

# Effect of Growth Factors on the Production of Mycelium-based Biofoam

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## Research Article

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# Abstract

Mycelium-based biofoam has the potential to become an alternative to petroleum-polymeric based-foam by utilising fungal mycelium and lignocellulosic material as the matrix and substrate, respectively. The lignocellulosic materials, which were rice husk, sawdust, and sugarcane bagasse, which is crucial for the production of biofoam, were tested as a substrate for *Pleurotus ostreatus* mycelium growth during the screening procedure. Three growth factors were varied during mycelium-based biofoam production: incubation temperature, spawn loading, and moisture content. In this study, rice husk was the ideal substrate in the production of mycelium biofoam compared to other fungi. The inhibition of *P. ostreatus* mycelium growth at 30°C incubation temperature was due to decay and contamination. On the other hand, by varying the growth factor of mycelium biofoam on rice husk, the optimum dry density of mycelium-biofoam was observed at 50% (w/w) moisture content (1.07 g/cm<sup>3</sup>), while the optimum compressive strength was observed at 40% (w/w) spawn loading (1.350 MPa). These results showed that varying the growth factor could influence the mechanical behaviour of the material. The morphology of the biofoam was also observed through a scanning electron microscope (SEM). Short and highly entangled tube-like structures and compact filaments forming a material were seen, responsible for the lightness characteristic of the material. The functional group of the biofoam was also determined using a Fourier transform infrared (FTIR) spectrophotometer. A new band of proteins and lipids was detected at 1633 cm<sup>-1</sup> and 3280 cm<sup>-1</sup> in the biofoam. It clearly shows that the chemical nature of feeding substrate responsible for the changes of material spectra. Therefore, this study highlighted that the biodegradable mycelium biofoam of *P. ostreatus* using rice husk as a substrate is a promising alternative to polymeric foam.

## Introduction

Bio-based material or bio-composite has been extensively explored since it can replace the petroleum-based polymeric foam, such as expanded polystyrene (EPS) and polyurethane, which is used in a huge variety of shape and application in the industry (Bruscato *et al.*, 2019; Yang *et al.*, 2017). Petroleum-based polymeric foam is a non-biodegradable material, which means it will take many years to degrade if left in the environment and cause environmental pollution by releasing organic compounds such as benzene and styrene (Bruscato *et al.*, 2019). This pollution problem creates concern and awareness among researchers and related industrial players, thus sparks interest in developing a sustainable material such as biofoam to replace petroleum-based polymeric foam. It has become the current highlight in biomaterial engineering for its zero pollution and renewability during the formation and after treatment process (Attias *et al.*, 2017). This material could offer a promising solution as a 'green material' for the environmental problem caused by rapid population growth and accompanying discard culture (Ghazvinian *et al.*, 2019). This biomaterial has shown the potential to develop products with various applications, such as packaging, household, building materials, and a broad variety of design objects and furniture (Karana *et al.*, 2018). Another attractive factor of the mycelium biofoam is the possibility to exploit the agricultural wastes and/or lignocellulosic biomass. The waste and residues are valorised

rather than discarded, which lead to the circulation of the economy. Maroušek et al. (2015) reported that building material produced from biowaste and recovered electricity, provides a minimal cost which contributes to its novelty. Therefore, biofoam can be produced with lower price due to cheaper raw material and also provides an economical and environmental advantage since the process did not use any additional electricity, fuels, hazardous reagents or rare catalysts. In the production of mycelium biofoam process, the organic substances will be inoculated with an individual strain of fungi to form the mycelium biofoam. As the fungus grows, it forms a network of branching hyphae called mycelium that binds together the nutritive substrate and create a vast three-dimensional matrix (Islam *et al.*, 2018). This mycelium secretes hydrolytic enzymes that will break down the substrate into an easily absorbable substrate and transport nutrients like sugars for further extension. The mycelium growth will stop when it reaches complete substrate colonisation that will render inert material and allow the evaporation of any residual water (Elsacker *et al.*, 2018). Heating at a minimum temperature of 60°C will permanently kill the fungus, while the drying method offers regrowth when moisture conditions become favourable again. Furthermore, mycelium biofoam can be shredded and added to the home compost pile after being used because of its biodegradable properties.

The production of mycelium biofoam is simply a combination of fungal mycelium and lignocellulosic substrate. Nevertheless, the technical and experiential qualities of the resulting material depend on the type of fungus and substrate, growing conditions, and the processing of the material (Karana *et al.*, 2018). Previous work by Appels *et al.* (2019) demonstrated that fungal species and the type of substrate would impact fungal skin thickness and stiffness of the material. *Pleurotus* spp is one of the most extensively studied white-rot fungi for its exceptionally ligninolytic properties (Bellettini *et al.*, 2019). Compared to brown-rot fungi, this genus has the ability to cleave cellulose, hemicellulose, and lignin from wood efficiently, whereas the brown-rot can only cleave cellulose and hemicellulose (Machado *et al.*, 2016). López-Nava *et al.* (2016) reported that *Pleurotus* spp. obtained lower value of compressive strength compared to *Ganoderma* spp. due to the mycelial feature, which is significantly softer when introduced wheat straw as a substrate. However, Haneef *et al.* (2017) reported that the investigation on *Postreatus* and *G.lucidum* revealed that *Postreatus* was stiffer than *G.lucidum*. The suggestion was made based on its higher polysaccharide content, making it stronger in compressive strength. Hence, they concluded that a substrate is strongly affected by the mycelial composition in polysaccharide, lipids, and chitin, as well as the overall mechanical and morphological properties (Haneef *et al.*, 2017).

Elsacker *et al.* (2019) reported on the types of substrate that influence the colonisation of mycelium. Further improvement on the mechanical properties was observed when the substrate particle size is less than 5 mm and pre-compressed prior to the incubation period. Evaluation on different mixing protocols by Yang *et al.* (2017) demonstrated higher dry density, elastic moduli, and compressive strength was achieved for a densely packed substrate compared to the loose packing condition. These researches, however, focused primarily on varying the type of fungus, substrate, and processing of the material. Since the colonisation level and mycelium skin thickness affect the characteristics of the resulting biomaterial, a bottom-up investigation on the factors affecting mycelium growing condition should be performed as well. Previous studies reported that mycelium species (Jones *et al.*, 2018), substrate selection (Haneef *et*

*al.*, 2017), interaction between fungus and substrate, and process variables, especially in growth factor (Elsacker *et al.*, 2019) are among the factors affecting the production of biofoam.

Therefore, at the end of this study, better insight into the actual mycelium growth performance, the evolution of the growing mycelium under different growth conditions, and characterisation of the forming biofoam were presented, and a methodology was established to evaluate the suitability of the process variable for the production of a mycelium-based biofoam for future study.

