Introduction of Mycelium-Based Composites in the Portuguese Industry

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The majority of contemporary economic outputs make use of scarce valuable resources with little regard for their end-of-life or environmental impact. As a result, the industry must take a stand and demonstrate a greater commitment to environmental stewardship now more than ever. In March, the European Union presented the new European Green Deal, providing new barriers for businesses as they adjust to new green laws and levies. Considering this, it's clear that demand for new environmentally friendly and sustainable materials is increasing. Mycelium composites are a biodegradable and sustainable material that adheres to the circular economy principle. Due to mycelium's ability to transform organic leftovers into a variety of new materials, this dissertation will examine two Portuguese companies' by-products and how they can be used as a substrate. This will be performed by incorporating mycelium into the substrates, and examining several variables such as substrate particle size, additives, and mycelium species used.

Keywords: Mycelium- based composites, sustainability, circular economy.

1 Introduction

We are living in an era in which the circular economy concept is gaining traction. With each passing day, it becomes more critical to shift the paradigm and abandon linear thinking. If we are to have a green economy and a functional society, we must create new solutions that are circular and sustainable in nature [1]. Taking this into account, the purpose of this thesis is to introduce a new sustainable material into the Portuguese industry that adheres to the circular economy concept. This material is referred to as mycelium-based composites.

One of the principal impulses for this sustainable transition is the European Green Deal announced in March 2020. The Green Deal is a European plan to make the EU's economy sustainable. It intends to make Europe climate neutral by 2050, meaning the achievement of net-zero greenhouse gas emissions for EU countries [2]. Another notable feature of the Green Deal is the Circular Economy Action Plan.

The transition towards a circular economy is already underway, with front-runner businesses, consumers, and public authorities in Europe embracing this sustainable model. The Circular Economy Action Plan presents measures to make sustainable products the norm in the EU, empower consumers, focus on the sectors that use the most resources and where the potential for circularity is high, such as packaging, and ensure less waste [3].

For many years, mycelium-based composites have been under study and have recently gained interest due to its circular and sustainable nature. Mycelium can be thought of as the mushroom's roots. These "roots" grow and penetrate organic substrates, forming a tangle of branching fibers which is referred to as a mycelium-based composite. This substance may exhibit similar characteristics to plastics and even wood. The mechanical properties of this material are mainly determined by the fungus species, the organic substrate, and the optimal growing conditions used [4]. As such, the focus of this thesis will be to contact various companies in the Portuguese industry, including those in the wine, rice, and mushroom sectors. After collecting samples from these companies' organic by-products, we will investigate the possibilities of using those by-products as a substrate to create mycelium-based composites. The outcome of these composites is typically materials similar to styrofoam used in packaging, which is why this thesis will focus on the packaging sector.

The first company contacted is Delta Cafés. Delta is a Portuguese brand and firm that specializes in coffee roasting and packaging. After meeting with Delta's Senior Innovation Manager, it was found that the company already has an effective end-of-life strategy for its coffee grounds. They have a contract with Nãm Mushroom, a small company. Nãm creates edible mushrooms by utilizing used coffee grounds from Delta Cafés. Thereafter, Nãm Mushrooms was approached to ascertain its organizational structure. It was discovered that their mushroom cultivation produces a significant amount of by-product, which is now being used as compost for several small local farms. Even though their residues follow a sound end-of-life strategy, they were intrigued by the prospect of enriching their value chain into the coffee grounds by-product. Nãm mushrooms is a promoter of sustainability and circular economy, and as such, they were thrilled to be able to convert their residues into a new material capable of replacing one of the most harmful products on the market, plastics packages. Another company contacted is Sogrape, a Portuguese company devoted to wine cultivation, manufacturing, and export. The Head of Research and Development expresses concern about the end of life of their most valuable by-product, pruning wood. Due to the lack of use for their by-product, hundreds of tonnes of pruning wood are burned each year. As a result, Sogrape is keen to discover a solution that adds value to their by-product. Rice husks are a by-product of the rice cultivation process. Is frequently utilized as soil compost or as a livestock feed supplement. However, it can be employed as a valuable resource in the production of mycelium-based composites. This way not only may the by-product be increased in value, but also a material capable of replacing harmful plastics can be developed.

Mycelium-based composites are a material that addresses several of the industry's most significant challenges. However, an important drawback of mycelium-based composites is the manufacturing

process and, in some situations, the cost. Because mycelium is a living organism, the manufacturing process can be lengthy and challenging to adapt to.

