PROGRAMMED GROWTH

A Living Mycelium and Clay Composite

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Abstract. Through 3D printing of a mixture of clay and natural fibres, this paper proposes a first design experimentation to understand the parameters that influence mycelium materials' growth, including inoculation (IS) and fabrication strategies (FS). Given the broad spectrum of complex, interrelated variables, the aim is to provide insight based on many empirical experiments and visual inspection through a series of prototypes. The growth behaviour of mycelium and the visual inspection highlight that the inoculation strategy with a post-inoculation of mycelium in the substrate is the one that assures maximal mycelium colonization. Through empirical testing, we demonstrated control over the growth process by guiding the manual insertion of mycelium spawn with undulated areas on the printed surface generated through an image sampling algorithm. Moreover, such a strategy is viable for future programmed growth artefacts once the interactive insertion of mycelium spawn is substituted with a multi-material technique allowing for controlled growth in selective areas induced by the fabrication technique. In terms of resource optimization, the FS that optimizes the use of the material is the most favourable since it was possible to obtain the same final colonized geometry with less material.

Keywords. Additive Manufacturing, Mycelium And Clay Composite, Material Experimentation, Biological Fibres, Visual Inspection.

1. Introduction and State of the Art

The construction sector needs a shift in emphasis toward grown and reusable materials instead of excavated resources utilized in building systems (Hebel & Heisel, 2017). In this context, fungi, microorganisms that have been used for thousands of years as nourishment, have gained momentum in recent years due to their potential use as 100% biological binders for biobased materials (Barzee et al., 2021). As it grows and branches out in space searching for food, the mycelium can digest lignocellulosic biomass forming very light, natural composites and fire resistant (Jones et al., 2018).

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Today, there are examples in the literature that use this living being to assemble insulation materials or for interior applications, paving the way towards broader use of biotechnology in the field of design and architecture (Camere & Karana, 2018; Robertson et al., 2020). To broaden the scope of application, the subsequent stage for building with mycelium is to use free-form additive manufacturing mycelium-based materials rather than growing them into moulds (Goidea et al., 2022, Bhardwaj et al., 2020; Elsacker et al., 2021; Soh et al., 2020). In some of these examples, clay is added as viscous material to assure the printability of the mixture (Jauk et al., 201). With 3D printing of mycelium-material, where mycelium is in a combination of clay and natural fibres, it is possible to reach the desired stage of a fully natural composite that is totally "cultivated" and that is responding to local resource availability, from the primary material to the binder itself, while optimizing the quantity of material used. However, in all the analysed cases, the living mycelium is inserted as spawns or liquid culture before printing, without the possibility of control, or better, "program" the growth of the mycelium itself to respond to design necessity.

2. Research Challenges and Objectives

Indeed, what are the parameters that could affect the programmed growth of mycelium? The State-of-the-art highlighted the research gap on different inoculation and fabrication strategies that could affect the 3D printing parameters of the living extrudable matter. By challenging the standard approach of simply inoculating the mycelium spawn in the printing paste, the purpose of this paper is to provide comprehension of all relevant challenges, processes, production steps and underlying growth divers of extrusion-based 3D-printing of mycelium materials. The biocomposite production can be summarized in three stages: injection, development and deactivation (Carcassi et al., 2022). In this research, only the inoculation and incubation of 20 days are considered since the aim is to understand the parameters that could affect the mycelium growth while printing. As a matter of fact, in this work, we: a) developed an extrusion paste specifically for 3D printing, b) investigated three mycelium inoculation strategies to test the growth rate, c) analysed four fabrication approaches to understand the printing factors affecting the mycelium growth of the most promising inoculation strategies, d) proposed a solution that could guide the user to control the mycelium growth, better called design drivers. Given the broad spectrum of complex, interrelated variables, the aim is to provide a first insight based on many empirical experiments and visual inspection of eight prototypes.

