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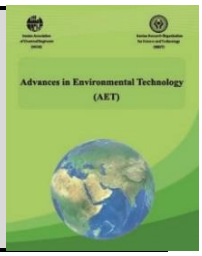

Preparation of Kissiris/TiO_2/Fe_3O_4/GOx biocatalyst: Feasibility study of MG decolorization

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ABSTRACT

Titanium dioxide (TiO_2) and Fe_3O_4 magnetite particles were coated on spherical Kissirises; glucose oxidase (GOx) enzyme was immobilized on Kissiris/Fe_3O_4/TiO_2 by physical adsorption. This catalyst was analyzed by a scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and energy dispersive X-ray (EDX) measurements. The performance of the prepared biocatalyst in the decolorization of Malachite Green dye was investigated. The optimal operation parameters were 20 mg/L, 20 mM, 5.5 and 40˚C for initial dye concentration, initial glucose concentration, pH and temperature, respectively. Under these conditions, a 95% Malachite Green decolorization efficiency was obtained after 150 min of reaction by using 1 g of prepared heterogeneous bio-Fenton catalyst. In this process, in contrast to a conventional Fenton's reaction, external hydrogen peroxide and ferrous ion sources were not used. The effect of various reaction parameters such as initial concentration of dye, amount of catalyst, concentration of glucose, pH value and temperature on MG decolorization efficiency was studied.

1. Introduction

Synthetic dyes are used in many industries such as textile, leather tanning, paper, plastics, pharmaceuticals, and foods[1]. There are more than 100,000 types of mercantile dyes and over 7×10^5 tons of dyestuff are produced per year [2, 3]. Malachite green (MG) is a triphenylmethane dye that is extensively used in the above mentioned industries. MG has some damaging effects on the ecosystem and its contact with skin leads to irritation, redness, and pain [4, 5]. Therefore, the elimination of MG from industrial wastewaters has become environmentally important. Many methods such as advanced oxidation, ozonation, adsorption, reverse osmosis, ion exchange, and membrane filtration have been utilized to remove dyes from waste effluents [6-10]. Fenton's reaction is a well-known method for the elimination of organic pollutants [11]. Fenton and Fenton like reactions can be demonstrated according to Equations (1) and (2) [10]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-} \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH^{\cdot} + H^{+} \quad (2)$$

Hydroxyl radicals have high oxidizing potential (E^0 =2.8 v) in terms of oxidizing various organic materials such as dyestuffs [12]. H_2O_2 is widely used as a source of hydroxyl radicals. Recently, in-situ production of hydrogen peroxide within the reaction medium has been developed in order to increase the efficiency of wastewater treatment and decrease the risk of hydrogen peroxide transportation and storage [13, 14]. Glucose oxidation, a simple enzymatic reaction, is catalyzed by glucose oxidase and applied for the in-situ generation of hydrogen peroxide. Therefore, simultaneous bio-Fenton's reactions can be done to decolorize dyes via Eqs. (1-4) [15].

$$C_6H_{12}O_6 + H_2O + O_2 \xrightarrow{GO_X} C_6H_{12}O_7 + H_2O_2 \quad (3)$$

$$OH^{\cdot} + Ppllutant \rightarrow Oxidation\ products \quad (4)$$

To increase the efficiency of the bio-Fenton process, a proper carrier should be prepared to enhance GOx loading, activity, and stability as well as to decrease the enzyme usage cost in continuous reactors [17]. The high surface