2 Experimental Methodology

2.1 Introduction

This experimental work is carried out to examine the possibility of developing mycelium-based composites. To obtain the desired material, it's necessary to carry out the work steps, already described in the state of art:

- **Matrix**. Acquire the mycelium spawn.
- **Substrate.** Collect the substrates
- **Substrate Preparation.** Two out of the three substrates (Sogrape and Nãm), presented large and heterogenous particle sizes, giving inadequate characteristics to obtain the desired material mechanical properties. Therefore, it is necessary to use a grinding machine to have smaller and homogenous particle sizes. The rice substrate is not ground because it already has an adequate particle size and is homogeneous. This phase also consists in adding the right amounts of water and additives into the substrate. When preparing the mixture, the goal for each substrate is to reach a "moist to the touch" moisture. The final ambition is to be able to grab a bit of substrate and hold tight in the hand, if the mixture holds together and no water comes out, then the substrate has a good amount of water.
- **Sterilization.** An autoclave is used to sterilize the substrate. The mixture is placed in the polypropylene bags. The bags are left for 15 minutes at 121ºC in the autoclave. After the sterilization is completed, the mixture must cool down to a temperature lower than 30ºC.
- **Inoculation.** Once the necessary amount of water is incorporated and the substrate is sterilized, it is possible to safely introduce the mycelium spawn on the substrate. Since this step needs to be performed in a sterilized environment to avoid contamination, a laminar flow cabinet is used to assure clean conditions. The material obtained after the inoculation is labelled as pre-material. It was decided to add 10% of mycelium spawn into the mixture. After placing the mycelium spawn into the bag with the sterilized substrate, it is necessary to shake well the bag until everything is homogenized. The bag is then sealed with a plastic sealer.
- **Growing phase.** In this phase, the mycelium is left to grow and expand in the substrate under controlled conditions. To achieve these conditions, the samples are left to grow in a laboratory oven. The right growing conditions for the mycelium to grow were considered the following:
    - Humidity: 90-95%.
    - Luminosity- None.
    - Temperature: 22ºC – 24ºC.
    - Controlled airflow.

This phase is divided into three different stages: 1st Stage of Development, 2nd Stage of Development and 3rd Stage of Development.

1st Stage of Development

Perform the inoculation on special filtered polypropylene bags. As soon as the bags are inoculated and sealed, they will be ready to be placed on a laboratory oven. For this growing stage, the pre-material will be left to grow inside the polypropylene bags for seven days.

2nd Stage of Development

On the stage of development, the pre material will be taken out of the polypropylene bags and will be placed on the different molds. It is critical to clean and disinfect the molds prior to placing the pre-material on them. To avoid contamination, a sterilized plastic cuff with small holes is placed on top of each mold. This stage lasts different days depending on the sample.

3rd Stage of Development

On the third stage of development, two different approaches will be used: the first approach is to take the pre-material is out of the molds and leave them to grow. Due to the fact that this approach might not be the most adequate for every sample, a second approach is used. The moulds are cut on the bottom and sides to ensure that not only the top layer receives air and has a higher chance of developing. Additionally, a weight is placed on top of the sample to compact it further. This method results in a sample that is more compact and has air entry in every section. This approach provides a more enclosed atmosphere, which ensures that the mould retains more moisture.

Throughout the stages of development, numerous parameters such as species, particle size, substrate, water content, and additives will be analysed to determine how they affect the mycelium's growth.

- **Material Processing.** This is the final phase in mycelium production, where the fungus is killed by applying a heat treatment. Once the fungus is dead, the mycelium growth stops. As a result, the material can be safely used without worrying about the further development of mushrooms. This phase is performed at a home oven. The samples are be left for four hours at 60°C home oven. After this phase is completed, the final material is obtained.

The phases of the experimental work described are portrayed in the flowchart in Figure 2.1.

All the experiments were performed in the *Recycling Laboratory*, *Chemistry Laboratory,* and *Bio-technology Department* at *Instituto Superior Técnico.* These experiments required materials, ancillary materials, and equipment, which will be described below

2.2 Materials Used

The substrates, additives and mycelium species used in these experiments are summarized in Table 2-1.

