Journal of Leather Science and Engineering

RESEARCH Open Access

Laccase-functionalized magnetic framework composite enabled chlorophenols degradation, a potential remediation for fungicides residues in leather

Min Cao[1†], Jie Yu[2†], Xing Zhang[1], Yamei Lin[1] and He Huang[1,2*]

Abstract: Chlorophenols, used as the fungicides in leather, are strictly limited in leather products. In this work, a metal–organic framework material, zeolitic metal azolate framework-7 (MAF-7), was first used to encapsulate laccase (Lac) to prepare MAF-7/Lac bio-composites with 98.5% immobilization yield. Afterward, Lac/MNP@MOM was formed by introducing the magnetic nanoparticles (MNPs) into the Lac@MOM. MAF-7 with better hydrophilicity and stronger pH buffering ability, exhibits good compatibility with laccase, which can reserve the activity of laccase after immobilization. Moreover, the porous structure of MAF-7 is favorable for the sufficient contact between laccase and substrates. Lac/MNP@MOM exhibited excellent activity when exposed to high temperature, extreme pH, and organic solvents, which also simplified complex recovery steps. Furthermore, the degradation rate of 2,4-dichlorophenol (2,4-DCP) could reach as high as 97% within 24 h by immobilized laccase, and after nine consecutive cycles of operation, enzyme activity could remain over 80%, which gives it the potential for practical applications.

Keywords: Zeolitic metal azolate framework-7 (MAF-7), Laccase, Magnetic nanoparticles (MNPs), Lac/MNP@MOM, 2,4-dichlorophenol (2,4-DCP)

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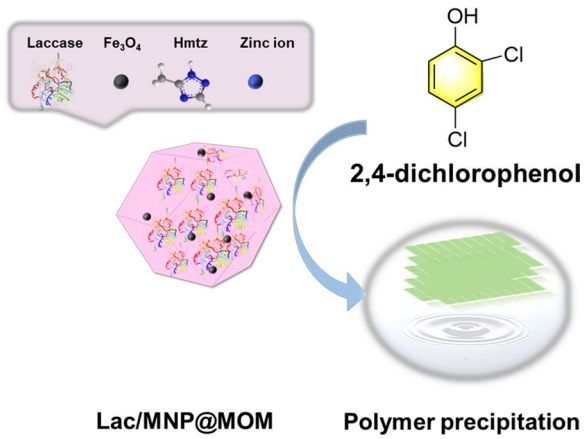
Graphical abstract

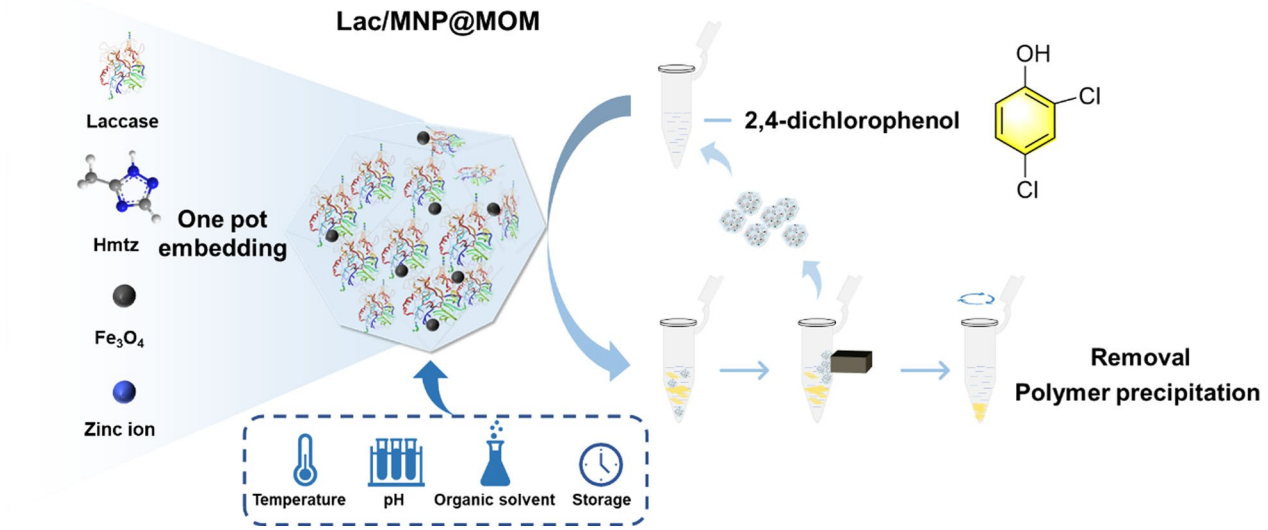


1 Introduction

Phenols and their derivatives are mainly used as pesticides in industry, such as leather, textile and plastics [1]. However, the residues from the phenolic matrix were identified to threaten the environment and organisms [2, 3], and included as a priority pollutant in the list issued by the United States Environmental Protection Agency (USEPA), requiring that the content of phenol in drinking water must be less than 1 μg/L [4]. Therefore, it is meaningful to exploit efficient methods for removing such low-concentration, highly toxic, and difficult-to-degrade pollutants. At present, physical adsorption [5, 6], chemical oxidation [7, 8], and microbiological involved methods [9, 10] are common pathways applied for the treatment of phenolic pollutants, which may inevitably cause secondary pollution or time–cost [11]. Gradually, the enzyme treatment method emerges as a useful tool for phenolic pollutants treatments, with advantages of mild operating conditions, nontoxic by-products, and excellent degradation efficiency [12].

