The Synergistic Utilization of Plantation Forestry Wastes: Edible Fungi Cultivation Coupled with Hemicellulose Extraction by Liquid Hot Water

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A two-step statistical method was developed with two main objectives. One objective was to utilize plantation forest wastes for edible fungi (*Pleurotus ostreatus* CICC 14012) cultivation and the second was to maximize the laccase activity in the substrates to facilitate hemicellulose extraction. A total of eight forestry wastes were studied using this method. Among these, *Pinus massoniana* sawdust, *Acacia confusa* leaves, and *Populus tomentosa* leaves were selected and optimized to formulate an ideal culturing substrate. As a result, a laccase activity of 125.7 U/g and mycelium growth rate of 1.19 mm/d were achieved with a 30-d cultivation period and without the addition of foodstuffs or agricultural residues. The physical and chemical changes in the cultivated substrate were measured through a combination of morphology and spectroscopy analyses. The findings led to the selection of a liquid hot water treatment, which was optimized through comparative analysis among different conditions. With this technique, a maximum hemicellulose recovery ratio of 68.8% was achieved with treatment at 170 °C for 50 min. The number average and weight average molecular weights of the extracted hemicellulose were 1920 and 4289, respectively.

Keywords: Forestry wastes; Hemicellulose; LHW; Pleurotus ostreatus; Laccase

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INTRODUCTION

According to statistics published in March 2018 by the China National Forestry and Grassland Administration (Youdong 2018), the national plantation forests of the country comprise 69.33 million ha, which accounts for 51.36% of the global resource. However, restricted by natural and technical conditions, fast-growing and low-valued trees have been planted on a large scale by local governments and private enterprises. The policy encouraging quick reforestation by fast-growing plantation monocultures has raised controversial ecologic problems, such as a decline in the soil fertility and a potential sabotage of the biodiversity (Bremer and Farley 2010). There is increasing awareness of the issue demands for financially feasible means to replace fast-growing plantations with biodiverse ecosystems. The effort has led to applied research initiatives and engineering for the exploitation of fast-growing forests. With funding from the Chinese government, considerable progress has been made, especially in the fields of material technology and pharmaceutical sciences (Kanowski 1997). However, industrial applications can cause environmental problems of their own, such as discarded waste from plywood factories and extracted residues produced from the medicine, flavor, and fragrance industries.

The purpose of this study was to explore the possibility of comprehensive utilization of the wastes by integrating various industrial practices. In the local mushroom industry, efforts had been made to add the plantation wastes in cultivation substrates to replace crops ingredients. However, due to the lack of scientific support, the results of such efforts are often quite ambiguous, if not controversial. In an effort to shed some light on this subject, statistics were introduced into the design of substrates. A total of eight wastes were studied to assess their characteristics in fungal cultivation. Among the edible fungi commonly found in the daily food market of the Far East, *Pleurotus ostreatus* specifically is widely cultivated for its flexible nutritional requirements and culture conditions (Sánchez 2010). Nevertheless, the species has its own preference and aversion to culture substrates. Thus, a two-step statistical method was developed to optimize integrated industrial wastes for fungal cultivation. As a by-product, cultivated substrates were studied using morphology and spectroscopy analyses to measure the physical and chemical changes in the substrates. The findings led to further bio-refinery processes, among which liquid hot water (LHW) extraction was considered to have complementary advantages for hemicellulose extraction, due to the enzymes activities induced by cultivation (dos Santos 2018).

In the LHW treatment, relatively high pressures are applied to keep the heated water in a liquid state. The advantage presented by this approach is that, with no addition of chemicals, water molecules that serve simultaneously as solvents and catalysts would split spontaneously into $[H]^+$ ions and $[OH]^-$ radicals. Then, the $[H]^+$ ions break down the β-1,4-glycosidic bonds among the polysaccharides, while the $[OH]^-$ radicals catalyze the hydrolysis of ester bonds between the ferulic acid and hemicellulose molecules (Rogalinski *et al.* 2008). Thus, hemicellulose molecules could be released from the lignin/phenolic-carbohydrate complexes (LPCC) under mild conditions without the addition of chemicals.

There have been numerous studies on LHW pretreatments to enhance the enzymatic digestibility of lignocellulose biomasses (Liu and Wyman 2005; Dien *et al.* 2006; Ko *et al.* 2015), but few have studied the effects of biological pretreatment on LHW pretreatment. Moreover, the LHW process has usually been used as a pretreatment for herbaceous biomass, such as wheat straw and sugarcane bagasse (Mosier *et al.* 2005; Pérez *et al.* 2008). In this study, the approach was applied to more complex structures, which were forestry residues.

EXPERIMENTAL

Microorganism and Inoculum Preparation

The *Pleurotus ostreatus* strain used in this study was CICC 14012, which was supplied by the China Center of Industrial Culture Collection (CICC). The strain was grown and maintained on Czapek-Dox medium at 25 °C for 9 d.

