Lab on a Chip

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Design and Build a Green Tent Environment for Growing and Charactering Mycelium Growth in Lab

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Abstract

Mycelium-based materials have seen a surge in popularity in the manufacturing industry in recent years. This study aims to build a lab-scale experimental facility to investigate mycelium growth under a well-controlled temperature and humidity environment and explore how substrates of very different chemical and mechanical properties can affect the microscopic morphology of the mycelium fibers during growth. Here, we design and build a customized green tent with good thermal and humidity insulation for controlling the temperature and humidity and monitor the environmental data with an Arduino chip. We develop our procedure to grow mycelium from spores to fibrous networks. It is shown that a hydrogel substrate with soluble nutrition is more favorite for mycelium growth than a hardwood board and leads to higher growing speed. We take many microscopic images of the mycelium fibers on the hardwood board and the hydrogel substrate and found no significant difference in diameter (~3 μm). This research provides a foundation to explore the mechanism of mycelium growth and explore the environmentally friendly and time-efficient method of its growth.

Introduction

Mycelium has been recognized as an environmentally sustainable material with a great potential for various applications. Baked and pressed into a dense composite, mycelium has proved to be lightweight, fire-resistant, soundproof, and strong, which makes it highly sought-after in engineering, construction,

packaging, and architecture [1]. These properties, combined with its biodegradability and customizability, have garnered a lot of interest in mycelium-based materials [2]. Mycelium plays a crucial role in soil health and plant growth. By secreting enzymes and acids, mycelium helps to break down organic matter and increase nutrient availability. Additionally, mycelium forms networks of hyphae that improve soil structure by binding soil particles together, resulting in better soil aeration, water retention, and nutrient availability [3]. As the main body of fungi, mycelium is growing in a dark and humid environment. It is a rapidly spreading network of thin hyphae tubular structures that absorb nutrients from the surrounding environment. The mycelium can continue to grow and spread, forming a dense and interwoven network of hyphae that can persist for years [4]. It is non-toxic, safe for use in human and animal contact, and a good insulator with thermal and acoustic properties [5].

The mycelium network is the vegetative part of a fungus, consisting of a mass of branching, thread-like hyphae [6], [7]. The topology of the mycelium network is complex and varies depending on the species of fungus and the environmental conditions. Mycelium networks typically consist of interconnected nodes, each representing a branching point where hyphae intersect. These nodes can be dense, with multiple hyphae crossing simultaneously. In some species of fungi, the mycelium network can span great distances, with interconnected fibers extending for up to several kilometers [6], [8], [9]. This long-range connectivity is thought to be facilitated by specialized structures known as "rhizomorphs," which are bundles of hyphae that grow together in a linear, root-like form [10]. Rhizomorphs are thought to be vital in transporting nutrients and water over long distances, allowing the mycelium network to absorb resources from a wide area [10]. The mycelium network can also absorb nutrients from a wide area rather than being limited to the immediate vicinity of the growing mycelium. This is because the mycelium network can secrete enzymes and acids that break down organic matter in the soil, making nutrients available for absorption [11]. In addition, the mycelium network can form symbiotic relationships with other organisms, such as plants, exchanging nutrients in a mutually beneficial relationship [12]. Overall, the topology of the mycelium network is complex and dynamic, with a series of interconnected hubs and long-range fibers that allow for the absorption of nutrients from a wide area. The ability of the mycelium network to form symbiotic relationships and break down organic matter also makes it a key player in nutrient cycling and ecosystem functioning.

The growth of mycelium in nature is always hidden underground or inside the rotten woods, preventing from direct observation and experiment. It is crucial to develop the lab facilities that enable to control the conditions to mimic the natural environments and allow to directly observe the mycelium growth without damaging its structure. The most suitable environment for most mycelium to grow is in a low-light environment with a temperature of 20-25 °C and humidity level for 93-95 %RH [13]–[15]. While the incubation periods for the fungus ranged from 12 to 32°C [16]. In order to build the environment and grow mycelium in the lab, a real-time climate control system is needed.

It is shown in literature that the chemical composition of the substrate where mycelium is growing on can significantly affect the growing speed and the diameter of mycelium fibers of certain species [17], [18]. Having a substrate that enables the mycelium fibers to grow quickly with higher mechanical strength can make it attractive for sustainable building materials. As there are many different kinds of nutrition that provide carbon source for mycelium growth, e.g. agar (with yeast extract and malt extract) and cellulose-potato dextrose broth (PDB) [17], it is important to understand how different substrate may affect the morphology of mycelium fiber.

