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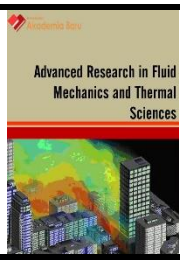

Preparation and Characterization of Mycelium as a Bio-Matrix in Fabrication of Bio-Composite

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Mohammad Aliff Shakir[1], Baharin Azahari[2], Yusri Yusup[1], Mohd Firdaus Yhaya[3], Ali Salehabadi[1], Mardiana Idayu Ahmad[1,*]

[1] Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Gelugor, Pulau Pinang, Malaysia
[2] Bio-resource, Paper and Coatings Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Gelugor, Pulau Pinang, Malaysia
[3] Biomaterial and 3D Imaging (BioM3D) Laboratory, School of Dental Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

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ABSTRACT

The objective of this work is to access the possibility of mycelium as a new reinforcement and inexpensive bio-matrix in production of bio-composite board. In this study, mycelium has been obtained from different substrate, inoculation time, and heating time processing. Various properties of the mycelium bio-matrix, either chemical or physical, were measured using Fourier Transform Infrared (FTIR) spectroscopy, Thermogravimetry (TG/DTG), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM), Light Microscopy, and flexural strength test. The structural analysis of the samples indicates that the chitin content in the mycelium increases as inoculated with either cellulosic substrate or starch substrates, but with prolonged inoculation time. The TGA and DSC thermograms reveal that the thermal stability and glass transition (T_g) temperature are improved with prolonged inoculation time. The morphological observations confirm the presence of mycelium networks which can be used as a potential bio-matrix in bio-composites. The mechanical properties of the samples, at pressing times of 20 and 40 min, show an enhanced flexural strength of mycelium bio-composite board from 1.82 MPa to 3.91 MPa.

Keywords:
Mycelium; thermal; bio-matrix; bio-composite

1. Introduction

Mycelium is a natural fibre polymer composite (NFPC) composed of chitin, cellulose, protein, etc. Mycelium is an effective unprocessed binder which can be exploited by the companies in order to fabricate wood composite products [1]. Mycelium, as a bio-matrix, can improve the wood composite properties in terms of environment, economy, energy consumption, and biodegradability [2, 3]. However, some deficiencies have prevented wide range applications of mycelium like poor mechanical properties as compared to conventional engineered composites. The mycelium bio-

* *Corresponding author.*
E-mail address: drmia707@gmail.com (Mardiana Idayu Ahmad)

matrix is still capable of being used in semi-structural applications such as furniture, and decking [4-6].

A single fibrous mycelium is called hyphae. The elongation of the mycelium occurs through the extension of the cell membrane and cell wall at the hyphal tip. The hyphae growth direction can stimulate to the location of nutrients. This mechanism is responsible for shear thickening at the hyphae tip, which contains chitin synthase and glucan synthase enzymes. Chitin is rigid polymers which can synthesize in the cell wall of mycelium. A large amount of chitin can enhance the mechanical properties of the mycelium bio-matrix during the production of bio-composite [7]. During mycelium growth, chitin and glucan are continuously assembled, which can crosslink, and impart rigidity to hyphae [8, 9].

It is reported that the formation of mycelium is higher when inoculate using glucose, starch, maltose, and lactose [10]. Although starch is a polysaccharide, it still can be consumed by mycelium in a short period of time by breaking into small monomers unit or simple sugar (glucose). The mycelium can also grow on polysaccharides such as cellulosic materials by penetrating and secreting digestive enzymes [11]. Moreover, the physical and mechanical properties of mycelium as a matrix depend on the consuming time during inoculation process [4, 12].

Mycelium, in the production of bio-composites, can be used instead of synthetic composite board [7, 13, 14]. As mentioned before, mycelium is a natural binder with different properties which can be enhanced during inoculation process [15, 16]. The longer inoculation time improves the rigidity of the mycelium structure and also producing a smooth surface on the bio-composite board [1]. Water repellence and fire retardant properties of mycelium during bio-composite production are two key advantages as compared to synthetic binders [6, 7].

