International Journal of Biomass Utilization and Sustainable Energy
Volume 2, 2024 [42-53]

Effect of Cold Exposure on the Biofoam Produced from Different Types of Oyster Mushroom

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ABSTRACT

Mycelium-based biofoam is a sustainable material derived from the growth of fungal mycelium on lignocellulosic agricultural waste substrate, as it has potential use in a variety of applications. The main objective of this research is to advance the sustainable alternatives for various application by investigating the mycelium growth of the biofoam produced from Pleurotus florida and Pleurotus sajor-caju on rice husk substrate, in improving the properties of the biofoam through innovative cold exposure. This study showed P. florida can produce mycelium biofoam at a faster rate, 7.022mm/day compared to P. sajor-caju 6.08mm/day). By cold exposure at 0°C and 10°C for 3 hours, every 2 days and 5 days, respectively until the mycelium are fully grown in the substrate, sample exposed to the latter condition for P. florida exhibits a faster growth rate at 7.3037 mm/day. However, cold exposure on biofoam produced from P sajor-caju had not improved the mycelium growth rate. Cold exposure samples at 0°C every 5 days and 10°C every 2 days have demonstrated capability in water (103.51%) and oil absorption (143.23%), proving their effectiveness in absorbing pollutants for the purpose of environmental remediation. The FTIR analysis confirmed the presence of hydrophilic and oleophilic characteristics in the biofoam, indicating its capability to absorb water and oil. By subjecting biofoam to cold exposure, its properties can be altered, broadening its potential applications.

Keywords: Mycelium biofoam, *Pleurotus sp*., rice husk substrate, cold exposure.

1. INTRODUCTION

The proliferation of petroleum-based plastics has indeed led to a significant global pollution crisis. However, the use of plastics cannot be eliminated even though they contribute to issues in the environment and health due to their durable and resistant behavior. This has prompted novel approaches and technologies in producing mushroom-based biofoam to tackle this issue. The production of biofoam is also as a solution to environmental issues caused by improper management of agricultural waste such as rice husk. When the mycelium is incorporated with substrate matrix, it creates a type of biofoam that is renewable and biodegradable. It is reported that they are lightweight, have good insulation properties, and can be grown using agricultural waste as a substrate (Indarti *et al*., 2023; Iriani *et al*., 2020). The repurposing of rice husk as a substrate allows mushroom biofoam to grow within the matrix that contains lignocellulosic components for mycelium. These lignocellulosic components povide a structural and nutrient-rich matrix to mycelium in the form of a carbon source.

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The fibrous nature of rice husk can contribute to the physical structure of the growth medium, providing a scaffold for mycelial colonization. Mycelium networks grow in a map structure with a dense network of interconnected threads. Under specified growth conditions, the mycelium produces a natural adhesive, binding the threads together and forming a solid biofoam (Hazarika & Thakur, 2020). The nutritional profile also includes rice bran to provide a balanced nutrient composition while supporting the mycelium's metabolic process and promote healthy growth. Calcium carbonate serves as a buffer, and calcium ions contribute as nutritional content to the medium, forming a robust biofoam structure.

The tailored formulation medium is specific to the *Pleurotus sp.*, commonly known as oyster mushrooms. The *Pleurotus sp.* are widely used in several studies and experiments by researchers because of their relatively fast mycelium growth compared to other fungi. This characteristic is advantageous in the context of biofoam production, where rapid colonization of the substrate is often desirable for efficient and timely cultivation (Hoa & Wang, 2015). *Pleurotus sp.* demonstrates adaptability, allowing for the use of diverse agricultural byproducts and waste materials, making them suitable candidates for sustainable and cost-effective biofoam production. However, research and studies may vary in exploring the optimization of growth media formulations, substrate ratios, and cultivation conditions to enhance *Pleurotus sp.* mycelium production for biofoam applications (Girmay *et al*., 2016).

