Use of waste mushroom beds for the production of value-added biodegradable fiber sheet

Walaiporn Suksai[1], *Chaniga* Chuensangjun[2,*], *Jittimon* Wongsa[3], *Vilai* Rungsardthong[1], *Savitri* Vatanyoopaisarn[1], *Benjawan* Thumthanaruk[1], *Rungsima* Yeetsorn[4], and *Buddhi* P. Lamsal[5]

[1]Department of Agro-Industrial, Food and Environmental Technology, Faculty of Applied Science, Food and Agro-Industry Research Center, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand.
[2]Science and Technology Research Institute, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand.
[3]Department of Agricultural Engineering for Industry, Faculty of Industrial Technology and Management, King Mongkut's University of Technology North Bangkok, Prachinburi 25230, Thailand.
[4]The Sirindhorn International Thai-German Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand.
[5]Department of Food Science and Human Nutrition, Iowa State University, 536 Farm House Lane, Ames, IA 50011-1054, USA.

Abstract. This research focused on the utilization of waste mushroom beds (WMB) after the harvesting of oyster (WMB-O) and lingzhi mushrooms (WMB-L) for the preparation of eco-friendly materials, fiber sheets. The WMB were sterilized and determined for their chemical compositions. The dry fiber of the sterilized WMB were pretreated by a steam explosion, comparing with alkaline pretreatment before the fiber sheet forming process. The results showed that α-cellulose contents of the WMB were in the range of 27-35% by dry weight basis. The fiber from WMB-L treated by alkaline at 13.5% w/w of NaOH for 120 min showed better fiber sheet appearance, compared to the steam explosion. Afterward, tapioca starch was added as a natural binder during the fiber sheet forming and their physical properties were determined. The analytical results indicated that an increase of NaOH concentration in the pretreatment led to an increase in the toughness and water absorption of the fiber sheet. The additional tapioca starch promoted the interaction between cellulose fiber networks, corresponding to the decrease of water absorption and a compressed appearance after water immersion. These finding results disclosed a potential use of the WMB as eco-friendly materials, e.g. biodegradable packaging, packing materials, cultivation vase in the future.

Keyword. Bio-material, Eco-friendly material, Lingzhi mushroom, Oyster mushroom, Pretreatment, Waste mushroom bed

1 Introduction

Mushroom are popularly consumed in many countries because of their texture, aroma, taste as well as their high nutritional composition. Mushrooms indicate high fiber content, low energy, high nutrition, and low calories. Several varieties of mushrooms contain bioactive compounds and present medicinal properties and health benefits. Generally, mushrooms are grown on timber rotting decay, manure, or compost. Some species need specific nutrients for their growth. Moreover, most species of mushrooms require high humidity up to 70-90%, especially during their caps opening. Water holding capacity of planting containers and humidity control are important factors for mushroom cultivation [1, 2]. Nowadays, mushroom farmings are widely expanded. There are three types of mushroom cultivations in Thailand including the cultivation in the wooden pickup, in the plastic bag, and on timber. The plastic bags for mushroom cultivation are commonly used because they have heat resistance, require a small cultivation area with convenient preparation and easy removability [2]. Lignocellulosic materials such as rice

bran and sawdust are generally used as raw materials for mushroom cultivation in the plastic bag because they are abundant agricultural waste resources and degradable under the natural environment [3]. After the first mushroom cultivation, the nutrients in the culture medium were reduced and some types of mushroom beds could be reused with the addition of necessary nutrients, while some species of edible mushroom bed could be reused up to 5 times [4]. The mushroom beds after cultivation still contain some components or nutrition needed for another cultivation, especially fiber cellulose, hemicellulose, and lignin [5]. The waste mushroom beds (WMB) could be utilized as raw materials for the production of reducing sugars for biodiesel production [6], planting crops [7], and hydrogen production for the growth of *Clostridium thermocellum* [8]. According to the information obtained from the Thai Intellectual Learning Center Samutprakarn (Samutprakarn Province, Thailand), one of the large mushroom farms in Thailand, the oyster and lingzhi mushrooms are the two most popularly cultivated as edible, and medicinal mushrooms in the market, respectively. Generally, after-harvesting in mushroom

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* Corresponding authors: chaniga.c@stri.kmutnb.ac.th

cultivation, the WMB are discarded rather than reused due to the insufficient nutrients for cultivation to the growth of the mushrooms. Consequently, there are a lot of WMB and the discarded plastic bags in many mushroom farms presently. The wastes are underuse and require waste management or utilization into more valuable products. Substitution of plastic planting containers using degradable materials is one expected alternative to reduce the waste plastics in mushroom farms.