Waste Preparation and Culture Conditions

Sawdust was desiccated at 60 ℃ for 12 h and then ground to an approximately 2-mm particle size. Leaves and needles were boiled in water (5% w/v) for 60 min without covering to remove the aromatic volatiles. Then they were desiccated and ground using the same procedures as for the sawdust. The sawdust, extracted leaves/needles, and deionized water with $CaCO_3$ (0.2% w/v) were mixed as designated. The mixtures were kept overnight before being transferred into polypropylene bags (15 cm × 9 cm). The mixture bags were

sterilized at 121 ℃ for 30 min. One gram of Czapek-Dox medium with the cultivated strain was inoculated in each bag, and then cultivated at the designated temperature for 30 d.

Screening of the Forestry Wastes

The Plackett-Burman design (PBD) was used to screen for select forestry wastes. *Cinnamomum cassia* sawdust, *C. cassia* leaves, *Pinus massoniana* sawdust, *Pinus massoniana* needles, *Acacia confusa* sawdust, *A. confusa* leaves, *Populus tomentosa* sawdust, *Populus tomentosa* leaves, water (dummy1), temperature (dummy2), and pH (dummy3) were each selected as variables to evaluate the contribution of each main component to the *Pleurotus ostreatus* growth rate (Table 1). The data analysis was performed using Design Expert Software 11.0.4.0 (Stat-Ease Inc., Minneapolis, MN, USA).

Table 1. PBD for Screening of the Forestry Wastes

Factor	Name	Type	Minimum	Maximum	Coded Low	Coded High
A	*C. cassia* sawdust	Mixture	20.0%	33.3%	-1.00	+1.00
B	*C. cassia* leaves	Mixture	20.0%	33.3%	-1.00	+1.00
C	*Pinus massoniana* sawdust	Mixture	20.0%	33.3%	-1.00	+1.00
D	*Pinus massoniana* needles	Mixture	20.0%	33.3%	-1.00	+1.00
E	*A. confusa* sawdust	Mixture	20.0%	33.3%	-1.00	+1.00
F	*A. confusa* leaves	Mixture	20.0%	33.3%	-1.00	+1.00
G	*Populus tomentosa* sawdust	Mixture	20.0%	33.3%	-1.00	+1.00
H	*Populus tomentosa* leaves	Mixture	20.0%	33.3%	-1.00	+1.00
J	Water	Categoric	55.0%	60.0%	-1.00	+1.00
K	Temperature	Categoric	25.0 °C	28.0 °C	-1.00	+1.00
L	pH	Categoric	5.8	6.5	-1.00	+1.00

Response	Unit	Obs.	Ana.	Min.	Max.	Mean	St. Dev.
Growth Rate	mm/d	12	Factorial	0.6	1.15	0.8983	0.1824

Ana. – analysis; Min. – minimum; Max. – maximum; and St. Dev. – standard deviation

Optimization of the Culture Substrates

The components that were screened and selected by the PBD were optimized through a randomized simplex centroid mixture design, using the laccase activity in the substrates and fungal mycelium growth rate as dependent variables to measure the efficiency of the selected culture substrates. The ratio of each component in the mixed culture medium ranged from 0% to 100%. Similarly, the total proportions of all the components in each experimental run added up to 100% ($A + B + C = 100\%$; where A is

Pinus massoniana sawdust, *B* is *A. confusa* leaves, and *C* is *Populus tomentosa* leaves). The mixture design matrix is shown in Table 2, with the experimental and predicted values for each dependent variable. Linear, quadratic, special cubic, and full cubic models were considered to estimate the synergistic effects among the components, as shown by the following equations:

$$Y = \sum_{i=1}^{p} \beta_i x_i \quad (1)$$

$$Y = \sum_{i=1}^{p} \beta_i x_i + \sum\sum_{i<j}^{p} \beta_{ij} x_i x_j \quad (2)$$

$$Y = \sum_{i=1}^{p} \beta_i x_i + \sum\sum_{i<j}^{p} \beta_{ij} x_i x_j + \sum\sum\sum_{i<j<k}^{p} \beta_{ijk} x_i x_j x_k \quad (3)$$

$$Y = \sum_{i=1}^{p} \beta_i x_i + \sum\sum_{i<j}^{p} \beta_{ij} x_i x_j + \sum\sum_{i<j}^{p} \delta_{ij} x_i x_j (x_i - x_j) + \sum\sum\sum_{i<j<k}^{p} \beta_{ijk} x_i x_j x_k \quad (4)$$

where Y is the response for the enzyme activities, β_i, β_{ij}, and β_{ijk} are the linear, quadratic, and cubic coefficients, respectively, δ_{ij} is the parameter of the full cubic model, $\beta_i x_i$ is the linear mixture portion.