Here, we design and build a fully customized green tent, meticulously designed to offer proper thermal and humidity in the tent for mycelium growth. Our goal for the green tent is to provide us with complete control over the temperature and humidity levels within the tent. We integrate an Arduino chip to monitor and regulate environmental data to achieve this effect. Moreover, the Arduino chip can help us to record the data for analysis. We use agar as the substrate and king oyster mushroom as the injection to

prepare the mycelium in the green tent. Once the mycelium fully occupied the Petri dish, we migrated a small part of the mycelium onto a new agar substrate and put hardwood aside from it. Once the mycelium grows on agar substrate and hardwood board, we use a freeze dryer to get the dry sample for microscopic imaging. Our experiment analyzes how different substrates will affect the mycelium structure, such as the diameter of the thread-like hyphae.

Method

Circuit design for sensing, storing data and device control.

A small change in the environment will lead to mycelium growth failure. An Arduino Mega 2560 circuit with sensors is used to monitor the temperature, humidity, and CO2 levels of mycelium growth to control the environment in the green tent. The scanning frequency during the operation is 115200 Hz. The accuracy of the sensors is the DHT22 Temperature-Humidity Sensor & DS18B20 Temperature Sensor (resolution of 0.1℃ and 0.1%RH respectively), and Gravity Infrared CO2 Sensor (resolution of 1PPM). All the data is written to a log file in a SD card for every 5 second through a data logger module (DS1307 V03 Real Time Clock Module and MicroSD Card Adapter) by using an Ethernet cable.

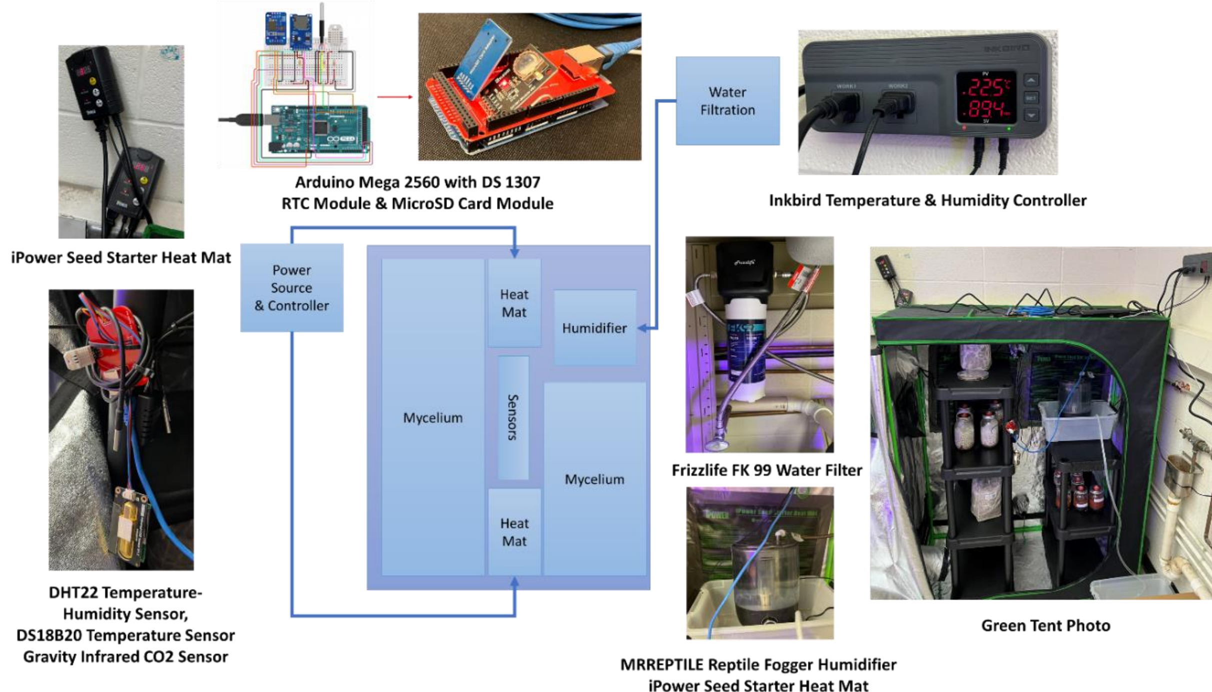

Figure 1. (A). Green tent appearance, a schematic of Arduino connection, a 3D circuit board, a placement chart of the system overview

Electric device for heat and humidity generation.