In the current study, mycelium was successfully extracted from cellulose (rubberwood), starch (corn grain), and glucose. The extracted mycelium was used in the production of bio-composite board. The bio-composites were formed by incubation of rubberwood sawdust (RWS) and mycelium. Though the inoculation time does not affect the properties of composite board, however it can involve in cost and time during the production process. Finally, the properties of mycelium as a bio-matrix were investigated at different inoculation time on board performances at different pressing time during production.

2. Experimental

2.1 Materials

The mycelium tissue used in this study was inoculated and extracted from different types of substrates, such as cellulose, starch, and glucose. For cellulose and starch substrates, the mushroom block made from rubberwood sawdust (RWS) and corn grain were used, respectively. Both substrates were sterile and inoculated with mycelium. For the glucose substrate, the mycelium was inoculated using sterile 10 % glucose agar in a petri dish. The grown mycelium after 14 days for each type of substrates was carefully extracted for analysis using the cutting method. For glucose substrate, the mycelium was extracted by continues boiling and filtering using dry weight method in order to obtain pure mycelium [17]. Additionally, on a starch substrate, the mycelium tissue was prepared by increasing the inoculation time from 14 days to 45 days. Figure 1 shows the raw mycelium tissues obtained from cellulose, starch, and glucose substrates.

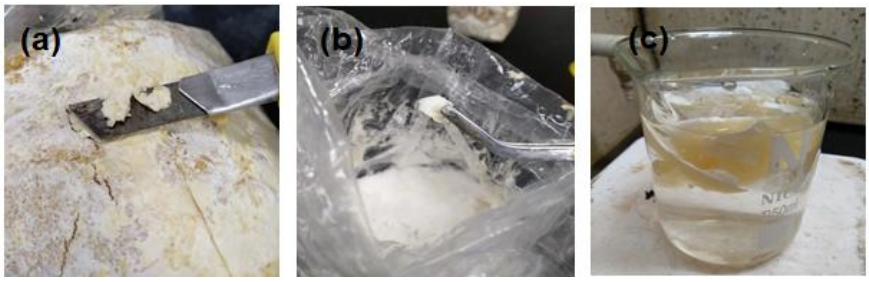

Fig. 1. Mycelium tissues from (a) cellulose, (b) starch, and (c) glucose substrates

2.2 Fabrication of Mycelium Bio-Matrix

Mushroom substrate from rubberwood sawdust (RWS) was obtained and disintegrated. Appropriate amount of RWS was placed into a sterile plastic mold with dimension of 21 cm × 15 cm × 0.6 cm with a target density of 0.80 g/cm^3. The RWS was allowed to incubate in the chamber at room temperature (~25 ^{o}C) with humidity of 80% for 45 days. This allows mycelium to inoculate and bind fibres together. The species of mycelium used in this study was *Pleurotus Ostreatus* (white-rot fungi) [11]. The fully grown mycelium bio-composite board were withdrawn from the mold, and the fabrication process was continued using a hot-pressed machine at 130 °C with a pressure of 5 MPa for 20 min and 40 min (Figure 2). The composite board was cut and tested according to the Japanese Industrial Standard JIS A 5908:2003[18].

Fig. 2. Self-assembly bio-composite from RWS inoculated with mycelium before hot press

2.3 Fourier Transforms Infrared Spectroscopy (FTIR)

The functional groups in the mycelium were identified using a Nicole infrared spectrophotometer (Avatar 360 FTIR E.S.P) analyzer in the range of 4000 cm^{-1} and 470 cm^{-1} with a resolution of 4 cm^{-1}. Approximately 5 mg of mycelium was mixed with 95 mg of finely ground potassium bromide (KBr) and pressed into pellets of about 1mm in thickness. The significant transmittance peaks at particular wavenumbers were measured using the “find peak tool” provided by Nicolet OMNIC 5.01 software.

2.4 Thermogravimetry (TG)

The thermal stability of mycelium was measured using a Perkin Elmer 7 thermogravimeter at the temperature range from 30 °C to 800 °C. Practically, approximately 10 mg of the samples were placed in an aluminium pan in a heating rate of 20 ^{o}C min^{-1} under nitrogen atmosphere.

2.5 Differential Scanning Calorimetry (DSC)

DSC thermogram of mycelium was recorded using a Perkin-Elmer Pyris 7 under nitrogen purge at a heating rate of 10 °C/min. Approximately 10 mg of dry sample was transferred into a hermetic aluminium pan and sealed. The sample was placed inside the chamber and an empty hermetic aluminum pan as a reference. The sample was heated in nitrogen flux from room temperature to 400 °C. The thermal profile of the sample was recorded continuously over the temperature and time intervals.

