TÉCNICO LISBOA

Introduction of Mycelium-Based Composites in the Portuguese Industry

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Thesis to obtain the Master of Science Degree in

Industrial Engineering and Management

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December 2021

Acknowlegments

O meu profundo agradecimento à professora Fernanda Margarido, por todo o apoio e dedicação no desenvolvimento desta tese. Apesar de todas as dificuldades a professora sempre se manteve do meu lado a ajudar-me com tudo o que conseguia.

Aos meus pais pelo apoio incondicional, pela educação que sempre lutaram para me dar e por me ensinarem a acreditar em mim mesma.

Ao meu namorado pela inspiração e apoio.

Aos meus amigos que me acompanharam ao longo dos anos, pelas boas memórias e recordações.

Abstract

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

Sustainability; Circular economy; Mycelium-based composites.

Resumo

Uma grande parte da produção na economia atual usa recursos valiosos e finitos sem considerar nem o seu fim de vida, nem o seu impacto ambiental. Logo, agora mais do que nunca, a indústria tem a obrigação de cuidar do meio ambiente. Em março, a União Europeia lançou o novo acordo, "European Green Deal", onde estableceu novos desafios para as empresas que terão de se adaptar a uma nova legislação verde, e a novos impostos que serão implementados. Evidentemente, a necessidade de novos materiais verdes e sustentáveis irão aumentar. Esta tese utilizará compósitos à base de micélio, um material de base biológica, biodegradável e sustentável, que respeita o conceito de economia circular. Uma vez que com micélios é possivel transformar resíduos orgânicos em diferentes tipos de novos materiais, iremos estudar os sub-produtos e residuos de várias empresas portuguesas para analisar se é possível adaptar os processos à base de micélios à indústria portuguesa.

Palavras Chave

Sustentabilidade; Economia Circular; Compósitos de micélios.

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Acronyms

RHS^{A} - GL + PO	*Ganoderma Lucium* and *Pleurotus Ostreatus* species on Rice Husks substrate with additives.
RHS^{A} - GL	*Ganoderma Lucium* species on Rice Husks substrate with additives.
RHS^{A} - PO	*Pleurotus Ostreatus* species on Rice Husks substrate with additives.
RHS - GL	*Ganoderma Lucium* species on Rice Husks substrate without additives.
RHS - PO	*Pleurotus Ostreatus* species on Rice Husks substrate without additives.
SS$_{4}$ - GL	*Ganoderma Lucium* on Sograpе substrate without additives, grinded with 4mm sieves.
SS$_{4}$ - PO	*Pleurotus Ostreatus* on Sograpе substrate without additives, grinded with 4mm sieves.
SS$_{4}^{A}$ - GL + PO	Ganoderma Lucium and Pleurotus Ostreatus on Sograpе substrate with additives, grinded with 4mm sieves.
SS$_{4}^{A}$ - GL	Ganoderma Lucium on Sograpе substrate with additives, grinded with 4mm sieves.
SS$_{4}^{A}$ - PO	Pleurotus Ostreatus on Sograpе substrate with additives, grinded with 4mm sieves.
SS$_{2}$ - GL	Ganoderma Lucium on Sograpе substrate without additives, grinded with 2mm sieves.
SS$_{2}$ - PO	Pleurotus Ostreatus on Sograpе substrate without additives, grinded with 2mm sieves.
SS$_{2}^{A}$ - GL	Ganoderma Lucium on Sograpе substrate with additives, grinded with 2mm sieves.
SS$_{2}^{A}$ - PO	Pleurotus Ostreatus on Sograpе substrate with additives, grinded with 2mm sieves.
NSN - GL	Ganoderma Lucium on Nãm substrate without additives, non-grinded.
NSN - PO	Pleurotus Ostreatus on Nãm substrate without additives, non-grinded.
NSN^{A} - GL	Ganoderma Lucium on Nãm substrate with additives, non-grinded.
NSN^{A} - PO	Pleurotus Ostreatus on Nãm substrate with additives, non-grinded.
NS$_{10}$ - GL	Ganoderma Lucium on Nãm substrate without additives, grinded with 10mm sieves.
NS$_{10}$ - PO	Pleurotus Ostreatus on Nãm substrate without additives, grinded with 10mm sieves.
NS$_{10}^{A}$ - GL + PO	Ganoderma Lucium and Pleurotus Ostreatus on Nãm substrate with additives, grinded with 10mm sieves.
NS$_{10}^{A}$ - GL	Ganoderma Lucium on Nãm substrate with additives, grinded with 10mm sieves.

NS_{10}^{A} - PO	Pleurotus Ostreatus on Nãm substrate with additives, grinded with 10mm sieves.
NS_{6} - GL	Ganoderma Lucium on Nãm substrate without additives, grinded without 6mm sieves.
NS_{6} - PO	Pleurotus Ostreatus on Nãm substrate without additives, grinded with 6mm sieves.
NS_{6}^{A} - GL	Ganoderma Lucium on Nãm substrate with additives, grinded with 6mm sieves.
NS_{6}^{A} - PO	Pleurotus Ostreatus on Nãm substrate with additives, grinded without 6mm sieves.
GL - ½ $SNS_{4,6}^{A}$	Ganoderma Lucium species on 1/2 Sograpе substrate, grinded with 4mm meshs, and 1/2 Nãm substrate, grinded with 6mm sieves, with additives.
PO - ½ $SNS_{4,6}^{A}$	Pleurotus Ostreatus species on 1/2 Sograpе substrate, grinded with 4mm meshs, and 1/2 Nãm substrate, grinded with 6mm sieves, with additives.
GL - RNS_{10}^{A}	Ganoderma Lucium species on 2/3 of Rice Husks substrate and 1/3 of Nãm substrate grinded with 10mm sieves, with additives.
PO - RNS_{10}^{A}	Pleurotus Ostreatus species on 2/3 of Rice Husks substrate and 1/3 of Nãm substrate grinded with 10mm sieves, with additives.
GL - RNS_{6}^{A}	Ganoderma Lucium species on 2/3 of Rice Husks substrate and 1/3 of Nãm substrate grinded with 6mm sieves, with additives.
PO - RNS_{6}^{A}	Pleurotus Ostreatus species on 2/3 of Rice Husks substrate and 1/3 of Nãm substrate grinded with 6mm sieves, with additives.
GL - RSS_{2}^{A}	Ganoderma Lucium species on 2/3 of Rice Husks substrate and 1/3 of Sograpе substrate grinded with 2mm sieves, with additives.
PO - RSS_{2}^{A}	Pleurotus Ostreatus species on 2/3 of Rice Husks substrate and 1/3 of Sograpе substrate grinded with 2mm sieves, with additives.
GL - RSS_{4}^{A}	Ganoderma Lucium species on 2/3 of Rice Husks substrate and 1/3 of Sograpе substrate grinded with 4mm sieves, with additives.
PO - RSS_{4}^{A}	Pleurotus Ostreatus species on 2/3 of Rice Husks substrate and 1/3 of Sograpе substrate grinded with 4mm sieves, with additives.
GL - 2/3 $SNS_{4,6}^{A}$	Ganoderma Lucium species on 2/3 Sograpе substrate, grinded with 4mm meshs, and 1/3 Nãm substrate, grinded with 6mm sieves, with additives.
PO - 2/3 $SNS_{4,6}^{A}$	Pleurotus Ostreatus species on 2/3 Sograpе substrate, grinded with 4mm meshs, and 1/3 Nãm substrate, grinded with 6mm sieves, with additives.

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. 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.

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 determined by the fungus, the organic substrate, and the optimal growing conditions used.

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 of these companies' organic by-products, we will investigate the possibilities of using mycelium to create new sustainable materials.

1.1 Problem Context

Over the last few years, most global production processes have encountered numerous environmental issues. However, the industry's major issues at the time were a lack of resources, waste output, and CO_2 emissions.

A separate but related issue is how many businesses choose to address these issues. Numerous businesses employ greenwashing to appear ecologically friendly rather than implementing truly sustainable practices.

Scarce Resources

By 2060, the global population is predicted to exceed 10 billion [1], and as nations get wealthier, the need for additional resources will arise. Between 1970 and 2017, material resource use increased by 89 billion tonnes. Additionally, experts predict that worldwide consumption of materials such as biomass,

fossil fuels, and metals will quadruple over the next forty years as the world's population rises and the economy expands [2]. All the raw materials mentioned previously are limited resources. Thus, as the population rises, it will be impossible to maintain a balanced economy and a fair society without developing novel sustainable solutions.

Waste Production

Nature employs a cyclical, cradle-to-cradle biological system in which waste does not exist because in this structure, waste means food. Industry's imposition altered the natural equilibrium of materials. Men developed the ability to extract substances from the earth, concentrate and synthesize them into vast amounts of material that cannot be safely returned to the soil [3]. Poorly managed and unmanaged waste endangers our way of life by causing flooding, transmitting diseases, increasing respiratory problems because of inhaling waste particles, and harming animals that consume the waste.

Throughout decades of economic expansion, the waste problem was ignored, creating an urgent need for action [4]. By 2050, waste is expected to increase by 70% to 3.4 billion tonnes per year [4]. Until 2015, approximately 8 billion tonnes of plastic were consumed [5]. Only about 9% of plastics are recycled, and only 10% of those are recycled multiple times. 12% have been incinerated, and approximately 60% of all plastics ever manufactured are discarded and accumulated in landfills or in the natural environment [5]. The packaging sector accounts for most of the plastic production at 146 million tonnes, followed by building and construction at 65 million tonnes and textiles at 59 million tonnes [6]. Although, while building and construction materials have a life expectancy of about 12 to 35 years, packaging has a life expectancy of less than a year since it is typically used as a single-use product [6].

The European Union has made significant efforts to address the issue of waste. We will discuss the EU's strategies for addressing these types of issues in greater detail.

As a result, the significance of mycelium-based composites is obvious. Not only does this save waste by repurposing material, but it is also biodegradable. One of the pioneers and the most prominent company is Ecodative, founded in 2007 in the United States, which started by developing packaging. Ecovative packaging is now used by Dell, Puma SE, and Steelcase, and have recently partnered with IKEA. They also have been developing building materials, textiles, and design creations [7].

CO_2 Emissions

Over the last 60 years, the annual rate of increase in anthropogenic carbon dioxide has been approximately 100 times faster than natural increases, and as is well known, this amount of CO_2 released into the atmosphere is causing a dramatic increase in the earth's average annual temperature

[8]. As illustrated in Figure 1.1, industry processes account for 5.2 percent of greenhouse gas emissions, waste accounts for 3.1 percent, and agriculture, forestry, and land use account for 18.4 percent. The chart's largest slice is devoted to the energy sector, which includes the largest subsector of greenhouse gas emissions, industrial energy [9].

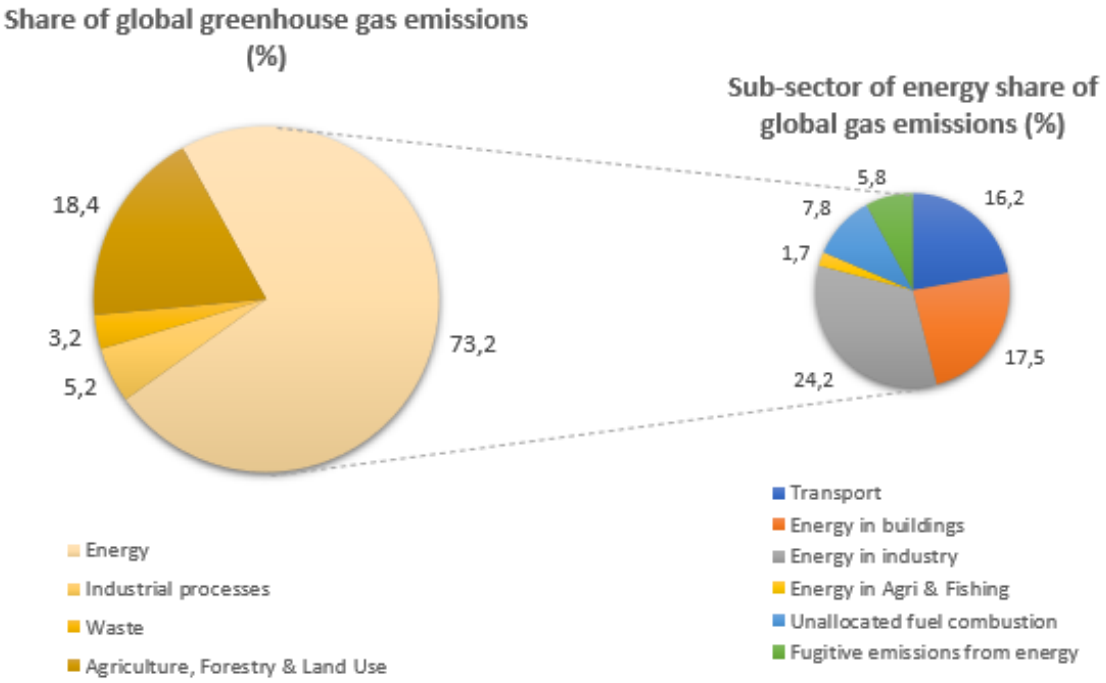


Figure 1.1 Global Greenhouse Emissions by Sector and Sub-sector of Energy [8]

Greenwashing

According to ING Research's most recent global survey [10], consumers are more willing to avoid brands that do not prioritize sustainability or environmental issues. Numerous studies, however, have revealed that the average consumer is unwilling to pay extra for a sustainable product. Consumers will continue to engage in the convenience economy despite this increased demand for sustainable products. As a result, the only plausible way to effect change is for businesses to start a transition within themselves. The primary issue is that many large corporations are meeting consumer demand for sustainable products by utilizing the greenwashing concept. Greenwashing is a term that refers to a strategy used to promote speeches, actions, propaganda, or campaigns that promote environmental correctness, eco-friendliness, greenness, and sustainability. It is essentially spreading the perception of being environmentally ethical while failing to take any practical measures and occasionally even having a detrimental effect on the environment. It is a practice that can be carried out by businesses and industries, non-governmental organizations, governments, and public leaders. Volkswagen's 2015 campaign is an excellent illustration of the greenwashing concept. Volkswagen launched an advertising campaign emphasizing its environmental stewardship. They spent $77 million dollars advertising its diesel cars in the American market, frequently emphasizing their environmental friendliness. Engineers

at the German automaker rigged 11 million of the company's ostensibly clean diesel engines with software that cheated emissions tests, allowing the vehicles to emit far more pollutants than permitted by law [11]. While this is a clear case of greenwashing, others are more difficult to identify. Ford, for example, advertises its products as being asbestos-free while using antimony sulphide, a more potent carcinogen than asbestos. Another example is when, in 2019, several of the world's largest plastics producers established a non-profit organization and pledged $1.5 billion dollars to assist in the ocean's plastic waste clean-up [12]. Today, the same companies intend to invest $400 billion in new petrochemical plants in anticipation of increased demand for plastic [13].

There are also numerous ways to confound consumers and failing to educate them is one of the simplest. Frequently, complicated "green" terminologies are used on product labels. For instance, deciphering the difference between bio-based and biodegradable materials can be challenging. Numerous brands tout their products as environmentally friendly due to the use of bioplastic in their packaging. The term "bio-based" refers to carbon that originates from organic sources such as plants and agriculture, rather than fossil fuels such as oil or coal, but does not necessarily imply that the material is biodegradable. Thus, the waste issue raised in the previous chapter persists, and the product is not as environmentally friendly as it appears at the end of its life cycle.

These examples are intended to illustrate this concept, as the subject is significantly more complicated than what is presented. However, we can conclude that it is critical for consumers to be educated about these issues and for large multinational corporations to practice truly sustainable morals.

1.2 Problem Definition

Concerns about resource consumption, waste management, and plastic production are escalating. As a result, the world is progressing forward, with numerous laws and regulations designed to hasten the transition to a sustainable economy. This will have a significant influence on businesses and society. As a result, many corporations must adapt their structures to this new sustainable method of doing business.

Seven Portuguese companies were contacted to have a better understanding of how the Portuguese industry is adapting to sustainable practices. It was observed that many of them generate enormous amounts of organic waste without having a sufficient end-of-life strategy. As a result, it was determined that there is a significant gap in the utilization of these by-products as raw materials. Solving this issue will resolve a slew of existing industry issues, including scarcity of resources, waste, and CO_2 emissions.

Delta Cafés is one of the businesses contacted. Delta is a Portuguese brand and firm that specializes in coffee roasting and packaging. It is active in 40 countries, both directly and indirectly. 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 Lisbon based small company. This business 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.

1.3 Motivation

Current industrial society consumes more natural resources than nature can regenerate and emits more carbon dioxide into the atmosphere than ecosystems can absorb. It is necessary to meet the limits of our planet by designing materials and processes capable of using a circular economy rather than a "Take-make-disposal" economy. The use of fungal mycelium materials can be a part of this transition. Therefore, this thesis will interact with different industry sectors in Portugal by analysing how to use local waste and provide it with a new application using mycelium-based composites.

One of the principal impulses for this 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 [14]. So, it aims to boost the economy through green technology. A European Climate Law,

currently in development, strives to write into law the goal set out in the European Green Deal. This law aims to ensure that all EU policies contribute to this goal and all sectors of the economy and society play their part [13]. 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 Commission will make sure that the circular economy transition delivers opportunities for all, leaving no one behind. The Circular Economy Action Plan is presented today as part of the EU Industrial Strategy. It 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, plastics, textiles, construction and building, and others, and ensure less waste [15].

1.4 Objectives and Research Questions

The goal of this thesis is to develop a sustainable material out of mycelium-based composites. By utilizing low-energy sources, mycelium-based composites can convert organic waste into a wide range of products. As a result, an attempt will be made to leverage this feature to establish a circular process for transforming local waste into new materials. The research questions for this thesis will be as follows:

1) **Can we optimize the production of mycelium?**

2) **Is it possible to use the mycelium waste from the mushroom production industry to develop mycelium-based products?**

3) **Can we integrate mycelium technology in the Portuguese industry in a circular, sustainable way while still being economically feasible?**

Our main objective is to determine whether it is possible to develop mycelium-based composites within the Portuguese industrial sector by collecting samples from various organic by-products and examining the possibility of converting them into a suitable substrate to produce these composites.

Mycelium growth occurs at a very localized and specialized level. Fungi are living organisms that must adapt to their environment, and sometimes this adaptation is not successful. If a company wishes to use its by-products to create mycelium-based composites, a thorough trial and error procedure must be conducted to attain the best mechanical properties and production efficiency. The same fungus species may grow at varying speeds on various substrates. For instance, the consequence of growing fungi in a substrate, say, pine, will be different depending on whether the pine is from the United States or Portugal. Additionally, because this product is intended to reduce CO_2 emissions, importing it from other nations would jeopardize sustainability, as transportation CO_2 emissions might be exceedingly high. Additionally, we aim to ensure that production is as efficient and effective as possible.

This allows us to identify a solution for Sogrape, as they can add value to their by-product. Nãm mushroom can expand their business by promoting sustainable packaging. Finally, the rice sector now has a better understanding of how to transform its by-product into a more valued commodity.

2 State of Art

There are many terms and definitions around the sustainability concept. So, before starting to address mycelium, it's important to identify and clarify some of the essential terminologies. These terms will be defined mostly given a European Framework. Once the basic concepts are defined it's easier to start looking deeper into the mycelium sphere.

2.1 Sustainability and Circular Economy

Despite the importance of sustainability and circular economy in current society, the definition of these terms and the relationship between them is still not clear. To facilitate this reading, from now on the term sustainability will be defined as:

- *"A situation in which human activity is conducted in a way that conserves the functions of the earth's ecosystems, a transformation of human lifestyle that optimizes the likelihood that living conditions will continuously support security, well-being, and health, particularly by maintaining the supply of non-replaceable goods and services or an indefinite perpetuation of all life forms"* [16].

