

Decolorization of Disperse Dyes Using Immobilized Laccase Enzyme on Nano Zinc Ferrite from Single and Binary Systems

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Abstract

In this paper, immobilized laccase enzyme on nano zinc ferrite was used in order to decolorize disperse dyes from single and binary systems. In this case, disperse dyes such as Disperse red 60 (DR60), Disperse blue 56 (DB56) and Disperse yellow 54 (DY54) were selected as model dyes. Several parameters such as enzyme concentration, pH and dye concentration and their effect on decolorization of dyes from single and binary systems were studied. According to the experimental results, the optimized immobilized laccase enzyme concentration, reaction time and pH for decolorization of DR60, DB56 and DY54 from single and binary systems were 500 mg/L (for DR60 and DY54) and 400 mg/L (for DB56), 20 min and 3, respectively. Moreover, Dye decolorization kinetics followed Michaelis-Menten Model. Finally, the results showed that enzymatic process using immobilized laccase enzyme on nano zinc ferrite was effective method to decolorize disperse dyes from single and binary systems.

Keywords

Enzymatic Decolorization, Immobilized laccase Enzyme, Single and Binary Systems, Michaelis-Menten Model, Disperse Dyes, Nano Zinc Ferrite

1. Introduction

Dye removal of industrial effluent has been a major concern in wastewater treatment, especially for textile and dyestuff plants.

Enzymatic processes are frequently applied to decolorize textile and dyestuff wastewater due to the cost effectiveness [1] [2] [3].

Alternatively, enzymatic oxidation of dye using Laccase has received great attention in recent years due to the high efficient dye decolorization [4].

Laccases from white rot fungi are potential industrial enzymes in various applications such as pulp delignification, wood fiber modification, chemical or medicinal synthesis and waste water treatment [5].

Different factors such as highly polluted waste water and mediator-by products have significant effect on decreasing of stability and catalytic ability of free enzyme. Therefore, finding suitable solution to overcome this problem is important subject [6].

Laccase enzymes from white rot fungi with high enzymatic activity have found many applications in various industries, such as pulp delignification, wood fiber modification, chemical or medicinal syntheses, and wastewater treatment [7].

The stability and the activity of free enzyme are reduced in the presence of highly polluted wastewater. Thus, finding suitable solutions to overcome this difficulty is an important subject [8]. The immobilization of enzymes is one approach to this; it increases the stability and prolongs the shelf-life of the enzyme in solution [9].

Different methods can be used for the immobilization of enzymes on different substrates:

A) Activation of the enzyme by suitable chemical reactions before immobilization on the carrier.

B) Modifying the carrier before the immobilization process.

C) Using cross-linking agent such as glutaraldehyde to mediate between the carrier and the enzyme functional groups [10].

Immobilized substrate based on the last method retains high enzymatic activity and is effective in dye degradation over a wide range of pH and temperature [8] [9]. Different mechanisms (e.g., adsorption, entrapment, encapsulation, and covalent coupling) can be used for immobilization of free laccase enzyme on various carriers [11]. The performance of immobilized enzyme largely depends on the structure of the support. The support can be inorganic (e.g., nanoTiO₂) or a modified polymer (e.g., polypropylene) [12].

In this paper, enzymatic decolorization of dye using from single and binary systems was studied.

Disperse red 60 (DR60), Disperse blue 56 (DB56) and Disperse yellow 54 (DY54) were used as a model dyes. The effect of several parameters such as enzyme concentration, pH and dye concentration on decolorization of dyes from single and tertiary systems was evaluated.

2. Experimental

2.1. Materials

Disperse red 60 (DR60), Disperse blue 56 (DB56) and Disperse yellow 54 (DY54) were obtained from Youhao company. The chemical structure of dyes was shown in **Figure 1**. Free laccase enzyme (Denilite II S) was provided by Novo Nordisk Company. γ -Amino propyl triethoxysilane and glutaraldehyde were obtained from Sigma-Aldrich. All other chemicals were of analytical grade and purchased from Merck Company (Germany).