2.6 Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) - LEO Supra 50 Vp, Field Emission SEM, Carl-Zeiss SMT, Oberkochen, Germany - was used in order to analyse the morphology of the mycelium and mycelium-based bio-composite. The SEM micrographs were obtained from the surface of 0.5 cm × 0.5 cm of experimental panels. Prior to that, the samples were coated with gold using an ion sputter coater (Polaron SC 515, Fisons Instruments, UK) was used for microscopic study.

2.7 Light Microscopy

Mounted mycelium specimens were examined using optical microscopy (Olympus GX41 microscope with Pixera Pro 150ES camera and Olympus SZX16 microscope with ColorView camera). For each sample, four to five specimens were prepared, and the most precise images of these specimens were obtained.

2.8 Flexural Strength Testing

Three replicated specimens for flexural strength test with the dimension of 5 cm × 15 cm were prepared from mycelium bio-composite board for the evaluation of mechanical properties according to the Japanese Industrial Standard JIS A 5908:2003 [18]. The mechanical analysis was measured using an Instron Testing System UTM-5582 equipped with a load cell capacity of 1000 kg.

3. Results and Discussion

3.1 Spectroscopic Analysis

The structural unites (functional groups) can be assessed with respect to their chemical nature, that is discussed in this section based on their FTIR measurements. FTIR spectra of the four different types of mycelium are shown in Figure 3. Remarkable bands for all samples can be observed at 1650 cm^{-1} and 1550 cm^{-1}, which are raised from amide I and II in protein, respectively. It is obvious that a band at 1650 cm^{-1} for the cellulosic feeding substrate after 14 days inoculation is more intense as compared to other 14 days carbohydrate feeding substrates [7]. However, with the same feeding substrate, mycelium feeding starch has stronger intensity, as inoculation time is prolonged from 14

days to 45 days. The strong intensity is most probably due to the production of higher amount of protein in mycelium during inoculation process [19]. The vibration frequency of chitin can be observed at 1374 cm^{-1}. After 14 days inoculation time, the cellulosic feeding substrate shows more intense chitin band as compared to the other feeding substrates. Cellulose is the most difficult substrate to be penetrated and digested by the mycelium during inoculation, which led to synthesize more chitin to perform. Similarly, the starch feeding substrate after 45 days inoculation was showed an intense band in chitin zone. A weak vibrational frequency at 2900 cm^{-1} (ranging from 3000 cm^{-1} to 2800 cm^{-1}) is attributed to lipid-based structure. In general, the feeding substrates such as starch and glucose are easy to get absorbed by the mycelium as compared to the cellulose; therefore, the mycelium tends to stimulate biosynthesis of more lipid instead of chitin [7]. The stretching vibration frequencies of polysaccharide can be observed in the range of 1200 cm^{-1} to 900 cm^{-1}. Finally, a broad band centred at 3400 cm^{-1} is attributed to the hydroxyl groups of carbohydrates, the main component of cell walls in the mycelium.