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area to volume ratio provided by the nanoparticles, such as TiO_2 and Fe_3O_4, favors high binding capacity and high catalytic specificity of the conjugated enzyme [16]. However, the main problem of the suspended biocatalyst is in the separation of nanoparticles after the treatment [18]. To solve the separation problem of carriers, this study used Kissirisfor the TiO_2/Fe_3O_4/GOx immobilization. Kissiris is formed by foam thickening of volcanic lava and has micro-pores which are irregularly distributed throughout the surface. This natural mineral with a highly porous structure, good mechanical strength and stability toward chemical agents could be a very attractive material for enzyme immobilization [19-22]. In order to provide a better medium for the GOx enzyme, this research used TiO_2 nanoparticles due to their chemical internees, rigidity, thermal stability, good adhesion to carriers, and high surface area [23]. Fe_3O_4 was coupled with TiO_2 to make a TiO_2/Fe_3O_4 adequate composite for GOx immobilization, the capacity of which is usually higher than pure TiO_2 and pure Fe_3O_4 [24]. Moreover, Fe_3O_4 was a ferrous source for the heterogenic Fenton's reaction and the adherence property of TiO_2 nanoparticles caused Fe_3O_4 nanoparticles to be strongly linked to Kissiris carriers. Therefore, the prepared carrier had the advantage of being enzyme compatible and included a ferrous source for the commencement of bio-Fenton reactions.

2. Materials and methods

2.1. Materials and equipment

TiO_2 nanoparticles (commercial Degussa P25) were a mixed phase containing 80% anatase and 20% rutile with an average crystal size of 21 nm. Methanol (99.9%), glucose oxidase (EC 1.1.3.4, from Aspergillus niger), β-D-glucose, and Malachite Green oxalate (MG) were obtained from Sigma Aldrich.Iron (III) chloride tetrahydrate, iron (II) sulfate heptahydrate, ammonia trihydrate (Merck) and nitrogen gas were used to prepare magnetite nanoparticles. All the solutions were prepared using distilled water. A UV–vis spectrophotometer (1700 UV–vis Shimadzu, Japan) was used to determine dye concentration. Scanning electron microscopy (SEM) images and EDX analysis were taken by MIRA3FEG-SEM (TescanBrno, Czech Republic). FTIR spectrums were obtained by Tensor 27 (Bruker, Germany). A Sonoplus Ultrasonic Homogenizer HD 2200 (Germany) was used for sonication.

2.2. Coating TiO_2 and Fe_3O_4 on Kissiris

A suspension of 0.5 g TiO_2 nanoparticles in 25 mL of methanol was sonicated for 15 min. The desired amount of HCl (1 N) was added to the solution to reach apH value of 5. The suspended solution was poured onto 5 g of Kissirises at 90˚C and was allowed to dry at the mentioned temperature for 5 h [25]. The coated Kissirises were washed with distilled water to remove weakly attached particles. The deposited amount of TiO_2 was measured by the difference in the mass of Kissirises before and after TiO_2 deposition. Fe_3O_4 nanoparticles were synthesized using a chemical co-precipitation method [26]. Kissirises, coated with TiO_2 were dispersed in 100 mL of deionized water and then 2.7 g of $FeSO_4.7H_2O$ and 5.4 g of $FeCl_3.4H_2O$ were added. The mixed solution was continuously stirred under nitrogen gas at 80˚C for 1 h. Subsequently, 30 mL of $NH_3.3H_2O$ was rapidly added to the mixture and stirred for another 1 h. Finally, the carriers were washed with 100 mL of deionized water to remove impurities and allowed to dry in a vacuum condition for 1 h.

2.3. Immobilization of GOx on Kissiris/TiO_2/Fe_3O_4

1 g of the carrier was dispersed in 10 ml of GOx solution in a phosphate buffer at a pH value of 5.5 by stirring in a shaker incubator for 2 h at 30˚C. The prepared heterogeneous bio-Fenton catalyst was separated from the solution and washed with distillated water to remove weakly attached GOx [17].

2.4. Decolorization

Experiments were carried out at a certain temperature and a constant stirring rate of 160 rpm in a 100 mL Erlenmeyer flask, which contained 20 mL of reaction mixture. The reaction mixture contained a certain concentration of MG and glucose with the desired amount of bio-Fenton catalyst to measure MG concentration in the sample solutions. The dyeabsorbance was measured at a maximum wavelength (λ_{max}=617 nm). The decolorization percentage was calculated by the following equation:

$$\text{Decolorization}\ (\%) = \left(1 - \frac{C}{C_0}\right) \times 100 \quad (5)$$

Where C_0 and C are the concentrations of the sample solution at times 0 and t, respectively.

