Optimization of Poplar Wood Shavings Bio-pretreated with *Coriolus versicolor* to Produce Binderless Fiberboard Using Response Surface Methodology

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Free formaldehyde is released due to the addition of aldehyde-based adhesives during the production of fiberboard. This is harmful to human health and pollutes the environment, and for that reason binderless fiberboard has become a research hotspot. There have been reports about pretreatments with white-rot fungi or lignocellulase to produce binderless fiberboard, but there have been no such reports about optimizing the bio-pretreatment conditions. In this study, poplar wood shavings were used for fiberboard production, and the bio-pretreatment conditions with *Coriolus versicolor* were studied using response surface methodology. After single-factor optimization, the central levels of bran, molasses, and magnesium sulfate were obtained. Further optimization was carried out using Box-Behnken design to study the influence of the factors. A second-order polynomial equation was obtained, and the low *p*-value (0.001) implied that the model was highly significant. The optimized bio-pretreatment conditions for modulus of rupture (MOR) of the fiberboard were obtained by ridge analysis as 3.021 g of bran, 8.907 g of molasses, and 0.27 g of magnesium sulfate. Under the optimized conditions, MOR of fiberboard reached 27.21±0.64 MPa, which was 2.2 times that of the control fiberboard. Bio-pretreatment with *C. versicolor* should be a good choice to produce a high-strength binderless fiberboard.

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INTRODUCTION

Fiberboard with appropriate adhesives is a type of artificial board made from wood fibers or other plant fibers. Such fiber materials have advantages such as environmental friendliness, renewability, biodegradability, and easy modification (Seki *et al.* 2022; Frahat *et al.* 2023). They mainly come from logging residues, such as small diameter wood and branches, as well as wood resource processing residues, such as shavings and sawdust. Therefore, fiberboard can fully utilize the residue of wood resources and efficiently utilize wood resources (Hartono *et al.* 2023). However, adhesives are often regarded as indispensable materials in traditional fiberboard production processes (Zhong *et al.* 2024). The problem is that free formaldehyde is released due to the addition of aldehyde-based adhesive during the production of fiberboard (Jin *et al.* 2023). The release of free formaldehyde can cause harm to human health and also pollute the environment (Liang *et al.* 2016). In order to reduce the problem of a large amount of free formaldehyde release during the production and application of fiberboard, researchers have conducted many explorations on formaldehyde adhesives (Liu *et al.* 2024). Despite achieving many valuable results, free formaldehyde cannot be completely eliminated (Zhao *et al.* 2024). Binderless fiberboard by wood self-bonding has become a research hotspot (Shi *et al.* 2023).

Studies on the self-bonding properties of wood fibers mainly have focused on the physical, chemical, and biological pretreatment of wood fibers. Chemical activation methods mainly include acid treatment, alkali treatment, *etc*. The surface of wooden materials is treated with acidic substances (such as acetic acid) to partially degrade lignin or carbohydrates. The resulting products are bonded between the wood fiber interfaces through chemical cross-linking reactions (condensation) during the hot pressing process, which results a self-bonding fiberboard (Pan *et al.* 2007). The surface of wooden materials is treated with alkaline solution to activate their interfacial and surface chemical properties, followed by formation of the material into fiberboard by hot-pressing (Geng *et al.* 2006). The various physical and mechanical properties of the alkali-treated fiberboard are good, but their water absorption and thickness swelling properties are not satisfactory. Adding certain natural ingredients, such as soy protein, chitosan, and lignin to replace aldehyde-based adhesives (Xu *et al.* 2022; Charii *et al.* 2024; Zhong *et al.* 2024), has attracted the attention of more and more researchers. Considering lignin as an example, after enzymatic hydrolysis and acid-base treatment, lignin would yield abundant low molecular lignin, which can provide abundant bio-based raw materials for the preparation and reshaping of numerous polymer materials, such as wood adhesives (Shuai *et al.* 2016; Brodin *et al.* 2017; Mallinson *et al.* 2018). Biological activation methods with lignocellulase or enzyme-producing fungi can regulate the physical and chemical structure of the biomass, promote the condensation reaction between lignin degradation products, cellulose and hemicellulose degradation products by hot pressure, increase chemical bonding and hydrogen bonding during the bonding process, and improve the comprehensive performance of fiberboard (Wu *et al.* 2011). The biological activation method is currently the most environmental-friendly method for producing binderless fiberboard.