Unique and innovative approaches to improve biofoam growth involves the application of stress physically (Roshita *et al*., 2017). Stress-induced methods aim to enhance productivity, quality, and other desirable characteristics of the mycelium during cultivation. By exposing the mycelium biofoam to cold temperatures below its optimal growth range, stress is induced, thus triggering metabolic adjustments as the mycelium responds to adapt to the cold stress. These might create a more compact and structurally robust biofoam due to the densification of the mycelium network (Hoa & Wang, 2015). To unlock the potential of cold exposure to biofoam cultivation with desired outcome and without compromising growth, optimizing parameters is a major challenge. Thus, it is a fresh idea to cultivate mushrooms through cold exposure with different variables in temperature and time intervals (Fu *et al*., 2016).

This study focused on integrating *Pleurotus florida* and *Pleurotus sajor-caju* by inducing stress through cold exposure effects with variables designed on cold exposure temperature of 0°C and 10°C for 3 hours, every 2 and 5 days. This is to create mycelium-based biofoam that is adaptable to various applications, having water and oil absorption capabilities characterized by water and oil absorption capability tests.

2. MATERIALS AND METHODS

2.1 Substrate Preparation

Rice husks used as the substrate in the mycelium biofoam cultivation was collected from a rice mill located in Kuala Perlis, Perlis, Malaysia. First, the rice husk was washed and ground, followed by mixing with rice bran and calcium carbonate following the ratio of 100:10:1 (Majib *et al*., 2023). Distilled water was added into the mixed substrate to turn it into clumpy features to provide sufficient moisture content to the mycelium growth. The clumpy substrate was compacted into an 8 oz container until 75% filled. The container was placed into an autoclave machine for 20 minutes at 120°C.

2.2 Biofoam Production

According to Majib *et al*. (2023), about one spatula of fungal spawn was added to each container with substrate. The containers are then covered up with a lid with hole but filled up with cotton wool, labelled and stored in several dark boxes for cultivation. Different species of oyster

mushroom were cultivated in separate containers to avoid cross contamination. After all the samples were fully grown within the substrate (samples with or without cold exposure), the samples were then immediately dried using a convection oven at 60°C for 24 hours. Moisture content in the biofoam was removed during this process, and thereafter terminate the growth of biofoam and avoid the formation of fruiting body (Costa *et al*., 2019). Dry conditions prevent contamination and the growth of mold in the biofoam. Figure 1 illustrates the processing steps in biofoam fabrication.

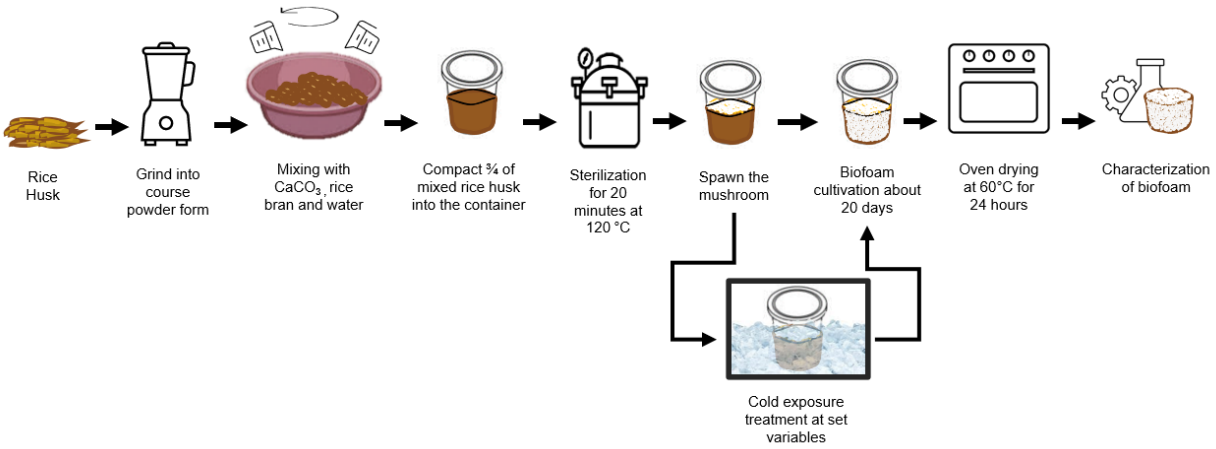

Figure 1: Process flow illustration showing the method to produce mycelium biofoam with or without cold exposure.