Cellulose fibers from the lignocellulosic material and agricultural wastes, such as mulberry inner bark, rubberwood sawdust, spent coffee grounds, straw, etc. are reported for the preparation of high value-added eco-friendly materials, such as natural paper, the substrate for biorefining process, natural fiber-reinforced composites or degradable cultivation vase [9-12]. Eco-friendly fiber sheets prepared from cellulose fibers or agricultural wastes were reported, such as the use of Phyllostachys (bamboo) to produce carbonaceous fiber sheets for preparing the gas diffusion layers of proton exchange membrane fuel cells [13] and production of the bio-composite films from cassava starch by using cellulose extracted from durian rind as reinforcement agent [14]. However, there were no the published reports about the preparation of fiber sheets from WMB. Therefore, the preparation of water-absorbent and biodegradable fiber sheets from WMB would be interesting for further application in the future. The high water holding capacity and biodegradability of obtained fiber sheets are expected for forming the mushroom cultivation vase, lead to reduced frequency for watering, and promote zero waste in the mushroom farms.

Cellulose ($C_6H_{10}O_5$) is a polysaccharide that contains D-glucose linked together by ß-1,4-glycosidic bonds [15-16]. Each chain of cellulose is interacted by hydrogen bonds, resulting in high strength structure of the cellulose network [17]. Natural cellulose can be extracted from the plant (wood and non-wood), animal, and some microorganisms [15], therefore, cellulose is the most abundant lignocellulosic materials in the world [18]. Moreover, there are many reports on the properties of natural cellulose fiber including its advantages, potential use for renewable resources, strong and tough structure, and water absorbability [19-21]. Cellulose has been used in several industries including food, pharmaceutical, and textile industries [22]. However, the pretreatment of raw materials is an important process to remove lignin, hemicellulose, and other extractives from the raw materials to obtain high purity of cellulose [23]. For the preparation of fiber sheets from agricultural waste, high cellulose fiber contents and remained fibrous morphology are main characteristics of the pretreated raw materials [13], especially for the production of fiber sheets with high water absorption. Alkaline pretreatment and steam explosion could break the strong interaction between hemicellulose, lignin, and cellulose, resulting in an increase of porous structure in the cellulose fiber [24].

In this study, the potential use of WMB from oyster (WMB-O) and lingzhi mushrooms (WMB-L) cultivation as fiber sheets, the biodegradable material was investigated. Both fibers were pretreated by a steam explosion and compared to the alkaline treatment. The fiber sheets were formed and their physical properties such as toughness, water absorption, swelling, and porosity were measured according to the Thai Industrial Standard (876-2547) [10, 25] to evaluate their potentials for further applications in the future.

2 Materials and methods

2.1 Materials

Both WMB after harvesting for 7 days, WMB-O from oyster (*Pleurotus ostreatus*) and WMB-L from lingzhi (*Ganoderma lingzhi*) cultivation (Fig. 1.) were supplied from Thai Intellectual Learning Center (Samutprakarn Province, Thailand).
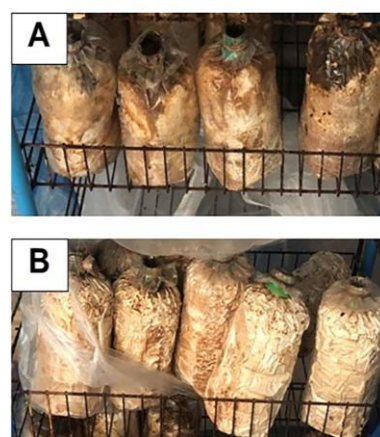

Fig. 1. Waste oyster (A) and lingzhi (B) mushroom beds after harvesting for 7 days.

Tapioca starch to be used as a natural binder for preparing the fiber sheet was derived from Tongchan Co., Ltd. (Bangkok, Thailand). Sodium hydroxide (NaOH) to be used for the alkali pretreatment was the commercial-grade product obtained from Chemiall Co., Ltd. (Bangkok, Thailand).

2.2 Methods

2.2.1 Preparation of natural fibers from WMB-O and WMB-L

The sterilization of WMB-O and WMB-L was carried out in an autoclave at 121ºC for 1.5 h. Thereafter, the sterile WMB-O and WMB-L were dried in a hot air oven at 60ºC for 24 h. The dried WMB samples were ground and kept in closed plastic bags at room temperature until the composition analysis and preparation of the fiber sheets.

2.2.2 Chemical composition analysis

The chemical composition analysis including moisture content, ash, solvent extractives, lignin, holocellulose

(alpha-cellulose and hemicellulose) of the dried WMB were determined according to the Technical Association of the Pulp and Paper Industry (TAPPI) [26], as followed.

