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Immobilizing laccase to cellulose-biochar composite beads for removing phenol from an aqueous system

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ABSTRACT

Novel modified cellulose/biochar beads (MCBBs) were prepared and utilized as support to immobilize laccase to remove phenol from an aqueous system. Nano $CaCO_3$ was introduced in CBBs prepared stage to produce proper pore structure by reacting with HCl solution. CBBs were then modified with maleic anhydride to introduce carboxyl groups. By adsorption and covalent bonding, the laccase was immobilized on the MCBBs. The pH, laccase concentration, and contact time were chosen as the main parameters in the laccase immobilizing experiment and the effects of these parameters on immobilization rates and recovered activity were studied. The results demonstrated that the best conditions are: pH 4.0, concentration 8 g L^{-1}, and contact time 6 h. The thermal and operational stability of the immobilized laccase has attended a good performance under these conditions. The immobilized laccase was used to degrade phenol in an aqueous system and 40.97% removal was achieved after 72 h. The results suggest that as a new green material, MCBBs can be good support for immobilizing laccase. This technique has potential advantages in removing phenol from wastewater.

Keywords: Phenolic compound pollutants; Modified cellulose/biochar beads; Adsorption; Laccase immobilization

1. Introduction

Environmental pollution control has always been the focus of scientific research. In recent years, more and more environmental problems have been exposed to the economic development, especially wastewater pollution. Chemical plants with phenol or phenolic compounds as raw material produce a large amount of phenol wastewater in the production process [1]. Effluents from the pulp and paper industry are especially problematic because the effluent normally contains high concentration phenolic compounds and deep color materials, lignin, and its derivatives, which are released during pulping and bleaching processes [2]. Phenol, also known as carbolic acid, is an important chemical raw material [3]. Phenol is a cytoplasmic toxin and contains highly toxic, and it can enter the human body through the respiratory tract and skin [4]. It can also lead to serious pollution problems in water and soil if a large amount of phenol is directly discharged into the environment without strict standardized treatment [5].

At present, there are four main methods to treat wastewater containing phenol: physical method [6], chemical method [7], the biological method [8], and adsorption [9]. Biological method has advantages of environmental friendliness and no secondary pollution [10], and it plays an increasingly prominent role in treating environmental pollution caused by phenolic substances in recent years [11,12]. Laccase is a polyphenol oxidase containing four copper ions [13], which can degrade many kinds of phenols and phenolic compounds by catalytic oxidation [14]. Copper ions

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contained in laccase can transfer electrons. So, they can remove electrons from the hydroxyl groups of phenolic compounds to generate free radicals. In this reaction, phenolic compounds are oxidized and the product is water. Laccase can undergo redox reactions with many stable substrates to produce water without intermediate products. Therefore, laccase was widely applied in the field of environmental governance and it can significantly reduce toxic pollutant discharge [15,16]. But, on the other hand, laccase is hard to reuse in the wastewater treatment process because it is easily dissolved in water, which limits its application in the actual wastewater treatment process [17,18]. The immobilization technology of laccase is an effective way to solve the deficiency mentioned above, and it can greatly improve the stability of laccase and reduce the cost [19].

Biochar is a solid residue produced by biomass pyrolysis [20]. Recent studies have shown that biochar as a biosorbent can be used as a useful material for adsorbing heavy metal ions from contaminated water, and it can also be used as a medicine to fix metal ions in metal-contaminated soil [21]. For example, Marek et al. [22] verified that modified biochar (saturated with $FeCl_3$ solution, and then neutralized with NaOH solution) can adsorb significant amounts of phosphorus from wastewater. Due to its developed pore structure, strong adsorption capacity, and high environmental stability [23,24], biochar is feasible as a carrier for immobilizing laccase [25]. In our lab, two kinds of granular biochar have been investigated for laccase immobilization [20]. At present, there are few studies on the adsorption and fixation performance of powdered biochar as a laccase carrier and its application in phenols wastewater pollution control [26,27].

In this study, powdered biochar and cellulose, known as green, degradable, and easy to modified material [28,29], were used to produce a novel adsorbent for laccase immobilizing. Nano $CaCO_3$ was introduced into the cellulose/biochar beads (CBBs) to generate appropriate pore structure, then the CBBs were modified by maleic anhydride to prepare MCBBs, and carboxyl groups were achieved on the beads. The beads with porous structure and functional groups have the function of adsorbing laccase and covalently bonding with laccase. MCBBs were used as a carrier of laccase to obtain immobilized laccase. The effects of immobilization conditions on the immobilization yield and recovery activity were studied. Finally, the effectiveness of immobilized laccase for degradation of phenol in an aqueous solution was investigated. From the perspective of wastewater treatment, this technology has the potential to remove phenol from wastewater.

2. Materials and methods

2.1. Materials

$Na_2HPO_4{\cdot}12H_2O$, anhydrous citric acid, maleic-anhydride, urea, coomassie brilliant blue (CBB), 95% ethyl alcohol, 85% H_3PO_4, and NaCl were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd., (Tianjin, China). Bovine serum albumin (BSA), sodium hydroxide, $CaCO_3$ powder (≤30 μm), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and phenol were purchased from Sigma Chemical (China). Ionic liquid used in this experiment is 1-allyl-3-methyl imidazolium chloride which was purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences. Laccase (50 U mg^{-1}) was purchased from Shanghai Zeye Biotechnology Co., Ltd., (Shanghai, China). Biochar was produced in the lab. The cellulose was obtained from Filter paper (i-Quip®, Q5780 Qualitative Filter Paper, Slow Flow, Filtration Speed 70~140 s, Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China).

