote mold growth [5]. Once again, the essence of the developing mycelium is not controlled with this process. No mycelium was introduced in the preparation, only the conditions of the environment allow the growth of mycelium of a certain species.

Another technique consists of first developing mycelium in petri plates by sprinkling water and cellulose powder on the soil in the plates. These plates are then cover by a wet filters before being stored at 28°C for 7-10 days. Then sterilised plastic bags (Heat sterilised) were taken, and substrates were added into plastic bags. Formalin was added to sterilise the substrates and to prevent unwanted fungal growth. Fungal colonies grown on PDA plates were inoculated into the plastic bag and kept in incubation for 7-15 days at 28°C [6]. This process in 2 steps, first developing the mycelium then colonizing the substrate with the mycelium, is long but allows to control the first development of the mycelium before incorporating it into the brick's moulds.

The mycelium act as a binder in the brick, J Maximino observed a presence of natural fibre from mycelium in the stereoscopic microscope [4]. It acts as a bond between the particles of the material that makes up the matrix.

The mycelium also improves the water resistance of the material into which it is introduced. Bio stabilized earth bricks with mycelium show better water resistance than class earth bricks. Compared to earth brick without mycelium, the bricks which underwent a dry cure to develop mycelium reduced sorptivity by around 28 % and rain-induced mass loss by around 64 %, while extending immersion time before deterioration [5]. Lily Walter and her team also show that with certain substrate, there were no mass loss during the spray test that simulates a tropical rain.

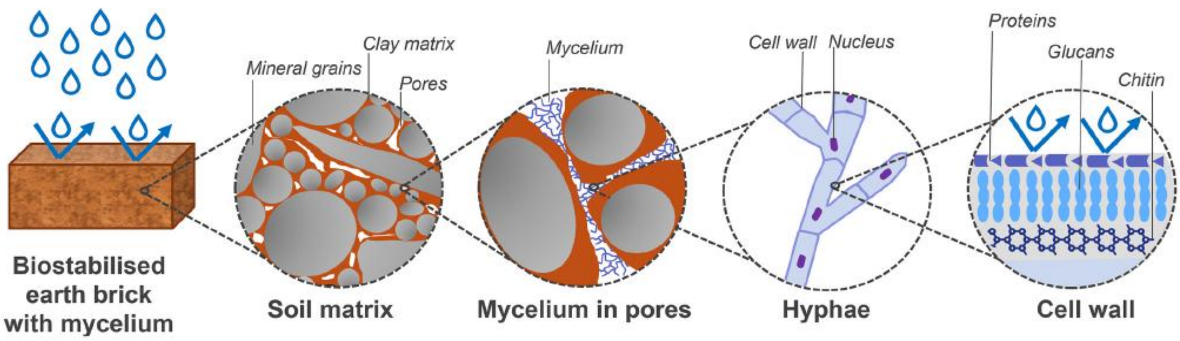

Figure 2: Proposed mechanism for mycelium-enhanced water resistance; rights: Lily Walter

The mycelium appears to improve water resistance of earth bricks [5]. However, on her study, Lyli Walter does not control the type of fungi that is developed in the bricks unlike Jaswant Kandikanti, that uses petri plates to control mycelium development [6]. The objective is to see how the mycelium develops into earth bricks construction controlling the type of fungi and preventing unwanted fungal growth. If the mycelium succeeds to develop and based on the studies cited above, **we hypothesise that the mycelium could improve the water resistance and mechanical properties of earth bricks and reduces shrinkage and cracking during the drying process.**

This study evaluated the mycelium development into earth bricks and its impact on the mechanical properties after heat inactivation. Earth bricks were produced using a certain type of soil (FAC) and a substrate (hemp and sawdust). Different protocols were tested to see the impact on mycelium's development. All samples were finally dried at 80 ◦C to inactivate the mycelium. Physical properties and mechanical properties were evaluated in samples to determine the best protocol and formulation. This research contributes to developing sustainable and cost-effective materials for earth construction with sufficient water resistance. The project incorporates a sustainable vision, with the creation of a brick made from earth, mycelium and a substrate, resulting in a material with a low carbon footprint. Very often the earth is stabilized with cement to obtain good mechanical properties but this incorporation of cement in the earth increases the impact on the environment of the material. The use of mycelium would make the brick a biomaterial and 100% compostable. Unlike recycling that requires energy to produce new material, biodegradation is a natural process that requires no energy or facilities. This material is therefore part of the circular economy approach and would make the construction sector more sustainable.

This project is realised in the context of a research and development initiation project as part of my studies at **INSA Lyon** in the **Civil Engineering and Urban Planning** program. This project aims to develop virtuous materials and brings together the company **Fungimaker**, the **GEOMAS** and **MATEIS** laboratories, and of course the INSA Lyon engineering school.

Fungimaker is a company specializing in the development and production of mycelium-based materials, known as mycomaterials. These innovative biocomposites are created by combining organic substrates with fungal mycelium, resulting in sustainable, biodegradable materials that have potential applications in various industries like construction or even art.

The **GEOMAS** laboratory (GEOmaterials Mechanics and Structures) is a research group at INSA Lyon, specializing in the mechanics of geomaterials and structures. The lab focuses on understanding the mechanical behavior of natural and artificial materials under different conditions in geotechnical engineering, soil mechanics, and structural analysis.

The **MATEIS** laboratory (Materials: Engineering and Science) is an interdisciplinary research center at INSA Lyon, focusing on the study of materials from a scientific and engineering perspective. The lab's expertise covers a wide range of materials, including metals, ceramics, polymers, and biomaterials.

2. Methodology, Materials and methods

2.1 Methodology

To achieve the stated objectives, the following methodology was applied:

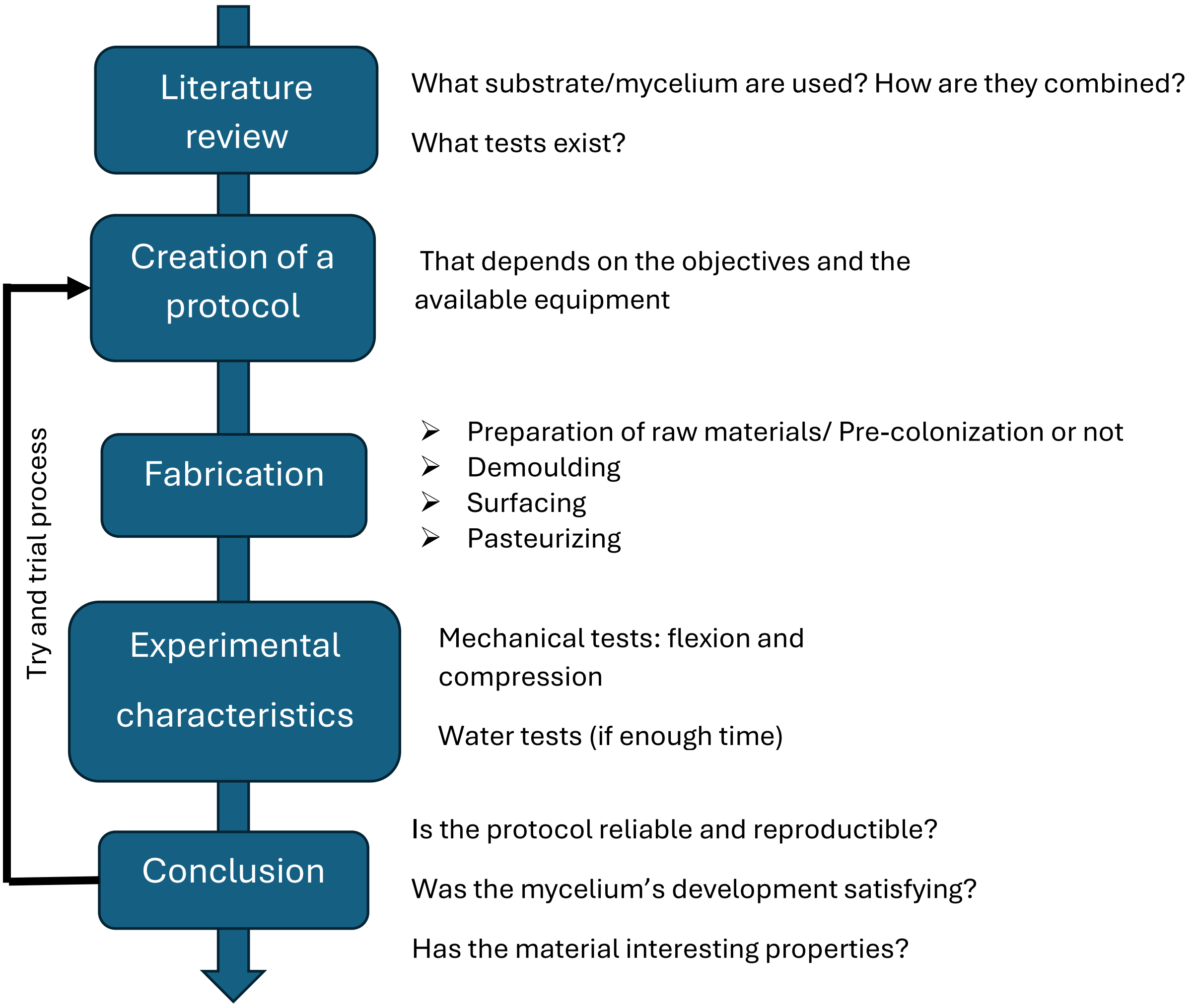

Figure 3: Methodology of the project

2.2 Materials: mycelium, earth and subtracts

2.2.1. The mycelium

The species of mycelium used for this study is *Ganoderma Lucidum*, which is available on cereals grains. The purchase of this mycelium is available online. The grains must be stored in the refrigerator to prevent mycelium's development. The cold stops or at least greatly slows down growth without killing the fungus, which enters a kind of hibernation. As a human, fungus breathe in O2 and breathe out CO2 to live. Its O2 consumption is not very high and sometimes the air already present in the bag is sufficient for its survival. However, if the mushroom remains in the refrigerator for a long time, air must be allowed to enter and CO2 to escape. To do this, a system must be used that does not allow spores from outside to enter, as the mushroom could be contaminated by other species. Special bags are available that only allow air to pass through.

Figure 4: mycelium (Ganoderma Lucidum) on grain

2.2.2. The earth

The quarries of Boulonnais produce aggregates of various sizes for cement concrete. These grains are washed to remove fine clay and limestone particles that would interfere with the concrete's performance. The washing sludge represents 8 million tonnes of fine clay and limestone particles that are currently unused. This coproduct is called FAC for Fine Argilo Calcaire in French. Fine clay-limestone soils are very fine. The size of the largest particles is 0.1 mm. The main advantage of using this type of earth is that we master the particle size distribution. The granulometric curve of the fine clay-limestone particles was obtained by sieving and sedimentometry. The proportion of clay (<0.002 mm) is 37% [8]. They are mainly composed of carbonates such as calcite and dolomite, but also contain smaller amounts of quartz, muscovite and kaolinite. The liquidity limit defines by Ugo de Filipis for the FAC is 32% [9]. This value will be used during the experimentations.

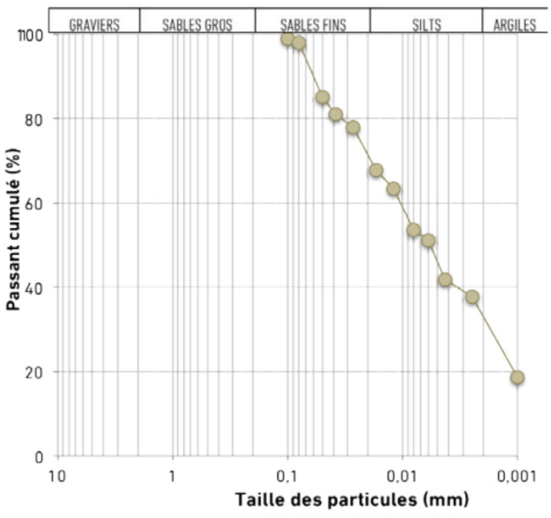

Figure 5: Granulometric curve of FAC, Rights: Moevus-Dorvaux,Mariette [8]

2.2.3. The subtracts

Two types of substrates are used: hemp and sawdust.

The harvested and dried hemp plant yields two co-products after the plant's stems are defibred and crushed: fibres (from the outer shell of the stem) and wood particles called hemp shives. Their bulk density is around 110 kg/m3 and their thermal conductivity is 0.048 W/m.K, which is very low. Hemp shives are composed of 90% cellulose, hemicellulose and lignin. Air occupies 92% of the total volume of bulk shives particles. This air is largely trapped within the particles, which have 78% internal porosity. This allows the hemp shives to absorb up to four times their weight in water [8]. The size of the particle is close to 10mm.

The sawdust is collected from David Perret who supplies us with the mycelium. The types of wood include conifers and broadleaf trees. Therefore, the exact composition of the sawdust will not be known in this study. The particles are fine and do not exceed 2mm. The water contained in the substrate was removed by passing them through a drying oven at 50 °C, resulting in a loss of 8% of the initial mass.

Figure 6: hemp shives dry (left) and sawdust dry (right)

2.2.4. Water and addition

Water is an important element, as its quantity has a significant influence on mycelium development and the final mechanicals properties of the brick. It is also the main vector of contamination. Tap water, which contains natural minerals, was preferred for this study. The French regional health agency reports a pH of 7.4 for the water in Villeurbanne, the municipality where the experiments took place.