In China, the planting area of poplar is very large, and poplar wood shavings (PWS), the waste of poplar wood processing, are very easy to be obtained. In the previous research, PWS were used for binderless fiberboard production by cellulase treatment (Wu *et al.* 2020b). Nevertheless, the usage of cellulase in treatment led to the increase in the production cost of fiberboard. In order to reduce the cost of enzyme, the bio-pretreatment of PWS with fungi *Coriolus versicolor*, which can secrete strong lignocellulase, also achieved a good result (Wu *et al.* 2020a). However, the bio-pretreatment conditions have not been optimized yet. It is necessary to optimize the pretreatment conditions in order to improve the bio-pretreatment effect of *C. versicolor* on PWS. The Box-Behnken design in response surface methodology is an alternative for the optimization of analytical methods (Ferreira *et al.* 2007; Djimtoingar *et al.* 2022). Therefore, the design was used for the optimization of bio-pretreatment conditions in this study. The result should provide technical support for the production of binderless fiberboard for decoration purposes, which has a high economic value.

EXPERIMENTAL

Materials and Agents

PWS was purchased from a wood processing factory in Huai'an, China, and soybean meal and bran were purchased from COFCO Group Co., Ltd, China. PWS, soybean meal and bran were all dried in oven with 60 °C to constant weight, and then converted to particles using a grinder. PWS was passed through a 60-mesh sieve, and soybean meal and bran were passed through a 100 mesh sieve. Molasses was purchased from Hunan Wanhui Biotechnology Co., Ltd, China. Glucose, starch, magnesium sulfate, copper chloride, manganese sulfate, sodium hydroxide, and yeast extract were all analytical grade and purchased from China National Pharmaceutical Group Chemical Reagent Co., Ltd, China.

Strain and Cultivation

The strain *Coriolus versicolor* was preserved at Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake in Huai'an, China. Two or three agar blocks (1cm × 1cm) from the preserved test tube slant of the strain were inoculated into 100 mL of potato dextrose liquor in a 250 mL round flask. After culturing for 3 days at 29 ℃ on a reciprocal shaker with 150 r/min, 5 mL of culture was inoculated into 100 mL of potato dextrose liquor in a 250 mL round flask for the second culture under the same conditions.

Single-factor Optimization of Bio-pretreatment with *C. versicolor*

Single-factor optimization was necessary to be conducted to provide appropriate variables and levels for response surface methodology design. The single-factor optimization included the selection of optimal variables and optimal variable levels.

Selection of optimal variables: The first step was to dissolve 6 g of carbon source (glucose, molasses, or starch), 2 g of nitrogen source (soybean meal, bran, or yeast extract), or 0.2 g of inorganic salts (magnesium sulfate, copper chloride, or manganese sulfate) in 90 mL of deionized water. This was adjusted a pH to 6.0 with 0.1 mol/L NaOH solution, mixed well with 60 g of PWS, then divided the mixture into two 250 mL round flasks, and sterilized at 121 ℃ for 30 min. 10 mL culture of *C. versicolor* was inoculated into every flask for bio-pretreatment. The bio-pretreatment kept still at 29 ℃ for 21 days.