2.2. Preparation of cellulose-biochar beads

Biochar was ground and filtered first, and then the powder with a size between 150 and 200 mesh was collected. Before producing the beads, the biochar powder was washed 3 times with distilled water to remove the impurities and then put in the oven for 24 h at 110°C. The above process is shown in Step 1 of Fig. 1.

As shown in the second step of Fig. 1, 4 g filter paper was cut into small pieces (almost 1 mm × 1 mm) first and then put into 6 g ionic liquid and stirred using a magnetic stirrer (IKA, RT15, Germany) for 20 min at 50°C, 4% concentration cellulose solution was obtained. Then follow the illustration in Step 3, 2 g biochar and 1 g $CaCO_3$ powder were added into the cellulose solution and stirred for 10 min. Through the same operation, the mixtures with different amounts of $CaCO_3$ powder (1, 2, 3, 4, and 5 g) were obtained. A 20 mL syringe with a flat needle (inner diameter 0.51 mm) was utilized to produce the beads by dropping the mixture into 1 M hydrochloric acid solution. $CaCO_3$ in the CBBs reacted with the HCl immediately and the reaction was kept until no more CO_2 bubbles released from the CBBs surface.

The generation of CO_2 inside the CBBs promotes the formation of the pore and channel structures in the beads. Then the CBBs were washed with distilled water 3 times to remove the $CaCl_2$ generated in the reaction and placed in the freeze-dryer (LYOALFA, Ningbo Xinzhi Biotechnology Co., Ltd., China) for 24 h to remove water.

2.3. Modification of CBBs

The process of modification is shown in Fig. 2. 2 g maleic anhydride dissolved in 20 mL acetone and then reacted with CBBs at 25°C for 4 h [30]. After the reaction, the CBBs were placed in a vacuum oven at 55°C for 0.5 h to remove the acetone, then the beads were washed 3 times with the distilled water and put into the freeze-dryer for 24 h. In this section, additional –COOH groups were generated because the hydroxyl group in cellulose reacted with maleic anhydride. As a result, the MCBBs were obtained. The modified beads were successfully grafted with a carboxyl group, which can react with the amino group on laccase and so the laccase was immobilized in MCBBs.

2.4. Hydroxyl groups determination

According to a titration method, an auto titrator (ZDJ-4B, Micromeritics Instrument (Shanghai) Ltd., China) was used to determinate the substitution degree of hydroxyl groups on cellulose with the carboxyl group of MCBBs [31].

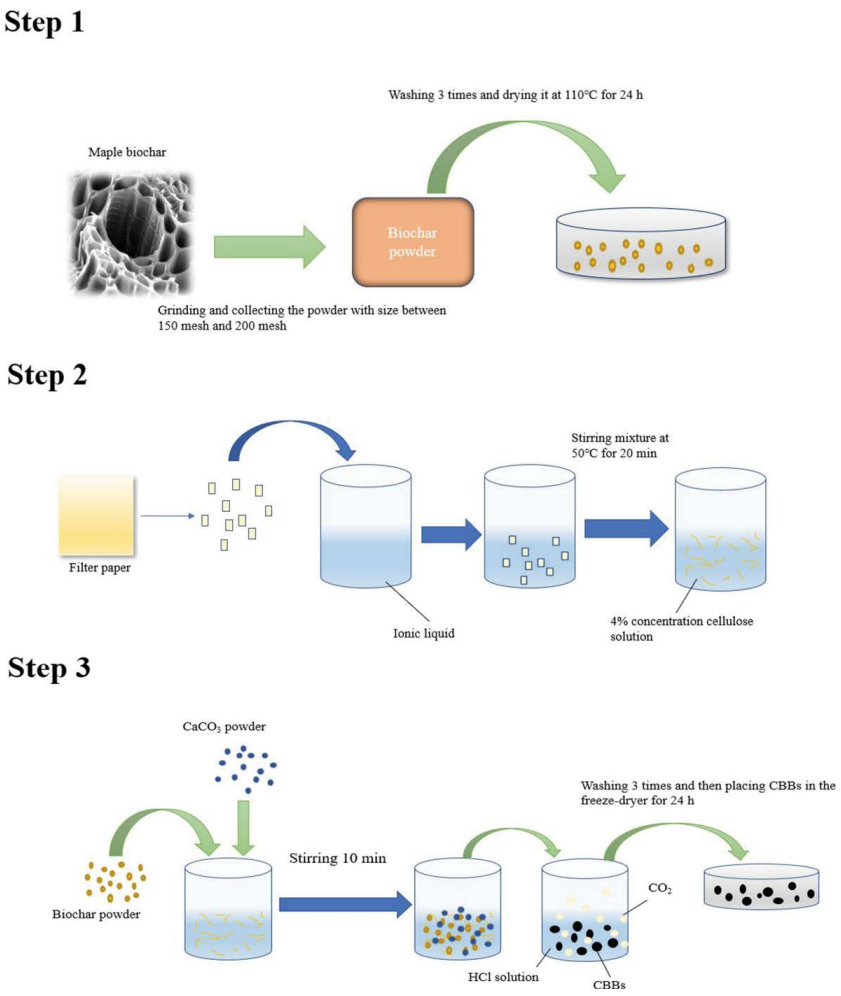

Fig. 1. Preparation of cellulose/biochar beads (CBBs).

