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Removal of 2,4,6-trichlorophenol by laccase immobilized on nano-copper incorporated electrospun fibrous membrane-high efficiency, stability and reusability

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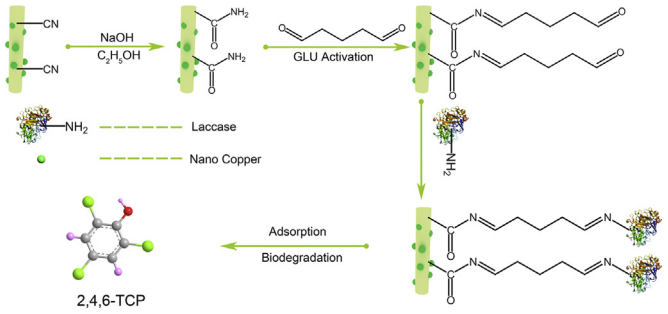
HIGHLIGHTS

- The PAN/PVdF membrane was immobilized by laccase and nano-copper.
- The relative activity of the immobilized laccase is enhanced.
- The removal efficiency for 2,4,6-TCP of immobilized laccase reaches high value.
- The laccase immobilized on PAN/PVdF/Cu NFMs exhibits high reusability.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, nano-coppers were incorporated into an enzyme carrier, polyacrylonitrile/polyvinylidene fluoride (PAN/PVdF) electrospun fibrous membranes (EFMs). Laccase immobilized on PAN/PVdF/Cu EFMs prepared was used for the removal of 2,4,6-trichlorophenol from water. The physical and electrochemical properties of PAN/PVdF/Cu EFMs, as well as the efficiency of laccase immobilization and 2,4,6-TCP removal were investigated in detail. Scanning electron microscopy and transmission electron microscopy images show that the diameters of PAN/PVdF/Cu EFMs ranged from 200 nm to 500 nm. The immobilized laccase had a broader application pH and temperature range compared with free laccase. Laccase immobilized on PAN/PVdF/Cu EFMs showed significantly better performance on 2,4,6-TCP removal (95.4%) than that without nano-coppers (82.2%) ($p < 0.05$). It was because the incorporation of nano-coppers into the fibers facilitated the electron transfer during the process of catalytic reaction with laccase. In addition, it was demonstrated the lac-PAN/PVdF/Cu EFMs prepared in this work possessed high reusability. Therefore, the lac-PAN/PVdF/Cu EFMs have promising potential in industrial application for the removal of pollutants from water.

1. Introduction

Chlorophenols are a class of typical organic compounds widely found in the effluents of different industrial waters [1,2], among which 2,4,6-trichlorophenol (2,4,6-TCP) is a representative characterized by toxic, mutagenic, and carcinogenic effects. Recently, enzyme-based treatments for the removal of chlorophenols attract wide attention from scientific researchers due to their remarkable properties in physical and chemical processes, such as mild treatment condition, high efficiency for substrate removal, and ability to handle large volumes of effluents [3–5].

Laccase alone, or with redox-mediators, has been reported to oxidize a variety of organic pollutants [6], such as diphenols

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polyphenols, diamines, aromatic amines [7], bisphenol A [8], and endocrine disrupting chemicals [9]. The laccase monomer generally requires four copper atoms for functional catalytic activity and the mechanism for laccase-induced treatment involves the oxidation of pollutants to free radicals or quinones that subsequently undergo polymerization and partial precipitation [10]. However, in most cases, free laccase lacks stability, reusability, and recyclability for industrial application. Therefore, it is very necessary to find an effective way to make it applicable in different reaction environments.

Immobilization of enzymes on EFMs is considered an effective method for pollutant removal due to their desirable characteristics, such as easy separation and recycling, prominent adsorption capacity, and reusability. Our previous research work found that pollutant removal by enzymes immobilized on EFMs should be attributed to two mechanisms: adsorption by the carrier and biodegradation by the enzyme [11]. The results showed immobilized laccase retained 72% activity of free laccase, meanwhile, more than 85% of the TCP was removed under optimum conditions [3]. To further increase the removal efficiency of TCP up to 90% and make the immobilized laccase more stable and reusable, we considered introducing some additive ingredients with catalytic properties into the laccase immobilized EFMs.

Polyacrylonitrile (PAN) is a suitable carrier material with excellent stability and adhesive properties and has been used in filtration and adsorption [12,13]. Polyvinylidene fluoride (PVdF), a hydrophobic polymer, has been widely studied for application in rechargeable batteries [14,15] and normally possesses strong chemical resistance [16]. Therefore, these two polymers are suitable materials for preparing EFMs, which may be potentially used to process industrial effluents.

Metallic nanomaterials have many remarkable properties including small size effect, quantum effect, and macro quantum tunnel effect, as well as wide applications in pharmaceutics, chemosynthesis, and electronics [17–22]. Nano-copper was the most commonly used metallic nanomaterial because of its low price and stable physicochemical property [23]. It has been applied in various areas, such as inkjet printing [24], lubrication oil additives, [25], and electrochemistry [26,27]. However, it is less reported in the field of environmental engineering. Considering the copper atoms contained in the structure of laccase, which are involved in catalyzing the oxidation of chlorophenols, we chose nano-copper as an additive ingredient to improve the biodegradation of TCP by immobilized laccase.

