Chemical Engineering Journal 347 (2018) 703–710

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Polydopamine tethered CPO/HRP-TiO_2 nano-composites with high bio-catalytic activity, stability and reusability: Enzyme-photo bifunctional synergistic catalysis in water treatment

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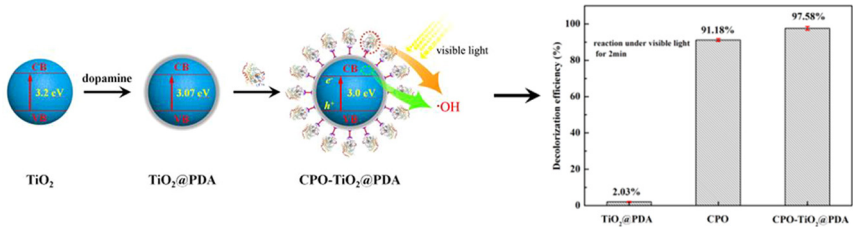
HIGHLIGHTS

- Enzyme-TiO_2 composite with high catalytic activity, stability at harsh reaction conditions.
- Enzyme-photo bifunctional synergistic effect was observed when applied in water treatment.
- CPO/HRP-TiO_2@PDA can be used under visible light irradiation instead of ultraviolet light.
- HRP-TiO_2@PDA is excellent in reuse, with no loss of activity when used for 15 cycles.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Bifunctional catalysis
Synergistic effect
CPO/HRP enzymatic catalysis
TiO_2 photocatalysis
Water treatment

ABSTRACT

An enzyme–TiO_2 composite with high catalytic activity, stability at harsh reaction condition, and good reusability was prepared via modification of TiO_2 by polydopamine (PDA) to tethering with chloroperoxidase (CPO) or horseradish peroxidase (HRP) through glutaraldehyde. TiO_2 played both roles of solid carrier and photocatalyst in the application of soluble dyes decolorization, and in this way, the loss of enzymatic activity during enzyme immobilization can be compensated. Because the enzyme and TiO_2 were included in same composites, an enzyme-photo bifunctional synergistic catalysis was observed due to the interaction between enzyme and TiO_2, resulting in that the formation of catalytic intermidiate, ·OH, increased and the band gap energy of TiO_2 became narrow. The light absorption by TiO_2 was extended from the UV region to the visible region through coated by PDA and CPO/HRP so as that CPO/HRP-TiO_2@PDA can be used under visible light irradiation instead of ultraviolet light irradiation, which can avoid the decrease of enzyme activity by ultraviolet light irradiation. HRP-TiO_2@PDA composite had even the best resuability of enzyme immobilized on solid support compared with the data in reference, in our best knowledge. There was no activity loss of HRP-TiO_2@PDA after 15 times use, and more than 90% activity can be remained after 25 times use. Even after 40 times use, the relative activity of HRP-TiO_2@PDA was 63.6% of that in the first run. The difference of reusability between CPO and HRP have relationship with the distribution of function group among the surface of enzyme molecule.

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https://doi.org/10.1016/j.cej.2018.04.083
Received 11 December 2017; Received in revised form 5 April 2018; Accepted 13 April 2018
Available online 24 April 2018
1385-8947/

1. Introduction

Recent years, enzymatic catalysis has emerged as an important green technology in the synthesis of pharmaceuticals and fine chemicals because enzymatic processes are generally conducted under mild conditions in water with high efficiency and selectivity. Moreover, the use of enzymes generally obviates the need for functional group protection and/or activation, affording cost-effective synthetic routes and generating less waste compared with conventional organic syntheses [1–3], However, industrial application of enzymes is often hampered by the lack of operation stability and difficulty of re-use. These drawbacks can generally be overcome by immobilization of the enzyme on solid support. But binding enzyme on a support often results in the decrease of enzymatic activity when compared with that of a free enzyme due to either enzyme deactivation during immobilization, or increased transfer resistance of substrate in the support. So, preparation of immobilized enzyme with both stability and high activity is interesting, but still a challenge.

The support for enzyme immobilization is generally required to have high chemical stability, low toxicity, and bio-compatibility. Besides, it often has suitable pores to contain enzyme, for example, SBA-15 and SBA-16 mesoporous silicas materials [4,5], or materials with functional groups ready for combining with enzyme, such as graphene oxide [6–10]. However, all these reported supports were used only as a support rather than a catalyst working together with the enzyme to compensate the enzymatic activity loss in the immobilization process.

TiO_2 is an optimal candidate for immobilization of enzyme due to its high chemical stability, low toxicity, and high bio-compatibility [11,12]. Meanwhile, TiO_2 is a versatile material and its applications from energy to engineering cover several sectors and final users. Besides the popular photo-catalytic activity used for the treatment of organic pollutants in wastewater [13,14], TiO_2 is also used as electrodes in sodium batteries and in aqueous solar cells [15–22]. However, TiO_2 surface is difficult to be modified to link with enzyme, so it is a challenge to provide sufficient linkages between TiO_2 and enzymes. Recently, some modified TiO_2 for enzyme immobilization was reported. For instance, Wu et al. modified nano-porous TiO_2 to immobilize enzyme through the electrostatic attraction and self-assembly technique between M-TiO_2, ε-Poly-L-lysine (EPL) and enzyme [23]. Zhuang et al. reported the surface of mesoporous TiO_2 was modified by (3-aminopropyl) triethoxysilane (APTES) to immobilization adenosine deaminase, and Wang et al. immobilized γ-glutamyltranspeptidase on silylated mesoporous TiO_2 whiskers through covalent combination [24].

