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# Decolorization of dyes using laccase enzyme from single and binary systems

Khashayar Mohajershojaei[a], Alireza Khosravi[a], Niyaz Mohammad Mahmoodi[b,*]

[a]*Department of Polymer Engineering and Color Technology, Amirkabir University of Technology, Tehran, Iran*
[b]*Department of Environmental Research, Institute for Color Science and Technology, Tehran, Iran*
*Tel. +98 021 22969771; Fax: +98 021 22947537; email: mahmoodi@icrc.ac.ir*

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ABSTRACT

In this paper, enzymatic decolorization of dyes using laccase from single and binary systems was studied. Direct red 31 (DR31) and Acid blue 92 (AB92) were used as model dyes. The effect of several parameters, such as enzyme concentration, pH, dye concentration, and salt (sodium chloride, sodium carbonate, sodium bicarbonate and sodium sulfate) on decolorization of dyes was evaluated. The optimized enzyme concentration, reaction time, and pH for decolorization of DR31 and AB92 were 500 mg/L, 10 min, and 5, respectively. The presence of salt in solution does not have significant effect on decolorization of dyes. However, addition of sodium chloride salt has significant effect on decreasing decolorization of dyes because of its inhibitory effect on enzymatic processes. Dye decolorization kinetics followed Michaelis–Menten Model. The results showed that the enzymatic process using laccase was an effective method to decolorize dyes from single and binary systems.

*Keywords:* Enzymatic decolorization; Laccase; Single and binary systems; Michaelis–Menten Model

## 1. Introduction

Increasing rate of population leads to manufacture of more dyes and more pollution. Nowadays, pollution concerns are increasing because the strict policies for pollution control are obligatory [1–14]. Due to the wide range of dyes used in textile and other industries, large amounts of dyes are released into aqueous ecosystems. This trend has led to alter the pH, biochemical oxygen demand, chemical oxygen demand, and gives the rivers intense colorations. In addition, it leads to the death of marine creatures and environmental pollution. In these cases, without adequate treatment, these dyes will remain in the environment for an extended period of time [6–9]. The stability and xenobiotic nature of many dyes (especially azo dyes) make them recalcitrant. Thus, they are not totally degraded by conventional wastewater treatment methods [8,12,14,15]. It underlines the need for largely unspecific processes for treating textile wastewater. Nowadays, different techniques have been used for wastewater treatment, such as physical, chemical, and biological methods [1–4,8–11,13,15].

The enzymatic processes of wastewater treatment are simpler and more efficient than traditional methods. Some characteristics of enzymes are viable to treat colored wastewater. They are biodegradable

*Corresponding author.

catalysts that operate at low and high substrate concentrations over a wide range of pH, temperature, and salinity. Moreover, these processes have a reduced sludge formation and they are simple and easy to control. However, enzymes are sensitive to additives, heavy metals, and salts. Moreover, the enzymes production processes are time consuming and expensive [16,17].

In these cases, enzymes are able to treat pollutant in selective manner at mild conditions of temperature, pressure and pH [10–12,16]. Enzymes have different sources, such as plants, fungi, or bacteria. Laccase is one of the most important enzymes that is used for the decolorization of dyes. It has different sources, such as plants, fungi, and bacteria [12,13]. It belongs to the group of phenoloxidases. These copper-containing enzymes are oxidative enzymes detected in many plants and secreted by numerous fungi [14,15].

Fungi from the basidiomycetes group, known as white rot fungi are a heterogeneous group of micro-organisms. This kind of fungi area main origin of different enzymes (such as free laccase (Denilite II S) and lignin peroxidases, etc.). The white rot fungi are the most efficient liginiolytic micro-organisms. They are able to degrade a wide variety of recalcitrant pollutants including various types of lignin, phenol, non phenol compounds, and different dyes [7,10,15,16]. Most of the information on the biodegradation of synthetic dyes by ligninolyic fungi have been obtained with *Phanero Chaete Chrysosporium* [8,15,16]. The decolorization of textile dyes using Denilite II S enzyme as a ligninolytic enzyme is an attractive treatment technology. Laccase is able to catalyze the oxidation of various aromatic compounds (particularly, phenols) with the concomitant reduction of oxygen to water [7,8,11].