The titration agent was 0.01 N of NaOH solution. Repeat the experiment three times and take the average.

2.5. Characterization of MCBBs

In order to determine the existence of carboxyl groups on the MCBBs, Fourier-transform infrared (FT-IR) (Frontier, PerkinElmer, USA) was utilized to analyze the chemical structures of MCBBs and that of CBBs. A scanning electron microscopy (SEM) was used to observe the surface structure of MCBBs (JEOL 6400, JEOL, Tokyo, Japan). The MCBBs were first placed in liquid nitrogen for 15 min, then pinched off with tweezers to form a cross-section. Before measurement, gold was coated on the samples first. The specific surface area of MCBBs were determined with Brunauer–Emmett–Teller (BET, ASAP2020, Micromeritics Inc., USA).

2.6. Determination of enzyme activity

The immobilized and free laccase were reacted with 0.4 mM ABTS to determinate the laccase activity at 25°C ± 1°C [32]. 0.1 mL free laccase solution was dropped into 2.9 mL ABTS solution and the UV absorbance was determined at 420 nm (S420 = 36,000 m^{-1} cm^{-1}) by UV-visible spectrophotometer (UV-1200, PerkinElmer, USA). Recorded the data of the UV absorbance every 30 s and persisted 5 min. All the data was utilized to form a kinetic curve, and then measure the slope of initial linear portion of the curve. Enzyme activity was determined by using the following equation [33]:

$$\text{U/mL} = \frac{\left(A_{\text{abs/min}} \times f_{\text{dil}} \times V_r \times 10^6\right)}{\left(\varepsilon \times V_{\text{lac}}\right)} \quad (1)$$

The unit of free laccase activity is U mL^{-1}, 1 U is defined as the amount of free enzyme needed to oxidize1 μmol substrate per minute under specific conditions (25°C). Here, $A_{\text{abs/min}}$ represents the change in absorbance value within a certain period of time, f_{dil} is the dilution factor of the sample, V_r is the volume of reaction, ε is the ABTS molar absorption coefficient (3.6 × 10^4 M^{-1} cm^{-1} at 420 nm), V_{lac} is the volume of free laccase before dilution.

2 mL ABTS solution and 7 mL citrate-phosphate buffer were mixed in a 20 mL tube, and then 0.1 g support-laccase was added to the mixture solution to determine the immobilized laccase activity. Every 2 min, 2 mL of the mixture solution was withdrawn from the tube and was measured

the absorbance at 420 nm by the UV-Vis spectrophotometer (UV-1200, PerkinElmer, USA) and was poured back to the tube immediately after analysis. Repeat the operation for 8 times and use the data to form a kinetic curve and the slope of the initial portion was obtained. Immobilized laccase activity was calculated by the following formula:

$$\text{U/g} = \frac{\left(A_{\text{abs/min}} \times f_{\text{dil}} \times V_r \times 10^6\right)}{\left(\varepsilon \times m_{\text{MCBBs}}\right)} \quad (2)$$

Unit of immobilized enzyme activity was denoted in U mg^{-1}. Where U g^{-1} is the enzyme amount per mass unit of a carrier that is capable to oxidize 1 μmol of ABTS per minute, m_{MCBBs} is the mass of immobilized laccase added to the solution (g).

2.7. Immobilization of laccase

The conditions for immobilizing laccase on MCBBs were optimized first. A total of 10 mg of MCBBs were incubated in 10 mL of laccase solution containing 2–8 g L^{-1} of enzyme in a 20 mL bottle at varying pH from 3.0 to 6.0 at the room temperature. The bottles with the solutions were stirred on a magnetic stirrer. At the optimized conditions of pH and enzyme concentration, the effect of contact time was evaluated in the range from 12 to 96 h. After immobilization, the support was collected by filtration and washed three times with 0.1 M of phosphate buffer (pH 4.0) (±100 mL each wash). The immobilization process is shown in Fig. 3. The filtrate was kept for enzyme activity measurements. The immobilized enzyme activity was measured as described above.

To research the primary influence conditions on immobilizing laccase on MCBBs, a response surface methodology (RSM) was devised and analyzed by the Box–Behnken design of Design-Expert software (8.0.6). The pH value, contact time, and laccase solution concentration were chosen as the main factors for the immobilization experiment [34,35]. Table 1 shows the immobilization conditions of pH (X_1), contact time (X_2), and laccase concentration (X_3) with the details of lower and upper limit values. For creating response surfaces, the experimental data obtained based on the above design were fitted to second-order polynomial equation of the form.

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1^2 + A_5X_2^2 + A_6X_3^2 + A_7X_1X_2 + A_8X_1X_3 + A_9X_2X_3 \quad (3)$$

where Y is the response in immobilized yield; A_0 is a constant; A_1, A_2, and A_3 are the linear coefficients; A_4, A_5, and A_6 are the quadratic coefficients; A_7, A_8, and A_9 are the cross-product coefficients.