Two of the heat mats provide enough heat in the green tent system. They are installed on the back wall center area. The iPower GLHTMTM Durable Waterproof Seedling Heat Mat has a size of 20 inch by 20 inch, and the target temperature control range is 40-108 °C. The operating power is 96 W for two heat mats. We use an regular ultrasonic humidifier in the green tent to tune the humidity level inside. The humidifier has a 4 liters water tank with 210 ml/h maximum mist output. It is controlled by the humidity sensor and only be turned on once the humidity level drops 90 %RH. The average water consumption is Approx. 1.5 liter per day. The water in the humidifier is filtered by an external water filter (Frizzlife FK99).

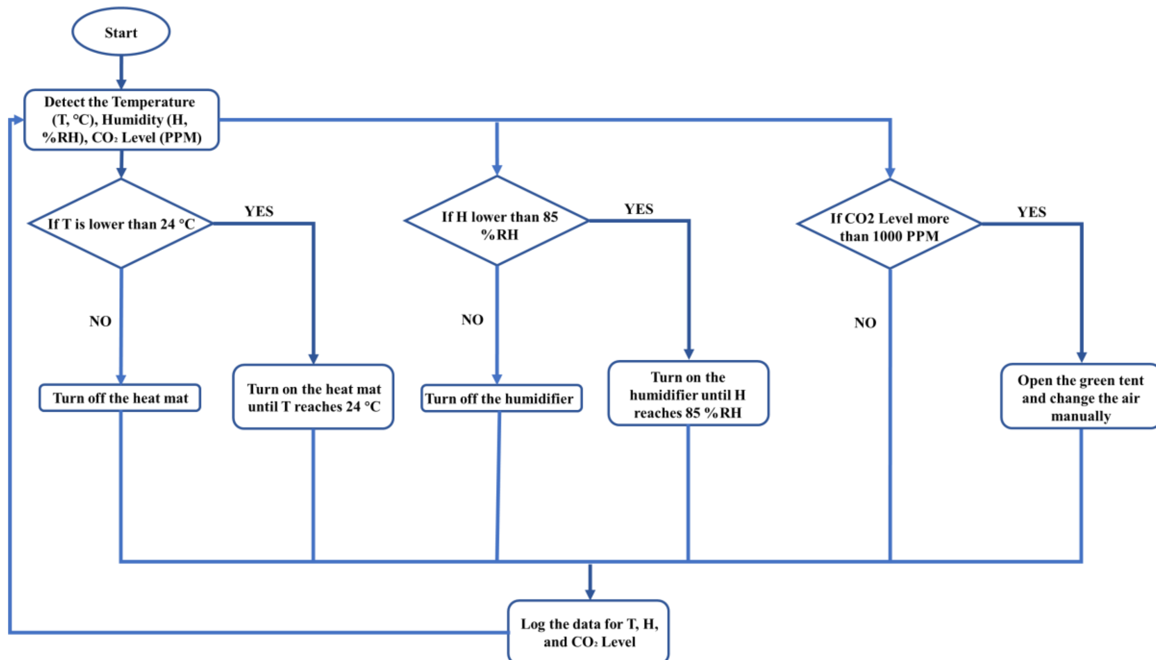

Figure 2. A schematic information flow chart from the sensor to Arduino and controllers.

Preparation of agar plate for mycelium growth.

We use the agar plate to culture the liquid *Pleurotus eryngii*, which is known as king oyster mushroom to better understand the mycelium microstructure. Moreover, we set an obstacle of a basal wood to observe the mycelium fiber growth behavior. Analysis of the SEM imaging results to recognize mycelium diameter in the different positions to understand how the basal wood can affect the mycelium microstructure.