## Materials And Methods

### 2.1 Substrates preparation and strain culture

The biomass residues of sugarcane bagasse, sawdust, and rice husk were obtained from the local agricultural site. All collected substrates were left to dry under the sun for five days to remove excess water and ground into a smaller size. The ground substrates were then kept in a sealed plastic container until further use. Other materials such as commercial sorghum spawn of *Pleurotus ostreatus*, calcium carbonate (CaCO<sub>3</sub>), and rice bran were obtained from the local mushroom farm. The *P. ostreatus* strain was inoculated onto Potato Dextrose Agar (PDA, Oxoid) medium using the commercial spawn. The cultures were incubated at 25°C for 7 days to obtain sufficient mycelial growth for the following procedure in substrates screening.

### 2.2 Substrates screening process

1 gram of sugarcane bagasse, 3 grams of sawdust and 5 grams of rice husks were poured into an autoclaved plastic bag, respectively. Each substrate was separately supplemented with 2% (w/v) calcium carbonate (CaCO<sub>3</sub>) and 10% (w/w) rice bran, which was based on the dry weight basis and was thoroughly mixed. Next, the moisture content was set to 50% (w/w) according to Equation 1.

$$\text{Moisture content}(\%) = \frac{\text{Weight of wet substrate} - \text{Weight of dry substrate}}{\text{Weight of wet substrate}} \times 100 \quad (1)$$

The substrates mixture was autoclaved at 121 °C for 15 minutes to sterilise. After cooling, each substrate mixture was poured into a petri dish in a laminar flow and sanitised with propyl alcohol beforehand to avoid contamination. One piece of 1 cm<sup>2</sup> mycelium plug was placed in the middle of the petri dish. The mycelium was allowed to grow in dark condition at room temperature for 7 days. The mycelium growth rate for each substrate was observed and recorded every day by calculating the area colonised by the mycelium (cm<sup>2</sup>/day) (Sardar *et al.*, 2015). All procedures were done under a sterilised condition in order to avoid mould contamination.

### 2.3 Production of mycelium biofoam

A mixture of rice husk, 2% (w/v) CaCO<sub>3</sub>, and 10% (w/w) rice bran was used as the substrate to fabricate mycelium biofoam. The substrate mixture was prepared and autoclaved according to the method mentioned in Section 2.2 under sterilised condition. The moisture content was set to 50% (w/w), and 20% (w/w) commercial spawn of *Postreatus* was added into the mixture after cooling. After thorough mixing, it was then poured into a rectangular plastic container (10 cm × 10 cm × 5 cm) and incubated at different temperatures (20, 25, 30, 35, and 40°C) for the mycelium to grow. The effect of moisture content was determined by adding different amount of water to the rice husk substrate according to Equation 1. The moisture content was varied at 30, 40, 50, 60, and 70% (w/w) based on the dry weight basis. For the spawn loading, the substrate mixture was added with different spawn amount, which ranges from 5, 10, 20, 30, 40, and 50% (w/w). The growing condition of mycelium was observed every two days, and the process was stopped on the 11th day of the growing period. The mycelium biofoam was dried in an oven at 100°C for 24 hours (Appels *et al.*, 2019).

#### ***2.4 Characterisation and mechanical testing***

The density of the biofoam was determined by measuring the final weight and volume accomplished by using an electronic balance and a calliper. A JSM-6390LV (JEOL, USA) scanning electron microscope (SEM) was used to examine the surface morphology of the mycelium biofoam. The sample was sputter-coated with a thin layer of gold to avoid electrostatic charging during the examination with an accelerating 10–15 kV voltage. Different types of functional groups in the sample were identified using the Fourier transform infrared (FTIR) spectrophotometer model Nicolet IS5 (Thermo Fisher Scientific, USA). A small amount of sample was then placed on top of the sample holder made from zinc selenide (ZnSe) crystal. The related functional groups present in the absorbents were further identified using the OMNIC operating system (Version 7.0, Thermo Nicolet). The experiment was carried out over spectral range, varying from 4000 to 400cm<sup>-1</sup>. Finally, a compression test was conducted using the Zwick Roell Z020 testing machine to identify the mechanical behaviour of the mycelium based biofoam. The pre-load was set at 5N with a rate of 10 mm/min and was stopped when it reached 50% deformation.

## **Results And Discussion**

#### ***3.1 Characterisation of mycelium growth on different substrates***

The type of substrates greatly influences the mycelium growth since the hyphae is in direct contact with the substrate and extract the essential nutrients from it (Belletini *et al.*, 2016). Mycelium would colonise faster and prevail over other contaminants if the substrate could provide the desired growth condition, hence produced biofoam with higher quality and quantity (Elhami and Ansari, 2008). Rice husk, sawdust and sugarcane bagasse were selected as substrates in the screening process due to their availability and bio-compatible with the selected white-rot fungi, *Postreatus*. The growth performance of mycelium on three different substrates was visually inspected every two days to determine the compatibility of *Postreatus* with the selected substrate, as shown in Fig. 1.

From Fig. 1, faster growth of mycelium was observed over the agar plate containing the rice husk as substrate, where the mycelium was fully covered on the 8<sup>th</sup> day. The slow growth of mycelium was observed over the agar plate that contained sawdust and sugarcane bagasse substrate. A dense white fungal biomass layer has formed over the rice husk agar plate. Different results from different substrates probably due to the difference in chemical compositions found in each substrate, which could feed the mycelium during cultivation. Fig. 2 shows the growth curve data of the mycelium *P. ostreatus* on rice husk, sawdust, and sugarcane bagasse to correlate with the visual inspection in Fig. 1.

Fig. 2 shows that *P. ostreatus* on rice husk was adapting to the new growth condition and experienced a lag phase on the 1<sup>st</sup> day, while other substrates experienced lag phase on the 1<sup>st</sup> day to 3<sup>rd</sup> day. The fungus started to enter the log phase, where it started to feed off the nutrients in the substrate to grow and elongate its mycelium. The nutrients in the substrate were in excess, which enables the extension of the mycelium to occur at a constant rate at day 8. As for the rice husk, the mycelium expanded more than 100 cm<sup>2</sup> on the 8<sup>th</sup> day. A study by Obadai *et al.* (2003) suggested that the *P. ostreatus* would colonise faster using rice husk as substrate. On the other hand, sawdust and sugarcane bagasse experienced a lag phase until day two followed by slower colonisation afterwards. Elsacker *et al.* (2019) experienced slow growth as well when sawdust and flax dust were used as substrate. This situation occurs due to implication of different chemical compositions in the substrates, thus making it difficult to digest during colonisation. Ghazvinian *et al.* (2019) reported that the glucans forming in sawdust are more complex than those with straw, such as rice husk, resulting in slow digestion by the mycelium and slow growth.