The immobilization yield was calculated as follows:

$$\%\text{immobilization} = \frac{\text{original enzyme concentration} - \text{enzyme concentration after immobilization}}{\text{original enzyme concentration}} \times 100\% \quad (4)$$

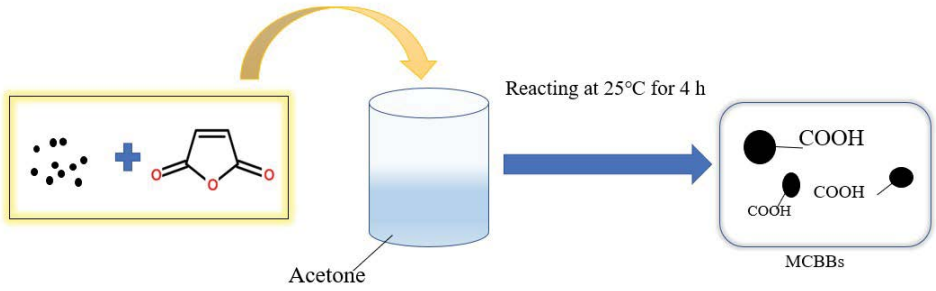

Fig. 2. Modification of CBBs.

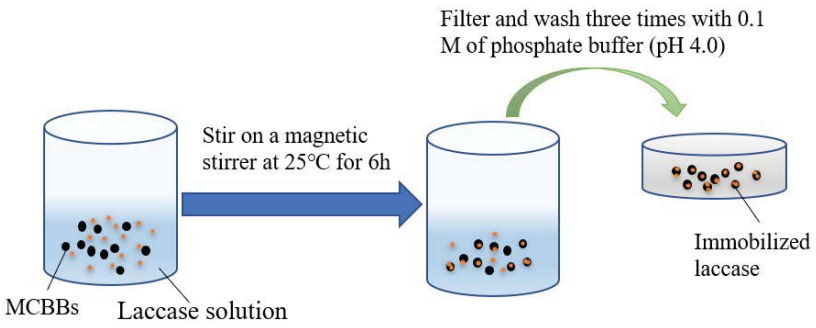

Fig. 3. Process of immobilizing laccase.

Table 1
Factors and levels in Box–Behnken design

	Coded-level variables		
Variable	–1	0	1
X_1: pH	3	4	5
X_2: Contact time (h)	2	5	8
X_3: Laccase concentration (g L^{-1})	2	5	8

Measurement of enzyme concentration referred to the Bradford method [36,37].

The recovered activity was determined by using the following equation:

$$\%\text{recovered activity} = \frac{\text{immobilized enzyme activity}}{\text{activity of same amount of free enzyme}} \times 100\% \quad (5)$$

2.8. Thermal and operational stability of immobilized laccase

An experiment of the thermal stability evaluation was proceeded under the following conditions: pH = 4.0, T = 60°C. The experiment lasted 6 h, and the enzyme activity of the free laccase and immobilized laccase was detected every 1 h and the initial activity was set as 100%.

0.2 g MCBBs-Laccase was used to evaluate the operational stability, the experiment condition is: 2 mL ABTS solution and 7 mL citrate-phosphate buffer at pH 4.0, T = 25°C ± 1°C. 7 cycles experiments were operated. The immobilized laccase activity in the first cycle was set as 100%. After each cycle, the MCBBs-Laccase was collected by filtration and washed twice with 25 ml of pH 4.0 buffer solution before the next cycle.

2.9. Degradation of phenol by immobilized laccase

In order to prepare phenol aqueous solution, 100 mg phenol was added in 50 mL of deionized water in volumetric flask, then the final volume was adjusted to 1,000 mL. The degradation yield of the phenol was evaluated by introducing 50 mg MCBBs-Laccase into 10 mL of phenol aqueous solution and 10 mL of pH 4.0 phosphate buffer. In order to measure the degradation yield, the concentration of phenol was measured per 12 h, and the whole experiment was lasted for 96 h. The concentration of phenol aqueous solution can be calculated from the standard curve of absorption at 269 nm. Degradation yield (%) was defined as follows:

$$\%\text{degradation} = \frac{\text{original phenol amount} - \text{final phenol amount}}{\text{original phenol amount}} \times 100\% \quad (6)$$

A control experiment, the amount of free laccase equal to 50 mg MCBBs-Laccase was added into 10 mL of phenol aqueous and 10 mL of pH 4.0 phosphate buffer, was operated at the same time.

2.10. Statistical analysis

For each assay duplicate or triplicate measurements were conducted, and all data were presented as mean ± SD.

3. Results and discussion

3.1. Optimal calcium carbonate concentration for preparing CBBs

BET surface area of CBBs at different calcium carbonate concentrations is shown in Fig. 4. In the process of preparing CBBs, $CaCO_3$ played an important role. This role was to react with acid to release carbon dioxide gas, thereby creating the pore structure inside the cellulose spheres. According to the data of BET, as the initial concentration of $CaCO_3$ increased from 0% to 30%, the specific surface of CBBs increased from 21.8895 to 49.1976 m^2 g^{-1}. However, when the concentration of $CaCO_3$ exceeded 30%, the BET value was lowered. The results indicated that the use of $CaCO_3$ was favorable to the formation of pore structure, but excessive use may lead to the collapse of CBB pores, this result was in line with the study of Li et al. [30].

