Robust magnetic laccase-mimicking nanozyme for oxidizing o-phenylenediamine and removing phenolic pollutants

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ABSTRACT

In this study, we report a novel magnetic biomimetic nanozyme (Fe₃O₄@Cu/GMP (guanosine 5′-monophosphate)) with high laccase-like activity, which could oxidize toxic o-phenylenediamine (OPD) and remove phenolic compounds. The magnetic laccase-like nanozyme was readily obtained via complexed Cu²⁺ and GMP that grew on the surface of magnetic Fe₃O₄ nanoparticles. The prepared Fe₃O₄@Cu/GMP catalyst could be magnetically recycled for at least five cycles while still retaining above 70% activity. As a laccase mimic, Fe₃O₄@Cu/GMP had more activity and robust stability than natural laccase for the oxidization of OPD. Fe₃O₄@Cu/GMP retained about 90% residual activity at 90°C and showed little change at pH 3–9, and the nanozyme kept its excellent activity after long-term storage. Meanwhile, Fe₃O₄@Cu/GMP had better activity for removing phenolic compounds, and the removal of naphthol was more than 95%. Consequently, the proposed Fe₃O₄@Cu/GMP nanozyme shows potential for use as a robust catalyst for applications in environmental remediation.

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Introduction

Nanoparticle-based enzyme mimics, known as nanozymes, have drawn considerable attention due to their potential as more stable and cost-effective alternatives to natural enzymes (Wei and Wang, 2013; He et al., 2014; Gao and Yan, 2016; Zhou et al., 2017). So far, various nanomaterials, such as nanoceria (Xu and Qu, 2014), iron oxide nanoparticles (Gao et al., 2007), and gold nanoparticles, have been reported to mimic catalase, oxidase, peroxidase (Manea et al., 2004; Han et al., 2015), superoxide dismutase (Liu et al., 2015, 2016) and glucose oxidase (Luo et al., 2010). Efforts have been made to improve the specificity and activity of nanozymes via strategies such as surface modification (Liu and Liu, 2015; Wang et al., 2016a; Fan et al., 2017; Zhang et al., 2017a, 2017b). Despite this progress, an important challenge is to immobilize nanozymes so that they can be reused and better controlled. Immobilization of protein enzymes is an important field, which has applications in biosensor development, catalysis, and environmental protection (Kumar et al., 2018; Zdarta et al., 2018). Development on this front for nanozymes is, however, still lagging behind. This can be attributed to the fact that nanozymes are made from inorganic nanoparticles, and attaching ligands to nanozymes might block their...
surfaces and thus their activity (Liu and Liu, 2017). To avoid this problem, we reasoned that growing a layer of nanozyme on a substrate could be a viable approach. Among the different types of substrates that can be used for enzyme immobilization, magnetic nanoparticles are particularly attractive since the immobilized enzymes can be readily separated with a magnet (Xu et al., 2007; Gawande et al., 2013). In this work, our goal was to prepare a model nanozyme immobilized on magnetic iron oxide nanoparticles.

Laccase is a type of copper-glycoprotein enzyme with four copper ions catalyzing oxidation of a variety of compounds (e.g. ortho and para dihydroxy or diaminobenzene derivatives). In the same process, molecular oxygen is reduced to water with a four-electron reduction (Galli et al., 2013; Jones and Solomon, 2015; Mate and Alcalde, 2015). Laccases do not require or produce toxic H2O2 and are considered green catalysts. Laccases have been used for the oxidation of α-phenylenediamine (OPD) and the remove of phenols, and also have been employed for several biotechnological applications such as inorganic synthesis for the oxidation of functional groups, and coupling of phenols (Chandra and Chowdhary, 2015; Lin et al., 2017; Fathali et al., 2019). While laccases have highly important applications, they are very costly to produce biologically. To mimic laccase activity, many copper-containing complexes have been prepared with different types of organic ligands (Tanaka et al., 2004; Zhou et al., 2006; Veronica Rivas et al., 2011; Ulhmann et al., 2013; Ge et al., 2015) and carbon dots (Ren et al., 2015). Nucleotides with water solubility, biocompatibility, and chirality have been a significant focus of research as ligands, and they can produce materials with different nanostructures (Nishiyabu et al., 2009; Liang et al., 2016; Lopez and Liu, 2017). Nucleotide-coordinated Cu2+ complexes also have laccase-like activity (Zhou et al., 2015; Liu et al., 2017). Such coordination polymers might be immobilized on iron oxide surfaces to form Fe3O4@Cu/nucleotide nanoparticles (Frey et al., 2009; Liang et al., 2015).