2.2.2.1 Moisture content

Moisture cans were weighed, then 1 g of the WMB-O and WMB-L sample was added into each moisture can. The samples were dried in a hot air oven at 105ºC for 24 h after they were cooled down in a desiccator. The moisture content (%) of the samples was calculated according to equation (1).

$$\text{Moisture content (\%)} = [(A-B)/A] \times 100 \qquad (1)$$

when A is the weight of the sample before drying (g) and B is the weight of the sample after drying (g).

2.2.2.2 Ash

A crucible was burnt at 550ºC for 15 min, cooled down in the desiccator, and weighed. A 1 g (dry matter basis) of the WMB-O, and WMB-L was added into the crucible. The samples were burnt at 550ºC until the white ashes were obtained. The samples were cooled down and weighed. The ash content of the samples was calculated according to equation (2).

$$\text{Ash (\%)} = (A/B) \times 100 \qquad (2)$$

when A is the weight of ash (g) and B is the weight of the sample (g).

2.2.2.3 Solvent extractives

Extractives which are the plant resin, wax, alkaloid, and flavonoid that could be dissolved in an organic solvent were extracted from both WMB-O and WMB-L (3 g dry matter basis) using a soxhlet extractor for 4 h. A mixture of ethanol and benzene (1:2) was used as solvents for the extraction. The solvents were then vaporized from the extracted samples overnight followed by the drying at 105ºC in a hot air oven and cooled down in a desiccator before their weight measurement. The solvent extractives were calculated according to equation (3).

$$\text{Solvent extractives (\%)} = [(A-B)/C] \times 100 \qquad (3)$$

when A is weight of extracted sample (g), B is weight of blank (solvent) (g) and C is weight of the WMB (g).

2.2.2.4 Lignin

The extracted samples (3 g) were secondly extracted for 4 h using 200 mL of the 95% ethanol. Samples were left in a fume hood at room temperature overnight and then filtered with a Buchner funnel. The precipitates were washed several times with distilled water and left in the fume hood at room temperature (25 ± 2ºC) for 2 h before drying in a hot air oven at 60ºC for 24 h. The extractive-free samples of 0.35 g were added to the beaker in an ice

bath. The concentrated H_2SO_4 (72% w/w) of 5.21 mL was gently added into the beaker. The mixture was stirred every 15 min for 2 h, at room temperature. Subsequently, the sample was added into the distilled water of 139.13 mL, and the total volume was adjusted to 200 mL by distilled water. Sample solutions were condensed for 4 h by a reflux technique, after that the solutions were poured into a beaker and left for 16 h at room temperature. The sample was filtered and dried at 105ºC for 3 h. The beaker containing the sample was cooled down in the desiccator and weighed. The lignin was calculated according to equation (4).

$$\text{Lignin (\%)} = (A/B) \times 100 \qquad (4)$$

when A is the weight of lignin (g) and B is the weight of the sample (g).

2.2.2.5 Holocellulose

The extractive-free samples of 3 g were suspended in a mixture of water (350 mL), acetic acid (1.1 mL), and NaCl (3.3 g). The sample suspension was incubated in a water bath shaking at 70ºC for 3 h. The acetic acid of 1.1 mL and NaCl of 3.3 g were added into the suspension every hour (3 times). At the end of the reaction, the suspension was cooled down to 10ºC in the ice bath before the filtration. The precipitates were dried at 105ºC for 16 h and then cooled down in a desiccator before the weight measurement. The holocellulose was calculated according to equation (5).

$$\text{Holocellulose (\%)} = (A/B) \times 100 \qquad (5)$$

when A is the weight of holocellulose (g) and B is the weight of the sample (g).

2.2.2.6 Alpha-cellulose and hemicellulose

The holocellulose of 1.5 g was mixed with 75 mL of NaOH (17.5% w/v). The mixture was cooled down to 2.5 ± 0.2ºC in the ice bath and stirred with a magnetic bar for 30 min. The magnetic bar was washed with 2.5 mL of NaOH (17.5% w/v) and then adjusted the total volume of the mixture to 100 mL by distilled water. The mixture was continuously stirred for 30 min at 2.5 ± 0.2ºC. The sample mixture was filtered and washed with distilled water until the neutral pH was obtained. The acetic acid (10% v/v) of 40 mL was then added to the sample precipitates and filtered. The precipitates were dried at 105ºC for 16 h and cooled down in the desiccator before being weighed. The alpha-cellulose was calculated according to equation (6).

$$\text{Alpha-cellulose (\%)} = (A/B) \times 100 \qquad (6)$$

when A is the weight of alpha-cellulose (g) and B is the weight of the holocellulose (g).