Selection of optimal variable levels: 3, 6, 9, or 12 g of molasses mixed with 90 mL of deionized water (adjusting the pH to 6.0) was added into 60 g of PWS, and mixed well, then divided the mixture into two 250 mL round flasks, respectively. 1, 2, 3, or 4 g of bran mixed with 90 mL of deionized water was added into 60 g of PWS and mixed well. Then the mixture was divided into two 250 mL round flasks. 0.1, 0.2 0.3, or 0.4 g of magnesium sulfate mixed with 90 mL of deionized water was added into 60 g of PWS and mixed well. The mixture was divided into two 250 mL round flasks. A specimen without the addition of additives ("0" in Fig. 1) served as the control. The flasks were sterilized at 121 ℃ for 30 minutes. 10 mL culture of *C. versicolor* was inoculated into every flask for bio-pretreatment. The bio-pretreatment kept still at 29 ℃ for 21 days.

Response Surface Methodology Optimization of Bio-pretreatment with *C. versicolor*

Factors (bran, molasses and magnesium sulfate) were optimized by response surface methodology (RSM), and the Box-Behnken design of experiment is shown as Table 1 (the Level 0 was determined by single-factor optimization).

Table 1. The Level of Variables for the Box-Behnken Design of Experiment

Factor	Code	Level		
		-1	0	1
Bran (g)	x_1	1	3	5
Molasses (g)	x_2	6	9	12
Magnesium sulfate (g)	x_3	0.2	0.3	0.4

Preparation of Fiberboard

PWS were manually well-distributed and loaded into a stainless mold (100 mm 100 mm × 3 mm), then pressed flat into a fiberboard by a R-3202 Hot-Press Model (Wuhan Qien Science and Technology Development Co., Ltd., Hubei, China). Hot pressing was conducted under a pressure of 12 MPa at 170 ℃ for 24 min. The fiberboard was stored at room temperature in a dryer with a humidity of around 60%.

Properties of Fiberboard

The density of fiberboard was measured by dividing the fiberboard mass (kg) by its volume (m^3). The fiberboard was cut into fiberboard specimens of about 100 mm in length and 20 mm in width, respectively. The modulus of rupture (MOR) of fiberboard specimens was determined by performing the three point flex test at a crosshead speed of 3 mm/min using a micro-computer control electron universal testing machine (Model ETM104B, Wance Group, Shenzhen, China). The MOR could be calculated as Eq. 1. The water swelling ratio (WSR) was measured from the different thickness (h) before and after immersing in water for 24 h, and the WSR could be calculated as Eq. 2. The length, width, and thickness of fiberboard specimens were measured accurately by a micrometer.

$$MOR = \frac{3FL}{2bh^2} \tag{1}$$

$$WSR(\%) = \frac{h_2 - h_1}{h_1} \times 100\% \tag{2}$$

where F is the maximum force to break the fiberboard specimens (N), L is the span length (60 mm), b is the width of fiberboard specimens (mm), h is the thickness of fiberboard specimens (mm), h_2 is the final thickness of fiberboard specimens after immersing in water (mm), and h_1 is the initial thickness of fiberboard specimens before immersing in water (mm).

Statistical Analysis

Test Pilot software (Wance Group, Shenzhen, China) was used to calculate MOR and modulus of elasticity (MOE), ORIGIN PRO 8.0 (OriginLab Corp., Massachusetts, USA) was used to analyze the data and draw bar graphs, and Statistical Analysis System 9.4 (SAS 9.4) (SAS Institute Inc., North Carolina, USA) was used to design experiment and analysis the data of RSM. The error bars in all figures corresponded to standard errors of three sample test, and each sample test was determined for five times. ANOVA was used for analysis of significant.

RESULTS AND DISCUSSION

Single-Factor Optimization of Bio-pretreatment with *C. versicolor*

The effects of different carbon sources, nitrogen sources, and inorganic salts on the preparation of binderless fiberboard by bio-pretreatment with *C. versicolor* are shown in Fig. 1.

