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Simultaneous adsorption–degradation of organic dyes using $MnFe_2O_4$/calcium alginate nano-composites coupled with GOx and laccase

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ABSTRACT

In this study, glucose oxidase and laccase enzymes were immobilized on $MnFe_2O_4$ nanoparticles, which were synthesized by co-precipitation method. The nanocomposites of glucose oxidase/$MnFe_2O_4$/calcium alginate, laccase/$MnFe_2O_4$/calcium alginate, and $MnFe_2O_4$/calcium alginate were prepared by trapping enzyme/nanoparticles in calcium alginate. The size, morphology and crystallite phase of $MnFe_2O_4$ nanoparticles were investigated by SEM and XRD techniques, respectively. FT-IR spectroscopy was used to examine the functional groups of the nanoparticles before and after the immobilization of each enzyme. Also, prepared absorbents were applied for the decolorization of three model dye wastewaters of methylene blue, indigo and acid red 14. Decolorization of these dyes at pH 7 by laccase/$MnFe_2O_4$/calcium alginate during 1 h was observed 82.13, 25.09, and 20.42%, respectively. The maximum amount of decolorization of indigo and acid red 14 dyes at pH 5 and 3 by glucose oxidase/$MnFe_2O_4$/calcium alginate for 1 h were 44.03 and 46.5%, respectively; and decolorization of methylene blue at pH 9 after 3 h reached 93.46%.

1. Introduction

Most of the dyes used in various industries are toxic and their carcinogenic and mutagenic effects have been observed in humans and aquatic organisms [1,2]. For example, the cationic dye methylene blue, which is mostly used for dyeing cotton, wool, and silk can cause eye irritation, methemoglobinemia, cyanosis, convulsions, dyspnea, skin sensitivity, and tachycardia in humans [3]. Dyes usually have complex and aromatic structures and, due to their chemical structure, they are stable against aerobic decomposition, light, heat, and oxidizing reagents [2,4]. Also, owing to their good solubility, the discharge of wastewater containing dyes into natural streams and rivers leads to serious problems. Thus, removing dyes is one of the important necessities in wastewater treatment before the discharge of these materials in nature.

Although there are various methods for removing dyes such as degradation by UV/O_3, sound decomposition, membrane separation, flocculation, biological treatment and photo-catalytic decomposition with good efficiency, the operational cost and cost of raw materials used in these processes are high [5–7]. Adsorption of pollutants is superior to other methods of wastewater treatment in terms of low cost of raw materials, simplicity of design, and ease of operation [8–10]. Also, activated carbon is mostly used for removing dyes from wastewater, which is due to its high adsorption potential [11]; but its use is limited due to the high cost. So, other inexpensive and effective absorbents such as sawdust [12], zeolite [13–15], chitosan [16], bentonite and clay [17], Fe–Mn binary oxide[18], etc. have been developed.

Recently, magnetic adsorbents are used for adsorbing dyes and pollutants in aqueous solutions. These particles not only have strong adsorption activity, but also are easily collected and separated from aqueous environments owing to their magnetic property. In recent decades, magnetic particles such as iron oxide nanoparticles, especially magnetite, hematite, and spinel ferrite have been used for this purpose [19–21]. Biological adsorbents are another type of adsorbents that are prepared with low cost, simple design, and easy operation with biological materials such as chitosan and alginate [22]. Alginate is a polysaccharide, which is widely used for removing heavy metals and organic dyes from wastewater [23].

The preparation of nanocomposites of different adsorbents and degradation of pollutants may cause a synergistic effect on their

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property [24]. Laccase is an enzyme that can be used to oxidize a wide range of chemical compounds such as diphenols, polyphenols, diamines, aromatic amines, benzenethiols, substituted phenols [25–27], and a large group of dye pollutants [28,29]. Glucose oxidase (GOx) is one of the most widely used available enzymes which is known as oxidoreductase. It leads to the production of in-situ hydrogen peroxide during electron transfer reaction from substrate (glucose) to oxygen. Immobilization of enzymes on insoluble support provides the possibility of their continuous or multiple uses. Immobilization of enzymes on porous-support through adsorption is a simple method and it is one of the first enzyme immobilization techniques [30].

The purpose of this study is providing nanocomposite ferrite-biopolymer coupled with oxidase enzymes for simultaneous adsorption and degradation of various dye pollutants such as indigo (IG), methylene blue (MB) and acid red 14 (AR14) from aqueous solutions. The effect of various parameters such as initial pH of solution, initial dye concentration, and contact time on the adsorption of dyes with $MnFe_2O_4$ and ferrite-biopolymer nanocomposites as well as their enzymatic decomposition was studied.

2. Materials and methods

2.1. Materials

GOx (EC.1.1.3.4*Aspergillusniger*), glucose, NaOH (99%), HCl, $FeCl_3{\cdot}6H_2O$, $MnCl_2{\cdot}4H_2O$ and $CaCl_2$ were obtained from Merck. Alginic acid sodium salt and laccase from Sigma–Aldrich, and indigo, methylene blue, as well as acid red 14 were purchased from Buyakhsazan Company.