The unique spongy structure of a rice husk substrate promotes good absorption of water and high retention of water which will stimulate better growth for the mycelium (Siwulski *et al.*, 2011). This structure will help prevent the rice husks substrate from drying since it has a high holding water capacity. Besides that, rice husk substrate possesses an antiseptic property that will help to inhibit competing organism that grew within it (Dawidowicz *et al.*, 2018). Thus, the yield of mycelium-based biofoam is guaranteed as a fast overgrowth of fungus mycelium was promoted on the rice husk substrate. Therefore, no further test was conducted with the sawdust and sugarcane bagasse. Rice husk was chosen as the most suitable substrate to be applied in the mycelium biofoam production for *P.ostreatus*.

### **3.2. Effect of variables on mycelium growth**

The growth of the *P. ostreatus* mycelium that has been incubated at five different temperatures, 20, 25, 30, 35, and 40 °C, and was visually inspected for every two days, is shown in Fig. 3. From the observation, white mycelium patches can be seen starting on day 3, and on day 11, a fully grown *P. ostreatus* mycelium was found covering the whole area when it was incubated at a temperature of 20, 25, and 30°C.

As shown in Fig. 3, the mycelium grew the fastest at 30°C, resulting in a highly dense white fungal biomass layer on the rice husk on day 11. This is because *P. ostreatus* grew naturally in the subtropical climate at an average temperature of 29°C. Therefore, it is easier to adapt to an environment similar to

their natural growing condition (Marino *et al.*, 2003). The result was consistent with findings by Tesfaw *et al.* (2015) which reported the optimum temperature for *P. ostreatus* to grow was at 30°C and moderate growth of the mycelium is at 20°C and 25°C. Conversely, inhibition of growth would occur by further elevation of temperature. Slow mycelium growth was observed at 35°C, while there is no growth of mycelium at 40°C. Dawidowicz *et al.* (2018) also reported that temperature above 35°C would cause the mycelium to decay. The substrate would be dry at said temperature and inhibit the growth of mycelium due to loss of moisture (Patel *et al.*, 2009).

Moisture content in the substrate plays an important role, as it provides large turgor pressure, which is needed for the hypha tip of the fungus to have better penetration into the solid substrate. Fig. 4 shows the growth of the *P. ostreatus* mycelium at moisture content of 30, 40, 50, 60, 70% (w/w) on rice husk.

Fig. 4 shows a dense white fungal biomass layer forming on the substrate that contained 40% (w/w) and 50% (w/w) moisture content. Shen *et al.* (2008) reported that 55% substrate moisture gave the highest yield of *Lentinula edodes*. The optimum moisture content of synthetic logs during the spawn run was different by species. However, the natural optimum moisture content for hyphal elongation is in the range of 55–70% (Shen *et al.*, 2008). Sufficient moisture content in the substrate is crucial as water uptake enables the elongation of hyphae cells and extension of mycelia within the whole substrate (Arijit *et al.*, 2013).

On the other hand, Fig. 4 shows that at 60% and 70% (w/w) of substrate moisture content, the mycelium growth was inhibited and was prone to contamination by black mould and white worms. This occurrence occurred due to excess water. Narh *et al.* (2011) reported that the water would set at the bottom of the substrate, which later promotes mycelium's surface growth, which leads to bacteria contamination at the bottom area. Excessive moisture content would lead to the depletion of oxygen and nutrients due to the leaching process, which results in the decrease of enzymatic activities as the anoxic condition developed. According to the work of Ryu *et al.* (2015), there was a possibility for diseases and competing material such as moulds to occur since the level of moisture is high. On the other hand, the growth rate of the *P. ostreatus* mycelium at different spawn loading (5, 10, 20, 30, and 40% (w/w)) is illustrated in Fig. 5.

In general, the spawn consists of fungi mycelium and its supporting medium or substrate which provides nutrition for fungi growth, also known as inoculum (Satpal *et al.*, 2017). The result shows that optimum growth rate was obtained when the rice husk was inoculated with 40% (w/w) spawn loading. Through visual inspection, the mycelium almost covered the whole area of rice husk substrate on the agar plate at day 5. In contrast, inoculation by using 5% (w/w) of spawn loading took 9 days to outgrow rice husk substrate on the agar plate. Sabu *et al.* (2005) and Patel *et al.* (2009) reported a similar situation with lower spawn loading and suggested slow mycelium growth was due to low laccase activity to extract nutrient to initiate the growth of mycelium. Idowu *et al.* (2016) also reported that the lowest *P. ostreatus* spawn level (3%) takes the longest day to complete substrate colonisation compared with the highest spawn level (13%). Increasing the quantity of spawn may reduce the competitiveness between organisms present in the substrate (Idowu *et al.*, 2016). However, increasing spawn loading above limits (> 60%) may

reduce laccase production due to the fast depletion of nutrients. This will result in a decrease in metabolic activity. Patel *et al.* (2009) reported that increasing spawn loading at an optimum amount could enhance the utilisation of substrate, thereby improving laccase activity, which leads to extracting more nutrients for mycelium growth. Moreover, a shorter colonisation time of substrate by mycelium could also be obtained.

### ***3.3 Physical and Mechanical Characterisation***

In the production of mycelium-based biofoam, maintaining the density of a material is one of the main challenges. The effect of incubation temperature on the physical properties of the mycelium biofoam is shown in Table 1 and Table 2. Referring to Table 1, the optimum dry density for each parameter was obtained when the biofoam incubated at 30°C, 50% (w/w) moisture content and 40% (w/w) spawn loading, which produced biofoam with dry density of 0.387 g/cm<sup>3</sup>, 1.07 g/cm<sup>3</sup>, and 0.437 g/cm<sup>3</sup>, respectively. On the other hand, the lowest dry density were observed for biofoam that was incubated at 40°C, 30% (w/w) moisture content and 5% (w/w) spawn loading, with dry density of 0.290 g/cm<sup>3</sup>, 0.286 g/cm<sup>3</sup>, and 0.314 g/cm<sup>3</sup>, respectively. Similarly, Table 2 shows a same trend for the compressive strength result. The highest compressive strength was obtained for biofoam incubated at 30°C with 0.975 MPa, 50% (w/w) moisture content with 1.070 MPa, and 40% (w/w) spawn loading with 1.350MPa. Subsequently, the lowest compressive strength for each parameter was observed at 40°C with 0.277 MPa, 70% (w/w) moisture content with 0.376 MPa, and 5% (w/w) spawn loading with 0.893 MPa.

Table 1: Average dry density of biofoam at different parameter.



Factors	Parameters	Average dry density, (g cm <sup>-3</sup> )
Temperature (°C)	20	0.314 ± 0.005 <sup>a</sup>
	25	0.350 ± 0.019 <sup>a</sup>
	30	0.387 ± 0.013 <sup>a</sup>
	35	0.309 ± 0.005 <sup>b</sup>
	40	0.290 ± 0.001 <sup>c</sup>
Spawn loading (wt %)	5	0.314 ± 0.005 <sup>b</sup>
	10	0.336 ± 0.007 <sup>c</sup>
	20	0.356 ± 0.021 <sup>d</sup>
	30	0.369 ± 0.011 <sup>e</sup>
	40	0.437 ± 0.010 <sup>a</sup>
Substrate moisture content (w/w %)	30	0.286 ± 0.005 <sup>b</sup>
	40	0.326 ± 0.035 <sup>a</sup>
	50	0.409 ± 0.023 <sup>a</sup>
	60	0.358 ± 0.010 <sup>a</sup>
	70	0.347 ± 0.014 <sup>c</sup>

Table 2: Compressive strength of biofoam at different parameter.