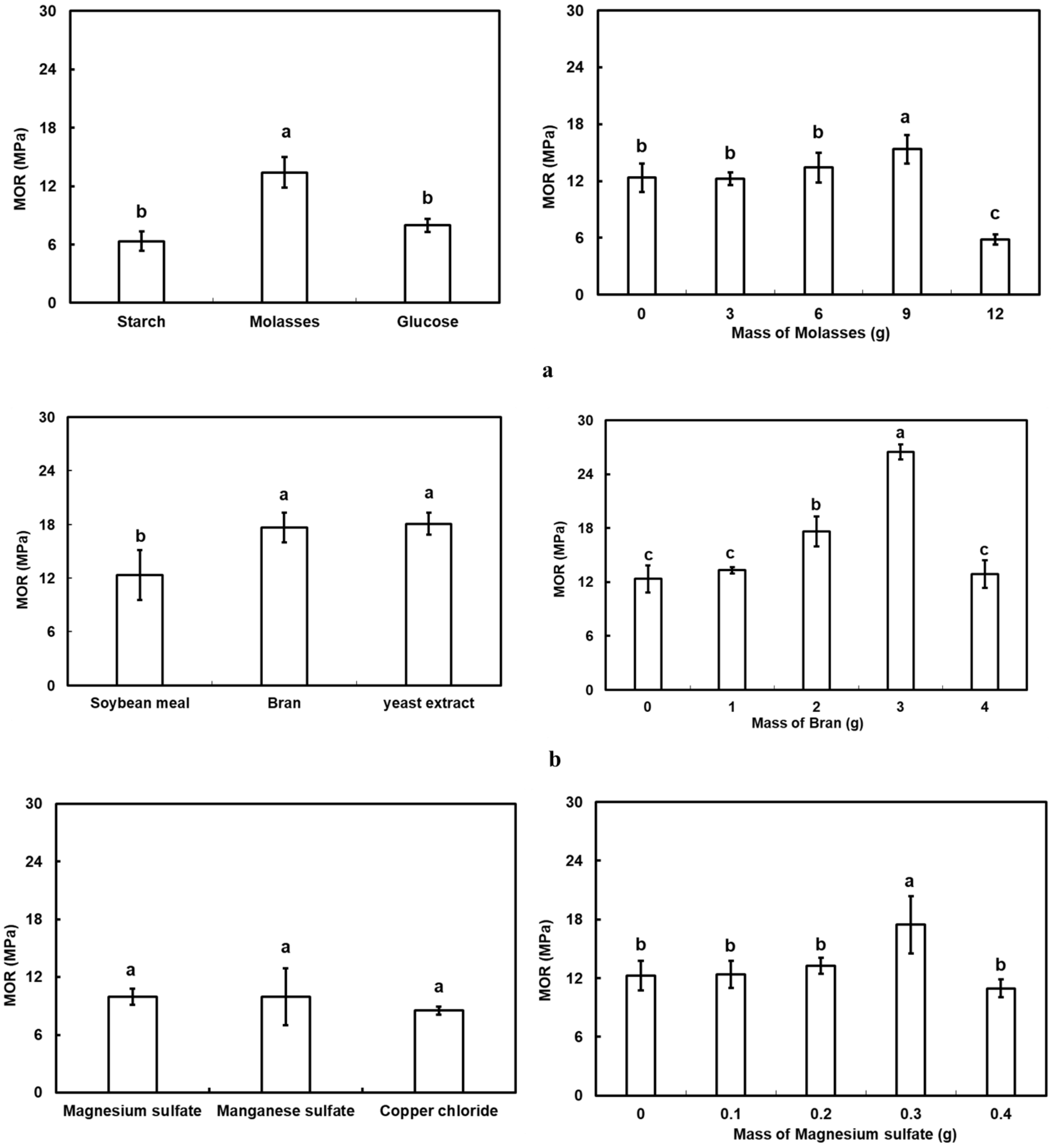

Fig. 1. Single-factor optimization of bio-pretreatment. (a. the effect of carbon sources and carbon source level in 60 g PWS on MOR of fiberboard; b. the effect of nitrogen sources and nitrogen source level in 60 g PWS on MOR of fiberboard; c. the effect of different inorganic salt and inorganic salt level in 60 g PWS on MOR of fiberboard)