Hemicellulose was calculated according to equation (7).

$$\text{Hemicellulose (\%)} = A-B \qquad (7)$$

when A is the percentage of holocellulose and B is the percentage of the alpha-cellulose.

2.2.3 Optimization of the fiber pretreatment

The optimal conditions for an alkaline pretreatment of the WMB fibers were investigated using the sterilized WMB-O and WMB-L fibers as the raw materials. The concentration of NaOH was studied at 1.5, 5.5, 9.5, and 13.5% w/w (g NaOH / g dry fiber) in this study. The fibers (30 g dry basis) were boiled in the NaOH solution for variable times, namely 5, 60, 90, and 120 min. In addition, a steam explosion technique was used to compare with the alkaline pretreatment. Briefly, the fibers (30 g dry basis) were steamed in an autoclave at 121ºC for 5, 60, 90, and 120 min. The pretreated fibers from both methods were used for the preparation of the natural fiber sheet.

2.2.4 Preparation of fiber sheet

The pretreated pulps from WMB-O and WMB-L were soaked in water (1 g dry basis:30 mL) overnight, and then the pulp suspension was agitated homogeneously. The binder, tapioca starch was dissolved in water to obtain 10% w/v. The pulp was mixed with the tapioca starch solution at the weight ratio of 60:40 [10]. The mixture was thoroughly spread on a mold (21.59 cm × 27.94 cm), and the natural fiber sheet was then dried under the sunlight for 1 day. Thereafter the fiber sheets obtained from each WMB were peeled carefully from the mold. The significant properties of the fiber sheet, such as water absorption and swelling, were measured with the standard method of Thai Industrial Standards Institute (876-2547) [25]. The mulberry paper and printing paper (80 grammages) with the same thickness were used for the comparison of their properties.

2.2.5 Characterization of fiber sheet

2.2.5.1 Toughness

The fiber sheet was cut into pieces (50 × 50 mm). The top side of the fiber sheet was held on a shoulder pole whereas the bottom side of the fiber sheet was bound vertically to a plastic bag. The sand was slowly added into the plastic bag until the fiber sheet started to tear. The toughness of the fiber sheet was measured as the weight of the sand was put in the plastic bag. All measured data were given as mean values standard deviation (SD) of triplicate measurements.

2.2.5.2 Water absorption and swelling

The three pieces of fiber sheet (50 × 50 mm) were weighed and immersed in 120 mL of water and kept at room temperature. The fiber sheet piece was removed from the water every 30 min, dabbed with absorbent tissue paper to remove the surface moisture, and

weighed until a constant weight was observed. The absorption was calculated according to equation (8).

$$\text{Absorption (g/g)} = (W_a - W_b)/W_b \qquad (8)$$

when W_a is the weight of the fiber sheet after immersion (g) and W_b is the weight of the fiber sheet before immersion (g) [27]. For the determination of swelling, the thickness of the fiber sheet (50 × 50 mm) was measured using a hand-held manual micrometer to prevent compression, after that the fiber sheet was immersed in water for 180 min at room temperature. The fiber sheet piece was removed from the water, determined for its thickness and swelling ability according to equation (9)

$$\text{Swelling (\%)} = [(T_a - T_b)/T_b] \times 100 \qquad (9)$$

when T_a is the thickness of the fiber sheet after immersion (cm) and T_b is the thickness of the fiber sheet before immersion (cm) [28].

2.2.5.3 Porosity

The volume of the fiber sheet (V_s) was measured using a micrometer and its density (ρ_b) was calculated according to [28]. The fiber sheet was ground, added into a volumetric flask (250 mL), and weighed. Then 120 mL of water was added into a volumetric flask and the suspension of the sample was mixed thoroughly. The sample was incubated in a water bath at 80ºC until the steam was observed and then the sample was cooled down in an ice bath. Water was added into the volumetric flask to adjust the volume, and the flask was weighed again [25]. The porosity (ε) was calculated according to equation (10)-(13), respectively.

$$V_w = (m_2 - m_1)/\rho_w \qquad (10)$$

$$V_s = V_F - V_w \qquad (11)$$

$$\rho_s = m_s/V_s \qquad (12)$$

$$\varepsilon = [1 - (\rho_b/\rho_s)] \times 100 \qquad (13)$$

when m_1 is weight of the volumetric flask contained sample (g); m_2 is weight of the volumetric flask contained suspension after incubation (g); m_s is mass of sample (g); V_w, V_s, V_F are volume of water suspension, sample and volumetric flask (mL); ρ_w, ρ_s, ρ_b are density of water (at 80ºC), sample and fiber sheet (g/cm³), respectively.