In this study, we prepared a magnetic core and Cu/nucleotide nanozyme shell structure achieving immobilization, recycling and stabilization properties. The prepared magnetic Cu/nucleotide coordination polymers exhibited excellent laccase-like activity, as they could catalyze the oxidation of OPD and remove phenol pollutants. Additionally, the biomimetic nanozyme showed outstanding environmental tolerance and high stability, and it is easy to separate and recycle, indicating that the Fe3O4@Cu/nucleotide nanoparticles can potentially be considered as a replacement for natural laccase in practical applications.

1. Materials and methods

1.1. Materials

Guanosine 5′-monophosphate (GMP) disodium salt hydrate, ferric chloride, ferrous chloride, copper dichloride, OPD, catechol, hydroquinone, naphthol and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were obtained from Aladdin Inc. (Shanghai, China). Laccase was purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). 2-(N-morpholino)ethanesulfonic acid monohydrate was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other common chemicals were of analytical grade and purchased from Beijing Chemical Works. Milli-Q water was used to prepare all the buffers and solutions.

1.2. Preparation of Fe3O4@Cu/GMP nanozyme

Magnetic Fe3O4 nanoparticles (NPs) were prepared via the coprecipitation of FeCl3·6H2O and FeCl2·4H2O by addition of NH3·H2O as reported previously (Hou et al., 2015a). The Fe3O4 NPs and Cu/GMP complexes were prepared by mixing 200 μL of 25 mmol/L GMP, 100 μL of 5 mg/mL Fe3O4 NPs, 600 μL of HEPES buffer (10 mmol/L, pH 8.0), and 100 μL of CuCl2 (50 mmol/L). The samples were separated using an external magnet and washed with milli-Q water three times.

1.3. Characterization

The complexes prepared above were dispersed into milli-Q water by vortex mixing. Transmission electron microscopy (TEM) was performed on a Hitachi H-800 transmission electron microscope (Hitachi, H-800, Hitachi, Japan). The sample for TEM was prepared by pipetting a spot of the suspension onto a copper grid and drying with filter paper. The coordination complexes were freeze-dried for X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) analysis. XRD patterns were obtained using an X-ray diffractometer (D8 ADVANCE, Bruker, Germany) using Cu-Kα radiation (λ = 1.5178 Å, 40 kV × 40 mA). The 2θ scanning range was from 10° to 70° with a scanning speed of 0.1°/sec. FTIR was performed on a Nicolet MODEL 205 (Nicolet MODEL 205, Thermo Fisher Scientific, USA) with a wavelength range from 400 to 4000 cm−1. The same method was applied to prepare Fe3O4 NPs and Cu/GMP complex samples for FTIR.

1.4. Oxidation of OPD by laccase and nanozyme

The precipitated complexes were re-dispersed in pure water, and the typical oxidation reaction of OPD in the presence of the as-prepared complexes was performed under the following experimental procedure. A 100 μL portion of the nanoparticles (1 mg/mL) was mixed with 800 μL of 2-(N-morpholino)ethanesulfonic acid hydrate (MES) buffer (30 mmol/L, pH 6.8), and then 200 μL of oxidized substrate OPD (1 mg/mL) was dispersed into the above solution. After 60 min, the mixture was separated by an external magnet. The absorbance of the supernatant was detected at a fixed wavelength (418 nm). Kinetic measurements were performed at a fixed concentration of Fe3O4@Cu/GMP nanozyme or Laccase (1 mg/mL) in 30 mmol/L MES buffer (pH 6.8) by varying the concentration of OPD (20, 40, 60, 80, 100, 150 μg/mL).