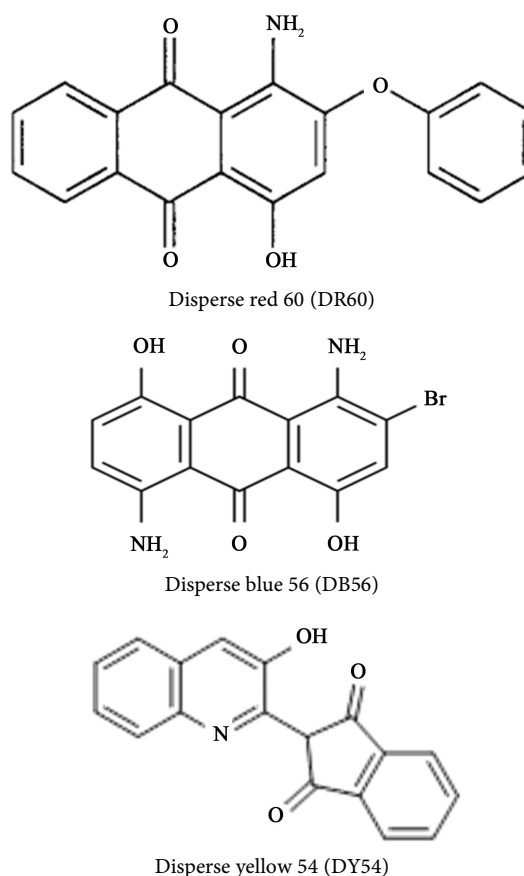


Figure 1. The chemical structure of disperse dyes.

2.2. Laccase Immobilization

Zinc ferrite nanoparticle was synthesized in previously published paper [13]. Zinc ferrite nanoparticle was silanized by immersing in a 4% (v/v) γ -amino propyl triethoxysilane in acetone solution at 45°C for 24 h. The silanized zinc ferrite nanoparticle was washed thoroughly with distilled water and then immersed in 2% (v/v) of aqueous glutaraldehyde solution for 2 h at room temperature. After washing of obtained nanoparticle with distilled water, they were dried at 60°C for 1 h. Thereafter, 5 g obtained nanoparticles were immersed in given amount of free laccase for 48 h at room temperature.

2.3. Characterization Methods

FTIR and SEM were used for analyzing compositions, dimensions and morphology of samples. FTIR spectroscopy was used for determining functional groups of materials.

PHILIPS scanning electronic microscope was used for studying morphology of materials

2.4. Dye Decolorization

Experiments were carried out in a batch mode reactor with total capacity of 250 mL. Decolorization of dyes was performed using a 100 mL solution containing

specified concentration of dye using immobilized Laccase enzyme on nano zinc ferrite.

The solution pH was adjusted using HCl or NaOH. Samples were withdrawn from sample point at certain time intervals and analyzed for dye degradation.

Dye degradation was checked and controlled by measuring the absorbance at maximum wavelength (λ_{\max}) of dyes at different time intervals using UV-vis spectrophotometer (Perkin-Elmer Lambda 25 spectrophotometer).

The effect of free laccase enzyme concentration on dye degradation was investigated by contacting 100 mL of dye solution (20 ppm) at 45°C and pH 3. Different enzyme concentrations (200, 300, 400 and 500 ppm) were applied.

The effect of pH (3 - 9) on dye degradation was investigated by contacting 100 mL of dye solution (20 ppm) and enzyme concentration (500 ppm) at 45°C.

The effect of initial dye concentration (10 - 50 ppm) on dye degradation was investigated by contacting 100 mL of dye solution and enzyme concentration (500 ppm) at 45°C and pH 3.

In single system, concentration of dye and its variation during enzymatic processes are measured due to the Beer-Lambert law.

$$A = \varepsilon LC \quad (1)$$

where ε , L and C are extinction coefficient (L/mg cm), path length (cm) and dye concentration (ppm), respectively.