In addition, wheat bran is added to enhance the growth of mycelium. Wheat bran positively affected fungal biomass yield, but elevated water absorption ability. Irbe Ilze explains that it improves compressive strength in the sawdust samples but decreased it in the hemp samples [10]. In our case the proportion of wheat bran is low compared to the mass of hemp and the impact on the compressive strength is negligible because the earth will have a structural role and not only the hemp like it is the case in the study of Irbe Ilze. The wheat bran was bought in an organic shop.

Sugar was used for 3 formulations to enhance mycelium's development. It is classic white beet sugar purchased in a shop.

2.3. Methods

2.3.1. Pre-colonization

Before incorporating the mycelium on grain in the brick's mixture, the substrate can be colonized by the mycelium. It consists of putting together mycelium on grains, hemp shives, wheat brand and water in a plastic bag in an environment with constant temperature of 22°C. At day 2 piercing the bag is necessary to provide O2 to the mycelium and let out the CO2 produced. After five days, the hemp is colonized, and the mixture is ready for use. For the pre-colonization the mass ratio between hemp (S like substate) and mycelium (M) is 1 and $\frac{16}{100}$ for the wheat bran (WB). The mass ratio for the water (W) is 2,8.

Table 1: ratio for the pre-colonization

Ratio	
$\frac{WB}{S}$	$\frac{16}{100}$
$\frac{M}{S}$	$\frac{1}{1}$
$\frac{W}{S}$	$\frac{280}{100}$

Then the pre-colonized mixture is added with the soil mixture that consist of FAC and water.

2.3.2. Fabrication of bricks

Here is the timeline that shows fabrication of the bricks. Each step is described further below.

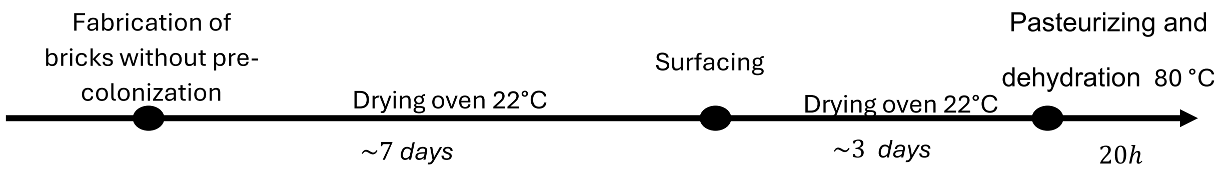

Figure 7: timeline of the protocol

Mixture preparation and moulding

First, it’s very important that all materials and work surfaces are sterilized with pure alcohol to prevent contamination during fabrication and casting. The mixture with FAC and water and the other with the substrate and mycelium are prepared separately.

To start, the wheat bran and the hemp are mixed at speed 1 (60t/min) during 1min. Then, the water is added if the formulation includes water for the mycelium which is not always the case because the mixture with FAC already contains water. The preparation is mixed during 1min30 at speed 1(60t/min). Then, the mycelium is added, and the preparation is mixed during 1min30 at speed 1(60t/min). The mixture with FAC is obtained by adding a certain quantity of water which corresponds to 32% of FAC weight. Finally, both mixtures are mixed during 2 min at speed 1(60t/min) in desired proportions. For the formulations with sugar as addition, the latter is mixed into the water used for the FAC preparation.

Each mixture is casted into polystyrene test specimens measuring 4 x 4 x 16 cm³. One mould is used to make 3 bricks with the same formulation, resulting in the preparation of three samples for each formulation. The volume of one test specimen is 0.256 liters (0.000256 m³). Samples are realized with compaction. A plastic tool measuring 4cm by 4cm is used to compact the mixture into the mould and make sure that the mixture is homogeneous and take the shape of the specimen to have a brick with regular surfaces.

Once the mixture is poured into the three sterilized polystyrene test specimens, the mould needs to go into a drying oven at the constant temperature of 22°C. However, there is a constant flow of air in the oven that prevent mycelium to grow. Indeed, fungi are really sensible to air flow. The mould has to be protected from this air flow. For this, 2 protocols were tested.

The first one consists of covering the mould with a plastic film (figure 8 on the left) with small holes to regulate CO2 and O2 during the growth phase. The second consist of putting the mould into a sealed plastic bag (figure 8 on the right) with enough air inside to avoid piercing the bag.

Figure 8: differences of protocol for the mycelium development

Demoulding

The demoulding step is delicate and requires careful attention. The water content in the bricks after the growing period remains nearly the same as the initial one, it means that bricks are malleable. To make this step easier, after 5 days, during the growing phase, a cut-out is made with a cutter to separate the brick surfaces from the mould walls. After, the growing period, the cut out is made again before breaking the mould to pull out the brick. The bottom surface remains untouched. To remove the brick, it is essential to use a cutter to separate the bottom surface from the mould. If the demoulding is poor, bricks do not have a regular shape and are and are unusable for mechanical tests.

Figure 9 : A cut-out with a cutter (left), a poor demoulding (right)

Surfacing

Only the surface in contact with the air or the film is colonized by the mycelium. To obtain the same result as the top surface, the surfacing step is necessary. The bricks are placed upside down (the surface in contact with the air for 7 days is now in contact with the oven plate) in flat plastic basins (cleaned with alcohol). They are then covered by a plastic film to protect the brick from the air flow. The film is pierced with a needle to enable the mycelium to breathe. Then, plastics basins are placed in the drying oven for the next 3 days at 22°C. For some reasons explained later and problems of planning, the value of these 3 days is subject to change

Figure 10 : bricks in plastic basin covered by a film for the surfacing

Dehydration

The last step of the protocol is the dehydration. It's an essential step for 2 reasons. First, it allows to evacuate water in the bricks and create strong bonds between grains. Secondly, it allows to stop mycelium's growth. The result is an inert brick that does not release spores that are harmful to health.

Plastics films are removed and the basins are placed in the dry oven at 80°C for at least 20h.

2.3.3. Samples characterization

Mechanical tests are conducted to determine the bending and compression resistances of the material. For the bending test, a 3-point bending test is performed with a 100 mm gap between the lower supports.

It is essential to perform the bending tests before the compression tests, as compression affects the internal structure of the material. The SyntX software allows us to control the modular press, with a speed set at 5 mm/min for bending and 4 mm/min for compression.

For the compression tests, each sample are cut with a circular saw to form an approximately 40 x 40 x 40 mm^3 cube (figure 11). During cutting, the specimen must be cut slowly to avoid tearing the fibres.

Figure 11: cubic specimens for compression test (left) and the circular saw (right)

The tests were terminated when a break or tear occurred in the material during bending tests corresponding to a drop in recovery efforts. The compression tests were terminated when the material's deformation reached 30%. The top surface is placed facing the window because it is the surface that presents the most irregularities.

Figure 12: bending test (left) and compression test (right)

3. Results and discussion

Various samples were created. Each time something changed, the ratio, the protocol, the substrate or the addition. A qualitative analysis is made to characterize mycelium's development, and a qualitative analysis is realized thanks to the mechanical tests.