Factors	Parameters	Average compressive strength, (MPa)
Temperature (°C)	20	0.647 ± 0.033 <sup>b</sup>
	25	0.761 ± 0.013 <sup>a</sup>
	30	0.975 ± 0.106 <sup>a</sup>
	35	0.529 ± 0.001 <sup>c</sup>
	40	0.277 ± 0.075 <sup>d</sup>
Spawn loading (wt %)	5	0.839 ± 0.053 <sup>a</sup>
	10	0.929 ± 0.026 <sup>a</sup>
	20	1.005 ± 0.036 <sup>a</sup>
	30	1.125 ± 0.035 <sup>a</sup>
	40	1.350 ± 0.198 <sup>a</sup>
Substrate moisture content (w/w %)	30	0.746 ± 0.035 <sup>a</sup>
	40	0.789 ± 0.005 <sup>a</sup>
	50	1.070 ± 0.000 <sup>a</sup>
	60	0.475 ± 0.004 <sup>e</sup>
	70	0.376 ± 0.011 <sup>f</sup>

The values obtained for dry density and compressive strength were in correspondence with the preceding growth, where the formation of a dense white mycelium fungal skin occurred at the highest dry density and compressive strength. According to Owaid *et al.* (2015), high value of density indicated good mycelium growth, whereas low dry density was due to the nonexistent component of the mycelium and shrinkage caused by moisture loss. Growth of mycelium under optimum condition will produced biofoam with good compressive strength and density (Ghazvinian *et al.*, 2019). Bruscato *et al.* (2019) also reported similar density values of biofoam for mycelium *P. sanguineus*, *P. albidus*, and *L. velutinus* when using sawdust as substrate, which are 0.32, 0.30 and 0.35 g/cm<sup>3</sup>, respectively. In general, the biofoam density was denser compared to the density of expanded polystyrene (EPS) which is 0.03 g/cm<sup>3</sup>. Higher density obtained by mycelium biofoam however could be attributed by the presence of the supporting medium from the spawn and types of substrate used. Therefore, a spawn with the same substrate medium used for biofoam production should be considered in future study instead of using the grain spawn. On the other hand, Jones *et al.* (2020) reported that mycelium-based biofoam shows comparable

compressive strength (0.17-1.1 MPa) towards polystyrene foam (0.03-0.69 MPa) and a slightly weaker strength compared to polyurethane and phenolic formaldehyde resin.

### **3.4 Characterisation of biofoam morphology**

The colonisation of mycelium in the substrate normally is non-homogenous, and thus SEM was used to analyse the surface feature of the mycelium biofoam. Fig. 6 shows the morphological of mycelium biofoam. Based on the SEM image, the short and highly entangled tube-like structures were observed during the initial growth phase, whereas compact filaments increase with time (Fig. 6a). A study by Bruscato *et al.* (2019) using *Pleurotus albidus* also shows a compact filament where a higher number of hyphae adhere to the substrate then adhered to each other. Haneef *et al.* (2017) reported that the two species belong to the same group, white-rot fungi, could have an identical morphology because of the ability to excrete similar enzyme during the production of mycelium biofoam. On the other hand, the diameter of the filaments also depends on the availability of nutrients such as cellulose in the substrate. Haneef *et al.* (2017) reported that a substrate rich in polysaccharides could change the hyphae morphology responsible for the lightness characteristics of the material. In Fig. 6(b), hyphae were observed to appear flatten on the SEM image. This occurred because the internal hydrostatic pressure no longer supported the filaments of the mycelium once the mycelium has stopped growing due to heat treatment (Girometta *et al.*, 2019).

### **3.5 Characterisation of the biofoam's functional group**

Fig. 7 presents two FTIR spectra of rice husk as the substrate and the produced mycelium biofoam. For the infrared absorption spectra of the rice husk, a wide broad peak that is the polysaccharides component was observed from 900 to 1200  $\text{cm}^{-1}$  spectrum, with an obvious peak of C-C stretching at 1033  $\text{cm}^{-1}$ . Gao *et al.* (2018) stated that a rice husk was made up of 35 to 45% of cellulose, 15 to 20% of hemicellulose, and 20 to 25% of lignin. However, the intensity of the polysaccharides band, observed in the spectra of the mycelium biofoam, has decreased (Fig. 8b) as the rice husk was degraded as a substrate for the growth of *P. ostreatus*. In comparison, new bands of proteins and lipids were detected at 1633  $\text{cm}^{-1}$  and 3280  $\text{cm}^{-1}$  when the mycelium grew in the biofoam. Haneef *et al.* (2017) reported that *P. ostreatus* biofoam sample shows a relative increase in proteins and lipids on PDB-cellulose substrate compared to those grown on pure cellulose. The intensity of the polysaccharide in mycelium biofoam spectra was also found reduced from a value of 0.08 for pure cellulose to 0.06 for PDB-cellulose. Haneef *et al.* (2017) further stated that the decreasing amount of rigid chitin from the cell wall probably related to the collapse of the central area of mycelia was during the growth of mycelium on PDB-cellulose. Therefore, this finding found that the chemical nature of the feeding substrate is also responsible for the changes in infrared spectra of mycelium biofoam. Apart from that, the infrared absorption spectra of mycelium can also be translated as useful biomolecules during the composting process. Those biomolecules, which include protein (amide I with the wavelength of 1700-1600  $\text{cm}^{-1}$ , amide II and III with the wavelength of 1575-1300  $\text{cm}^{-1}$ ), lipids with the wavelength of 3000-2800  $\text{cm}^{-1}$ , and polysaccharides with the wavelength of 1200-900  $\text{cm}^{-1}$  (Pena *et al.*, 2014).

# Conclusion

Mycelium biofoam was produced by reinforcing lignocellulosic fibres combined with a commonly cultivated fungus, *Pleurotus ostreatus*. An interwoven three-dimensional filamentous mycelium network was formed to bind the substrate into a solid yet lightweight material. The result shows that the growth of mycelium was dependent on the types of substrate used. Moreover, the mycelium of *P. ostreatus* was found to grow at its best on rice husk as substrate but poorly on sawdust and sugarcane bagasse. Mycelium was observed to grow faster and with thicker volume at a given condition. As more mycelium presence, the compressive strength of the forming biofoam becomes stronger. Overall, the above results inferred that the types of substrate, incubation temperature, moisture content, and spawn loading influence the growth rate of the mycelium and the final characteristics of the forming biofoam. The morphological of mycelium biofoam confirms that the entanglement present in the structure was responsible for the lightness characteristic of this material, while chemical changes found in FTIR confirm that the chemical present in feeding substrate was responsible for distinct changes in material spectra. Thus, the growth conditions of the mycelium should be tailored based on the types of substrate used and the desired properties of the final material.