1.5. Stability of laccase and magnetic nanozyme under different conditions

The pH stability of the Fe3O4@Cu/GMP nanozyme and natural Laccase were compared by the following procedure. A certain amount of free Laccase or Fe3O4@Cu/GMP nanozyme was incubated at varying pH (3.0–9.0) at room temperature for 8 hr. The enzymatic activities were determined and the relative
activity (%) was calculated by the ratio between the activity of each sample and the activity of the same sample at pH 7.0. The temperature tolerance of the Fe₃O₄@Cu/GMP nanozyme and natural Laccase was studied in the temperature range of 30–90°C for 30 min, and the relative activity (%) and the activity of the sample at 30°C was taken as the control. The storage stability was determined by measuring the residual activity of free Laccase or Fe₃O₄@Cu/GMP nanozyme dispersed in ultrapure water. Samples were stored at room temperature and the enzyme activity was measured at 418 nm every two days.

1.6. Effects of ions and organic solvents on the catalytic activity

The influence of metal ions (CuCl₂, MgCl₂, CaCl₂, BaCl₂, ZnCl₂, MnCl₂, NaCl, KCl) and salt ions (Na₂SO₄, Na₂CO₃, NaNO₃, NaCl) was determined at a final metal concentration of 100 μmol/L. Different aqueous solutions of metal ions (100 μL, 1 mol/L) and 700 μL of pure water were added to 100 μL of Fe₃O₄@Cu/GMP nanozyme (1 mg/mL) or laccase (1 mg/mL), followed by an addition of 100 μL of OPD (1 mg/ml). The reaction was carried out for one hour and the absorbance was measured at 418 nm. The activity of the catalysts without the addition of metal ions was measured as a control group and set to 100%.

1.7. Reusability of Cu/GMP and Fe₃O₄@Cu/GMP nanozyme

To test the recycling ability of Cu/GMP and Fe₃O₄@Cu/GMP, OPD (200 μL, 1 mg/mL) was added to a suspension of Cu/GMP or Fe₃O₄@Cu/GMP (1 mg/mL) in MES buffer (30 mmol/L, pH 6.8), followed by reaction at room temperature for 15 min. Cu/GMP were collected by centrifugation at 10,000 r/min for 2 min. Fe₃O₄@Cu/GMP were collected by an external magnet. Then, the absorption of the supernatant was measured at 418 nm. The precipitate was washed with ultrapure water and collected again. The precipitate was then subjected to the next catalytic cycle. The absorption of the supernatant at 418 nm for the first measurement was set as 100%.

1.8. Removal of phenolic pollutants

The removal activity of Fe₃O₄@Cu/GMP, laccase and TiO₂ for several phenolic compounds was studied by measuring the concentration of the phenolic compounds using high performance liquid chromatography (HPLC) (LC-20A, Shimadzu, Japan). A reverse-phase C18 column (Diamonsil, Dikma, China) was used for separation. The mobile phase was methanol (55%) and water (45%) at a flow rate of 0.6 ml/min. The injection volume was 10 μL. The ultraviolet (UV) (UV-5800H, Metash instrument Co., Ltd., China) detection was performed at 270 nm. The reactions for phenol removal were performed in 1 mL reaction mixtures consisting of aqueous phenol solution mixed with five kinds of phenolic substances (1 mg/mL) and 800 μL ultrapure water, reacted with 100 μL of 1 mg/mL catalyst (laccase, TiO₂ or Fe₃O₄@Cu/GMP) for 1 or 9 hr (TiO₂ reaction system included UV irradiation). The residual content of phenolic compounds in the system was determined by HPLC. The removal rate (η) was calculated according to the following formula:

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

where, C₀ (mg/mL) is the initial concentration of phenolic substrates, and Cₜ (mg/mL) is the concentration of the pollutants at different time intervals after the removal reaction, respectively.