On the other hand, the circular economy will be defined as:

- *"A regenerative system in which resource input and waste, emission, and energy leakage are minimized by slowing, closing, and narrowing material and energy loops "* [16].

These two concepts have very different origins, goals, motivations, system prioritisations, institutionalizations, beneficiaries, timeframes, and perceptions of responsibilities. The motives behind sustainability are based on past trajectories, are diffused and diverse, and often embrace reflexivity and adaptivity to different contexts. In contrast, the circular economy concept is mainly motivated by the observation that resources could be better used, and waste and emissions reduced with circular rather than linear make-use-dispose systems [16].

In short, the goal of sustainability is to benefit the environment, the economy, and society at large, while in circular economy the goal focuses more on economic factors, efficiency gains, and waste avoidance, considering mainly the resource input, waste, and emission output. Therefore, it's possible to conclude that sustainability, even being a more complex concept, can be a goal, or a societal idea, circular economy can be considered as a tool to achieve sustainability.

2.1.1 EU Waste Framework

In 2006 a legislative framework for the handling of waste was created to encourage [17]:

- The prevention or reduction of waste production and its harmfulness.
- The recovery of waste including recycling, reuse or reclamation, and the use of waste as a source of energy.
- Ensure that waste is recovered or disposed of without endangering human health and without using processes or methods which could harm the environment.
- Establish an integrated and adequate network of disposal installations.

In 2008 the Waste Framework Directive, or Directive 2008/98/EC [18], together with Hazardous Waste Directive [19] and Waste Oils Directive [20], repealed the Directive 2006/12/EC. The revised Directive 2008/98/EC sets the basic concepts and definitions related to waste management and lays down waste management principles such as the "polluter pays principle" or the "waste hierarchy". The waste hierarchy established by the waste framework in Figure 2.1, identifies five steps to manage waste and conserve resources.

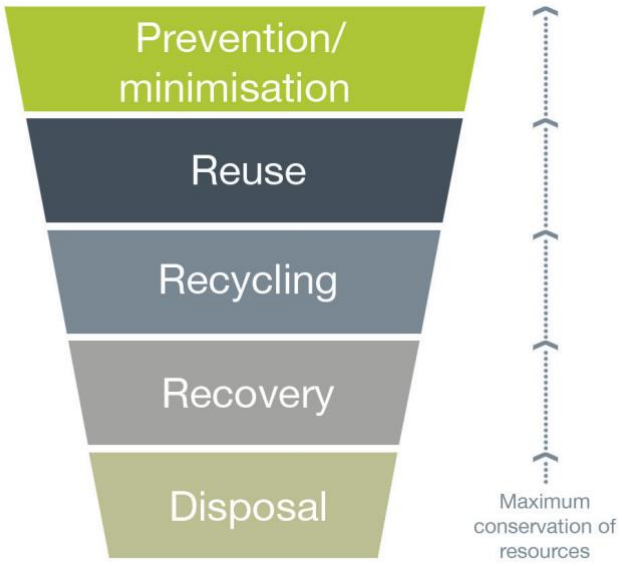


Figure 2.1 EU Waste Hierarchy [21].

An EU framework will be used for defining some of the most important notions for this thesis [18]:

- **Waste** means any substance or object which the holder discards or intends or is required to discard.
- **Bio-waste** means biodegradable garden and park waste, food and kitchen waste from households, restaurants, caterers and retail premises and comparable waste from food processing plants.

- **Prevention** means measures taken before a substance, material or product has become waste.
- **Re-use** means any operation by which products or components that are not waste are used again for the same or other purpose for which they were conceived.
- **Recycling** means any recovery operation by which waste materials are reprocessed into products, materials, or substances whether for the original or other purposes. It includes the reprocessing of organic material but does not include energy recovery and the reprocessing into materials that are to be used as fuels or for backfilling operations.
- **Recovery** means any operation the principal result of which is waste serving a useful purpose by replacing other materials which would otherwise have been used to fulfil a particular function, or waste being prepared to fulfil that function, in the plant or in the wider economy.
- **Disposal** means any operation which is not recovery even where the operation has as a secondary consequence the reclamation of substances or energy.

As part of the Thematic Strategy on the prevention and recycling of waste, the Commission introduced a way to tackle one of the issues around the waste definition. In the communication on waste and by-products [22], several terms are defined and, the business and environmental context around by-products are explained:

- **Product** all material created deliberately in a production process. In many cases it is possible to identify one (or more) "primary" products, which is the main material produced.
- **Waste** a material that is not deliberately produced in a production process, but it may or may not be wasteful.
- **By-product** a residue from production that is not waste.

2.1.2 Bioplastics

We realized that mycelium-based composites mainly concern the bioplastics sector. For this reason, we considered it important to scrutinize these terminologies to better understand the scope of mycelium and where it fits given the European context. There are several critical terms to understand while discussing sustainable products. To gain a better understanding, it's helpful to start with Figure 2.2, which depicts the four plastics families.

According to the EU standard EN16575 2014, and by the 2002 USDA Farm Bill (US Congress, 2002):

- **Bio-based** products are those that derive part or all the biomasses. The production process may undergo a physical, chemical, or biological treatment process.
- **Biodegradable** products are capable of degradation under the action of microorganisms in specific environments.

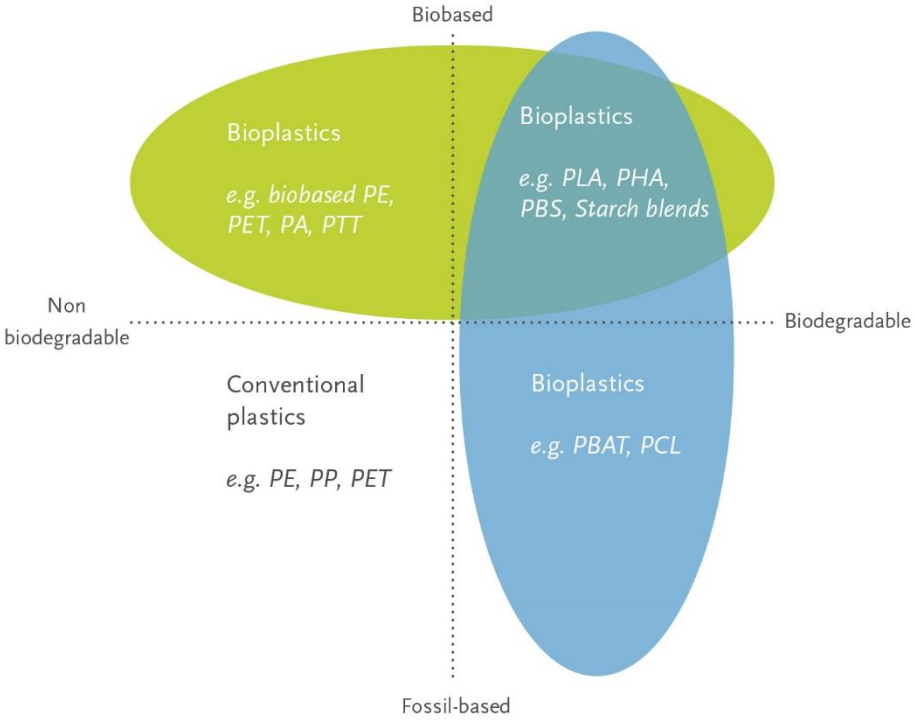


Figure 2.2 Families of Plastics [23].

Bio-based, non-biodegradable plastics made from renewable resources such as biomass, agri-food sector by-products, and bio-waste, include:

- **Bio-based:** polyethylene terephthalate (bioPET), polyethylene (bio-PE), polyamides (bio-PA), polyesters, and starchpolyolefin blends.
- **Bio-based materials under development:** bio-based: polypropylene (bio-PP), polyethylene furanoate (bio-PEF).
- **Bio-based, biodegradable, and compostable plastics**: thermoplastic starch (TPS), polylactic acid (PLA), polyhydroxyalkanoates (PHA), and polybutylene succinate (PBS).

The 2020 production of bioplastics was 2,171 million tons, and it is expected that this value will rise to 2,426 million tons by 2025 [24]. There are several bioplastics of an innovative nature, such as PP (polypropylene) and PHAs (polyhydroxyalkanoates), which are co-starting to grow. But, despite the great growth of bioplastics, this branch remains a niche in the industry. In 2019, 368 million tons of plastic were produced [25]. The production of bioplastics in 2019 was 1,952 tons [24], which represents around 0.5 percent of plastics on the market. Currently, biodegradable plastics occupy almost 60 percent of global bioplastic production. As in traditional plastics, the packaging industry continues to be the industry with the greatest application of bioplastics, holding 47 percent of global production, followed by consumer goods holding 12 percent and, 11 percent on textiles. The remaining 30 percent is represented by agriculture, automotive, and others [24].

Despite the apparent benefits of bioplastics and their growth, market penetration has been slow. The reason for this can be due to several factors. It has been estimated that the cost of bioplastics can be up to twice as high as conventional plastics. Despite their ability to be recycled, bio-based plastic can come to contaminate the recycling process if not separated from conventional plastics. It is also important to analyse where raw materials to produce bio-based plastics come from, as they are not always sustainable solutions if raw materials are exploited. Besides, the energy consumed for the transformation and the consequent CO_2 emissions by these processes must be considered, as well as its life cycle assessment analysis from production to end-of-life. As we mentioned, another problem is the confusion between terms and the fact that brands take advantage of consumers' ignorance to sell products under the label of "environmentally friendly". There is still a lack of legislation regarding the production, use, and waste management of bioplastics. There are still several methods used to determine the degradation and end of life of these materials. Therefore, it is necessary to standardize these details and legislate the use of raw materials, energy consumption, emissions, use, and waste management [26].

Bio-plastics end-of-life Strategies

Bio-based plastics, which are not necessarily biodegradable, are usually chemical and physical in nature identical to fossil-based plastics. So, the uncontrolled and improper disposal of bioplastics wastes also contributing to the problems like littering and, soil and water pollution. Many methods for bioplastics waste management exist that are going to be explained further. However, the bioplastics that enter the municipal waste stream makes some complications for existing plastic recycling systems, since it is having a variation in the base materials over conventional plastics.

There are several end-of-Life alternatives for bio-based plastics, but the most used method is mechanical recycling, which is defined as the reprocessing of waste material into a new product [27]. The mechanical recycling companies transform these sorted products by using processes like washing, density separation, and compounding, it mechanically crushes the plastic and remelts it into granulate [28]. Thus, producing recyclates that can be converted into plastics products, substituting virgin plastics. So, bio-based plastics that are not biodegradable, made of bio-based equivalents of conventional polymers (e.g. bio PE, bio-PET), can be introduced in recycling streams corresponding conventional plastics as they are chemically identical.

Another option for recycling is chemical recycling, while mechanical recycling preserves the molecular structure, chemical recycling splits polymer chains and supplies products such as crude oil, naphtha, or fuels. Chemical recycling agrees with the basis of circular economy, and many successful research projects have been conducted in recent years. Chemical recycling that concerns plastics and chemical it's only the recommended alternative over mechanical recycling when this last one is not viable technically. However, this method has some disadvantages, it's a very high-cost approach, the

technology is not ready to be used in all polymers, and its environmental footprint due to the complicated treatment of waste [21].

Organic recycling is only used by bio-degradable plastics, which occurs when the plastic has the capability to turn into a nutrient-rich soil amendment. These are plastics that biodegrade within a specific timeframe in clearly defined conditions (e.g. temperature, humidity, and the presence of microorganisms). The natural microorganisms can transform these matters into simpler compounds. If these compounds are decomposed into carbon dioxide and water, it's considered to be an aerobic decomposition, when it decomposed into methane and carbon, then it's an anaerobic decomposition [27].

The energy recovery end-of-life method is the last method used after mechanical, chemical, or organic recycling when these two last ones are not available for technical or economic reasons. Even though this method produces renewable energy, it destroys valuable resources, the post-consumer bio-based products that may be used in higher added value end-of-life routes. An incineration process is only a valuable option for bioplastics since it creates renewable energy, and because the CO_2 emitted by the incineration process are equivalent to the CO_2 requested by the plants used as raw material for the bioplastic [21].

The landfill is not an end-of-life option for biodegradable bio-based products, as these products may undergo anaerobic digestion producing the harmful greenhouse gas methane while valuable resources are destroyed. The landfill is the least desirable end-of-life option EU waste hierarchy and despite the efforts to avoid this option, this is still the most common waste disposal in many European countries [21].

2.1.3 Eco-labels

According to an EU-wide survey conducted by TNS Political and Social, at the request of the European Commission and, as we have mentioned before, European consumers consider environmental factors when making a purchase. But, as also previously mentioned in the greenwashing discussion, consumers are not fully informed about product characteristics. Therefore, it's easy for brands to enrol in misleading marketing. As a result, eco-labels are an essential tool to achieve sustainability and to regulate companies.

Current criteria in existing eco-labels now are, for sustainability [29]:

- Environmental criteria, such as sustainable sourcing of biomass, greenhouse gas emissions, toxicity, durability end-of-life strategies, added by considerations on reusability and reparability.
- Social criteria such as corporate social responsibility and fundamental principles at work.
- Economic criteria, regarding costs and efficiency.

And additional criteria to promote sustainable bio-based products:

- Percentage of bio-based product content.
- Percentage of bio-based content in packaging.
- Fitness for use.

According to the EU *"To qualify for the EU Ecolabel, products have to comply with a tough set of criteria. These environmental criteria, set by a panel of experts from several stakeholders, including consumer organizations and industry, take the whole product life cycle into account - from the extraction of the raw materials to production, packaging, and transport, right through to your use and then your recycling bin."* Acknowledging that 78 percent of European citizens have said that they trust eco-labels as being environmentally friendly [30], it's relevant to take into consideration these factors when creating new products.

2.2 Production of Mycelium-Based Composites

With the use of mycelium, it's feasible to transform organic residues into a valuable product. To better understand how to turn a by-product into a bio-based and bio-degradable material by using mycelium, a review on mycelium-based composites production will be performed in this item.

Mycelium

Mycelium is the vegetative part of a fungus, consisting of a complex network of interlaced, microscopic, pipe-shaped filamentous structures called hypha. It grows in both solid-grown and submerged cultures. It has been identified as the largest living organism on earth.

Mycelium can be classified into three kinds, depending on the benefits each kind offers in a relationship with organisms:

- **Saprophytic fungi** use enzymes to digest organic matter into molecules, small enough to be absorbed by other organisms. They are the most relevant group since they transform organic waste into a mycelial mass.
- **Pathogenic fungi** endanger the host's health and cause diseases in organisms.
- **Symbiotic fungi** form associations with plants in a mutually beneficial relationship.

Saprophytic fungi feed on dead organic matter and therefore have an essential role as decomposers in ecosystems. Saprophytic fungi have a natural ability to bind and digest lingo-cellulose fibers of plants. This ability provides an inherent bonding that forms a natural lightweight bio-composite. The fungal structure of this bio-composite functions as the matrix and holds the substrate together without using any synthetic adhesives. The type of characteristics that the saprophytic fungi hold make them very interesting for this research. So, from now on, only the saprophytic fungi are considered.

Subsequently, as fungal cell walls are presented in a hypha structure, they form an interwoven three-dimensional filamentous network that comprises a thick and complex fibrous network of chitin, and other polysaccharides, such as glucans, mannoproteins, chitosan, polyglucuronicacid or cellulose, and smaller quantities of proteins and glycoproteins [31]. It's possible to see an example of a hypha structure in Figure 2.3.

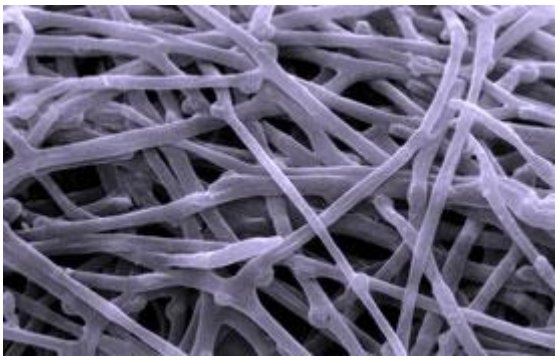


Figure 2.3 Fungal hyphae forming a mantel around a tree root [32].

During the mycelium's growth process, the fungi decomposes plant matter while gradually colonizing the substrate, covering it with this network of filamentous hyphae acting both as fiber and bonding material, resulting in a structure that's able to create properties similar to the ones in plastics, and even wood, if treated the right way [33]. Consequently, utilizing biological growth rather than expensive and energy-intensive manufacturing processes, mycelium composites can convert low-cost organic wastes into economically viable and environmentally friendly materials [34].

2.2.1 Factors affecting the production of mycelium

Mycelium-based materials are usually composites. Some materials use only pure mycelium sheets, but those applications are rarer. Composites are highly valued when the right combination that generates the desired material properties is found. When looking into a material, there are many properties that are important to balance, such as stiffness, strength, weight, high-temperature performance, corrosion resistance, hardness, or conductivity. These properties might not be able to adjust in singles entities. Because of that, keeping in mind all the factors that affect mycelium production is essential to creating the right material.

So, there are many factors that affect the production of mycelium and, consequently, their mechanical properties. Some of these factors are: 1) The matrix (fungal species), 2) The substrate (feedstock selection), 3) Sterilization, 4) Inoculation, 5) Growing Phase, and 6) Material Processing [33]. These factors will be explained carefully.

Table 2.1 summarizes several scientific articles in which diverse fungi species are tested on a variety of substrates.

Table 2-1 Substrates and fungal species depicted in literature

Article	Fungal Specie	Substrate
[N. Attias et al. (2017)]	Pleurotus Ostreatus	Woodchip of Eucalyptus
	Pleurotus Salmoneostramineus	Woodchip of Oak
	Pleurotus Pulmonarius	Woodchip of Pine
	Aaegerita Agrocibe	Woodchip of Vine
		Woodchip of Apple
[N. Attias et al. (2019)]	Ganoderma Lucidum	Woodchip of Vine
	Trametes Multicolor	Woodchip of Apple
	Trametes Versicolor	
[R. Alves et al. (2018)]	Pleutorus Ostreatus	Pine Sawdust
	Hypsizygus Ulmarius	Coffee grounds
	Trametes Versicolor	Mixed Sawdust
[H. Muhammad et al. (2017)]	Pleutorus Ostreatus	Cellulose
	Ganoderma lucidum	Cellulose/potato-dextrose
[F.V.W.Appel et al. (2018)]	Pleutorus Ostreatus	Beech Sawdust
	Ganoderma Lucium	Rapeseed Straw
[C. Bruscato et al. (2019)]	Trametes Multicolor	Pine Sawdust
	Pycnoporus Sanguineus	Wheat Bran
	Pleurotus Albidus	Calcium Carbonate
[F.V.W. Appels et al (2018)]	Schizophyllum Commune	Static Liquid Culture
	Pycnoporus Sanguineus	Agar Minimal Medium
[M. Jonesa et al. (2019)]	Trametes Versicolor	Rice Hulls
		Wheat Grain Inoculum
[G. Holt et al. (2019)]	Ganomerma Lucium	Cotton
[35] [Z. Vidholdova et al. (2019)]	Trametes Versicolor	Woodchip of Pine
[36] [J. A. López Nava et al. (2016)]	Pleurotus Ostreatus	Wheat Residues

1) Fungal specie

The selected fungal species will significantly influence mycelium colonization and the mechanical properties of the bio-composite. Some of the factors that can depend on the selected fungi species are the colonization rate, hyphae thickness, branching trend, and surface topography.