## Declarations

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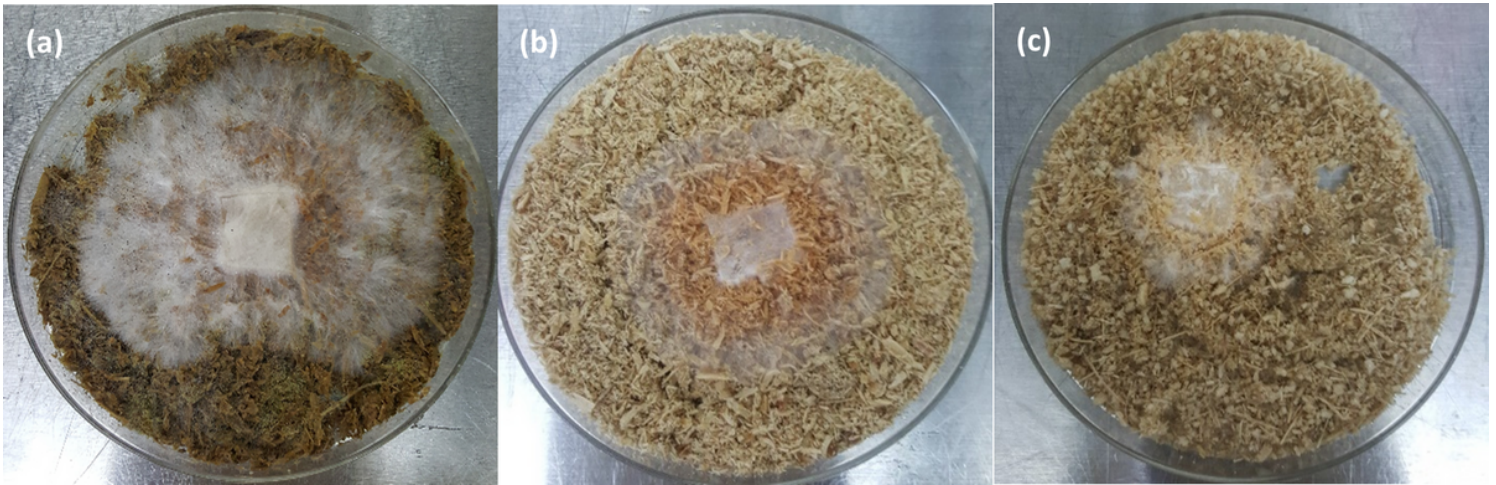
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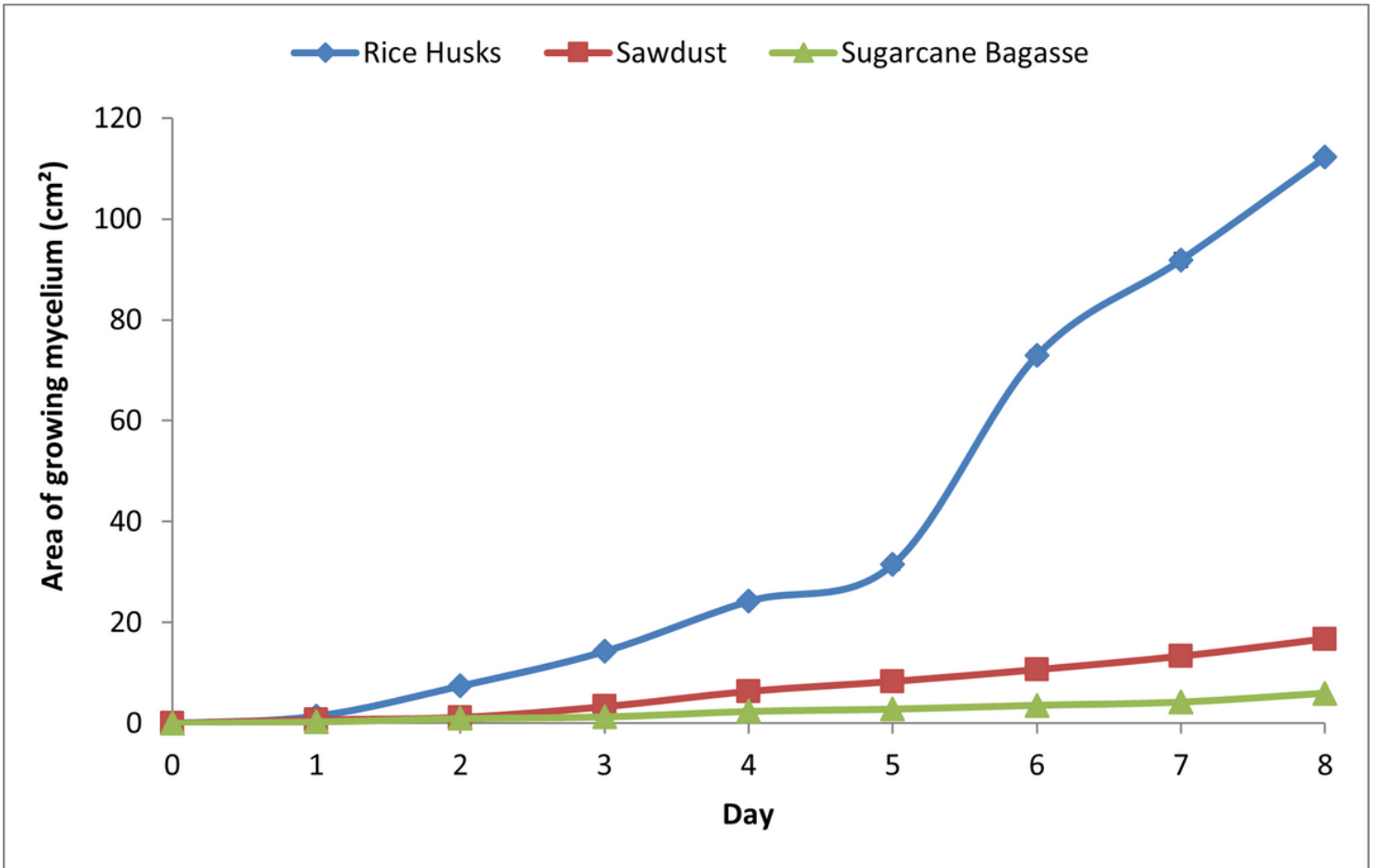
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## Figures



**Figure 1**

Mycelium growth of *Pleurotus ostreatus* on different lignocellulosic substrates: (a) rice husk, (b) sawdust, and (c) sugarcane bagasse.



