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ENZYMATIC *IN-SITU* GENERATION OF H_2O_2 FOR DECOLORIZATION OF ACID BLUE 113 BY FENTON PROCESS

Decolorization of Acid Blue 113 in an aqueous medium by bio-Fenton process has been investigated in this study. Enzymatic oxidation of glucose was performed for in-situ generation of H_2O_2, which was employed to react with Fe^{2+} for producing hydroxyl radicals. The effects of various parameters, including concentrations of AB 113, glucose, and $FeSO_4$, activity of glucose oxidase (GOx) and pH were assessed. The highest decolorization of AB 113 was achieved at Fe^{2+} concentration of 0.2 mmol/L, pH 4.0, glucose concentration of 0.018 mol/L, and glucose oxidase activity of 2500 U/L in the constant temperature (23 ±0.1 °C) and constant shaking rate (160 r/min), while the concentration of AB 113 was 40 mg/L. Under these conditions, the obtained AB 113 decolorization efficiency after 60 min was about 95%.

Keywords: Acid Blue 113; bio-Fenton; decolorization; glucose oxidase.

Synthetic dyes are widely used in many industries, such as textile dyeing, leather tanning, plastic and paper production and printing industries. They are major sources of environmental contamination, especially in water pollution [1]. Releases of the wastewater of these industries to the environment without refining steps are damaging and retrievable. The existence of chromatic materials in water reduces the influence of light, thus aquatic plants photosynthesis is reduced. The toxicity of dyes causes death of aquatic beings [2,3]. An additional difficulty is that these dyes are not easily degraded by common wastewater treatment systems. Therefore, the employment of these dyes must be managed and must be treated before being released into the environment [3,4].

Numerous processes have been proposed for industrial dyes wastewater treatment, which were classified as physical, chemical and biological methods. They are limited to special cases and cannot be effectively applied for all dyes [5,6]. Ozone oxidation and physical adsorption by activated carbon are accepted processes but they are adverse because of their high equipment and operating costs [7,8]. Some of the other methods such as flocculation and coagulation just transfer pollutants from the liquid phase to the other phase.

Azo dyes, the largest class of synthetic dyes are distinguished by containing one or more azo groups (–N=N–) that are bonded to the aromatic rings. It should be emphasized that due to their complicated and recalcitrant molecular structure, they are difficult to remove from wastewater by using common physical, chemical and biological treatment methods [9-11]. Therefore, it is essential to look for appropriate methods or techniques for the treatment of this kind of pollutants to reduce their environmental impact.

Advanced oxidation processes based on the generation of hydroxyl radicals ($^{\bullet}OH$) with great oxidizing potential (E = 2.8 V/SHE), can quickly and non-selectively oxidize a wide spectrum of diverse organic dyes [12,13]. Therefore, advanced oxidation processes can be a good selection to treat this kind of wastewaters because of their powerful oxidizing capability to oxidize dyes to non-toxic products CO_2 and H_2O [14]. This method doesn't have the problem of residuals and also, can be used in room temperature and atmospheric pressure [15-16]. The oxidation mechanisms of Fenton oxidation process are shown in the following equations [2-4]:

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

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$$Fe^{3+}+H_2O_2 \rightarrow Fe^{2+}+HOO^{\bullet}+H^{+} \quad (2)$$