3. Results and discussion

3.1. Characterizing of the catalysts

Mineral composition of the prepared support was analyzed using EDX (energy dispersion X-ray) patterns (Figure 1). According to the figure, the applied Kissiris contained Al_2O_3, SiO_2, CaO, etc. The sharp peak was assigned to Au since the samples were coated by gold in the analysis procedure. The appearance of Ti and Fe can be clearly observed in the Figure 1, implying the successful deposition of TiO_2 and the synthesis of Fe_3O_4.

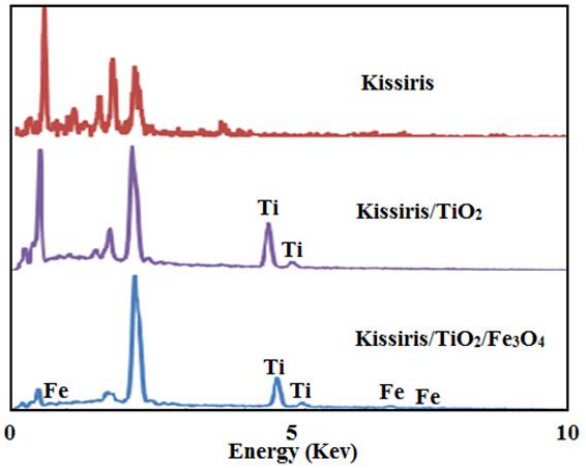

Fig. 1. EDX spectrum of Kissiris, Kissiris/TiO_2 and Kissiris/TiO_2/Fe_3O_4

Figure 2 presents the FTIR spectrum of Kissiris, Kissiris/TiO_2, Kissiris/TiO_2/Fe_3O_4, and Kissiris/TiO_2/Fe_3O_4/GOx. In the measured spectra, the bonds at about 3400-3500 cm^{-1} were attributed to the vibration of the OH group. The bending vibration of H_2O molecules was observed within 1400-1700 cm^{-1}[27]. In the spectrum of volcanic Kissiris, the peaks at 400-600 cm^{-1} were assigned to the vibration of Al-O bonds of Al_2O_3 and those at 600-900 cm^{-1} and 1100-1250 cm^{-1} corresponded to the vibration of Si-O-Si and Si = O, respectively [28]. The peaks at 400-700 cm^{-1} in the spectra of Kissiris/TiO_2 corresponded to the stretching vibration of Ti-O and Ti-O-Ti [28]. The peaks at 400-700 cm^{-1} were assigned to the vibration of Fe-O-Fe and Fe-O in the spectra of synthesized Fe_3O_4 [27]. In Figure 2, the Kissiris/TiO_2/Fe_3O_4/GOx represents the FTIR spectra of immobilized GOx on the carrier, in which the peaks at 1645.82 and 1513.17cm^{-1} correspond to amide I and amide II bonds of GOx, respectively [29]. Thus, the results proved the successful preparation of the bio-Fenton catalyst of Kissiris/TiO_2/Fe_3O_4/GOx. Scanning electron microscopy (SEM) images of the Kissiris, thin deposited layers of TiO_2/Fe_3O_4, and immobilized GOx were taken. Figure 3(b) and (c) clearly shows the attachment of TiO_2 and Fe_3O_4 nanoparticles on the Kissirises. Figure 3d shows that GOx was successfully immobilized on the carrier.

3.2. Catalytic activity of the Kissiris/TiO_2/Fe_3O_4/GOx

To ensure that the enzyme was acting, the new catalyst was used to decolorize 5 mg/L of malachite green aqueous solution with and without adding glucose. In the absence of glucose, the decolorization was only 33% at 60 min. The GOx enzyme did not produce H_2O_2 without substrate (e.g., glucose) and it was physical adsorption that caused the disappearance of dye in this case. When glucose was added to the reaction medium, the decolorization percentage increased to 99.9% at 60 min.