To prepare the agar plate petri dish, we use 20 g of agar powder, 20 g of malt extract, 2 g of yeast, and 1000 g of water, as shown in the first step in **Figure 3**. We use malt extract and yeast because both can provide the nutrition for mycelium growth, and the agar as a substrate can allow the mycelium growth on the surface. To successfully get the agar plate, we boil the water and put all the material into an Erlenmeyer flask, as shown in the second step in **Figure 3**. The thermal mixer keeps the mixture at a high temperature and solutes all the powder. Even though we boil all the mix in the water, some precipitate still cannot be solved. So, we use a funnel to filter the mixture into the other Erlenmeyer flask, as shown in the third step in **Figure 3**. Use aluminum foil to cover the Erlenmeyer flask and put it into an autoclave for high temperature (123 °C) and pressure (24 psi) sterilizing for 40 minutes. When the sterilizing is finished, take the Erlenmeyer flask to the clean room, and wait until the mixture temperature is cool down to around 45 °C. Use 75% alcohol wipes to clean the Petri dish and glass rod. We use a glass rod to guide the mixture into the Petri dish from the Erlenmeyer flask, as shown in step fifth in **Figure 3**. Wait until the mixture cools down to a solid, and then inoculate the liquid *Pleurotus eryngii* mushroom. Use the laboratory film to seal the Petri dish and put them into the green tent for 7 to 14 days; wait until the mycelium fully occupies the Petri dish and use it for the next step. Once the mycelium is fully occupied in the Petri dish, we cut a small piece, inoculate it into the new Petri dish, and put a long strip of balsa wood aside from it. Observer the mycelium growth state for 7 to 14 days and then use it for SEM imaging.

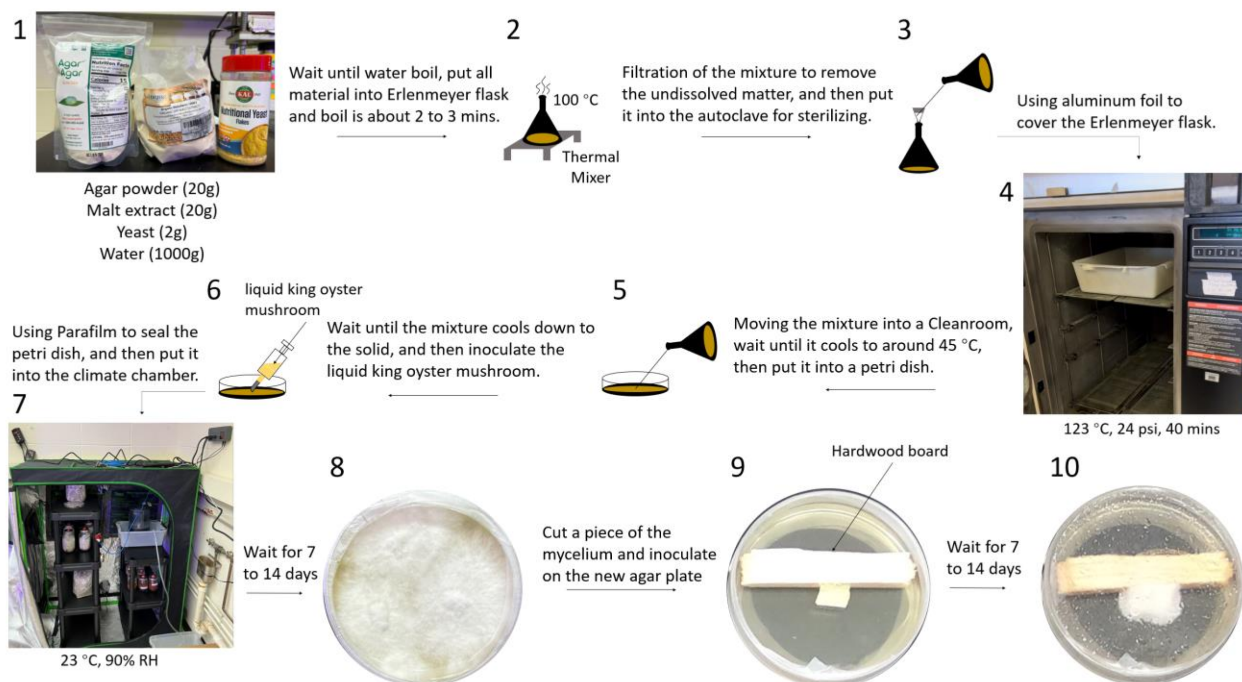

Figure 3. The general process of the agar substrate preparation and mycelium sample preparation.

