



## Study on improving the stability of adsorption-encapsulation immobilized Laccase@ZIF-67

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### ARTICLE INFO

#### Article history:

Received 11 September 2020

Received in revised form 14 October 2020

Accepted 1 November 2020

#### Keywords:

Zeolite imidazolate framework-67 (ZIF-67)

Laccase

Enzyme immobilization

Enzyme activity

Stability

### ABSTRACT

Laccase (*Trametes versicolor*) was immobilized onto/into zeolite imidazolate framework-67 (ZIF-67) for the first time, materials were performed by assisting one-pot synthesis strategy in aqueous solution at room temperature. The resulting laccase@ZIF-67 composite was characterized by powder X-ray diffraction (PXRD), fourier transform infrared (FT-IR) spectroscopies, field emission scanning electron microscopy (FE-SEM) and energy dispersive X-ray (EDX), thermal gravimetric analyses (TGA). The results showed that laccase could be well immobilized on ZIF-67 materials. Laccase@ZIF-67 was stable on storage stability and reuseability, retaining 88 % (15 days at 4 °C) and 59 % (five reaction cycles) of residual enzyme activity. Laccase@ZIF-67 exhibited relatively stable activity at pH 3–5. In addition, the thermal deactivation kinetic studies of laccase@ZIF-67 showed a lower  $k$  value, higher  $t_{1/2}$  and  $\Delta G$  values along with the enhancement of thermodynamic parameters than that of free laccase, and laccase@ZIF-67 had excellent level of thermostability.

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## 1. Introduction

Laccases (benzenediol: oxygen oxidoreductase EC 1.10.3.2) are a type of lignin-modifying enzymes that are able to oxidize a wide range of molecules. As a clean, effective and green biocatalyst, its theoretical research and technical application have been developed for decades. Due to their low specificity, several organic compounds such as polycyclic aromatic hydrocarbons [1], aromatic amines [2], phenolic compounds [3], etc. are the substrates of laccase [4]. Hence, laccases can be used for applications such as bleaching of deinked pulp [5], paper pulp [6], bioremediation [7], the removal of phenolic pollutants [8] and the biodegradation of industrial dyes [9]. Despite free laccase can offer excellent activities, the practical application is limited by its poor stability. In aqueous solution, the free laccase is difficult to recover and reuse, while immobilized laccases are more robust to a variety of complex environment [10], furthermore, the immobilized laccase exhibit good reusability and storage stability [11]. For instance, a laccase from *Lentinus polychrous* immobilized in barium

alginate could be reused for five reaction cycles in the degradation of acetaminophen contaminated in aqueous solution, the capability of removal and enzyme activity were retained above 70 % [12]. Immobilization means coupling the catalyst to an insoluble carrier matrix to maintain the proper geometry [13]. Laccase immobilization methods are generally classified into adsorption, covalent bonding, encapsulation and cross-linking [14]. The catalytic activity and stability of immobilized laccases may be affected by the immobilization procedure and the nature of the solid support. Each of these methodologies for laccase immobilization has limitations and disadvantages. Therefore, the application of immobilization of laccase by two methodologies in combination might properly obtain better results.

Metal organic frameworks (MOFs) are a class of porous, high surface area materials that have been investigated for a myriad of applications, especially for gas separation [15], gas storage [16], drug delivery [17], heterogeneous catalysis, and enzyme immobilization [18–21]. S.Nadar et al. have done a lot of research on enzyme encapsulation in MOF materials, the immobilized lipase they prepared showed up to 54 % of residual activity (7 cycles of reuse), whereas it retained 90 % of residual activity (25 days) [22]; the immobilized glucoamylase they prepared exhibited up to 57 % of residual activity after (6 consecutive cycles of reuse), whereas it retained 91 % of residual activity (25 days) [23]. Zeolitic

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imidazolate frameworks (ZIFs) are a class of MOFs in which metal centers like  $\text{Zn}^{2+}$  and  $\text{Co}^{2+}$  are linked by imidazole (Im), 2-methylimidazole (mIm) or functionalized imidazole (RIm) ligands [24]. ZIFs are easy to prepare, possess high porosity and surface area, high thermal and chemical stability [25]. There are hundreds of existing ZIFs [26], ZIF-8 ( $\text{Zn}(\text{mIm})_2$ , mIm = 2-methylimidazolate), which is the most common and widely investigated ZIFs [27]. The method to synthesize laccase@ZIF-8 composite has been reported in some literature [28–31]. ZIF-67 ( $\text{Co}(\text{mIm})_2$ , mIm = 2-methylimidazolate), which is also the most representative ZIF materials with a zeolite sod topology [32]. The difference from the chemical composition of ZIF-8 is that the metal centers of ZIF-67 is  $\text{Co}^{2+}$ , and both of them are the most representative ZIF materials with a zeolite sod topology [32]. In addition, other common ZIFs, such as ZIF-4, ZIF-zni, etc., are not suitable as carriers for immobilization of laccase due to harsh preparation conditions, high reaction temperatures, and poor adsorption properties. ZIF-67 has great potential in environmental protection applications, which exhibited superior adsorption capacity compared with common sorbents such as activated carbons. This makes it a potential adsorbent for the adsorptive removal of 1-naphthol from waste water [33]. Pan et al. reported that ZIF-67 was used for the removal of phenol from aqueous solutions via adsorption and shows high adsorption capacity for phenol [34]. Li et al. have demonstrated its fast kinetics and high adsorption efficiency towards chromium removal in water for the first time [35]. S. Nadar et al. have embedded  $\alpha$ -amylase onto ZIF-67 by single pot, they demonstrated that the immobilization process has been well done. And the recycling studies showed 32 % residual activity after 6 cycles [36]. To the best of our knowledge, ZIF-67 has not been used as a carrier for immobilization of laccase, here we reported a method that the laccase was adsorption-encapsulation immobilized onto/into ZIF-67 for the first time.