Different carbon sources had significant differences on MOR of fiberboards, and molasses was the optimal carbon source. MOR of fiberboard increased as the mass of molasses added increased. When the mass of molasses was 9 g, the maximum MOR of fiberboard reached 15.4 MPa. Further increasing the mass of molasses added resulted in a significant decrease in MOR of fiberboard. The reason might be that the content of sugars in the pre-treatment system was too high, which was not conducive to the secretion of lignocellulase, and thus was not conducive to the self-bonding of fiberboard (Reddy and Kanwal 2022). Bran and yeast extract were the optimal nitrogen sources, and the optimal nitrogen source was selected as bran based on an economic consideration. As the mass of bran added increased, MOR of fiberboard first increased and then decreased. When the mass of bran added was 3 g, MOR of fiberboard obtained its maximum. Magnesium sulfate, manganese sulfate, and copper chloride had little effect on MOR of fiberboard, which might be due to the low mass of inorganic salts (0.2 g). Further selecting the most economical inorganic salt (magnesium sulfate) for optimization of level, it was found that with the increase of the mass of magnesium sulfate added, MOR of fiberboard also showed a trend of first increasing and then decreasing. When the mass of magnesium sulfate added was 0.3 g, MOR of fiberboard reached its maximum. The interactions of molasses, bran, and magnesium sulfate might appear during the bio-pretreatment, which affected MOR of fiberboard. It was necessary to optimize these three factors by RSM.

Optimization by Response Surface Methodology

Box-Behnken design (BBD) in RSM (Li *et al.* 2019) was implemented to determine the optimal levels of the three selected factors (bran for x_1, molasses for x_2, and magnesium sulfate for x_3). The experimental design and results are shown in Table 2.

Table 2. Box-Behnken Design along with MOR as Response

Run	Variables Level			MOR (MPa)
	Bran (g) (x_1)	Molasses (g) (x_2)	Magnesium Sulfate (g) (x_3)	
1	1	6	0.3	23.65
2	1	12	0.3	23.23
3	5	6	0.3	23.69
4	5	12	0.3	22.98
5	3	6	0.2	24.45
6	3	6	0.4	24.68
7	3	12	0.2	24.64
8	3	12	0.4	24.29
9	1	9	0.2	25.26
10	5	9	0.2	25.54
11	1	9	0.4	24.95
12	5	9	0.4	24.89
13	3	9	0.3	26.38
14	3	9	0.3	26.97
15	3	9	0.3	26.62

The relationships between MOR and the factors were obtained by application of RSM. By employing multiple regression analysis on the experimental data, MOR and the tested factors could be related by the following second-order polynomial equation as Eq. 3,

$$Y = 3.3176 + 2.205 \times x_1 + 4.04 \times x_2 + 15.35 \times x_3 - 0.328 \times {x_1}^2 - 0.0121 \times x_1 x_2$$
$$-0.425 \times x_1 x_3 - 0.2175 \times {x_2}^2 - 0.4833 \times x_2 x_3 - 18.4583 \times {x_3}^2 \quad (3)$$

where Y is MOR of fiberboard, x_1 is the mass of bran, x_2 is the mass of molasses, and x_3 is the mass of magnesium sulfate.

The analysis of variance data for the selected quadratic polynomial model is listed in Table 3. The high model *F*-value (27.621) and low *p*-value (0.001) implied that the model was highly significant. The coefficient of determination R^2 was calculated as 98.03 %, which implied that the model was reliable for the MOR of fiberboard in present study.