2. Results and discussion

2.1. Characterization of Fe₃O₄@Cu/GMP nanozyme

Coordination polymers (CPs) containing nucleotides have been shown to efficiently encapsulate various guest molecules and nanomaterials (Nishiyabu et al., 2009; Zhou et al., 2015). These CPs could be formed under ambient conditions with a simple mixing step without the need for toxic initiators or UV light. Therefore, we suspected that we might achieve the desired core/shell structure using the reaction shown in Fig. 1a by mixing magnetic Fe₃O₄ NPs with GMP and Cu²⁺. The Fe₃O₄ nanoparticles were prepared by the coprecipitation method, and they had a relatively broad size distribution from 10 to 40 nm (Fig. 1c). We then mixed GMP and Cu²⁺, and observed features indicative of coordination polymer formation (Fig. 1a). For comparison of magnetic responsiveness, we also prepared Cu/GMP complexes in the absence of the Fe₃O₄ NPs (Fig. 1a, right inset photos). As illustrated in Fig. 1a, the Fe₃O₄@Cu/GMP nanozyme had excellent magnetic responsiveness, but the Cu/GMP complexes could not be attracted by magnets.

The Fe₃O₄@Cu/GMP nanozyme was synthesized as shown in Fig. 1a, and we observed a network Cu/GMP structure (Fig. 1d) and Cu/GMP complexes surrounding the Fe₃O₄ cores (Fig. 1e). Dynamic light scattering (DLS) (Helos-Sucell, Sympatec GmbH, Germany) showed that the average size of Cu/GMP and Fe₃O₄@Cu/GMP particles were over 1 μm, indicating agglomeration of Cu/GMP and Fe₃O₄@Cu/GMP, consistent with the above TEM data. We also measured the zeta (ζ)-potential of the free Fe₃O₄ NPs to be −27.67 ± 0.61 mV. Coating with Cu/GMP shifted the charge to 2.03 ± 0.79 mV (Appendix A Table S1). The change of ζ-potential also confirmed the formation of the Cu/GMP layer around the Fe₃O₄ NPs, and the low final charge can also explain the tendency of the material to agglomerate (Lopez and Liu, 2013). The magnetic properties of the synthesized magnetic Fe₃O₄ and Fe₃O₄@Cu/GMP were obtained using a vibrating sample magnetometer (VSM) (GWC-CHZ-1, Quantum Design, USA). As shown in Fig. 1b, the saturation magnetization values of bare Fe₃O₄ and Fe₃O₄@Cu/GMP were 60.5 and 35.5 emu/g, respectively. The significant decrease in the magnetic properties of Fe₃O₄@Cu/GMP in comparison to Fe₃O₄ alone was due to layers of Cu/GMP on the surface of bare Fe₃O₄. However, although the magnetic strength was decreased, it was still sufficient to support the separation of Fe₃O₄@Cu/GMP from the solution within 3 min using an external magnet (Fig. 1b, the inset photo).

To further characterize these materials, XRD analysis was used to identify the crystalline structures of the Fe₃O₄ sample and the prepared Fe₃O₄@Cu/GMP nanoparticles (Appendix A
Fig. S1). All the observed diffraction peaks were indexed to the expected structure of Fe$\text{O}_3$ with the Miller indices 220, 311, 400, 422, 511 and 440 (Ge et al., 2015). After being entrapped by Cu/GMP, the peak positions were retained although the intensities were weakened, indicating that the phase of the Fe$\text{O}_3$ core was maintained after embedding into Cu/GMP. No new diffraction peaks were identified, showing that the Cu/GMP structure was amorphous. The elemental mapping for Fe$\text{O}_3$@Cu/GMP (Appendix A Fig. S3) clearly showed the presence of C, Cu, Fe, N, O and P, and the content of each element characterized by energy-dispersive spectrometry (EDS) (Appendix A Fig. S4) was 23.12%, 18.24%, 29.86%, 1.46%, 26.34% and 0.98%, respectively.