Table 2. Mixture Design Matrix with the Experimental and Predicted Values of the Dependent Variables

Run	***A*** (%)	***B*** (%)	***C*** (%)	**Laccase (U/g)**		**Growth Rate (mm/d)**	
				Act.	Pred.	Act.	Pred.
1	16.67	66.67	16.67	109	109.41	0.94	0.91
2	66.67	16.67	16.67	122	122.84	1.15	1.15
3	0.00	100.00	0.00	90	92.20	0.65	0.67
4	0.00	50.00	50.00	105	103.15	0.81	0.82
5	50.00	0.00	50.00	121	119.00	1.07	1.07
6	33.33	33.33	33.33	117	118.34	1.00	1.04
7	0.00	0.00	100.00	95	95.90	0.75	0.75
8	0.00	100.00	0.00	94	92.20	0.69	0.67
9	100.00	0.00	0.00	117	116.63	1.13	1.14
10	100.00	0.00	0.00	116	116.63	1.15	1.14
11	50.00	50.00	0.00	123	120.30	1.13	1.10
12	16.67	16.67	66.67	108	110.21	0.95	0.93
13	50.00	50.00	0.00	119	120.30	1.06	1.10
14	0.00	0.00	100.00	97	95.90	0.75	0.75

Act. – actual; and Pred. – predicted

Enzymatic Assays

The laccase activity was measured by oxidizing guaiacol at 25 °C for 30 min (Sharma *et al.* 2018). One unit (U) of laccase activity was the change of 0.01 $mL^{-1} \cdot min^{-1}$ in absorbance at 470 nm.

Morphology and Spectrograph Analyses

The optimized culture medium was sampled before and after cultivation and desiccated at 105 °C for 4 h. The surface morphology of the samples was examined using

scanning electron microscopy (SEM) (Hitachi s-3400N, Hitachi, Tokyo, Japan) at a voltage of 15.0 kV. The porous characteristics were determined with a porous medium surface area analyzer (Micromeritics ASAP 2020, Micromeritics, Norcross, USA). This was done at a temperature of 77 K and using high purity nitrogen as the adsorbent. Chemical changes in the functional groups were measured with the help of Fourier transform infrared (FTIR) spectroscopy (Thermo Nicolet Magna-IR 550, Thermo Nicolet Corp., Madison, WI, USA). The measurements were done with KBr disks containing 1% (w/w) of the samples and a total of 32 scans were performed from 4000 cm^{-1} to 400 cm^{-1} at a resolution of 2 cm^{-1}.

Liquid Hot Water Extraction

After cultivation, LHW extraction was performed in a high-temperature homogeneous reactor (PAI LAN PLJF-8, Pengyi, Shanghai, China) with 110-mL polytetrafluoroethylene (PTFE) vessels. Five grams of each sample with 100 mL of deionized water were placed into the PTFE vessels and sealed with nitrogen gas. The reactor was then progressively heated to the designated temperature. The stirring rate was held at a constant 40 rpm during the process. Immediately after the temperature was reached, the vessels were removed from the reactor and chilled in ice water to terminate the process. The pH value was adjusted to 2.0 with the addition of 1 mol/L HCL, and then the contents were centrifuged at 6000 rpm for 3 min to remove the lignin components from the water-phase. After separation, 1 mol/L NaOH was used to adjust the pH value to 4.5 and 200 mL of ethanol were added for ethyl alcohol precipitation of the hemicellulose. The hemicellulose recovery ratio was calculated using the following equation,

$$Y_{\mathrm{rec}} = \frac{M_{\mathrm{hem}}}{M_{\mathrm{FRS}} \times C_{\mathrm{pret}}} \times 100\% \quad (5)$$

where Y_{rec} is the hemicellulose recovery ratio (%), M_{hem} is the mass weight of the hemicellulose (g), M_{FRS} is the mass weight of the optimized forestry residual substrates (g), and C_{pret} is the percentage of hemicellulose in the substrates (%).

Relative Molecular Weight Analysis

The hemicellulose components were dissolved in ultra-pure water. The relative molecular weight was measured with gel permeation chromatography (GPC) (Waters 2414, Waters Corporation, Milford, USA) using a refractive index detector and PL gel MIXED-B column (Waters 2414, Waters Corporation, Milford, MA, USA). The mobile phase was sodium phosphate buffer with 0.02 mol/L NaCl (pH = 7.5). The flow rate was 0.5 mL/min. The column temperature was 30 ℃. The injection volume was 20 μL and the concentration of the standard pullulan solution was 5 g/L (Sun *et al.* 1999).