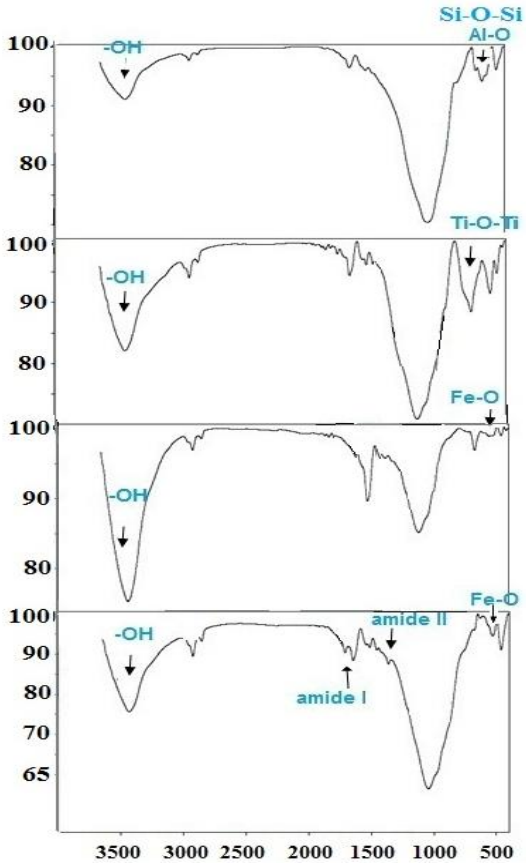

Fig. 2. FT-IR spectrum of Kissiris, Kissiris/TiO_2, Kissiris/TiO_2/Fe_3O_4, and Kissiris/TiO_2/Fe_3O_4/GOx**.**

A possible mechanism of decolorization can be outlined as the following reactions [30]. First, MG was adsorbed onto the catalyst surface, Eq. (6), where Me refers to the Fe or Ti on the surface of catalyst. The enzymatic reaction produced in-situ hydrogen peroxide according to Eq. (3). Then, Fenton and Fenton-like processes took place and active radicals such as hydroxyl and perhydroxyl radicals ($^{\cdot}OH$ and $^{\cdot}OOH$) were generated, as shown in Eqs. (1) and (2), respectively. Finally, the produced radicals oxidized the organic molecules of dye, leading to MG decolorization (Eqs. (7) and (8)).

$$MeOH + MG \rightarrow MeOMG + H^+ \quad (6)$$

$$MG + {}^{\cdot}OH \rightarrow CO_2 + H_2O \quad (7)$$

$$MG + {}^{\cdot}OOH \rightarrow CO_2 + H_2O \quad (8)$$

3.2.1. Effect of dye concentration

The experiments were conducted at an initial concentration of MG in the range of 20 – 100 mg/L, while maintaining agitation rate, catalyst loading, initial glucose concentration and temperature at 160 rpm, 1 g, 20 mM and 35°C, respectively. Figure 4 shows that MG disappearance efficiency decreased with a decrease in initial dye concentration. It can be seen that the decolorization rate was faster at the start of the reaction for each initial concentration, which was mainly due to the higher number

of available dye molecules for reaction and the rapid engagement of active radicals with organic molecules decreased the chance of undesired reactions between radicals in the first minutes of the reaction [31]. Table 1 shows the overall decolorization rate.

