A Novel Peroxidase Mimics Based on the Concave Octopod PtCu Alloy Nanoframes and Its Application in Phenolic Compounds Removal

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Abstract. Phenolic compounds are main water pollutant sources with strong toxic potential even at very low concentrations. In this study, a novel peroxidase mimics with excellent catalytic performance was developed for phenolic compounds removal from industrial wastewater based on the uniform concave octopod platinum-copper alloy nanoframes (PCNFs). The as-prepared PCNFs performed as peroxidase mimics and exhibited splendid catalytic activity for phenolic compounds degradation with H2O2 as an oxidant. The PCNFs possessed series of advantages such as simple fabrication technology, convenient batch production, low manufacture cost, less equipment input, stable chemical/catalytic performance even under severe conditions like high temperatures and/or extremes of the pH scale. Furthermore, the peroxidase mimics PCNFs showed better catalytic efficiency compared with the natural horseradish peroxidase (HRP) over a wider range of temperatures and pH values in the presence of H2O2. The PCNFs presented quite good phenolic compounds removal from trade effluent within a 30-minute reaction period. The extraordinary catalytic purification performance of the PCNFs for phenolic compounds degradation renders its practical application in sewage water treatment.

1. Introduction
Over the past decades, the rapid expansion of industrial manufactures produce varieties of organic contaminants. Among them, phenol and its derivatives are very important in many domains such as medicine, chemicals and rubber industry[1]. The productions like aspirin, herbicides, pesticides, nylon, paint and adhesives, are widely-used substances in daily life[2]. However, large amounts of phenolic compounds (PCs) were usually presented in the industrial wastewater from industrial processes like medicine preparation, coal conversion, dyes processing, plastic production and so on[3]. Most of PCs may pose great risks to environment and bring about fearful health threats to human beings even at very low concentrations. Meanwhile, the PCs have been already identified as a primary contaminant by the Environmental Protection Agency (EPA) of America, Environment Agency (ECA) of European and the National Pollutant Release Inventory (NPRI) of Canada, so the PCs must be eliminated before releasing to the natural environment[4]. Hence, effective measures for PCs removal and/or degradation should be advanced for the sewage disposal. Numerous dynamic technologies have been developed and applied for PCs treatment such as conventional physical adsorption, liquid-solid extraction, steam
distillation, coagulation process and biological degradation. Recently, sorts of advanced technologies, such as photo/electrochemical oxidation, Fenton reaction degradation and enzyme catalytic treatment, have been widely promoted for the elimination of PCs\textsuperscript{[5]}. Thereinto, the enzymic degradation have motivated much attention and been proved effectively for PCs treatment in wastewater\textsuperscript{[6]}. Furthermore, natural biocatalysts such as horseradish peroxidase (HRP) and glutathione peroxidase, are famous catalysts for various oxidation reactions and have been proved to be effective for phenolic wastewater treatment with the assist of the pro-oxygenic agents\textsuperscript{[7]}. However, the natural peroxidases might be limited by the reaction conditions and often presented poor performance in catalytic reaction. Especially, the enzymatic structure might be destructed and lost catalytic activity under sundry handicaps such as extreme pH and temperature\textsuperscript{[8]}. Therefore, investigation on new technologies for enzymatic abatement is of paramount importance. During the past decades, based on different nanomaterials, varieties of peroxidase-like catalyzers (named peroxidase mimics) have aroused great attention and been used in the degradation of phenolic effluent. Typically, nanostructure such as ferric oxide (Fe_{3}O_{4}, Fe_{2}O_{3}), Au, Pt, CuO and RuO_{2} nanospheres have been extensive researched and proved to be an effective media tool for the elimination of PCs\textsuperscript{[9]}. The mechanism of degradation by peroxidase mimics is mainly ascribed to the generation of reactive oxygen and/or radicals under the assistance of hydrogen peroxide. Among these peroxidase-like nanoparticles, copper-based materials were attracted great attention for their special advantages such as excellent reusability, high catalytic activity under wider pH ranges and higher temperatures and so on\textsuperscript{[10]}. Therefore, high activity peroxidase mimics should be cleverly designed and manufactured for advancing the catalytic performance. In this study, the microalloyed concave octopus-like PtCu nanoframes (PCNFs) with high peroxidase-like catalytic activity were fabricated for the removal of PCs in presence of pro-oxidant. Scheme 1 showed the phenol (one of the most common PCs) degradation by PCNFs with the assist of H_{2}O_{2}.