Laccase, first reported in 1883, belongs to the superfamily of multicopper oxidases (MCOs)-a group of enzymes comprising many proteins with different substrate specificities and diverse biological functions [13]. The presence of cupredoxin-like domains allows laccase to reduce oxygen to water without producing harmful byproducts, accompanied by the oxidation of the substrate, especially phenolic compounds [14]. Laccase oxidizes phenols to active radicals that polymerize to form dimers or polymers with reduced solubility and toxicity in water, allowing easy separation from water, which avoids the problems of secondary pollution and separation [15, 16]. Li et al. [17] described that catechol could be degraded by 88.4% within 2 h in the presence of free laccase; Zhang et al. [18] found that the removal efficiency of 2,4-dichlorophenol (2,4-DCP), 2-chlorophenol (2-CP), and 4-chlorophenol (4-CP) could reach 94%, 75%, and 69% within 10 h on laccase, respectively. In addition, laccase is considered as an efficient enzyme for dye degradation, and the application of laccase to degrade leather dyes enables cleaner production [19–21]. However, the environmentally sensitive, low stability, and unrecyclable nature of free laccase have limited its industrial application to a certain extent [22].

Enzyme immobilization is an ideal method to compensate for the deficiency of free enzymes, which contains the selection of suitable supports and reasonable application of the effects of support-enzyme interaction[23, 24]. Metal–organic frameworks (MOFs), with high encapsulation rate [25], porous crystallite structures [26], low cytotoxicity [27], and good biocompatibility [28], have been widely applied in enzyme immobilization [29]. Lin et al. [30] encapsulated laccase into ZIF-90 that was combined with adenosine triphosphate and designed an ultrasensitive online electrochemical detector with a detection limit as low as 0.12 nm; Wei et al. [31] encapsulated laccase into ZIF-8 to prepare a glucose biosensor, which maintained good stability after 15 h of continuous work. Among immobilized materials, MAF-7 has better hydrophilicity and stronger pH buffering ability [32], which was more compatible with enzymes and leads to retention of catalytic activity. In addition, the confined structure of MAF-7 could inhibit the undesirable aggregate or conformational change of laccase, and the size of MAF-7 channel is suitable for the transport of substrates and products. Thus, immobilization of laccase on MAF-7



Scheme 1 Schematic diagram for the synthesis of Lac/MNP@MOM

is still desirable although no researchers have encapsulated laccase in MAF-7.

Meanwhile, the introduction of magnetic nanoparticle Fe_3O_4 into enzyme immobilization system can simplify the operation and separation process, and facilitate the recovery of enzyme[33]. The electron spin state of the intermediate product can be affected and the activation of enzyme can be promoted by magneto-induced effect in the presence of Fe_3O_4 nanoparticles during the reaction process. The remediation of pyrene polluted soil was reported that achieved by iron nanoparticles and the removal rate of pyrene increased with the increase of iron nanoparticles [34]. Liu et al. [35] immobilized the laccase on Fe_3O_4@SiO_2-NH_2 nanoparticles, and the removal rate of 2,4-DCP is still more than 59% after six times of recycling.

In this study, it is the first time that laccase and Fe_3O_4 were both encapsulated in the MAF-7 by a mild and simple one-step synthetic approach, affording magnetically and catalytically active composite (Scheme 1). After immobilization, the crystalline structure with the dodecahedral rhombus structure of MAF-7 was destroyed, therefore, the material which used to encapsulate laccase was named MOM (Metal Organic Material) in the manuscript. Compared with the reported work and the typical reaction catalyzed by laccase, we found that the immobilized laccase displayed excellent catalytic activity and stability in harsh conditions, and retained a high percentage of its initial activity after a month. Also, the immobilized laccase included the unsurpassed removal rate of 2,4-DCP, and remained surprisingly activity after nine consecutive operations, which provided the possibility to degrade fungicides residues in leather manufacture.

2 Experimental section

2.1 Materials

Zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$), 3-methyl-1H-1,2,4-triazole (Hmtz), $FeCl_3{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$, and $NH_3{\cdot}H_2O$ (25%) were purchased from Sigma Aldrich (Shanghai, China). 2,4-Dichlorophenol (2,4-DCP), 4-aminoantipyrine (4-AP), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2-(*N*-morpholino) ethanesulfonic acid (MES) were obtained from Macklin (Shanghai, China). The laccase from *Aspergillus oryzae* (Lac) was supplied by SUNSON (Ningxia, China) and stored at 4 °C. A Bradford Protein Assay Kit for protein (0.1–1.5 mg mL^{-1}) was provided by Beyotime (Shanghai, China).

2.2 Synthesis method for magnetic nanoparticles (MNPs)

The magnetic nanoparticles (MNPs) were prepared by previously reported with slight modifications [36]. 80 mL of deionized water (DI water) and magnetic stirrers were placed in a 250 mL double-necked round-bottomed flask, and the oxygen present was purged with nitrogen for half an hour. Subsequently, $FeCl_3{\cdot}6H_2O$ (27 g, 0.010 mol) and $FeCl_2{\cdot}4H_2O$ (0.99 g, 0.005 mol) were added and reacted at 50 ℃ for 1 h. Under vigorous stirring, ammonia aqueous solution (10 mL, 25% w/w) was slowly added, and the reaction was continued at 70 ℃ for 3 h. MNPs were obtained by magnetic separation, washed with water for three times, and ethanol for

three times respectively, and finally stored at ethanol solution at -4 ℃.