3.1 Summary of the tests

Here is a quick review of the different tests made during the PIRD. Only tests with mycelium are considering, others test with only FAC were made to have a base to compare the mechanical results. Plus, some tests were made to see the mycelium's development. For example, the mycelium was not incorporated with the water and the FAC but placed only on the surface or on different layer of the bricks. We will see later the results of these tests.

Table 2: summary of the experimentations

Realisation	Type of substrate	Type of earth	Additive for Mycelium dev	Number of formulations tested
1	Hemp	FAC	Wheat bran	3
2	Hemp	FAC	Wheat bran	3
3	Hemp and Sawdust	FAC	Wheat bran	2 for each substrate
4	Sawdust/ None	FAC	Wheat bran	2
5	Sawdust	FAC	Wheat bran +sugar + agar agar	1 (but with 2 protocols)
6	Sawdust	FAC	Wheat bran	2
7	Sawdust	FAC	Wheat bran	3

This table allows to know how many tests were conducted and what type of material was used.

Just below, this is a more accurate table with the percentage of the different element. In the table, W represents the water, FAC is the name of the earth, WB is the Wheat Bran, M is the mycelium and finally S is the substrate. As explained before, 2 protocols were used for the mycelium's development. The first one with a plastic film (F) and the second one with a sealed bag (SB).

When in the name of the formulation, FAC is not mentioned, it means that the percentage indicated is a percentage relative to the mass of FAC and not to the total mass of dry materials. For example, in the formulation 5%M/10%Sawdust, the mass of mycelium corresponds to 5% of the mass of FAC and the mass of substrate corresponds to 10% of the mass of FAC.

The term MF represents the Mixture with Fac and water and the term MM represents the Mixture with the Mycelium, the substrate, the wheat bran and the additional water. The last column indicates the percentage of FAC relative to the total dry weight mass (Mycelium+FAC+Substrate+Addition).

Table 3: explanation of the different formulation

N°	Formulation	$\frac{W}{FAC}$(%)	$\frac{M}{FAC}$(%)	$\frac{S}{FAC}$ (%)	Extra W $\frac{W}{S}$ (%)	$\frac{WB}{M}$ (%)	Addition? $\frac{X}{FAC}$ (%)	Protocol	% of FAC/total dry mass
1	33% MF + 66% MMH	32	53	53	280	16	No	F + pre-colonization	46
	50% MF + 50% MMH	32	26,6	26,6	280	16	No	F+ pre-colonization	63
	66% MF + 33% MMH	32	13,3	13,3	280	16	No	F+ pre-colonization	78
2	33% MF 66% MMH	32	63	63	300	16	No	SB	42
	50% MF + 50% MMH	32	32	32	300	16	No	SB	59
	66% MF+ 33% MMH	32	16	16	300	16	No	SB	74
3	50% MF + 50% MM S	32	32	32	300	16	No	F	59
	66% MF+ 33% MM S	32	16	16	300	16	No	F	74
	50% MF+ 50% MMH	32	32	32	300	16	No	F	59
	66% MF + 33% MMH	32	16	16	300	16	No	F	74
4	66% MF + 33% MMS	32	21	21	200	16	No	SB	69
	90%FAC/10%M	32	11	0	No	16	No	SB	88
5	5%M/5%Sawdust (sealed bag)	32	5	5	No	16	No	SB	90
	5%M/5%Sawdust (film)	32	5	5	No	16	No	F	90
6	5%M/5%Sawdust+ sugar	32	5	5	No	0	Sugar/ 15 2g agar-agar	SB	87
	10%M + sugar	32	10	0	No	0	Sugar/ 15 2g agar-agar	SB	87
7	5%M/5%Sawdust	32	5	5	No	16	No	SB	90
	10%M/5%Sawdust	32	10	5	No	16	No	SB	86
	5%M/10%Sawdust	32	5	10	No	16	No	SB	86

Just above, we can notice that in the first 4 experiment, additional water was incorporated in the mixture containing the mycelium, the substrate and the wheat bran. It involves a ratio $\frac{M}{FAC}$ and $\frac{S}{FAC}$ much higher than the 3 last experimentations.

3.2 Qualitative analysis

The first thing we can evaluate is the mycelium development by looking on the surfaces and the aspect of the bricks. A difference is made between the top surface which is in contact with the air during the development period and the other surface which are in contact with the air during the surfacing period. First, we evaluate the mycelium development on the top surface after the 7 days development period for the 7 realisations.

Mycelium development after 7 days			
1			
	33% MF + 66% MMH	50% MF + 50% MMH	66% MF + 33% MMH
2			
	33% MF + 66% MMH	50% MF + 50% MMH	66% MF + 33% MMH

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<tr><td rowspan="2">3</td><td></td><td></td><td></td><td></td></tr>
<tr><td>50% MF + 50% MMH</td><td>66% MF + 33% MMH</td><td>50% MF + 50% MMS</td><td>66% MF + 33% MMS</td></tr>
<tr><td rowspan="2">4</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="2">66% MF + 33% MMS</td><td colspan="2">90%FAC/10%M</td></tr>
<tr><td rowspan="2">5</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="2">5%M/5%Sawdust (sealed bag)</td><td colspan="2">5%M/5%Sawdust (plastic film)</td></tr>
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<tr><td rowspan="2">6</td><td colspan="2"></td><td></td></tr>
<tr><td colspan="2">5%M/5%Sawdust+ sugar</td><td>10%M + sugar</td></tr>
<tr><td rowspan="2">7</td><td></td><td></td><td></td></tr>
<tr><td>5%M/5%Sawdust</td><td>5%M/10%Sawdust</td><td>10%M/5%Sawdust</td></tr>
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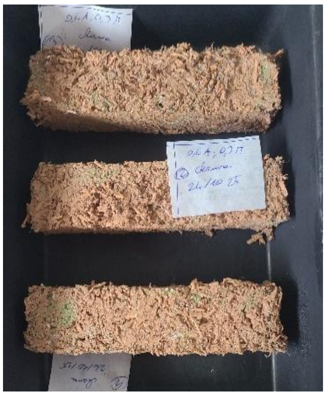
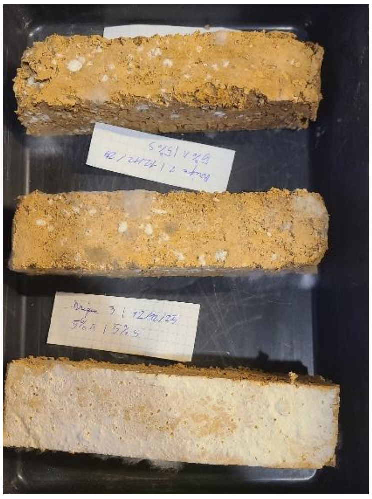
Mycelium development after the surfacing

1				
	33% MF + 66% MMH	50% MF + 50% MMH	66% MF + 33% MMH	
2				
	33% MF + 66% MMH	50% MF + 50% MMH	66% MF + 33% MMH	
3			No surfacing	No surfacing
	50% MF + 50% MMH	66% MF + 33% MMH	50% MF + 50% MMS	66% MF + 33% MMS

4		
	66% MF + 33% MMS	90%FAC/10%M
5 (21 days)		
	5%M/5%Sawdust (sealed bag)	5%M/5%Sawdust (plastic film)
6 (17 days)		
	5%M/5%Sawdust+ sugar	90%FAC/10%M + sugar

7 (5 days)			
	5%M/5%Sawdust	5%M/10%Sawdust	10%M/5%Sawdust

It is possible to draw several conclusions from these trials.