3. Methodology

The methodology is divided into four steps: material mixture design, inoculation strategies, fabrication strategies and design drivers. Pleurotus Ostreatus (P24) was purchased from ITALSPAWN Srl (Onigo di Pedderoba, Italy) as a rye grain spawn. According to the grain spawn producers, it is essential to mix the substrate and wet the mixture until there is a constant relative humidity (RH) of 70 maximum and a pH of 7-7.5. Therefore, the pH of the substrate was measured. The non-firing modelling clay (NFC) was purchased from Pébéo (Gémenos, France). This type of clay was chosen to avoid the firing stage, which could compromise mycelium growth and survival. The non-firing clay was chosen to be black so that the white filaments of mycelium would stand out more. Wooden sawdust fibres (WF) produced as waste during the wood panels manufacturing were chosen as fibrous materials in a circular economy logic. The sawdust comes from the first sawmill processing by Rainoldi Legnami (Castione Andevenno, Italy) of coniferous wood spruce from Switzerland. Once transported to the laboratory at Politecnico of Milan, they were sorted with a column of sieves. Only fibres obtained from the mesh number 20 were selected that corresponded to a length of less than a millimetre (around 0.8mm). A base mixture of wooden sawdust fibres and clay was first made. This relates to establishing a appropriate link between the elements to have at the absolute least, enough workability and constructability. To achieve the proper consistency for the printing paste, various ratios were examined. The wood sawdust fibres were supplied as a food source for the mycelium. To start, 30% w/w of threads were mixed with 70% w/w of clay. To the base mixture, 30% w/w of deionized water was added. This quantity of water was chosen to ensure the fruiting bodies grow at an RH of 70%, as suggested by the mycelium spawn producers. However, this concentration was not the most suitable because the consistency of the mixture needed to be more proper since the fibres and the clay were not bonded enough. Due to the low workability, a change in the concentration of the base mixture was assessed. First, 80% by weight of clay and 20% by weight of fibres, and second, 90% by weight of clay and 10% by weight of fibres. All three ratios were tested with a syringe with a diameter of 12 mm to simulate the extrusion process of the printer and later on with the 3D printer. However, only the third ratio, namely the 90% w/w clay and 10% w/w in fibres, was successfully extruded from the nozzle in terms of both the buildability and workability of the printed element. While extruding, the mycelium was manually inserted between layers, and after three days, the mycelium growth was observed. When working with mycelium materials, it is recommended to sterilize the substrate properly, here called the printing paste, to remove the microbial competition of existing bacteria and fungi in the material. The sterilization was done in an autoclave for 40 minutes at a temperature of 121°C. The heat rendered the clay workable by hand to homogenize and distribute the fibres in the mixture. The pH was also measured with a pH tester stripe and was performed three times on the final mix to ensure a pH of 7-7.5. The advised temperature for spawning is 25°C which will also be maintained during the incubation. The entire development of the mycelium in the compost is obtained at 25-28° C. Normally, after 14-17 days, the substrate is white and ideally incubated; hence, we chose 20 days of observation to give more time to the mycelium to develop fully. During this phase, it is essential to maintain the temperature and the humidity of the ambient constant at 80-90%. These temperatures were assured with a thermos comfort mat and measured with a hygrometer thermometer. Once the printing paste ratio was determined, three inoculation strategies (IS) were investigated to understand the best way to integrate mycelium spawn in the printing process. The percentage of each component was kept constant, namely clay 60%, sawdust 7%, deionized water 20%, and mycelium 13%, and only the timing of insertion of the sawdust fibres and mycelium changed. In IS 1, the mycelium spawn was already mixed in the printing paste; in IS 2, the mycelium was already mixed in the printing paste, whereas the sawdust fibres were added interactively while printing; in IS 3, the sawdust fibres were inserted in the printing paste, and the mycelium was post inoculated manually while printing. The aim was to understand the parameters that can control and maximize mycelium growth. In IS 1, both mycelium and the feeding substrate were inserted in the printing paste in a traditional way. In IS 2, by manually inserting the sawdust fibres, we expected the randomly inserted mycelium spawn to reach the fibres according to the manual distribution. IS 3, we expected the mycelium to reach out for food throughout all the printed objects. The results were visually analysed for 20 days. When scaling up clay and mycelium composites, the fabrication strategy (FS) is just as important as the different ways of inoculation. Mycelium growth depends on a damp clay substrate, which is problematic for additive manufacturing because of buckling induced by the own weight of the overlying material and consequent failures. So, the different FS given in the research enable changeable moisture content for mycelium development without compensating for growing circumstances (Figure 1).