2.3 Synthesis method for Lac/MNP@MOM, Lac@MOM, and MAF-7

The approach was reproduced as described in the literature with some modifications. Laccase and Zn $(NO_3)_2$ aqueous solution (1 mL, 0.12 mol L^{-1}) were rapidly mixed with an aqueous solution containing Hmtz (2 mL, 0.18 mol L^{-1}), MNPs, and $NH_3{\cdot}H_2O$ (10%). The solution changed from clear to opaque after 24 h of continuous agitation at room temperature. Finally, Lac/MNP@MOM was recovered from the sediment by magnetic separation, and washed with water for three times.

Following the procedure for Lac/MNP@MOM, Lac@MOM was obtained in the absence of MNPs and was gathered by centrifugation at 8000 rpm for 5 min. Pure MAF-7 was prepared by a similar method without MNPs and laccase.

2.4 Characterization

Scanning electron microscopy (SEM) was performed using a Hitachi SU8100 instrument. X-ray diffraction (XRD) data were obtained by Rigaku Smart Laboratory at 9 keV in Bragg–Brentano geometry using Cu κα radiation (λ = 1.5405 Å) as the X-ray source, and the scan speed was 10 min^{-1}, step 0.02°, and 2θ was in the range between 5° and 30°. The spatial distribution of fluorophore-tagged laccase within the MOF composites was determined using the CLSM technique (UP-Sigma, Rtec). Fourier transform infrared (FT-IR) spectra of the samples were recorded on a Bruker ALPHA FT-IR spectrometer (Bruker Vertex 70) using the KBr disk method. The X-ray photoelectron spectroscopy measurement (XPS) was obtained with Thermo Fisher Scientific K-Alpha. Thermal gravimetric analysis (TGA) data were obtained on a simultaneous thermal analyzer (STA) (TGA/DSC) from Linseis Thermal Analysis, then, samples were filled into an alumina crucible and heated in a continuous flow of nitrogen gas from room temperature up to 800 °C. A vibrating sample magnetometer (VSM Lakeshore 7404) was utilized to analyze the magnetic values of the samples.

2.5 Laccase activity assay and steady-state kinetic assay

Following the method in previously reported literature [37], the activity of laccase was determined by using ABTS as the substrate. Generally, the reaction mixture containing a suitable amount of laccase (free laccase or immobilized laccase) and ABTS (1 mmol L^{-1}) solution prepared by citrate buffer solution (0.1 mol L^{-1}) were reacted for 5 min under the respective optimum temperature conditions. The activity of laccase was measured at 420 nm using a UV–vis spectrophotometer, one unit (1 U) of activity was defined as the amount of laccase required to produce 1 μmol ABTS to green chelate within one minute. The pure MAF-7 sample without laccase was measured, and the result showed that MAF-7 exhibited no catalytic activity. All activities were measured three times in parallel, and the results were averaged.

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

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

The Michaelis–Menten kinetic parameters (K_m and V_{max}) were obtained by measuring the initial reaction rate with ABTS at 420 nm. The reactions were carried out at 30 ℃ in citrate buffer solution (pH 3.5), and the concentration of ABTS ranged from 0.1–3 mmol L^{-1}. The Lineweaver–Burk equation was employed to calculate K_m and V_{max}.

$$\frac{1}{V} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

[S]—substrate concentration (mM);V—reaction rate (mM·min^{-1}).

2.6 Laccase activity in harsh conditions

Free laccase, Lac@MOM, and Lac/MNP@MOM were incubated at 50 ℃, 60 ℃, 70 ℃, and 80 ℃ for 30 min to explore the thermal stability of laccase. The investigation of pH stability, free laccase, Lac@MOM, and Lac/MNP@MOM were incubated in solutions with different conditions (30 mM MES, pH 3, 4, 7, 9, 10) for 30 min. The polar and nonpolar solvent stability, Lac@MOM and Lac/MNP@MOM were stored in ethyl acetate (EA), dichloromethane (DCM), ethanol (EtOH), dimethyl sulfoxide (DMSO), and *N, N*-dimethylformamide (DMF) for 30 min. Then, the precipitates were recovered and washed three times with DI water, and the residual activity was analyzed. To investigate the storage stability, the free and immobilized laccases were stored in a refrigerator at 4 ℃ for 30 days, and the enzyme activity was measured every 5 days. The initial enzyme activity was defined as 100%.