Table 3. Analysis of Variance (ANOVA) for the Second-Order Polynomial Model

Source	DF	SS	MS	*F*-value	*p*-value
Model	9	19.663	2.185	27.621	0.001
Error	5	0.395	0.079	-	-
(Lack of Fit)	3	0.219	0.073	0.831	0.587
(Pure Error)	2	0.176	0.088	-	-
Total	14	25.281	-	-	-
R^2 =98.03%					

The coefficient estimates of model equation, along with the corresponding *p*-values, are presented in Table 4. The *p*-value was employed as a tool to check the significance of each coefficient, which also indicated the interactions between the variables. The corresponding coefficient was more significant as the *p*-value was smaller. It was observed from Table 4 that variables (${x_1}^2$ and ${x_2}^2$) were highly significant with *p*-values less than 0.001, and it was indicated that bran and molasses were the most effective factors.

Table 4. Regression Results of the Box-Behnken Design

Variables	estimate	Standard error	*t*-value	*p*-value
x_1	0.0013	0.099	0.013	0.990
x_2	-0.166	0.099	-1.672	0.155
x_3	-0.135	0.099	-1.358	0.233
$x_1 * x_1$	-1.312	0.146	-8.965	0.0003
$x_1 * x_2$	-0.073	0.141	-0.516	0.628
$x_1 * x_3$	-0.085	0.146	-0.604	0.572
$x_2 * x_2$	-1.957	0.146	-13.37	< 0.0001
$x_2 * x_3$	-0.145	0.141	-1.031	0.350
$x_3 * x_3$	-0.185	0.146	-1.261	0.263

The 3D response surfaces (Figs. 2) generated by SAS 9.4 were the graphical representations of the regression equation (Eq. 3). They could visualize the relationship between the response and each variable, and the interactions between two tested variables. The 3D response surfaces could also locate the optimum values of the factors for the maximum of the response (Wu *et al.* 2020b). Peaks were found in 3D response surfaces (Fig. 2), which indicated that there was indeed an optimal condition to get the maximum MOR of fiberboard.