A literature review showed that decolorization of dyes using laccase from binary system was not studied. In this paper, the enzymatic decolorization of dyes using laccase from single and binary systems was studied. DR31 and AB92 were used as model dyes. The effect of several parameters, such as enzyme concentration, pH, dye concentration, and salt on decolorization of dyes from single and binary systems was evaluated.

## 2. Experimental

### 2.1. Materials

DR31 and AB92 were obtained from Ciba. The chemical structure of dyes is indicated in Fig. 1. Denilite II S enzyme was provided by Novo Nordisk Company. All other chemicals were of analytical grade and purchased from Merck (Germany).

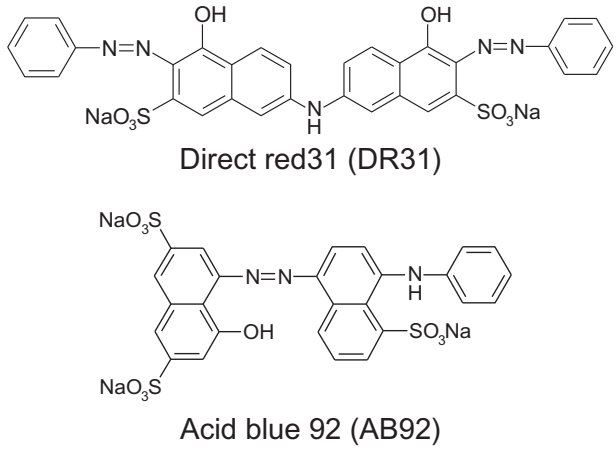



Fig. 1. The chemical structure of dyes.

### 2.2. 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 Laccase. 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 ($\lambda_{max}$) of dyes (524 nm for DR31 and 574 nm for AB92) at different time intervals using UV-vis spectrophotometer (Perkin–Elmer Lambda 25 spectrophotometer).

The effect of enzyme concentration on dye degradation was investigated by contacting 100 mL of dye solution (20 mg/L) at 45°C and pH 5. Different enzyme concentrations (100, 200, 300, 400, and 500 mg/L) were applied.

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

The effect of initial dye concentration (10–50 mg/L) on dye degradation was investigated by contacting 100 mL of dye solution and enzyme concentration (500 mg/L) at 45°C and pH 5.

The effect of salt on the percentage of dye degradation was studied. About 0.02 mol of different salts (NaCl, $Na_2SO_4$, $Na_2CO_3$, and $NaHCO_3$) was added to 100 mL of dye solution (20 mg/L) and enzyme concentration (500 mg/L) at 45°C and pH 5.

In single system, the concentration of dye and its variation during enzymatic processes was measured using Beer–Lambert law.

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

where $\varepsilon$, $L$ and $C$ are extinction coefficient (L/mg cm), path length (cm), and dye concentration (mg/L), respectively.

The dye concentration in binary system was calculated as follows: for a binary system of components $A$ and $B$ measured at $\lambda_1$ and $\lambda_2$, respectively, the optical densities of $d_1$ and $d_2$ are given as follows [4]:

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

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

where $k_{A1}$, $k_{B1}$, $k_{A2}$, and $k_{B2}$ are the calibration constants for components $A$ and $B$ at the two wavelengths $\lambda_1$ and $\lambda_2$, respectively.

### 2.3. Measuring enzyme concentration

According to the chemical state (powdered state) of Laccase enzyme, enzyme concentrations were measured based on the dry weight of powdered laccase enzyme (mg) in proportion to the volume of the solution (L).

## 3. Results and discussion

### 3.1. Enzyme concentration

The decolorization of dyes from single and binary systems at different concentrations of enzyme is shown in Fig. 2. The results show that with increasing enzyme concentration, the dye removal percentage increases gradually because of the existence of more enzyme molecules at the fixed amount of dye molecules [17]. At 500 mg/L enzyme concentration, the complete removal of DR31 (20 mg/L), AB92 (20 mg/L) and its binary system (20 mg/L) was observed. Therefore, their optimized enzyme concentration for decolorization was 500 mg/L.