N°1: The results in the first experimentation are not conclusive; the mycelium has developed very little. This is due to the plastic film that covered the moulds during the growing period of 7 days, it was misplaced and allowed air to pass through. It is important to note that air is constantly blown into the drying oven and that the mycelium does not like draughts to develop. Moreover, bricks are fragile, especially the 33% MF + 66% MMH formulation. The mycelium developed more on the surfaces in contact with the mould than the one in contact with the air. This proves that an environment without draughts is necessary for the mycelium to develop. The water content was low in the bricks; it explains the no-development of the mycelium during the surfacing. Finally, we can ask ourselves if the pre-colonization is necessary, to know the impact, the next experiment is realised without pre-colonization of the hemp.

N°2: The second experiment shows better results in term of mycelium development. Moulds were placed into sealed bag during the 7 days period and despite the absence of pre-colonisation the mycelium developed well on the top surface. It should be noted that growth is weaker for bricks with the 33% MF + 66% MMH formulation. Plus, 2 out 3 of these bricks broke during demoulding. The mechanical characteristics are too weak because the earth content is too low. This formulation will be abandoned for the next experimentations. As the moulds were in sealed bags, the water content is the same as the initial one, and the bricks stick to the moulds. This causes problems when removing them from the moulds, as some bricks are no longer uniform and cannot be used for mechanical testing. Using a cutter to trim the bricks during the mycelium development period could prove effective. Concerning the surfacing, it worked much better than the first experimentation especially for the bricks with 50% MF + 50% MMH formulation. This is due to the high rate of water.

Figure 13: problem of demoulding and bricks malformed

N°3: The third experimentation completely failed. A strong musty odour was released when the oven was opened. Another fungus contaminated all the bricks, and the incorporated mycelium did not develop at all. This could be due to a contamination during the experimentation or the mycelium incorporated was already contaminated. The mycelium developed on the fridge and a greenish liquid was present at the bottom of the sachet.

Figure 14: difference between good and bad mycelium

Plus, there was no difference before and after surfacing. The two moulds containing sawdust contained far too much water. The same water absorption rate was used to calculate the amount of water to be added to the sawdust, even though its absorption rate is lower than that of hemp. The bricks couldn't be removed from the mould, so they didn't underwent surfacing. However, we the sawdust as substrate, we obtain more homogeneous bricks.

N°4: In the fourth experimentation, we tried to incorporate the mycelium in the earth without substrate to nourish it. The mycelium developed a bit, but nothing compared to the other formulation. The bricks with the 66% MF + 33% MMS formulation are the easiest one to demould because the water rate of the bricks is lower. Indeed, the water absorption rate has been modified to 200% instead of 300%. However, the surfacing didn't work well, the mycelium didn't develop on the others surfaces.

N°5: For this experimentation, the rate of mycelium and substrate has been lowered. Plus, no water was added for the substrate and the mycelium. The goal was to see if the water present in the mixture with the earth was enough to allow mycelium development. We also wanted to compare the 2 protocols, the one with the film and the other with the sealed bag. First, the mycelium succeeded to growth without extra water. It's an improvement because the bricks were easier to demould. We can also observe on the pictures a clear difference in mycelium development between the two protocols. The protocol with the sealed bag seems to work better. This is not viable at huge scale production but instead of the plastic bag, a drying oven without air flow would lead to same result. The bricks stayed 21 days in the drying oven for the surfacing; it was done to see if the mycelium just needed more time to cover all the surfaces. The experience was not conclusive, a kind of fluff measuring few millimetres, consisting mainly of air, has developed all around the brick. The fluff has spread and extended to the walls of the plastic basin. This fluff seems to retain water and does not adhere to the brick. When touched, the fluff comes off and black spots can be seen on the brick. This is a sign of the presence of another fungus.

Figure 15: brick colonized by another fungus

By leaving the bricks for so long, the likelihood of contamination was higher. The aim is to encourage the growth of our mycelium so that the others do not have time to develop. We need to understand why it is not working and find another way to achieve the surfacing.

N°6: The idea of the sixth experimentation was to boost mycelium development by giving it sugar. The sugar was incorporated and mixed in the water to ensure that there was sugar everywhere throughout the brick. The "5%M/5%Sawdust+ sugar" formulation worked well during the mycelium development on the top surface. All the surface is white, and we can see that the layer of mycelium on top has small holes in it. In addition, bubbles have formed, similar to those that form when making caramel. This indicates that a reaction has occurred between the sugar and another material. Once again, the surfacing didn't work, only small white points are visible on the surfaces despite a 17-day surfacing period

Figure 17: bubble on the brick with sugar

On the picture we can observe the difference between the experimentation 5 and 6 for the 5%M/5%Sawdust formulation, the only difference in the formulation is the addition of sugar in the experimentation n°6.

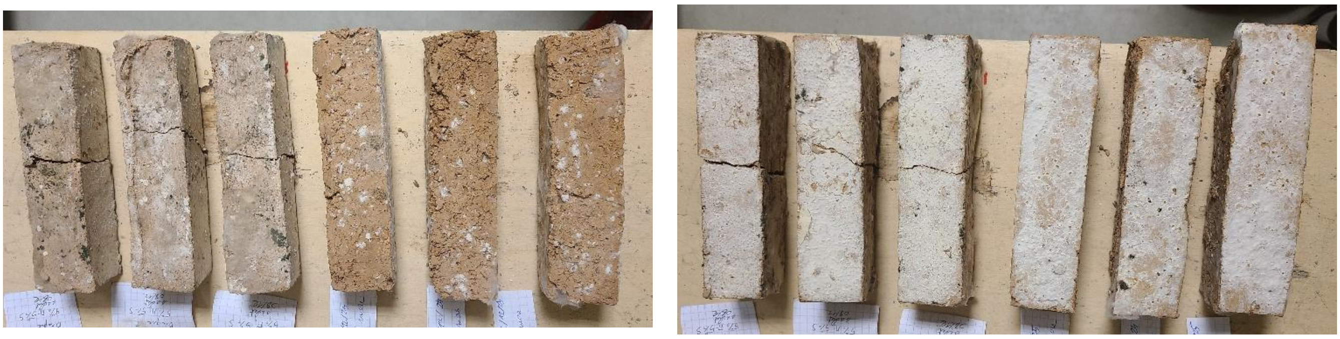


Figure 18: difference between brick with and without sugar

The "90%FAC/10%M + sugar" formulation didn't show conclusive results; the mycelium didn't develop and the bricks could not be removed from the mould. That's why they stayed in the moulds for 22 days. After this period the mycelium succeeded to develop a bit but didn't cover the entire surface. This confirms that a substrate is necessary to nourish the mycelium and allows it to grow.