2.3 Cold Exposure

Cold exposure was carried out by exposing the mycelium biofoam to temperatures at 0°C and 10°C for the allocated samples, once every 2 and 5 days for each temperature, with a duration of 3 hours per session. For samples that undergo cold exposure at 10°C, the samples were exposed to the cold environment in the refrigerator for 3 hours; for samples that undergo cold exposure at 0°C, the samples were exposed to ice cubes in an ice box for 3 hours. During the whole process of cultivating the biofoam, the height of mycelium in the substrate within the container was assessed every 3 days to determine the growth. Table 1 summarized the experiment sets of the biofoam samples.

Table 1: Experiment Sets of the Biofoam Samples

Group	Species	Interval time of	Exposure Temperature	Label
1	*P. florida*	Control	N/A	FC
2		Every 2 days	0°C	F2D0C
3			10°C	F2D10C
4		Every 5 days	0°C	F5D0C
5			10°C	F5D10C
6	*P. sajor-caju*.	Control	N/A	SC
7		Every 2 days	0°C	S2D0C
8			10°C	S2D10C
9		Every 5 days	0°C	S5D0C
10			10°C	S5D10C

2.4 Growth Performance

To describe the behaviour of mycelium growth performance, growth rate was obtained by using the Linear Regression Model in Equation 1 (Guadarrama-Mendoza *et al*., 2014). Growth data of mycelium growth height within the substrate in units (mm) on the container was recorded and used to generate growth profiles. The slope of the linear line is the growth rate of mycelium.

$$Y = mX + C \quad (1)$$

where Y represents mycelium growth height in y-axis, m is the slope represents growth rate of mycelium, and C is the intercept represents the initial growth of mycelium.

2.5 Water and Oil Absorption Capability Test on Biofoam

Three samples of each experiment set were tested to determine the water absorption capability. The initial weight of the dry biofoam samples were measured by using an analytical electronic balance. The samples were soaked in 250ml of water for about 24 hours. A similar procedure for the biofoam samples were prepared for the oil absorption capability test. The weight of the wet biofoam samples were measured and the absorption capability of water and oil were determined using Equation 2.

$$Absorption\ Capability\ (\%) = \frac{w' - w}{w} \times 100 \quad (2)$$

where w' is the weight of sample after soaked (g) and w is initial weight of dry sample (g) (Kumla *et al*., 2020).

2.6 Functional Group Analysis

The selected biofoam samples were prepared and inserted into the FTIR machine. The FTIR (Perkin Elmer, USA) spectrum was chosen within the range of 650 cm^{-1} to 4000 cm^{-1} with a resolution of 1 cm^{-1}.

3. RESULTS AND DISCUSSION

3.1 Mycelium Growth Performance of *Pleurotus Species*

The growth curve in Figure 2 shows that the mycelium of *Pleurotus florida* and *Pleurotus sajor-caju* had grown completely into biofoam at the same duration, which is 15 days. Both species of oyster mushroom initiated their growth with a slow rate from Day 0 to Day 2 by adapting to the substrate medium. From days 2 to 12, the mycelium entered an exponential growth phase where it started to grow after absorbing nutrients and water in the substrate. The mycelium reached a maximum growth height at 80 mm because fruiting was not required for this experiment, therefore the growth of mycelium was terminated before the fruiting phase (Appels *et al*., 2018). Besides, there was a difference in mycelium growth from day 2 to day 15 between *P. florida* and *P. sajor-caju*. During this period, *P. florida* made a tremendous growth, possibly due to substrate preferences (Elsacker *et al*., 2019). *P. florida* was reported to be able to grow within a short duration with less water and space, while the rapid growth of *P. florida* might be caused by its genetic makeup and its ability to efficiently utilize nutrients from the rice husk substrate (Kinge *et al*., 2016).