Scheme 1. Overview of phenol removal mechanism of PtCu nanoframes with the assist of H_{2}O_{2}

2. Materials and Methods

2.1. Materials

Cetyltrimethyl ammonium bromide (CTAB, 99%), copper acetylacetonate (Cu(acac)\textsubscript{2}, 99%) and platinum acetylacetonate (Pt(acac)\textsubscript{2}, 99%) were purchased from Sigma-Aldrich (USA). Phenolic compounds (phenol, 99.5%; 2-Methoxyphenol, 99%; 4-Methoxyphenol, 99%; 2-Aminophenol, 99.5%; 3-Aminophenol, 99%; 1,4-Benzenediol, 99%; 2-Chlorophenol, 99%; 4-Chlorophenol, 99.5% and Catechol, 98%) were supplied by Alfa Aesar Co., Ltd (China). 3, 3′, 5, 5′-tetramethylbenzidine (TMB, 99%) and horseradish peroxidase (HRP, enzymatic activity >250 U/mg) were bought from Sinopharm Chemical Reagent Co., Ltd (China). Other conventional reagents were obtained from Aladdin Co., Ltd (China) without any further purification.

2.2. Synthesis of PCNFs

The concave octopod PtCu alloy nanoframes (PCNFs) were fabricated through a solvothermal method\textsuperscript{[11]}. Briefly, 31.05 mg Pt(acac)\textsubscript{2} and 153.8 mg Cu(acac)\textsubscript{2} were added in a three-necked flask
which containing 25 mL oleylamine and 700 mg CTAB under magnetic stirring in protection of nitrogen. The mixture solution was then transferred into a teflon-lined stainless steel autoclave (50 mL) for the solvothermal reaction at 180 °C within a 20 hour period. Then the solution was cooled down to room temperature, and the black products were collected by centrifugation (5000 rpm, 6 min) and then redispersed in cyclohexane/deionized water (V/V = 1/1). The pH of above solution was adjusted to 4.0 with 0.1 M HCl and stirred with continuous magnetic stirring for 3 h. Finally, The PCNFs were collected by centrifugation (10000 rpm, 8 min) and dried in a vacuum drying oven.

2.3. Characterization of PCNFs
Typically, the TEM images of the obtained PCNFs nanoparticles were observed by transmission electron microscopy (TEM, LIBRA-200FEG, Zeiss, Germany), the SEM images of PCNFs were obtained by using a scanning electron microscopy (SEM, FEINova 400 Nano SEM, Philips, Netherlands), then the HAADF-STEM images and the EDS mappings were recorded by a high angle annular dark field scanning transmission electron microscopy (HAADF-STEM, Titan G2 30, FEI, America) with energy dispersive X-ray (EDX) detector system. The XRD pattern of PCNFs was recorded by an X-ray diffractometer (D/max-2500 PC, Rigaku, Japan).

2.4. Peroxidase mimics activity assay of PCNFs
The peroxidase mimics property of the PCNFs was assayed as following: Firstly, the PCNFs (10 mg) was dispersed in 1.0 mL of NaAc buffer (0.1 M) containing 0.9 mM TMB and 5 mM H2O2 in a 1 mL quartz cuvette. The mixture was measured under different reaction conditions (pH, temperature and H2O2 concentration) by monitoring the absorbance change at 652 nm for 30 min on a UV-vis spectrophotometer. Effects of the temperature on the catalytic activity of PCNFs were tested in buffer solutions at pH 7.0 at the temperature from 4 °C to 100 °C. Effects of the pH were recorded within the pH range from 3.0 to 11.0 at 25 °C for 30 min, respectively. Meanwhile, free HRP was used as control and then similar procedures were also performed for the free HRP.

2.5. Phenolic compounds removal by PCNFs
The removal of phenolic compounds was explored by using PCNFs as a peroxidase mimics catalyst in the presence of H2O2. Briefly, PCNFs was added in 1.0 mL of NaAc buffer (0.1 M, pH 3.5) containing 6 mM of phenol and 30 mM of H2O2, and to compare the peroxidase mimics activity of PCNFs with that of HRP, then 10 mg free HRP was used as control. Subsequently, other phenolic compounds were also explored on removal efficiency by PCNFs.

3. Results and discussion
The morphology of the as-synthesized PCNFs was observed by various electron microscopes. As shown in Figure 1, Figure 2 and Figure 3, the TEM, SEM and HAADF-STEM images show that the PCNFs are mono-dispersive nanoparticles with uniform morphology. Meanwhile, the HAADF-STEM (Figure 3) also indicates that the Pt and Cu elements are uniformly dispersed in an octopod PtCu nanoframe. The high-resolution transmission electron microscope image (HRTEM) of an individual tentacle of the PCNFs was shown in Figure 4, where terrace atoms were obviously observed oriented from the step edge area.
Further, the average diameter of the PCNFs was about 62.9 nm (Figure 5, n = 400), and the elemental ratio of Pt: Cu in a PtCu alloy nanoframe was around 1: 2 (Figure 6). Then the crystal structure of the PCNFs was further detected by X-ray diffraction (XRD) measure, the XRD pattern of PCNFs possessed characteristic peaks appear at 2θ = 40.2°, 46.7°, 68.3°, and 82.3° were consistently corresponded to the 111, 200, 220 and 311 planes, respectively (Figure 7). The composition of the PCNFs was detected using the X-ray photoelectron spectroscopy (XPS) method (Figure 8). Typically, peaks monitored around 72 eV and 940 eV were assigned to the 4f electron of Pt and 2p electron of Cu.