3. Results and discussion

3.1 Chemical compositions

The chemical compositions of WMB-O and WMB-L i.e. ash, extractives, lignin, holocellulose, alpha-cellulose, and hemicellulose, are presented in Table 1. The chemical compositions of WMB-O were significantly different from those of WMB-L, except extractives, as

shown in Table 1. The WMB-L contained a lower amount of ash, extractives, and lignin than the WMB-O. This implied that the WMB-L comprises fewer residue nutrients, compared to the WMB-O. According to the information from the mushroom farm (Thai Intellectual Learning Center, Samutprakarn Province, Thailand), the oyster mushroom bed could be reused as many times up to 5 times while the lingzhi mushroom bed is a disposable bed due to some insufficient substances for re-cultivation. In terms of holocellulose which is comprised of alpha-cellulose and hemicellulose, the WMB-O showed a higher amount of alpha-cellulose, but contained a lower amount of hemicellulose compared to the WMB-L. However, this result might due to the growth of bacteria and fungi in the lingzhi mushroom beds which could produce a cellulosic enzyme to degrade the cellulose [29]. In addition, the xylanase and laccase enzyme activity were also found in the oyster mushroom bed [30], resulting in possible degradation of hemicellulose and lignin in the mushroom beds too [31].

Table 1. Chemical compositions of waste mushroom beds.

Chemical compositions	Waste oyster mushroom bed (WMB-O) (% dry basis)	Waste lingzhi mushroom bed (WMB-L) (% dry basis)
Ash	16.01 ± 2.43^{a}	9.38 ± 0.74^{b}
Extractives	2.94 ± 0.99^{ns}	1.46 ± 0.48^{ns}
Lignin	15.13 ± 0.64^{a}	7.29 ± 0.87^{b}
Holocellulose	42.44 ± 0.69^{a}	46.36 ± 1.42^{b}
α-Cellulose	34.82 ± 1.85^{a}	27.33 ± 2.30^{b}
Hemicellulose	7.61 ± 1.43^{a}	19.03 ± 2.3^{b}

Values within the same row followed by the same letter are not significantly different ($p > 0.05$)
ns: not significantly different ($p > 0.05$)

3.2 Optimization of the fiber pretreatment

Fiber pretreatment aimed to remove undesirable elements such as lignin, hemicellulose, and extractives from the WMB before the forming process for the fiber sheet. Comparison of the methods for WMB pretreatment (steam explosion and alkaline solution) and type of WMB (WMB-O and WMB-L), demonstrated a different appearance of the pretreated fiber sheets as presented in Fig. 2. The pretreated fiber from the WMB-O showed a very rough surface, dark color, brittle, and unable to prepare as the fiber sheets by both pretreatment methods. This might be due to residual lignin after the pretreatment [32]. The results of steam explosion pretreatment of WMB-L at 121°C indicated that an increase in the pretreatment time could promote the fiber sheet forming (Fig. 2. L1-L4).

However, a slightly brittle fiber sheet was observed under the steam explosion at 121°C for 120 min. Comparing with alkaline pretreatment, the fiber sheets from WMB-L after boiling with NaOH at the concentration of 1.5% w/w for 120 min showed a better appearance including a smooth surface, light brown color, and increased toughness (Fig. 2. L8). Therefore, the effects of NaOH concentration were further investigated to optimize the fiber pretreatment of WMB-L. From Fig. 2. (L9-L11), the increase of NaOH concentration at 5.5, 9.5, and 13.5% w/w promoted better fiber sheet forming, especially with NaOH at 13.5% w/w for 120 min (Fig. 2. L11). Using a high concentration of NaOH at a high temperature and long time could completely remove lignin and hemicellulose from the raw fiber. These results corresponded to previous reports, for example, increasing alkali concentration gave the proper properties of the physic nut (*Jatropha curcas* Linn.) pulps (15% alkalinity) [33], and cornstalk paper (20% NaOH) [34]. Accordingly, the fiber sheets prepared by alkaline pretreatment of WMB-L at 9.5 and 13.5% w/w of NaOH for 120 min were selected and analyzed for their physical properties including toughness, water absorption, swelling, and porosity, respectively.
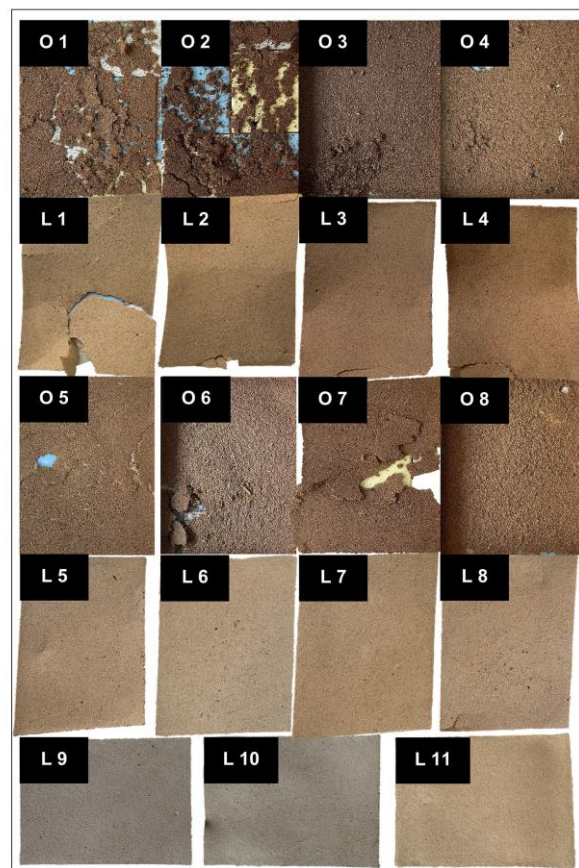