Dye concentrations were calculated as follows. For a binary system of components A and B measured at λ_1 and λ_2 , respectively, to give optical densities of d_1 and d_2 [3]:

$$C_A = (k_{B2}d_1 - k_{B1}d_2)/(k_{A1}k_{B2} - k_{A2}k_{B1}) \quad (2)$$

$$C_B = (k_{A1}d_2 - k_{A2}d_1)/(k_{A1}k_{B2} - k_{A2}k_{B1}) \quad (3)$$

where k_{A1} , k_{B1} , k_{A2} , and k_{B2} are the calibration constants for components A and B at the two wavelengths λ_1 and λ_2 , respectively.

3. Results and Discussion

3.1. Enzyme Concentration

Decolorization of dyes from single and binary systems at different concentrations of enzyme is shown in **Figure 2**. The results show that with increasing immobilized laccase enzyme concentration, the dye removal percentage increases gradually because of existence of more enzyme molecules at the expense of fixed amount of dye molecules [5] [14].

Furthermore, the optimized amount of immobilized enzyme laccase enzyme, reaction time and pH for decolorization of DR60, DB56 and DY54 from single and binary systems were 500 mg/L (for DR60 and DY54) and 400 mg/L (for DB56), 20 min respectively.

3.2. pH

Decolorization of dyes from single and binary systems at different pH is shown in **Figure 3**.