$$Fe^{3+}+HOO^{\bullet} \rightarrow Fe^{2+}+O_2+H^{+} \quad (3)$$

$$R-H+HO^{\bullet} \rightarrow R^{\bullet}+H_2O \quad (4)$$

$$R^{\bullet}+Fe^{3+} \rightarrow Fe^{2+}+R^{+} \quad (5)$$

$$R^{+}+H_2O \rightarrow ROH+H^{+} \quad (6)$$

$$Fe^{2+}+HO^{\bullet} \rightarrow Fe^{3+}+HO^{-} \quad (7)$$

$$H_2O_2+HO^{\bullet} \rightarrow H_2O+HOO^{\bullet} \quad (8)$$

$$HOO^{\bullet} \rightarrow O_2^{\bullet-}+H^{+} \quad (9)$$

$$HOO^{\bullet}+Fe^{2+}(+H^{+}) \rightarrow Fe^{3+}+H_2O_2 \quad (10)$$

$$HO^{\bullet}+HO^{\bullet} \rightarrow H_2O_2 \quad (11)$$

The aim of this study is the investigation of bio-Fenton process application in AB 113 decolorization using H_2O_2 which was in-situ produced from oxidation of glucose, catalyzed by glucose oxidase. The effects of variables including glucose, glucose oxidase, dye, and Fe^{2+} concentrations and also, the pH of the reaction were studied by changing each in turn while keeping the other parameters constant.

EXPERIMENTAL

Materials

Glucose oxidase (GOx) type II (EC 1.1.3.4, 25 U/mg, from *Aspergillus niger*), β-D-(+)-glucose, sodium acetate, acetic acid, sulfuric acid, sodium hydroxide, 2,2'-azino-di-(3-ethylbenzthiazolin-sulfonate), $FeSO_4$, and Acid Blue 113 (AB 113) of analytical grade were obtained from Sigma Aldrich.

Analytical methods

The absorption spectrum of AB 113 in aqueous solution was recorded in the range of 400-850 nm and it was found that the maximum wavelength was at 566 nm. Aqueous solutions of AB 113 with different concentrations were prepared and their absorption intensities at 566 nm were measured, and a calibration curve for finding dye concentration in decolorization experiments was plotted.

The decolorization of AB 113 at different reaction times was determined by measuring the absorption intensity of the solution at 566 nm. The decolorization efficiency of AB 113 was defined as follows [1]:

$$R = 100\frac{c_0 - c_t}{c_0} \quad (12)$$

where R is decolorization efficiency, c_0 is initial AB 113 concentration, and c_t is AB 113 concentration at time t.

A Biowave S2100-WPA UV-Vis spectrophotometer (made in England) was used for absorption measurements.

Experimental procedures

All experiments were carried out in a 100 mL flask bioreactor in a shaker incubator at 160 r/min. Temperature of the reaction mixture in all experiments was constant at 23±1 °C. The initial pH of solutions were adjusted by 1.0 mol/L sulfuric acid and 1.0 mol/L sodium hydroxide solutions using pH meter Labtron (PHT-110). The required concentrations of glucose, glucose oxidase, $FeSO_4$, and AB 113 were prepared in distillated water correspondingly.

GOx activity determination

GOx oxidizes β-D-glucose in the presence of oxygen to β-D-glucono-δ-lactone and H_2O_2. The produced H_2O_2 is then utilized to oxidize a chromogenic substrate in a secondary reaction in the presence of catalase and a resultant color change is monitored spectrophotometrically. 2,2'-Azino-di-[3-ethylbenzthiazolin-sulfonate] was used for this goal through forming a greenish-blue oxidized product that was measured spectrophotometrically at 420 nm. One unit of catalyst activity (U) is defined as the amount of GOx required to consume 1 μmol substrate in one min at 25 °C [18].

RESULTS AND DISCUSSION

Comparison of AB 113 absorption spectrum with the other materials

Water soluble bisazo dye AB 113 (Figure 1) (molecular formula C23H21N5Na2O6S2 and molecular weight of 681.65), a toxic and carcinogenic mater [19], was selected for decolorization by bio-Fenton reaction. Total bio-Fenton reactions which were used are:

$$C_6H_{12}O_6+H_2O+O_2 \xrightarrow{\text{Glucose oxidase}} C_6H_{12}O_7+H_2O_2 \quad (13)$$

$$M^{n+}+H_2O_2 \rightarrow M^{(n+1)+}+HO^{-}+{}^{\bullet}OH \quad (14)$$

where M is a transition metal like iron [20].