FTIR spectroscopy was also used to characterize and compare the synthesized nanoparticle Fe$\text{O}_3$, Cu/GMP and Fe$\text{O}_3$@Cu/GMP (Appendix A Fig. S2). This technique can reveal changes in functional groups that occur through modification reactions. Very weak absorption peaks at 574 cm$^{-1}$ were observed in the spectrum of Fe$\text{O}_3$@Cu/GMP compared with that of Cu/GMP, which can be ascribed to Fe–O stretching (Gao et al., 2013; Hou et al., 2015b). The new characteristic absorption is consistent with encapsulation of the Fe$\text{O}_3$ nanoparticles by Cu/GMP. The bands at 976 and 1081 cm$^{-1}$ were assigned to the phosphate groups of GMP, and the peak at 1484 cm$^{-1}$ corresponds to C–N stretching. The broad peak in the range 3200–3750 cm$^{-1}$ and the band at 1640 cm$^{-1}$ are related to (–OH) in GMP (Nishiyabu et al., 2009; Zhao et al., 2013). The results of TEM, XRD and FTIR were consistent with Fig. 1a, all confirming the formation of the desired Fe$\text{O}_3$@Cu/GMP nanoparticles.

2.2. Laccase mimicking activities

After characterizing the material, the laccase-like catalytic activity of the Fe$\text{O}_3$@Cu/GMP nanozyme was studied using an oxidation reaction. OPD is one of the simplest aromatic diamines and is mainly used in the manufacture of pesticide fungicides, reducing dyes, cationic dyes, polymer stabilizers, heterocyclic compounds, antifreeze, and copper anticorrosive agents, but large concentrations of OPD are harmful to the environment. Therefore, it is necessary to remove OPD or transform it into less harmful by-products prior to discharge into the environment (Arowo et al., 2016; Paranji et al., 2016; Lin et al., 2017). OPD is a substrate of laccase, and it can be oxidized to form a less toxic by-product (Fig. 2a), which display a characteristic absorption at 418 nm (Wang et al., 2016b). After mixing OPD with Fe$\text{O}_3$@Cu/GMP nanozyme, we indeed observed ultraviolet–visible (UV–Vis) peak at 418 nm, which was consistent with the reaction product from laccase (Appendix A Fig. S5). The activity of the Fe$\text{O}_3$@Cu/GMP nanozyme was compared with that of natural laccase and Fe$\text{O}_3$ (Fig. 2b). At the same mass concentration (0.1 mg/mL), Fe$\text{O}_3$ had almost no activity, but the Fe$\text{O}_3$@Cu/GMP nanoparticles exhibited higher activity than laccase.

To further characterize the mechanism of the laccase-like activity of Fe$\text{O}_3$@Cu/GMP nanozyme, reaction kinetic assays were carried out at various concentrations of OPD. Parameters including $K_M$ values (Michaelis–Menten constant) and $V_{\text{max}}$ values (maximum reaction rate) were measured (Appendix A Table S2). The $K_M$ of laccase was 18-fold smaller than that of the Fe$\text{O}_3$@Cu/GMP nanozyme, which meant that laccase had
better affinity toward the substrate. However, the $V_{\text{max}}$ of Cu/GMP was almost 4.2 times higher than that of laccase. The higher activity of the nanozyme might be related to its higher copper content (Liang et al., 2017). The values of $V_{\text{max}}$ proved that the nanoparticles indeed had the excellent catalyst characteristic of speeding up a reaction.