Results and Discussion

After setting up the Arduino controller for the green tent, we test it for 8 hours to ensure it runs correctly. **Figure 4** shows that the log data results refer to 8 hours. The temperature and humidity are the correct results based on the setting. We set the target value of temperature as 23 ℃ and the tolerance range as 21.5 ℃ [6], as shown in **Figure 4A** (red and magenta horizontal line.) The reason that we set this range is that it is a relatively good temperature for mycelium growth. When the temperature sensor detects the temperature achieved at 23 ℃, the controller will atomically turn off the heat mat. As the temperature decreases, when the temperature is lower than 21.5 ℃, the controller will turn on the heat mat to allow the inside temperature of the green tent to increase to 23 ℃. As with the temperature as shown in **Figure 4B**, the controller will turn on the humidifier when the humidity sensor detects the inside humidity of the green tent lower than 90% RH and turn off the humidifier when the humidity achieves 99% RH. Since the mycelium needs a relatively high-humidity environment to grow [7]. The CO_2 sensor detects the CO_2 level in the green tent is around 400 to 500 PPM as shown in **Figure 4C**. We did not set a target value and tolerance range for the CO_2. The only number we compared with is the average CO_2 in the air, around 420 PPM [8]. Since the mycelium is breathed when growing [9], the average value in the **Table 1** is higher than the CO_2 level in the air. Moreover, we calculate the mean value and standard deviation (SD) for the test results. To compare the results with the set value, our results can be acceptable. Using the Arduino controller, we can very well detect the environmental condition in the green tent.

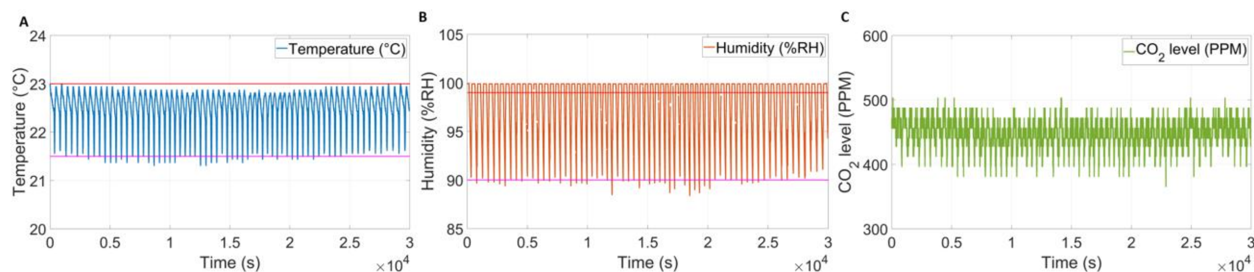

Figure 4. The plot of the **A.** Temperature, **B.** Humidity, and **C.** CO_2 history for the consequent 8 hours.

Table 1. The mean value and standard deviation (SD) of the temperature, humidity, and CO_2.

	T (°C)	RH (%)	CO_2 (PPM)
Mean value (μ)	22.5	97.66	449.28
Standard deviation (σ)	0.37	3.05	19.95

We also calculated the thermal insulation (R value) for the green tent. The fluctuation is slight due to the small range necessary to grow the mycelium. Given the DS18B20's accuracy of ± 0.5 ℃, the max value peaked at 27 ℃ and remained above 25 ℃. Using the data, we used thermodynamics principles to calculate the R-value of the green tent, which was 0.77 m²*K/W. Though we do not have the actual product value, we compared our computed tent R-value to other materials and found that it is higher than drywall but lower than polystyrene. This means that the tent retained the heat inside of it better than any common wall material, at a caliber that was high, but not higher than one of the best insulating materials. From this test, we find that the data is representative of the experiment performed - the tent's purpose is to create a stable environment, retaining the heat, which is what the temperature data proved.
We use the formula:

$$R = \frac{tA\Delta\mathrm{T}}{Pt_{on}} \quad (1)$$

to estimate the thermal resistance R value of the green tent to understand its energy efficiency. Here, A = 7 m^2 is the surface exposed area of the green tent, ℃ is the temperature difference between the lab temperature and target temperature inside the tent, P = 96 W is the total power of the heat mat, t = 28800 s is total testing Time and t_{on} =14400 s is the total amount of time that the heat mat is on. Using the numerical values, we obtain the thermal resistance R=0.77 $m^2 \cdot K/W$, as R4.4 of an imperial unit. Considering the layer thickness of the tent is only 0.8 mm, this corresponds to the thermal conductivity of 0.001038 $W \cdot m^{-1} \cdot K^{-1}$, which is better than the stand air at the temperature of 300 K (0.02614 $W \cdot m^{-1} \cdot K^{-1}$), mainly because of the reflective inner layer that prevents the radiant heat given by the heat mat from escaping the tent.