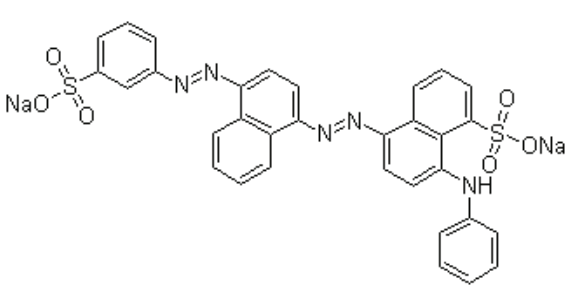

Figure 1. Molecular structure of Acid Blue 113 [20].

For determination of the efficiency and rate of decolorization, dye concentration measuring in different steps is necessary. For awareness of uninterference of the spectrum peaks of AB 113 with $FeSO_4$, glucose, and GOx, their absorption spectra were recorded in the range of 400-800 nm. Comparison of the results indicates that only AB 113 has a spectrum peak at 566 nm (Figure 2). Thus, AB 113 concentration easily can be calculated spectrophotometrically.

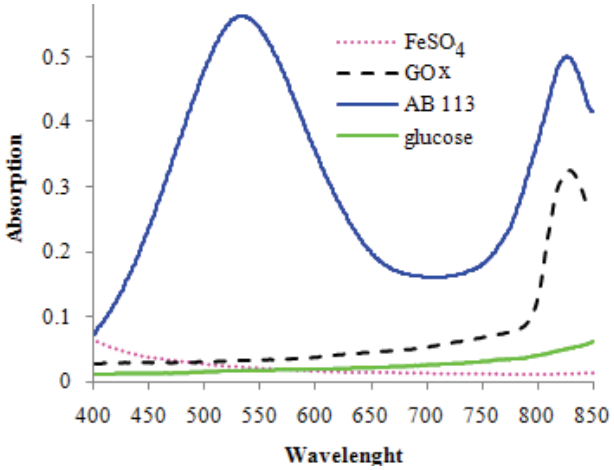

Figure 2. Comparison of the absorption spectrum of AB 113 in aqueous solution with glucose, GOx, and $FeSO_4$.

Effect of glucose concentration

H_2O_2 plays a very important role as a source of •OH generation in Fenton's reaction. In this study, H_2O_2 produced *in-situ* using oxidation-reduction reaction of glucose-O_2 in the presence of GOx. As can be seen from Eq. (13), the production rate of H_2O_2 in the reaction medium depends on glucose concentration. Due to this reason, the effect of glucose concentration on the decolorization of AB 113 was examined by varying initial concentration of glucose from 0.006 mmol/L to 0.022 mmol/L while concentration of dye (40 mg/L), Fe^{2+} (0.2 mmol/L), GOx activity (2000 U/L) and the initial pH (4.0), were constant. The results are shown in Figure 3. It can be observed that increasing the glucose concentration from 0.006 mmol/L to 0.018 mmol/L could enhance the decolorization of AB 113 from 76 to 90% within 60 min. However, further increase of the glucose concentration above 0.022 mmol/ /L didn't improve the decolorization rate of AB 113. This observation can be explained that in high concentrations of glucose, there is the scavenging effect of excessive produced H_2O_2 to •OH (Eq. (8)). In addition, with the recombination of hydroxyl radicals (Eq. (11)), amounts of •OH are declined and following it, the decolorization efficiency of AB 113 was reduced [9]. From the results, it was found that a suitable glucose concentration is about 0.018 mmol/L.

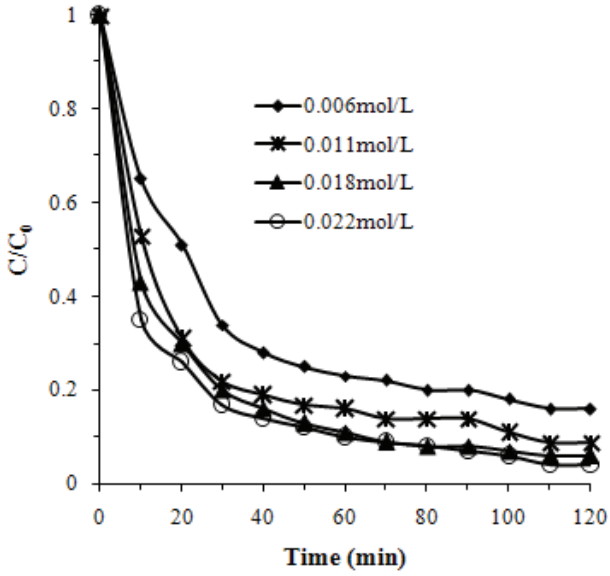

Figure 3. Effect of glucose concentration on the rate of decolorization.