There might be millions of fungi species in the world, and only around 100,000 are known. In this review, only about 24 species are studied. Therefore, the screening of additional fungal species under the subject of bio-composites is essential. The most common species used in literature are shown in Figure 2.4. Many different fungi have been studied over the years, being the most popular *Pleutorus Ostreatus*

and *Ganoderma Lucium*. A lot of publications do not specify the fungal species due to intellectual propriety restrictions.

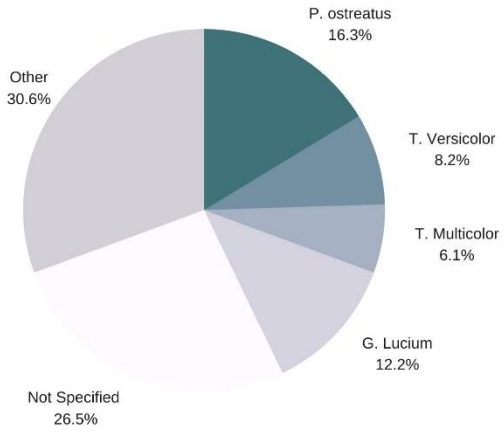


Figure 2.4 Pie chart of most used fungal species in literature

Mycelium hyphae are classified into generative, skeletal, and binding hyphae in fungus taxonomy. Generative hyphae are relatively undifferentiated and are capable of developing reproductive structures. They often have thin walls with occasional thickening, many septa (cell walls that divide the cells), and may contain clamp connections (i.e., the unique hook-like structure enabling hyphal cell growth). Skeletal hyphae are more densely packed, longer in length, and rarely branch. They feature a sparse septum and are devoid of clamp connections. Binding hyphae have a dense wall, are frequently solid, and are frequently branched. The mycelium network can be classified into three categories based on the three distinct hyphal types: monomitic, dimitic, and trimitic. Monomitic species are composed entirely of generative hyphae, dimitic species are composed of two types of hyphae (often generative and skeletal), and trimitic species are composed of all three hyphal types. These mycelium networks exhibit considerable structural and mechanical differences, with monomitic species exhibiting lower mechanical performance than dimitic and trimitic hyphal species. As an example, is possible to see in Figure 2.5 and 2.7, how the *Trametes Versicolor* species has a higher tensile strength (0,04 MPa) and flexural strength (0,22 MPa) than the monomitic species *Pleurotus Ostreatus*.

Prior studies have proven that *Pleutorus Ostreatus* and *Ganoderma Lucium* are the most stable to produce mycelium-based composites, due to the essential chemicals they produced, including a variety of enzymes that can efficiently degrade plant components difficult to hydrolyze, including lignin. These fungi are composed of trimitic and monomitic species. *Ganoderma Lucium* tends to have higher growth rates, growth density, and resilience to infections than *Pleutorus Ostreatus*. But even though these two species have shown great production potential, species like *Agaricus Bisporus* have been seen as especially stiff and strong compared to the previous two mycelia strains [37]. *Trametes Versicolor* has been reported to have competitive compressive strength when comparing to *Ganoderma Lucium* and *Pleutorus Ostreatus* [38]. Schizophyllum Commune is not one of the most popular fungi species, nut some articles have also shown a great potential for tensile strength [39].

The graphs in Figure 2.5, 2.6 and 2.7, illustrate a collection of results culled from literature. The tensile, compressive, and flexural strengths and stresses of various species will be analysed using these graphs. In Figure 2.5, it is possible to do a tensile strength and stress analysis. It is reasonable to conclude that *Trametes Multicolor* has comparable or perhaps superior values to *Pleutorus Ostreatus*. Tensile stress measurements for *Pleutorus Ostreatus* and *Ganoderma Lucium* are remarkably similar.

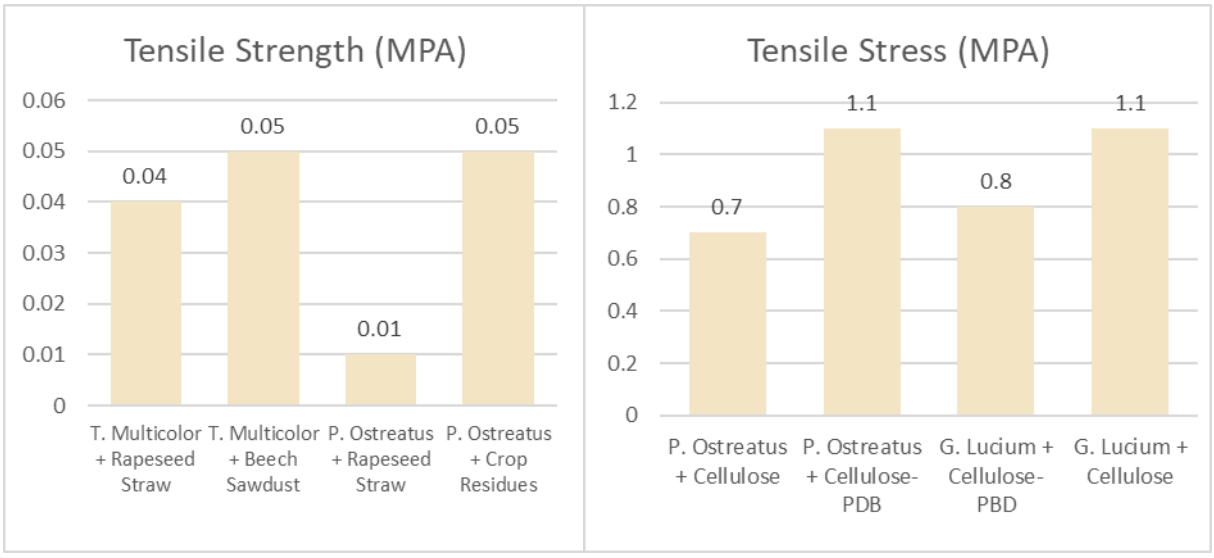


Figure 2.5 Tensile Strength and Stress Results of Various Literature

When compressive strength is compared in Figure 2.6, *Ganoderma Lucium* consistently outperforms *Pleutorus Ostreatus* [40]. Certain *Ganoderla Lucium* stength values are comparable to or exceed *Trametes Versicolo*r stress values, indicating that *Ganoderla Lucium* outperforms *Trametes Versicolor* in compressive strength [41].

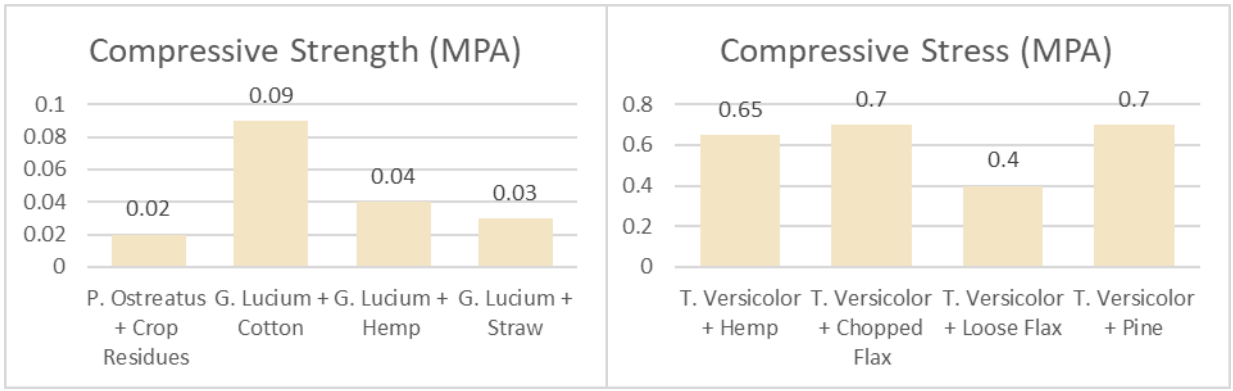


Figure 2.6 Compressive Strength and Stress Results of Various Literature

According to the results of the literature analysis in Figure 2.7, *Ganoderma Lucium* has the highest flexural strength values, followed by *Trametes Multicolor*, which has significantly lower values, and *Pleutorus Ostreatus*, which has even lower values [42].

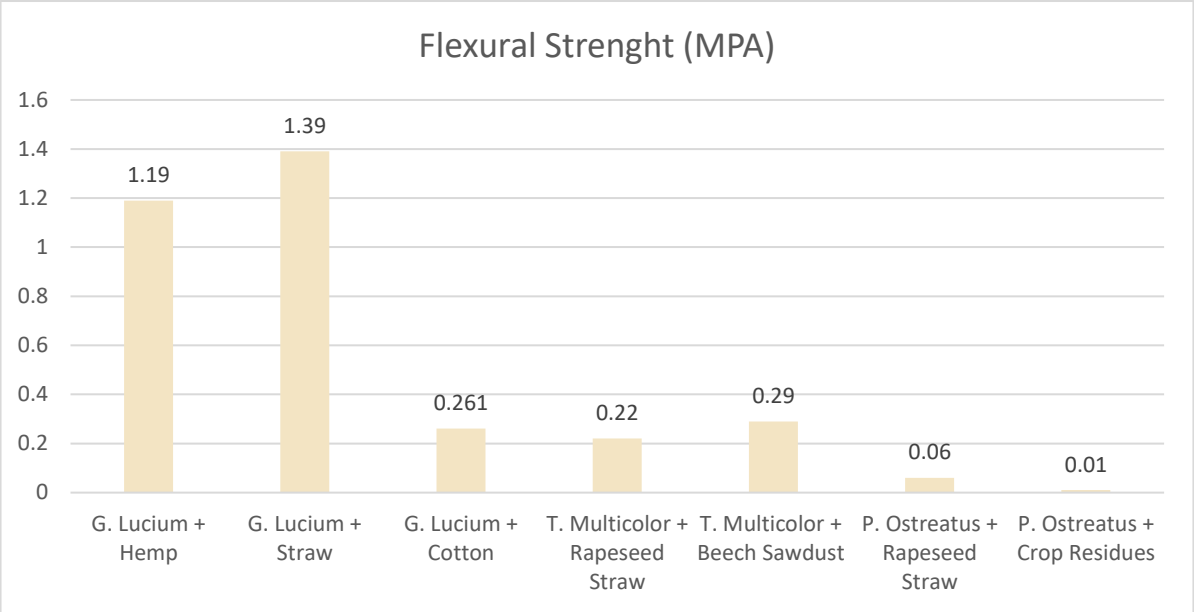


Figure 2.7 Flexural Strength Results of Various Literature

Even though the substrates for the samples displayed differ and thus alter the mechanical characteristics, based on the research conducted, it is feasible to conclude that Ganoderma Lucium is the fungus species with the best results for the majority of mechanical properties. Nonetheless, species such as Trametes Versicolor and Trametes Multicolor exhibit intriguing results.

2) Substrate

It has been shown that the production of mycelium-based composites and their mechanical properties are dependent on the fiber form and size. Most substrate mixture described in literature consists of ground agricultural crop waste such as cotton, corn, wheat, hemp, kenaf, flax residues. In terms of the shape of the substrate, usually, it comes in the form of fiber, woodchip, and sawdust.

Certain studies have demonstrated that the formation of mycelium-composites and their mechanical properties are fiber type dependant. Substrates such as dust flax and dust straw exhibited poor growth. This inconsistency could be a result of nutritional deficiency and the absence of air spaces inside the composite. A combination of fibers can alter the strength of the material depending on the type of fiber and its dimensions. Smaller fibers would make the sample more brittle, while a longer fiber would spread the force over a larger surface area.

Concerning particle size, it is notable that for more porous compounds water absorption will be higher. In Figure 2.8, we can see this effect after 168 hours of a water absorption test. Cotton husk substrate is used from several blends: 28–5, 12–28, 0.1–12, 12–51, 0.1–12 and 28–51, 0.1–51. The recipe for each blend was identical except for the particle size range of the cotton-based materials. When the substrates have dimensions between 28 and 51, the highest rate of water absorption is 198.1 percent. When

smaller grain dimensions (0.1-12, 28-51) are inserted into the bend, the water absorption values drop. The lowest water absorption rating corresponds to the tiniest mix dimensions (0.1-12) [34].

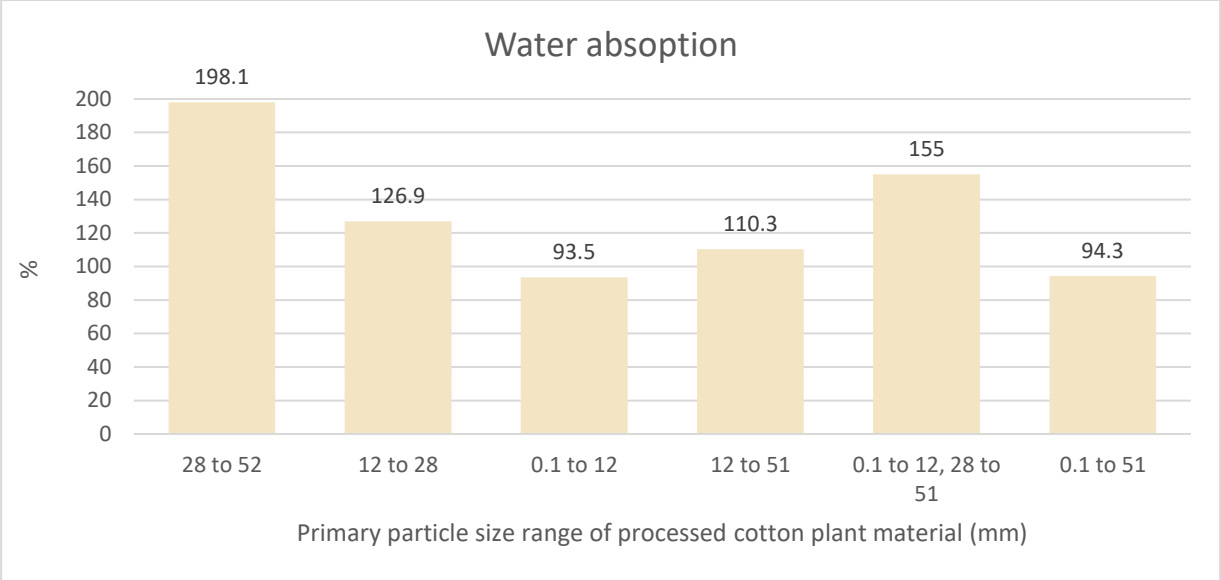


Figure 2.8 Water Absorption and Particle Size

There is an interesting correlation that can be observed between water absorption, density, and compression strength. *Ganoderma Lucium* is a fungus whose densities, when combined with woodchips, are relatively high. *Ganoderma Lucium* has the highest density when mixed with apple substrate and the highest water resistance when mixed with vine substrate. On the other hand, *Trametes Versicolor* has the highest density and water absorption when combined with vines. Knowing that the result with higher density and less water absorption is the combination of the Ganoderma fungus with the vine substrate, it's observed that it also has the greatest compression force. On the other hand, the fungus *Trametes Versicolor* combined with the apple substrate shows the lowest density and highest water absorption and, consequently, the lowest compression force [43].

3) Substrate Preparation

Mycelium thrives in a humid environment therefore, adding water into the substrate is one of the essential steps to be certain the mycelium has the right conditions to grow. Although, excessive moisture inhibits satisfactory gas exchange, therefore is very important to control the moisture content of the substrate. The additives are used to boost fungi growth.

Fungal cells rely on macro-nutrients and, optimizing fungal nutrition is quite challenging. But it's known that carbon is the energy source for fungi, there for substrate mixtures are often enriched by carbohydrates to stimulate the development of the fungi. The most common additives used are wheat flour since it contains sugar in the form of carbohydrates. So, adding these nutrients is a way to stimulate

the growth of the spawn and feed them. It's usually added around 3 to 10 percent of the weight of the wet substrate.

4) Sterilization

Contamination is one of the most common forms of failure when cultivating mushrooms. It is important to provide the mycelium with the best environment in which to thrive. When cultivating mushrooms, it's essential to limit the number of microorganisms competing for the same resources. Contamination can come from our breath, clothing, and skin. Therefore, it is prudent to take the necessary measures to avoid contamination. Some ways to do this is to use clean protective clothing, a face mask, and gloves. Sterilizing the tools is also an important step to avoid contamination. It's necessary to sterilize the substrate to make sure it's not contaminated by other fungi or organisms that can harm the development of the mycelium. The sterilization is usually done by placing the substrate in an autoclave. Different articles report appropriate times and temperatures like: 115ºC for 28 minutes, 121ºC for 20 minutes. Not all articles sterilize the substrate, although it appears to seem an important step of the process.

5) Inoculation

The inoculation must be performed in sterile conditions. The spawn added to the substrate is usually around 3-10 percent [44], [45].

6) Growing Phase

Three phases can be seen after the inoculation:

- **Lag phase** is a period of low population growth since the inoculated cells grow accustomed to their new chemical and physical environment. The duration of this phase varies by species.
- **Exponential phase** in the right conditions, the fungal cells will grow exponentially, and proportionally biomass will increase.
- **Stationary phase** this phase happens only if essential nutrients are exhausted, or if contamination happen, the exponential phase will cease, turning the specific growth rate into zero and biomass remains relatively constant. If this phase remains cells may begin to die.

Fungal growth required high levels of humidity, and the right level of humidity is around 90 percent [45]. With reduces water activity the cells can be affected, and the fungal growth would be negatively affected. Water activity is most important during the lag phase, while only having a slight effect on growth rate post lag phase.

The incubation period goes from 1 to 21 days in literature, with temperatures around 21ºC to 28ºC [46]. Temperature plays an important role. Decreased temperature results in increased lag phase duration and reduced exponential phase growth rate, high temperatures are especially important for the initial

growth rate. But if temperatures are too high the growth and the enzymes will be inactive [34]. These factors depend mostly on the specimen of fungi and materials involved.

In terms of defining the optimal pH level is difficult, different works of literature contradict each other so there isn't a good conclusion [46], [47].

It is advisable to minimize the lag phase and ensure that optimal environmental conditions and abundant nutrients are available to maximize growth rate and yield and prevent growth from entering the stationary phase prematurely [34].

Following an exhaustive review of the literature, it is determined that the ideal growth phase should follow three distinct stages 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.

7) Material Processing

Additionally, the substrate can support a variety of treatments. The most popular method is to just allow the mycelium to sit and grow for the duration of the predicted time. However, it has been reported that re-mixing the substrate with the inclusion of additives during the growing phase results in a more homogenous dispersion of the mycelium development [44]. Pre-compressing the substrate has a

beneficial effect on its mechanical properties. Compressive mechanical characteristics are enhanced, while the Young´s modulus is increased [45].

The drying process is performed to kill the fungus for it to stop growing. In the drying process is important that the right amount of water is removed to make sure that the fungi die, otherwise when the fungi will only stay in a "hibernated" state, allowing the fungi to restart growing when moisture conditions are met. According to the literature, the drying conditions are met by heating the mycelium composite in a conventional oven around 60ºC to 105ºC, for 4 to 6 hours. Nevertheless, there are other treatments for mycelium-based composites, such as heat pressing and cold pressing. When cold pressing the result is usually a foam with higher mechanical properties. When heat pressing the final mycelium-based composites have similar properties as a board material [48].

In Figure 2.9, as described below the graph, he results displayed with the darker colours are non-pressed, while the results depicted with the lightest colour are those that were heat pressed. Is possible to see that the heat and cold pressed composites show superior compressive strengths. Although, the results in the heat pressing process are a lot higher than the other processes.
When analysing flexural strength, the values can go as high as 4 MPa [48].