Table 2-1 Materials Used: Substrates, Additives and Species

Substrates	Sograpе Pruning Wood	Nãms Mushroom Production Residues	Rice husk
Additives	Wheat Flour	Wheat Bran	Flax Flour
Mycelium Spawn	Ganoderma Lucium	Pleurotus Ostreatus	

The pruning wood was kindly provided by Sogrape. Two kinds of wood were collected, Alvarinho and Arinto. These samples come from the “Quinta da Romeira” farm in Bucelas. Hand-cutting and collecting the pruning wood samples was required. The mushroom farming residues were provided by Nãm Mushrooms. The mushrooms growth is carried out in large bags, filled with substrate and one mycelium species. The substrate used coffee grounds, provided by the coffee company Delta, and straw.

The species used is *Pleurotus Ostreatus*. Each bag can be used up to two or three times to grow good-quality mushrooms. After use, the bag needs to be disposed. The samples of rice husk were obtained at a rice factory in Alcácer do Sal. The additives were acquired from the supermarket. The mycelium spawn was bought from *Mycelia.be*, a Belgium company. The strains acquired had the following codes: *Ganoderma Lucium* (9726) and *Pleurotus Ostreatus* (2191).

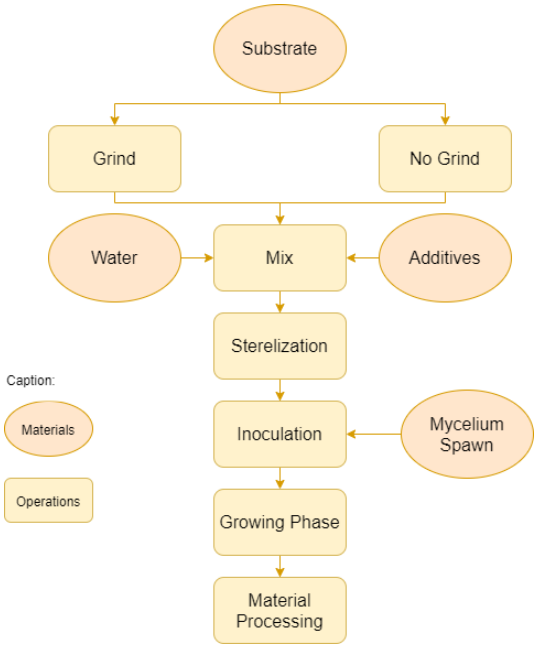

Figure 2.2.1 Flowchart of experimental work

Ancillary materials

Because the sterilization is carried out in an autoclave, it's necessary to use 60 microns polypropylene bags. These bags also have a 0.2 microns filter which will provide to the bag the capacity to be used in the first stage of development since these filters prevent the contamination of the contents inside the bag. On one of the stages of the growing phase, the pre-material was placed in moulds with different shapes to perform the growing. The propylene bags were acquired from a Portuguese mushroom producer and, the moulds were obtained at the supermarket. A plastic bag sealer, acquired at the *Recycling Laboratory*, is also necessary.

2.3 Equipment

1) Grinder

The grinder used is from Erdwich mod, EWZ 2000. The grinder has a rotor with ten disks each one containing three claws. A discharge grid, inserted below the fragmentation chamber, defines the particle

size of the fragmented material. The sieves used as discharge grids have different meshes, and the ones used in this experiment were: 10, 6, 4 and 10 millimetres.

2) Autoclave

The *Chemistry Laboratory* kindly provided us with an autoclave. The brand of the autoclave was *Sanyo Labo Autoclave MLS 3020*. An autoclave is an equipment used to carry out industrial and scientific processes requiring elevated temperature and pressure in relation to ambient pressure/temperature, with the aim of sterilizing equipment and supplies.

3) Laminar Flow Cabinet

The *BioTechnology Department* at Instituto Superior Técnico, provided the laminar flow cabinet *Aura 2000 M.A.C from Bioair instruments*.

4) Laboratory Oven

All the stages of the growing phase were completed on the laboratory oven, *Memmert U10 oven*.

5) Home Oven

A home oven is sufficient to perform the necessary heat treatment on the fungi. The brand of the oven in question is Indesit.

3 Results and Discussion

3.1 Substrate Characterization

The substrates as received can be seen in Figure 3.1 from a macroscopic perspective. As illustrated in Figure 3.1 a), the pruning wood as received lacks the required properties for usage in the development of mycelium-based composites. As a result, it is important to grind the Sograpе substrate to create a homogeneous substrate with reduced particle sizes. Since the Sograpе substrate was harvested directly from the vineyard, the branches remained extremely green. Following the Sograpе substrate, it can see the rice husk substrate in Figure 3.1 b). This is a very homogeneous and dry material. In contrast to the Nãm substrate depicted in Figure 3.1 c), which has multiple small branches of varying diameters.