N°7: For the final experimentation, 3 formulations were tested by changing the percentage of mycelium and sawdust. The 3 formulations present a good mycelium development after only four days. To obtain a good surfacing, some water was sprayed on the bricks after the demoulding. We thought that maybe the mycelium didn't have enough water on the surfaces to grow. Apparently, we were wrong, after 5 days the surfaces weren't white, while at that time the top surface were already totally white. The formulation where the surfacing worked the best is the 10%M/5%Sawdust.

3.3 Quantitative analysis

Compression and flexion tests were conducted for each formulation when it was possible. To compare the influence of the mycelium and the substrate, a total of 5 bricks with only FAC was made. 3 of these bricks were put in a sealed bag for 10 days and then were heated with others bricks with mycelium at 80°C for several hours. The 2 others were put directly in the drying oven without sealed bag or plastic film, so the bricks dried during 10 days before going at 80°C. We can see on the graphics that the 2 protocols induce slightly different results.

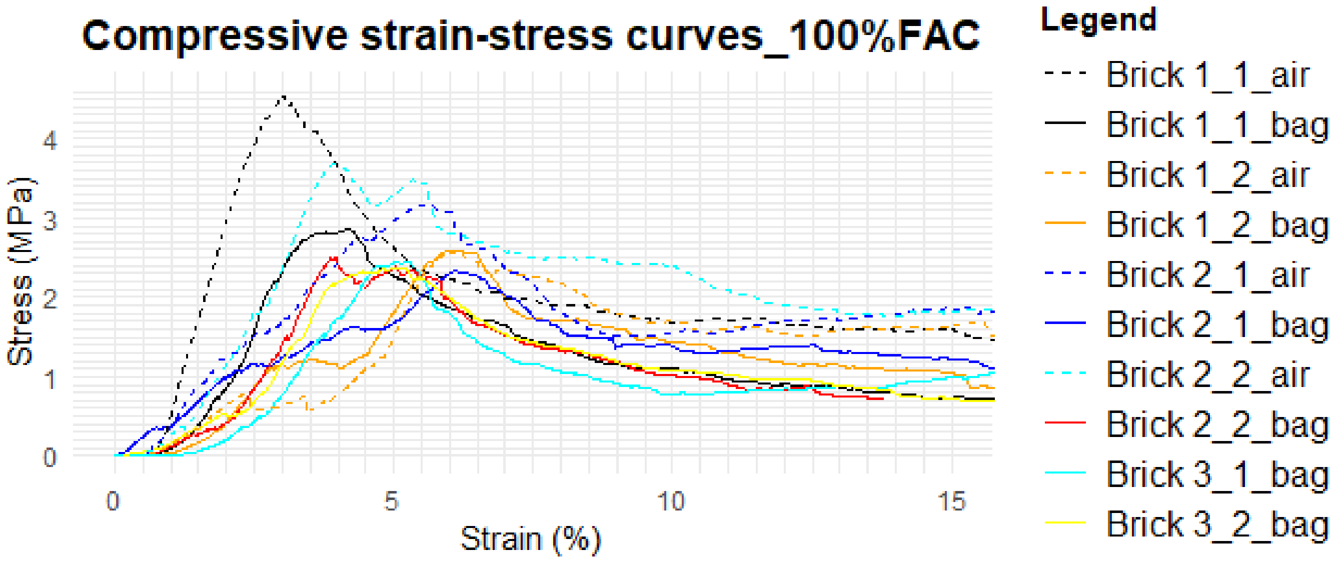


Figure 19: compressive strain-stress curves 100% FAC

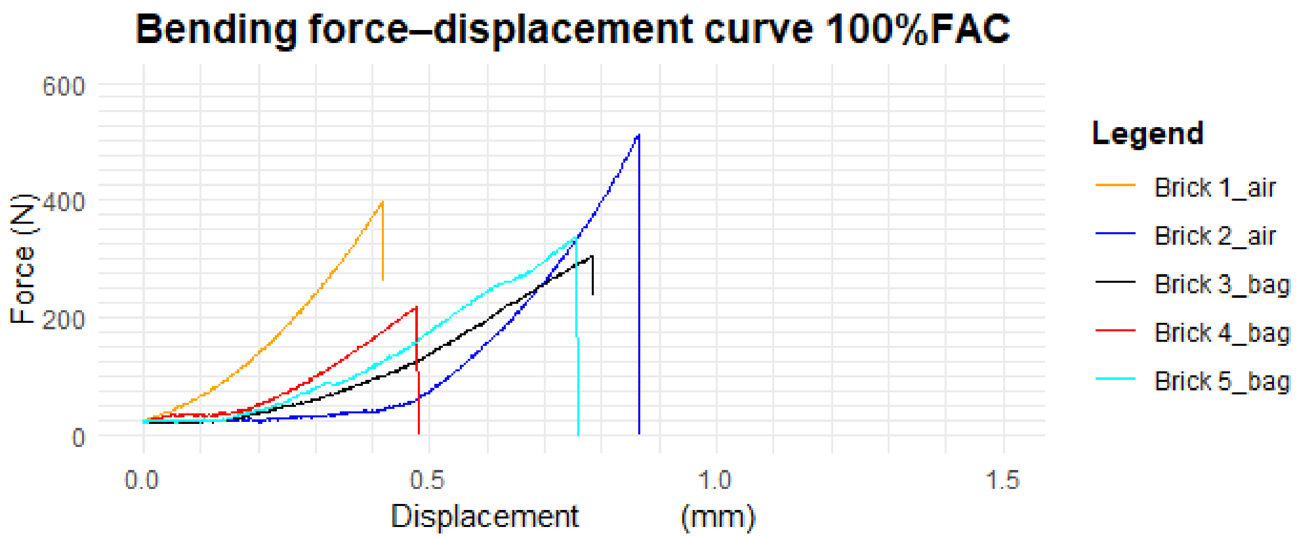


Figure 20: bending force displacement curves 100% FAC

Table 4: mechanical properties of 100%FAC bricks

	Compression strength max (Mpa)	Bending strength (Mpa)	Density (kg/m^3)
Min	2,34	0,667	1788
Max	2,85	1,029	1819
Average	2,52	0,879	1803

Bricks without the bag have slightly better mechanical characteristic than bricks that remained in the bag. This means that drying affects mechanical properties; in our case, a longer drying at a lower temperature result in better properties. It's the case for the bending and compression strength. Even if the results are a bit different, the average we'll be used to compare the mechanical properties of bricks that contain mycelium. Concerning the shrinkage, it's equivalent for the drying method

The maximal value of compression strength is used for the comparison. However, the initial experiments produced compression curves without peaks because the samples had a high compressibility capacity. In these cases, the stress value corresponding to 10% deformation is selected. The table below, show the results of mechanical tests for each formulation.