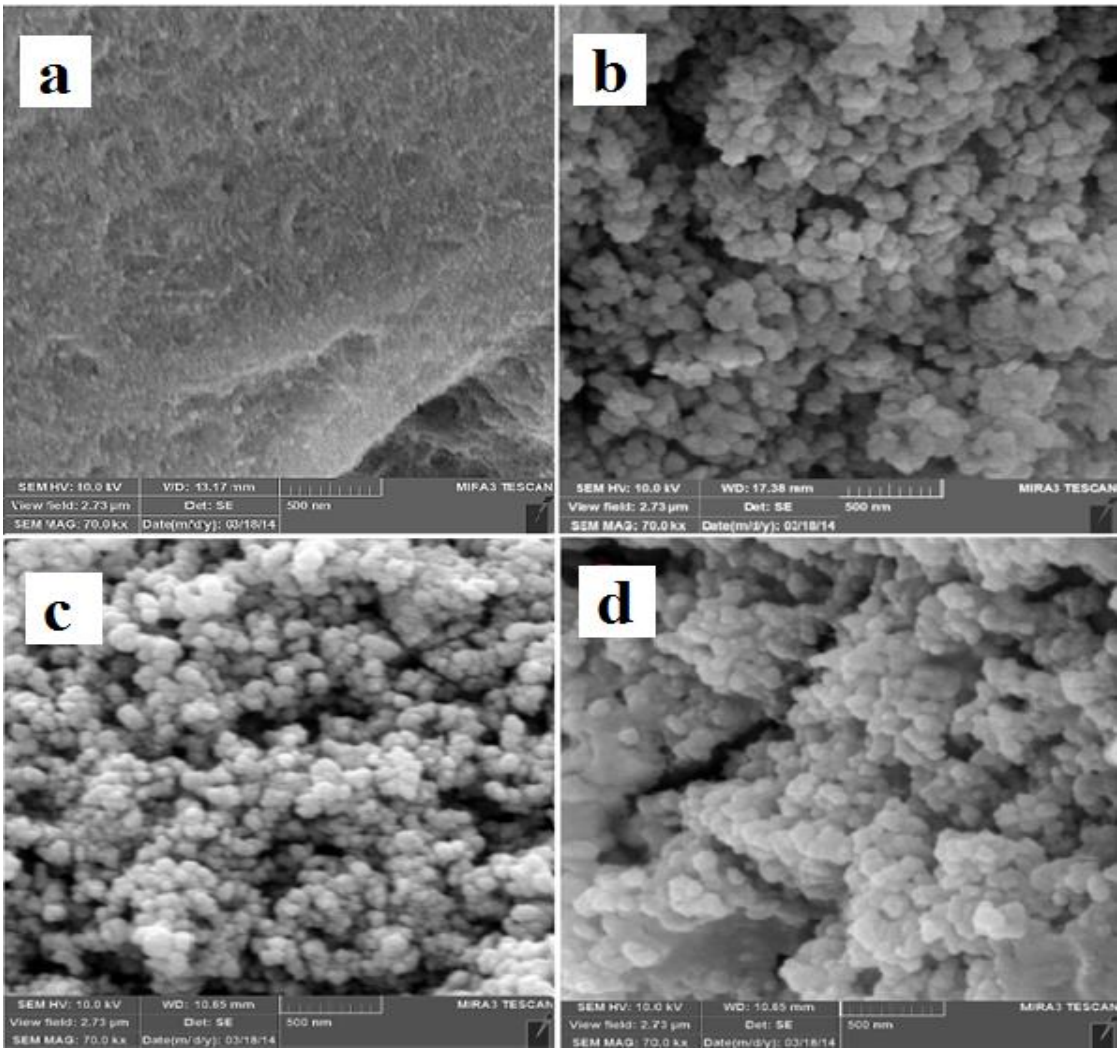

Fig. 3. SEM images of (a) Kissiris, (b) Kissiris/TiO_2, and (c) Kissiris/TiO_2/Fe_3O_4, (d) Kissiris/TiO_2/Fe_3O_4/GOx.

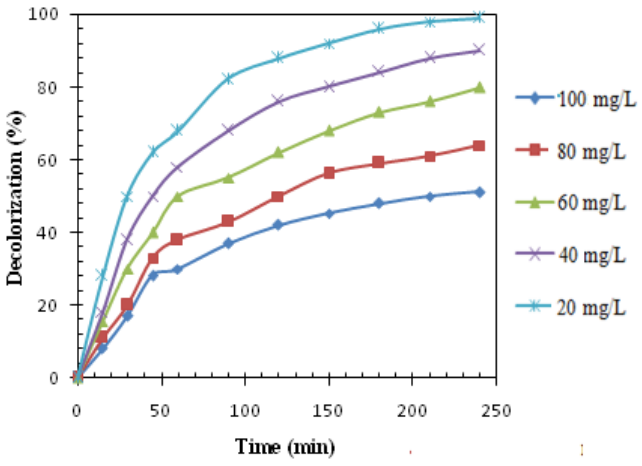

Fig. 4. Effect of initial concentration of dye on pseudo-first order rate constant during heterogeneous bio-Fenton oxidation treatment; $[glucose]_0$=20 mM, 1 g Kissiris/Fe_3O_4/TiO_2/GOx, T= 35˚C.