The BET surface area was related to the laccase immobilization rate. A larger surface area means more porous structures within biochar. Also, a large surface area could support more opportunities for CBBs to contact with laccase, which was a benefit for immobilizing laccase and increasing the adsorption rate. Therefore, the optimal calcium carbonate concentration was 30%. In this condition, the BET surface area and micropore volume were 49.1976 and 0.024457, respectively.

3.2. Characterization of MCBBs

3.2.1. FTIR spectra analysis

Fig. 5 shows the FTIR spectra of MCBBs and CBBs.

Because of the stretching of the O–H group, strong adsorption emerged at 3,440 cm^{-1}. The peak of 2,902 cm^{-1} was related to the vibration of C–H which is from cellulose. In the comparative of the spectra of MCBBs and CBBs, it can be observed that the bands appearing at 1,722 and 1,653 cm^{-1} were the most changes. The peak of 1,722 cm^{-1} correlated with the carboxyl groups (C=O), while the band at 1,653 cm^{-1} was due to the vibration of the vinyl groups (C=C). The FTIR spectra confirmed the maleic anhydride has been grafted onto the cellulose surface successfully, which was owing to the existence of the adsorption peaks from carboxyl and vinyl groups. The amount of carboxyl groups was defined by titration, and the result indicated that the carboxyl concentration of MCCBs was 1.1975 mmol g^{-1}.

3.2.2. SEM analysis

The inner structure of the MCBBs was measured by scanning electron microscopy. Quite a lot of hollow structures and channels were formed inside the beads, as Fig. 6 shows. It investigated that a poly porous material was successfully achieved by the method mentioned above. The formation of carbon dioxide promoted the formation of pores and channels in the sphere due to the reaction of calcium carbonate in the CBBs with the HCl solution.

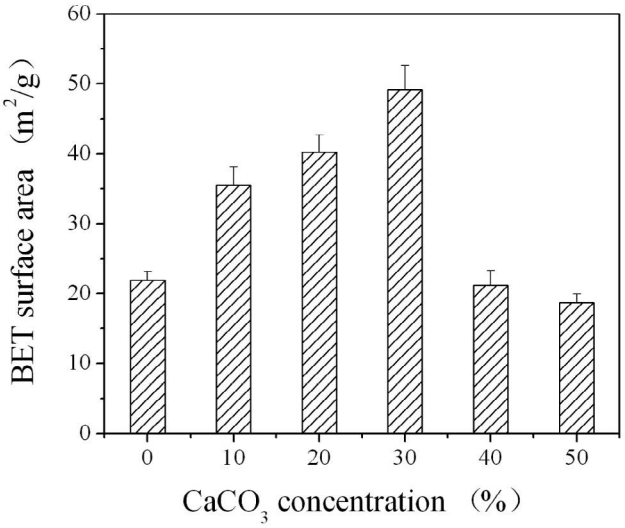

Fig. 4. BET surface area of CBBs at different calcium carbonate concentrations.

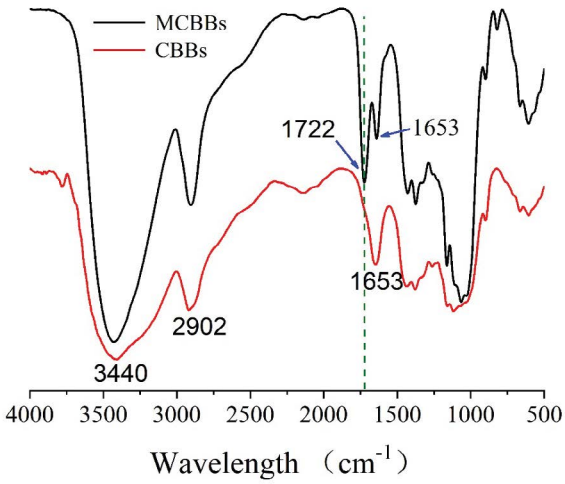

Fig. 5. FT-IR of MCBBs and CBBs.

3.3. Optimum laccase immobilization conditions

Box–Behnken design (BBD) was applied to optimize the immobilization parameters in the immobilization of laccase on modified cellulose/biochar beads (MCBBs). Immobilization conditions of pH (X_1), contact time (X_2), and laccase concentration (X_3) were optimized with the immobilization yield collected as the response. The immobilization yield from the experiments were collected from range of 14.87% to 27.21%. Immobilization of laccase on MCBBs was described with the quadratic polynomial model. The coefficient of determination (R^2) of 0.9957 and adjusted coefficient of determination (R^2_{adj}) of 0.9902 were recorded by the model indicated that both observed and predicted value of immobilization yield were in a good range. Moreover, 0.9745 of predicted R^2 showed that the model obtained was applicable to predict the optimum immobilization condition to maximize the immobilization of laccase on MCBBs. The regression equation was used to predict the response by factors in terms of coded level as shown in Eq. (7):

$$Y = 25.47 + 0.35X_1 + 2.22X_2 + 2.73X_3 - 2.57X_1^2 - 2.00X_2^2 + 0.10X_3^2 - 0.26X_1X_2 + 0.20X_1X_3 - 0.73X_2X_3 \quad (7)$$

ANOVA is shown in Table 2. We can find that the model was highly significant. The value of determination coefficient (R^2 = 0.9957), a small P-value, and no significant lack of fit, indicated good relation between the predicted and experimental values. The results demonstrated that the model developed was successful.