This study aimed to incorporate the nano-coppers into EFMs and investigate the physical, chemical, electrochemical properties of EFMs as well as the enzyme-based EFMs. The enzyme-based material was then used for the removal of organic pollutant 2,4,6-TCP. In addition, the reaction conditions were optimized with response surface methodology. It is expected that the study would provide a new application potential for the removal of 2,4,6-TCP from water, especially industrial wastewater.

2. Materials and methods

2.1. Materials

PAN (*M*w = 150,000), N,N-dimethylformamide (DMF), Coomassie brilliant blue (G250), citrate phosphate buffer solution (CPBS), 2,2′-azinobis-(3-ethylbenzthizaoline-6-sulphonate) (ABTS), glutaraldehyde (GLU, $C_5H_8O_2$), laccase from *Trametes versicolor* (enzyme activity $\geq$ 0.5 U/mg) and 2,4,6-TCP were obtained from Sigma-Aldrich. PVdF was purchased from Arkema, China. Sodium hydroxide (NaOH), disodium hydrogen phosphate (Na_2HPO_4), and phosphoric acid (H_3PO_4), were obtained from Sinopharm Chemical Reagent Co. Ltd, China. Deionized water was used in all experiments and all chemicals used were of analytical grade.

2.2. Preparation of Nano-copper

Nano-copper was prepared using the liquid phase reduction method, namely $CuSO_4$ was reduced to Cu by KBH_4. The detailed preparation process was as follows: Firstly, 0.26 g KBH_4 was dissolved in 200 mL water and placed in a conical flask, and 2 mol/L NaOH solution was then added into the former solution to adjust the solution to pH 12.0. Secondly, 2.50 g $CuSO_4{\cdot}5H_2O$ was dissolved in a solvent to prepare a 0.1 mol/L $CuSO_4$ solution (the solvent was a mixture of absolute ethyl alcohol and water, the volume ratio of which was 2:3). A suitable amount of PVP was then added into the $CuSO_4$ solution. Thirdly, the former KBH_4 solution was added dropwise with continuous stirring. Finally, the precipitates were washed six to eight times using distilled water, and then dried under vacuum at 40 °C for 12 h to obtain nano-copper. The prepared nano-copper was then stored for later use.

2.3. Preparation of PAN/PVdF/Cu EFMs

The electrospinning apparatus used in this study was as follows: a high voltage supplier, a capillary tube with stainless steel needle (inner diameter, 1.2 mm), a syringe pump and a rotating cylinder collector wrapped with aluminum. A total of 0.8 g PAN was dissolved in 9.2 g DMF and stirred for 8 h at 60 °C to form a homogeneous solution. Then, 0.5 g PVdF was added into 4.5 g DMF and stirred for 5 h at 60 °C to form a homogeneous solution. About 3 g of 8% PAN and 1 g of 10% PVdF solution was mixed together and stirred for half a day at 60 °C. About 0.02 wt %–0.14 wt% nano-copper was added into the solution above and sonicated for 30 min to evenly disperse the nano-copper into the mixture. The PAN/PVdF/Cu sol–gel was placed into a plastic capillary with the following electrospinning parameters: a voltage of 16 kV, a tip-to-target distance of 15 cm, and a relative humidity of 40% ± 5%. The flow rate was 0.8 mL/h. The PAN/PVdF/Cu EFMs were collected for 8 h and dried under vacuum at 60 °C for 12 h to remove the residual organic solvent and moisture. It was found the EFMs possessed the optimum tensile-strength, enzyme loading and activity when the concentration of nano-copper was 0.1 wt%. As a result, we chose 0.1 wt% of nano-cooper to continue the following experiments.

2.4. Characterization of prepared nano-copper and EFMs

Scanning electron microscopy (SEM) measurements were performed on a field emission XL-30 SEM conducted at 30 kV. Transmission microscopy (TEM) experiments were performed on a JEM-2011 electron microscope operated at 200 kV. X-ray diffraction (XRD) images were recorded with the D8 Advance X wide-angle XRD scanner within the range of 10°–80°.

2.5. Electrochemical measurements

The electrochemical tests were carried out in a standard three-electrode system controlled with a PGSTAT302N electrochemical working station. The working electrode was formed using 10 mg of the samples pressed onto a nickel grid with a surface of 1 cm^2. Platinum wire and Ag/AgCl served as the counter electrode and reference electrode, respectively. The electrolyte solution was composed of 5 mM $K_3[Fe(CN)_6]$ $K_4[Fe(CN)_6]$ and 1 mM KCl. The cyclic voltammetry (CV) data was obtained at a scan range of −0.3 V to 0.6 V with a scan rate of 5 mV/s.