## 2. Experimental

### 2.1. Materials

Laccase from *Trametes versicolor* (0.89 U/mg, from Sigma Chemical Company), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS,  $\geq 98\%$ ), coomassie brilliant

blue G-250 (Sigma Chemical Company).  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  ( $\geq 97\%$ ), 2-methylimidazole (2-MI,  $\geq 99\%$ ) were purchased from Sinopharm Chemical Reagent Co., Ltd.

### 2.2. Preparation

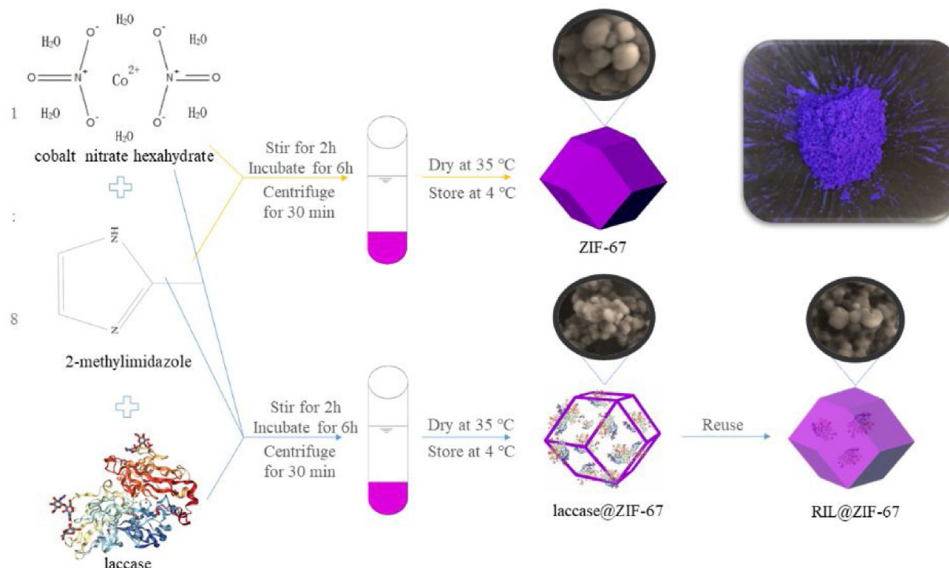
The pure rhombic dodecahedral ZIF-67 microcrystal was synthesized according previous report [36,37], the molar ratio of  $\text{Co}^{2+}$  to 2-methylimidazole is about 1:8 [34]. To the best of our knowledge, this is the first report of the immobilization of laccase within ZIF-67. Typically, 2 mL of an acetic acid-sodium acetate (AA-SA) buffer solution of laccase (20 mg/mL) and cobalt nitrate hexahydrate (0.045 M, 5 mL) were mixed with 2-methylimidazole water solution (0.36 M, 5 mL), then the obtained mixture was stirred at room temperature for 2 h, the reaction mixture was incubated at room temperature for 6 h. Finally, the purple solid was collected by centrifugation at 4000 rpm for 30 min, and the obtained purple powder sample was dried at 35 °C and stored at 4 °C (Scheme 1). The pure carrier synthesis was carried out in the same way except for the fact that no laccase was added.

### 2.3. Characterization

Powder X-ray diffraction (PXRD) analysis were performed through a Bruker D8 Advance x-ray diffractometer using  $\text{Cu-K}\alpha$  radiation at  $2\theta$  from 5 to 35°. Fourier transform infrared (FT-IR) spectroscopies were carried out on a Bruker TENSOR II infrared spectrometer in the range 400–4000  $\text{cm}^{-1}$ . Field emission-scanning electron microscope (FE-SEM) studies at various magnifications were carried out in a Nova 400 NanoSEM at 20 kV, the energy dispersive X-ray (EDX) results obtained from a INCA IE 350 PentaFET X-3 EDS analysis. Thermal gravimetric analyses (TGA) were performed on a STA449 thermoanalyzer, samples were filled into an alumina crucible and heated in a continuous-flow of  $\text{N}_2$  from 30 up to 900 °C.