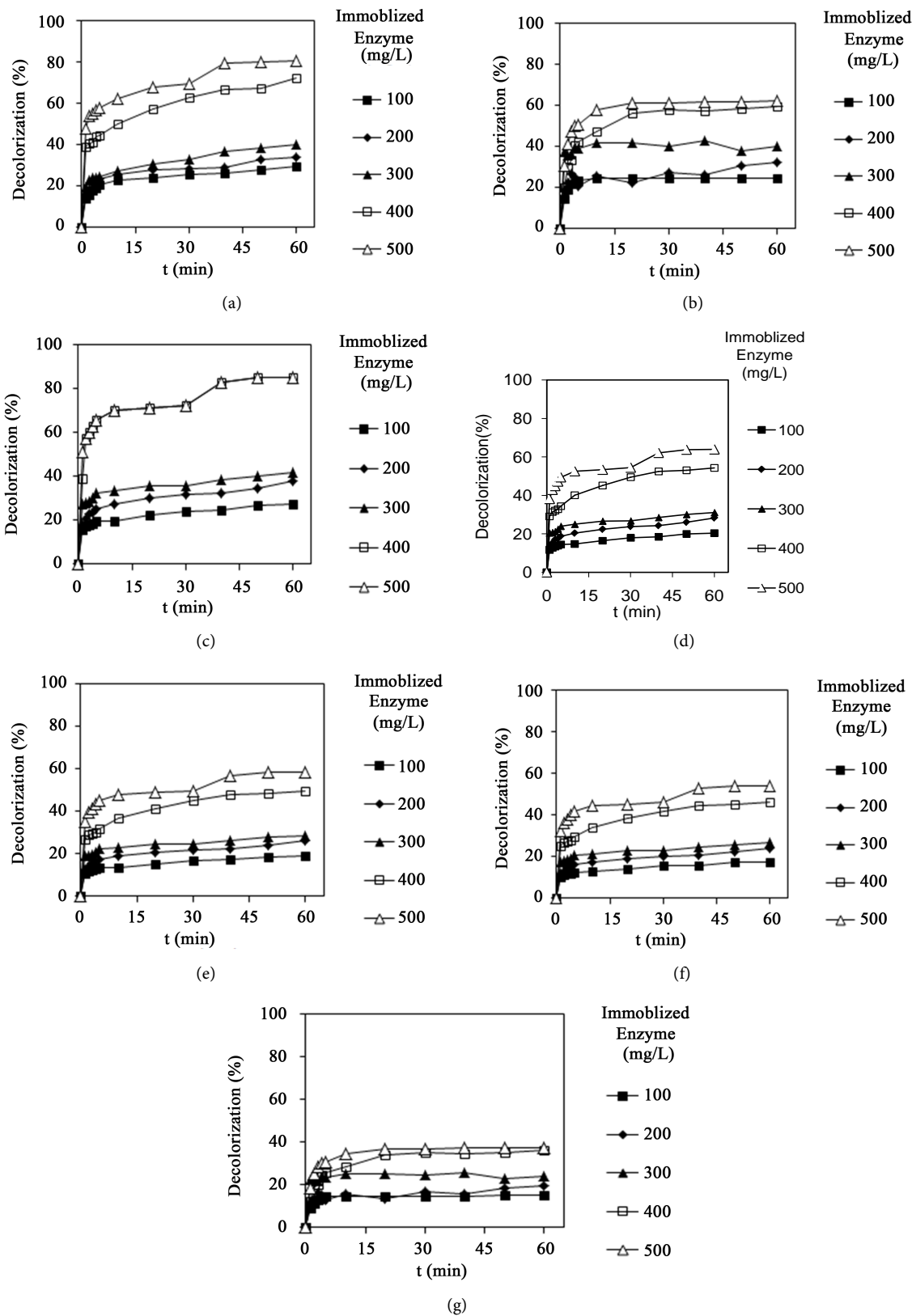


Figure 2. The effect of immobilized laccase enzyme concentration on enzymatic decolorization of dyes from single and binary systems. ((a)-(c): single system; (e): DR60-DB56, (f)-(g):DR60-DY54). (a) DR60; (b) DY54; (c) DB56; (d) DR60 in binary system (DR60-DB56); (e) DB56 in binary system (DR60-DB56); (f) DR 60 in binary system (DR60-DY54); (g) DY 54 in binary system (DR60-DY54).