RESULTS AND DISCUSSION

Screening of the Wastes

Eight plantation forestry wastes were screened using the PBD to identify the principle factors and their contribution to the growth rate of *Pleurotus ostreatus* CICC 14012. In Table 3, the PBD model *F*-value of 7.11 implied that among the wastes, *Pinus massoniana* sawdust, *A. confusa* sawdust, and *Populus tomentosa* leaves were found to be favorable by the strain with a 93.28% confidence level. The priority of the favorable wastes, measured by their influence on the mycelium growth rate, was as follows: *A.*

confusa sawdust (27.55%) > *Pinus massoniana* sawdust (25.59%) > *Populus tomentosa* leaves (20.99%).

Table 3. PBD Observations of the Growth Rate

Source	Sum of Squares	Mean Square	*F*-value	*P*-value	Contribution
Model	0.3476	0.0435	7.11	0.0672	
C. cassia sawdust	0.0008	0.0008	0.14	0.7364	0.23%
C. cassia leaves	0.0560	0.0560	9.17	0.0564	15.31%
Pinus massoniana sawdust	0.0936	0.0936	15.32	0.0296	25.59%
Pinus massoniana needles	0.0033	0.0033	0.55	0.5137	0.91%
A. confusa sawdust	0.1008	0.1008	16.50	0.0269	27.55%
A. confusa leaves	0.0096	0.0096	1.58	0.2982	2.63%
Populus tomentosa sawdust	0.0065	0.0065	1.07	0.3772	1.78%
Populus tomentosa leaves	0.0768	0.0768	12.57	0.0382	20.99

Optimization of the Substrates

The mycelium growth rate and laccase activity were selected as the dependent variables. Linear, quadratic, special cubic, and full cubic models were established, and the fitness of each model was examined. The quadratic polynomial (Eq. 2) was selected for modelling by maximizing the coefficient of determination (R^2) (0.9601 and 0.9013, respectively). The final equations in terms of the three favorable wastes are shown in Table 4.

Table 4. Final Equations Representing the Growth Rate and Laccase Activity

Y (Growth Rate)	Equation
+1.13943	*Pinus massoniana* sawdust
+0.674192	*A. confusa* leaves
+0.752990	*Populus tomentosa* leaves
+0.753724	*Pinus massoniana* sawdust × *A. confusa* leaves
+0.487481	*Pinus massoniana* sawdust × *Populus tomentosa* leaves
+0.416053	*A. confusa* leaves × *Populus tomentosa* leaves
***Y* (Laccase Activity)**	**Equation**
+116.62704	*Pinus massoniana* sawdust
+92.19847	*A. confusa* leaves
+95.89837	*Populus tomentosa* leaves
+63.55772	*Pinus massoniana* sawdust × *A. confusa* leaves
+50.96585	*Pinus massoniana* sawdust × *Populus tomentosa* leaves
+36.39442	*A. confusa* leaves × *Populus tomentosa* leaves

For a visual representation, the final equations were pictured as triaxial diagrams and contour plots. Figure 1 shows the mycelium growth rate with different waste proportions. The peak of the surface was observed at triaxial point A (*Pinus massoniana* sawdust) and indicated that a high proportion of *Pinus massoniana* sawdust in the substrates was more desirable for mycelium growth compared with the other two components. However, the peak laccase activity in Fig. 2 was biased towards, but not reached at triaxial point A, which suggested a synergistic effect may have existed among

the three components. Five replicates of the validation experiments with Student's t-test confirmed that however moderate the improvement (4.26% to 7.89%) in the laccase activity, it was unlikely ($p < 0.02$) the improvement occurred by chance.