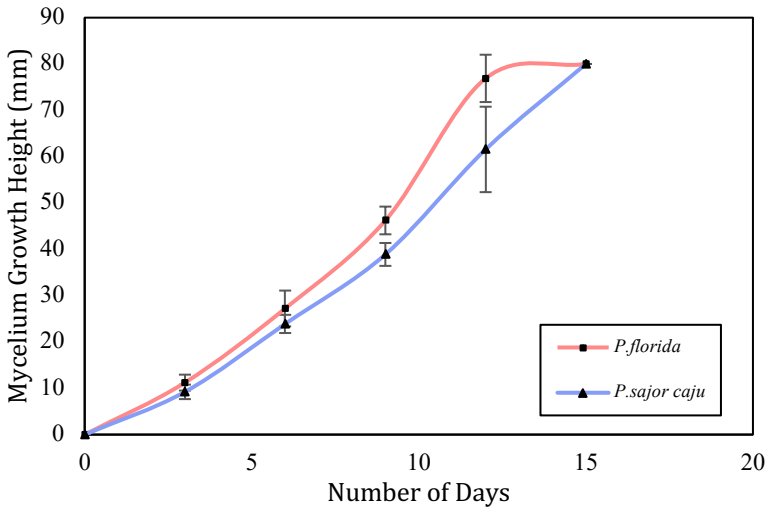

Figure 2: Mycelium growth height (mm) vs number of days for *P. florida* and *P. sajor-caju*

Table 2 shows the average growth rate of *Pleurotus* sp. cultivated in rice husk substrate. The growth rate of mycelium biofoam cultivated in rice husk for *P. florida* at 7.02 mm/day was comparatively higher than *P. sajor-caju,* at 6.08 mm/day.

Table 2: Average growth rate of *Pleurotus* species cultivated in rice husk substrate

Species	Growth rate (mm/day)
P. florida	7.0222
P. sajor-caju	6.0815

This result suggested that *P. florida* is preferable in mycelium biofoam fabrication in terms of growth rate when cultivated in rice husk substrate. Previous research showed that *P. sajor-caju* had higher growth rate than *P. florida* when using different materials as substrate such as wheat extract agar and potato dextrose agar (Kumar *et al*., 2018). According to Nashiruddin *et al*. (2022), rice husk is a good source of nutrients for mycelial growth as it contains about 35-45% cellulose, 15-20% hemicellulose, and 20-25% lignin. These components provide necessary carbon and energy sources for the fungus to thrive.

3.2 Mycelium Growth Performance with Cold Exposure

Cold exposure has been demonstrated to impact the growth and properties of fungal mycelium by inducing new protein expression, which is influenced by the species, growth factors, and growth mediums (Yang *et al*., 2017). Figure 3 shows the mycelium growth of *P. florida* samples has similar growth rate from day 0 to day 3. Control sample for *P. florida* (FC) and both 5-day interval samples have a higher growth rate after day 3 to day 12. *P. florida* samples exposed to 0 °C and 10 °C every 5 days (F5D0C, F5D10C) showed a positive influence on its mycelium growth at different periods of cultivation when compared to the control sample, while the other cold exposure conditions had a negative growth impact. Nevertheless, F5D10C and F5D0C demonstrated only a marginally higher growth rate in different periods when compared to the control sample. The F5D0C sample had the highest growth from day 3 to day 9; F5D10C had the highest growth in day 9 to day 15. F2D0C had a linear growth rate, but a significantly slow growth. Nevertheless, it was fully grown on day 15, similar to other samples.