2.2. Preparing of nanocomposites

$MnFe_2O_4$ nanoparticles were prepared by co-precipitation phase inversion method from $FeCl_3{\cdot}6H_2O$ and $MnCl_2{\cdot}4H_2O$ with the molar ratio of Fe^{3+}:Mn^{2+} = 2:1. 100 mL of the solution including 0.1 M Mn^{2+} and 0.2 M Fe^{3+} were added dropwise to 100 mL of 3 M NaOH solution at 95 °C on the magnetic stirrer and stirring was continued for 2 h. The precipitation was washed for several times with distilled water and dried for 12 h at 60 °C [31]. 0.2 g of it was used in each adsorption test.

To prepare $MnFe_2O_4$/calcium alginate, 0.2 g of $MnFe_2O_4$ in 10 mL of distilled water was mixed with 20 mL of 2 wt.% sodium alginate solution. The mixture was transferred dropwise to 2 wt.% solution of calcium chloride to obtain insoluble granular calcium salt. For preparing the enzymatic nanocomposites of glucose oxidase and laccase, the same process was followed, except that the considered enzyme was immobilized on $MnFe_2O_4$.

2.3. Immobilization of enzymes on $MnFe_2O_4$ and assay of activities

To immobilize the enzymes on the support, 0.2 g $MnFe_2O_4$ was added to 10 mL laccase solution (850 U/L) in 0.1 M acetate buffer (pH 4.5) or glucose oxidase (882 U/mL) in 0.1 M acetate buffer (pH 5.5). Then, it was stirred in the incubator shaker at 120 rpm and 30 °C for 60 min [32,33]. Immobilization amount was calculated by measuring the difference between the initial activity of solution and the supernatant.

Activity of laccase at 30 °C was determined by 10 mM guaiacol as the substrate in 100 mM acetate buffer containing 10% volume of acetone [34,35]. Adsorption changes of the reaction mixture containing guaiacol at 470 nm with (ε = 6740 M^{-1} cm^{-1}) were followed for 5 min by incubation. One unit of activity is the amount of enzyme which catalyzes the conversion of 1 μmol substrate in 1 min at 25 °C.

According to Eq. (1), glucose is oxidized by glucose oxidase. The activity of glucose oxidase can be measured in the presence of glucose by spectrophotometer analyzer, where the produced H_2O_2 is measured by Eqs. (2) and (3) [36].

$$C_6H_{12}O_6 + O_2 + H_2O \xrightarrow{GOx} H_2O_2 + C_6H_{12}O_7 \quad (1)$$

$$H_2O_2 + 2H^+ + 2I^- \xrightarrow{Mo(VI)} I_2 + 2H_2O \quad (2)$$

$$I_2 + I^- \rightarrow I_3^- \quad (3)$$

In this technique, 100 μL of enzyme solution was mixed with 20 mM glucose solution and incubated for 1 min. Solution A (33 g of KI, 1 g of NaOH, 0.1 g of ammonium molybdate tetra hydrate) and B (10 g of KHP) with the ratio of 1:1 were immediately added to the mixture of glucose and the enzyme, and I_3^- was read in the mixture (λ_{max} = 351 nm) by UV–vis spectrophotometer. H_2O_2 concentration was determined by a standard calibration curve (which is not shown here). One unit of enzyme activity is defined as the amount of enzyme required to produce 1 μmol H_2O_2 in 1 min at 25 °C.

2.4. Point of zero charge of the $MnFe_2O_4$ nanoparticles

Determining the point of zero charge pH (pH_{pzc}) of $MnFe_2O_4$ nanoparticles at 30 °C in the 0.1 M $NaNO_3$ solutions was done by Mehdizadeh et al. method with a slight change [37]. 20 mL of 0.1 M $NaNO_3$ solution was poured in 100 mL Erlenmeyer and their initial pH (pH_i) was set from 2 to 9 by HNO_3 (0.1 M) and NaOH (0.1 M) using a pH-meter. Then, 0.1 g of $MnFe_2O_4$ adsorbent was poured in each of the 100 mL flasks and put in the incubator at 120 rpm and 30 °C for 24 h. The final pH (pH_f) of all the solutions was read and the ΔpH (pH_i–pH_f) curve was drawn versus the initial pH (Fig. 1).

2.5. Decolorization process

The experiments were done in 250 mL Erlenmeyer flasks, containing 50 mL dye solution with certain concentration and a specific amount of different nanocomposites at the given temperature of 30 °C and 120 rpm. In catalyst (GOx/$MnFe_2O_4$/calcium alginate) containing GOx enzyme, 80 mM glucose was added as the substrate to the solution. Absorption of aqueous solutions of IG, MB, and AR14 was read at the wavelengths of 657, 663, and 514 nm, respectively, which have been determined as the wavelengths of maximum dye absorption, at specified intervals using UV–Vis spectrophotometer (1700 UV–vis, Shimadzu, Japan). The percent of decolorization was calculated by Eq. (4):

$$\text{Decolorization}\ (\%) = \frac{A_0 - A_t}{A_0} \times 100 \quad (4)$$

where A_0 and A_t are the absorbance of the sample solution at times 0 and t, respectively.

3. Results and discussion

3.1. Characterization of the nanoparticles

Phase identifications were performed on a powder X-ray diffractometer D5000 (Siemens AG, Germany) using CuKα radiation. The morphology of the obtained materials was examined with a field emission scanning electron microscope (MIR-A3FEG-TescanBrno, Czech Republic). Brunauer–Emmett–Teller (BET) surface area was determined in a chembet-2400 Quantachrome instruments. The particle size distribution of prepared