We use the green tent to grow our *Pleurotus eryngii* samples. It is easy to grow, have high yield and is the same genus as the *Pleurotus ostreatus*, which is more widely used for material developments [17]. The mycelium is allowed to grow on the petri dish inside the tent for 14 days, and then put the petri dish into the freeze dryer for 48 hours to dry the sample for use in the SEM imaging. The sample is taken out of the freeze dryer after 48 hours, weighed it, and then put back to dry for three more hours to ensure that the model is completely dry. We marked six different positions on the Petri dish to analyze the diameter. The six positions mainly represent the mycelium growing on the wood and mycelium growing on the agar plate. The SEM imaging results of 6 positions are shown in **Figure 5A**. The results mainly show the unique structure of the mycelium fiber, which is the clamp connection. We chose 10 mycelium fibers for each position, randomly measured the fiber's diameter several times, and made the histogram, as shown in **Figure 5B**. Moreover, to better analyze the diameter distribution, we use normal distribution curve fitting to get the mean value of the diameter for each position, as shown in **Table 2**. It is shown that the average diameter value of mycelium from the wood and the agar plate is relatively the same. Apparently, the wood substrate reduces the growing speed of the mycelium network (**Fig. 3**), making the growth slower than the network on agar substrate. However, such a reduction in the network growth seems not appliable to the fiber diameter, according to the many measurements (**Fig. 5B**).

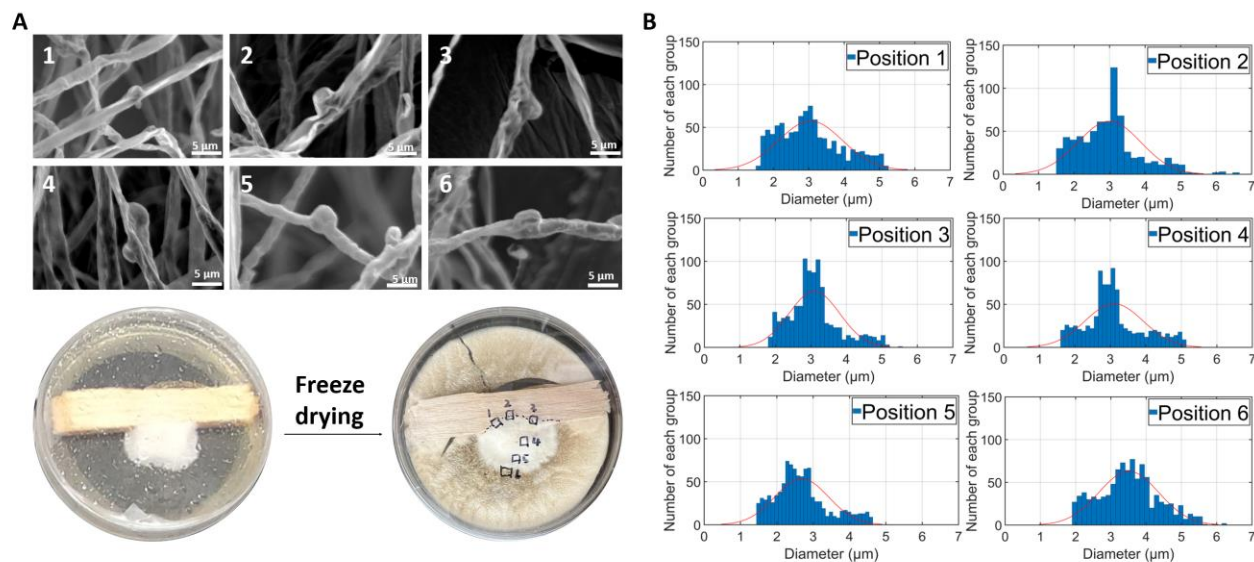

Figure 5. (A). Picture of dried petri dish before and after taking the samples and SEM pictures for 6 different positions **(B).** Diameter measurement histograms for each position.

Table 2. The mean and standard deviation of the histogram curve fitting for 6 different positions.