### 3.2. pH

The effect of pH on decolorization of dyes from single and binary systems is shown in Fig. 3. The results showed that the pH significantly influenced the laccase action during dye decolorization. Dye decolorization was found to improve with an increase in aqueous phase pH until it reached a value of 5.0 and thereafter, an increase in the aqueous phase pH from 5.0 to 9.0 caused the efficacy of the enzymatic decolorization process to decrease. The aqueous phase pH of 5.0 had a significant effect on the rate of dye decolorization compared with other pH conditions. Thus, the aqueous phase pH plays a significant role in enzymatic reactions and many enzymes exhibit maximum activity at one particular pH. In addition, the pH-activity relationship of any given enzyme depends on the acid–base behavior of enzyme and substrate as well as many other factors that are usually difficult to analyze quantitatively [18]v.

### 3.3. Effect of salts on dye decolorization

The decolorization of dyes from single and binary systems in the presence of salts (sodium sulfate, sodium bicarbonate, sodium carbonate, and sodium chloride) is shown in Fig. 4. The results show that addition of 0.02 M salts to dye solution from single and binary systems have slight effect on dye decolorization. It can be attributed from competition between salt and dye for adsorbing to enzyme active sites. However, addition of sodium chloride has significant effect on decreasing dye decolorization because of its inhibitory effect on enzymatic processes and inactivation of enzyme [19,20].

The ionic strength of the sodium chloride solution affected enzymatic performance as well as the solubility of reaction products. High ionic strength can totally inhibit laccase activity. The effect of salts such as NaCl on free laccase activity was determined as AB92 and DR31 decolorization rate. The decolorization rates of AB92 and DR31 in single and binary systems are shown in Table 1.

### 3.4. Dye concentrations

The decolorization of dyes from single and binary systems at different dye concentrations is shown in Fig. 5. The results show that the increasing of dye concentration leads to the reduction in dye decolorization because of the existence of more dye molecules. Additionally, dye concentration influences the enzyme activity. When the amount of enzyme concentration was kept constant and the substrate (dye) concentration was gradually increased, the velocity of the reaction increased until it reached the maximum. After obtaining the equilibrium state, further addition of substrate (dye) did not alter the rate of reaction [21].

### 3.5. Kinetics of dye decolorization processes

Kinetics 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,

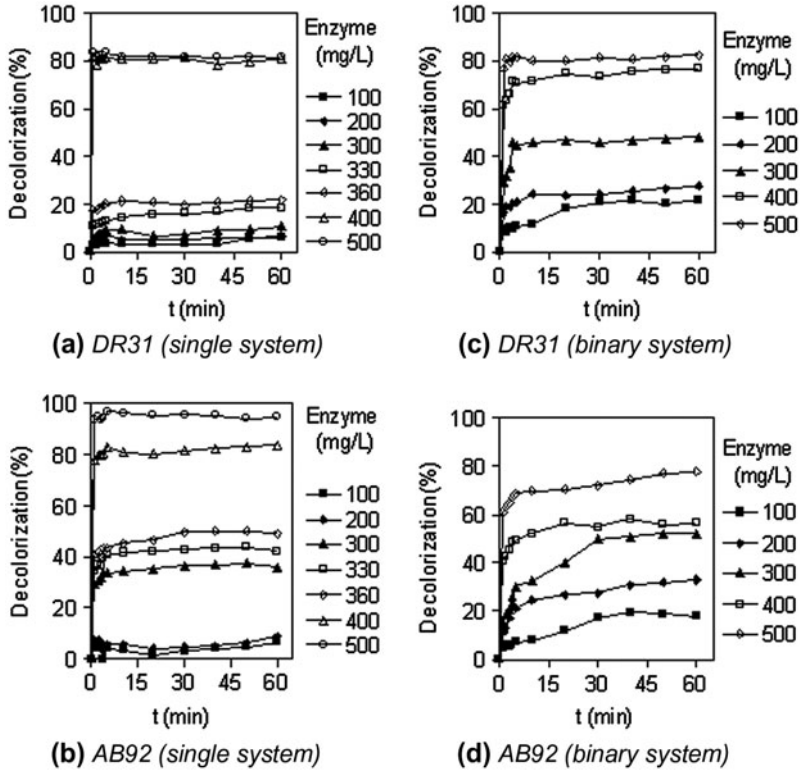



Fig. 2. The effect of enzyme concentration on enzymatic decolorization of dyes from single and binary systems.