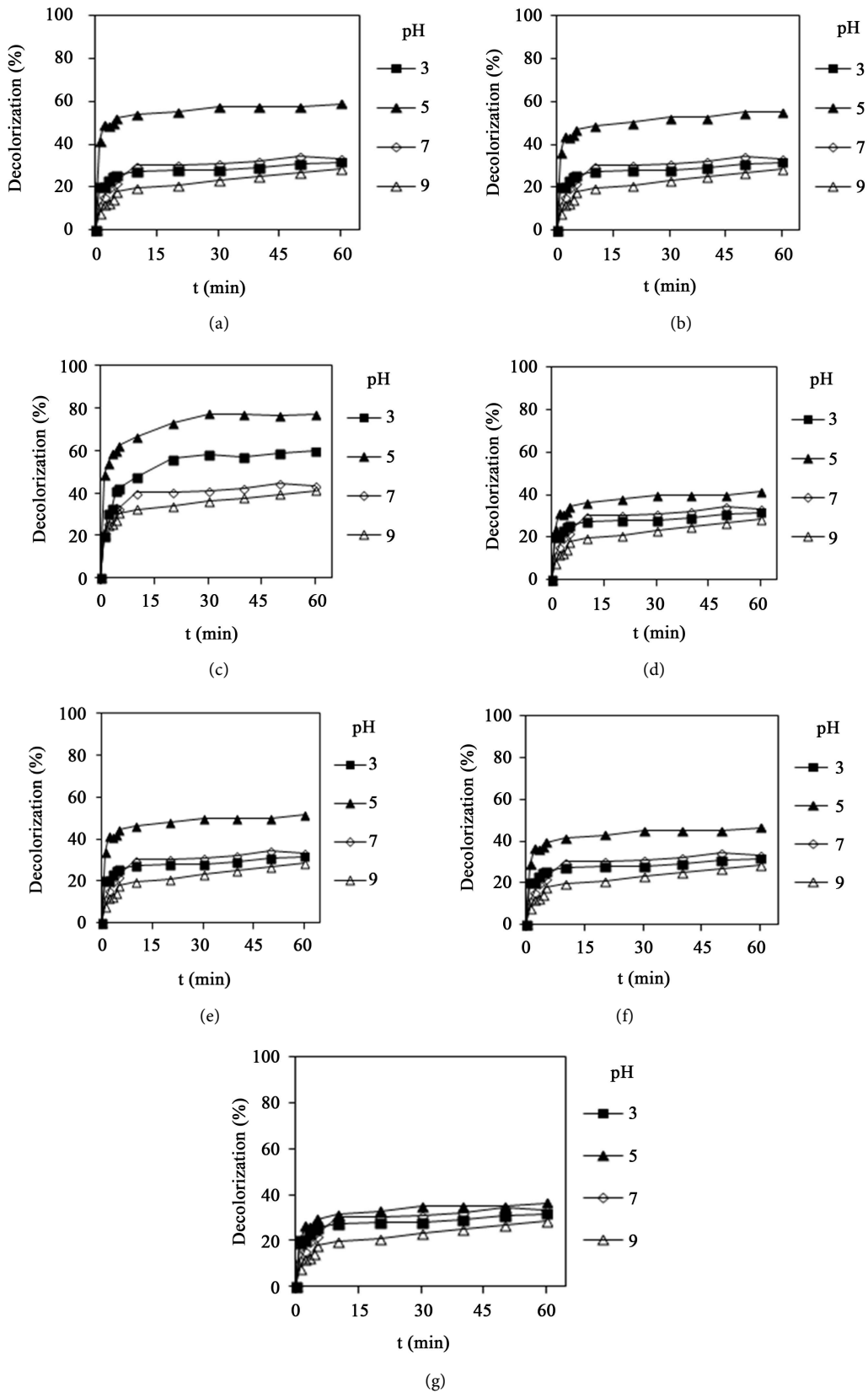


Figure 3. Effect of pH on enzymatic decolorization of dyes from single and binary systems. ((a)-(c): single system; (e): DR60-DB56; (f) and (g): DR60-DY54). (a) DR60; (b) DY54; (c) DB56; (d) DR60 in binary system (DR60-DB56); (e) DB56 in binary system (DR60-DB56); (f) DR60 in binary system (DR60-DY54); (g) DY54 in binary system (DR60-DY54).

The results showed that the pH significantly influenced the immobilized laccase enzyme action during dye decolorization. Dye decolorization was found to improve with an increase in aqueous phase pH until a value of 5.0 and thereafter an increase in the aqueous phase pH from 3.0 to 9.0 caused the efficacy of enzymatic decolorization process to decrease.

The aqueous phase pH of 5.0 had significant effect on the rate of dye decolorization compared to other pH conditions. Thus, aqueous phase pH plays a significant role in enzymatic reactions and many free enzymes exhibit maximum activity at one particular pH. In addition, the pH-activity relationship of any given free enzyme depends on the acid-base behavior of free enzyme and substrate as well as many other factors that are usually difficult to analyze quantitatively [5] [15].

3.3. Dye Concentrations

Decolorization of dyes from single and binary systems at different dye concentrations is shown in **Figure 4**. Results showed that increasing of dye concentration leads to reduce dye decolorization because of existence of more dye molecules. This phenomena show that the aqueous phase dyes concentration influenced the immobilized enzyme activity. When the amount of immobilized laccase enzyme concentration was kept constant and the substrate (dye) concentration was gradually increased, the velocity of reaction increases until it reached the maximum. After obtaining the equilibrium state, any further addition of substrate (dye) did not alter the rate of reaction [16].

3.4. Kinetic of Dye Decolorization Processes

Kinetic of enzymatic decolorization of dyes has been studied according to the substrate (dye) absorption and enzymatic reaction rate. To investigate the mechanism, a Michaelis-Menten constant has been used to fit the experimental data. The kinetic constant, Michaelis-Menten (K_m), Maximum decolorization rate (V_{max}) and catalytic constant (K_{cat}) of laccase were determined for dyes from single and binary systems by linear regression and Hanes-Woolf plots (**Table 1**) [16].

Table 1. Kinetic constants of enzymatic decolorization of dyes from single and binary systems using immobilized Laccase.