2.3. Stability of Fe$_3$O$_4$@Cu/GMP nanozyme and laccase under different conditions

We researched the stability of natural laccase and the Fe$_3$O$_4$@Cu/GMP nanozyme by subjecting the catalyst to different pH values, temperatures, metal ions and salt ions. The pH

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**Fig. 2** – (a) Oxidation reaction of OPD in the presence of catalyst and (b) the enzyme activity of laccase, Fe$_3$O$_4$@Cu/GMP nanozyme and Fe$_3$O$_4$.

**Fig. 3** – Performance of free laccase and Fe$_3$O$_4$@Cu/GMP nanozyme after incubation at different (a) pH and (b) temperatures; Stability of Fe$_3$O$_4$@Cu/GMP nanozyme and laccase in different (c) metal ions and (d) salt ions.
stability of Fe₃O₄@Cu/GMP nanoparticles and free laccase is compared in Fig. 3a. The free laccase almost completely lost activity at pH 3.0 and decreased to almost 80% of the relative activity at pH 9.0, which showed that solutions with extreme pH can distinctly reduce the catalytic activity of natural laccase. However, the Fe₃O₄@Cu/GMP nanozyme maintained high laccase-like activity without significant loss.

To examine the effect of temperature on the catalytic activity, Fe₃O₄@Cu/GMP nanoparticles and free laccase were subjected to temperatures of 30–90°C for 30 min, and then their activities were compared (Fig. 3b). At low temperatures (30–50°C), the enzyme activity of neither laccase nor the Fe₃O₄@Cu/GMP nanozyme changed much with the increase of temperature. At high temperatures (60–90°C), the activity of the natural laccase reduced sharply with the increase of temperature, attributable to enzyme denaturation. Interestingly, the activity of Fe₃O₄@Cu/GMP dropped very little with the increase of temperature, and the activity was about 90% retained at 90°C. This indicated that changing the temperature had little effect on the activity of the Fe₃O₄@Cu/GMP nanozyme.

Since Cu²⁺ is a critical component of laccase, we then studied the effect of metal ions (Fig. 3c). In the presence of different metal ions (Cu²⁺, Mg²⁺, Ca²⁺, Ba²⁺, Zn²⁺, Mn²⁺, Na⁺, K⁺), the activity of natural laccase was clearly affected, and it was decreased by more than 50% and even became completely inactive. Thus, addition of exogenous metal ions might easily lead to the inactivation of enzyme proteins (Lu et al., 2012; Shankar and Nill, 2015). On the other hand, the activity of the Fe₃O₄@Cu/GMP nanozyme was even increased to approximately two-fold higher than the control, except for Cu²⁺. The activity in the presence of Cu²⁺ ions was lower than the rest, because copper ions had a strong influence on the UV–Vis absorption (Mesu et al., 2005).

Since almost all the metal ions had the same activity, the question was raised whether chloride ion has any effect on the nanoparticles. Therefore, we carried out experiments to explore the effects of different salt ions. At the same final
concentration of Na⁺ (100 mmol/L), different concentrations of salt ions were compared. Fig. 3d shows that chloride ion had a great influence on the nanozyme (almost 200%), while the other anions had no obvious effect. By contrast, laccase almost completely lost its activity in the presence of the salt, which demonstrated that salt ions did harm to the natural enzyme. Fig. 3c and d shows that chloride ions could significantly facilitate the chromogenic reaction. It had been discovered that chloride could accelerate the copper-based Fenton reaction (Shan et al., 2016; Wang et al., 2017a). It was demonstrated that Cu(II), serving as Fenton catalyst, reacted with oxygen or peroxide to produce hydroxyl radical (•OH) (Allen et al., 2013; Bokare and Choi, 2014), and chloride anions acted as scavengers to decrease the •OH yield.

2.4. Long-term storage and recycling

Generally, natural enzymes cannot be stored for a long time at room temperature, which limits their practical application. To compare the storage stability of laccase and the Fe₃O₄@Cu/GMP nanozyme, we tested the activity of laccase and Fe₃O₄@Cu/GMP nanozyme stored at room temperature for 13 days. As shown in Fig. 4a, the laccase gradually lost its activity day by day to reach zero on the last day, while Fe₃O₄@Cu/GMP kept nearly full activity.