### 2.4. Laccase activity

Dissolve 10 mg of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate acid) (ABTS) in 73 mL of ultrapure water to prepare ABTS standard solution (0.25 mM). The activity of free laccase was



**Scheme 1.** Schematic representation and photograph of the synthesis of the pure ZIF-67, immobilized laccase (laccase@ZIF-67) composite and reused immobilized laccase (RIL@ZIF-67).

determined spectrophotometrically at 420 nm [38,39]. Briefly, 2 mL of ABTS standard solution (0.25 mM) and 1 mL of laccase/laccase@ZIF-67 solution (10 mg/mL) were mixed in a cuvette at room temperature ( $25 \pm 3$  °C). ABTS as a substrate, laccase catalyzed oxidation of ABTS to  $\text{ABTS}^+$  (Scheme 2) [40], the absorption coefficient of  $\text{ABTS}^+$  at 420 nm is much higher than that of ABTS. Therefore, the change of absorbance value can reflect the activity of laccase in a certain period of time, one unit (U) of laccase activity was defined as the particular specific variation of the absorbance per minute. The pure ZIF-67 sample without laccase were determined, the results revealed that ZIF-67 possessed no catalytic activity.

The measured enzyme activity at the first time was recorded as the initial enzyme activity (100 %) and the relative enzyme activity was calculated by the ratio between the measured enzyme activities and the initial enzyme activity, as shown in the following equation:

$$\text{Relative enzyme activity (\%)} = \frac{\text{measured enzyme activity}}{\text{initial enzyme activity}} \times 100 \%$$

### 2.5. Storage stability and reusability

The enzyme activity of immobilized laccases was measured per five days, and the relative enzyme activity within fifteen days reflects the storage stability. The reusability of laccase@ZIF-67 was investigated in the catalytic oxidation reaction of ABTS with laccase for five reaction cycles, the resulting laccase@ZIF-67 were separated by centrifugation (4000 rpm, 5 min) and dried at room temperature, then the structure of the reused immobilized laccases (RIL@ZIF-67) were characterized by FT-IR. The above experiments are performed in AA-SA buffer (pH 5) at room temperature.

### 2.6. pH stability

In order to investigate the pH stability, free and immobilized laccase were incubated in AA-SA buffer solution (pH range of 3–8) for 2 h at room temperature, and then the residual enzyme activities were determined per half hour. The acidity and alkalinity of the AA-SA buffer solutions were varied by using HCl or NaOH solutions (0.1 M).

### 2.7. Thermostability and thermal deactivation kinetics model

Free laccase and laccase@ZIF-67 were incubated at 40, 50, 60, 70 and 80 °C for 2 h, and monitored for their residual activity at the interval of every 0.5 h to assess thermostability. The thermostability of the immobilized laccases at a given temperature were investigated by using thermal deactivation kinetics model [41]. Common thermal deactivation kinetics models are one-step model and multi-step model [42]. The one-step model is also called the first-order model, the multi-step model is divided into the synchronous model and the tandem model. The synchronous

model considers that all enzyme molecules can be divided into several components with different thermostability, each of which conforms to the one-step model; the tandem model assumes that the complete deactivation of the enzyme requires multiple intermediate transition states [43–45].

The thermal deactivation kinetics equation of one-step model:

$$\ln \frac{A_t}{A_0} = \ln A = -kt \quad (1)$$

The thermal deactivation kinetics equation of synchronous model:

$$\ln \frac{A_t}{A_0} = \ln A = (-kt) \ln \sum x_i \quad (2)$$

The thermal deactivation kinetics equation of tandem model:

$$\ln \frac{A_t}{A_0} = \ln A = (-k_{d1}t) \ln \sum \alpha + (-k_{d2}t) \ln \sum \beta \quad (3)$$

The equation of half-life:

$$t_{1/2} = \frac{\ln 2}{k} \quad (4)$$

The equation of gibbs free energy:

$$\Delta G = -RT \ln \left( \frac{kh}{k_b T} \right) \quad (5)$$

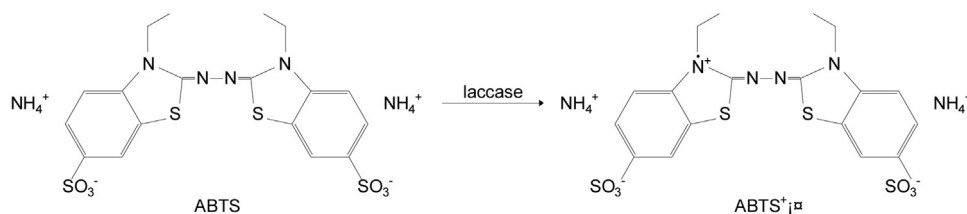
Where,  $A_t$  is the specific enzyme activity at time  $t$ ,  $A_0$  is the initial specific activity,  $A$  is the relative specific enzyme activity at time  $t$ ,  $k$  is deactivation rate constants,  $t_{1/2}$  is half-life, molar gas constant  $R$  is 8.314472 J/(mol K), planck constant  $h$  is  $6.62607015 \times 10^{-34}$  J·s, the constant of boltzmann  $k_b$  is  $1.3806505 \times 10^{-23}$  J/K.