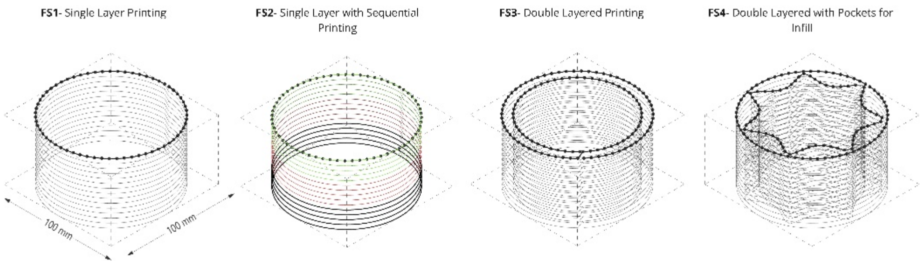


Figure 1. The four different fabrication strategies (FS) to integrate mycelium in clay additive manufacturing. From the left: FS1, FS2 , FS3 and FS4.

To test the hypothesis, a cylinder with a diameter of 10 cm and height of 6 cm, with a layer height of 4 cm, was used to determine the maximum printing height while still enabling mycelium growth. The computational methods were set up to match the fabrication parameters to the material properties. In the case of FS, the moisture content is lowest amongst the four FS, allowing the volume to be printed at once. FS2 enables high water content, but the fabrication steps are spanned over different periods, allowing the lower printed parts to cure to construct on top without facing buckling failures. FS3 reproduces the mould and cast scenario where the two double layers act as an encapsulating layer to host the mycelium spores. At last, FS4 allows printing parameters to hint at inoculating areas in print. The results were visually analysed for 20 days. A fabrication aware system from geometry influences design drivers, allowing for 360-degree growth exposure, which poses a concern of uncontrolled development. To demonstrate process control, a 2-D map of desired growth area was sampled and filtered using an image function to make a 3D array of voxels to undulate 3D printing curves of the cylindrical volume. These undulations have been used in FS4 to guide the embedding of mycelium in print. Experiments were conducted with the Delta WASP 40100 Clay 3D printer (WASP, Italy). The printer has a nozzle of 9mm, and pressure was kept constant at 0.1 MPa throughout the printing. The computational system comprises the design of the geometry and the generation of the code. The geometry to be printed was designed in Rhinoceros 7 and Grasshopper 3D-modelling engines. To validate the feasibility of printing with a living system, a simple design scheme was preferred, namely a cylinder. From the geometry, the printing path was generated by keeping a layer height of 4 mm.

4. Results

The pH of the clay, water and fibre mixture was equal to 7, therefore in line with the recommended condition for the mycelium to grow. The mixture stability while assured while printing for all 14 layers printed for both the strategies to integrate mycelium in clay. The mycelium growth was assessed by visual inspection to verify the degree of mycelial hyphae colonization (Schritt et al., 2021) for the three IS. Each sample was inspected every four days for a total of 20 days, and the results are collected in Table 1. IS 3 is the one that assures the maximal mycelium colonization, as it is possible to see after 20 days when comparing the three pictures. Here, mycelium spawns were inserted along the contour of the printing path to ensure their ability to breathe. On the contrary, IS 1 and IS 2 are comparable in terms of low colonization. In IS 1, the mycelium spawn is randomly dispersed in the mixture, reducing the likelihood of fresh air contact, which is one of the main drivers for mycelium growth (Elsacker et al., 2019). The same can be said for IS 2. In the latter, not only does the mycelium have trouble contacting fresh air, but it must also search for its principal food supply, which consists of wooden fibres that have been dissolved between two successive layers, making it more difficult for the mycelium to access them. In Figure 2, not only the mycelium growth is analysed, but other factors such as growth control, the 3D printing process control and the scalability are compared to provide an overview of the parameters affecting the fabrication of the three strategies.