The reusability of an enzyme mimic is critical for its potential application in industry. We compared Fe₃O₄@Cu/GMP nanozyme with and without Fe₃O₄ to investigate the role of Fe₃O₄ in reusability. As presented in Fig. 4b, the residual activity of Cu/GMP declined to under 50% after reusing it five times, while the Fe₃O₄@Cu/GMP nanozyme retained above 70% of its initial activity. The good stability of the Fe₃O₄@Cu/GMP nanozyme demonstrated that Fe₃O₄ could effectively adsorb Cu/GMP to ameliorate the loss of Cu/GMP after several reaction circles.

2.5. Removal of phenolic pollutants

Phenols released by various industries have become common organic pollutants, which pose great harm to the environment and human body (Dayana Priyadharshini and Bakhthavatsalam, 2016; Vela et al., 2018). Therefore, it is significant to remove the phenolic pollutants. Next, we studied the removal performance of Fe₃O₄@Cu/GMP for phenolic compounds, compared with natural laccase and titanium dioxide (TiO₂), which have been proved to be effective methods to remove organic pollutants (Carbonaro et al., 2013; Adamu et al., 2016; Wang et al., 2017b). The chromatograms of tested phenolic compounds are shown in Appendix A. The removal of the tested phenolic samples was determined by HPLC using 0.1 mg of laccase, Fe₃O₄ NPs, Fe₃O₄@Cu/GMP, or TiO₂ with UV-irradiation (TiO₂ + UV), respectively. The removal efficiency of the three catalysts toward mixed polyphenol compounds is shown in Fig. 5. After reaction for 1 hr (Fig. 5a), Fe₃O₄ NPs exhibited no removal ability. Laccase showed good removal effects for naphthol, hydroquinone, and catechol, compared to Fe₃O₄@Cu/GMP and TiO₂. Under UV light irradiation, TiO₂ showed the best phenol decomposition activity, and Fe₃O₄@Cu/GMP displayed better ability to remove naphthol and hydroquinone than TiO₂ + UV.

As shown in Fig. 5b, when reacted for up to 9 hr, laccase could degrade almost all of the catechol, hydroquinone and naphthol, but only removed about 10% of the phenol. The phenol, catechol, hydroquinone and naphthol removal by Fe₃O₄@Cu/GMP was markedly stronger, and in particular the removal of naphthol was increased to more than 95%. The above results show that if the reaction time is prolonged, naphthol, catechol, and hydroquinone can be completely removed by Fe₃O₄@Cu/GMP. Though TiO₂ + UV performed better at removing phenol and catechol, it is much simpler and more convenient to use Fe₃O₄@Cu/GMP without UV light. Overall, Fe₃O₄@Cu/GMP has potential application in the removal of phenolic contaminants from wastewater.

3. Conclusions

To conclude, we used a simple and facile method to develop a magnetic nanozyme with Fe₃O₄ nanoparticles embedded in supramolecular networks based on GMP-coordinated Cu²⁺. The Fe₃O₄@Cu/GMP NPs showed satisfactory laccase-like activity for oxidation of OPD. In addition, the magnetic nanozyme showed robust temperature stability and remarkable pH tolerance compared with natural laccases. Furthermore, the nanozyme maintained its initial activity after long-term storage under ambient conditions. This work presented a novel recyclable and reusable biomimetic catalyst for oxidation of OPD. In addition, Fe₃O₄@Cu/GMP showed good removal ability toward phenolic pollutants, and in particular exhibited high naphthol removal ability, with a removal ratio of 95% after 9 hr. The attractive features of the Fe₃O₄@Cu/GMP nanozyme make it potentially useful in developing new principles of chemical and biological detection, and the new material can be applied in the removal of phenolic pollutants in wastewater.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.07.008.

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