## 3. Results and discussion

### 3.1. Samples characterization

As shown in Fig. 1, the synthesized pure ZIF-67, laccase@ZIF-67 were characterized by powder X-ray diffraction. The PXRD patterns of synthesized pure ZIF-67 material and laccase@ZIF-67 samples did not show any significant differences when compared to that of published simulated ZIF-67 structure data [46], indicated that the immobilization process of laccase did not affect the crystal structure of ZIF material [47].

Fig. 2 showed the FT-IR spectra of free laccase, pure ZIF-67 material, laccase@ZIF-67 and the reused immobilized laccase (RIL@ZIF-67). Since the composition of ZIF-67 contained 2-methylimidazole, the vibrational bands of pure ZIF-67, laccase@ZIF-67 and RIL@ZIF-67 in the range of 700–1500  $\text{cm}^{-1}$  correspond to the characteristic stretching and bending modes of the 2-methylimidazole. Specifically, the vibrational bands at 750  $\text{cm}^{-1}$  are corresponding to the out-of-plane bending of 2-methylimidazole rings, whereas specific peaks in the range from 1027 to 1438  $\text{cm}^{-1}$  can be attributed to the in-plane bending of 2-methylimidazole. The peaks of spectra of laccase, pure ZIF-67, laccase@ZIF-67 and RIL@ZIF-67 are all shown as 1612  $\text{cm}^{-1}$



Scheme 2. Mechanism of catalytic oxidation of ABTS.

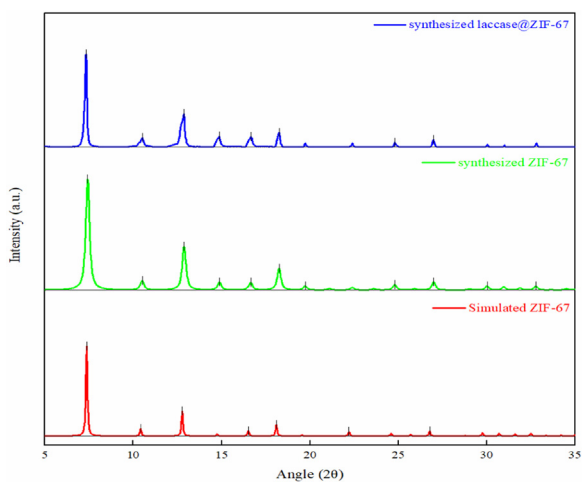


Fig. 1. PXRD patterns of pure ZIF-67, laccase@ZIF-67 and RIL@ZIF-67.

(amide-I band) which attributed to N—H bending and C—N stretching. This indicated an interaction between laccase and ZIF-67 material while coordinating the carbonyl group of the laccase with the  $\text{Co}^{2+}$  cation of ZIF-67 [48]. Moreover, the aliphatic and aromatic C—H stretching of the 2-methylimidazole can be attributed to the peaks at  $3190\text{ cm}^{-1}$ . The spectra of the free laccase exhibited a special absorption bands at  $3440\text{ cm}^{-1}$ , on the contrary, the pure ZIF-67 spectrum did not exhibit the absorption band at  $3440\text{ cm}^{-1}$ . However, the band was visible in the laccase@ZIF-67 (including RIL@ZIF-67) spectrum, confirming the presence of the laccase within the ZIF-67.

SEM images (Fig. 3) revealed the morphology and size of pure ZIF-67, laccase@ZIF-67 and RIL@ZIF-67. Moreover, the energy dispersive X-ray (EDX) results were obtained from SEM analysis (Fig. 3), the presence of Co in laccase@ZIF-67 (including ZIF-67) could be clearly observed.

Furthermore, in order to further prove the presence of laccase in laccase@ZIF-67, thermogravimetric analysis (TGA) curves were performed for pure laccase, ZIF-67 material and laccase@ZIF-67

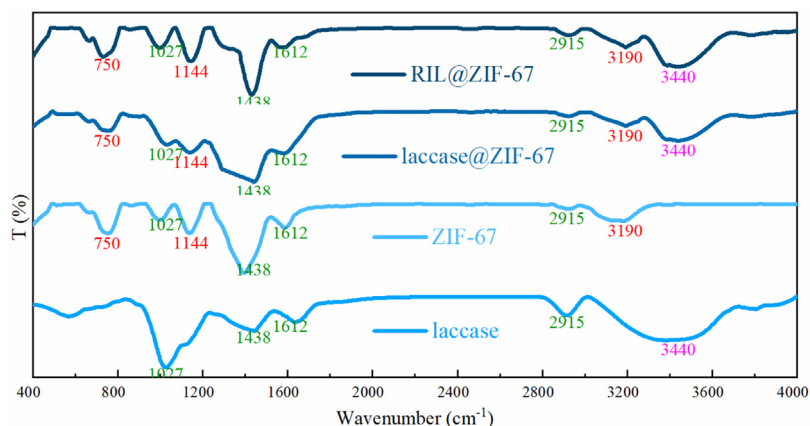


Fig. 2. FT-IR spectra of free laccase, pure ZIF-67, laccase@ZIF-67 and RIL@ZIF-67.