2 reference bricks with 100% FAC (figure 21, the 2 on the right) and one reference brick with FAC, sawdust and sugar (figure 21, brick on the left) were created but the bricks were unexploitable for mechanical tests. They had no cohesion; the bricks had broken on their own. Plus, the bricks without sawdust the bricks have expanded from 2 to 4mm from the top surface. There has been a reaction between FAC and sugar, the sawdust seems to prevent or at least decrease the effects of this reaction.

Figure 21 : control bricks with 100% FAC+ sugar (the 2 bricks on the right) + sawdust (brick on the left)

Table 5: mechanical properties of all the formulation

		Compression strength			Bending strength			
N°	Formulation	Min (MPa)	Max (MPa)	Average (MPa)	Min (MPa)	Max (MPa)	Average (MPa)	Density (kg/m^3)
1	33% MF + 66% MMH	0,10	0,2	0,15	0,020	0,030	0,026	X
	50% MF + 50% MMH	0,03	0,12	0,077	0,044	0,054	0,049	339
	66% MF + 33% MMH	0,08	0,21	0,14	0,068	0,204	0,134	531
2	33% MF + 66% MMH	X	X	X	X	X	X	355
	50% MF + 50% MMH	0,10	0,15	0,14	0,115	0,141	0,127	495
	66% MF + 33% MMH	0,22	0,34	0,30	X	X	X	704
3	50% MF + 50% MMH	0,16	0,30	0,22	0,083	0,155	0,115	424
	66% MF + 33% MMH	0,30	0,60	0,43	0,259	0,313	0,281	701
	50% MF + 50% MMS	0,27	0,39	0,34	0,17	0,25	0,210	692
	66% MF + 33% MMS	0,35	0,45	0,39	0,30	0,43	0,34	874
4	66% MF + 33% MMS	0,45	0,55	0,50	0,43	0,51	0,46	1420
	90%FAC/10%M	0,88	1,31	1,10	0,50	1,05	0,63	875
5	5%M/5%Sawdust (SB)	1,78	2,38	2,14	1,14	1,50	**1,31**	1350
	5%M/5%Sawdust (F)	1,50	2,14	1,80	0,77	1,01	**0,88**	1322
6	5%M/5%Sawdust + sugar	2,34	3,61	**2,95**	1,44	2,86	**2,09**	1436
	90%FAC/10%M + sugar	X	X	X	X	X	X	X
7	5%M/5%Sawdust	1,36	1,87	1,60	1,28	1,34	**1,31**	1337
	10%M/5%Sawdust	1,32	1,66	1,5	0,83	0,88	**0,85**	1244
	5%M/10%Sawdust	2,25	2,86	**2,62**	1,37	1,47	**1,42**	1302

It should be remembered that some formulations have the same name, but as additional water may or may not have been added to the substrate, they do not have the same proportions of material in the final brick. Plus, the cross in the table indicate that some bricks were unexploitable for the mechanical tests due to theirs shapes or their consistency.

First, we observe a particularity during the compression test, When the sample has reached decay, a pyramid is formed, the base of which is the upper surface that remains intact. The mycelium therefore provides cohesion to the soil particles (photo in the Appendix).

Bricks with additional water for the substrate (experimentation 1 to 4) have much weaker mechanical properties than those without the addition of water (experimentation 5 to 7), this is due to a higher ratio of earth in the bricks. The additional water seems useless for the mycelium's development as seen before but also reduces the mechanical properties by lowering the FAC ratio in the bricks.

We notice that only 2 formulations have a better compression strength in average than the 100% FAC bricks, the 5%M/10%Sawdust formulation and the 5%M/5%Sawdust + sugar formulation. There is a noticeable difference between the mechanical properties of bricks with sugar and those without. Maybe the incorporation of sugar allows to align the clay tiles, which translates into better mechanical properties. With the control brick, we can suppose that a reaction between the sugar and the FAC exist. This reaction involves a swelling that can be prevent by the presence of sawdust. However, the control brick with sawdust didn't have any cohesion and the one with the mycelium had, so we can suppose that the mycelium brings cohesion between the grains of FAC but in the meantime the "90%FAC/10%M + sugar" formulation bricks were unexploitable. It proves the necessity of a substrate. Maybe the cohesion is provided by the mycelium, but the latter needs substrate to develop and bind the grains together.

Another point is that addition of substrate or the mycelium allows to obtain better bending strength, the rupture is less fragile with these bricks. We should have made bricks with only substrate and FAC to compare the real impact of the mycelium.

In the experimentation n°5 and 7, the exact formulation was tested. The bending strength is similar, but the compression strength is 34% higher for the formulation n°5 while the density is similar. It shows that these results should be taken with a pinch of salt. Maybe the small slenderness close to 1 affects the results, sometimes the width is 45mm and other time it's only 35mm. Or maybe, the mycelium developed well inside the bricks with a higher rate of substrate and provides a matrix that links the soil particles, resulting in improved mechanical characteristics.

3.4 Additional analysis

Other types of experiments were conducted to observe the mycelium development. Two plates were produced using the formulations: 90%FAC/10%M and 66% MF + 33% MMS.

On the 66% MF + 33% MMS bricks, the mycelium developed well on the top surface but not on the 90%FAC/10%M bricks. However, on the 2 plates, the mycelium development is good. Some grains with mycelium were added on the half of the plates with 2 wheat bran trails.

Figure 22: photos of the mycelium plates

The mycelium developed more on the half surface with the added grains, but the growing is uniform. The growing condition were similar. The difference in results could be explained by compaction: in the mould, there was little air in the brick for the mycelium to develop to the surface, whereas in the plate, there was no real compaction, it was easier for the mycelium to reach the surface. Plus, there is more surface in contact with the air and since the mycelium needs air to grow, it found a more favourable environment on the plate than in the mould.

Another experiments was conducted by putting the mycelium on different layer of the bricks. One brick had mycelium on the top and bottom surface, another one had mycelium on the central layer, and the last one had a layer at ~5mm from the top.

M on the top/bottom

M in the middle

M at 5mm from the top

Figure 23: bricks with mycelium at different level

We can see that the mycelium succeeded to reach the surface even when it was in the middle of the bricks. However, it was difficult to put the layer of mycelium on a flat surface.

Figure 24: mycelium development on different layers

The mycelium didn't develop on the bottom surface of the brick; the lack of air is the most probable reason.

Finaly, some grains with mycelium were put on a plate only made of FAC to measure its expansion.