Substrate	K_m (μM)	V_{max} (mg/L min)	K_{cat}
Disperse red 60 in single system	1.848	0.00136	0.00221
Disperse blue 56 in single system	1.8121	0.00118	0.00198
Disperse yellow 54 in single system	3.052	0.00143	0.00128
Disperse red 60 in DR60/DB56	6.09	0.00326	0.00180
Disperse blue 56 in DR60/DB56	1.946	0.00117	0.00176
Disperse red 60 in DR60/DY54	6.731	0.00357	0.00187
Disperse yellow 54 in DR60/DY54	3.35	0.00147	0.00122

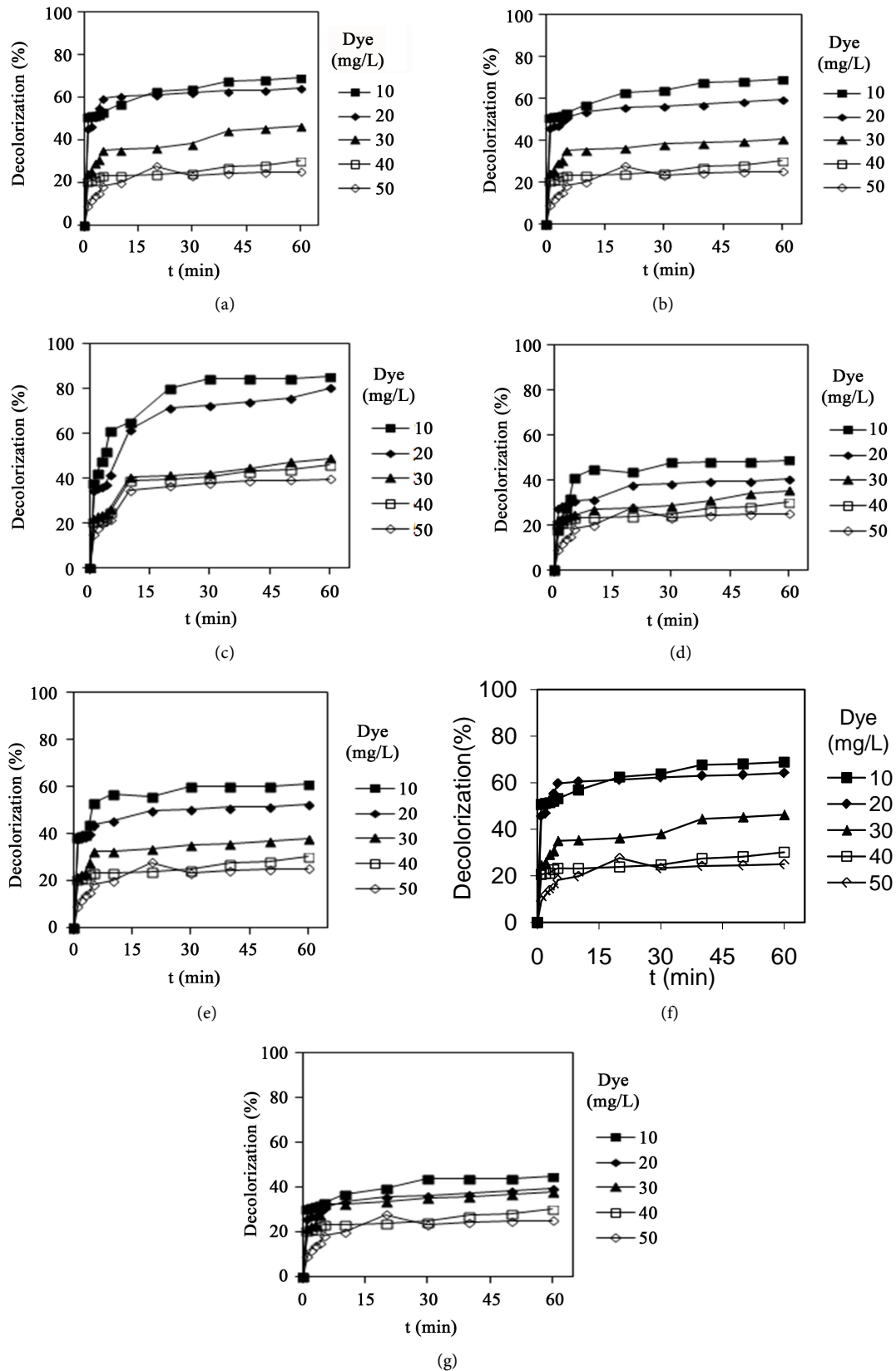


Figure 4. Effect of dye concentration on enzymatic decolorization of dyes from single and binary systems. ((a)-(c): single system; (e): DR60-DB56; (f) and (g): DR60-DY54). (a) DR60; (b) DY54; (c) DB56; (d) DR60 in binary system (DR60-DB56); (e) DB56 in binary system (DR60-DB56); (f) DR 60 in binary system (DR60-DY54); (g) DY 54 in binary system (DR60-DY54).

