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Preparation of laccase-graphene oxide nanosheet/alginate composite: Application for the removal of cetirizine from aqueous solution

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ABSTRACT

Graphene oxide (GO) nanosheet was synthesized by modified Hummer's method. Laccase was immobilized by adsorption method on GO and was followed by entrapment method in the alginate matrix. Characterizations of GO, alginate, and laccase-GO/alginate composite were studied by FT-IR, XRD, and SEM analysis. Laccase activity was increased by immobilization due to the improving the electron transfer between the laccase and substrate. Laccase-GO/alginate composite application for removal of cetirizine as a model pharmacetucal pollutant was evaluated and effect of variables including; pH of cetirizine solution, reaction temperature, and cetirizine concentration were studied. Results indicate laccase-GO/alginate composite have high storage and thermal stability and reusability with high removal efficiency of cetirizine.

1. Introduction

The widespread production and consumption of pharmaceutical and personal care products in the last decades has raised growing concern [1–3]. These compounds entered in water and wastewater through the pharmaceuticals manufacturing plants, landfills, hospitals, household, and human excretion via urine and feces [4]. Due to the hydrophilic nature of most pharmaceuticals, they undergo incomplete biological degradation. Pharmaceutical pollution is a new challenge and an important issue in water treatment process because these compounds tend to concentrate by water recycling through the biosphere. Therefore, releases of these wastes to environment without suitable treatment processes have retrievable effects on human health and environment. So the employment of pharmaceuticals and personal care products and their wastes must be managed. Considering the demand of water quality standards for dis-charge of industrial wastewaters to the environment and on the other side water shortage, different methods were investigated to the treatment of polluted waters. Numerous methods such as ozonation [5], solar photo fenton [6], photo oxidation [7], adsorption on carbon nanotube and graphene nanoplates [8], sonophotolysis [9], adsorption on activated carbon [10], membrane filtration [11] and nanofiltration [12,13] have been tested for removing of these contaminants. Nevertheless, expensive plant requirements, high operational costs, regeneration problem and secondary pollutants are some technological and economical disadvantages of these methods.

Enzymatic removal of organic pollutants found the great and significantly increasing attention. The some of the advantages of enzymatic treatment of organic pollutants comprise; absence of toxic effects, shorter reaction times, easy and simple process, lack of unforeseen products due to their high specificity, lack of acclimatization period, less energy requirement, operation under mild conditions, and operability over a wide range of pH, temperature, and salinity and also operability at low and high concentrations of pollutants [14,15]. Overall can be said, enzymes provide environmental friendly method to this public health concern.

Laccase (EC 1.10.3.2., *p*-diphenol: dioxygen oxido-reductase) belongs to a large group of blue multicopper enzymes [16]. Laccase is one of the oldest enzymes ever described [17]. The range of laccase substrates is very wide. Basically any substrate with similar structure to the *p*-diphenol can be oxidized by laccase with the concomitant reduction of oxygen molecule (as an electron acceptor) to water. Laccase catalyzes the oxidation of many aromatic and inorganic substances including; endocrine disrupters and polycyclic aromatic hydrocarbons, phenols, trichlorophenols, organophosphorus pesticides and dyes [18–22]. Among, laccase can eliminate pharmaceuticals and personal care product from water and wastewaters alone or in the presence of a redox mediator [23–25]. Laccase mechanism is degradation. In this study reaction products and reaction path were not investigated.

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According to Sutar and et al. [14] report, laccase first converts cetirizine to (2-(4-(4-chlorophenyl) phenylmethyl) piperazine-1yl) ethanol which further degraded into 1-benzyle-4-chlorobenzene and 2-(piperazine-1-yl) acetaldehyde.

When enzymes applied in their free form for the treatment of contaminated waters, enzymes face basic operational problems such as rapid denaturation, lack of reusability, and requirement of large quantities which will enhance the overall cost of their use and consequently limit their applications [26–30]. Immobilization and insolubilization of enzymes is one of the most effective techniques to overcome the mentioned disadvantages.

In this research laccase was immobilized on graphene oxide (GO) nanosheets and followed by entrapment method using alginate for easy separation and reusability. Laccase-GO/alginate composite preparation and application for water treatment was investigated for the first time in this study according to our knowledge. The GO nanosheets have two main role in this immobilization process. Functional groups of GO enhance the interfacial interaction between graphene oxide and a polymer matrix and improve considerably the mechanical and thermal properties of alginate [31]. Also, GO such as other carbonaceous compounds increases the activity of laccase [32].

Sodium alginate is a water soluble linear polysaccharide and a natural occurring carbohydrate polymer composed of α-L-guluronate and β-D-mannuronate residues and has hydrophilic, biocompatible and nontoxic properties. By exchanging of sodium ions from the guluronic acid residues with the divalent cations the solidification is occurred. Calcium alginate has been used to immobilize different nanoparticles for easy separation and usage. In this work it was used to produce easily separable beads.