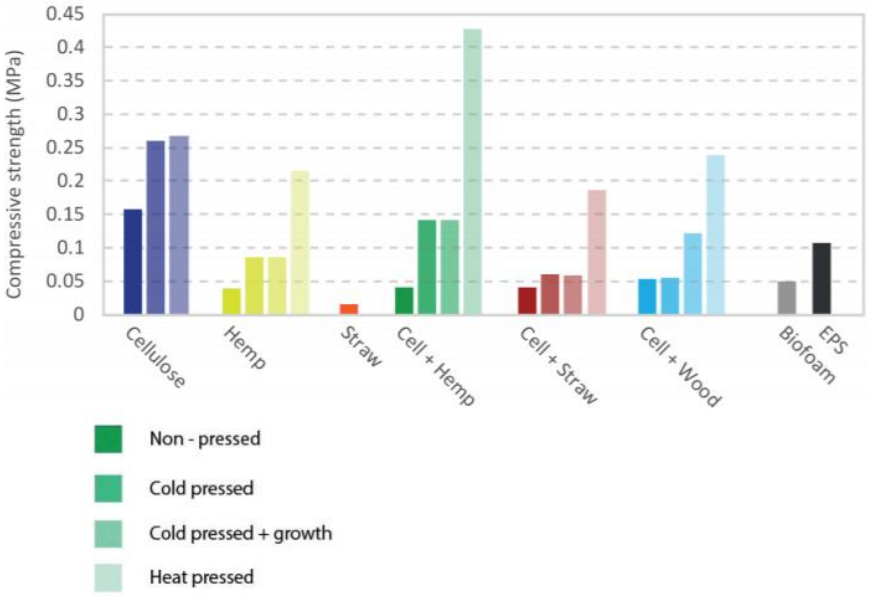


Figure 2.9 Overview of Compressive Strength of Different Fibers and Processes [48]

2.2.2 Application of mycelium bio-composites in the industry

As mentioned before in the greenwashing discussion, just because a material is bio-based doesn't necessarily mean it is bio-degradable therefore, the waste issue remains. Mycelium-based materials are not only bio-based but also bio-degradable and it can be used as compost closing a loop of circular thinking like demonstrated in Figure 2.10. This causes the production of mycelium-based products is far more sustainable and helpful to the environment than most plastic solutions in the market.

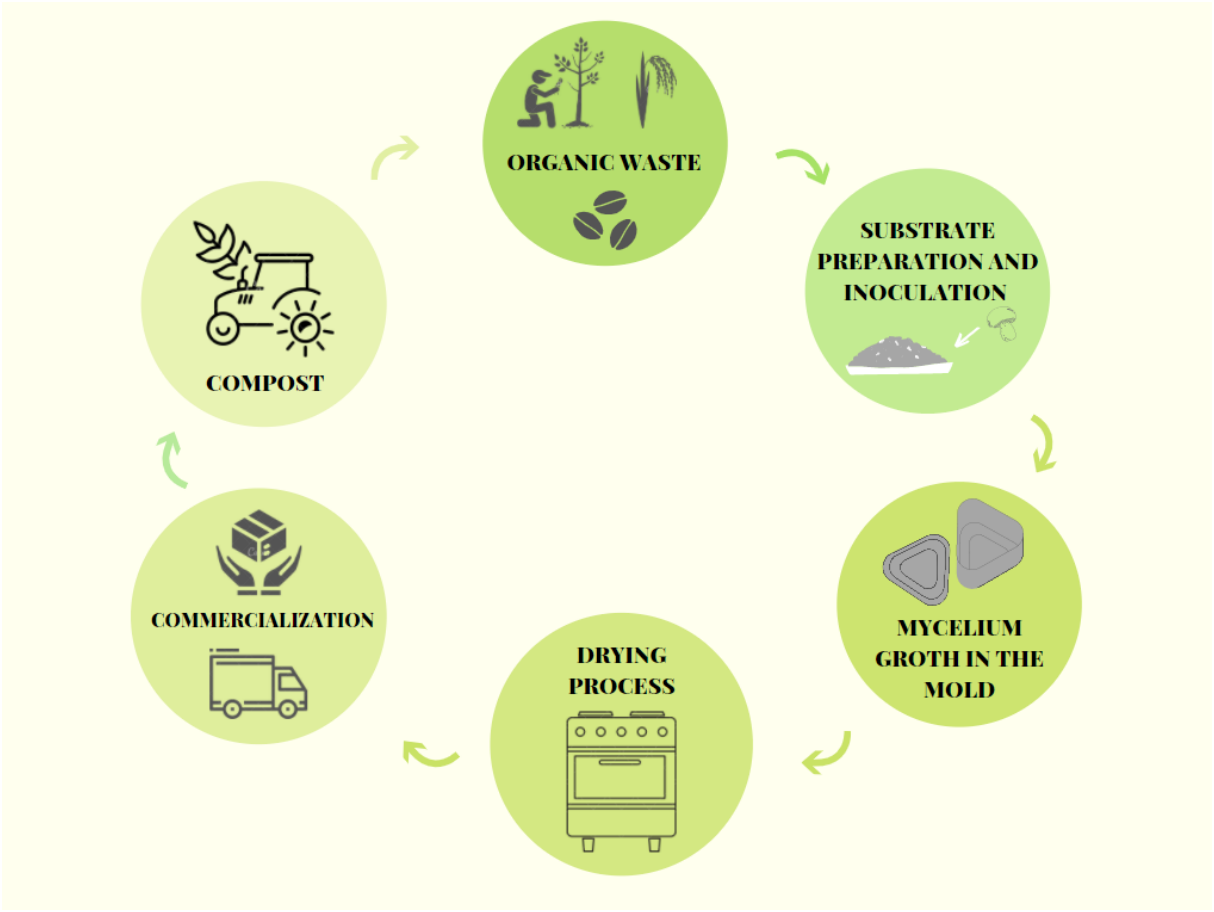


Figure 2.10 Diagram of life cycle of mycelium-based materials

Now, most of the mycelium research happens in the USA and China. Very few companies are producing and selling these products. There are a few more companies in Europe developing mycelium-based materials such as Mogu, which develops acoustic and floor products [49], and Grown.bio, which develops packaging and design parts, such as tables and lamps [50]. Also, some patents describe the use of mycelium to substitute petroleum-based products in the automotive industry. Ford has been developing methods for the injection moulding process, to produce components for vehicle interiors automotive parts such as bumpers, side doors, and dashboards [51].

1) Packaging

There have been concerns about the rising volume of packaging wastes, so the redefinition and redesign of conventional products in the packaging industry has been in great demand. Polystyrene (EPS) or styrofoam is nowadays, the commonly used solution for packaging, due to its low prices. Styrofoam is a petroleum-based non-biodegradable foam. It is composed of benzene and styrene, both of which are known human carcinogens, and its production is energy-intensive, creating large amounts of greenhouse gases [52]. It takes at least five hundred years to decompose, and in the meanwhile, it remains on earth as a piece of toxic waste to humans, wildlife and marine life, food supply, and our environment while costing taxpayers millions in clean up and mitigation costs. While EPS is technically "recyclable" there is, to date, no meaningful recycling of expanded polystyrene (EPS) or styrofoam due to high food contamination rates and a very weak market to clean, handle and process the material [53].

Mycelium can be grown in a mould to form different shapes for different items and, in the right conditions, they grow quickly into a dense material. After its useful life as a packaging material, mycelium-based materials can be left out in your backyard and decomposes within a few weeks. Despite the production still being limited to a few companies in the world, the productive process is improving quickly, resulting in high-quality materials at reasonably low costs. One of the biggest challenges of mycelium is manufacture since there is still a lot of investigation to be made around the material. Production is still very limited, but with the improvement of production and with economies of scale, the production could be a lot cheaper and more sustainable.

2) Construction Industry

Mycelium-based composites have been used as a substitute for energy-intensive synthetic construction materials. As we have seen before, mycelium composites have customizable material properties based on their composition and manufacturing process. These composites can replace foams, timber, and plastics for applications, such as insulation, door cores, panelling, flooring, cabinetry, and other furnishings. Due to their low thermal conductivity, high acoustic absorption, and fire safety properties outperforming traditional construction materials, such as synthetic foams and engineered woods, they show particular promise as thermal and acoustic insulation foams [54].

Mycelium composites that contain high-performance natural insulators such as straw and hemp fibers, have low densities, around 57–99 kg/m^3, and thermal conductivities, around 0.04–0.08 W/m.K [47]. This makes them excellent insulation materials, able to compete with conventional commercial thermal insulation products, such as glass wool (57 kg/m^3, 0.04W/m.K) and extruded polystyrene insulation (XPS, 34 kg/m^3, 0.03 W/m.K) [55]. Mycelium itself is also an excellent acoustic absorber. It shows great inherent low-frequency absorption (<1500 Hz) and may outperform cork and commercial ceiling tiles in road noise attenuation [56]. Additionally, mycelium composites of agricultural residue can also provide

a broader range of acoustic absorption with 70–75 percent absorption [57]. Mycelium has no notable or useful fire-retardant properties but incorporating substrates or fillers that are rich in natural phenolic polymers, such as lignin, significantly improved thermal degradation, fire reaction, and safety properties can be exhibited. Mycelium composites comprising low-weight substrates are competitive in terms of weight, with common synthetic insulation foams, such as polystyrene, polyurethane, and phenolic formaldehyde resin foams [47].

Mycelium composites are also cost-competitive with both synthetic foams and wood products, taking into consideration that, the cost of agricultural and industrial by-products, are usually much lower than the sail price of constituting the cost of the agricultural and industrial by-products used to make them, much lower than the wholesale price of polystyrene polyurethane, phenolic formaldehyde resin, foams, and plywood, softwood and hardwood products [47]. Mycelium composites do however have a significant advantage in terms of fire safety over traditional synthetic insulation materials, such as polystyrene and polyurethane foams, which are very flammable [47].

So, mycelium composites are best suited to compete with synthetic foams and wood products in thermal or acoustic insulation applications, where their combination of low density, low-cost, and fire resistance provides them a significant improvement in the market. The main disadvantage of mycelium composites for insulation applications would be moisture uptake, which is much higher than of polystyrene, polyurethane, and phenolic formaldehyde resin foams. This could be a serious problem in leaking walls or roof cavities. Another difficulty is the fact that mycelium still has a very slow manufacturing process, even though companies and researchers are developing every day better approaches, it's still an area of development.

3) Design and Architecture

There have been developed many different ideas for mycelium products in the spectrum of design and architecture. For example, in 2014, the Living Studio in New York worked with Ecodative on a project to build a pavilion with mycelium bricks showed in Figure 2.11 a) [58]. The pavilion was later dismantled, and the bricks used as compost. There have also been created structures for an exposition in Milan's fashion week 2019 [59], Figure 2.11 b).

Ecovative and Mogu, have been creating interior design pieces such as lamps, tables, plant pots, and other objects [49]. There have been attempts of using 3D printing with mycelium. One of the firsts designers to use 3D technology in mycelium is designer Eric Klarenbeek [60] . He 3D-printed a chair using living fungus, Figure 2.11 c), which then grows inside the structure to give it strength. As the chair didn't suffer the drying process, mushrooms grew out of it as a decorative item. Some small studios [61] [62], have been developing the technology to design pieces with 3D printing, but as it's still a very unexplored sector, so it hasn't been used in any industrial application, only in design.

Figure 2.11 a) Pavilion Build with Mycelium Bricks [58]; b) Milan's fashion week 2019 [58]; c) 3D-printed a chair using living fungus [60].

3 Experimental Methodology

3.1 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
- **Preparation of the substrate.** 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. 10% of additives are added.
- **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.

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.

- **Materials 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 3.1.

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

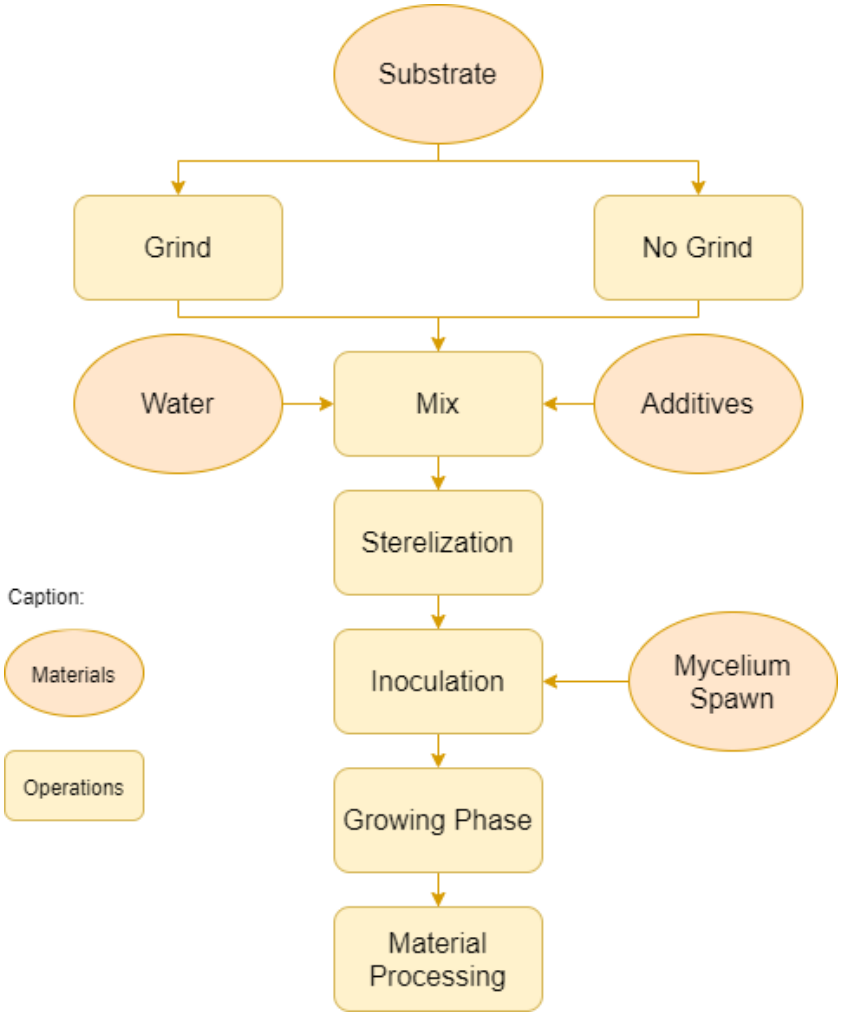


Figure 3.1 Flowchart of experimental work

3.2 Materials Used

The substrates, additives and mycelium species used in these experiments are summarized in Table 3-1.

Table 3-1 Materials Used: Substrates, Additives and Species

Substrates	Sogrape 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, Figure 3.2 a). 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, Figure 3.2 b), 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 3.2 a) Quinta da Romeira farm; b) Nãm mushrooms grown in bags

Ancillary materials

Because the sterilization is carried out in an autoclave, it's necessary to use 60 microns polypropylene bags, Figure 3.3 a). These bags also have a 0.2 microns filter, Figure 3.3 b), 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.

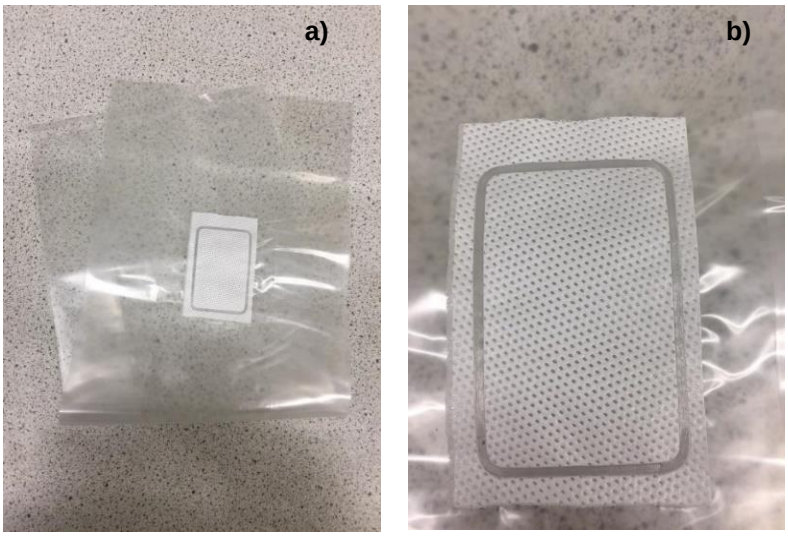


Figure 3.3 a) Polypropylene bags used; b) Bag 0.2 microns filter.

Nine types of moulds are used in this experiment:

Mould	Dimensions (cm)	Material
Type 1	3 x 2.5	Plastic
Type 2	45 x 25	Plastic
Type 3	13 x 8.5	Plastic
Type 4	7 x 7	Metal
Type 5	18.5 x 11.5	Plastic
Type 6	20 x 12	Metal
Type 7	20 x 9	Metal
Type 8	ϕ8	Plastic
Type 9	23 x 18	Plastic

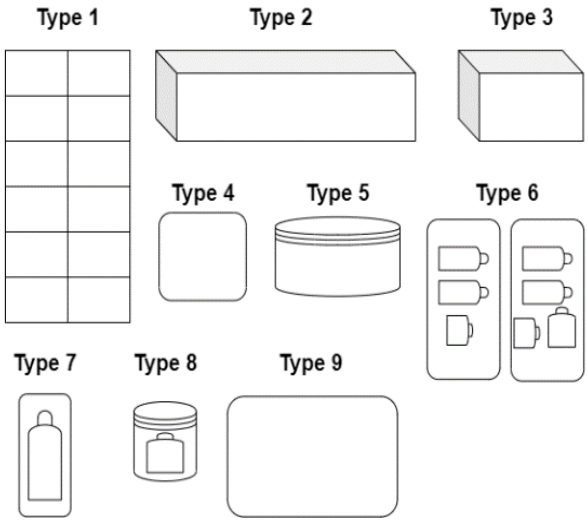


Figure 3.4 Moulds used for the experiment

3.3 Equipment

1) Grinder

The grinder used is from Erdwich mod, EWZ 2000, as seen in Figure 3.5 a). 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, and can be showed in Figure 3.5 b).

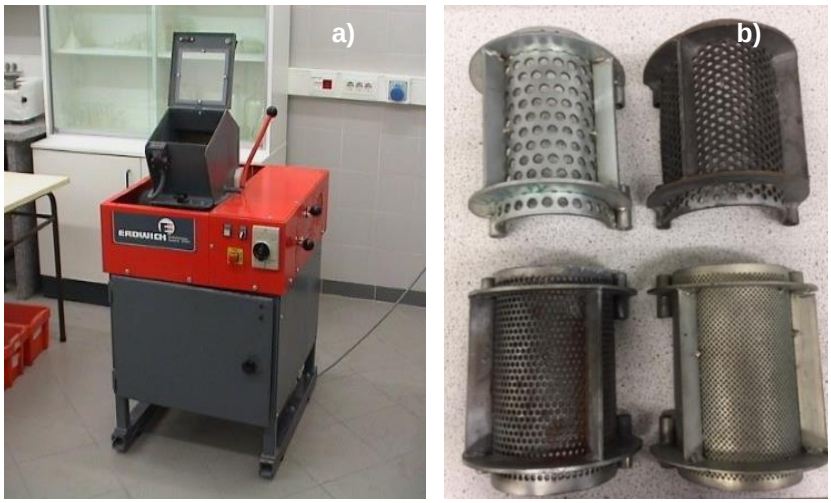


Figure 3.5 a) Grinder EWZ 2000 used to grind the substrate; b) Different sieves with diameters of 10, 6, 4 and 2 mm.