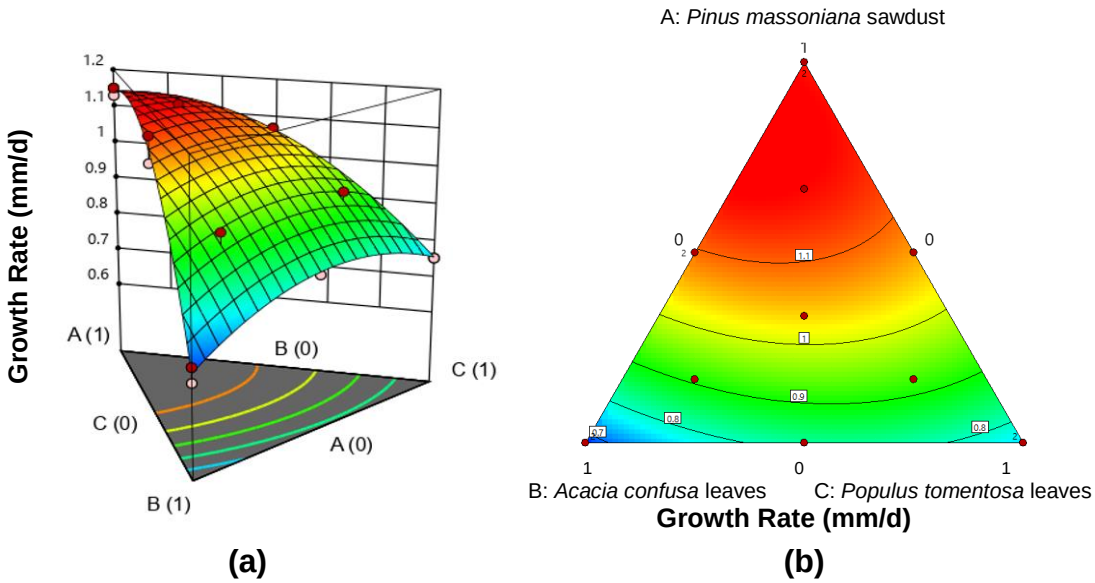

Fig. 1. Triaxial diagram (a) and contour plot (b) of the mycelium growth rate

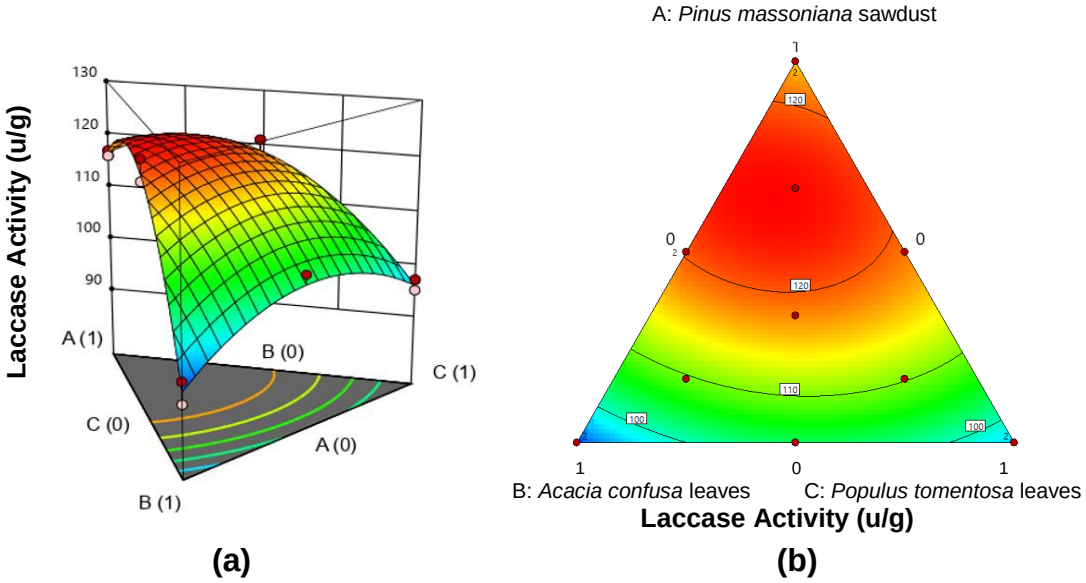

Fig. 2. Triaxial diagram (a) and contour plot (b) of the laccase activity

Numerical optimization by the Myers and Montgomery method (Myers *et al.* 2004) was used to maximize both the mycelium growth rate and laccase activity in the substrates. The results of this analysis are represented in Table 5. The proportions of each component in the substrates were as follows: 65.8% *Pinus massoniana* sawdust, 23.6% *A. confusa* leaves, and 10.6% *Populus tomentosa* leaves. Five replicates of the validation experiments using the optimized substrates resulted in an average laccase activity of 125.71 U/g (with

a standard deviation of 3.51 U/g) and mycelium growth rate of 1.19 mm/d (with a standard deviation of 0.07 mm/d).

Table 5. Numerical Optimization Solutions

Number	*Pinus massoniana* Sawdust	*A. confusa* Leaves	*Populus tomentosa* Leaves	Laccase Activity	Growth Rate	Desirability
1	0.658	0.236	0.106	123.000	1.150	1.000
2	0.692	0.308	0.000	122.649	1.157	0.995

Morphology and Spectrograph Analyses

The morphology structures of the substrates, photographed by SEM before and after cultivation, are shown in Fig. 3. Under 500x magnification, the lignocellulose structure before cultivation (Fig. 3a) was compactly arranged in order, with the tubular form of the cellulose tightly covered by the lignin components. After 30 d of cultivation, the compact configuration was comprehensively disorganized as the surface of the lignocellulose structure peeled and decomposed into filamentous debris (Fig. 3b).