2.7 Removal of 2,4-dichlorophenol

To investigate the degradation capacity of immobilized laccase towards hazardous phenolic compounds, 2,4-DCP was selected as object to compare the degradation effects of free laccase. The biological removal of 2,4-DCP was performed by adding immobilized enzyme (0.1 mL, 1 mg mL^{-1}) to a phenolic mixture (1 mL) containing 2,4-DCP (0.1 mg mL^{-1}) configured with MES buffer solution (30 mM). The supernatant was obtained by magnetic separation or centrifugation, and the concentration of 2,4-DCP was determined by high-performance liquid chromatography (HPLC) on a C18 reversed-phase column (Inertsil ODS-4, 150 mm × 4.6 mm, 3 μm particles). The detection conditions were as follows: the mobile phase was V (methanol): V(water) = 30: 70, the flow rate of 0.8 mL min^{-1}, and the detection wavelength was 226 nm. Before HPLC analysis, the samples were filtered through a 0.22 μm filter to remove insoluble compounds. The removal efficiency (RE) for 2,4-DCP can be computed by the following equation:

$$RE(\%) = (C_o - C_t)/C_0 \times 100\%$$

C_0—initial concentration of 2,4-DCP (mg mL^{-1});C_t—concentration of 2,4-DCP at t h (mg mL^{-1}).

To investigate the effect of pH on the biological removal of 2,4-DCP by free or immobilized laccase, phenol mixture with different pH values (3–10) were used to catalyze the reaction at 30 °C for 24 h. The influence of temperature was studied by evaluating the RE values of 2,4-DCP in the mixture catalyzed by free or immobilized laccase at different temperatures (25–55 °C) for 24 h. Then, the 2,4-DCP mixture was removed by Lac@MOM and Lac/MNP@MOM for 24 h at the respective optimal pH and temperature, and the RE values were measured. The reusability of Lac@MOM and Lac/MNP@MOM under optimal reaction conditions were evaluated by continuously and repeatedly removing phenolic mixtures. After each cycle, the samples were washed with deionized water three times and then added to the next reaction, initial laccase activity was defined as 100%. Moreover, the influence of substrate concentration was tested for Lac/MNP@MOM under optimal reaction conditions.

3 Results and discussion

3.1 Characterization of as-prepared composites

MAF-7, Lac@MOM, and Lac/MNP@MOM composites were investigated by SEM. As shown in Fig. 1a-c, the appearance of MAF-7 was to be a dodecahedral rhombus structure with a diameter of 2 μm despite their rough edges, which is consistent with that reported in literature [38]. While the morphology of Lac@MOM and Lac/MNP@MOM composites became more spherical, the particle sizes were unevenly distributed after the enzyme was immobilized, possibly due to laccase-mediated aggregation growth dynamics [39–41]. Additionally, XRD patterns were performed for MAF-7, Lac@MOM, and Lac/MNP@MOM (Fig. S1). Bare MAF-7 exhibited diffraction peaks at 2θ values of 7.34°, 10.38°, 12,72°, 14.72°, 16.46°, and 18.04°, which is consistent with the previously published data for MAF-7 crystal, confirming that MAF-7

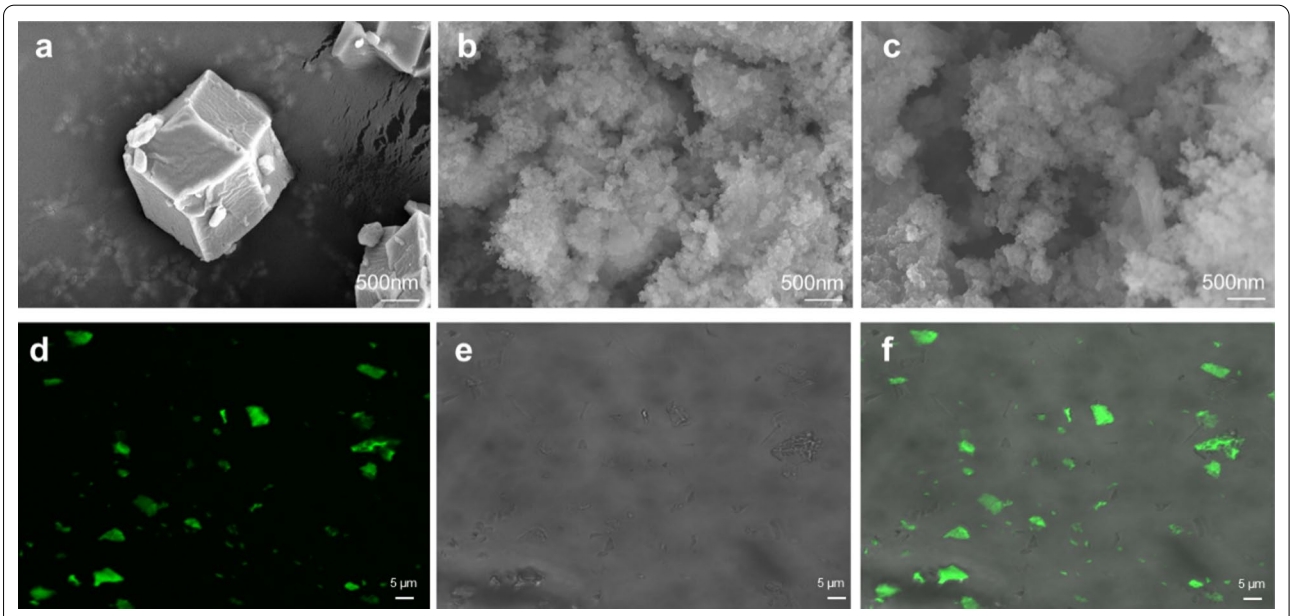


Fig. 1 SEM images of MAF-7 **a**, Lac@MOM **b**, and Lac/MNP@MOM **c**; Confocal laser scanning micrographs showing the fluorescence **d**, bright field **e**, and overlay **f** images of Lac/MNP@MOM

had a pure sodalite topology. Compared with MAF-7, the peak intensities of Lac@MOM and Lac/MNP@MOM significantly decreased, as the site of MAF-7 is occupied by enzyme molecules, thereby destroying the integrity of its crystal structure.