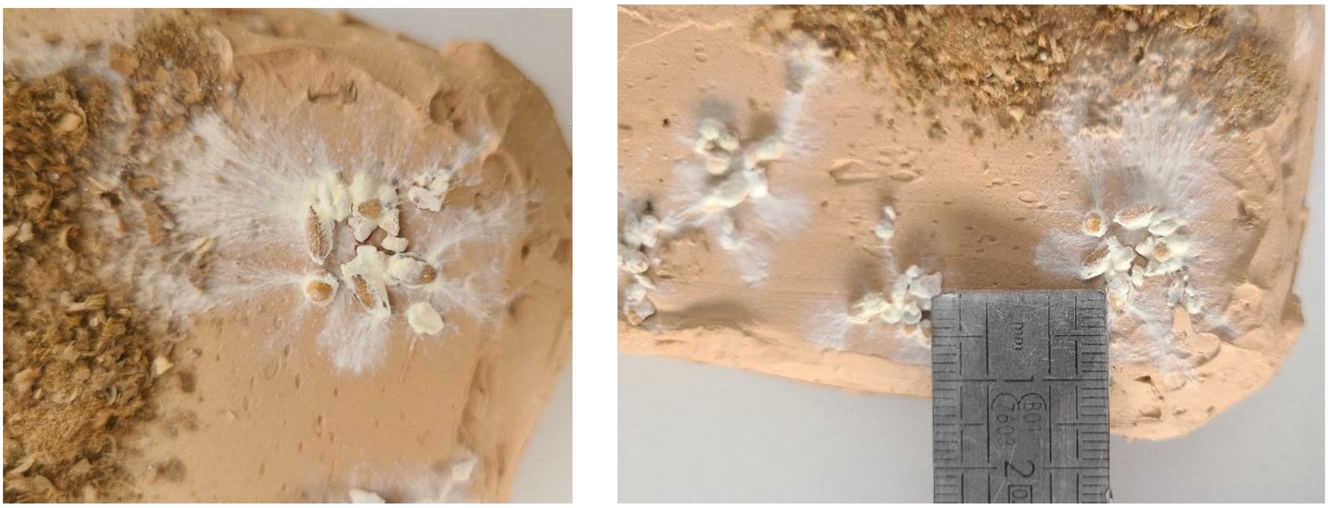


Figure 25: expansion of the mycelium

It shows that the mycelium is aware of its environment and will develop favourably in a direction where it can feed itself. Plus, the expansion of the mycelium can reach more than 1 cm from one grain.

N°	Formulation	Loss of volume due to shrinkage (%)	Gain compared to 100% FAC bricks	% of FAC/total dry mass
	100% FAC	**23,38**	0	
1	33% MF + 66% MMH	X	X	46
	50% MF + 50% MMH	10,83	-12,55	63
	66% MF + 33% MMH	7,50	-14,88	78
2	33% MF + 66% MMH	5,00	-18,38	42
	50% MF + 50% MMH	3,75	-19,63	59
	66% MF + 33% MMH	8,75	-14,63	74
3	50% MF + 50% MMH	3,10	-20,28	59
	66% MF + 33% MMH	4,13	-19,25	74
	50% MF + 50% MMS	7,97	-15,41	59
	66% MF + 33% MMS	15,15	-8,23	74
4	66% MF + 33% MMS	14,04	-9,34	69
	90%FAC/10%M	18,22	-5,16	88
5	5%M/5%Sawdust (SB)	11,61	-11,77	90
	5%M/5%Sawdust (F)	10,66	-12,72	90
6	5%M/5%Sawdust + sugar	10,27	-13,11	87
	90%FAC/10%M + sugar	X	X	87
7	5%M/5%Sawdust	12,34	-11,04	90
	10%M/5%Sawdust	6,75	-16,63	86
	5%M/10%Sawdust	9,14	-14,24	86

It was hypothesised that the mycelium reduced shrinkage. Indeed, it can be seen that all bricks have less shrinkage than bricks containing only FAC. Bricks with hemp have the lowest rate of FAC, it is therefore logical that they have the lowest shrinkage. The interesting result that needs to be to be explored further is the low shrinkage of the “10%M/5%Sawdust” formulation. Compared to the “90%FAC/10%M” formulation, the shrinkage is much lower, there are 2 explanations possible. First, the sawdust absorbed water. Secondly, the mycelium developed well in the bricks thanks to a higher rate of mycelium and the presence of sawdust and the micellar network created made it possible to reduce shrinkage.

4. Conclusion

Firstly, experiments have shown that it is possible to control the species of mycelium that develops in bricks. By promoting its rapid development, the appearance of other fungi can be prevented. During the first experiments, we constated that an environment without air flow is necessary for the mycelium to develop. However, the mycelium develops better if it is in contact with the air and not plastic.

Secondly, we can conclude that a presence of a substrate promotes greatly the mycelium development. The ratio of the substrate can be low, only 5% of substrate relative to the FAC mass are necessary. A pre-colonization of the substrate is not necessary. Plus, it allows to reach higher bending strength compared to the bricks made only of FAC, which present a more fragile behaviour at the break. Adding water for the substrate and the mycelium is absolutely not necessary, the water used to make the mixture with the FAC is sufficient to allow the development of the mycelium.

Regarding surfacing, it would be interesting to understand why it does not work well. Changes in atmosphere between the drying oven and the outside air during demoulding could be the cause. However, this seems unlikely because the mycelium is stored in the refrigerator and still manages to grow on the surface from one experiment to the next. The relevance of the surfacing can be aborded. If the objective is to create a building material, only one surface will be in contact with the outside. This could be the top surface of the mould. Other surfaces do not need to be protected from moisture. If only one surface needs to be covered by mycelium, this would greatly simplify the manufacture of bricks. Indeed, the main problem of manufacturing is that we want to keep the water content in the brick high so that the surfacing works, but this makes it difficult to remove from the mould because the material has not dried and therefore sticks to the mould walls. This manipulation is probably the critical point. We have shown with the plate that the mycelium can develop well on several surfaces, but when a manipulation is realised, the mycelium develops much less. It is as if the fungus felt attacked and stopped growing.

Finaly, as the mechanical resistance does not appear to be higher in bricks with mycelium, it would be interesting to observe on the microscope if the mycelium developed well inside the bricks and creates a matrix that links the soil particles and increase mechanical properties. Plus, control samples containing only soil and substrate must be produced in order to provide a basis for comparison. The presence of mycelium inside the brick could also explain the decrease in shrinkage. Moreover, to confirm the relevance of incorporating mycelium into bricks, tests will need to be conducted to see whether bricks with mycelium are more water-resistant than bricks without mycelium.

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Appendix

Here is an example of the quantities to use for the “5%M/5%Sawdust” formulation. The total weight of 1500g is the minimum weight to make 3 bricks, with these quantities, losses are minimized. As explained before, the quantities are calculated in relation to the mass of FAC.

Caracteristics	
Limit of liquidity of FAC	0,32
% M/FAC	5
M/S	1
% mass of wheat bran/mycelium	16

Total weight (g)	1500
Water + FAC	1386,55
FAC	1050,4
Water	336,1
Mixture with mycelium	113,45
Mycelium	52,5
Wheat bran	8,4
Sawdust	52,5

Figure 26: pyramid remaining after compression test