Figure 1. TEM image of the obtained PCNFs.
Figure 2. The SEM image of the as-synthesized PCNFs.
Figure 3. HAADF-STEM and EDX mapping images of an individual PtCu nanoframe.
Figure 4. High resolution TEM image of one foot of the PtCu nanoframe.
Figure 5. Particle size of the obtained PCNFs.
Figure 6. EDX line-scanning map of an individual PCNF.
To confirm the peroxidase mimics activity of PCNFs for the PCs removal, TMB was chosen as the chromogenic substrate for catalytic oxidation reaction with the assistance of H\textsubscript{2}O\textsubscript{2}. It is fairly well known that TMB can be oxidized to a blue ox-TMB with a characteristic absorbance at 652 nm. As shown in Figure 9, the TMB + H\textsubscript{2}O\textsubscript{2} + HRP group shows a sharp characteristic peak at 652 nm, and similarly, identical absorption peak were monitored at 652 nm in the TMB + H\textsubscript{2}O\textsubscript{2} + PCNFs group, indicating that the PCNFs are effective peroxidase mimics in the presence of H\textsubscript{2}O\textsubscript{2}. Further, the peroxidase mimics behavior of PCNFs under different conditions (pH, temperature and H\textsubscript{2}O\textsubscript{2} dose) were detected in our study, as shown in Figure 10 and Figure 11, the PCNFs present much higher catalytic activity than natural HRP in wider pH and temperature ranges, the maximum catalytic efficiency can be found around pH 5.0 and 55 °C. Meanwhile, the H\textsubscript{2}O\textsubscript{2} dose-dependent study was performed with an increasing proportionality of H\textsubscript{2}O\textsubscript{2}/PCs (phenol was chosen here), the result indicates that the PCNFs perform more effective at most of H\textsubscript{2}O\textsubscript{2}/PCs ratios (Figure 12). Next, the phenol (100 ppm) containing wastewater was treated with different concentrations of PCNFs (0.1-10.0 mg/mL) for 30 min, the degradation of phenol could achieve over 80% (Figure 13). Moreover, the regenerative and reusable character study was taken to ensure the recycling possibility of the PCNFs. Figure 14 shows that the PCNFs retained about 70% of its initial activity after 10 cycles, but the HRP remained only 25% of its original activity after 10 cycle.

Figure 7. XPS spectra of the as-prepared PCNFs.

Figure 8. XRD pattern of the as-synthesized PCNFs.

Figure 9. UV-vis absorption of PCNFs in the presence of H\textsubscript{2}O

Figure 10. Peroxidase mimics behaviour of PCNFs under deferent pH.

Figure 11. Peroxidase mimics behaviour of PCNFs under deferent temperatures.

Figure 12. Peroxidase mimics behaviour of PCNFs under deferent H\textsubscript{2}O\textsubscript{2} doses.
To explore the potential possibility of PCNFs in the removal of PCs from phenolic wastewater, eight PCs were tested in this study. By contrast, these PCs were treated with the same amount of HRP as a control. The removal efficiency for the phenolic compounds that treated with the PCNFs were summarized in Table 1. The oxidation degradation of the PCs is probably determined by the steric effects and the inductive effects imparted by substituents on the benzene ring.

Table 1. Removal efficiency of PCs by using the same amount of PCNFs and HRP.

| PCs           | Removal efficiency (%) |
|---------------|------------------------|
|               | PCNFs                  | HRP        |
| Phenol        | 82.1 ± 6.1             | 69.8 ± 5.9 |
| 4-Methoxyphenol | 81.5 ± 5.4             | 91.6 ± 7.2 |
| 2-Methoxyphenol | 86.4 ± 6.7             | 94.5 ± 6.6 |
| 4-Chlorophenol | 31.2 ± 2.8             | 22.6 ± 2.4 |
| 2-Chlorophenol | 26.5 ± 3.5             | 17.3 ± 1.9 |
| 3-Aminophenol | 49.3 ± 3.8             | 22.6 ± 2.7 |
| 2-Aminophenol | 86.7 ± 5.5             | 81.4 ± 5.4 |
| 1,4-Benzenediol | 77.9 ± 5.8             | 61.3 ± 6.0 |

4. Conclusion

In summary, a novel strategy was advanced for phenolic compounds removal from wastewater based on the as-prepared PCNFs. The peroxidase mimics activities of the as-synthesized PCNFs were explored compared with the natural HRP, and the PCNFs exhibit favourable peroxidase mimics behaviors over wide pH and temperature ranges. Eventually, the peroxidase mimics PCNFs presented excellent recycling performance for PCs degradation even after 10-cycle repeated uses. Taken together, the easy-preparation, high reusability, advanced stability of the PCNFs give its great potential in the degradation of industrial phenolic wastewater and other environmental protection application like water purification, water filtration, etc.

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