The typical response surfaces are shown in Figs. 7 and 8. The maximum response can be obtained from point A

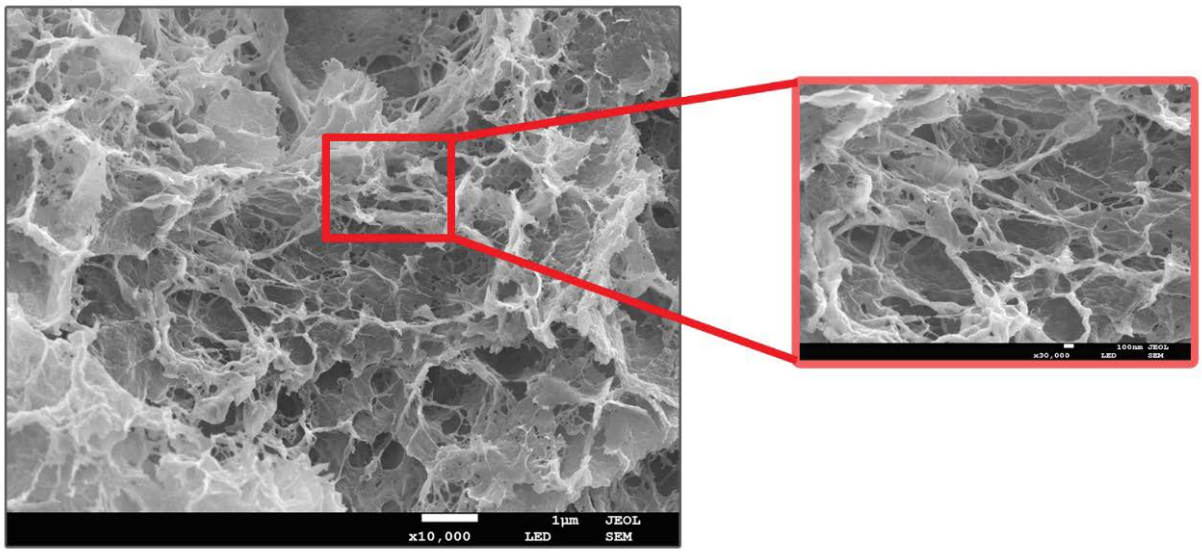

Fig. 6. Scanning electron micrograph of interior of MCBBs.

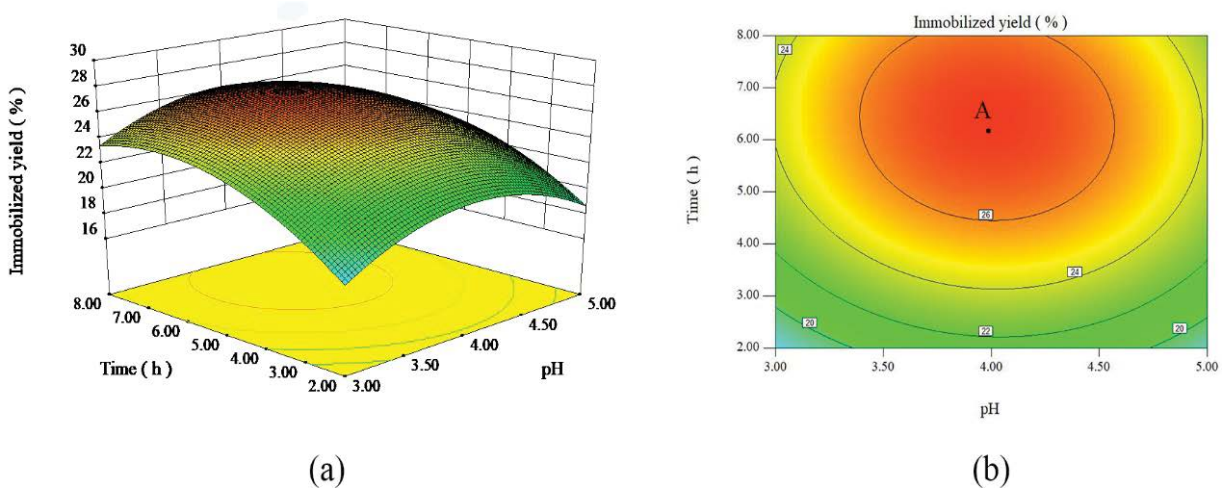

Fig. 7. Response surface (a) and contour plot (b) of pH value and time against the yield of laccase immobilization.