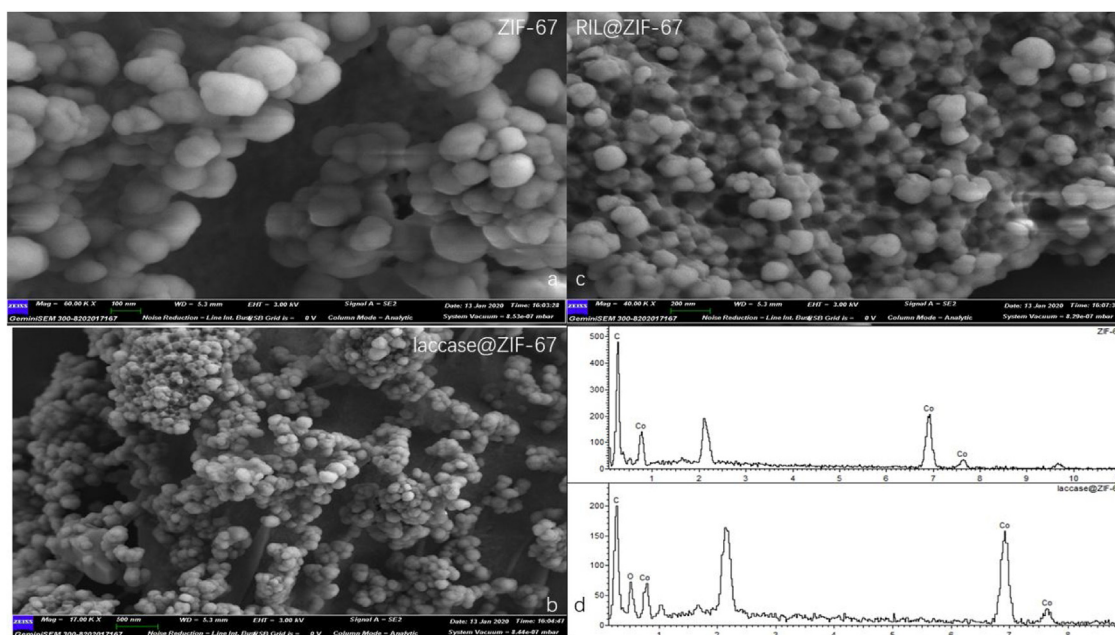
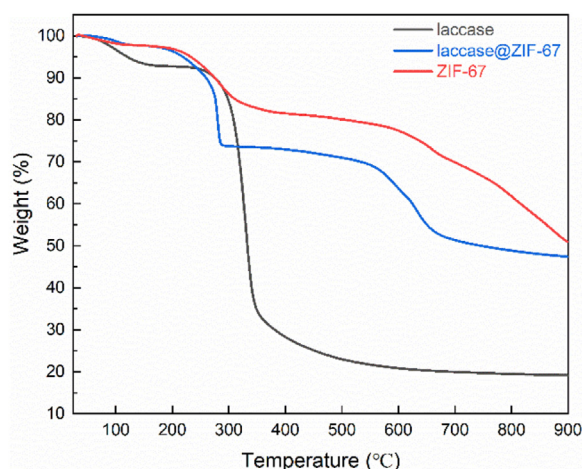


Fig. 3. FE-SEM images of (a) ZIF-67, (b) laccase@ZIF-67 and (c) RIL@ZIF-67. (d) Energy dispersive X-ray spectrum of ZIF-67 and laccase@ZIF-67.



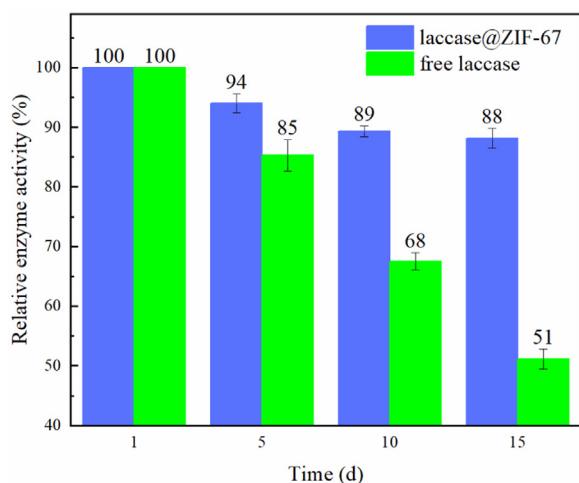


**Fig. 4.** TGA curves of pure laccase (black), ZIF-67 (red) and laccase@ZIF-67 (blue) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(Fig. 4). There were two weight-losses from 30 to 150 °C and 250–400 °C for the free laccase, which were corresponding to the removal of structural water and the pyrolysis of laccase, respectively. It also can be seen that the weight-losses of laccase@ZIF-67 can be divided into 3 stages: 30–300 °C, 300–650 °C and >650 °C. The first stage is mainly the loss of free water and crystallization water in the structure. The second stage is mainly the pyrolysis of laccase which was adsorbed-immobilized onto the outer surface of the carrier. The internal structure of the ZIF-67 has not changed much. The pyrolysis reaction of ZIF-67 occurred in the third stage, the internal structure collapsed and reorganized, and the generated gas evaporates at high temperature.