Though there are some reference mentioned that TiO_2 was used as photocatalyst to promote enzymatic reactions, for example, Zhang et al. combined photocatalytic water oxidation and enzymatic oxyfunctionalizations for selective aerobic oxidation reactions [25]; Romero-Arcos et al. immobilized enzyme on TiO_2 nanoparticles to detect phenol compounds as amperometric biosensors [26]. They point out TiO_2 can facilitate electron transference between surface of the electrode and the enzyme without loss of biological activity. However, in most reference, TiO_2 is only employed as a support to load enzyme molecules on its surface instead of as a photocatalytic materials.

On the other hand, it is reported that dopamine (DA) has been demonstrated to be able to polymerize and stick on both organic and inorganic surfaces through the formation of covalent and noncovalent bonds. The resulting polydopamine (PDA) has emerged as versatile for applications in cell adhesion, biomineralization and nanoparticle stabilization *etc* [27]. On the basis of this function, Wu *et al.* proposed PDA as a bioadhesive cross-linker to tether enzyme so as to form nano-sized particles, facilitating the repeated use of enzyme [28]. Inspired by this reports, in this work, we modified the surface of anatase TiO_2 through self polymerization of dopamine. Then, the core-shell TiO_2@PDA, having many functional groups ready for binding, was used for immobilization of heme protein, chloroperoxidase(CPO) or horseradish peroxidase(HRP) by glutaraldehyde cross-linking. As expected, the obtained CPO/HRP-TiO_2@PDA composite can combine enzyme catalysis with photo-catalysis in the application of decolorization of dyes or degradation of 2,4-Dichlorophenol. Moreover, an enzyme-photo bifunctional synergistic catalysis, like 1 + 1 > 2, was found. The catalytic activity and stability at elevated temperatures and in the presence of organic solvent as well as the reusability of the CPO/HRP-TiO_2@PDA composites were evaluated. The mechanism about the synergistic effect was investigated.

2. Materials and methods

2.1. Materials

Caldariomyces fumago was cultured according to the method established by Morris and Hager [29]. Then CPO was purified from this the culture medium. Horseradish Peroxidase (HRP) was purchased from sigma. Anatase TiO_2 with average diameter of 30 nm, dopamine hydrochloride, and hexamethylenetetramine were purchased from Alfa Aesar. Sodium hydroxide, terephthalic acid, methanol, acetonitrile, N,N-dimethylformamide (DMF), crystal violet, aniline blue, ethyl alcohol absolute, and glutaraldehyde (30% in aqueous solution), were all purchased from Sinopharm Chemical Regent Co. Ltd. $NH_3{\cdot}H_2O$, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, hydrogen peroxide (30% in aqueous solution) and other chemicals were obtained from Xían Chemical Co. Ltd. All chemicals were used without further purification and all solutions were prepared using ultrapure water ($R > 18.25\,M\Omega\,cm^{-1}$).

2.2. Preparation of the CPO-TiO_2@PDA and HRP-TiO_2@PDA

TiO_2@PDA was formed by the in situ polymerization of dopamine (DA) on the surface of TiO_2. Then, CPO or HRP were covalently attached onto TiO_2@PDA using glutaraldehyde as cross linking agent. Specifically, TiO_2@PDA was ultrasonically dispersed in phosphate buffer (pH 5.8) followed by 5.8 ml glutaraldehyde (25%) solution and activated at room temperature for 2 h. The glutaraldehyde activated TiO_2@PDA was washed and then centrifugated, and then soaked in a CPO phosphate buffer (pH 4.5) for 12 h. The CPO-TiO_2@PDA was then rinsed with buffer to remove any non-immobilized CPO. HRP-TiO_2@PDA was prepared in the same way, except the pH was controlled at 7.0.

2.3. Catalytic performance of free and CPO/HRP-TiO_2@PDA

The catalytic performance of the CPO-TiO_2@PDA was evaluated in terms of the decolorization efficiency of soluble aniline blue and crystal violet under visible light irradiation at ambient temperature. The decolorization efficiency (%) was measured by monitoring changes of the λ_{max} of dyes at λ_{max}: [30]

$$\text{Decolorization efficiency}(\%) = \frac{A_0 - A_t}{A_0} \times 100\% \quad (1)$$

A_0: Absorbance of initial solution of dye at λ_{max}; A_t: Absorbance of dye solution at λ_{max} at t min. Equation (1) was used based on the calibration curves of absorbance of dyes (soluble aniline blue or crystal violet) at λ_{max} to its concentration in the concentration range of 0–0.25 $mmol{\cdot}L^{-1}$ with the linear correlation coefficient R^2 was higher than 0.99.

10 mg CPO-TiO_2@PDA, free CPO with same amount enzyme as that in CPO-TiO_2@PDA, were added respectively into a 5 ml buffer containing 0.05 $mol{\cdot}L^{-1}$ dye aqueous solution, stirring continually in dark for 1 h. After reaching a complete adsorption–desorption equilibrium, it was exposed to visible light irradiation offered by 50 W Xe lamp with a 420 nm cut-off filter. The absorbance at λ_{max} was measured by an UV–visible spectrophotometer (UV-1750, Shimadzu, Japan).