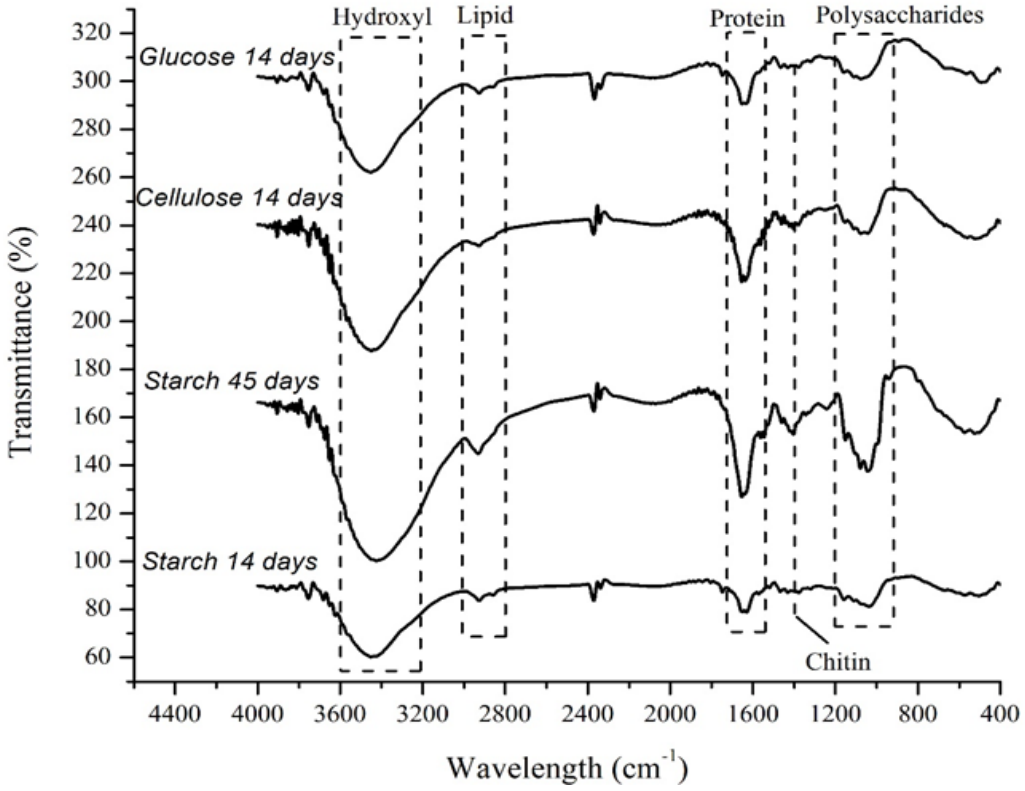

Fig. 3. FTIR spectra of mycelium at different inoculation days and feeding substrates

3.2 Thermal Stability

The TG-DTG thermograms of the samples were investigated in order to study the thermal decomposition profile**.** Figure 4 shows the thermal profile of mycelium, with three consecutive thermal decomposition steps. However, there are few differences between thermal degradation characteristic of mycelium inoculated for 14 days and 45 days. The mycelium inoculated for 45 days was possessed a higher thermal degradation profile (at 255 °C) as compared to mycelium inoculated for 14 days (at 237 °C). This phenomenon reveals that the thermal stability of the mycelium is improved by increasing inoculation times.

In the TG-DTG thermograms, the first stage degradation was occurred at 30 °C - 200 °C, attributed to the evaporation of free and chemically bonded water. The second stage at thermal degradation

range of 230 °C to 350 °C can be presumably due to the organic constituents such as chitin, amino acid, lipid, and polysaccharide, including polymerization, decomposition of acetylated and de-acetylated unit of chitin in the mycelium. These large molecules start to degrade by breaking down into the low molecular weight volatiles [6, 12, 20]. The high degradation temperature illustrates that the mycelium bio-matrix is thermally stable and can be used in the production and processing of bio-composite. In the final step of thermal degradation (from 400 °C to 800 °C), a small mass releasing was occurred for the samples, which is due to the formation of CO, CO_2, and H_2O as a result of char formation [21].