Saturation curve for immobilized enzyme showing the relation between the concentration of substrate and rate of dye decolorization has been indicated in **Figure 5**. According to **Figure 5**, increasing dye concentration at lower concentration increases rate of dye decolorization linearly, but from given dye concentration, increasing dye concentration has not any specific effect on increasing dye decolorization rate (Maximum rate of dye decolorization) [16].

Hanes-Woolf plots were made from the initial rates obtained at varying dye concentrations while amount of enzyme was held constant (**Figure 5** and **Figure 6**). According to the data of **Table 1**, the lower K_m value was estimated for oxidation of DB56 by immobilized laccase in single system, suggesting that this compound is well susceptible to immobilized laccase enzyme attack in compare with DR60 and DY54 [16]. Furthermore, the lower K_m value was estimated for oxidation of DB56 and DY54 by free laccase in DR60-DB56 and DR60-DY54 systems, suggesting that DB56 and DY54 are well susceptible to immobilized laccase enzyme attack in DR60-DB56 and DR60-DY54 [16].

4. Conclusions

Immobilized Laccase enzyme has a significant effect on decolorizing of DR60, DB56 and DY54 from single and Binary systems. Furthermore, the optimized laccase enzyme concentration, reaction time and pH for decolorization of DR60,

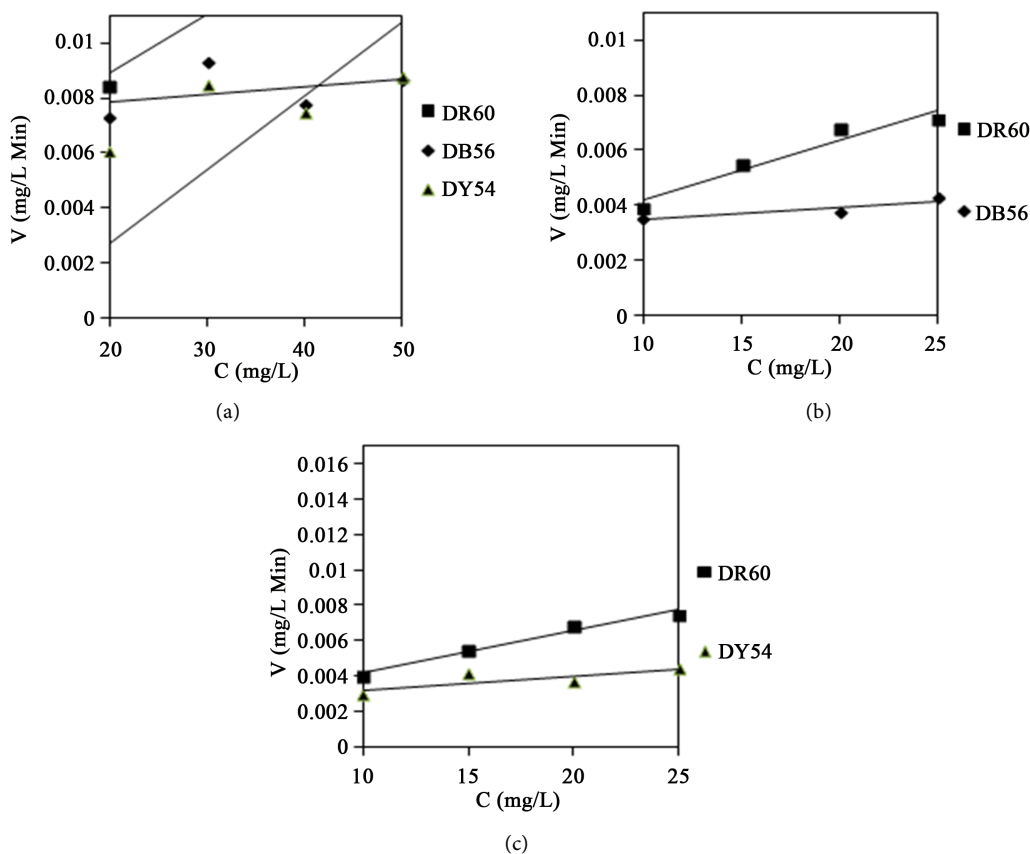


Figure 5. Saturation curve of enzyme during the enzymatic decolorization of dyes from single and binary systems. ((a) single system; (c): binary system).