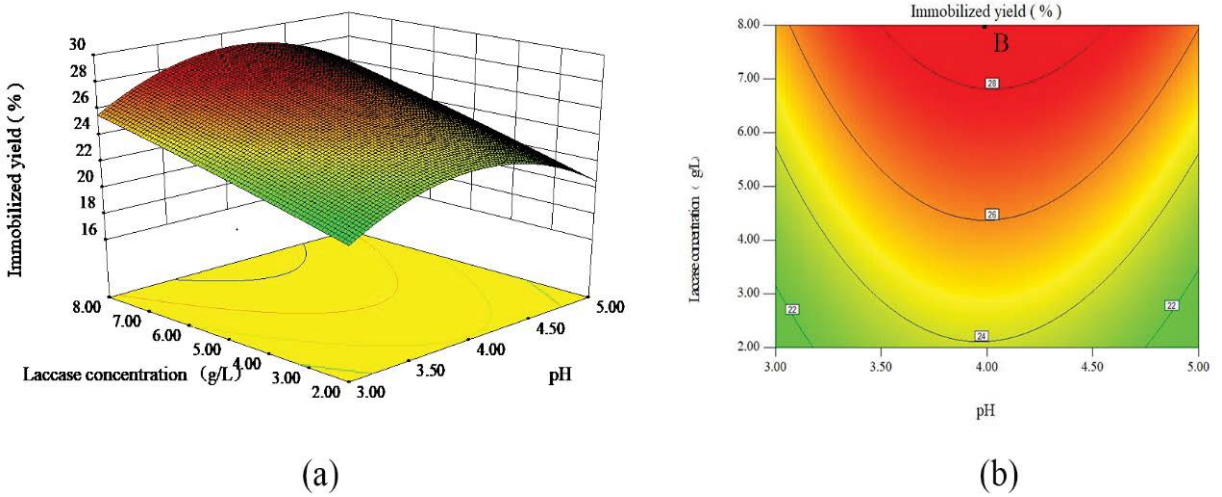

Fig. 8. Response surface (a) and contour plot (b) of laccase solution concentration and pH value against the yield of laccase immobilization.

in Fig. 7b and point *B* in Fig. 8b where the immobilized yield achieved 27.71%. Fig. 7 shows the interaction of pH value and contact time. As the pH raised from 3.0 to 4.0 and the contact time increased from 2 to 5 h, the immobilized yield increased. This indicated that the increase of contact time had enhanced the immobilization process by providing sufficient contact time between the enzyme and the support thus increase the amount of enzyme interacted with the support. However, as pH value varied from 4.0 to 5.0 and contact time raised from 5 to 8 h, the immobilized yield was reduced. At this phase, the desorption of the enzyme might be occurred due to the saturation of immobilization capacity on the surface of MCBBs. According to Fig. 8, the interaction between laccase concentration and pH value increased with the addition of laccase concentration. The reason for this result may be that the fixed amount of adsorbent can fully contacted more laccase when the system has a large amount of laccase under the same conditions.

In conclusion, the immobilization parameters for the adsorption of laccase on MCBBs were analyzed and the optimum conditions for each factor were predicted: pH value 3.81, reaction time 6.15 h, and laccase concentration 7.93 g L^{-1}. However, in order to better control the experimental conditions, the pH value, reaction time and laccase concentration were adjusted to 4.0, 6 h, and 8 g L^{-1}, respectively.

3.4. Thermal and operational stability of immobilization laccase

Thermal stability and operational stability are two significant parameters that affect the application of immobilized laccase. The thermal stability is shown in Fig. 9, and the operational stability is shown in Fig. 10. In order to assess the thermal stability of the immobilized laccase, the remaining enzyme activity of free laccase and immobilized laccase was measured at 60°C for 6 h. The optimal experimental conditions for immobilized laccase were:

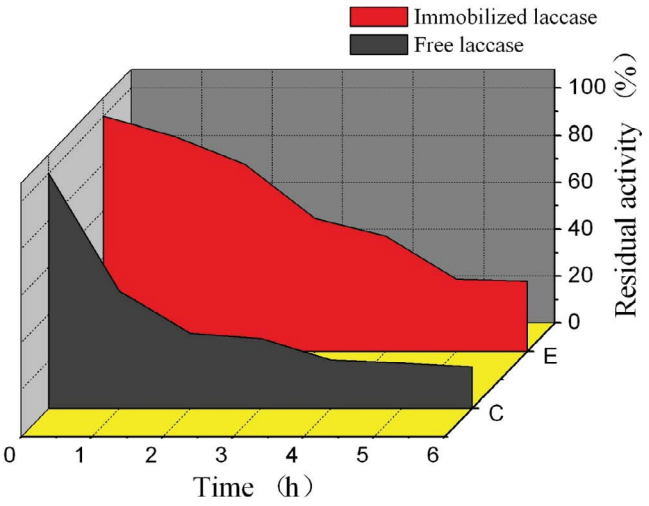

Fig. 9. Thermal stability of free laccase and laccase immobilized on MCBBs by absorption at pH 4.0, 8 g L^{-1} initial laccase concentration, and 6 h contact time.