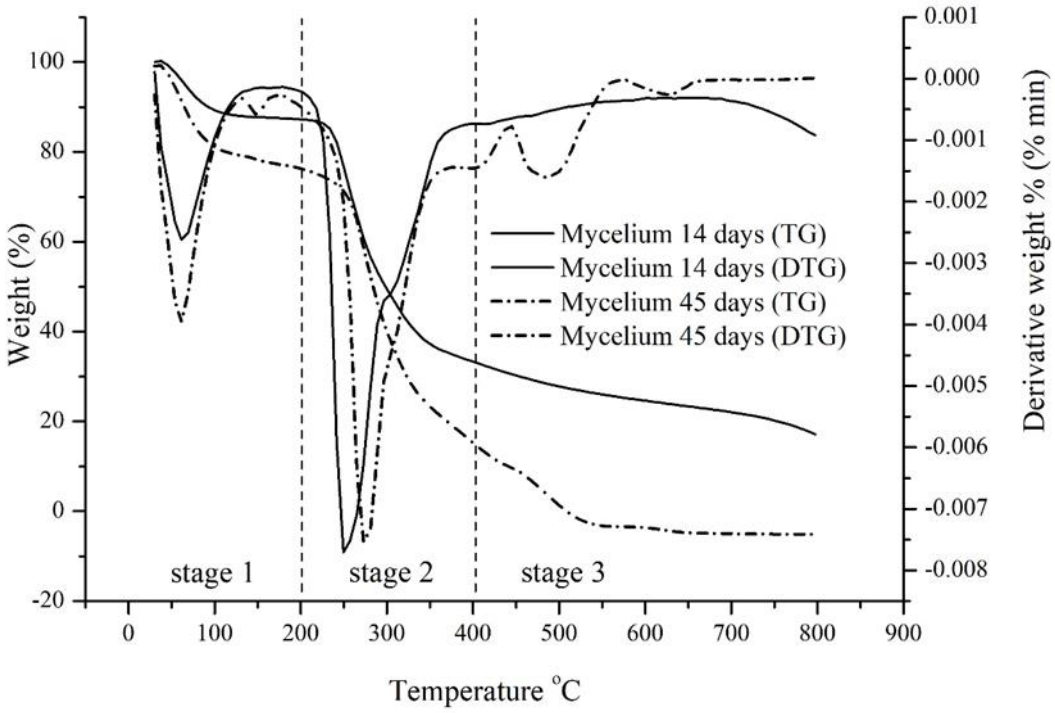

Fig. 4. TG-DTG thermograms of mycelium inoculated with starch at 14 and 45 days

3.3 Thermal Transitions

Thermal transitions of the samples were investigated using differential scanning calorimeter (DSC) (Figure 5). It could detect differences in fungal cell walls of mycelium at different inoculation days, which might have similar chemical compositions, but in a different amount that led to increasing the mycelium density. A higher density of mycelium can be attributed to the appearance of the thermal transitions in the higher temperature range [19]. From the result, it can be observed that the 45 days treated mycelium has a higher density as compared to the mycelium after 14 days of treatment. This variation is most probably due to the production of chitin. As mentioned before (FTIR and TG thermograms), the chitin content is higher in the mycelium inoculated for 45 days, where thermally more stable. The chitin can impart rigidity into the mycelium, which can further affect the density [7]. It can be deduced that multi-steps thermal transitions are most probably occurred in the mycelium cell walls [19, 22].

The DSC profiles of the mycelium samples were exhibited two endothermic transitions in the temperature range from 95 °C to 126 °C (for the sample inoculated for 14 days) and 149 °C to 166 °C (for the sample inoculated for 45 days), which could be due to the glass transition temperature (T_g) of chitin in mycelium [23]. The transition was due to the local relaxation of the backbone chain of the chitin [24]. The samples also show a weak transition peak (293 °C to 328 °C) for the sample after 14

days inoculation, and a broad transition (257 °C to 311 °C) for the sample with 45 days inoculation. The exothermic peaks can be related to the decomposition of acetyl-glucosamine unit of chitin [25]. Chitin is thermally stable due to the high density and hydrogen bonding. The exothermic peak emerging at 388 °C are presumable from the evaporation of volatile low molecular product during depolymerization [21].