Fig. 2. Photographs of natural fiber sheets prepared from the waste mushroom bed of oyster mushroom (WMB-O) and lingzhi mushroom (WMB-L) with different pretreatment. O1-O4: WMB-O with the steam explosion at 121°C for 5, 60, 90, and 120 min; L1-L4: WMB-L with the steam explosion at 121°C for 5, 60, 90, and 120 min; O5-O8: WMB-O with NaOH at 1.5% w/w for 5, 60, 90, and 120 min; L5-L8: WMB-L with NaOH at 1.5% w/w for 5, 60, 90, and 120 min; L9-L11: WMB-L with NaOH at 5.5, 9.5, and 13.5% w/w for 120 min.

3.3 Toughness

The toughness of the fiber sheet prepared by alkaline pretreatment of WMB-L at the concentration of 9.5 and 13.5% w/w NaOH for 120 min was compared with the sheet of mulberry paper and printing paper (Fig. 3.). The results revealed that the fiber sheet from WMB-L pretreated with NaOH at 9.5 and 13.5% w/w, could support the sand weight of 50.75±5.22 and 76.47±18.35 g, respectively whereas, the printing paper and mulberry paper could support the sand weight of 1522.58±152.00 and 1585.91±376.90 g, respectively. These results presented that the fiber sheets from the pretreated WMB showed less toughness as compared with the commercial paper (the mulberry and printing paper). Due to the multi-step for commercial papermaking (i.e. pulp extraction, digestion, bleaching, paper formation), almost lignin and hemicellulose were removed from the raw materials [35]. The cellulose content of the mulberry paper and the printing paper from *Eucalyptus* has been reported as over 62-90% by oven dry weight [9, 36], which was higher than the WMB-L. This demonstrated that the higher cellulose content could promote the higher toughness of the paper. From this result, the tapioca starch was therefore applied as a natural binder to further improve the production of fiber sheets from the WMB.
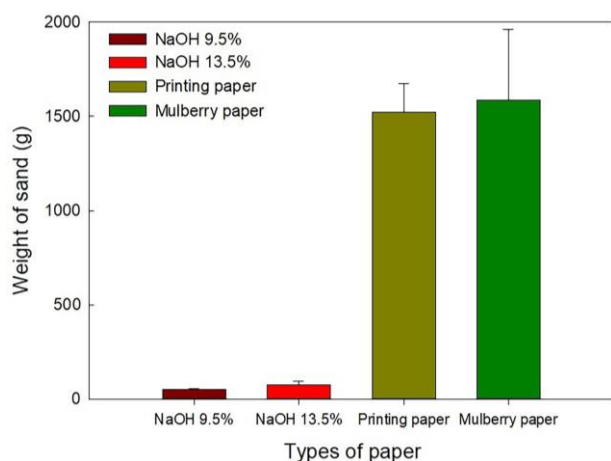

Fig. 3. Toughness of fiber sheet prepared from waste lingzhi mushroom bed after pretreatment with 9.5 and 13.5% w/w NaOH for 120 min, compared with the sheet of printing paper and mulberry paper.

3.4 Water absorption and swelling

The fiber sheet prepared from WMB-L pretreated with NaOH at 9.5 and 13.5% w/w showed the water absorption after water immersion for 30 min at 4.61±0.13 and 5.14±0.14 g/g, respectively (Fig. 4.). Their water absorption was higher than that of the printing paper (1.93±0.05 g/g), but lower than the mulberry paper (7.99±0.15 g/g). The addition of 40 wt% of natural binder (i.e. tapioca starch) into the fiber sheet prepared from WMB-L treated with NaOH at 13.5% w/w could reduce its water absorption to 3.47±0.03 g/g. The water absorption of all samples was quite constant,

even though the water immersion time increased. The results were corresponding with other reports that an increased amount of cellulose in the composite materials could improve water absorption due to the binding of the hydroxyl group in cellulose to the hydrogen bonds of water [37-40]. However, into the commercial process for printing paper production, surface strengthening, surface sizes or hydrophobic agents (e.g. starch) are usually added [41] to improve resistance level to the water absorption of water. Meanwhile, the printing paper needs a liquid holding capacity to quickly absorb and retain water or inks by micro capillarity [36].