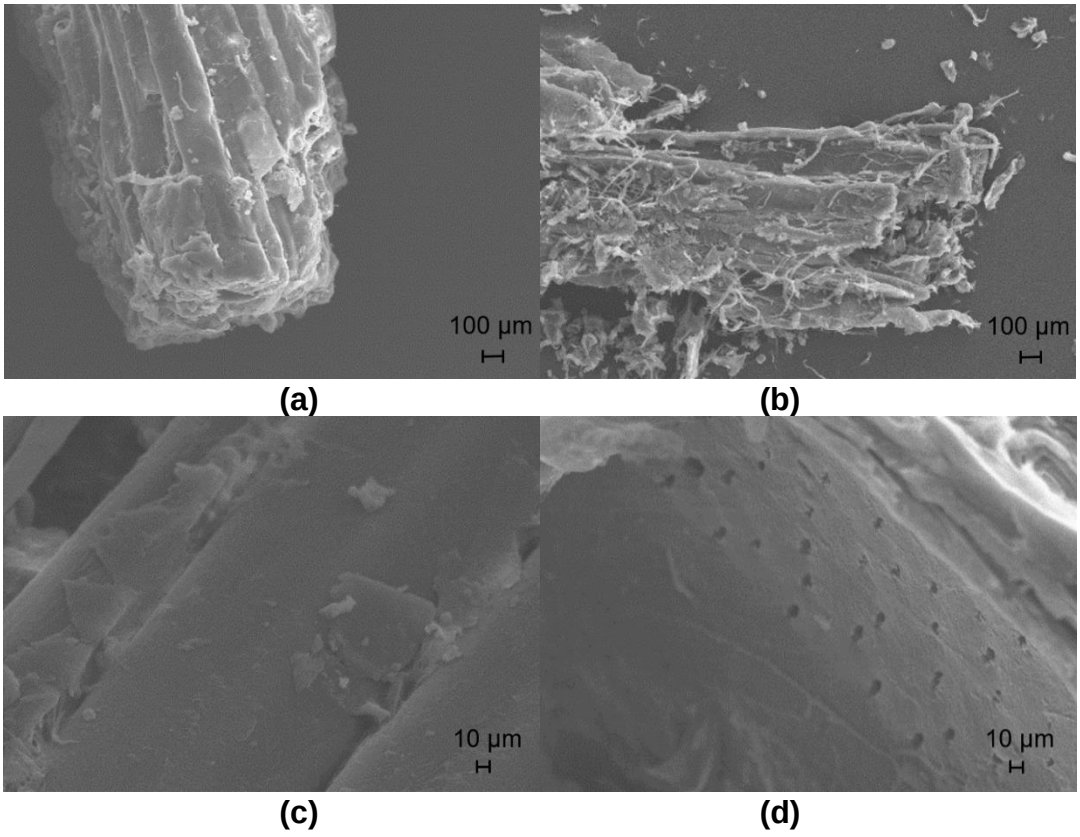

Fig. 3. Morphology images of the substrates before and after cultivation: (a) before cultivation at 500x magnification; (b) after cultivation at 500x magnification; (c) before cultivation at 3000x magnification; and (d) after cultivation at 3000x magnification

Further analysis under 3000x magnification revealed the surface structure with more details. Before cultivation, the lignin components were smooth and intact, which were linked up as a seamless plate (Fig. 3c); in contract, after 30 days' cultivation of *Pleurotus*

ostreatus, it seems the growth process had not only wrinkled the surface structure, but also bored pores into it (Fig. 3d). The formation of a porous structure was attributed to the laccase activity. The same phenomenon has been observed in other laccase-treated industrial lignins (Ortner *et al.* 2015). However, in this case, the pore structures observed were more significant, both in size and number. Judging by the diameter of the pores, it was possible that the later desiccated mycelium had grown into them, which in turn facilitated the decomposition of the lignin components.

The laccase-induced porous structure could have made the carbohydrate components more accessible during the LHW extraction process. To understand the structure better, porous medium surface area analysis was applied to measure the porosity characteristics in terms of the specific surface area, total pore volume, average pore size, and pore diameter distribution. The results are as shown in Table 6. Compared with the non-cultivated substrates, the specific surface area, total pore volume, and average pore size of the 15-d-cultivated substrates increased by 46.0%, 22.6%, and 20.4%, respectively. Similarly, the 30-d cultivation increased the specific surface area, total pore volume, and average pore size by 64.1%, 40.2%, and 21.4%, respectively. Because the specific surface area and total pore volume increased by 12.4% and 14.3% from the 15-d to 30-d cultivation samples, respectively, the average pore size did not increase significantly (< 0.01%). Therefore, after 15 d of cultivation, the pore structures in the substrates must have increased in number, but not in size. This conclusion was also supported by the fact that the pore diameter distribution patterns from the 15-d to 30-d substrates were essentially unchanged (Table 6).