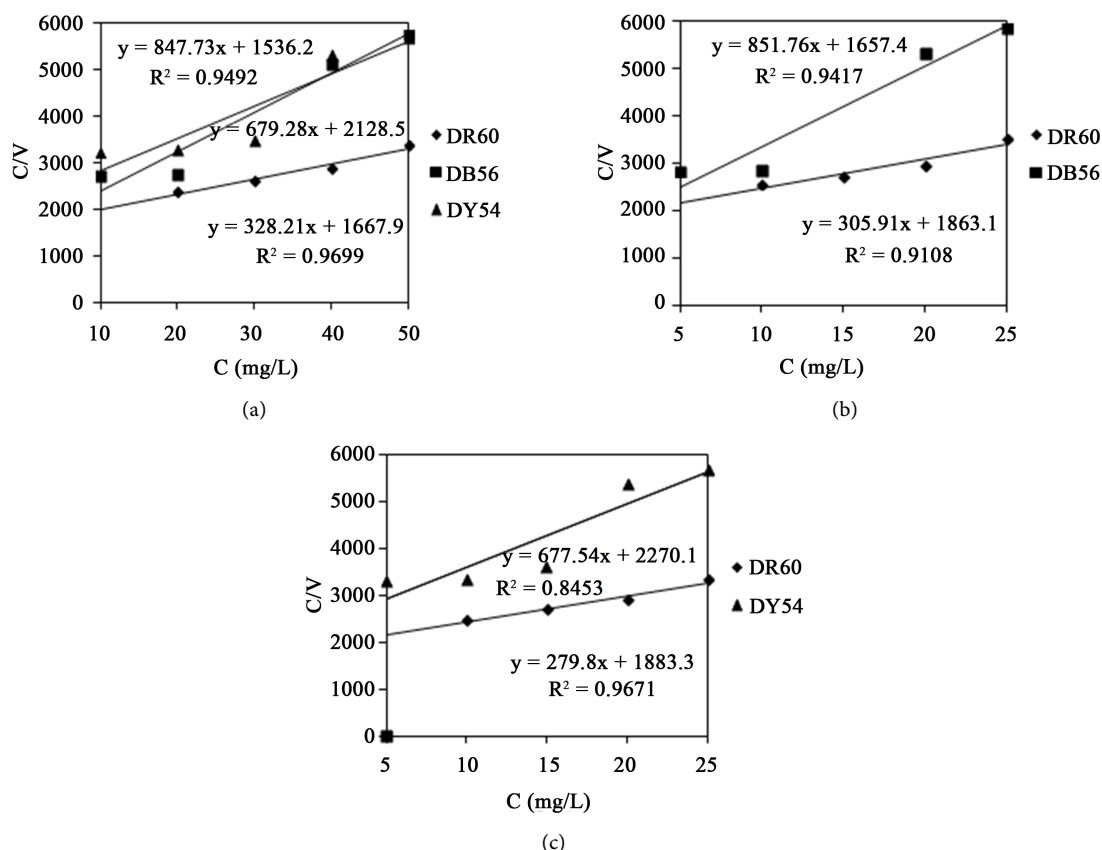


Figure 6. Linearization plots: A-Hanes-Woolf plot of enzymatic decolorization of dyes from single and binary systems. ((a) single system; (c) binary system).

DB56 and DY54 from single and binary systems were 500 mg/L (for DR60 and DY54) and 400 mg/L (for DB56), 20 min and 3, respectively.

Dye decolorization kinetics followed Michaelis-Menten Model. The results showed that enzymatic process using immobilized laccase was effective method to decolorize dyes from single and binary systems.

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