The results of the swelling analysis are shown in Fig. 5. The printing paper showed the highest swelling of 42.76%. Whereas the natural fiber sheet from the WMB-L with/without adding the natural binder and the mulberry paper showed contrary results, namely the compressed appearance and traces of decay due to water holding capacity after water immersion were observed (Fig. 6.). This phenomenon could be described that different raw materials and processes of pretreatment affected the structure of the fiber. The swellability depends upon the cell wall of the fiber, in other words, the higher microfibrils at the secondary wall of the fiber decrease swellability after water absorption but promote increasing strength [33-34, 14]. Moreover, the residual lignin after the pretreatment process, the addition of binder (such as starch), or introducing additives during the papermaking caused the different structure of the fiber, water absorption, and swelling properties [36-38].
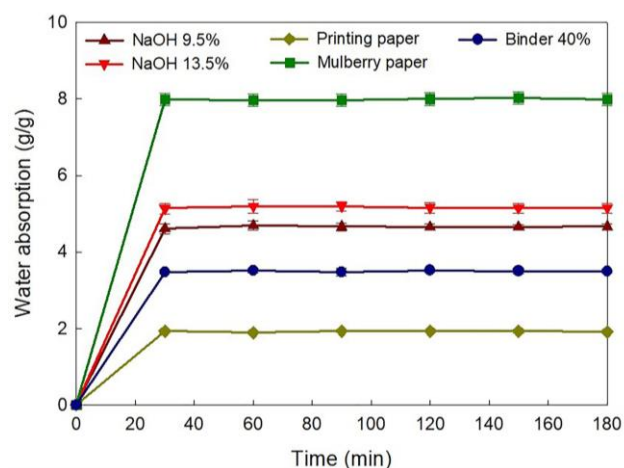

Fig. 4. Water absorption of fiber sheet prepared from waste lingzhi mushroom bed after pretreated with 9.5 and 13.5% w/w NaOH for 120 min, and fiber sheet with 40wt% binder compared with the sheet of printing paper and mulberry paper.

Although, good properties of the degradable cultivation vase from spent coffee grounds, shell lime, and rubberwood sawdust have ever been reported, their high water absorption and swelling, resulted in faster degradation (16 days) [10]. The strength of the cultivation vase is also one important property for production in the commercial. Moreover, the lifetime of the cultivation vase should be consistent with the growth time of cultivated plants, for example, the lingzhi and oyster mushroom require 75-90 days for their growth. Therefore, the optimal condition for fiber sheet forming

to obtain the suitable properties for mushroom cultivation vase production is required for further investigation.
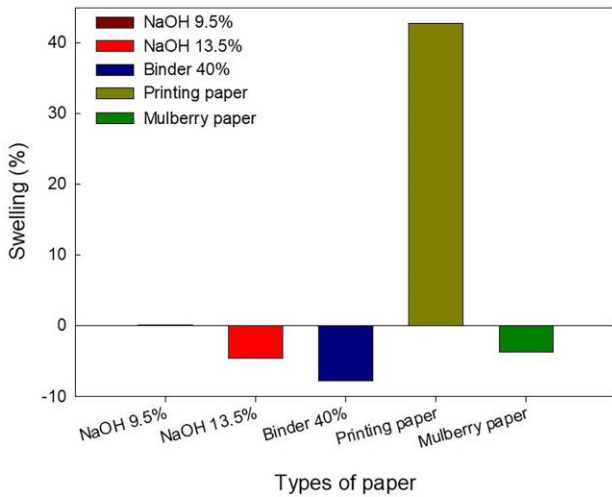

Fig. 5. Swelling of fiber sheet prepared from waste lingzhi mushroom bed after pretreated with 9.5 and 13.5% w/w NaOH for 120 min, and fiber sheet with 40wt% binder compared with the sheet of printing paper and mulberry paper.

Fig. 6. Photographs of the fiber sheets prepared from waste lingzhi mushroom bed after pretreated with 9.5% w/w NaOH for 120 min (A), and 13.5% w/w NaOH (B); C) fiber sheet with 40wt% binder; D) printing paper and; E) mulberry paper. The number of 1-3 mean the sample before soaking in water, after soaking in water and after soaking in water and left at room temperature for 16 h, respectively.