Table 6. Effect of Cultivation on the Porosity Characteristics of the Optimized Substrate

Cultivation Time (d)	Specific Surface Area (m^2/g)	Total Pore Volume (cm^3/mg)	Average Pore Size (nm)	Pore Diameter Distribution (%)		
				≤ 10 nm	10 nm ~ 50 nm	≥ 50 nm
0	1.211	6.787	6.535	69.25	21.43	9.21
15	1.768	8.324	7.871	51.69	26.98	21.33
30	1.987	9.516	7.932	50.14	27.36	22.50

In accordance with the study into the structural morphology of the substrates, the chemical components were analyzed by FT-IR spectroscopy (Fig. 4). The peaks were assigned according to the literature data (Kim *et al.* 2003). Specifically, the signal at 895 cm^{-1} represented β-glycosidic linkages between monosaccharide units. The slight decrease in the signal intensity indicated that only a fraction of the polysaccharides had been consumed by the fungi for cellular respiration and other energy metabolism processes. The signals at 1603 cm^{-1} and 1510 cm^{-1} represented skeletal vibration of lignin components, and both signals were significantly weaker after cultivation. This finding revealed that the lignin chemical structure collapsed. The signal at 1730 cm^{-1} represented acetyl ester groups of the hemicelluloses and carboxylic groups in the *p*-coumaric or ferulic acid. The signal strength remained almost identical after cultivation, which indicated that the cultivation process did not disrupt the LPCC and the hemicellulose components remained covalently linked with its lignin counterpart.

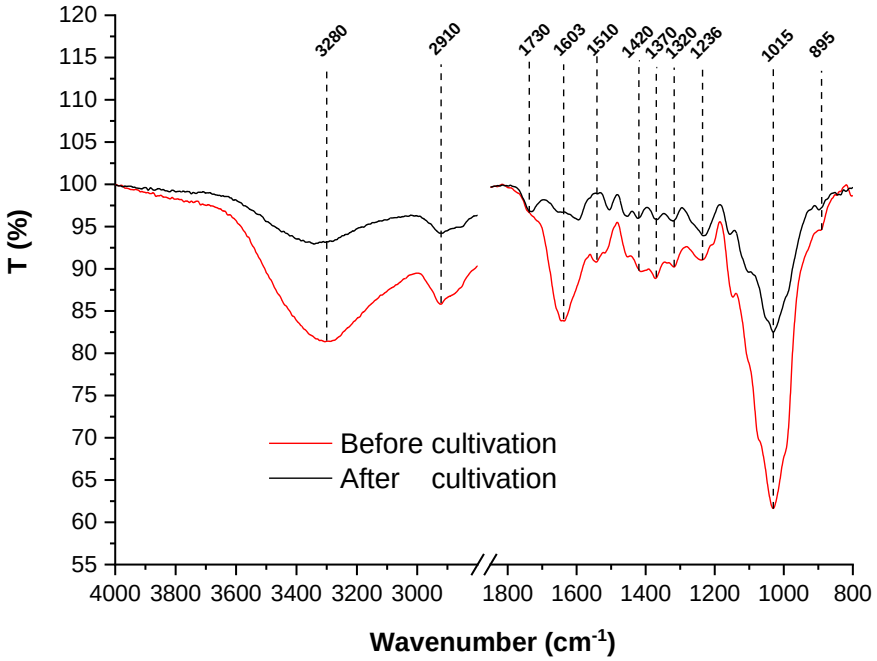

Fig. 4. FTIR spectra of the optimum substrates before and after cultivation

Liquid Hot Water Extraction

According to the morphological and spectrographic analyses above, the cultivation process physically disordered the compact configuration of the lignocelluloses, and enzymatically degraded the aromatic rings of the lignin. It also suggested that the ferulic groups, by which lignin and hemicellulose components covalently combined, remained intact. The LHW, which served simultaneously as a solvent and catalyst, was used to release polysaccharides from the lignin-hemicellulose compounds. In the process, as water molecules split into $[H]^+$ ions and $[OH]^-$ ions, the $[H]^+$ ions broke down the β-1,4-glycosidic bonds among the polysaccharides, while the $[OH]^-$ radicals catalyzed the hydrolysis of ester bonds between the ferulic acid and hemicellulose molecules. As a result, hemicellulose was extracted from the LPCC as polysaccharides with certain molecular weights. The effects of the reaction times and temperatures on the process are shown in Fig. 5. The hemicellulose yield ratio was above 65% after pretreatment at 170 ℃ for 50 min (68.8%) and 180 ℃ for 40 min (66.9%). Intuitively, the average yield ratio was higher at 170 ℃ and 50 min. However, the overlapping least significant difference (LSD) bars suggested that the yield ratio under the two conditions were not statistically different from each other. In either case, the prolonged reaction time beyond the optimum conditions resulted in a critical decrease in the yield ratio because of the depolymerization of hemicellulose and a further degradation of the saccharides. Compared with the non-cultivated substrates, the yield ratio of the cultivated substrates increased by 39.7% after treatment at 180 ℃ for 40 min and by 50.4% after treatment at 170 ℃ for 50 min. Because the hemicellulose yield ratio from the cultivated substrates were not statistically different between the two conditions, the explanation was that the two processes extracted hemicelluloses with a different degree of polymerization. A relative molecular weight analysis was performed to measure the distribution pattern of the extracted hemicellulose. According to the analysis