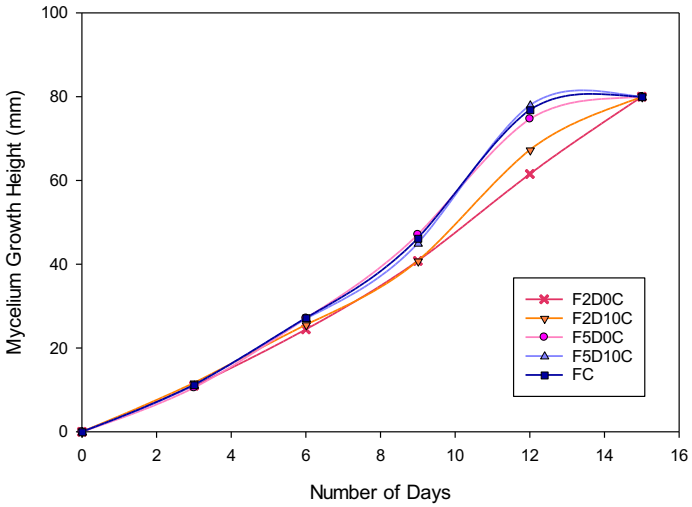

Figure 3: Mycelium growth profile of each cold exposure variable, in terms of temperature and day interval for *P. florida*, mycelium growth height (mm)

Based on Table 3, slight improvement on growth rate was obtained when the mycelium was treated to 10 °C, every 5 days (F5D10C), with 7.3037 mm/day.

Table 3: Average growth rate of *Pleurotus* species cultivated in rice husk substrate

Samples	Growth rate (mm/day)
F2D0C	5.8371
F2D10C	6.2148
F5D0C	6.7433
F5D10C	7.3037
FC	7.0222

Cold treated *P. florida* samples at 0 °C and 10 °C every 2 days (F2D0C, F2D10C) did not improve the mycelium growth rate, which is contrary to the experimental results obtained by Ibrahim *et al*. (2015). The results might possibly be affected by the surrounding growth factor in Control situation. The biofoam that did not undergo cold exposure were cultivated in an environment which was dark and air conditioned at 20-25°C, which probably is suitable for the mycelium growth (Yaazhini & Loganathan, 2021). The cold exposure treated samples for *P. sajor-caju* displayed only minor variations in mycelium growth rates as shown in Figure 4. From Day 0 to Day 9, S2D10C exhibited the highest growth rate compared to the SC sample. However, SC displayed the highest growth rate from day 9 until day 15 but not S2D10C. According to Figures 3 and 4, the results show that under cold exposure and non-cold exposure, both species of mycelium were fully grown in the rice husk substrate within 15 days. Note that there was no difference in the mycelium growth between *P. sajor-caju* and *P. florida* in terms of growth durations. Results in Table 4 show that there was no significant improvement in the mycelium growth rate under cold exposure for *P. sajor-caju*, but rather minor negative effect on the growth rate as the Control sample had the fastest growth rate. There might be a possibility where the optimal temperature to cultivate *P. sajor-caju* is relatively high as reported, which is approximately 27.5°C to 30°C and the low temperature about 15°C showed minimum radial mycelium growth (Kumar *et al*., 2018). The growth rate might be hindered when the mycelium was subjected to 0°C, due to adverse effects of low temperature on the metabolic process and physiological activities of mycelium cells, difficulties in nutrients uptake, protein synthesis and energy production (Zhao *et al*., 2020).

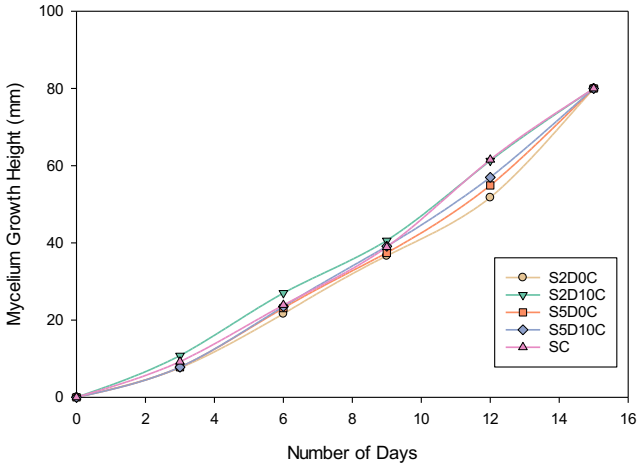

Figure 4: Mycelium growth profile of each cold exposure variable, in terms of temperature and day interval for *P. sajor-caju*, mycelium growth height (mm) vs number of days