3.5 Porosity

The porosity of the fiber sheet prepared by alkaline pretreatment of WMB-L with/without adding the natural binder, printing paper, and the mulberry paper are shown in Fig. 7. The porosity of the fiber sheet from the WMB-L without binder was approximately 168-172% and slightly decreased in the fiber sheet with the binder, due to the increase of interaction between cellulose and binder (starch) [42-43]. Corresponding to the water absorption results in Fig. 4, the decrease of capacity of water uptake was found in the fiber sheet with the binder. This implied that the high porosity of fiber sheets could promote the increase of capacity to absorb and retain water in their structure [36]. While the printing paper and mulberry paper showed higher porosity than the fiber sheet with/without binder due to their higher cellulose contents [9, 36].
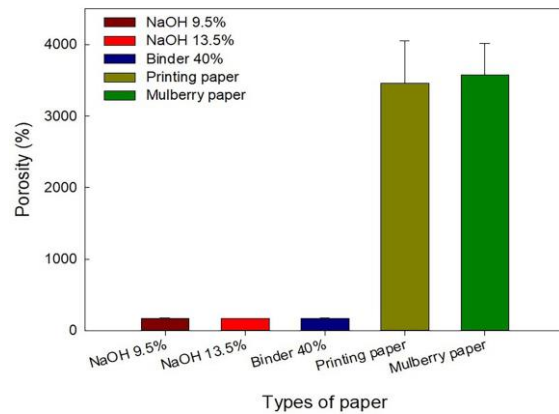

Fig. 7. The porosity of fiber sheet prepared from waste lingzhi mushroom bed after pretreated with 9.5 and 13.5% w/w NaOH for 120 min, and fiber sheet with 40wt% binder compared with the sheet of printing paper and mulberry paper.

From the results, the fiber sheet prepared from WMB-L demonstrated appropriate water absorption property and degradable behavior. This would be new alternative material for the possible production of value-added materials, such as a water-absorbent and biodegradable fiber sheet for mushroom cultivation vase production. In addition, the use of WMB for preparing as alternative materials could promote zero waste in the mushroom farms. The summary of finding results and expected applications from this study are also compared with the other lignocellulosic materials, as shown in Table 2.

4. Conclusions

The WMB-O and WMB-L samples after harvesting for 7 days showed different chemical compositions due to the different use-life of each mushroom bed. The amount of α-cellulose of the WMB were in the range of 27-35% dry basis, whereas, the lignin and hemicellulose were still high and might be the limitation of fiber sheet forming. The pretreatment processes by steam explosion

and alkaline solution were optimized for the removal of lignin, hemicellulose, and other extractives. The fiber sheets derived from the alkaline pretreatment of the WMB-L at 9.5 and 13.5% w/w of NaOH for 120 min disclosed an opportunity for the production of value-added materials, such as a water-absorbent and biodegradable fiber sheet for mushroom cultivation vase production. Natural binder, i.e. tapioca starch was added to improve the properties of the fiber sheet from the WMB-L. Although the finding results of fiber quantity/quality of the WMB-L have not yet qualified for papermaking as compared to commercial paper, such as mulberry paper and printing paper. Some physical properties results, such as water absorption, swelling, and porosity demonstrated the possibility for utilization in agricultural application. The high water absorption and traces of decay after water immersion of the fiber sheet from the WMB-L revealed a suitable water holding capacity for producing mushroom cultivation vase, and lead to zero waste in the mushroom farms. However, further study on the optimal condition for natural binder addition and the degradation behavior of the obtained fiber sheet from the WMB are required.

Table 2. Summary of the expected applications from several lignocellulosic materials.

Material	Application	Characteristic	Reference
Bamboo	Carbonaceous fiber sheets for use as the gas diffusion layers	- Fibrous morphology remained after carbonization. - Carbonaceous fiber sheet was able to electric conductivity.	Matsumura et al. [13]
Banana fiber	Cellulose nanofiber for use as reinforcing elements	- α-cellulose increased from 64% to 95% after pretreatment of banana fiber. - High aspect ratio, crystallinity, and thermal stability	Deepa et al. [44]
WMB of shiitake (*Lentinus edodes*)	Cellulose nanofiber preparation	- Cellulose content of WMB was 25.4%. - Dry films of cellulose nanofiber prepared from WMB were transparent.	Konno et al. [45]
WMB of lingzhi (*Ganoderma lingzhi*)	Fiber sheets preparation for further application in mushroom cultivation	- α-cellulose of WMB was 27.33% dry basis. - Fiber sheet from WMB showed high water absorption and biodegradability.	This study

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