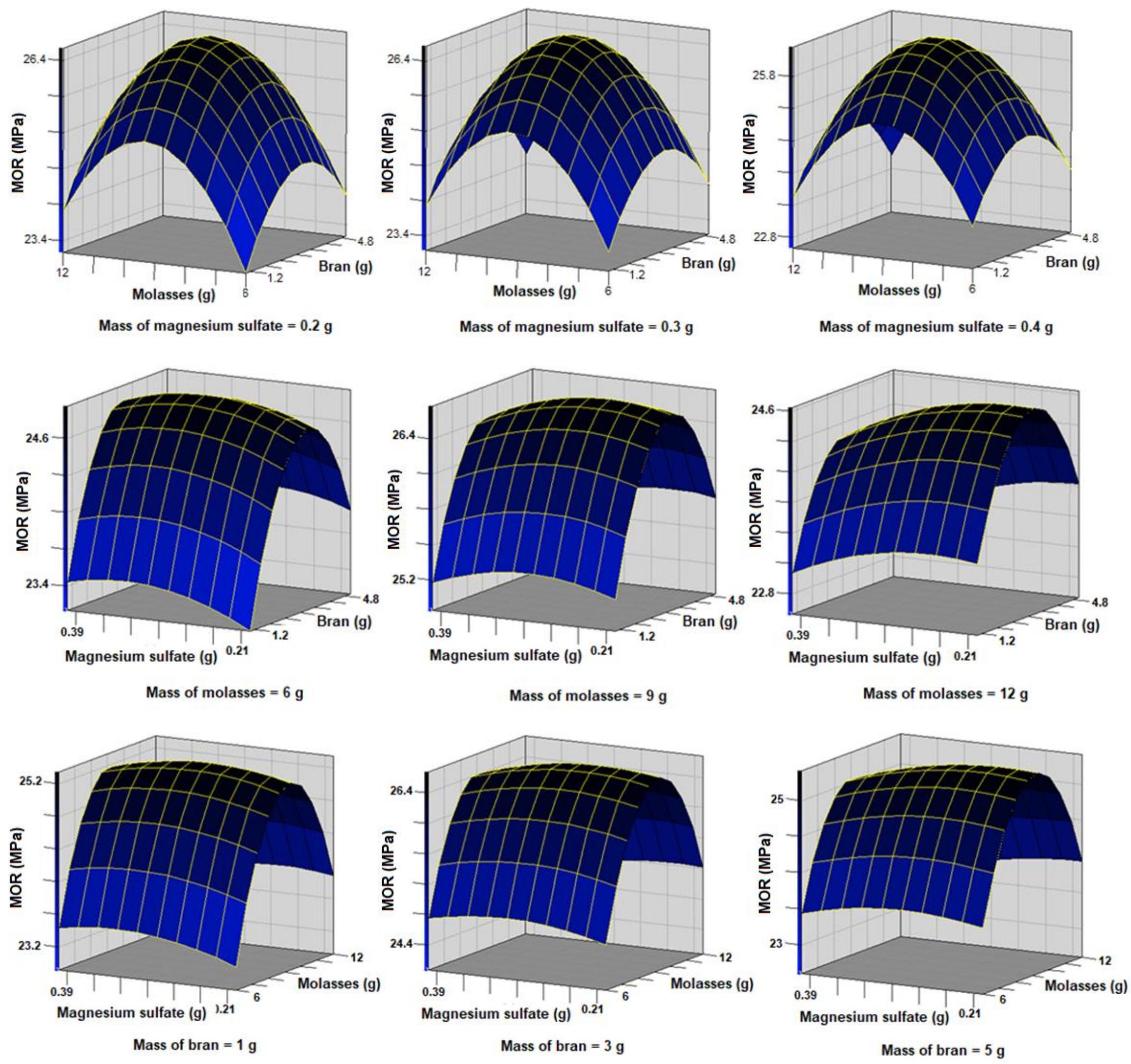

Fig. 2. 3D response surfaces showing the effects of bran, molasses, and magnesium sulfate on MOR of fiberboard

Optimization Results and Verification

The optimum conditions of bran, molasses, and magnesium sulfate could be determined by ridge analysis (Balamurugan *et al.* 2021), and the results are shown in Table 5. On the basis of ridge analysis, it was predicted that the maximum MOR of fiberboard could reach 26.683±0.0.158 MPa, when the mass of bran, molasses, and magnesium sulfate were 3.021 g, 8.907 g, and 0.27 g, respectively. To verify the predicted results, the validation experiments were performed in triplicate. Under the optimized condition, MOR and MOE of fiberboard reached to 27.21 ± 0.64 MPa and 3.81 GPa, respectively, which implied that experimental and predicted values (26.683 MPa) were in good agreement. In addition, MOR of the transversely cutting specimen, longitudinally cutting specimen, and 45° obliquely cutting specimen were 27.3, 27.9, and 26.8 MPa, respectively. So, the fiberboard could be approximated as an in-plane isotropic material. It was found that MOR of fiberboard using PWS bio-pretreated with *C. versicolor* under the optimized conditions was 2.2 times that of control fiberboard and 1.08 times that of fiberboard prepared by cellulase pretreatment (Wu *et al.* 2020b). It was further found that the strength of the fiberboard increased from 22.7 MPa (Wu *et al.* 2020a) to 27.21 MPa. The reason might be that the addition of bran and magnesium sulfate during the bio-pretreatment increased the enzyme activity of manganese peroxidase (Wu *et al.* 2020a), which improved the modification of lignin. The residual molasses in PWS also provided additional hydroxyl groups. Those led to an increase in the self-bonding active sites and improvement in the strength of fiberboard.