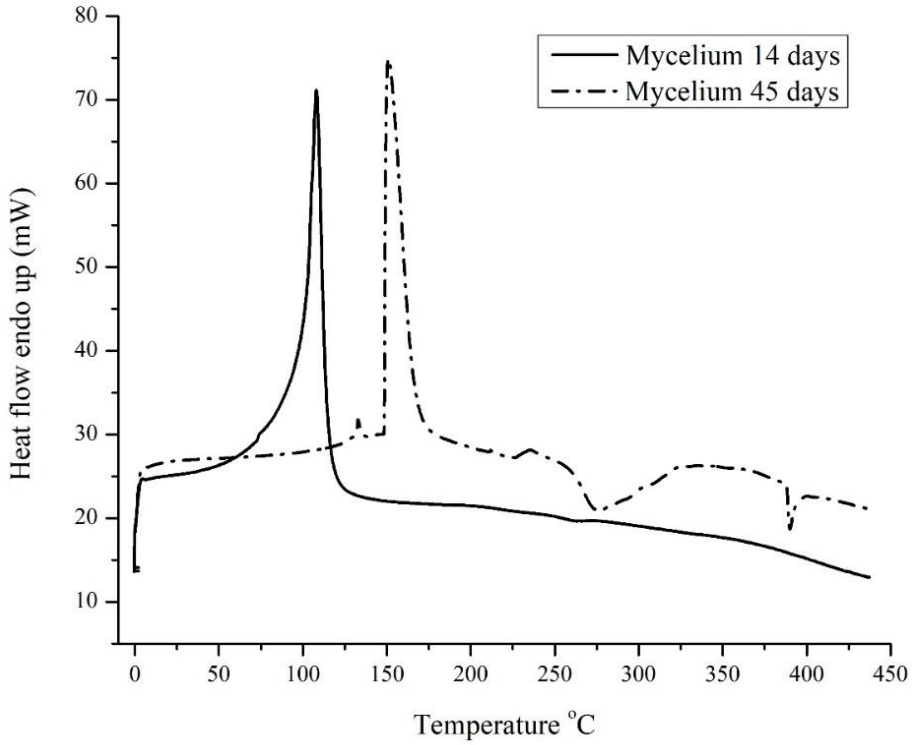

Fig. 5. DSC thermograms of mycelium inoculated with starch at 14 days and 45 days

3.4 Morphological Analysis

The light microscopy images and SEM micrographs of the mycelium (cellulose feeding substrate) are presented in Figure 6. The light microscopies (captured in 20x and 40x) were illustrated entanglement of the structure. This can prove the ability of mycelium bio-matrix in production of bio-composite [14]. The SEM micrograph of rubberwood particleboard without mycelium (blank sample) is shown in Figure 6(c). In this morphology, a complex and rocky structures with many morphological defects can be observed. This poor structure confirms the ability of binding performance of mycelium in production of self-assembled bio-composites. It is reported that the presence of voids and defects in the composites can reduce the mechanical and thermal properties of the product [26, 27]. The self-assembled mycelium based composite board is presented in Figure 6(d). It is obvious that the level of interaction in terms of binding between mycelium network and RWS fibre is much higher as compared to the sample without mycelium. This can be further reflected in thermal and mechanical properties of the bio-composite [5].

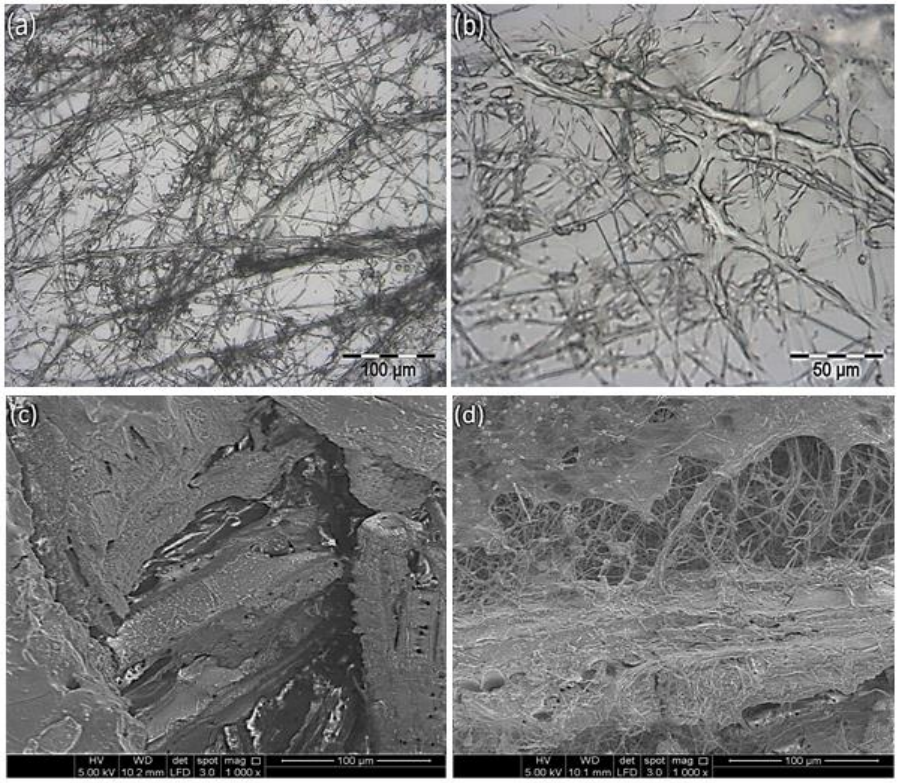

Fig. 6. Light microscope image for (a) – (b) two magnifications (20x and 40x) of mycelium bio-matrix, (c) SEM micrograph of the composite board without mycelium, and (d) composite board with mycelium