Furthermore, to inquire the location of laccase in Lac/MNP@MOM composites and ensure the successful incorporation of laccase into MAF-7, the fluorescent FLac/MNP@MOM was synthesized by applying FLac as the reactant for CLSM imaging. The confocal microscopy of FLac@MOM revealed a good colocalization of laccase (green fluorescence) and MAF-7 (Fig. 1d-f), indicating laccase is well dispersed in the backbone of MAF-7. Additionally, samples of the as-synthesized composites were also characterized by FI–IR as shown in Fig. 2a. As for MAF-7, the peak at 421 cm^{-1} may be due to Zn-N stretching. Lac@MOM and Lac/MNP@MOM showed two additional peaks at ~ 1544 cm^{-1} and 1654 cm^{-1}, which was attributed to amide II and amide I bands, respectively [42], implying the successful encapsulation of laccase in MAF-7. Moreover, the peak at 613 cm^{-1} for the MNP demonstrated the presence of Fe–O bonds, while the peak could not be seen in the MNP@MOM and Lac/MNP@MOM spectrums, it may be considered that MNPs were located inside of MAF-7 frame. Then, the XPS analyses (Figure. S2, Table. S1) showed that iron was evenly distributed in the immobilized enzyme system, which also indicated that the protein molecules of the enzyme were well coated in MAF-7. Meanwhile, the TGA analysis (Fig. 2b) confirmed the presence of laccase and MNPs in the composite material.

The magnetization of MNP@MOM and Lac/MNP@MOM were investigated by obtaining VSM measurements. The magnetization of MNP@MOM was approximately 0.99 emu/g, while the curve at Lac/MNP@MOM show a magnetization saturation value of 2.02 emu/g. Compared with MNPs, the curves of MNP@MOM and Lac/MNP@MOM exhibited a decrease in magnetization

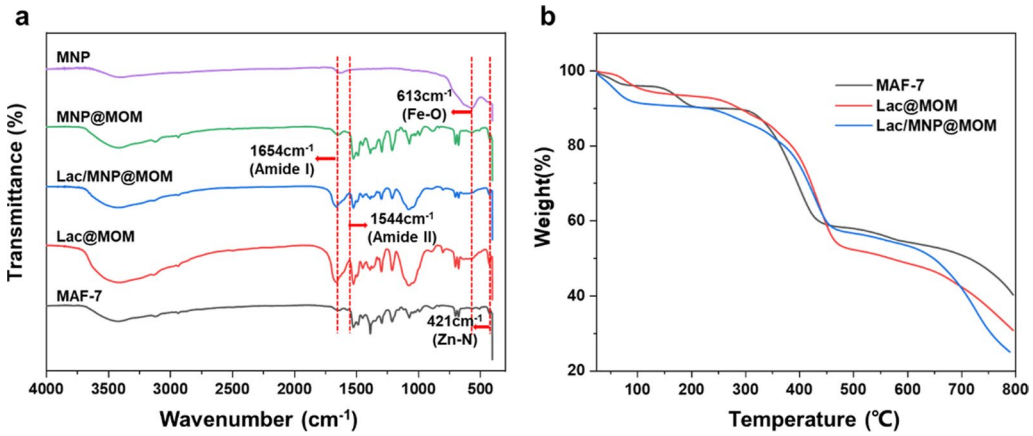


Fig. 2 **a** FT-IR spectra of MAF-7, Lac@MOM, Lac/MNP@MOM, and MNP@MOM; **b** TGA curves of MAF-7, Lac@MOM, and Lac/MNP@MOM

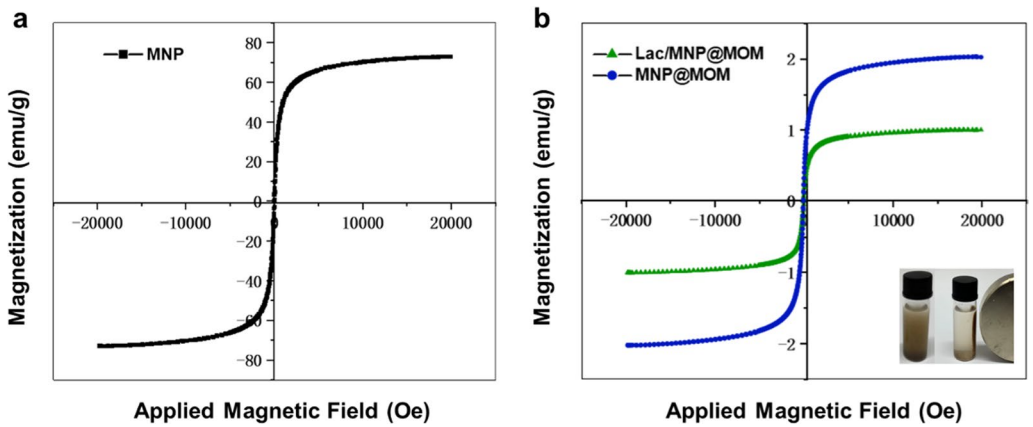


Fig. 3 VSM spectra of MNP (**a**), MNP@MOM and Lac/MNP@MOM (**b**)

saturation (Ms) values (Fig. 3). It could be reasoned that MAF-7 encapsulation results in a decrease in the surface moment of individual MNPs, eventually resulting in a decrease in the net magnetism [43]. Although the surface moments of MNP@MOM and Lac/MNP@MOM were slightly decreased, they still exhibit excellent superparamagnetic properties and can be quickly separated from the liquid in the presence of an external magnetic field.