Effect of the initial Fe^{2+} concentration

To study the role of initial concentration of Fe^{2+} on the decolorization of AB 113 by bio-Fenton oxidation, a series of experiments were performed with different initial Fe^{2+} concentration from 0.1 to 0.4 mmol/L while the concentration of dye (40 mg/L) and glucose (0.018 mol/L), GOx activity (2000 U/L), and the initial pH (4.0) were constant. The results have been presented in Figure 4. The decolorization efficiency of AB 113 was significantly changed over the initial Fe^{2+} concentration value of 0.1-0.4 mmol/L. The lowest and the highest decolorization efficiency were obtained at initial Fe^{2+} concentration of 0.1 and 0.2 mmol/L respectively after 60 min reaction time. Concentration of 0.3 mmol/L of $FeSO_4$ has no increasing in AB 113 decolorization efficiency. Much higher concentration of Fe^{2+} (0.4 mmol/L) could lead to excessive •OH value and the self scavenging of •OH radical by Fe^{2+} (Eq. (7)) would cause a decrease in decolorization rate of AB 113 [4].

Effect of the initial dye concentration

The initial dye concentrations ranging from 5 to 100 mg/L were investigated while glucose concentration (0.018 mol/L), Fe^{2+} concentration (0.2 mmol/L), initial GOx activity (2000 U/L), and the pH (4.0) were constant. The results are illustrated in Figure 5. It is observed that in the lower concentrations of the dye, decolorization is faster. The rise of the dye concentration in aqueous solution increases the number of

dye molecules in the solution while the number of hydroxyl radicals is constant [21,22], so despite of the increase in color removal rate, the decolorizing efficiency decreases at high concentration of dye.

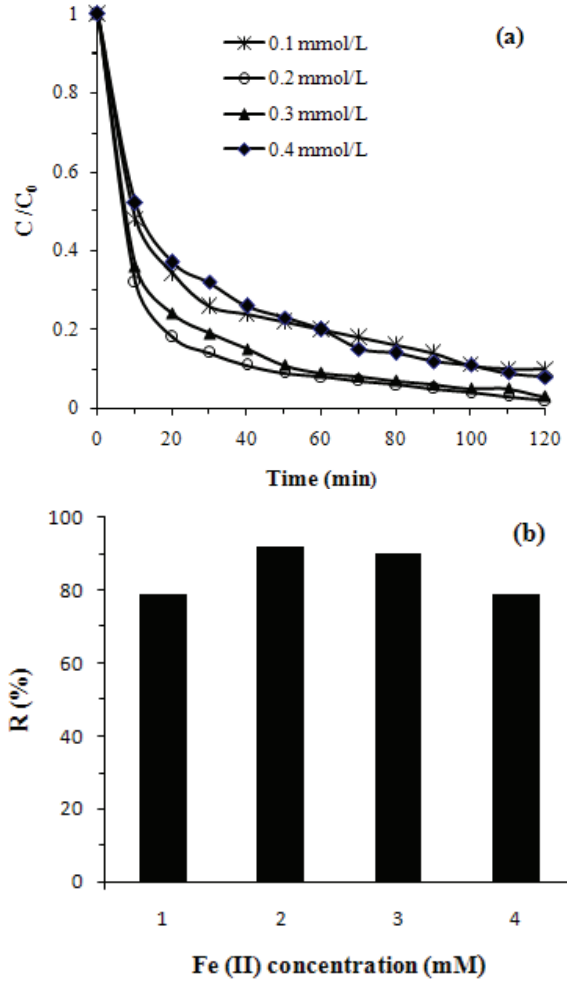

Figure 4. Effect of Fe (II) concentration on the decolorization rate (a) and decolorization efficiency (R%) (b) after 60 min.