### 3.2. Storage stability

Storage stability of immobilized laccases are an essential factor to be considered while developing robust biocatalyst. Storage stability was assessed by storing at 4 °C for 15 days, the residual activity of laccases was determined per 5 days. It was observed that the residual activity of free laccase was 51 %, and the residual activity of laccase@ZIF-67 was 88 % after 15 days as shown in Fig. 5. The result indicated that the immobilized laccases had better storage stability than free laccase, and the relative activity of the



**Fig. 5.** Storage stability of free laccase and laccase@ZIF-67.

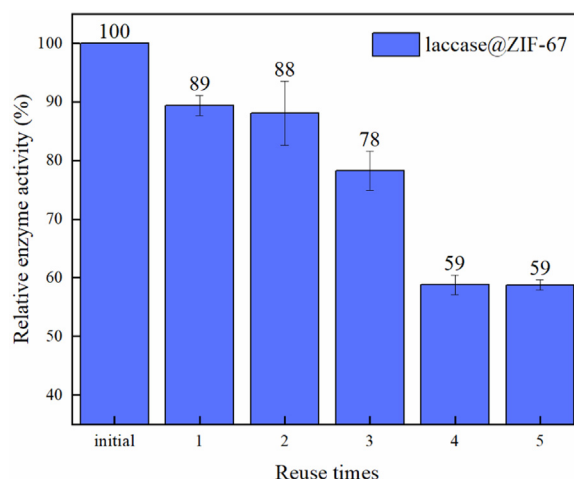
immobilized laccase did not be affected significantly. However, the relative activity of free laccase is only half of the original, which showed that ZIF-67 can give sufficient protection to the activity of laccase. Patil et al. studied the stability of laccase immobilized on ZIF-8, they found that about 80 % of residual activity was observed after storage at 30 °C in sodium acetate buffer (pH 4.5, 100 mM) for 3 weeks [28]. In addition, Naseri et al. stored the laccase@ZIF-8 and laccase@ZIF-zni at 4 °C for 15 days, they claimed that the residual activity of laccase@ZIF-8 and laccase@ZIF-zni were 94 % and 76 %, respectively [29]. S.Nadar et al. claimed that their lipase@ZIF-8 could retain 91 % residual activity after 20 days of long term storage [49].

### 3.3. Reusability

To evaluate the reusability of laccase@ZIF-67, the relative enzyme activity of laccase@ZIF-67 was assessed in 5 reaction cycles. As indicated in Fig. 6, the activity loss of laccase@ZIF-67 was 41 %. This may be due to the binding force between the laccase and the active site of ZIF-67 is not strong via adsorption. The decrease in enzyme activity from the third cycle to the fourth cycle may be due to the desorption of laccase adsorbed on the surface of ZIF-67. S.Nadar et al. reported that the lipase@ZIF-8 could retain 76 % of residual activity [49] and lipase@proline-MOF could retain 72 % of residual activity [50] after 6th cycles, respectively. Patil et al. studied the reusability of laccase immobilized on ZIF-8, they found that the residual activity after the 7th cycle was found to be 48 %, and about 60 % of residual activity was observed after 5th cycle [28]. Naseri et al. reported that laccase@ZIF-8 biocatalyst can retain about 12.6 % of residual activity after five reaction cycles [29], the difference in storage stability between this study may be due to the difference of storage conditions. Laccase shows high sensitivity to environmental factors, limiting the effective application and reuse of laccase. The immobilization treatment maintains the catalytic activity of laccase, enhances stability, and achieves repeated use of laccase. This excellent reusability could be own to the stable immobilization between the laccase and the ZIF carrier. After five reaction cycles, the RIL@ZIF-67 was monitored by FT-IR analysis (Fig. 2), confirmed the essential structure of laccase@ZIF-67 did not change.

### 3.4. pH stability

As shown in Fig. 7a, the relative enzyme activity of free laccase and laccase@ZIF-67 did not show significant difference within pH range of 3–8. Both free laccase and laccase@ZIF-67 reached the