3.2 Catalytic activity and Michaelis–Menten kinetic parameters of Lac@MOM, and Lac/MNP@MOM.

The activities of free laccase, Lac@MOM, and Lac/MNP@MOM were calculated to be 2542.2, 2694.7, and 2426.3 U g^{-1} protein (Additional file 1: Figure S3), indicating the encapsulation method has no significant effect on enzyme activity. As is shown in Table 1, the K_m, and V_{max} values of free laccase were 0.119 mM and 0.27 mM min^{-1} respectively. However, the K_m values for Lac@MOM and Lac/MNP@MOM were decreased to 0.080, 0.091 mM, respectively, which was probably caused by the triazole linkers of MAF-7 increasing the affinity between enzymes and substrates, thereby greatly increasing the reaction rate. The V_{max} values of Lac@MOM (0.25 mM min^{-1}), and Lac/MNP@MOM (0.23 mM min^{-1}) were lower than that of free laccase, especially the latter, the phenomenon may be caused by the mass transfer limitation for Lac/MNP@MOM composite tends to aggregate in the reaction. Besides, the k_{cat} value of free laccase was higher than that of the immobilized enzyme, the conversion rate of free enzyme is faster, which corresponded to the V_{max} value expressed. Consequently, contrast to free laccase, the enhanced k_{cat}/K_m for Lac@MOM, Lac/MNP@MOM indicated that immobilization of laccase on MAF-7 could have better catalytic efficiency.

3.3 Laccase activity in harsh conditions

3.3.1 Thermal deactivation studies

It can be seen that the activity of free enzyme, Lac@MOM and Lac/MNP@MOM gradually decreased with the increase of temperature, and the decrease rate of immobilized enzyme was much lower than that of free laccase (Fig. 4a, Additional file 1: Fig. S4). When the temperature increased to 80 °C, the remaining activity of the free enzyme was only 8.8%. However, the remaining activities of Lac@MOM and Lac/MNP@MOM were 42.74% and 38.30%, respectively. This is because MAF-7 can form an "armor" on the surface of laccase, preventing laccase from direct contact with high temperature environment, thereby avoiding the reduction of laccase activity caused by high temperature.

Table 1 Kinetic parameters of free laccase, Lac@MOM, and Lac/MNP@MOM

Sample	K_m (mM)	V_{max} (mM min^{-1})	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} mM^{-1})
Free Laccase	0.119	0.27	4.91	41.26
Lac@MOM	0.080	0.25	4.55	56.88
Lac/MNP@MOM	0.091	0.23	4.18	45.93

3.3.2 pH condition deactivation analysis

Enzymes are very sensitive to the pH of the processing system, and adverse conditions usually cause enzyme inactivation. As shown in Fig. 4b, both the free enzyme and the immobilized laccase maintained more than 70% activity under pH 7 (Additional file 1: Fig. S5). Notably, when the pH reached 9, the free laccase activity is reduced to 21.75%, while immobilized enzyme activity still retains more than 80%. Compared with free laccase, Lac@MOM and Lac/MNP@MOM showed higher tolerance to extreme pH and can function normally in a wider pH range. Moreover, MAF-7 affords a robust coating that offers protection, and its confined three-dimensional structure reduces enzymes undesirable aggregate or conformational changes in the basic solution to remain the enzyme activity, So, the immobilized laccase has stronger vitality than that of free laccase under alkaline conditions, which is consistent with the situation reported in the previous literature.

3.3.3 Organic solvent stability studies

Laccase has been used to repair the polluted environment by biological transformation, and the majority of substrates are soluble in organic solvents, which may significantly deactivate enzymes. Thus, enzyme-catalyzed reactions have been evaluated in a variety of polar and nonpolar solvents (Fig. 4c), including ethyl acetate (EA), dichloromethane (DCM), ethanol (EtOH), dimethyl sulfoxide (DMSO), and *N, N*-dimethylformamide (DMF). After treatment, Lac@MOM and Lac/MNP@MOM still showed excellent catalytic activity (>79%), the reason interpreted that MAF-7 has an Å-sized pore size, which prevents organic solvents from contacting the embedded enzyme. In addition, compared with Lac@MOM, Lac/MNP@MOM was more affected by organic solvents, which may be because MNPs change the pore size of MAF-7, thereby the probability of contacting organic solvents increases. Since the addition of MNPs involves recycling to a greater extent, the slight difference could be ignored.