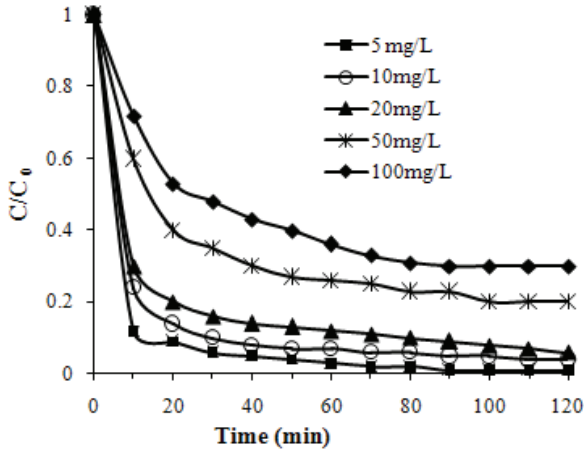

Figure 5. Effect of dye concentration on the rate of decolorization.

Effect of initial pH

The effect of initial pH on the decolorization of AB 113 by bio-Fenton process is shown in Figure 6. The initial tests show that dependence of the AB 113 absorbance is negligible in the pH range studied. The experiments were down at constant concentration of dye (40 mg/L), glucose (0.018 mol/L), Fe^{2+} (0.2 mmol/ /L), and initial GOx activity (2000 U/L) while the pH was varied from 3.0 to 7.0, that pH 3.0 and 4.0 were adjusted with H_2SO_4 and NaOH solutions (1.0 M) and pH 5.0 to 7.0 were adjusted with acetate buffer solution. The optimum pH was observed 4.0 and increasing or decreasing of the pH value had undesirable effects on the decolorization rate of AB 113. This is because the concentrations of Fe^{2+} and H_2O_2 depend on the pH, so correspondingly, pH affects the yield of the active $^{\bullet}OH$ and decolorization rate. The decolorization efficiency of AB 113 was decreased with the increase of pH from 4.0 to 7.0. This is mainly caused by the fact that $Fe(OH)_3$ is formed when pH is high [1], and inhibits the reaction between Fe^{2+} and H_2O_2, so only a small amount of the $^{\bullet}OH$ is generated. When the pH is lower than 4.0 approximately, GOx is become inactive and just a little amount of H_2O_2 is produced [23]. Thus, correspondingly, the rate and the efficiency of AB 113 decolorization have been decreased. In addition, when the pH of reaction medium is lower than 3.0, H_2O_2 reacts with excessive H^+ and forms hydronium ions (H_3O^+) [24] which do not have a positive effect on the decolorization.

Effect of GOx concentration

The experiments were performed at constant dye concentration (40 mg/L), glucose concentration (0.018 mol/L), Fe^{2+} concentration (0.2 mmol/L), and initial pH 4.0 while the GOx activity was varied from 500 to 5000 U/L. As shown in Figure 7, the dye decolorization rate increases with increasing GOx activity until to 2500 U/L. In the presence of too high GOx activity, due to high rate of H_2O_2 production, the reaction between active radicals and produced H_2O_2 is caused to decrease the AB 113 decolorization rate [25].

CONCLUSION

Most azo dyes are difficult to decompose by sample methods due to their complex and steady structure. The advanced oxidation process is an attractive method for decolorization of these dyes. In this study, a new Bio-Fenton process, involving enzymatic in-situ generation of H_2O_2, was introduced for AB 113 removal from water. Influence of different parameters which is effective on biological generation of H_2O_2 and Fenton process was investigated. The best