**Figure 2**

Growth curve of *Pleurotus ostreatus* mycelium on three different biomass substrates.

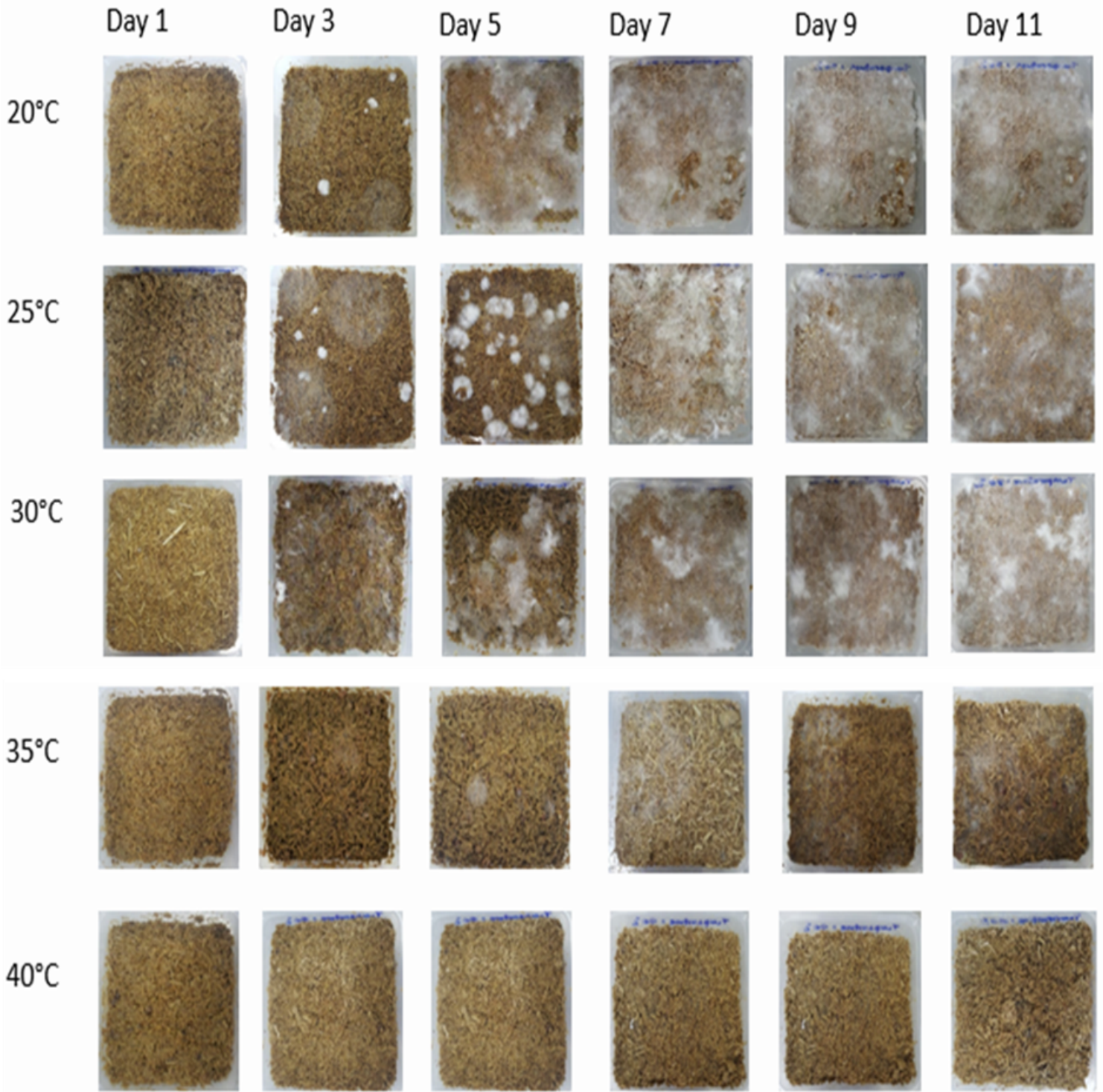
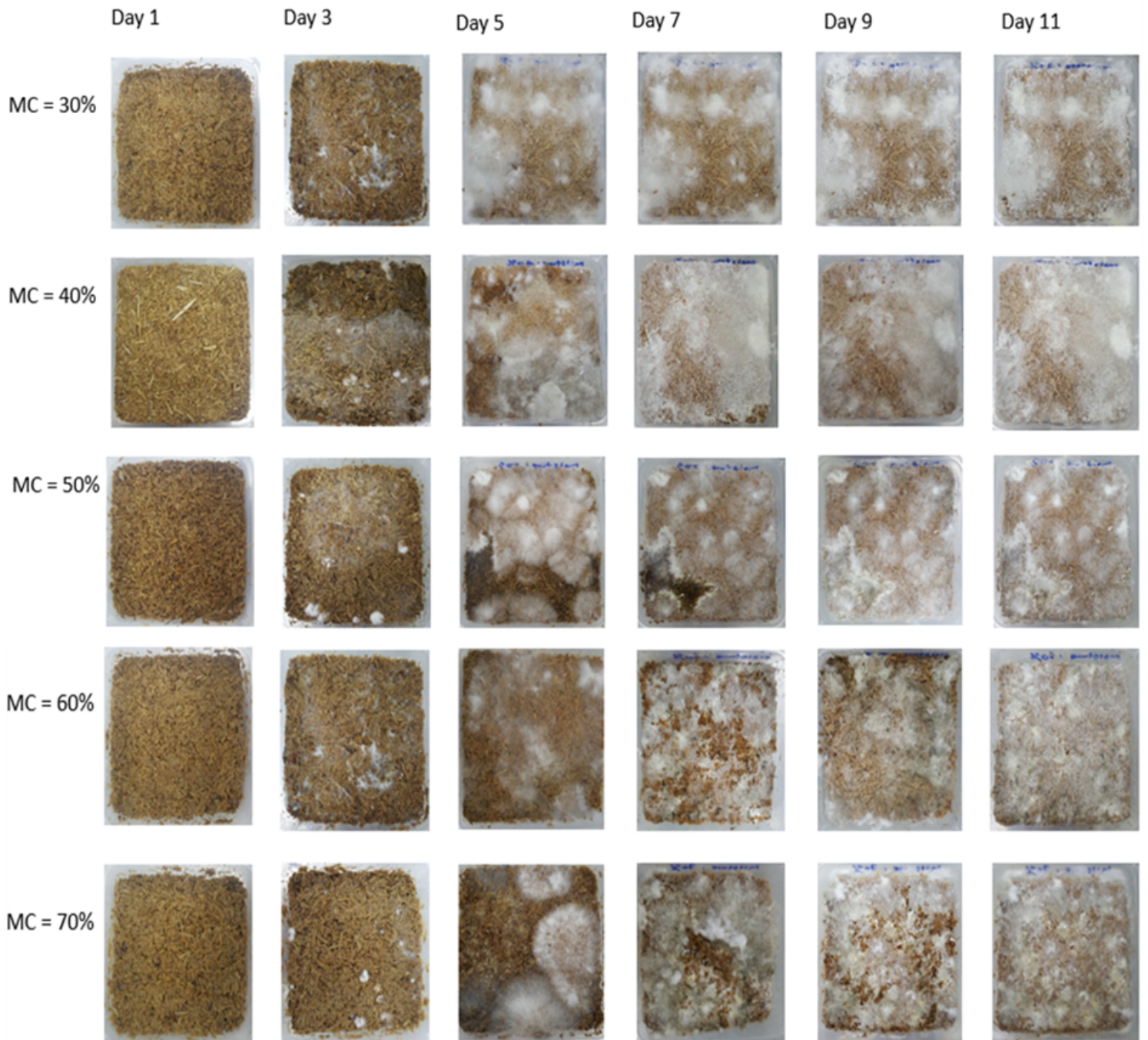