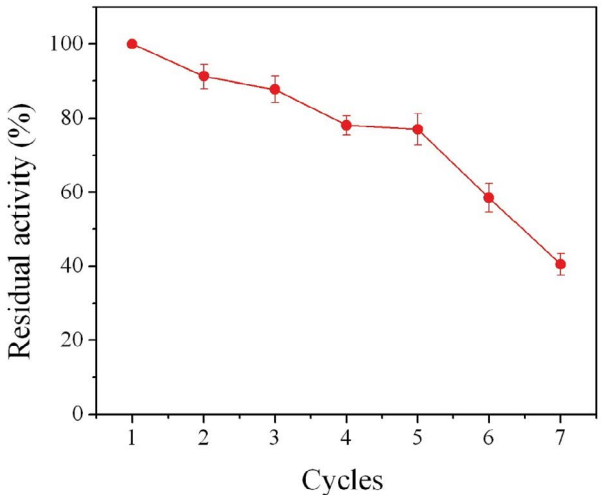

Fig. 10. Operational stability of laccase immobilized on MCBBs by absorption at pH 4.0, 8 g L^{-1} initial laccase concentration, and 6 h contact time.

laccase concentration 8 g L^{-1}, pH 4.0, and contact time 6 h. As shown in Fig. 9, the thermal stability of immobilized laccase at 60°C was distinctly better than that of free laccase. When the experiment finished, the primary activity of immobilized laccase was kept at 83%, and the retention rate of free laccase was only 56%. The increase of immobilized laccase activity is owing to the limited degree of freedom of immobilized laccase, which reduces the chance of drastic conformational changes and increases the stability of laccase [38]. Immobilization increased laccase rigidity and decreased laccase conformational flexibility [39].

Compared to free laccase, immobilized laccase has the advantage of being reusable which plays an important role in cost savings. The cyclic oxidative study of immobilized laccase was conducted under optimal conditions, and a 10-cycle carrier experiment was implemented to assess the stability of immobilized laccase. The result is shown in Fig. 10. At the end of the fifth cycle, the activity of the immobilized laccase was reduced to 63%. The physical adsorption immobilization exhibited weak binding forces between enzyme and carrier. Thus, the activity loss may have resulted from the laccase leaching during the washing stages [40]. Thereafter, the remaining activity tended to be gentle and retained about 60% of the original activity after 6 cycles. Although some results obtained in other studies were better than that in this work, which mainly because the support and immobilizing methods were different. Cellulose is a novel laccase immobilized carrier. The results of this study indicate that the laccase immobilized with MCBBs has broad prospects in the application.

3.5. Degradation of phenol by immobilized laccase

The immobilized laccase was utilized to degrade the phenol in wastewater. The results are shown in Fig. 11. At the 72nd hour, the removal of phenol by MCBBs-laccase and free laccase both reached the highest point and then began to decrease. The maximum removal was 40.97%. The reason for the decrease in removal is probably because the accumulation of decomposition metabolites may inhibit

Table 2
ANOVA for optimization of the immobilization parameters

Source	Sum of squares	Degree of freedom	Mean square	*P*-value
Model	149.51	9	16.61	0.0001
X_1 (pH)	0.99	1	0.99	0.0134
X_2 (Contact time)	39.39	1	39.29	0.0001
X_3 (Laccase concentration)	59.46	1	59.46	0.0001
X_1X_2	0.28	1	0.28	0.1243
X_1X_3	0.16	1	0.16	0.2290
X_2X_3	2.12	1	2.12	0.0020
X_1^2	27.86	1	27.86	0.0001
X_2^2	16.84	1	16.84	0.0001
X_3^2	0.042	1	0.042	0.5197
Lack of fit	0.20	3	0.065	0.6590

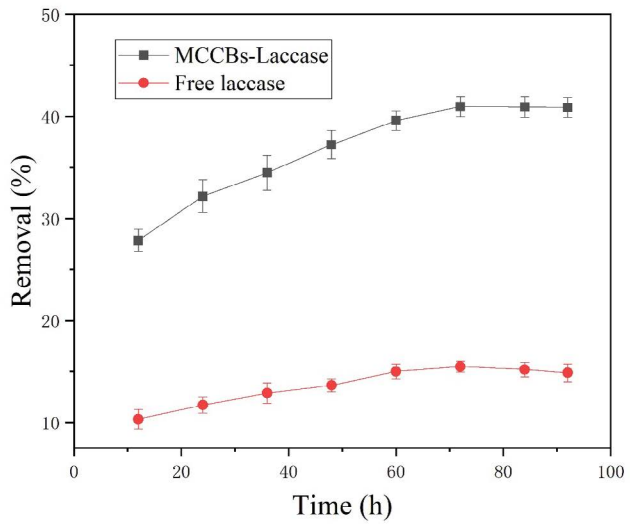

Fig. 11. Removal of phenol by immobilized laccase and free laccase at pH 4.0 room temperature.

the removal process, and a similar result has been reported by Yang et al. [41] in 2016.

The removal of immobilized laccase was obviously higher than that of the control group, indicating that phenol was effectively degraded by immobilized laccase. This further demonstrated that MCBBs have an adsorption effect on phenol. In summary, the removal of immobilized laccase probably due to the synergistic effect of enzymatic chemical degradation and adsorption of MCBBs.

4. Conclusion

In summary, CBBs were successfully prepared using maleic anhydride-modified cellulose and nano $CaCO_3$ powder as pore-forming agents and it was used to immobilize laccase in an attempt to degrade phenol. Stability analysis shows that MCBBs-laccase has good thermal stability, pH stability, storage stability, and reusability. As a green and environmentally friendly material, MCBBs can be used as a suitable carrier to immobilize laccase. The technology developed in this study has potential advantages in removing phenolic pollutants from wastewater.

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