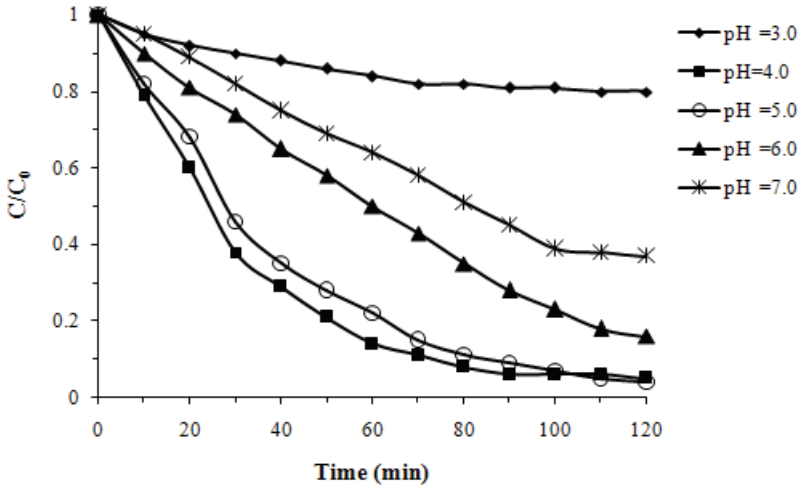

Figure 6. Effect of pH on the rate of decolorization.

conditions were achieved are; Fe^{2+} concentration 0.2 mmol/L, pH 4.0, glucose concentration 0.018 mol/L, and glucose oxidase activity 2500 U/L at constant temperature (23±0.1 °C) and constant shaking rate (160 r/min), while the concentration of AB 113 was 40 mg/L. Under these conditions, the obtained AB 113 decolorization efficiency after 60 min was about 95%.

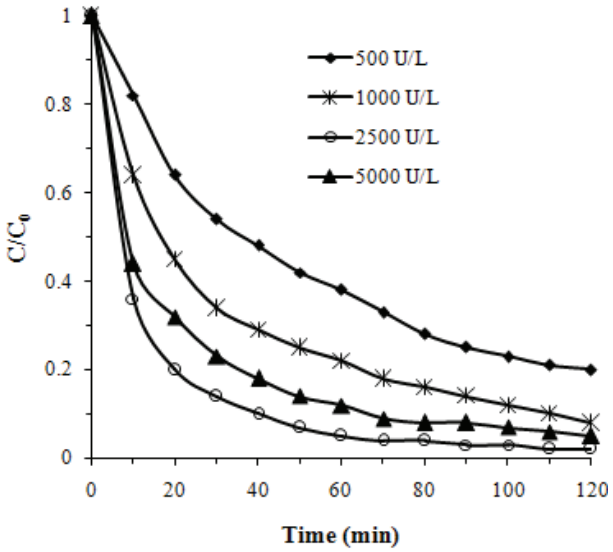

Figure 7. Effect of glucose oxidase activity on the rate of decolorization.

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NAUČNI RAD

ENZIMSKO *IN SITU* GENERISANJE H_2O_2 RADI DEKOLORIZACIJE BOJE ACID BLUE 113 FENTONOVIM PROCESOM

U ovom radu izvršena je dekolorizacija boje Acid Blue 113 u vodenom medijumu pomoću bio-Fenton-ovog procesa. Enzimska oksidacija glukoze je izvršena in situ, radi dobijanja H_2O_2, koji je dalje iskorišćen za reakciju sa Fe^{2+} kojom se dobijaju hidroksil radikali. Praćen je uticaj različitih parametara, uključuju koncentraciju boje Acid Blue 113, glukoze i $FeSO_4$, aktivnost glukozo oksidaze (Gox) i pH. Najviši stepen dekolorizacije boje Acid Blue 113 je postignut pri koncentraciji Fe^{2+} od 0,2 mmol/dm^3, pH vrednosti 4.0, koncentraciji glukoze od 0,018 mol/dm^3 i aktivnosti glukoza oksidaze od 2500 U/dm^3 na konstantnoj temperaturi (23±0,1 °C) i stalnoj brzini mućkanja (160 o/min). Pri tome je koncentracija boje Acid Blue 113 bila 40 mg/dm^3. U ovim uslovima, efikasnost dekolorizacije boje Acid Blue 113 od 95% je postignuta posle 60 min.Ključne reči:Acid Blue 113; bio-Fenton; dekolorizacija; glukozooksidaza.