3.3.4 Storage stability studies

As shown in Fig. 4d, due to the protective effect of MOFs on the active conformation of laccase, Lac@MOM and Lac/MNP@MOM had 78.7% and 74.1% residual activities after storage for 30 days at 4 °C. Compared

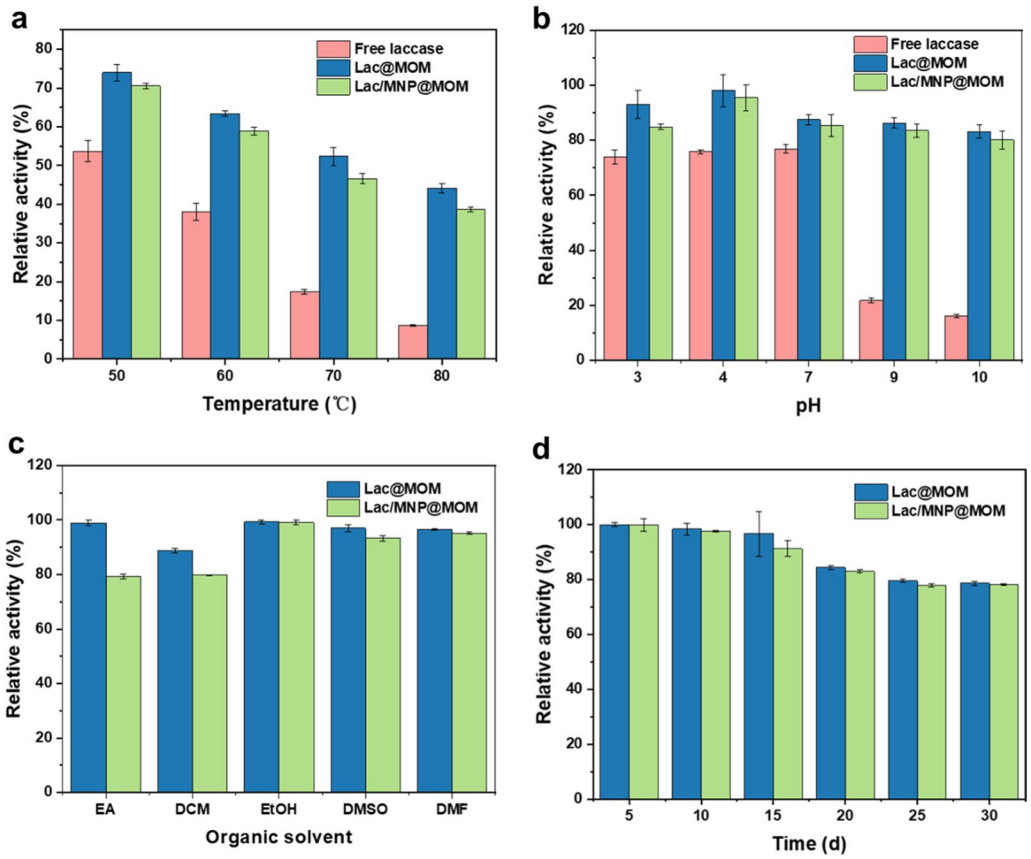


Fig. 4 **a** Thermal inactivation of free laccase (red), Lac@MOM (blue), Lac/MNP@MOM (green) at 50 ℃, 60 ℃, 70 ℃ and 80 ℃ for 30 min; **b** pH condition deactivation of free laccase (red), Lac@MOM (blue), Lac/MNP@MOM (green) at different pH conditions for 30 min; **c** Organic solvent stability evaluation of Lac@MOM (blue), Lac/MNP@MOM (green); **d** Storage stability of Lac@MOM (blue) and Lac/MNP@MOM (green) (4 °C)

with the reported literature, in which the laccase activity decreased significantly after prolonged storage(less than 20%) [44], the immobilized laccase in this work had higher storage stability. The increase in a residual activity fully implies the advantage of the metal–organic framework in further stabilizing the protein structure.

The above results showed that Lac@MOM and Lac/MNP@MOM were much more resistant to high temperature, alkaline pH and organic solvents compared to free laccase, and remained amazing activities after a month storage at 4 °C, indicating that the MAF-7 framework with high stability and special pore structure can effectively protect the activity of laccase from adverse environmental effects, thus providing the necessary stability for practical laccase applications.

3.4 Removal of 2,4 -dichlorophenol

It can be judged that the optimal pH of Lac@MOM and Lac/MNP@MOM for 2,4-DCP degradation is 4 and 5, respectively, and the temperature is 45 ℃ (Fig. S6-S7). Then, the degradation of 2,4-DCP in 24 h was studied by using the Lac@MOM and Lac/MNP@MOM under optimal reaction systems (Fig. 5a, Additional file 1: Fig. S8). After eight hours of reaction, the removal rate of Lac@MOM was 83.25%, which was slightly higher than that of Lac/MNP@MOM (76.68%), it may be caused by Lac@MOM having a better Michaelis constant and maximum reaction rate than that of Lac/MNP@MOM. After 12 h of reaction, the reaction rate of Lac/MNP@MOM and Lac@MOM slowed down, which may be reasoned by the formation of polymers blocked the pores in MAF-7 during the degradation of 2,4-DCP, which reduced the contact area with the substrate or prolonged the reaction and led to enzyme leakage. In short, compared with other immobilized laccase reported in the literature [44–46], Lac@MOM and Lac/MNP@MOM exhibit higher catalytic efficiency, due to the better compatibility of MAF-7 and laccase. Additionally, reusability is an important feature of immobilized biocatalysts and reduces the cost of industrial operations. Thus, the reusability of Lac@MOM and Lac/MNP@MOM were investigated to evaluate their performance in downgrading 2,4-DCP under optimal reaction conditions (Fig. 5b). The