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 *Bio-Engineering Department* at *Instituto Superior Técnico*, provided the laminar flow cabinet *Aura 2000 M.A.C from Bioair instruments*, Figure 3.6.

The cabinet disinfection is assured by a UV lamp. Its efficacy depends on the exposure time to the lamp radiations of micro-organisms to be destroyed. In this case, the UV lamp was used for thirty minutes. The working area is ventilated by a unidirectional filtered air flow whose rate is approximately 70% of the total handled air volume, whereas 30% creates an inward air flow at the access opening. The cabinet is equipped with a fan for handling 100% of the air volume to a plenum from which a portion of the air is supplied to the work area and the remainder is exhausted from the cabinet through an exhaust filter. The exhausted air volume corresponds to that which creates the inward air flow for personnel protection.

The filtration of both ventilation circuits assures sterility of the work area for products and of the exhaust air for ambient protection.

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.

Figure 3.6 Laminar flow cabinet aura 2000 M.A.C from Bioair instruments

3.4 Sample Nomenclature Identification

To obtain several samples, there are four major factors to consider:

1) Substrate

The rice husk samples are classified as Group A and identified as *RH*. The Sograpе samples are classified as Group B. Prior to any treatment, the sample is identified as *SS.* The Nãm substrate is identified as Group C and identified as *NS*. The mixture samples are classified as Group D, samples with a Sograpе and Nãm mixture are identified as *SNS*, samples with a rice husk and Nãm are identified as *RNS*, finally, samples with a rice husk and rice husk and Sograpе are identified as *RSS*. Group D is further classified as D1, D2, D3, and D4. D1 is a mix of Sograpе and Nãm substrates, this mixture contains 50% of each substrate, *½ SNS*. Group D2 is composed of two-thirds rice husk and one-third Nãm substrate, *RNS*. Group D3 is made up of two-thirds rice husk and one-third Sograpе substrate. D4 consists of two-thirds Sograpе substrate and one-third Nãm substrate *2/3 SNS*. The sole distinction between Groups D1 and D4 is the amount of substrate used in each group.

2) Particle size

Rice husk samples do not suffer from any grind treatment. Unlike the rice husk samples, Sograpе substrate is ground. The substrate ground with 4mm sieves is identified as SS_4, and 2mm sieves is identified as SS_2. Certain samples of the Nãm substrate are ground, whilst others grow on unground substrate. The unground samples are designated NS_N, the ground samples with 10mm meshes are designated NS_{10}, and the ground samples with 6mm meshes are designated NS_6. For D1 mixture, Nãm substrate is ground with 6 mm and Sograpе substrate is ground 4 mm meshes, $½\ SNS_{4,6}$. For group D2 some samples have the Nãm substrate ground with 10 mm meshes, RNS_{10}, and some with 6 mm meshes, RNS_6. For group D3 some samples have the Sograpе substrate ground with 4mm meshes, RSS_4, and some with 2mm meshes, RSS_2. Finally, group D4 has the Nãm substrate is ground with 6 mm and Sograpе substrate is ground 4 mm meshes, $2/3\ SNS_{4,6}$.

3) Additives

Rice husks samples containing additives are identified as RH^A. Sograpе samples containing additives are classified as SS_4^A *and* SS_2^A. The Nãm samples containing additives are classified as NS_N^A, NS_{10}^A, NS_6^A. Finally, the mixture samples containing additives are identified as $½\ SNS_{4,6}^A$, RNS_{10}^A, RNS_6^A, RSS_4^A, RSS_2^A and $2/3\ SNS_{4,6}^A$.

4) Species of mycelium

Additionally, the samples are classified according to the type of species employed. Samples inoculated with both *Ganoderma Lucium* and *Pleurotus Ostreatus* species are named RHS^A - *GL* + *PL*. The *RHS - GL* is inoculated with *Ganoderma Lucium* species, and the *RHS - PO* is inoculated with *Pleurotus Ostreatus* species. The same procedure is used for the remaining substrates.

Table 3-2, 3-3, 3-4 and 3-5 summarize this information.

Table 3-2 Group A Samples Acronyms and Meaning

Group	Acronym	Meaning
A	**RHS^A - GL + PO**	Ganoderma Lucium and Pleurotus Ostreatus species on Rice Husks substrate with additives.
	RHS^A - GL	Ganoderma Lucium species on Rice Husks substrate with additives.
	RHS^A - PO	Pleurotus Ostreatus species on Rice Husks substrate with additives.
	RHS - GL	Ganoderma Lucium species on Rice Husks substrate without additives.
	RHS - PO	Pleurotus Ostreatus species on Rice Husks substrate without additives.

Table 3-3 Group B Samples Acronyms and Meaning

Group	Acronym	Meaning
B	**SS_4 - GL**	Ganoderma Lucium on Sograpе substrate without additives, grinded with 4mm sieves.
	SS_4 - PO	Pleurotus Ostreatus on Sograpе substrate without additives, grinded with 4mm sieves.
	SS_4^A - GL + PO	Ganoderma Lucium and Pleurotus Ostreatus on Sograpе substrate with additives, grinded with 4mm sieves.
	SS_4^A - GL	Ganoderma Lucium on Sograpе substrate with additives, grinded with 4mm sieves.
	SS_4^A - PO	Pleurotus Ostreatus on Sograpе substrate with additives, grinded with 4mm sieves.
	SS_2 - GL	Ganoderma Lucium on Sograpе substrate without additives, grinded with 2mm sieves.
	SS_2 - PO	Pleurotus Ostreatus on Sograpе substrate without additives, grinded with 2mm sieves.
	SS_2^A - GL	Ganoderma Lucium on Sograpе substrate with additives, grinded with 2mm sieves.
	SS_2^A - PO	Pleurotus Ostreatus on Sograpе substrate with additives, grinded with 2mm sieves.

Table 3-4 Group C Samples Acronyms and Meaning

Group	Acronym	Meaning
C	**NS_N - GL**	Ganoderma Lucium on Nãm substrate without additives, non-grinded.
	NS_N - PO	Pleurotus Ostreatus on Nãm substrate without additives, non-grinded.
	NS_N^A - GL	Ganoderma Lucium on Nãm substrate with additives, non-grinded.
	NS_N^A - PO	Pleurotus Ostreatus on Nãm substrate with additives, non-grinded.
	NS_{10} - GL	Ganoderma Lucium on Nãm substrate without additives, grinded with 10mm sieves.
	NS_{10} - PO	Pleurotus Ostreatus on Nãm substrate without additives, grinded with 10mm sieves.
	NS_{10}^A - GL + PO	Ganoderma Lucium and Pleurotus Ostreatus on Nãm substrate with additives, grinded with 10mm sieves.
	NS_{10}^A - GL	Ganoderma Lucium on Nãm substrate with additives, grinded with 10mm sieves.
	NS_{10}^A - PO	Pleurotus Ostreatus on Nãm substrate with additives, grinded with 10mm sieves.
	NS_6 - GL	Ganoderma Lucium on Nãm substrate without additives, grinded without 6mm sieves.
	NS_6 - PO	Pleurotus Ostreatus on Nãm substrate without additives, grinded with 6mm sieves.
	NS_6^A - GL	Ganoderma Lucium on Nãm substrate with additives, grinded with 6mm sieves.
	NS_6^A - PO	Pleurotus Ostreatus on Nãm substrate with additives, grinded without 6mm sieves.

Table 3-5 Group D Samples Acronyms and Meaning

Group	Acronym	Meaning
D	**GL- ½ SNS$^{A}_{4,6}$**	Ganoderma Lucium species on 1/2 Sograpе substrate, grinded with 4mm meshs, and 1/2 Nãm substrate, grinded with 6mm sieves, with additives.
	PO- ½ SNS$^{A}_{4,6}$	Pleurotus Ostreatus species on 1/2 Sograpе substrate, grinded with 4mm meshs, and 1/2 Nãm substrate, grinded with 6mm sieves, with additives.
	GL- RNS$^{A}_{10}$	Ganoderma Lucium species on 2/3 of Rice Husks substrate and 1/3 of Nãm substrate grinded with 10mm sieves, with additives.
	PO- RNS$^{A}_{10}$	Pleurotus Ostreatus species on 2/3 of Rice Husks substrate and 1/3 of Nãm substrate grinded with 10mm sieves, with additives.
	GL- RNS$^{A}_{6}$	Ganoderma Lucium species on 2/3 of Rice Husks substrate and 1/3 of Nãm substrate grinded with 6mm sieves, with additives.
	PO- RNS$^{A}_{6}$	Pleurotus Ostreatus species on 2/3 of Rice Husks substrate and 1/3 of Nãm substrate grinded with 6mm sieves, with additives.
	GL- RSS$^{A}_{2}$	Ganoderma Lucium species on 2/3 of Rice Husks substrate and 1/3 of Sograpе substrate grinded with 2mm sieves, with additives.
	PO- RSS$^{A}_{2}$	Pleurotus Ostreatus species on 2/3 of Rice Husks substrate and 1/3 of Sograpе substrate grinded with 2mm sieves, with additives.
	GL- RSS$^{A}_{4}$	Ganoderma Lucium species on 2/3 of Rice Husks substrate and 1/3 of Sograpе substrate grinded with 4mm sieves, with additives.
	PO- RSS$^{A}_{4}$	Pleurotus Ostreatus species on 2/3 of Rice Husks substrate and 1/3 of Sograpе substrate grinded with 4mm sieves, with additives.
	GL- 2/3 SNS$^{A}_{4,6}$	Ganoderma Lucium species on 2/3 Sograpе substrate, grinded with 4mm meshs, and 1/3 Nãm substrate, grinded with 6mm sieves, with additives.
	PO- 2/3 SNS$^{A}_{4,6}$	Pleurotus Ostreatus species on 2/3 Sograpе substrate, grinded with 4mm meshs, and 1/3 Nãm substrate, grinded with 6mm sieves, with additives.

4 Results and Discussion

4.1 Substrate Characterization

The substrates as received can be seen in Figure 4.1 from a macroscopic perspective. As illustrated in Figure 4.1 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 4.1 b). This is a very homogeneous and dry material. In contrast to the Nãm substrate depicted in Figure 4.1 c), which has multiple small branches of varying diameters.

Figure 4.1 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 4.2, the rice husks and Nãm substrate are shown in greater detail. The rice husk, Figure 4.2 a), is between 6x2 and 8x2 millimetres. In Figure 4.2 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 4.2 a) Rice husk Substrate as received; b) Nãm Substrate as received

4.2 Preparation of samples

4.2.1 Substrate Preparation

Grinding Operation

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 4.3 a) and 4.3 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.

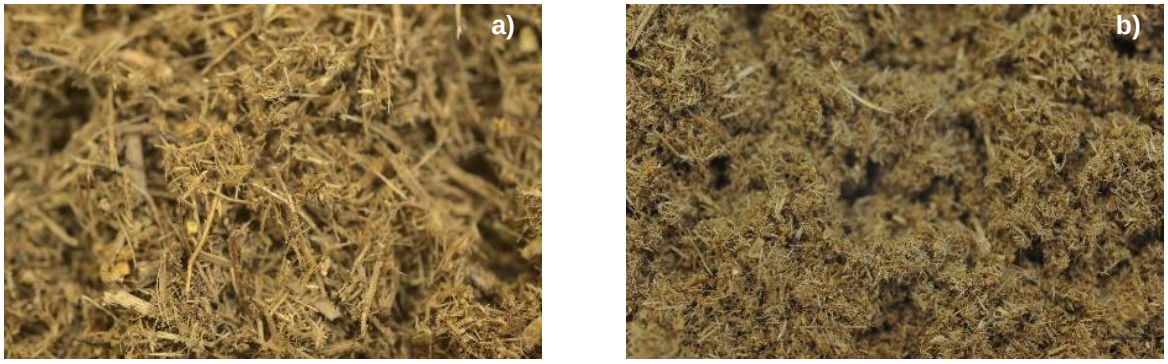


Figure 4.3 a) Sograpе substrate grinded with a 4mm sieve; b) Sograpе substrate grinded with a 2 mm sieve.

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 4.4 a) and 4.2 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 4.4 a) Nãm Substrate grinded with a 10mm sieve; b) Nãm Substrate grinded with a 6mm sieve.

Mix Operation

The weight of each sample, the additives, the amount of water applied to each sample, and the final water/substrate ratio are all listed in the tables 4-1, 4-2, 4-3 and 4-4. This data enables the analysis of the effect of water and the addition of additives on mycelium growth.

The information for Group A is presented in Table 4-1. Since the rice husk substrate is fully dry, rather large volumes of water are integrated. 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.

Table 4-1 Group A Preparation of the Mixture Experimental Data

Group A- Rice Husks					
Sample	**Weight (g)**	**Tot. Weight (g)**	**Additives (g)**	**Water (g)**	**Water / 1Kg Substrate**
RHS^{A} - GL + PO	80.50	194.00	12.20	367.30	1.89
RHS^{A} - GL	45.00		6.80		
RHS^{A} - PO	68.50		10.38		
RHS - GL	80.50	125.50	-	204.10	1.63
RHS - PO	45.00		-		

Table 4-2 summarizes the information pertaining to Group B. Since the Sograpе pruning wood was still green at the time of grinding, a significant amount of water drained from the branches, resulting in an extremely humid substrate. This resulted in the requirement for less water to attain the desired water content.

As observed, the SS_2^A - *GL* and SS_2^A - *PO* samples have a greater ratio, 0.9, than the other samples in this group. This could mean that the 2mm substrate requires more water than the 4mm substrate, or that the water was added incorrectly. Further investigation will be conducted to see whether having such a high water/substrate ratio has a negative or positive effect on the sample.

The information about Group C is presented in Table 4-3. Due to the fact that Nãm substrate is derived from earlier mushroom production, the process of adding water had already completed. Taking this into account, the amount of water necessary to maintain the moisture content is projected to be minimal. The samples NS_N^A - *GL* and NS_N^A - *PO*, show a higher ratio, 1.03, than the other samples. Further investigation will be conducted to determine whether such a high water/substrate ratio has a detrimental or beneficial effect on the sample.

Table 4-2 Group B Preparation of the Mixture Experimental Data

Group B- Sograpé						
Sample	**Sieve Size**	**Weight (g)**	**Tot. Weight (g)**	**Additives (g)**	**Water (g)**	**Water / 1Kg Substrate**
SS_4^A - GL + PO	**4 mm**	55.5	562.7	2.78	185.6	0.33
SS_4^A - GL		619		30.95		
SS_4^A - PO		61		3.05		
SS_4 - GL		45.5	320	-	141.8	0.44
SS_4 - PO		40		-		
SS_2^A - GL	**2 mm**	110	178	5.5	160.7	0.9
SS_2^A - PO		68		3.4		
SS_2 - GL		45	320	-	141.8	0.44
SS_2 - PO		45		-		

Table 4-3 Group C Preparation of the Mixture Experimental Data

Group C - Nãm						
Sample	**Sieve Size**	**Weight (g)**	**Tot. Weight (g)**	**Additives (g)**	**Water (g)**	**Water / 1Kg Substrate**
NS_N^A - GL	**No grind**	43	106	2.15	109.5	1.03
NS_N^A - PO		63		3.15		
NS_N^A - GL		43	86	-	43	0.5
NS_N^A - PO		43		-		
NS_{10}^A - GL + PO	**10 mm**	101.5	394	5.08	185.6	0.47
NS_{10}^A - GL		197.5		9.88		
NS_{10}^A - PO		95		4.75		
NS_{10}^A - GL		71	163	-	80	0.49
NS_{10}^A - PO		92		-		
NS_6^A - GL	**6 mm**	191.5	391	9.58	161	0.41
NS_6^A - PO		199.5		9.98		
NS_6^A - GL		78	156	-	76	0.49
NS_6^A - PO		78		-		

Table 4-4 shows the information about Group D. The water/substrate ratio is greater in rice husk samples. The Sograpé substrate samples have a higher ratio than the Nãm substrate samples. All the Sograpé and Nãm substrate samples show similar ratios, with the exception of the *1/2 $SNS^A_{4,6}$ - PO* sample has a much lower ratio (0.75) than the other samples in its group. With further results, an analysis will be performed, to understand whether having such a low value of the water/ substrate ratio will have a negative or positive impact on the samples.

Once all the mixtures are prepared, they are transferred into the polypropylene bags and labelled Figure 4.5. The bags are folded and taped to be able to be introduced in the autoclave.

Table 4-4 Group D Preparation of the Mixture Experimental Data

Group D- Mixture						
Sample	**Sieve Size**	**Weight (g)**	**Tot. Weight (g)**	**Additives (g)**	**Water (g)**	**Water / 1Kg Substrate**
1/2 $SNS^{A}_{4,6}$ - GL	**4 and 6 mm**	92.8	92.8	4.64	135.2	1.46
1/2 $SNS^{A}_{4,6}$ - PO	**4 and 6 mm**	92.8	92.8	4.64	70	0.75
RNS^{A}_{10} - GL	**10 mm**	50	50	2.5	100	2
RNS^{A}_{10} - PO	**10 mm**	50	50	2.5	97	1.94
RNS^{A}_{6} - GL	**6 mm**	50	50	2.5	77	1.54
RNS^{A}_{6} - PO	**6 mm**	50	50	2.5	75	1.5
RSS^{A}_{2} - GL	**2 mm**	50	50	2.5	105.4	2.11
RSSA2 - GL	**2 mm**	50	50	2.5	101	2.02
RSS^{A}_{4} - GL	**4 mm**	50	50	2.5	105	2.1
RSS^{A}_{4} - PO	**4 mm**	50	50	2.5	107	2.14
2/3 $SNS^{A}_{4,6}$ - GL	**4 and 6 mm**	50	50	2.5	74.5	1.49
2/3 $SNS^{A}_{4,6}$ - PO	**4 and 6 mm**	50	50	2.5	65	1.3

Figure 4.5 Labelled bags prepared to go into the autoclave.

4.2.2 Inoculation Operation

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, Figure 4.5. On Table 4-5 it is shown in detail information of the substrate and spawn weight.

Table 4-5 Substrate and Spawn Weight

Sample	**RHS^A - GL + PO**	**RHS^A -GL**	**RHS^A - PO**	**GL - RHS**	**RHS - PO**
Substrate Weight (g)	247.8	138.3	215	66	85.3
Spawn Weight (g)	24.8	13.8	21.5	6.6	8.5

Sample	**SS_2^A - GL**	**SS_2^A - PO**	**SS_2 - GL**	**SS_2 - PO**
Substrate Weight (g)	540.4	516.7	113.2	232.5
Spawn Weight (g)	54.0	51.7	11.3	23.3

Sample	**SS_4^A - GL + PO**	**SS_4^A - GL**	**SS_4^A - PO**	**SS_4 - GL**	**SS_4 - PO**
Substrate Weight (g)	199.2	110.1	284.8	62	79.8
Spawn Weight (g)	19.9	110.1	28.5	6.2	8.0

Sample	**NS_{10}^A - GL + PO**	**NS_{10}^A - GL**	**NS_{10}^A - PO**	**NS_{10}^A - GL**	**NS_{10}^A - PO**
Substrate Weight (g)	572.5	750.1	954.9	91	93.7
Spawn Weight (g)	57.3	75.0	95.5	9.1	9.4

Sample	**$NS6^A$ - GL**	**$NS6^A$ - PO**	**$NS6^A$ - GL**	**$NS6^A$ - PO**
Substrate Weight (g)	312.8	295.9	429	403.9
Spawn Weight (g)	31.3	29.6	42.9	40.4

Sample	**NS^A - GL**	**NS^A - PO**	**NS^A - GL**	**NS^A - PO**
Substrate Weight (g)	105.4	105.7	267.5	229.3
Spawn Weight (g)	10.5	10.6	26.8	22.9

Figure 4.6 Mycelium Spawn Being Transferred into the Bags.