Table 5. Ridge Analysis for MOR as Response

Radius	Bran (g) (x_1)	Molasses (g) (x_2)	Magnesium sulfate (g) (x_3)	**Predicted Response**	**Standard Error**
0	3	9	0.3	26.657	0.162
0.1	3.006	8.907	0.290	26.671	0.162
0.2	3.014	8.902	0.280	26.679	0.160
0.3	3.021	8.907	0.270	26.683	0.158
0.4	3.029	8.915	0.260	26.683	0.156
0.5	3.036	8.925	0.250	26.680	0.154
0.6	3.043	8.936	0.240	26.673	0.152
0.7	3.051	8.946	0.230	26.662	0.153
0.8	3.058	8.958	0.220	26.648	0.157
0.9	3.065	8.969	0.210	26.630	0.164
1	3.073	8.980	0.200	26.609	0.177

The density of the fiberboard reached 982 kg/m^3. The relationship between the density and strength of fiberboard was further analyzed, as shown in Fig. 3.

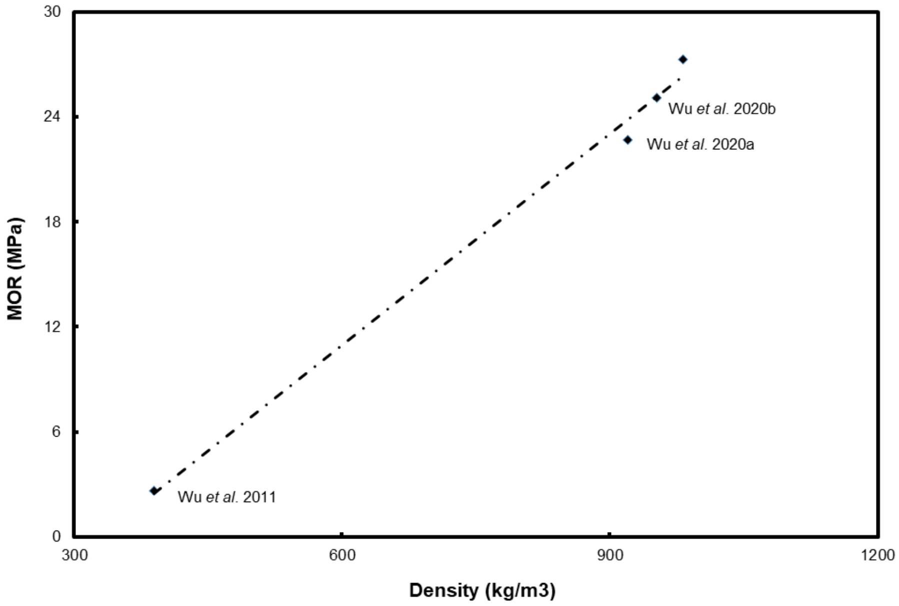

Fig. 3. The relationship between fiberboard density and strength

There was a significant positive correlation (R^2 > 0.99), which indicated that the strength of fiberboard might be improved by increasing the density of fiberboard. One of the methods to increase the density of fiberboard was to improve the self-bonding properties of PWS. In this study, the self-bonding property of PWS was improved by bio-pretreatment after optimization, which led to increasing the density of fiberboard and improving its strength. The WSR of the fiberboard was 21.4 %, which indicated that the fiberboard had a good water resistance performance. However, The WSR of the fiberboard was higher than that of early study (Wu *et al.* 2020a,b). It was perhaps due to the fact that the residual molasses in PWS led to the increase of WSR of the fiberboard. Consequently, bio-pretreatment should be a good choice to produce high-strength binderless fiberboard.

CONCLUSIONS

The optimization condition of bio-pretreatment was obtained as 3.02 g of bran, 8.91 g of molasses, and 0.27 g of magnesium sulfate. Under the optimized condition, the MOR and MOE of fiberboard reached 27.21±0.64 MPa and 3.81 GPa, respectively, and the MOR was 2.2 times that of control fiberboard. The density and WSR of the fiberboard reached 982 kg/m^3 and 21.4%, respectively. Bio-pretreatment with *C. versicolor* should be a good choice to produce high-strength binderless fiberboard.

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