Figure 3.1 a) Sograpе substrate as received from a far perspective. Arinto species on the left and Alvarinho species on the right side; b) Rice Husks substrate as received from a far perspective; c) Nãm substrate as received from a far perspective.

In Figure 3.2, the rice husks and Nãm substrate are shown in greater detail. The rice husk, Figure 3.2 a), is between 6x2 and 8x2 millimetres. In Figure 3.2 b), it's possible to see the brown coffee grounds encircling the straw fibers. Due to the substrate's previous use in mushroom cultivation, a white mycelium coating can also be seen around some of the fibers. As a living organism, the mycelium will continue to grow on the substrate. The rice husk material was fully dehydrated, indicating that additional water would be required to humidify the substrate. On the other hand, due to the Nãm substrate's

previous usage in mushroom production, water has been added to the substrate, resulting in a humid material.

Figure 3.2 a) Rice husk Substrate as received; b) Nãm Substrate as received

3.1.1 Substrate Preparation

After grinding Sograpе and Nãm with 2mm, 4mm, and 6mm sieves an analysis of the results is made, and the two particle sizes that are most appropriate for each substrate are chosen.

The 6mm substrate appeared to be too large for the Sograpе substrate, and hence was ruled out for this study. As illustrated in Figures 3.3 a) and 3.3 b), the variations between the 2mm and 4mm substrates are obvious. The 4mm particles have fibers that are longer and thicker. The smaller and shorter particles impart a "puffier" appearance to the 2mm substrate.

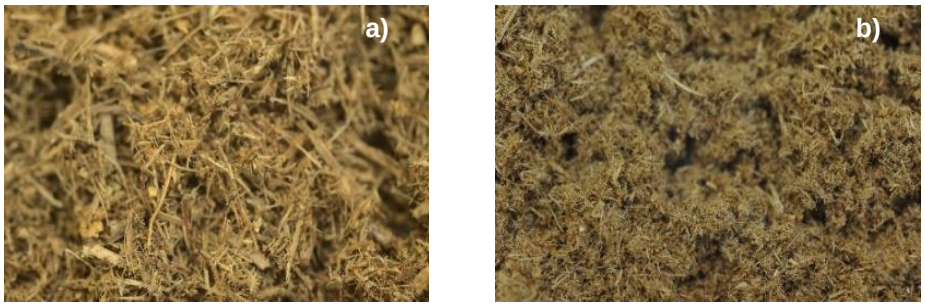

Figure 3.3 a) Sograpе Substrate grinded with a 4mm sieve; b) Sograpе Substrate grinded with a 4mm sieve.

After grinding the Nãm substrate and analysing the results, it was determined that the 4mm and 2mm sieves would not be used since the resulting substrate appeared to be too small. As a result, the trials agreed to continue utilizing the 10 mm and 6 mm sieves. By examining Figures 3.3 a) and 3.3 b), it is apparent that the straw fibers on the 10 mm substrate are already significantly smaller and more homogeneous than those on the unprocessed substrate. The straw fibers are significantly thinner, smaller, and more homogeneous on the 6 mm substrate.

Figure 3.4 a) Nãm Substrate grinded with a 10mm sieve; b) Nãm Substrate grinded with a 6mm sieve.

Due to the dry nature of the substrate, the water does not immediately absorb into it. As a result, the substrate is allowed to soak in the water for a period to absorb some of the moisture. After ten minutes, the substrate has absorbed all the water.

Once all the mixtures are prepared, they are transferred into the polypropylene bags and labelled. The bags are folded and taped to be able to be introduced in the autoclave.
The inoculation is performed by taking part of the mycelium spawn and weighting it on a scale. Once the right amount is introduced on the scale, the spawn is transferred into the labelled bag with the mixture.

3.1.2 Growing Phase

1st Stage of Development

Because this stage symbolizes the beginning of development, it is expected that it will exhibit slow growth, as the inoculated cells become accustomed to their new environment. As a result, the samples will pass through the lag phase during this stage. The lag phase period changes according on a variety of circumstances, therefore, following inoculation the samples are left to grow for seven days. On the seventh day, the samples are taken out for visual inspection. At this point, it is possible to infer that samples inoculated with *Ganoderma Lucium* have progressed more than the ones inoculated with *Pleurotus Ostreatus*. Additionally, samples with larger particle sizes provide the best outcomes; for example, samples with an unground Nãm substrate exhibit the best results, whereas Sograpе samples with a 2mm substrate exhibit the lowest results. The best substrate for mycelium development is the Nãm substrate, while the worst substrate for mycelium development is Sograpе.