Table 4: Average growth rate of *Pleurotus* species cultivated in rice husk substrate

Samples	Growth rate (mm/day)
S2D0C	5.8292
S2D10C	6.0630
S5D0C	5.8733
S5D10C	5.9300
SC	6.0815

3.3.3 Water absorption capability test

From Figure 5, mycelium biofoam displays varying water absorption capabilities ranging from 60% to 100% based on conditions, with distinct disparities observed between samples obtained from *P. florida* and *P. sajor-caju*.

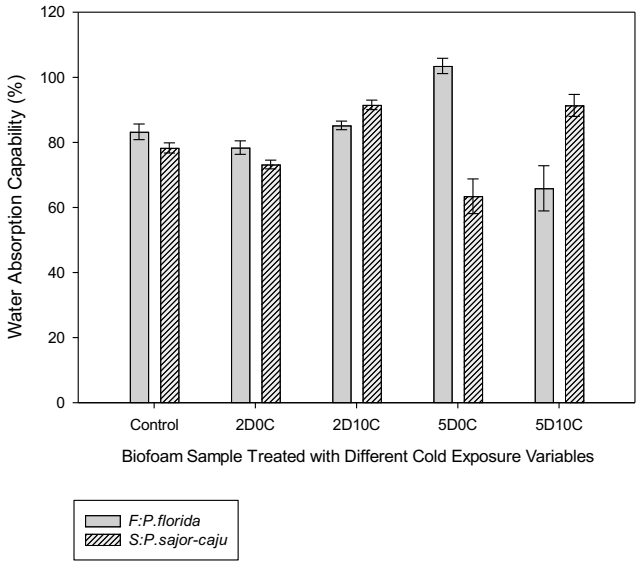

Figure 5: Water absorption capability for each type of biofoam according to cold exposure variables

In the case of biofoam derived from *P. florida*, the water absorption capability reached its peak at 100% when subjected to 0°C for 5 days (F5D0C), a higher value compared to the FC. The result indicates a substantial enhancement in water absorption under this specific condition. Sample F2D10C also had a higher water absorption capability than FC. Conversely, F2D0C and F5D10C showed a lower ability in water absorption compared to FC, suggesting that these conditions are less conducive to water absorption for *P. florida*. On the other hand, biofoam produced from *P. sajor-caju* demonstrated an increase in water absorption capability with treatments at 10°C for both 2 and 5 days (S2D10C, S5D10C), indicating that slightly warmer conditions increased the water absorption capability. The treatments at 0°C for both 2- and 5-days intervals (S2D0C, S5D0C) resulted in a lower water absorption capability than the control (SC), suggesting that freezing conditions contribute to a lower water uptake. However, in several applications that favor low water uptake, F5D10C and S5D0C were beneficial due to the lowest water absorption capability. According to Elsacker *et al*. (2019), water absorption capability of mycelium biofoam was also influenced by its hydrophobic nature of mycelium and hydrophilic nature of fibers in substrate.

3.3.4 Oil Absorption Capability Test

Figure 6 demonstrates an excellent oil absorption capability of the biofoam samples, ranging from 100 % to 145%.