Figure 3

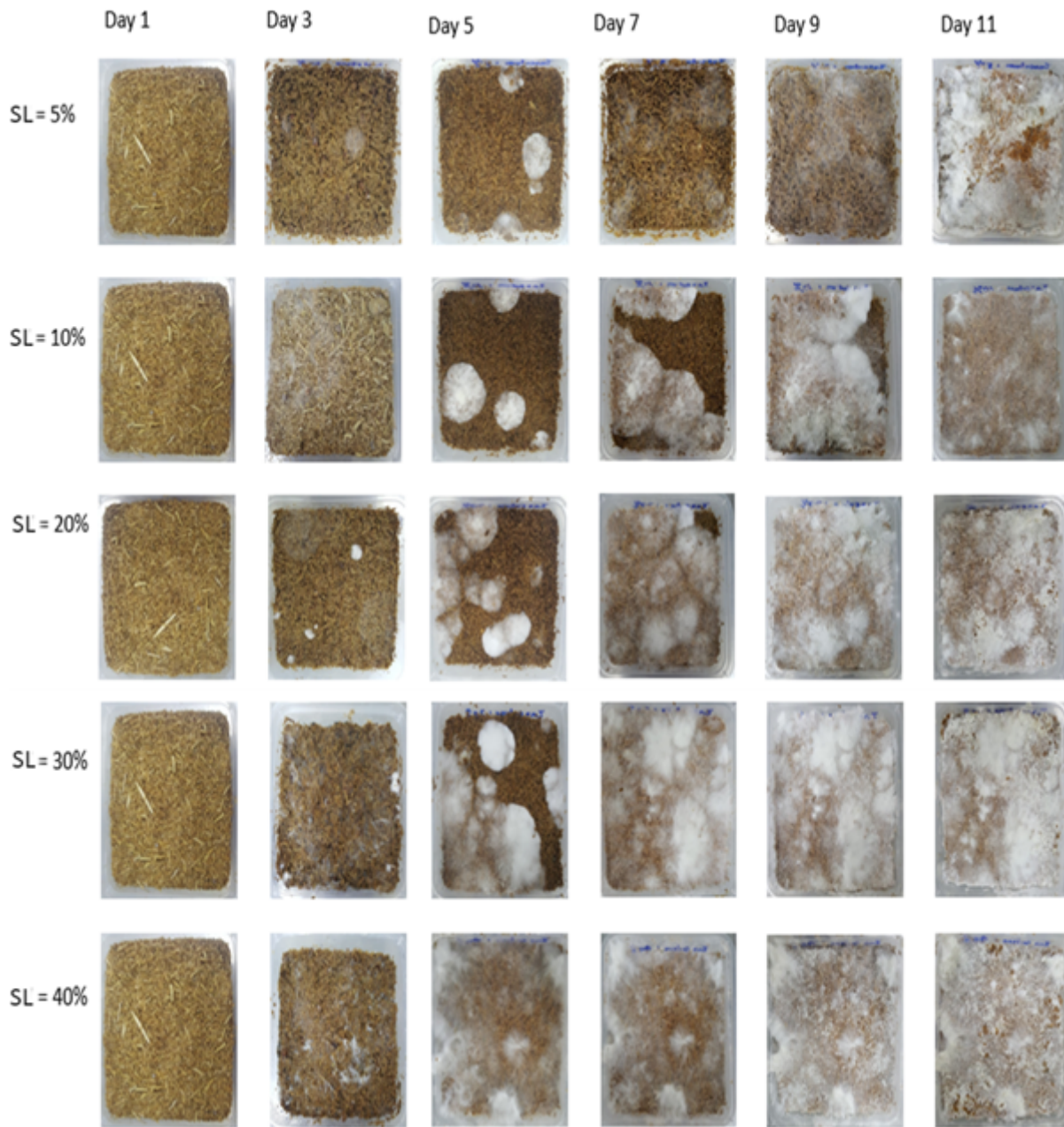
Mycelium growth of *Pleurotus ostreatus* mycelium at different incubation temperature.





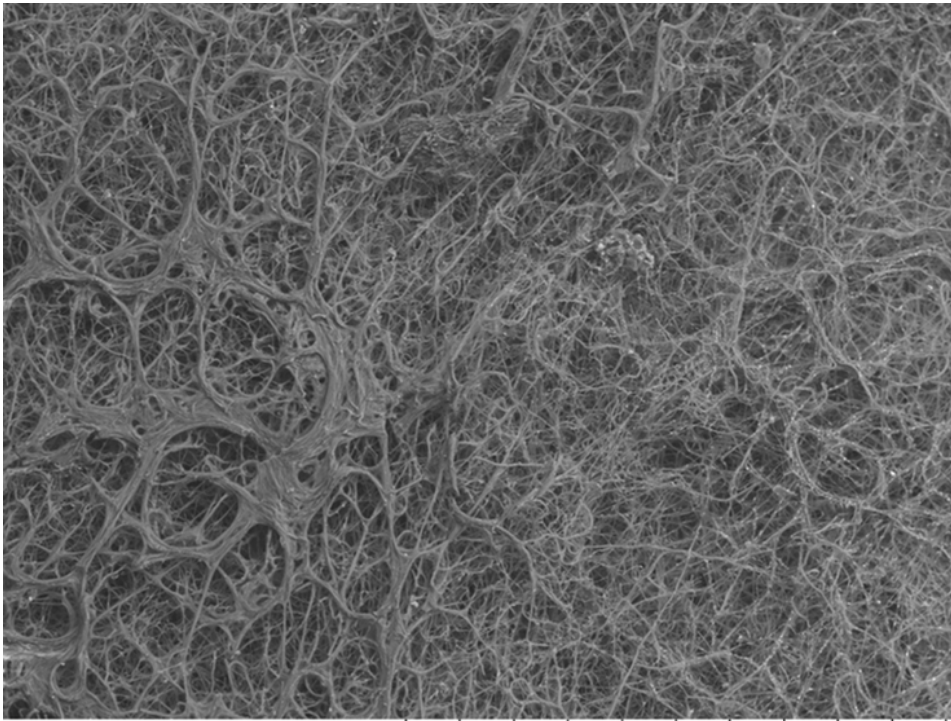
**Figure 4**

Mycelium growth of *Pleurotus ostreatus* mycelium at different moisture content.