that is shown in Table 7, the treatment at 170 ℃ for 40 min had higher number average (M_w) and weight average molecular weights (M_n), which suggested that treatment at these conditions was capable of releasing highly entangled hemicellulose molecules. Moreover, when comparing the polydispersity index (PDI, M_w/M_n), the distribution width was narrower at 170 ℃ than at 180 ℃. The evenly distributed molecular weight suggested that less polymers were degraded under the milder treatment conditions. The findings suggested that the extraction process with the higher temperature and smaller duration were more efficient at releasing molecules with a lower weight and looser entangled hemicellulose molecules. In contrast, the milder and longer process was more suitable for the comprehensive extraction of molecules with a higher weight.

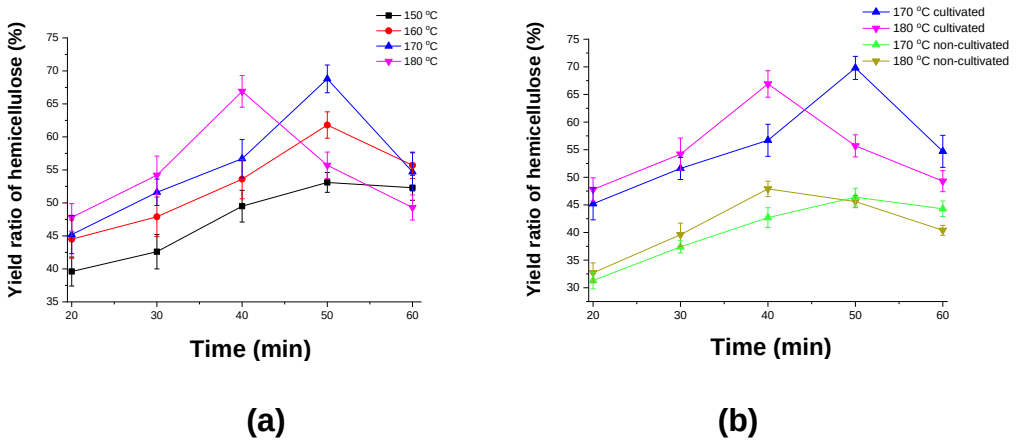

Fig. 5. Effect of the different conditions on the yield ratio from hemicellulose extraction

Table 7. Effect of the Reaction Temperature on the Relative Molecular Weight of the Extracted Hemicellulose

Temperature	M_n	M_w	PDI
180 °C	1510	4015	2.66
170 °C	1920	4289	2.23

CONCLUSIONS

1. From eight plantation forestry wastes, *Pinus massoniana* sawdust, *A. confusa* leaves, and *Populus tomentosa* leaves were statistically confirmed to have beneficial effects on the cultivation of *Pleurotus ostreatus* CICC 14012. Proportions of the three components were optimized as follows: 65.8% *Pinus massoniana* sawdust, 23.6% *A. confusa* leaves, and 10.6% *Populus tomentosa* leaves. A laccase activity of 125.71 U/g and mycelium growth rate of 1.19 mm/d were achieved after 30 d of cultivation on the optimum substrates.
2. The morphology and spectroscopy analyses revealed physical and chemical changes in the substrates during and after cultivation. Because the compact configuration of the lignocellulose structures was disrupted by the mycelium activities, the porosity of the substrates significantly increased in terms of the specific area (by 64.1%), average pore size (by 21.4%), and total pore volume (by 40.2%).

3. The LHW treatment conditions were optimized through a comparative analysis, and a maximum 68.8% hemicellulose recovery with a PDI of 2.33 was achieved with treatment at 170 °C for 50 min. With this method, more forestry wastes could be utilized for different edible fungi cultivation, followed by hemicellulose extraction and polysaccharide production.

ACKNOWLEDGEMENTS

This work was funded by the Fundamental Research Funds of Research Institute of Forest New Technology (Grant No. CAFYBB2017SY034), the Basic Scientific Research Project of Jiangsu Province Biomass Energy and Material Laboratory (Grant Nos. JSBEM-S-201802 and JSBEM-S-2017011), and the National Natural Science Foundation of China (Grant No. 31770636). The authors declare that they have no conflict of interest.

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Article submitted: January 22, 2019; Peer review completed: May 14, 2019; Revised version received: May 21, 2019; Accepted: June 1, 2019; Published: June 10, 2019.
DOI: 10.15376/biores.14.3.5862-5874