4.2.3 Growing Phase

1) 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, Figure 4.7.

Figure 4.7 1st Stage of Development Mycelium Growth

To classify the samples, a scale ranging from 0 to 5, created for this study, is employed. Each sample is assigned a classification, with 0 representing no visible mycelium growth and 5 representing those with the most growth. The classification of all samples is shown in Figure 4.8. Samples with a classification below 2.5 are deemed underdeveloped, those with a classification equal to 2.5 are regarded to be adequately developed, and those with a classification greater than 2.5 are considered to have good development.

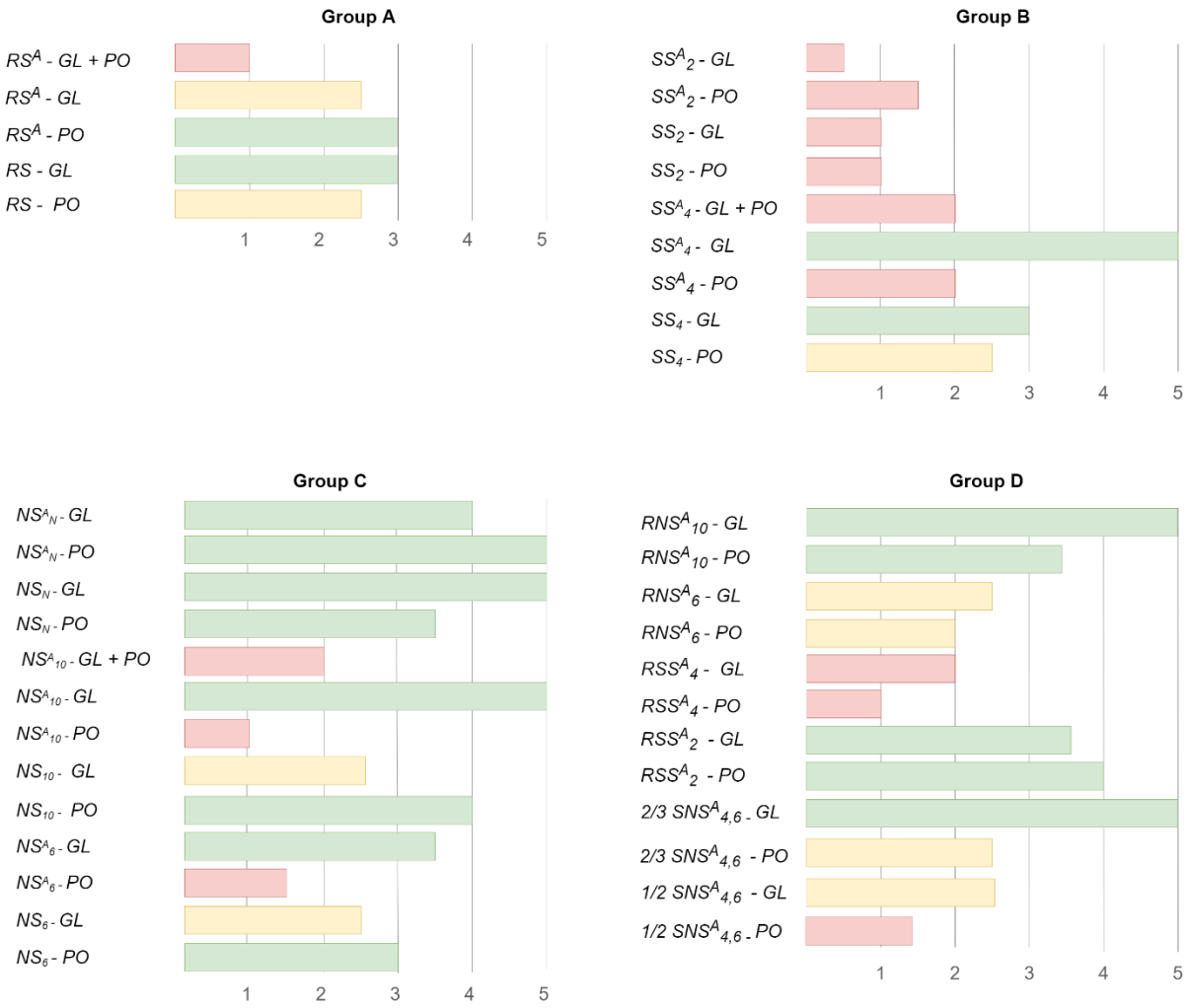


Figure 4.8 1st Stage of Development Diagram

From most groups, there is not a major growth rate difference between species, but there are some exceptions.

On Group A, the sample containing both species is clearly the weakest, but the samples containing only one species, produces similar results regardless of the species selected.

On Group B, the 2mm substrate sample yields no significant results; however, when 4mm substrates are analysed, those containing *Ganoderma Lucium* species yield a higher classification than the *Pleurotus Ostreatus* samples.

The same holds true for Group C, where the NS_{10}^{A} - *GL*, NS_{6}^{A} - *GL*, and *NS* - *GL* samples demonstrate that *Ganoderma Lucium* samples are significantly higher classified than *Pleurotus Ostreatus* samples. NS_{10} - *PO* is the only sample that demonstrates a significant higher classification with the *Pleurotus Ostreatus* species. The remainder of the samples demonstrates no significant variation across species.

On Group D the samples $GL - SNS^{A}_{4,6}$, $GL - RNS^{A}_{10}$, $GL - RSS^{A}_{4}$, $GL - SNS^{A}_{4,6}$ also show a significant higher growth rate than the *Pleurotus Ostreatus* species. The remainder of the samples demonstrates no significant variation across species.

After analysing the graphic on Figure 4.9, is possible to summarize the species results and see that the outcomes of combining the *Ganoderma Lucium* and *Pleurotus Ostreatus* shows the worst results. Comparing the *Pleurotus Ostreatus* and *Ganoderma Lucium* samples, it's noticeable that the *Ganoderma Lucium* samples have a tendency of getting higher classifications than the *Pleurotus Ostreatus* samples.

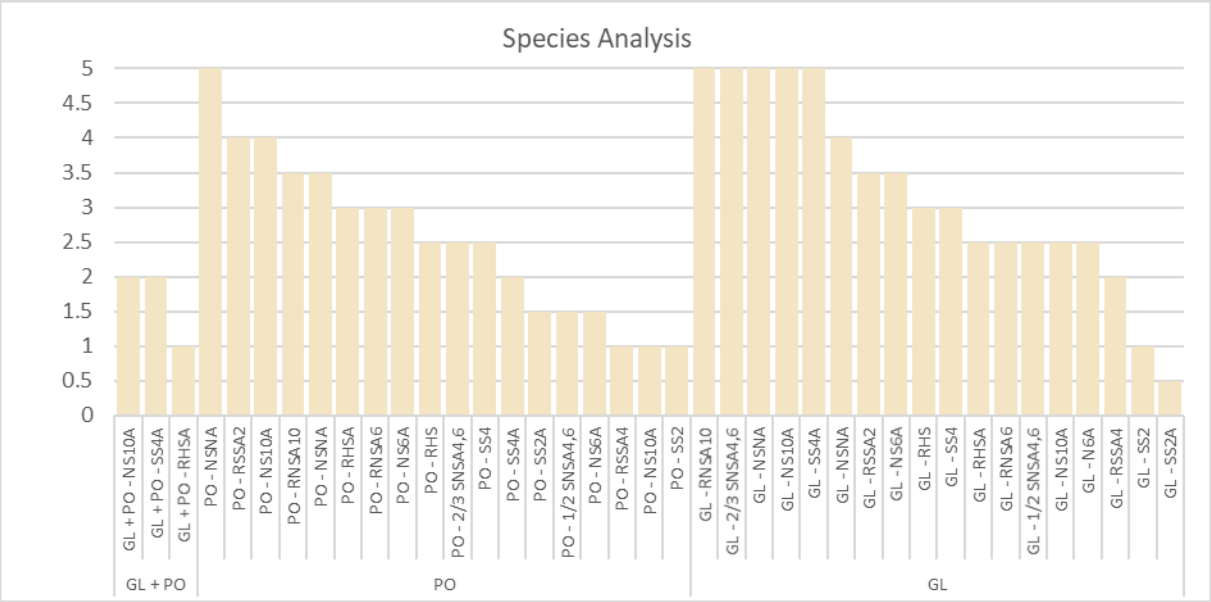


Figure 4.9 Species Classification Analysis

When the water content (Figure 4.10) is analysed, there is no obvious association between increased water content and mycelium development. Group A and Group D are the groups with the highest water content, and both exhibit somewhat typical growth or highly distinct growth within their respective groups. The same is true for Group B and C, where classifications vary significantly regardless of the samples' water content.

Rice husk particle sizes are regarded to be large in comparison to the particle sizes of the other substrates used in the experiment. Taking this into account, no significant conclusions can be drawn, as the result for these samples are normal.

All samples ground with 4 mm sieves perform better than samples ground with 2 mm sieves on the Sograpе substrate. The 2 mm samples exhibit very little development, and because their water/substrate ratios are relatively comparable, it appears as though the 2 mm sieves have a longer lag phase. On the other hand, the particles with a diameter of 4 mm exhibit normal mycelium development.

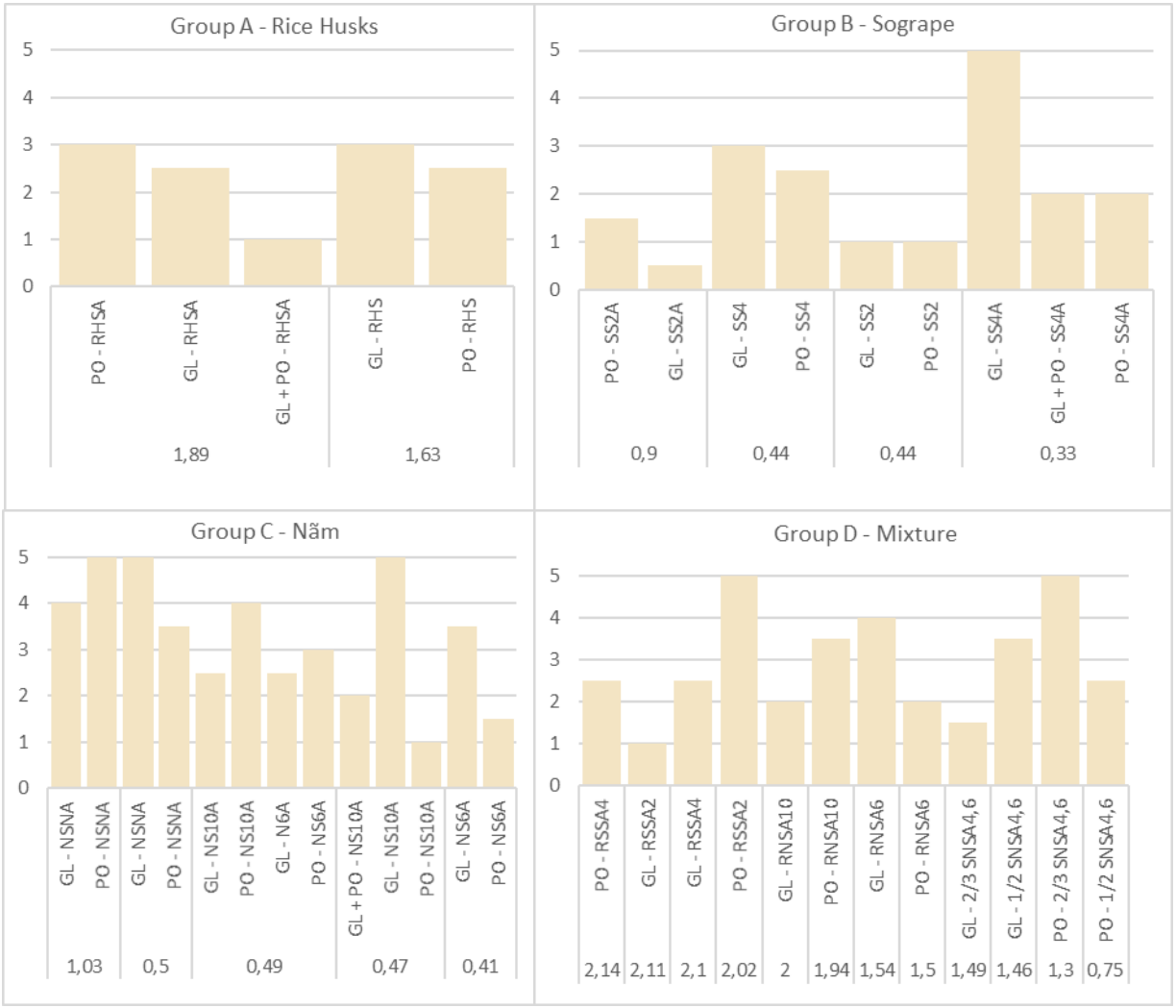


Figure 4.10 Water/ ratio analysis

All Nãm samples have a relatively comparable water/substrate ratio. The results for the 6 mm and 10 mm particle size samples are comparable, with the 10 mm sample performing somewhat better. However, non-grinded samples exhibit excellent results. As with the Sograpes substrate, it appears that larger particle sizes produce better results than smaller particle sizes, implying once again that smaller particle sizes may have a longer lag phase.

Three kinds of mixed samples were identified: rice husks and Nãm mix, rice husks and Sograpе mix, and Sograpе and Nãm mix.

When comparing the rice husks and Nãm mixtures RNS^{A}_{10} – *GL, PO* and RNS^{A}_{6} – *GL, PO*, it is clear that the 10 mm particle size sample performs better than the 6 mm particle size substrate, regardless of the species. However, because their water/substrate ratios fluctuate slightly, this group will be researched further.

The combination of rice husks and Sograpе produces intriguing outcomes. All of them exhibit a similar water/substrate ratio, although the *RSS$^{A}_{2}$ – GL, PO* samples perform better than the *RNS$^{A}_{4}$ – GL, PO* samples. Thus, when rice husks and Sograpе are combined, it appears that smaller particle sizes of the Sograpе substrate are more favourable for mycelium development. Although this result contradicts previous results for other samples in which larger particle sizes produce better results, because this analysis is referring to a mixture, having varying particle sizes (smaller in the Sograpе substrate and larger in the rice husks) may be beneficial for mycelium growth.

While both Sograpе and Nãm substrates are present in the $1/2\ SNS^{A}_{4,6}$ sample and the $2/3\ SNS^{A}_{4,6}$, the sample that contains more quantity of Sograpе than Nãm substrate has more successful results. This results doensn't mean necessarily that the Sograpе substrate show better growth results than the Nãm substates, since the particle size is different in both substrates.

The 2 mm substrate does not contain any samples grown with a classification greater than 2.5. Resulting in the poorest performance of all particle sizes. At 4 mm particle sizes, most samples are already matured, with only 40% remaining underdeveloped. The 6 mm particle size reveals that 50% of samples are fully developed, whereas 25% are underdeveloped. Rice substrates, with similar particle sizes to 6 mm Nãm, produce comparable outcomes to 4 mm and 6 mm particle sizes. Although, the rice husk samples are weaker than those obtained with the 4 mm and 6 mm substrates. The 10 mm particle size appears to have the same impact as the 4 mm particle size. However, the generated 10 mm samples exhibit somewhat superior results than the 4 mm samples. The unground samples produce the best results. Each sample is highly developed and has a high rate of development.

Thus, smaller particle sizes appear to have a longer lag phase, whereas larger particle sizes appear to have a fairly short lag phase showing more developed samples on the 1st stage of development.

Regarding the interactoin with the substrate, it is evident that the substrate with the poorest results is the Sograpе substrate, with the majority of its samples (67%) being undeveloped. On the other hand, the Nãm substrate produces the best outcomes, with 62% of samples indicating good development. Both rice husk and mixture substrates produce a high percentage of developed samples.

The impact of additives is determined across samples. In Figure 4.11 samples with the same properties, except for the additives, are compared side by side. When comparing the growth rate of the samples with additives and without additives is hard to get to a conclusion since all samples show very distinct results. For example, when analysing the rice husks samples there is not a clear conclusion. The *Ganoderma Lucium* sample with additives shows worst results than the *Ganoderma Lucium* sample with no additives, while the *Pleurotus Ostreatus* sample with additives show better results than the *Pleurotus Ostreatus* sample with no additives.

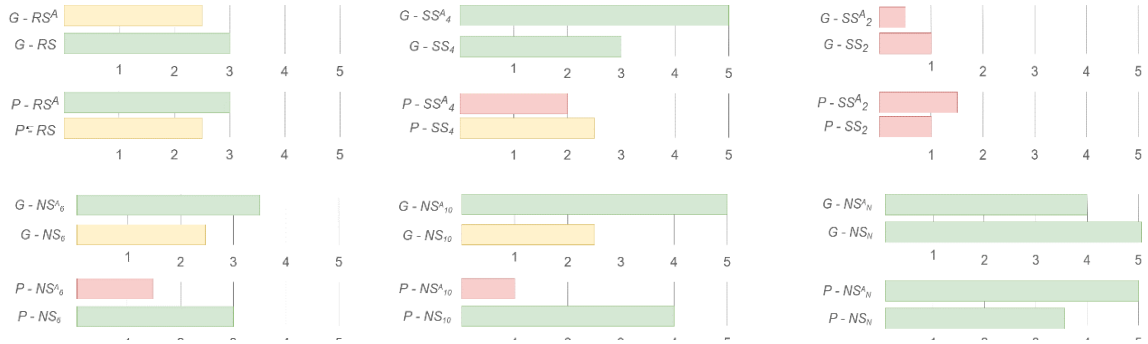


Figure 4.11 Additives Diagram

The only variant that differs is the water/ substrate rate. In this case the samples with additives have a higher rate, but given the results is hard at this point to conclude the effects of the water content. The same happens with the 2 mm Sograpе samples. The *Ganoderma Lucium* sample with additives shows worst results than the one without the additives. But the opposite occurs with the *Pleurotus Ostreatus* samples. Taking these factors in consideration is hard to make any assumptions for the effect the additive has on mycelium growth.

We considered that the species that could already be in a exponencial phase were: SS^A_2 - *GL* , SS^A_2 - *PO*, SS^A_4 - *GL* , SS_4 - *GL*, NS^A_{10} - *GL*, NS_{10} - *PO*, NS^A_6 - *GL*, NS_6 - *PO*, NS^A_N - *GL*, NS^A_N - *PO*, NS_N - *GL*, NS_N - *PO* , SNS^A_{10} - *GL* , SNS^A_{10} - *PO*, RSS^A_2 - *GL*, RSS^A_2 - *PO*, $SNS^A_{4,6}$ - *GL.* From all these samples, the majority are inoculated with *Ganoderma Lucium*, and the majority of samples still on the lag phase are *Pleurotus Ostreatus*. This leeds to belive that *Ganoderma Lucium* has a smaller lag phase than *Pleurotus Ostreatus*.

2) 2nd stage of development

The placing system used is depicted in Annex I, where is possible to see where all pre-materials are placed. 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, most samples became contaminated, as illustrated in Figure 4.12. Until now, the uncontaminated samples are shown in Annex II, in the moulding design. This study will be continued using the uncontaminated samples that were retained.

Figure 4.12 Contaminated Samples

The samples will be subjected to a visual inspection to determine how each sample developed over the first three and six days of development. The categorization is identical to that used for the first stage of development, with each remaining sample being assigned a value between 0 and 5 (less developed to more developed) Figure 4.13.

It is expected that during this phase, samples that were previously in the lag phase will enter an exponential phase and expand sufficiently to be taken to the third stage of development.