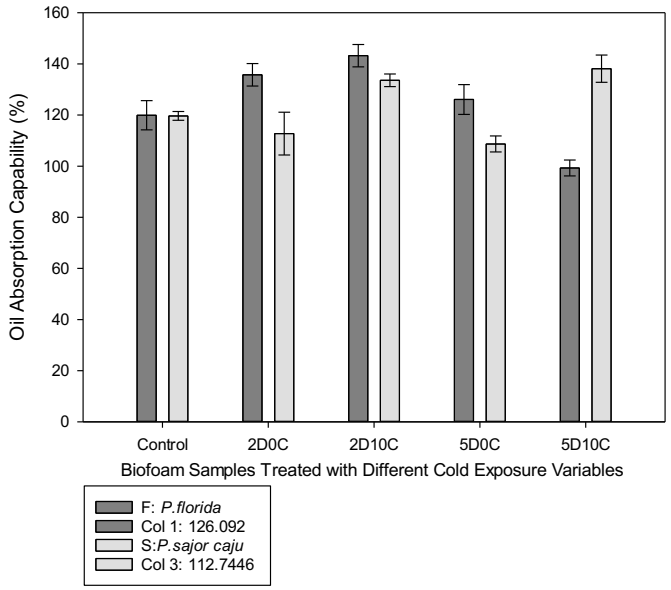

Figure 6: Oil absorption capability for each type of biofoam according to cold exposure variables

In terms of control samples, both *P. florida* and *P. sajor-caju* biofoams exhibited an oil absorption capability of 120%, indicating no difference between the two oyster mushroom species in Control condition. Specifically, for *P. florida* biofoams, F2D10C displayed the highest oil absorption capability. Only F5D10C showed the lower ability in oil absorption (approximately 100%) compared to FC. Results showed that the biofoams produced by *P. florida* exhibited excellent oil absorption capability after subjected to frequent cold exposure. On the other hand, *P. sajor-caju* biofoams showed the highest oil absorption capability (around 140%) in the sample treated at 10°C for every 5 days (S5D10C). SC and S2D0C samples had similar oil absorption capability values, whereas the sample treated at 0°C for every 5 days (S5D0C) exhibited the lowest oil absorption capability. Effective cold exposure at 10°C to the *P. sajor-caju* biofoam contributes to higher oil absorption capability, which offers the potential use in bioremediation application (Li *et al*., 2013). Additionally, oleophilic nature combined with the porous structure of biofoam can possibly facilitate the absorption and retention of oil molecules within the biofoam (Zhang *et al*., 2016).

3.4 Chemical Structure of Mycelium Biofoam

FTIR analysis was conducted within the range of 650 cm^{-1} to 4000 cm^{-1} to ascertain the chemical composition of the mycelium biofoam produced through the cultivation of *P. florida* and *P. sajor-caju* in rice husk substrate. The resulting spectra in Figure 7 provide insight into the chemical compositions and structural changes in the samples. Each of these spectrums reveals distinct absorption bands corresponding to various functional groups and chemical bonds present in the biofoam. Three key regions that stand out within the spectrum which are polysaccharide band, protein and lipids, and other functional groups. The polysaccharide band is the evidence representing the presence of carbohydrates structure; protein peaks are typically associated with amino acid residues, while the lipid peaks indicate fatty acid chains.