Table 1. Overall pseudo-first order kinetic rate constant for different dye concentration

MG concentration	k_{app}(min^{-1})	R^2
20	0.0029	0.9074
40	0.0041	0.9439
60	0.0063	0.9714
80	0.0093	0.9802
100	0.0182	0.9935

3.2.2. Effect of glucose concentration

To study the effect of glucose concentration on MG degradation, different concentrations of glucose (5-25) mM were tested (Fig. 5). The experiments were performed at a fixed initial Malachite Green concentration of 20 mg/L, 1 g of heterogeneous bio-Fenton catalysts, and a temperature of 35˚C. The increase in decolorization rate, shown in Figure 5, was related to the H_2O_2 generation rate, which was produced by enzymatic reaction using immobilized GOx. This in-situ production of H_2O_2 was the main advantage of

the immobilized enzyme. By the increase of glucose amount, the in-situ production and consumption rate of hydrogen peroxide increased. The decolorization efficiency was raised, as a result of the increased glucose concentration from 5 to 20 mM. However, at 25 mM, the degradation efficiency decreased; this can be due to the adverse reactions caused by hydrogen peroxide scavenging (Eqs. (10)- (11)) at such a high concentration of H_2O_2 [32].

$$H_2O_2 + OH^{\cdot} \rightarrow HO_2^{\cdot} + H_2O \quad (10)$$

$$HO_2^{\cdot} + OH^{\cdot} \rightarrow H_2O + O_2 \quad (11)$$

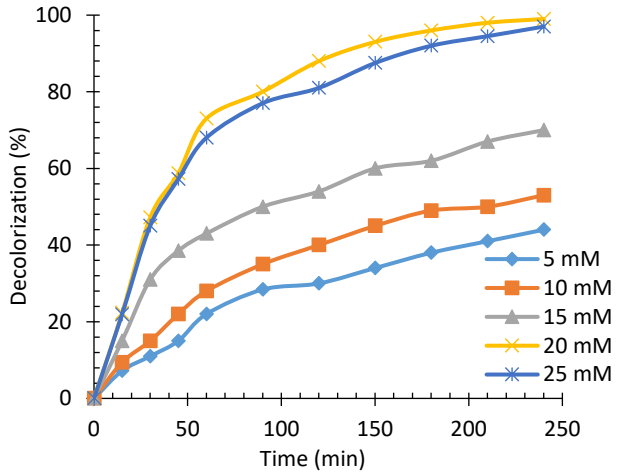

Fig. 5. Effect of initial glucose concentration on pseudo-first order rate constant during heterogeneous bio-Fenton oxidation treatment; $[MG]_0$= 20 mg/L, 1 g Kissiris/Fe_3O_4/TiO_2/GOx, T= 35˚C.

3.2.3. Effect of pH

As can be seen in Figure 6, the MG disappearance efficiency was influenced by the pH value of the solution. The efficiency increased by raising the pH value from 3.5 to 5.5 and decreased significantly above a pH of 5.5. These changes of decolorization efficiency can be attributed to the stability of H_2O_2 in the acidic medium. The decrease in efficiency at pH values higher than 5.5 was due to Fe $(OH)_3$ formation; in this form, iron disintegrated hydrogen peroxide to water and oxygen [31]. On the other hand, the higher and lower pH values disrupted the enzymatic reaction, so that there was an optimal pH value of 5.5 for enzyme activity and decolorization process.

3.2.4. Effect of temperature

To determine the effect of temperature on MG decolorization, experiments were conducted at a temperature range of 298 to 318 K. According to Figure 7, the decolorization efficiency increased by increasing temperature to 20˚C; this was due to an increase in GOx activity by temperature which consequently enhanced the hydrogen peroxide production rate. Also, the reaction of hydrogen peroxide with Fe ions was accelerated by an increase in temperature. Decolorization efficiency decreased above 20˚C, which was due to the denaturation of the protein molecules in the structure of the enzyme. Thus, excessive temperature deactivated glucose oxidase and disrupted its bio-catalytic activity; as a result, the in-situ generation of hydrogen peroxide decreased and consequently, decoloirzation efficiency decreased.