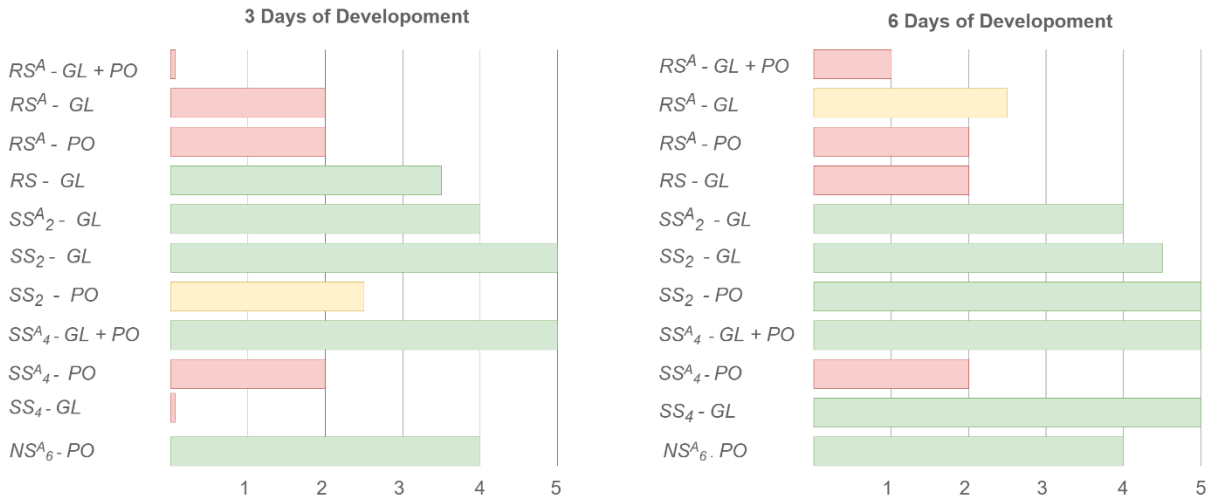


Figure 4.13 2nd Stage of Development Diagram

As illustrated in Figure 4.13, the most developed samples are *GL-SS$_2$, PO-SS$_2$, GL+PO-SS$^A_4$, GL-SS$_4$, PONS$^A_6$.* The *GL-RS* sample developed normally for the first three days, but subsequently appears to have plateaued. The *GL-SS$_4$* sample did not expand significantly over the first three days but has shown

remarkable growth after then. Contamination occurred in the *GL-SS$^{A}_{2}$* and *PO-SS$^{A}_{4}$* samples. The remainder had no significant development.

Only thirty-six percent of samples created during the first stage of development are free of infection. 36% of the species that survived are inoculated with *Ganoderma Lucium*, seven percent with *Pleurotus Ostreatus, and fourteen percent with both species. On the first stage of development, the percentage of samples inoculated with Ganoderma Lucium that developed rapidly was much higher than other species and,as a result, it's unsurprising that Ganoderma Lucium is also demonstrating some of the most prom isig results at this point. What is unexpected is that the samples inoculated with both Ganoderma Lucium and Pleurotus Ostreatus developed the slowest of all the species in the first stage, but two of the three samples inoculated with this species* survived, with one of them being one of the most developed samples in this stage. The samples inoculated with *Pleurotus Ostreatus* performed extremely poorly. Given the number of samples inoculated with this species during the first stage of development, very few survived after three days, and only one survived on the sixth days.

Given this, the specie with the highest rate of survival is *Ganoderma Lucium* and *Pleurotus Ostreatus*, with two out of three species remaining during the first stage of development. Following that, *Ganoderma Lucium* had seven out of eighteen samples remain. Finally, *Pleurotus Ostreatus* was found to be non-infected in one of eighteen samples.

It is evident that most samples that did not become infected have a substrate with a particle size of 2mm. Thirty eight percent of the surviving samples have 2 mm substrates, twenty three percent have 4 mm substrates, eight percent have 6 mm substrates, and thirty one percent have rice husk substrates. As the ground substrate concentration increases, more samples get contaminated.

The most successful size for development is likewise the 2 mm substrate, with seventy one percent of samples of this size receiving a classification of four or higher. This result is unexpected, given that the particle size with the least increase during the first stage of development was the 2 mm substrate. Additionally, a conclusion drawn during the first stage of development is that as particle size increases, the samples appear to have a shorter lag phase, indicating a faster development. At this point, it is possible to deduce that as the particle size of the sample increases, the sample becomes more susceptible to infection.

Sixty four percent of uncontaminated samples contain Sograpе substrate, twenty nine percent rice husks, and seven percent Nãm substrate. All the mixture samples became contaminated. This result is once again surprising, as the substrate that produced the best outcomes during the first stage of development was the Nãm substrate, while the substrate that produced the lowest results was the Sograpе substrate. In contrast to the outcome of this stage.

Due to the fact that a large number of samples were lost, it is extremely difficult to draw any conclusions about the water substrate ratio during this phase. However, it is worth noting that sample *GL – SS^{A}_{2}* is the sample with the greatest results and has the highest water substrate ratio. However, once again, due to a paucity of data, conclusions are difficult to draw. The same is true for additives.

3) Sample Analysis

Lag Phase and Exponential Phase

All samples that were not affected during the second stage of growth were either underdeveloped or developed normally in comparison to other samples. For instance, samples such as *RS^{A} - GL + PO, SS^{A}_{2} - GL, SS_{2} - GL, SS2 - PO, SS^{A}_{4} - GL + PO* and *NS^{A}_{6} - PO* demonstrated extremely poor development during the first stage, but samples such as *RS^{A} - GL*, *RS - GL*, *RS^{A} - PO*, *SS_{4} - GL* and *SS_{4} - PO* shown normal development. The samples with the poorest development during the first stage had longer lag phases, as it appears to take them longer to adjust to their new surroundings. On the other hand, the remaining samples that developed normally adapted to their surroundings better, growing at a faster rate and with a shorter lag phase.

It was hypothesized that more mature samples would be less prone to contamination due to the stronger mycelium. In this case, the sample with the least development on the first stage (*GL – SS^{A}_{2}*) proved to be one of the most successful on the second stage. On the first stage, the samples with the 2 mm Sogrape pre-material developed the least, followed by those with the 4 mm Sogrape pre-material. The bulk of these examples exhibit excellent second stage growth. The only samples that did not develop normally during the second stage were those that were only inoculated with *Pleurotus Ostreatus* species and, reinforcing the notion that *Pleurotus Ostreatus* species are more susceptible to contamination than *Ganoderma Lucium* species.

All samples will now be analysed:

GROUP A

RS^{A} - GL + PO **GANODERMA LUCIUM AND PLEUROTUS OSTREATUS SPECIES ON A RICE HUSKS SUBSTRATE WITH ADDITIVES**	
	*6 days of development* While many samples grew greatly within the first six days after being placed in the mould, this sample grew a patch of mycelium on the bottom corner only after six days of development. This demonstrates that the lag phase is significantly long in comparison to other samples.
	*10 days of development* The mycelium developed rapidly following the appearance of the first spot, indicating that the sample had reached the exponential phase. For the first ten days, the substrate is moist.
	*24 days of development* However, while the entire sample was covered in mycelium by the twenty fourth day, the pre-material is extremely dry, which inhibits mycelium development. It was determined that the moisture conditions were most likely not satisfied, resulting in the sample being extremely dry. As a result, the sample proceeds into the second approach of moulding. The sample proceeded to the third stage of development on the seventeenth day of development. Even though this sample has a developed top layer, when it's removed from the mould, the sample's other layers are extremely underdeveloped. Because of this, on attempting to remove the sample from the mould, it fell apart. As a result, the sample is removed from consideration for the study.

The pre-material placed in type 4 mould did not begin to produce results until day 36, which was unusual. Unfortunately, did not progress farther from that point. As a result, the sample was removed from consideration for the study.

RS^{A} - GL *GANODERMA LUCIUM* SPECIES ON A RICE HUSKS SUBSTRATE WITH ADDITIVES	
	*3 days of development* This sample grew rapidly during the first stage of development, indicating that the lag phase may have ended, and the sample was in the exponential phase at that point. Given this, it was anticipated that the second stage of development would yield positive outcomes. Only three days, mycelium began to grow in type 3 mould,
	*6 days of development* This sample substrate retained a significant amount of moisture, which is favourable for mycelium development. Even though the top layer of mycelium is not completely covered by mycelium on the sixth day of development, the sample appeared to have progressed sufficiently to progress to the next stage of development.
	*6 days of development* When entering the third phase, this sample appears to perform well. These samples were removed from the mold after six days of development. The samples from type 3 mould appear moist immediately upon removal, and the mycelium is rather white and bright**.**
	*15 days of development* Despite this, after 15 days of development, this sample began to lose its brightness; the rice husks have lost their color, indicating dryness and presenting the perception that the required humidity levels havenot been met. Due to the absence of development in all of the samples from this pre-material, this could signal that they are reaching a stacionary phase. This is essential since the stage's objective of completely covering all samples with mycelium has not been reached. Due to the extreme dryness of the rice husks, it is assumed that this is occurring since leaving the samaple entirely open does not appear to allow for moisture conditions to prevail. As a result, after 15 days of development, the type of moulding is changed to the second approach.
	*35 days of development* For the next twenty days, no significant growth is found in the sample following this change in mouling. The sample appear to be similar to those observed twenty days earlier. As a result, the mycelium has entered the stationary phase. This phase can be triggered by a multitude of circumstances, the most common of which is a lack of moisture. The samples are transferred to the Material Processing stage after 35 days

The examination of the remaining samples reveal that the behavior of the sample in type 4 mould is similar to that of the preceding sample. When the type 4 mold sample is removed, it appears bright and moist at first, but after a few days, it becomes increasingly dry and dull. On the other hand, the type 1 mold sample appears to be even less developed than the previous two. It also looks to be less developed and compact on subsequent days.The remainding development is very similar to the type 3 sample.

RS - GL *GANODERMA LUCIUM* SPECIES ON A RICE HUSKS SUBSTRATE	
	*6 days of development* This sample demonstrated favourable results at the first stage of development, the highest in Group A. This indicates that this sample may have already entered the expansion phase. This sample started by developing uneven after 6 days of development
	*10 days of development* Based on the top layer, it appears as though this sample is not progressing much over time after 10 days
	*15 days of development* After 15 days, the "old" mycelium formed is decaying while new spots of live mycelium continue to appear. Certain patches of mycelium are clearly growing upward rather than inside the substrate. This pattern of behaviour is also evident in the type 8 sample.
	*24 days of development* After 24 of development, the mycelium continued to grow in upward, instead of penetrating the substrate. This behaviour has never been seen in *Ganoderma Lucium* inoculated samples. This sample became infected, rendering it incapable of progressing to the next stage.

The sample in type 8 mould exhibits normal growth. One notable observation is that the mycelium is growing out of the sample. As seen after 15 days of development, certain patches of mycelium are clearly growing upward rather than inside the substrate. This pattern of behaviour is also evident in the type 3 sample. Unfortunately, this sample became infected after a few days.

RS^{A} - PO *PLEUROTUS OSTREATUS* SPECIES ON A RICE HUSKS SUBSTRATE	
	*3 days of development* This sample was already in the exponencial phase on the first stage of development, indicating that the lag phase had ended. As a result, it's expected to develop properly once placed in the mould. Despite the fact that it was supposed to grow rapidly, the mycelium developed in an extremely uneven manner. After three days, an uneven mycelium growth can be observed.
	*6 days of development* On the sixth day of growth a more uniform layer of mycelium begins to appear, but growth remains quite uneven.
	*15 days of development* The changes between six and fifteen days, are not notable, indicating that the samples had entered a stacionary stage, at which point the exponencial phase would cease. As a result, it's determined that the growing circumstances for this sample were inadequate.
	*19 days of development* The sample is already demonstrating progress on the nineteenth day of development, nine days after the alteration. Mycelium is developing much more uniformly and with a healthy white coating
 	*24 days of development* After twenty-four days, the sample is clearly in an exponential phase. The mycelium is quite vivid and well developed. So, the second approach is introduced into this sample in order to produce a more compact and humid sample. Because the entire sample, not just the top layer, is now under control, this action of altering the type of moulding is considered a transition into the third stage of development. When comparing the results of this moulding to those of the previous system (just the top side with an air opening), it is clear that this method produces superior outcomes with an equal growth pattern across the sample and improved moisture conditions.

28 days of development

On the twenty-eighth day, Figure 4.20 b), the mycelium begins to develop in a unique manner. There appear to be two stages of mycelium development: a bottom layer later in the process, where the mycelium is less visible, and a more consistent and thicker layer at the top. This thick top layer is interesting, and because it has the potential to expand further, it's decided to grow it for a few more days in order to analyse it.

48 days of development

No more growth is anticipated so, the sample is transferred to the Material Processing phase.

GROUP B

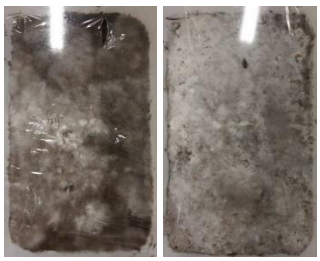
SS^{A}_{2} - GL
GANODERMA LUCIUM SPECIES ON A SOGRAPE SUBSTRATE WITH 2MM AND ADDITIVES

3 days of development – 1st Attempt

After three days of placing the pre-material on the mold, the top layer appears to be developing normally, despite the uneven coating of mycelium.

6 days of development – 1st Attempt

Mycelium totally covers the top layer of the sample in only six days. This result is quite intriguing since this sample previously had extremely poor results on the first stage of development. It was the least developed sample and appeared to be in the lag phase when placed in the mould. Unfortunately, some traces of contamination are emerging around the corners, and the sample cannot be permitted to grow for an extended period of time. As a result of the contamination, the growth process is halted and the material processing phase is initiated.



3 days of development – 2nd Attempt

As with the first attempt, this sample demonstrates excellent mycelium development. Both samples are showing encouraging development only three days after being placed in the mould.

8 days of development – 2nd Attempt

Both are covered in mycelium after eight days, with the M2 sample being slightly more developed than the M1. This time, none of the samples exhibit symptoms of contamination.

Both samples are removed from the mould after eight days of development. Both exhibit excellent development on the bottom and side layers. M2 continues to be slightly more developed than M1. The mycelium is white, and the sample is humid, indicating that it is developing normally.

12 days of development – 2nd Attempt

The mycelium has definitely expanded after twelve days. However, in order to generate a thicker mycelium layer, the second approach to moulding is used on M2, while the first approach is kept used on M1. This allows for an analysis of whether the second strategy is more appropriate.

17 days of development – 2nd Attempt

On the seventeenth day, it becomes very evident that the disparities are enormous. M2 sample generated a fairly thick layer of mycelium, whilst M1 sample exhibited no change from 5 days prior. This concludes that the second approach of moulding is far more appropriate than the first. Even with these results, we chose to continue growing both samples, transferring the M1 sample to the second approach.

After 21 days, the M2 sample did not grow significantly more; the only noticeable variation is that the sample's color became more brown. Something unusual about this sample is that the letters and numbers from the mold have been etched into the mycelium, this result can be seen in more detail in Annex VI. Assumedly, the sample is not improving, which is why it was transferred to the Material

Processing stage. On the other hand, the M1 sample was designed for the worst-case scenario. After 21 days, a portion of the sample developed a dark brown color. However, a new dot of new mycelium begins to appear in the center of the sample. After 28 day, the brown spots began to vanish and the entire sample took on a light brown tone. The white spot that appeared in the sample's center has become more developed, and newer spots are forming across the sample. This could be a sign of a mushroom emerging, however it's interesting to see if it can grow into a thick white coating. After 39 days of development, what could have been a new layer failed to develop sufficiently to be relevant for the sample to continue developing. Thus, the sample advances to the following process, Material Processing. This results can also be seen in detail in Annex VI.

The results of the remaining samples can be seen in Annex VI. The sample in type 1 mould has developed well. After eight days it has covered the entire top layer so, it’s decided to take the sample into the third stage of development. The samples grew normally covering the remaining layers the sample.

For mould type 4, even though the outer top layer of this sample was highly developed after eight days, the remaining layers were significantly less developed. This is possibly because the interior lacked access to air, resulting in a slower rate of development. So, it’s decided to remove the sample from the mould and allow it to grow on all sides. This was not the ideal decision, as it is discovered that this strategy is not optimal for meeting the growth criteria. This is because, despite the fact that some growth occurred after 12 days of development, no significant indication of development is observed. As a result, it's decided to switch to the second approach and use a different type of moulding to ensure that the sample retained more moisture. After 28 days, the mycelium retained nearly the same amount of mycelium, indicating that mycelium growth had ceased. It appeared as though there was no way to restart the mycelium growth, and the sample has entered the stationary phase. Although, something intriguing is found. The top layer of the sample appeared to be quite distinct from the other layers. On the top left side, a mushroom appeared, and a variety of brown colours emerged. The appearance of the mushroom may indicate that the mycelium should stop growing; otherwise, mushrooms may continue to grow, which is not goal intended. So, this sample is taken into the Material Processing phase after twenty-eight days.

Regarding the sample in type 5 mould, a plastic wrap is placed around the sample and beneath it to prevent contamination from coming into contact with the mould. Some patches of mycelium seem to have appeared after three days, however, mycelium growing all over the surfaces of the mould can be seen. After six days the sample is clearly more developed, but still isn’t covering the full surface. The bottom layer is expanding well after eight days of development, and a fine coating of mycelium is beginning to cover it. On the other hand, despite its healthy growth, the top layer is beginning to exhibit brown patches and an odd development in some areas. It's fascinating to observe how the mycelium has grown all around the plastic sheet and formed the plastics shape. It’s decided to take the sample out to the third stage of development. After 21 days, the top layer deteriorated significantly in

appearance. Black holes begin to develop, and the odour is peculiar. On the upper side of the sample, a small mushroom appears to be forming. The bottom layer continues to grow and has a more appealing look than the top layer. It's also fascinating to note that the cuts made on the mould are still visible in the mycelium. Mycelium grows in such a detailed manner that it appears to take on any shape. After twenty-eight days, the top layer continues to look dreadful. The mushroom appears to have begun to grow even larger, which is not a good indication. On the other side, the bottom layer appears to be healthy, more developed, and even the small label letters of the mould that previously contained ice cream are visible on the mycelium sample. While the bottom layer appears to be developing normally, the side and upper layers are highly uneven.

SS_2 - GL *GANODERMA LUCIUM* SPECIES ON A SOGRAPE SUBSTRATE WITH 2MM	
	*3 and 8 days of development* Three days after placement in the mould, the mycelium had covered the majority of the surface area. It is still not completely covered, since some regions have more prominent white spots than others. This result is surprising because throughout the first stage of development, this sample demonstrated extremely little mycelium growth. After eight days of growth inside the mould, the sample demonstrates an extremely developed top layer of mycelium. As a result, it was agreed to withdraw the sample and go on to the third stage of development.
	*8 days of development* When the sample is removed from the mould, it is immediately apparent that the sample is in an exponential phase with excellent development. Mycelium appears bright, and the sample has moisture.
	*21 days of development* Regrettably, the sample did not develop substantially after 21 days. The mycelium became weaker and less vibrant, and the sample lost its humidity. This is assumed to be because, as seen with previous samples, the first approach to moulding is ineffective since it exposes the sample excessively. Following these observations, it's decided to subject the sample to the second approach of moulding.
	*35 days of development* However, it appears that the sample has already reached a stationary phase, as the sample showed no difference after 35 days. Following that, the sample is sent to the Material Processing phase.