3.5 Flexural Strength

The flexural strength of the mycelium-based bio-composite board is presented in Figure 7. The boards were fabricated at 130 °C for 20 and 40 min using a hot-pressed machine. It can be clearly observed that upon increasing the pressing time from 20 min to 40 min, the flexural strength increased.

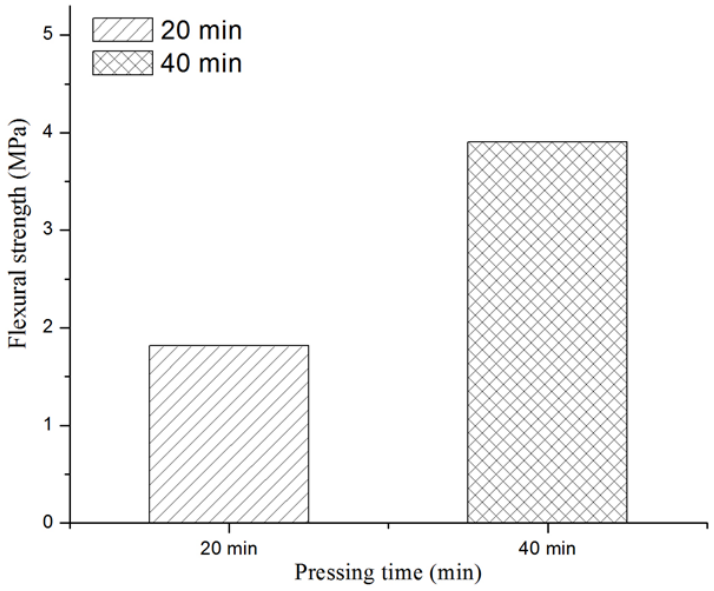

Fig. 7. Flexural strength versus pressing time

The flexural strength of the mycelium-based composite board was measured to be around 1.82 MPa and 3.91 MPa after 20 and 40 min pressing, respectively. The modulus of rupture for the composite board does not meet the requirement of the Japanese Industrial Standard. The weak bending strengths of the composite boards can be attributed to the self-hydrogen bonding between RWS fibre particles [28].

The texture of RWS is highly pack (Figure 8), therefore the air cannot flow through the gaps between the particle, which further prevent the penetration of oxygen into the composite board [29]. The bonding between fibres during board formation can be due to the pre-curing of lignin during pressing [30]. It seems that upon increasing pressing time from 20 to 40 min, the pre-cure of lignin, and the flexural strength of the composite board are simultaneously improved. Insufficient pressing time is one of the factors which can reduce the mechanical performance of the board due to high moisture content in the board as a result of incomplete hydrogen bonding between fibre particles [28].

Fig. 8. A cross section of the mycelium-based composite board

4. Conclusions

Primarily, mycelium was successfully obtained from different feeding substrates; cellulose, starch, and glucose, which further used in the production of bio-composite board. From the results, it can be concluded that the inoculation with different feeding substrates and different times can affect the thermal and mechanical properties of the mycelium. It was also observed that the mycelium can inoculate with the feeding substrate with difficulty in digestion such as cellulose; therefore, it can further increase the production of chitin in mycelium. Longer inoculation time was increased the chitin content, which can directly affect the properties of the product, such as thermal properties of mycelium to be used in the production of bio-composite in a with range of temperature profiles. However, the effect of mycelium as a bio-matrix in the mycelium bio-composite board was found to be contradicted in this study. Based on the modulus of rupture and board image, it was found that the mycelium was unable to inoculate inside of the board, therefore fails to bind fibres. This can be attributed to the small particle size of the RWS, which restricted the penetration of oxygen in the board as it is a crucial factor needed for the mycelium to inoculate. The production of bio-composite using mycelium as a binder can be improved using an optimum particle size during the inoculation and forming process.

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