Position number	μ (μm)	σ (μm)
1 (wood)	3.05	0.90
2 (wood)	3.00	0.88
3 (wood)	3.1	0.71
4 (agar)	3.11	0.82
5 (agar)	2.68	0.74
6 (agar)	3.51	0.85

It is intriguing to discuss how the diameter of the mycelium fiber is affected by the substrate type. As the literature shows, some research chose cellulose-potato dextrose broth (PDB) as the substrate for growing *P. ostreatus*. The study suggests that the failure of *P. ostreatus* filaments grown on PDB-cellulose substrate is likely due to a loss of internal hydrostatic pressure, reflected in the filaments' reduced width [17]. Here we separate other diameter data into two groups; group 1 is the mycelium grown on the wood, and group 2 is the mycelium grown on the agar plate. We obtain the mean and standard deviation of the fiber diameter of the two groups as shown in **Figure 6**, which shows that the two groups' medians are almost identical, around 3 μm. Since the normal distribution analysis results cannot distinguish the diameter difference between the mycelium growth on the wood and agar plate, we use Analysis of variance (ANOVA) to test the difference between two or more means which can let us know better how different substrates will affect the mycelium diameter [23]. We obtain a P value of 0.1301 by comparing the mean vale of these two groups by performing multiple comparison tests to determine which group differs from the others in terms of mean diameter [11]. Based on our setup of the mycelium growth on the wood and agar plate, the two groups diameter are equivalent. To ensure this conclusion is applicable to other mycelium species, we preform test and measure the diameter of mycelium grown alone on agar plates and hardwood, as shown in **Figure 7**. We migrate the *Pleurotus eryngii* mycelium on the agar plate on a new agar plate and the hardwood separately. **Figure 7 A** shows the samples after the freeze drying for SEM imaging. We use Image J to measure the diameter for a thousand times. **Figure 7 B** shows the diameter measurement histograms for the mycelium on the agar and hardwood. We used normal distribution curve fitting to determine the mean diameter value for the results to study the diameter distribution, as shown in **Table 3**. It is clearly

shown that mycelium from the wood and the agar plate has a roughly similar average diameter value of about 3 µm. We employed the ANOVA to compare two or more means to understand better how different substrates may impact the mycelium diameter as shown in **Figure 7 C**. By comparing the means of these two groups on several occasions, we arrive at a P value of 0.266. The result suggests that the mycelium hyphae grown on the wood and agar plate are of very similar diameter.

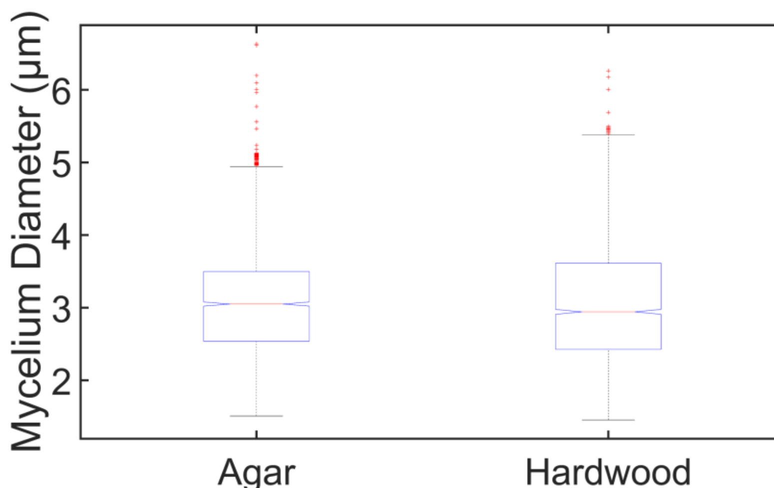

Figure 6. Distribution of two groups data (diameter of mycelium fiber on agar versus wood surface), the ANOVA test suggests that the mycelium diameters of the two groups are equivalent.