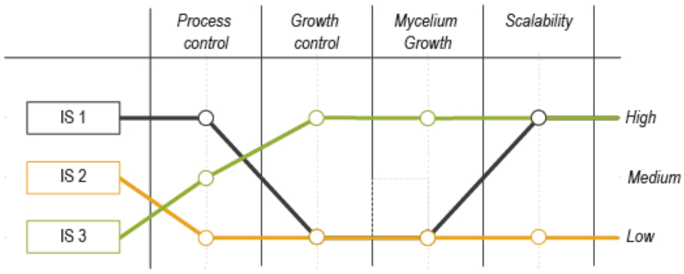


Figure 2. Three inoculation strategies (IS) comparison of four different parameters, namely process control, growth control, mycelium growth and scalability. These parameters are analysed according to a qualitative score (high, medium, low) from the visual inspection proposed in Table 1.

The growth control implies how much the typology of mycelium inoculation, i.e. mixed with the substrate or manually inserted along the contour of the printing path, impacts the growth velocity. The process control indicates the current level of the printer setup. The scalability parameter specifies which technology could be developed at a larger scale, namely with the substitution of the manual insertion of fibres or mycelium spawn with a dual nozzle system for reliable multi-material 3D Printing. IS 1 all the material is extruded together and therefore has a high score on process control and scalability. However, the low mycelium growth and growth control leave fewer chances of fully developing. IS 2 is the least encouraging, considering the process control and mycelium growth difficulties. IS 3 outperforms in terms of mycelium growth, growth control and scalability. This strategy could represent the most promising future programmed growth artefacts once the manual insertion of mycelium spawn is substituted with a multi-material technique. Strategy 3 was consequently used for inoculation of mycelium spawning.

Mycelium colonization status: low moderate high

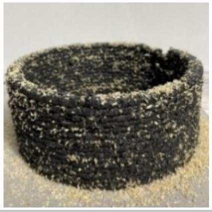
Day	IS 1	IS 2	IS 3
1			
18			
12			
20			

Table 1. Visual inspection of the three inoculation strategies (IS) for a period of 20 days.

At this point, different fabrications strategies (FS) were visually inspected to evaluate their influence on mycelium programmed growth. Each of the four samples was inspected every four days for a total of 20 days, and the results are collected in Table 2. FS 1 has the exact parameters of strategy three, analysed in the previous subchapter. Therefore, it was used as the starting point. Due to the resultant drying of 3 different portions of the element, FS 2 was characterized by a shrinkage phenomenon, especially the first part that was dried three times, resulting in a conical shape. FS 3 is similar to FS 1, but it has two layers of the cross-section. Hence more material is used. With FS 4, a different cross-section was tested, and a wooden fibre infill was also proposed to provide more feeding substrate to the mycelium. However, the additional fibres didn't affect the growth of mycelium, as it is possible to see after 20 days.

Mycelium colonization status: low moderate high

Day	FS 1	FS 2	FS 3	FS 4
1				
8				
12				
20				

Table 2. Visual inspection of the three fabrication strategies (FS) for a period of 20 days.

In Figure 3, the four FSs were compared according to the process control, the quantity of material used, and the scalability.

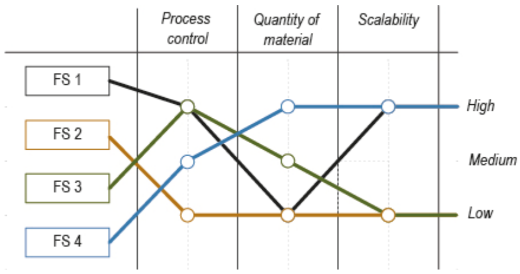


Figure 3. Four inoculation strategies (FS) comparison of three different parameters, namely process control, quantity of material and scalability. These parameters are analysed according to a qualitative score (high, medium, low) from the visual inspection proposed in Table 2.