Regarding the sample in type 8 mould, this sample has a very similar behaviour to the previous sample. It stars with a very good development, followed by a stagnation of growth due to too much exposure to the environment, ending up in a stationary phase, followed by entering the next phase Material Processing. The results can be seen in Annex VII.

SS_2 - PO *GANODERMA LUCIUM* SPECIES ON SOGRAPE SUBSTRATE WITH 2MM	
	*3 and 8 days of development* As with the other 2mm Sograpе samples, this one demonstrated poor outcomes during the initial stage of development and a lengthy lag phase. What makes this sample unique is that, unlike other 2 mm Sograpе samples that performed poorly in the first stage but well in the second, this one performed poorly in both phases. Even at the second stage, it appears as though this sample will never reach the exploratory phase. This sample demonstrates multiple patches of mycelium on the top layer during the first three days. After eight years of evolution, these patches have grown slightly in size but have remained relatively unchanged. In comparison to previous samples, this one is quite underdeveloped. After only a few days, the sample became contaminated.

SS^A_4 - GL + PO *GANODERMA LUCIUM* SPECIES ON SOGRAPE SUBSTRATE WITH 2MM	
	*3 and 6 days of development – 1st Attempt* This sample showed very good rate of mycelium growth. After three days, Figure 4.29 a), the top layer is already very developed. After six days, Figure 4.29 b), the top layer was completely covered by mycelium.
	*6 days of development- 1st Attempt* Unfurtunally, some signs of contamitation showed up around the corner so, the sample could not be left to grow anymore time, Figure 4.28. Due to this contamination, the sample skips the 3rd stage by stopping the growing process and proceeding into the next phase *Material Processing.*
	*3 days of development – 2nd Attempt* This sample shown, by far, the best results in terms of mycelium growth. After only three days the top later was almost completely covered in mycelium in both moulds. After eight days of being places in the mould, we considered the mycelium to be grown enough to be taken into the 3rd stage of development.

When removed from the mould, this sample demonstrates outstanding results, with the majority of all layers covered in mycelium, indicating that this sample is extremely robust.

12 days of development

However, as with previous samples, when this sample is exposed to the first approach of moulding, it begins to lose moisture. After 12 days, the sample does not improve so, it is determined to mould it using the second approach.

17 days of development

After 17 days, the sample has not improved in terms of mycelium growth, but it is exhibiting signs of mushroom development.

35 days of development

Regrettably, the white spots were actually forming mushrooms, leaving no space for mycelium to grow. At this point, the sample has been allowed to grow for an extended period of time and is transferred to the next phase of Material Processing.

SS_4 - PO
GANODERMA LUCIUM SPECIES ON SOGRAPE SUBSTRATE WITH 2MM

3 days of development

This sample developed normally throughout the first step. Given that this sample may have been in an exponential phase, or very close to it, during the first phase, the results from the second phase were surprising, given the sample's limited development.

This sample did not exhibit significant development throughout the first three days. In contrast to other Sograpе samples, this one exhibits some mycelium development paths. After a few days, the sample became contaminated

SS_4 - GL *GANODERMA LUCIUM* SPECIES ON SOGRAPE SUBSTRATE WITH 4MM	
	*8 and 11 days of development* Due to some complications of the experience, this sample was placed before the others. This sample was already in the exponential phase during the first step of development, thus positive outcomes during this phase are predicted. After eight days, the sample exhibits excellent mycelium development, with the top surface equally covered by mycelium.
	After 11 days, it is possible to see the formation of some lumps. When these lumps appear, it is usually an indication that a mushroom is forming. As a result, it is determined to move forward to the third stage of development.
	*20 days of development* This sample began with positive indicators of development, but then stagnated in growth as other samples did. After 20 days of development, and with no evidence of mycelium development, the second technique is applied in this sample.
	*41 days of development* Regrettably, no additional evidence of development are observed after 41 days, and hence the sample is sent to the Material Processing phase.

GROUP C

NS_6 - PO *PLEUROTUS OSTREATUS* SPECIES ON NÃM SUBSTRATE WITH 6MM	
	*3 and 6 days of development* After three days, this sample has developed a large number of spots throughout the substrate. Three days later, mycelium had completely covered the top layer. Outside, the mycelium is rapidly growing.

15 days of development.

On day fifteen, the sample continued to grow outward rather than within. This behaviour has been observed in other *Pleurotus Ostreatus*-inoculated samples. As a result, it is decided to remove this sample and flip it over, providing more oxygen to the sample's other sides. This action is regarded as progressing this sample to the next stage.

15 and 19 days of development

When the sample is turned over, it is obvious that the other layers did not obtain as much oxigen as the top layer. After only four days, the top layer exhibits significantly more mycelium development, demonstrating the critical nature of all layers having access to oxigen.

24 days of development

After twenty-four days of development, the sample continues to demonstrate development on the top layer, Figure 4.36 c), but it is decided to submit the sample to the second approach of moulding, because, while this sample is demonstrating excellent development, it has been demonstrated with other samples that the second approach has better results.

28 days of development

After twenty-eight days, mushrooms began to develop, indicating that the sample is approaching the end of its growth cycle (4.36 d).

39 days of development

After thirty-nine days, the sample continued to develop e), but at this point, the sample is transferred to the Material Processing step to prevent the appearance of additional mushrroms.

4.2.4 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.

Following treatment, all samples are significantly more dry and light. Table 4-6 shows the weight of each sample.

Table 4-6 Samples Weight after Material Process

Espécie	Attempt	Moulding Type	Weigth (g)	Code
RS^A - GL	1st	4	17	2
RS^A - GL	1st	3	25	4
RS - GL	1st	3	19	4
SS^A_2 - GL	2nd	3	31	6
SS^A_2 - GL	2nd	3	39	6
SS^A_2 - GL	2nd	3	23	6
SS^A_2 - GL	2nd	1	2	6
SS^A_2 - GL	2nd	1	3	6
SS^A - GL	1st	4	23	6 1st
SS^A_2 - GL	1st	5	71	6
SS_2 - GL	1st	3	23	8
SS_2 - GL	1st	1	2	8
SS_2 - GL	1st	8	22	8
SS^A_4 - GL + PO	1st	3	34	10 1st
SS^A_4 - GL + PO	2nd	3	22	10
SS^A_4 - GL + PO	1st	1	3	10 1st
SS_4 - GL	1st	3	19	13
NS^A_6 - PO	1st	1	2	21

After being removed from the oven, the sample *NS^A_6 - PO* in type 9 mould came apart. This sample never had a lot of development on the inside because, as previously stated, the mycelium tended to grow out of the sample rather than into it. As a result, the sample lacked mycelium linkages that held the substrate together.

The rice sample are very loose, the some rice husks fall out of the sample. Theses samples nedded fot more mycelium growth. While some smaples maintained the white mycelium color, some turned brown.

In Figure 4.14 is possible to see the final inspection of the remaining samples after the material processing phase.

Figure 4.14 Samples Final Look after Material Processing

5 Conclusions

The answer the first research question, 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. Since all Nãm samples got contaminated is not possible to take conclusions regarding its growth rate. 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.

On the first stage of development, Nãm samples were showing good mycelium growth and promising results. Due to the machine malfunction on the 2nd stage of development, all samples got exposed to an unclean environment and, consequently, almost all Nam samples got contaminated. Given that some samples from other substrates survived, this leads to conclude that samples that have been previously used in mushroom production are more prone to contaminations than substrates that have never been inoculated with mycelium. What is left unknown is if this substrate would have thrived if the samples had a “normal” and adequate environment.

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, it is once again difficult to take any conclusions. That being said, 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 sample, 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 answer the second question, it is hard to make conclusions due to the contamination rate on the Nãm substrate. Nevertheless, this substrate showed great potential on the first stage of development.

To answer the third question and 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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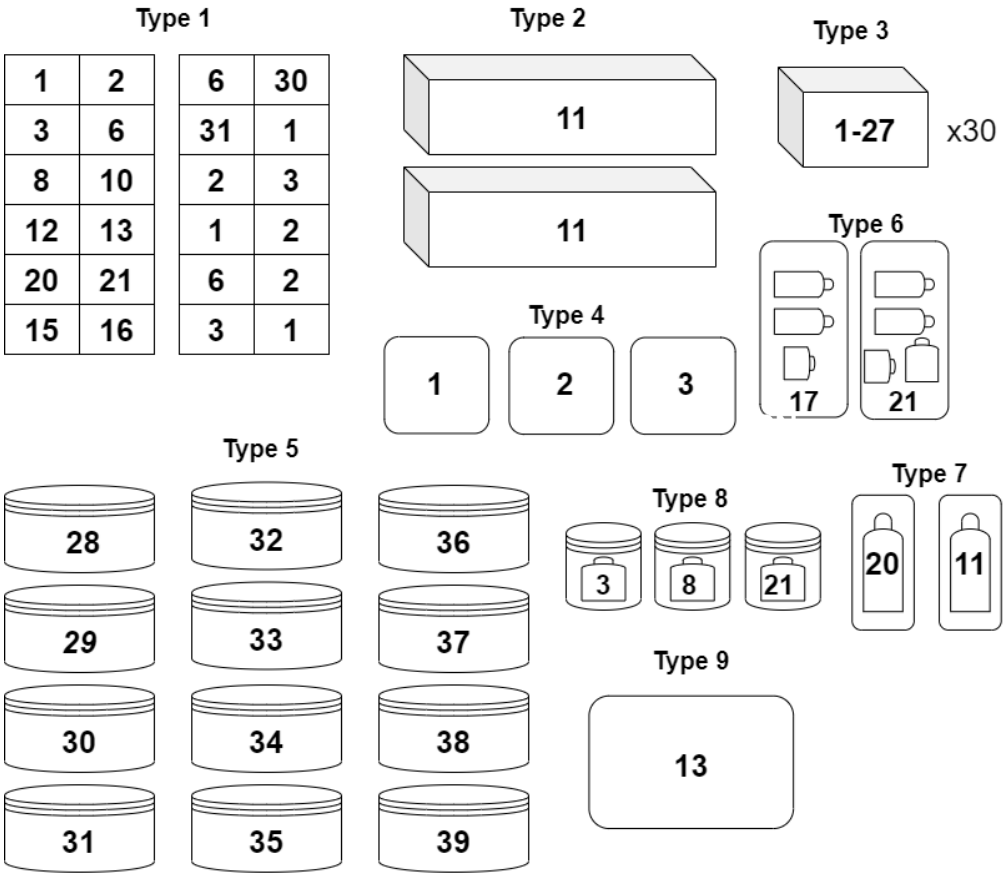
Annexs

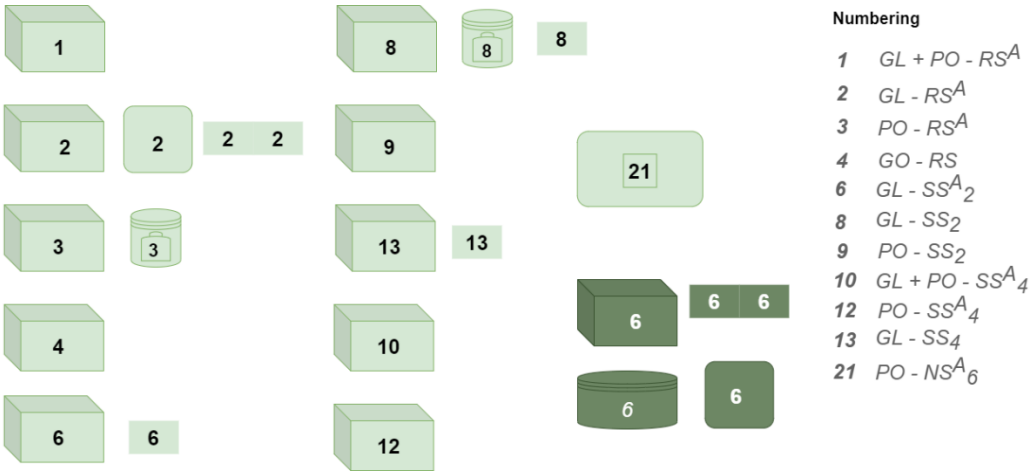
Annex I – Moulding system and all pre-materials applied



Numbering

1	$GL + PO - RS^A$	**14**	$PO - SS_4$	**27**	$PO - NS_N$
2	$GL - RS^A$	**15**	$GL + PO - NS^A{}_{10}$	**28**	$PO - SNS^A{}_{10}$
3	$PO - RS^A$	**16**	$GL - NS^A{}_{10}$	**29**	$GL - RNS^A{}_6$
4	$GO - RS$	**17**	$PO - NS^A{}_{10}$	**30**	$PO - RNS^A{}_6$
5	$PL - RS$	**18**	$GL - NS_{10}$	**31**	$GL - RSS^A{}_2$
6	$GL - SS^A{}_2$	**19**	$PO - NS_{10}$	**32**	$PO - RSS^A{}_2$
7	$PO - SS^A{}_2$	**20**	$GL - NS^A{}_6$	**33**	$GL - RSS^A{}_4$
8	$GL - SS_2$	**21**	$PO - NS^A{}_6$	**34**	$PO - RSS^A{}_4$
9	$PO - SS_2$	**22**	$GL - NS_6$	**35**	$GL - SNS^A{}_{4,6}$
10	$GL + PO - SS^A{}_4$	**23**	$PO - NS_6$	**36**	$PO - SNS^A{}_{4,6}$
11	$GL - SS^A{}_4$	**24**	$GL - NS^A{}_N$	**37**	$GL - SNS^A{}_{4,6}$
12	$PO - SS^A{}_4$	**25**	$PO - NS^A{}_N$	**38**	$PO - SNS^A{}_{4,6}$
13	$GL - SS_4$	**26**	$GL - NS_N$	**39**	$GL - SNS^A{}_{10}$

Annex II – Uncontaminated Samples



Annex III– Development of *RS^A - GL + PO*

2nd stage of development – Type 4

Figure 1 36 days of development

Annex IV – Development of *RS^A – GL*

2nd stage of development Type 4

Figure 2 3 days of development

3rd stage of development Type 1

Figure 3 a) 6 days of development; b) 16 days of development; c) 35 days of development

3rd stage of development – Type 4

Figure 4 a) 6 days of development; b) 15 days of development; c) 36 days of development

Annex V – Development of *PO - RS*^{A}

2nd stage of development -

Figure 5 a) 3 days of development; b) 10 days of development; c) 15 days of development.

Annex VI – Development of *GL – SS*$^{A}{}_{2}$

2nd stage of development - Type 1

Figure 6 a) 3 days of development; b) 8 days of development

3rd stage of development - Type 3

Figure 0.1 a) 21 days of development M1 sample; b) 28 days of development M1 sample; c) 39 days of development M1 sample.

Figure 0.2 21 days of development M2 Sample

2nd stage of development - Type 4

Figure 7 a) 3 days of development; b) 8 days of development.

3rd stage of development - Type 4

Figure 8 a) 12 days of development; b) 21 days of development; c) Bottom view of 28 days of development; d) Top view 28 days of development

2nd stage of development - Type 5

Figure 8 a) 3 days of development; b) 6 days of development; c) 8 days of development.

3rd stage of development - Type 5

Figure 9 8 days of development a) Sample top view b) Sample bottom view.

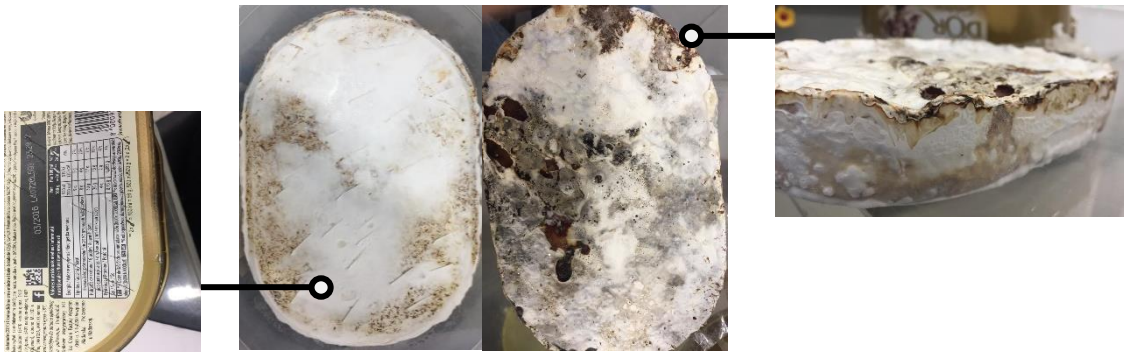


Figure 10 21 days of development a) Bottom view; b) Top view.

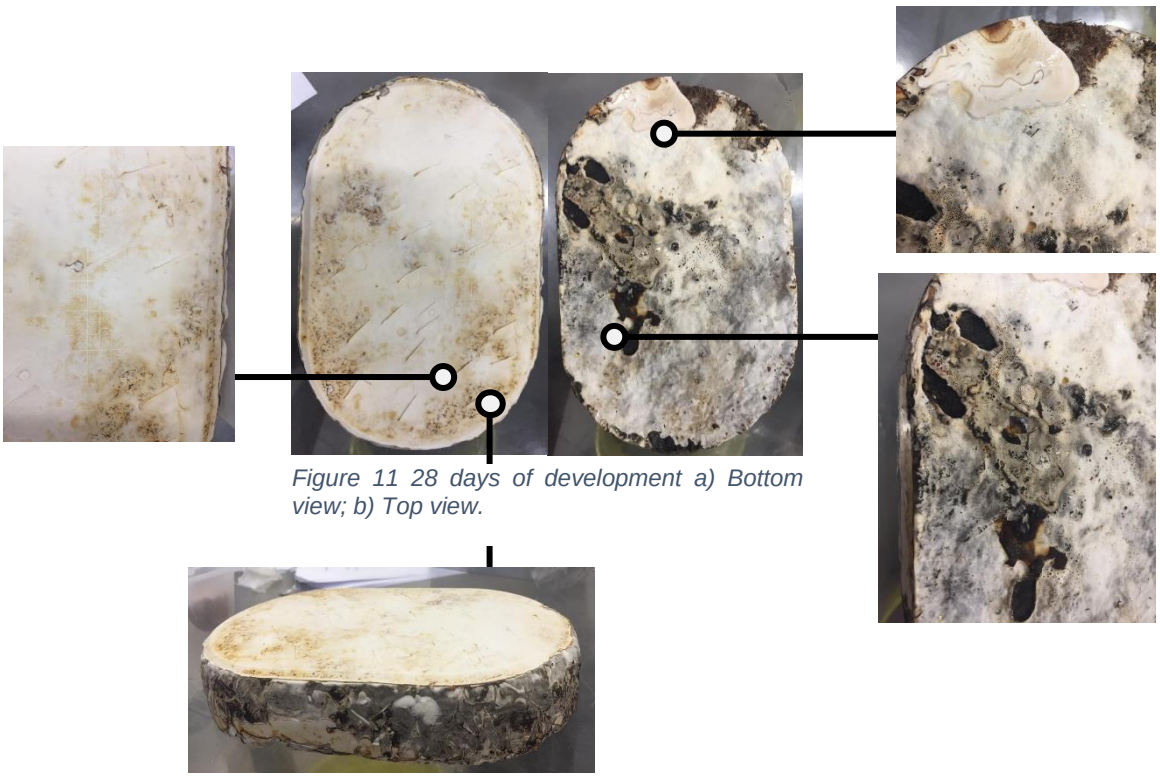


Figure 11 28 days of development a) Bottom view; b) Top view.

Annex VII - Development of *GL – SS_2*

3rd Stage of Development – Type 8

Figure 0.3 a) 8 days of development; b)15 days of development; c) 35 days of development bottom view; d) 35 days of development top view.