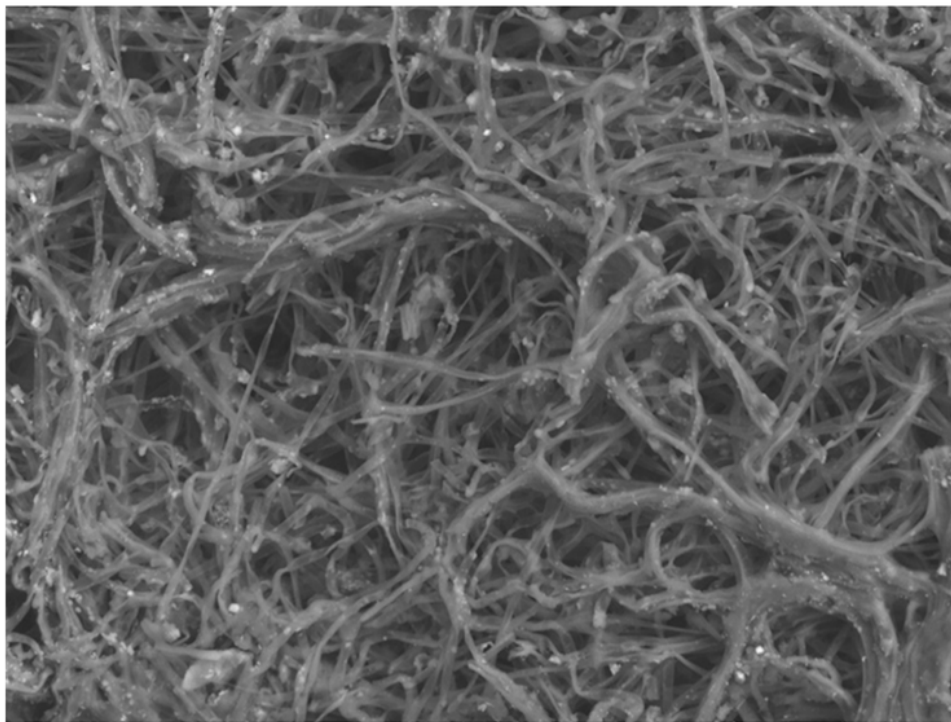


**Figure 5**

Mycelium growth of *Pleurotus ostreatus* mycelium at different spawn loading.



SEM AL x200 500 um



SEM AL x1.0k 100 um

**Figure 6**

SEM micrographs of mycelium biofoam.

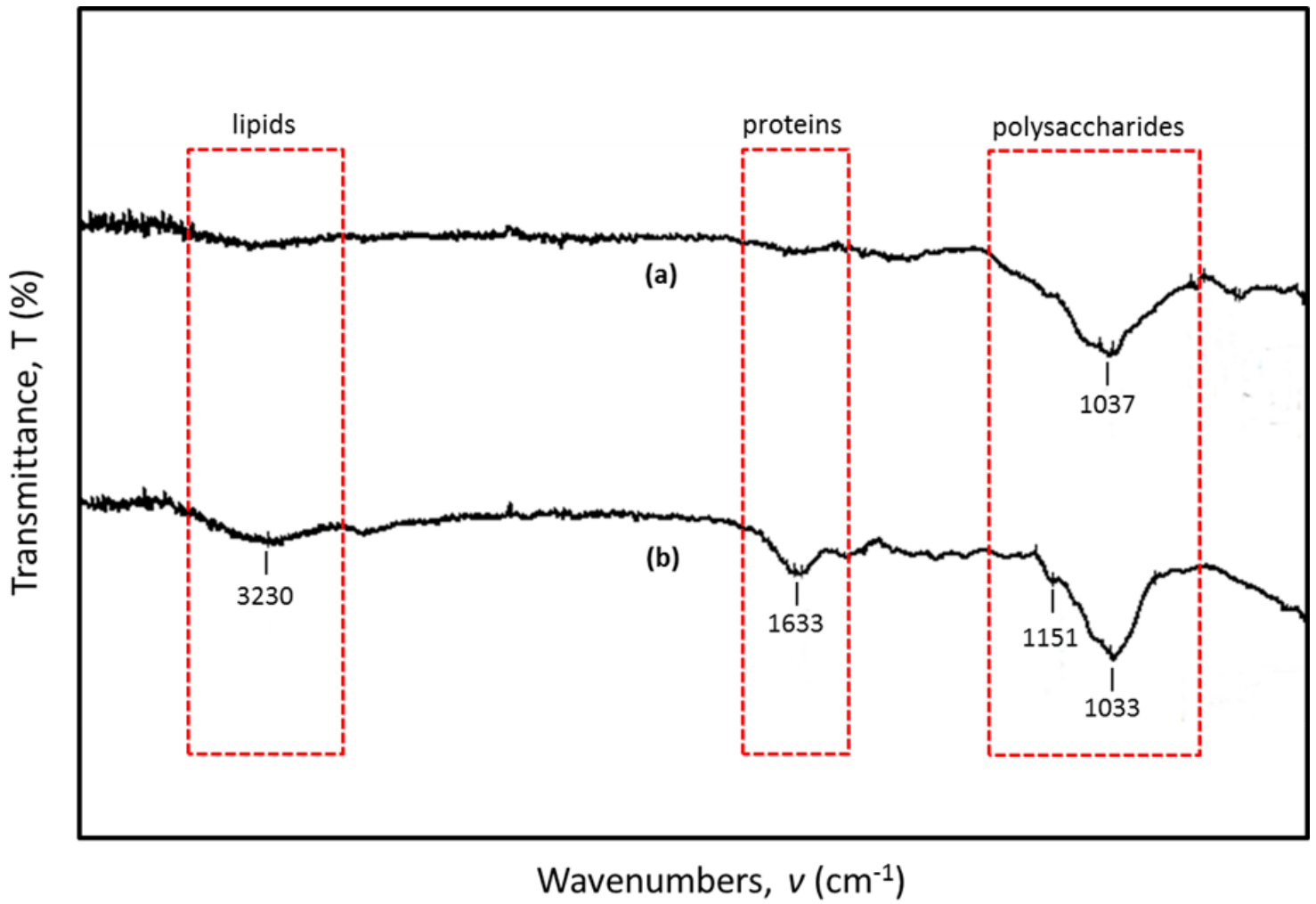


Figure 7

FTIR spectra of (a) rice husk substrate and (b) mycelium biofoam.