2nd Stage of Development

Regrettably, an incident occurred in the laboratory, during which the oven ceased to function. This was crucial, as mycelium requires a unique environment to survive. Fortunately, the Chemistry Department kindly permitted us to use their oven. However, mycelium requires a stable, ultra-clean environment. This transfer was fairly unhygienic, as there was not time to properly clean and disinfect the area. Regrettably, this indicator had a significant impact on the samples, contaminating the majority of them. As a result, this study will be continued using the uncontaminated samples that were retained. However, even after this incidence, it was possible to analyze not only the remaining samples, but also their

resilience. It was observed that the majority of surviving samples were inoculated with Ganoderma Lucium and used a 2mm Sograpе substrate. The most polluted substrate was Nãm. As was the case throughout the first stage of development, samples inoculated with *Ganoderma Lucium* demonstrated the best outcomes. Although, at this stage, the 2mm Sograpе substrate shown the highest development. Unlike the first stage of development, the Sograpе substrate exhibited the best growth during this stage.

3rd Stage of Development

At this stage, it was possible to verify that the first approach is inadequate, as samples following this approach appear unable to hold moisture, whereas samples following the second way demonstrated significantly more mycelium development. The remainder of the results are very similar to those from the Second Stage of Development.

3.1.3 Material Processing

After material processing phase, several samples continued to exhibit fungal activity. As a result, the samples were left at 60ºC for an additional four hours. So, four hours is insufficient time to ensure that the fungus is killed. Thus, a total of eight hours of drying was completed. Is possible to see in Figure 3.5 the end results of the remaining samples.

Figure 3.5 Remaining Samples after Material Processing

4 Conclusions

There have been found improvements in the production of mycelium- based composites. The majority of samples that survived and performed well were those that used the Sograpе substrate. This implies that this substrate is not only the most conducive to development but also the most resilient to contamination. Following that, some samples of the rice husk substrate survived, indicating that it may be a substrate with a minimal risk of contamination under normal conditions. So, Sograpе substrate showed the best results, followed by the rice substrate. All Nãm sample got contaminated, and as a result, we believe that Nãm samples are more susceptible to contamination. Sograpе appeared to be the most resilient substrate, with less contaminated substrates, followed by the rice substrate, and being Nãm the sample with more contaminations.

Regarding the species, it is easy to conclude that the Ganoderma Lucium species have a higher resilience and better growth rate than Pleurotus Ostreatus.

Considering particle size, the rice particle size was too large. Since the husks sizes were so big, the samples weren't well compacted. The space left between the rice husk particles was very large and

some mycelium connections were hard to make, the material was too porous. A grinding procedure should be done. Sograpе samples had the best results long term with the smallest particle size, 2mm. As for the Nãm substrate, the larger particle size substrates exhibited the best results in the first stage of development.

Concerning the different growing stages, is possible to conclude that while the 1st stage of development was an important and necessary stage, to break the hypha apart and distribute it on the substrate, the 2nd and 3rd stage of development were not ideal. We realized that on the second stage of development, leaving only the top layer of the sample in contact with oxygen didn't allow for the mycelium to grow evenly around the whole sample, resulting in only one layer of the sample having good development. The first approach of the 3rd phase of development was too harsh for the samples, making them not able to maintain the moisture. We figured out during the experiment, that the 2nd approach was way more adequate, since it allowed to sample to keep the moisture in a closed moulding system, while also getting oxygen com the cuts around the whole mould.

To finally conclude, it is, unquestionably, a possibility for businesses that generate organic waste to utilize this bi-product and transform it into a value-added asset for the company's value chain. Not only are mycelium-based composites eliminating waste but are also developing a product that addresses the serious issue of single-use packaging, among others. It is very important that the company studies its substrates properties and finds the most efficient production formula.

Future work

We propose additional research on previously utilized substrates in mushroom culture, as these samples demonstrated high potential for development. Additionally, investigate how the rice husks substrate would react if the particle size were reduced. Finally, we suggest following the stages of development recommended in the conclusions.

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