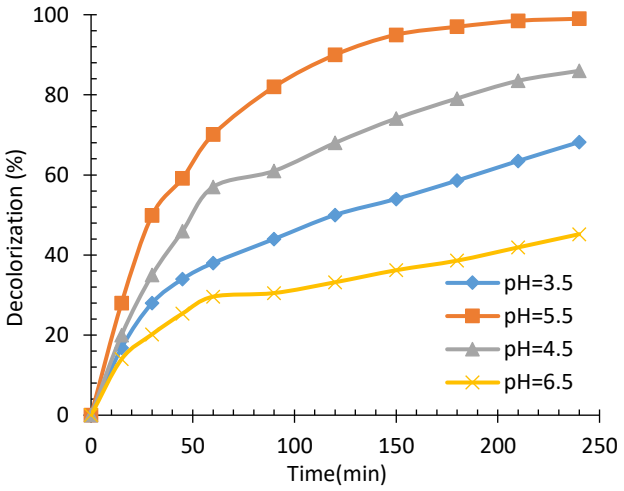

Fig. 6. Effect of pH value on pseudo-first order rate constant during heterogeneous bio-Fenton oxidation treatment; $[MG]_0$=20 mg/L, $[glucose]_0$=20 mM, 1 g Kissiris/Fe_3O_4/TiO_2/GOx, T= 35˚C.

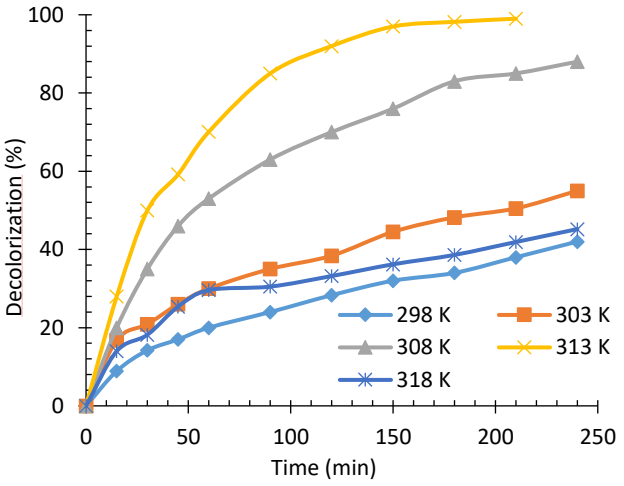

Fig. 7. Effect of temperature on pseudo-first order rate constant during heterogeneous bio-Fenton oxidation treatment; $[MG]_0$=20 mg/L, $[glucose]_0$=20 mM, 1 g Kissiris/Fe_3O_4/TiO_2/GOx, pH=5.5.

4. Conclusions

Bio-Fenton is a newly introduced method for the elimination of organic pollutants. In this technique, hydrogen peroxide is generated by an enzymatic reaction and reacts with a Fe^{2+} ion to produce a hydroxyl radical. The glucose oxidase enzyme was immobilized on the Kissiris/TiO_2/Fe_3O_4 carrier to enhance enzyme reusability. The results of EDX, FTIR, and SEM analyses showed that the synthesis of the hybrid heterogeneous bio-Fenton catalyst (e.g.,Kissiris/TiO_2/Fe_3O_4/GOx) was successful. The best MG

decolorization efficiency, by using 1 g of prepared heterogeneous bio-Fenton catalyst, was 95% after 150 min at an optimal operating condition of 20 mg/L MG initial concentration, 20 mM glucose, a pH value of 5.5 and a temperature of 40 ˚C. It was observed that Kissiris/TiO_2/Fe_3O_4/GOx is a promising catalyst for the bio- *P*-values of 0.0020 and 0.0066, respectively. In other words, increasing pH had a greater influence on the removal efficiency.

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