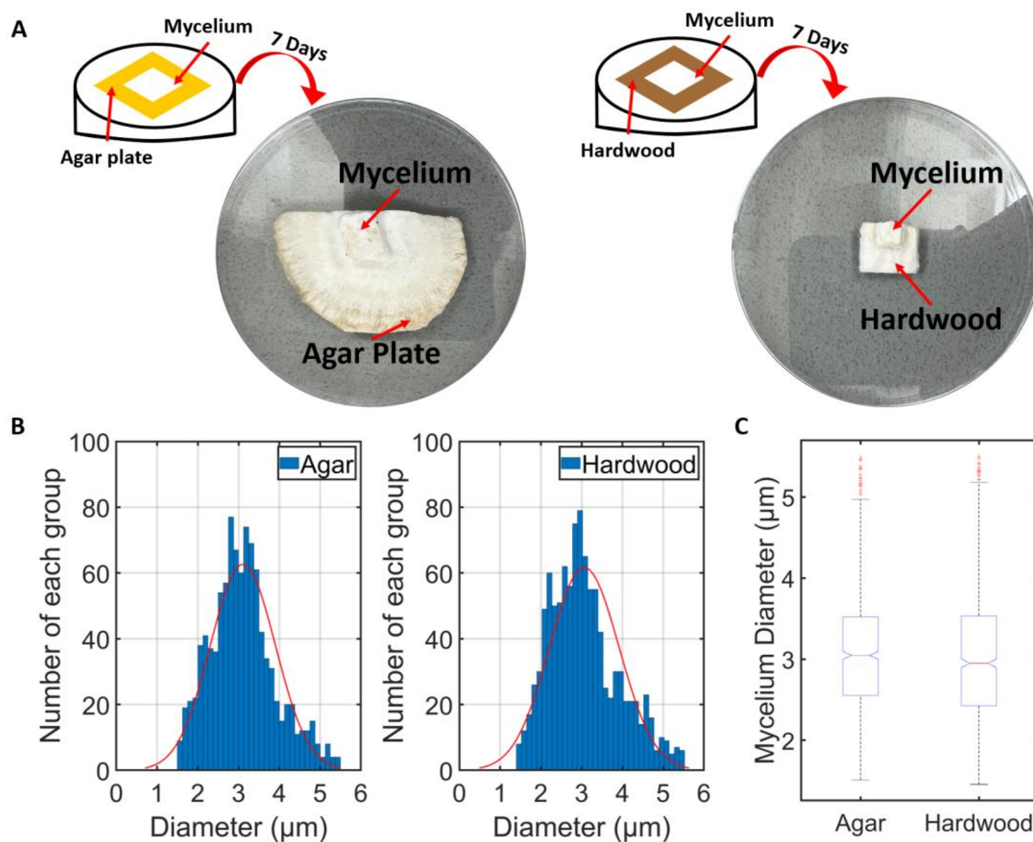

Figure 7. A. The freeze-drying sample of mycelium grown alone on agar plates and hardwood. B.

Diameter measurement histograms for each sample. C. Distribution of the diameter of mycelium fiber grown on agar versus wood surface.

Table 3. The mean and standard deviation of the histogram curve fitting for mycelium growth on the agar and hardwood.

	μ (μm)	σ (μm)
Agar	3.11	0.79
Hardwood	3.06	0.86

Conclusion

In conclusion, the SEM imaging results show that the diameter of the mycelium growth on the wood and the agar plate is around 3 μm. Even though the substrate for the mycelium growth is different, the microstructure cannot be affected easily.

Our green tent was environment-stable and well-suited for the mycelium to grow. This paper mainly focuses on the mycelium growth on the agar plate and illustrate its applications. However, our recent work has also demonstrate that the tent can be used to prepare mycelium-based bio-composite material. For example, after growing in a Petri dish, we can migrate the mycelium into a glass jar for grain spawn, and then migrate the mycelium into the bag to inoculate with the bulk substrate inside the tent (Fig. 1). We use the product to make samples for further mechanical and thermal tests and thus the green tent is helpful in preparing mycelium-based bio-composites. Moreover, the Arduino sensor system served as an important tool when tracking the environmental data when growing mycelium for a long time. Once the temperature drops or humidity is lost, the data will clearly show the time and number to track the issue of the green tent. This logger system can also be introduced to other environmental monitor equipment to better control and save the data. The whole customized green tent is able to replace the commercial climate chamber, which is a more budget-friendly and cost-saving choice for small environment tests and individual use.

Green tents are essential for indoor mycelium growth by providing specific environmental conditions for mycelium to thrive. The tent traps moisture and warmth inside, preventing it from escaping and providing a consistently high humidity level. Moreover, different mycelium species require different temperatures and humidity levels to grow. Thus, this device helps identify optimal environmental conditions for mycelium to grow. Finally, the controlled environment provided by a green tent can also protect the mycelium from contaminants that could impede growth, such as mold or bacteria, and it also prevents the spore from escaping and contaminating the lab environment.

Author contributions

Z.Q. proposed, designed, and supervised the research. L.Y., R.X., A.J. and M.A. assembled the tent and develop controlling and logging devices. R.X., Z.Q. and M.A. collect the data during the operation of the tent. L.Y. and A.J. prepared the mycelium experimental samples and took the microscopic images. L.Y. analyzed the measurement results. N.S. and B.D. provided oversight and critical review of the work. L.Y., R.X. and Z.Q. wrote the initial draft and revision with the inputs from all authors. All the authors approved the manuscript.

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