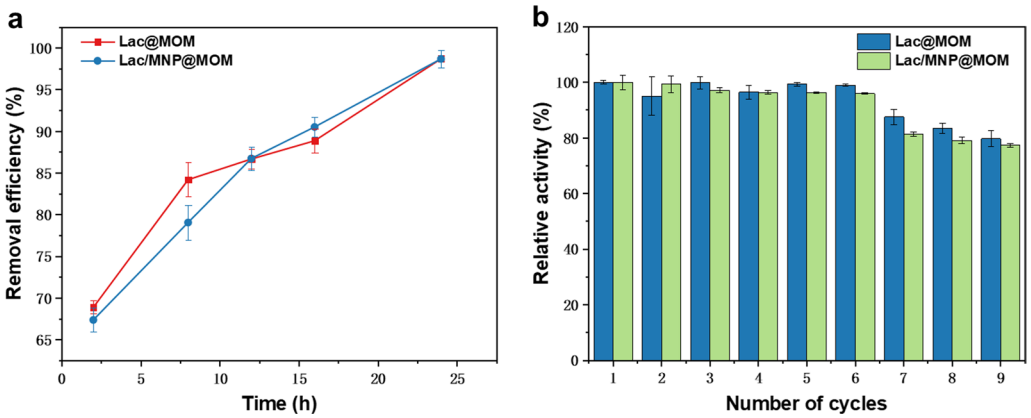


Fig. 5 **a** Removal efficiency of 2,4-DCP over 24 h; **b** Reusability of Lac@MOM (blue) and Lac/MNP@MOM (green)

residual activity remained above 80% after 9 cycles of continuous reuse, and the reduced activity may be attributed to the production of polymers that were bound to laccase or continuously adsorbed on the MOFs shell, or the leakage of enzyme molecules during use. Anyway, the immobilized laccase exhibits potential for industrial applications with a simple recovery process. Moreover, the degradation efficiency decreased with the concentration of 2, 4-DCP increased, however, even at concentration of 0.6 mg/mL, the degradation efficiency of 2, 4-DCP was still 58% (Additional file 1: Table S2).

4 Conclusion

A one-step process was developed to encapsulate laccase and MNPs into MAF-7 to form Lac @MOM and Lac/MNP@MOM, which eliminates the complex synthesis process, avoids the low load rate hindrance, and enables the recyclability of laccase. The morphology and crystal structure of the immobilized laccase were determined through a series of characterization methods, such as XRD, SEM, and FT-IR. Compared with free enzymes, the synthesized magnetic framework composite has high catalytic efficiency and recyclability and would be more stable in high temperature, extreme pH, and polar solvent environments. In conclusion, Lac/MNP@MOM showed high degradation activity and excellent reusability, meanwhile, apparently reducing the processing costs, which might be expected to be used in industry (e.g. leather manufacture and textile production).

Abbreviations

2-CP: 2-Chlorophenol; 4-CP: 4-Chlorophenol; 2,4-DCP: 2,4-Dichlorophenol; CLSM: Confocal laser scanning micrographs; FT-IR: Fourier transform infrared; Lac: Laccase; MAF-7: Zeolitic metal azolate framework-7; MCOs: Multicopper oxidases; MNPs: Magnetic nanoparticles; MOFs: Metal–organic frameworks; Ms: Magnetization saturation; RE: Removal efficiency; SEM: Scanning electron microscopy; TGA: Thermal gravimetric analysis; USEPA: The United States Environmental Protection Agency; XPS: The X-ray photoelectron spectroscopy measurement; XRD: X-ray diffraction.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42825-022-00094-3.

Additional file 1. **Fig. S1**. XRD patterns of MAF-7 (black), Lac@MOM (red), and Lac/MNP@MOM (blue). **Fig. S2**. XPS spectra of MAF-7, Lac@MOM, and Lac/MNP@MOM (a); XPS spectra of Zn 2p for Lac/MNP@MOM (b); XPS spectra of Fe 2p for Lac/MNP@MOM (v); XPS spectra of Fe 2p for Lac/MNP@MOM (d). **Table. S1**. Surface element ratios of Lac/MNP@MOM. **Fig. S3**. Effect of laccase concentration on laccase immobilization. **Fig. S4**. Temperature effect on laccase activity. **Fig. S5**. pH effect on laccase activity. **Fig. S6**. Effect of pH on removal of 2,4-DCP. **Fig. S7**. Effect of temperature on removal of 2,4-DCP. **Table. S2**. Effect of substrate concentration on removal of 2,4-DCP. **Fig. S8**. The UV-Vis absorption of composites catalyzed 2,4-DCP.

Acknowledgements

This work was financially supported by the National Key Research and Development Project (Grant No. 2021YFC2100100).

Author contributions

MC performed the experiments, analyzed the results and revised the manuscript. JY performed the experiments and wrote the initial draft. YL, XZ and HH reviewed and edited the manuscript. All authors read and approved the final manuscript

Funding

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Competing interests

The authors declare that they have no competing interests.

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Received: 9 April 2022 Revised: 13 May 2022 Accepted: 24 May 2022
Published online: 25 July 2022

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