Because of the importance of pharmaceutical wastewaters and their treatment using enzymatic methods, in this study, the removal of cetirizine dihydrochloride as a pharmaceutical model pollutant was studied. Cetirizine dihydrochloride, with $C_{21}H_{25}ClN_2O_3$ formula and molecular mass of 388.89 g/mol, is a second- generation antihistamine pharmaceutical that was used in the treatment of hay fever, allergies, angioedema, and urticarial. Cetirizine is frequently detected in wastewater samples [14].

2. Experimental

2.1. Materials

Graphite, $KMnO_4$, H_2SO_4, NaOH, and HCl were purchased from Merck. Laccase from aspergillus niger was obtained from Sigma Aldrich Company. Cetirizine dihydrochloride was bought from Shahre Daruo Company (Tehran, Iran).

2.2. Measurement of cetirizine dihydrochloride

The absorbance of cetirizine dihydrochloride aqueous solution was recorded in the range of 190–800 nm and 230 nm was found as the maximum absorbance wavelength. Using known aqueous solutions of pharmaceutical with different concentrations and their absorbance at 230 nm, the calibration curves was plotted. This curve was used to convert absorbance data to pharmaceutical concentration in the solutions. The removal efficiency of pollutant was defined using Eq. (1):

$$R(\%) = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

where R is pharmaceutical removal efficiency, C_0 (mg L^{-1}) is initial concentration of pharmaceutical, and C_t (mg L^{-1}) is its concentration at t min.

2.3. Laccase activity assay

laccase activity was measured with 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) as a substrate. The oxidation of 1 mM ABTS in 0.1 M phosphate buffer (pH = 4.5) at 420 nm considering $\varepsilon = 36 mM^{-1} cm^{-1}$ was followed using a spectrophotometer [33]. One unit of laccase activity (U) is defined as the amount of laccase required to consume 1 μmol of substrate in one min at 25 °C.

2.4. Synthesis of GO nanosheets

GO was synthesized using graphite as precursor by a modified Hummers method [34]. 2.5 g graphite was added to a mixture containing 115 ml H_2SO_4 (98% w/v), 15 g $KMnO_4$, and 2.5 g $NaNO_3$ that was kept in an ice bath. The mixture was kept at 0 °C for 24 h and then was stirred at 35 °C for 30 min and slowly diluted with deionized water. The reaction temperature was rapidly increased to 98 °C and kept for 15 min. Then H_2O_2 30% was added to the mixture which leads to colour change to yellow. Finally the mixture was centrifuged and washed with HCl (5%) and distilled water several times and was dried at room temperature. Its characterization was studied by FT-IR, XRD, and SEM analyses.

2.5. Laccase immobilization

Fig. 1 illustrates the preparation procedure of laccase-GO/alginate composite. The technique applied in this study was based on the adsorption of laccase on GO nanosheets that was followed by entrapment method using alginate biopolymer. For this mean 0.01 g GO was immersed in 10 ml of 200U/ml laccase solution in acetate buffer at pH 4.50 with shaking in a shaker-incubator for 2 h in the controlled temperature of 10 ± 0.5 °C.

The alginate solution was obtained by dissolving the sodium alginate in distilled water as 2% (w/v) solution at water bath 60 °C. After cooling the alginate solution to room temperature, the prepared laccase-GO suspension was slowly dropped into the viscous sodium alginate gel and was constantly stirred for 30 min using a magnetic stirrer to form a homogeneous mixture. The obtained viscous solution was transferred to a syringe and was dropped to the $CaCl_2$ solution (2%w/v) in an ice bath (4 °C) and was kept for 30 min in the ice bath. Finally the beads were washed with distilled water and were remained in buffer (pH = 4.5) at refrigerator until application.

The efficiency of laccase immobilization was calculated from two methods. 1. The amount of immobilized laccase was estimated by subtracting the amounts of laccase recovered in the supernatants and washing buffer from the amount of laccase initially was used. 2. The amount of immobilized laccase was calculated by measuring the activity of certain amount of the immobilized laccase.

The characterization of alginate, GO and laccase-GO/alginate were studied by fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscope (SEM). The structural characterizations were carried out by FT-IR using a Bruker Tensor 27, Germany and KBr pellets and XRD analysis using a Simens D-500 (Germany) with Cu K_a radiation at a wavelength of 0.15406 nm. The morphologies of alginate, GO, and laccase-GO/alginate were studied by a SEM (MIRA3 FEG-SEM Tescan, Czech).

2.6. Thermal and storage stability of free and immobilized laccase

1 ml of free laccase and 10 g of immobilized laccase in 5 ml acetate buffer pH = 4.5 were kept at 4 °C and the residual activities of samples were determined every day until 10 days. Thermal