**Fig. 6.** Reusability of laccase@ZIF-67 up to 5th reuse cycle.

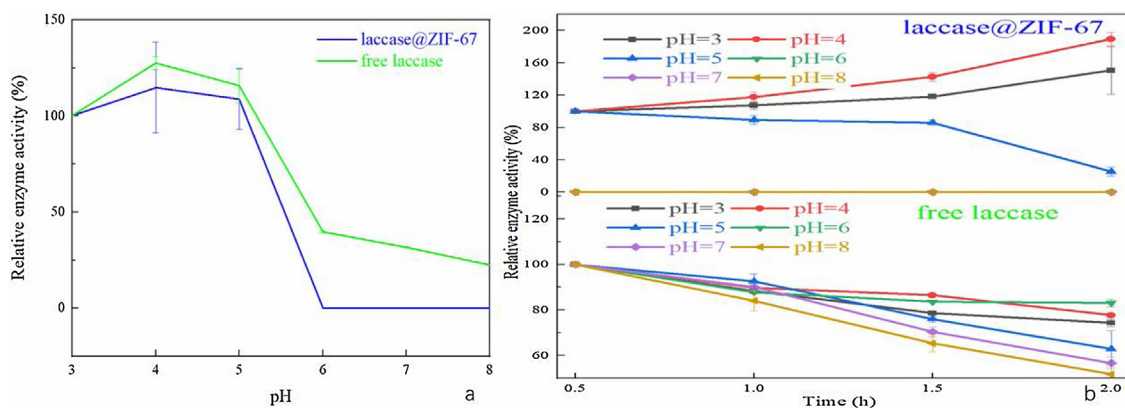


Fig. 7. (a) The relative enzyme activity of free laccase and laccase@ZIF-67. (b) The deactivation curve of free laccase and laccase@ZIF-67.

maximum enzyme activity at pH of 4. In addition, Fig. 7b showed the relative enzyme activity of free laccase and immobilized laccases over time in the range of pH. 3–8. It could be observed that no matter what the pH was, the relative enzyme activity of free laccase was reduced over time. However, the relative enzyme activity of laccase@ZIF-67 was increased over time at pH of 3 and 4. Moreover, laccase@ZIF-67 was inactivated at  $\text{pH} \geq 6$ , this may be due to the inappropriate pH of the solution, cause the amino acid originally inside the laccase to be exposed to the environment, which leads to a decrease in laccase activity. It also could be indicated that the laccase was deactivated gradually under alkaline condition, and more suitable for acidic conditions [51]. In general, the immobilized laccase maintained relatively stable enzyme activity after 2 h, exhibited higher tolerance under extreme acid condition compared to the free laccase.

Laccase is mainly composed of a single polypeptide, activity center of copper ion, polypeptide chain and sugar ligand. The general polypeptide contains 18 kinds of amino acids, which constitute the structural main body of the laccase. The amino acid contains considerable charges; therefore, the structure of the laccase was affected with the pH of the aqueous solution environment. The amino acid linked to the active center of copper ion, due to its own characteristics, the suitable pH of solution is favorable for the formation of redox potential energy and the maintenance of electron transfer process and electrostatic equilibrium in the laccase catalytic reaction [52].

### 3.5. Thermostability and thermal deactivation kinetics model

Enzymes are thermosensitive and may irreversibly lose their catalytic activity at elevated temperature due to permanent

conformational alteration occurring in structure [53]. Since there was no significant change in laccase activity from room temperature to 40 °C, we determined the relative enzyme activity change at 40–80 °C (Fig. 8a), it was observed that the immobilized laccases showed a noticeable increased stability compared to free laccase at the temperature from 40 up to 70 °C, yet, both free laccase and immobilized laccase inactivated at 80 °C without exception. At 70 °C, the relative enzyme activity of the free laccase was only 1.2 %, whereas laccase@ZIF-67 retained 21.9 %. The results clearly showed that the immobilization process of laccase on ZIF-67 material can prevent their conformational transition at high temperature, and enhance the thermostability of laccase. As shown in Fig. 8b, the linear fit shows the tendency of enzyme activity to decrease with time, it could be judged that the laccase deactivation was consistent with the first-order deactivation mechanics model (one-step model). It could be observed that the free laccase was inactivated after being incubated at 60 °C for 0.5 h, and the catalytic activity of laccase@ZIF-67 didn't lose until 80 °C. The above results showed that laccase@ZIF-67 displayed a better performance than free laccase in thermostability, which could protect laccase from harsh environment temperature outside to some extent.

Table 1 depicted the comparison of deactivation rate constants ( $k$ ), half-life ( $t_{1/2}$ ), and the  $\Delta G$  value of free and immobilized laccase at 40, 50, 60, 70 and 80 °C. At each temperature, the  $k$  of laccase@ZIF-67 were much lower when compared to free laccase, laccase@ZIF-67 conferred several folds enhanced half-life at each temperature. Table 1 also depicted the comparison of  $\Delta G$  value, where more energy was required to inactivate laccase. The higher  $\Delta G$  value for laccase@ZIF-67 designated would require a greater amount of energy to permanently deform its active conformational structure,