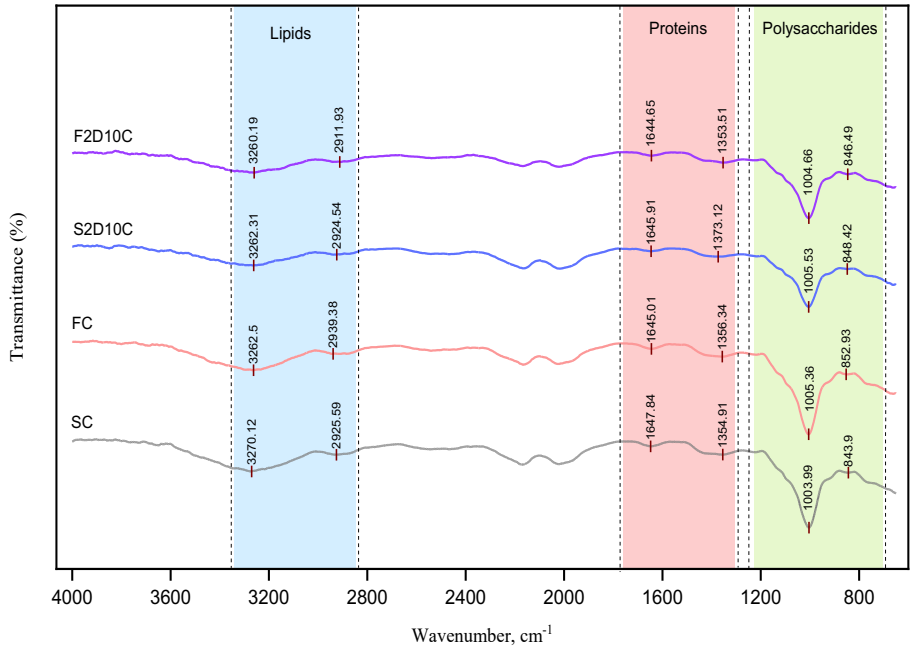

Figure 7. FTIR spectrum of mycelium biofoam samples for F2D10C, S2D10C, SC and FC

The O-H and N-H stretching vibrations, observed between 3260 and 3271 cm^{-1} indicate the presence of lipids, proteins and polysaccharides such as chitin in the biofoam sample. Peaks at approximately 2924-2939 cm^{-1} correspond to C-H stretching vibrations in waxes and oils, confirming lipid content as noted in the literature (Nashiruddin *et al.*, 2022). In the protein region, the amide I and II bands of protein shown at the peak range of 1644.65 to 1647.84 cm^{-1} confirm the presence of proteinaceous materials within the mycelium biofoam. Peaks around 1373-1356 cm^{-1} are due to C-H bending in cellulose and hemicellulose, as well as chitin, suggesting a polysaccharide composition which are major components of the mycelium cell walls (Javier-Astete & Zolla, 2021). Peaks at approximately 1003-1006 cm^{-1} are attributed to C-O stretching in cellulose, while peaks near 843-852 cm^{-1} are associated with C-H bending of the mode of vibration aromatic component potentially from lignin or other aromatic components of the mycelium or substrate. In between, functional groups such as O-H/N-H stretching, amide bonds, C-H bending and C-O stretching in cellulose are hydrophilic which have the affinity to attract water by forming hydrogen bonds with water. On the other hand, oleophilic functional groups which are C-H stretching vibrations in waxes and oils and the aromatic C-H bending interact well with nonpolar oil molecules.

The FTIR analysis reveals that the mycelium biofoams are composed of a complex mixture of lipids, proteins, polysaccharides (cellulose and chitin), and aromatic compounds. According to Haneef *et al.* (2017), the decrease in the polysaccharide peaks and the increase of protein and lipid peaks suggest that the formation of mycelium has degraded the rice husk substrate and incorporated its own biomass into biofoam structure. Samples (F2D10C, S2D10C, SC, and FC) shows consistent peaks

across the spectra, indicating similar chemical compositions with different cold exposure treatments. However, the treatments do not drastically alter the fundamental chemical composition, possibly due to the primarily affected the mycelium's metabolic physical properties such as texture or structure without significant alter its chemical composition.

4. CONCLUSION

Biodegradable mycelium biofoams have been successfully derived from *Pleurotus florida* and *Pleurotus sajor-caju* using rice husks as a substrate within 15 days. Without cold exposure, *P. florida* (7.022 mm/day) can produce mycelium biofoam at a faster rate compared to *P. sajor-caju* (6.08 mm/day). Physical stress by cold exposure to the biofoam at 0°C and 10°C for 3 hours once, applied at intervals of 2 and 5 days respectively until the mycelium fully grown in the substrate suggested that F5D10C for *P. florida* exhibits a faster growth rate of 7.3037 mm/day. However, cold exposure on biofoam produced from *P sajor-caju* had not heavily improved the mycelium growth rate. The FTIR analysis confirmed the presence of hydrophilic and oleophilic characteristics in the biofoam. Specifically, the F5D0C and F2D10C have demonstrated capability in water (103.51%) and oil absorption (143.23%), highlighting their potential in absorbing pollutants for the application in environmental remediation.

ACKNOWLEDGMENT

The authors would like to thank Universiti Malaysia Perlis (UniMAP) for providing the facilities to carry out this work.

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