The lower the material used, the better the solution is in terms of deployment of natural resources; therefore, FS 1 outperforms because it creates the same stable geometry and mycelium colonization by using half of the material. FS 2 is the less favourable one because the shrinkage effect creates more instability in the subsequential layers deposition, and to be scaled up; it would need a drying system that would account for more instability in the deposition of subsequential layers. To be scaled up, it would need a drying system that would account for more energy to obtain the final shape. FS 4 is still difficult to control with the current setup, and it uses three times the volumes of the other double-layer solution, namely FS 3. FS 1 and 3 can be considered the most efficient in process control and scalability. Additionally, the fibre addition didn't increase the ingrowth rate nor benefit in terms of stability compared with the currently tested geometry. At last, Figure 4 shows the attempt to guide the manual insertion of mycelium spawn to guide its growth according to the design requirements thoroughly.

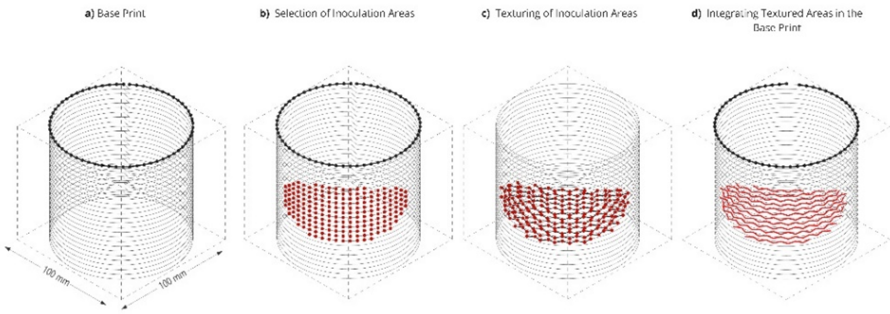


Figure 4. From the left: 3D model in the Rhino/Grasshopper environment highlighting the texture to guide the manual inoculation of mycelium spawns.

Thanks to the pattern, it was straightforward for the user to inoculate the living system when needed. Besides, with the application of a texture, the mycelium presence visibly declared opening the possibility of exciting living and functional patterns on the surfaces of the designed objects (Figure 5).

Figure 5. From the left: manual inoculation of mycelium spawn according to the printed texture; final results.

5. Conclusions and Outlook

According to these preliminary tests, the finalized printing paste ratio for the printing paste is composed of 60% w/w of clay, 7% w/w of sawdust fibres, 20% w/w deionized water, and 13% w/w mycelium spawn. The most promising solution for the inoculation strategy is IS 3, where mycelium spawns are manually inserted along the contours while printing the object. In this way, the mycelium is more in contact with fresh air rather than being immersed in the printing mixture, and it has a faster growth rate, according to the visual inspection done. The different FSs provide first guidelines according to the desired final element. According to the aim of this project, the possibility of minimalizing the material quantity, because of resource optimization, the FS 1 is the most promising since it was possible to obtain the same final colonized geometry with less material. In future works, when more complex geometries will be tested, other FS will be more suited, and optimization between the final geometry and the material used should be verified. Moreover, specific tests, such as chemical characterization, must be carried out to validate the visual inspection with the actual internal colonization of the printed object, and more experiments can be conducted to verify other parameters during the deactivation step that could affect mycelium colonization. These tests serve as a first collection of results to demonstrate design and growth parameter control and their interoperability for a living composite.

Three primary features of the tests have been identified for the subsequent testing phase: 1) Using local resources to design individualized growth prospects, 2) Integration of inoculation into the manufacturing process to obtain greater control over the deposition of mycelium spawns without human involvement. This could be accomplished by adding a second extruder that carries loose mycelium spawns and is actuated by motor vibrators; 3) utilize machine vision to monitor the growth to interfere with following procedures such as rehydrating the mycelium nutrients. Once these processes can be digitally controlled, Programmed Growth will be capable of expanding its application and reaching complicated geometries.

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