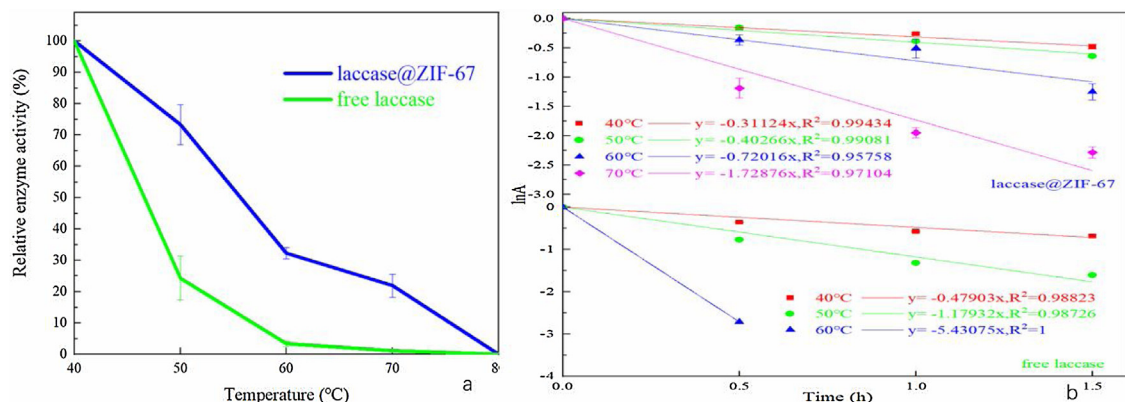


Fig. 8. (a) The relative enzyme activity of free laccase and laccase@ZIF-67. (b) Thermal deactivation kinetic studies of free laccase and laccase@ZIF-67.

**Table 1**  
Thermal deactivation constant (k), half-life ( $t_{1/2}$ ) and gibbs free energy ( $\Delta G$ ) of free laccase, laccase@ZIF-67.

Method	Temperature(°C)	40	50	60	70	80
laccase@ZIF-67	k(h <sup>-1</sup> )	0.3112	0.4027	0.7202	1.7288	-
	$t_{1/2}$ (h)	2.2271	1.7214	0.9625	0.4010	-
	$\Delta G$ (KJ/mol)	79,865	81,808	82,813	82,885	-
Free laccase	k(h <sup>-1</sup> )	0.4790	1.1793	5.4308	-	-
	$t_{1/2}$ (h)	1.4470	0.5878	0.1276	-	-
	$\Delta G$ (KJ/mol)	78,742	78,920	77,217	-	-

which ultimately leads to a better thermostability. It was correlated to the framework wrapped around laccase, which mainly protects the catalytic activity by maintaining its tertiary conformation in unfavorable environments. Compared with free laccase, the k of immobilized laccase were lower, the  $t_{1/2}$  were higher, and the  $\Delta G$  were much higher, these parameters could fully confirmed that the porous material ZIF-67 are robust and stable enough to protect laccase from harsh environment outside to some extent [54], and the laccase@ZIF-67 can effectively improve thermostability [55].

Appropriate temperature will accelerate the movement of the molecule and increase the probability of laccase binding to the substrate. However, excessively high temperature will destroy the secondary bond which could maintains the spatial structure of the laccase molecule [56], this condition would lead to a decrease in laccase stability. As the temperature increases, the fluorescence intensity of tryptophan residues gradually decreases, and fluorescence quenching occurs [52]. Moreover, excessively high temperature induces aggregation or precipitation of laccase, the decrease in laccase activity may be due to the loss of laccase activity and the decrease of dissolved oxygen due to excessive system temperature, which is not conducive to enzyme reaction [57].

#### 4. Conclusion

In conclusion, we have pioneered described a rapid and facile approach to immobilize laccase (*Trametes versicolor*) on the hybrid framework of ZIF-67, the enzyme activity and stability of laccase@ZIF-67 was studied. The results showed that laccase could be well immobilized on ZIF-67 materials, laccase@ZIF-67 was stable on storage stability and reuseability, retaining 88 % (15 days at 4 °C) and 59 % (five reaction cycles) of residual enzyme activity. In addition, laccase@ZIF-67 exhibited relatively stable activity in the range of pH. 3–5, this is similar to free laccase. Moreover, the thermal deactivation kinetic studies of laccase@ZIF-67 showed a lower k value, higher  $t_{1/2}$  and  $\Delta G$  values along with the enhancement of thermodynamic parameters than that of free laccase. As far as we know, this is the first work what the ZIF-67 material was used for laccase immobilization, the results showed that ZIF-67 had excellent performance as a novel carrier for laccase immobilization. In addition, ZIF-67 was a potential adsorbent for the adsorptive removal of phenol, 1-naphthol and chromium from waste water because of the superior adsorption capacity compared with common sorbents. Therefore, the laccase@ZIF-67 has an excellent removal ability for many organic pollutants by using the efficiently adsorption-catalysis-degradation method. The present study paves the way to use the porous ZIF-67 material as a novel carrier of immobilized laccase for environmental applications.

#### CRedit authorship contribution statement

**Zhaobo Wang:** Conceptualization, Methodology, Writing - original draft, Data curation. **Dajun Ren:** Conceptualization, Methodology, Writing - review & editing. **Hongyan Yu:** Investigation. **Shan Jiang:** Investigation. **Shuqin Zhan:** Writing - review & editing. **Xiaoqing Zhang:** Writing - review & editing.

#### Declaration of Competing Interest

The authors report no declarations of interest.

#### Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No 41571306), the Project of Excellent Fund in Hubei (Grant No 2018CFA067), the Major Project of Science and Technology research Program of the Hubei Provincial Department of Education (Grant No D20181101), the National Natural Science Foundation of China (Grant No 41501537) and Hubei Key Laboratory for Efficient Utilization and Agglomeration of Metallurgic Mineral Resources (Grant No 2017zy003).

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