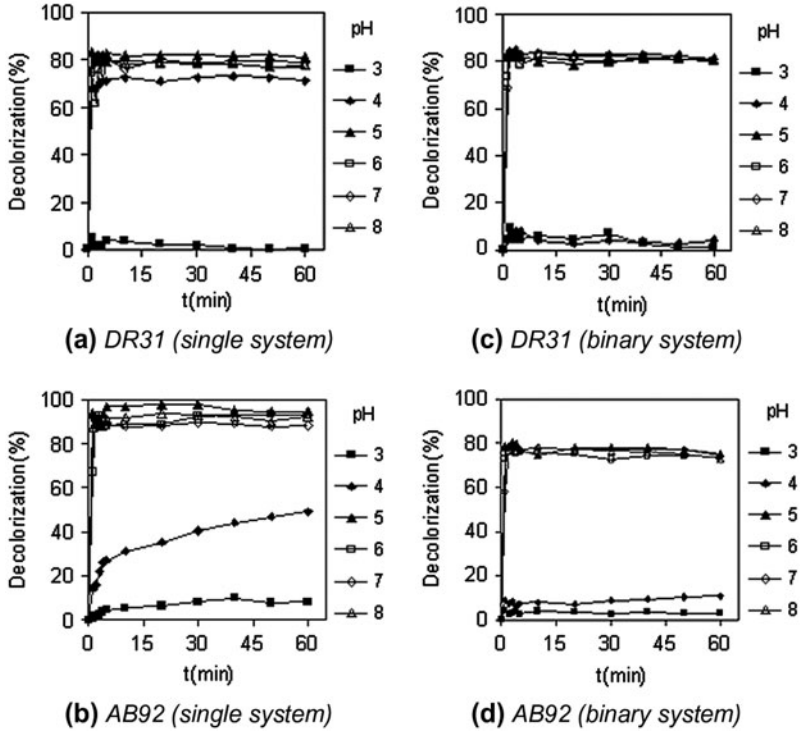



Fig. 3. Effect of pH on enzymatic decolorization of dyes from single and binary systems.

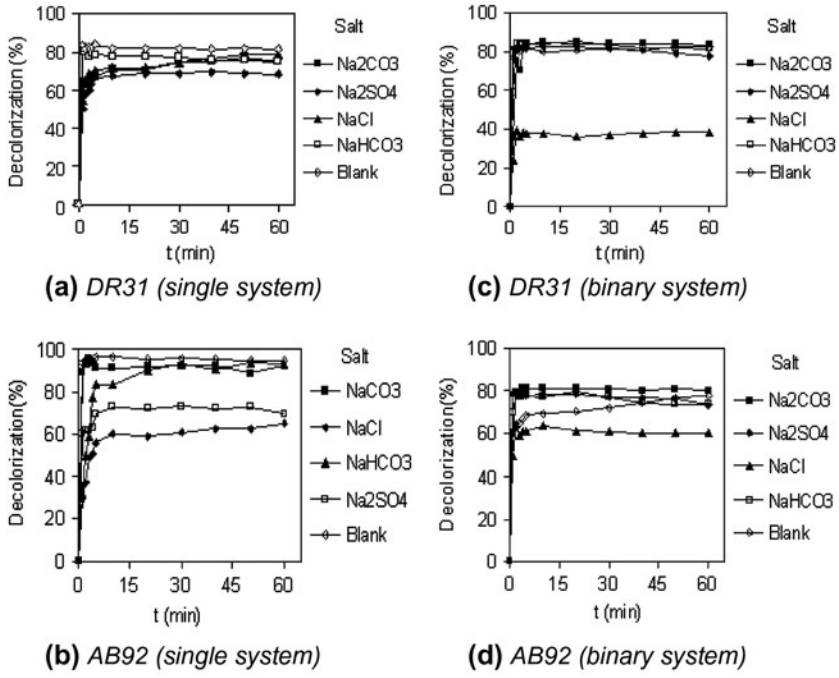



Fig. 4. Effect of salts on enzymatic decolorization of dyes from single and binary systems.

Table 1
Decolorization rates of DR31 and AB 92 in single and binary system with/without NaCl

| | Decolorization rate in single system (mg/L min) | | Decolorization rate in binary system (mg/L min) | |
|---|---|---|---|---|
| Dye | Without NaCl | With NaCl | Without NaCl | With NaCl |
| DR31 | 0.0048 | 0.0003 | 0.0009 | 0.0002 |
| AB92 | 0.0041 | 0.0007 | 0.0043 | 0.003 |

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 2) [21].

Saturation curve for an enzyme showing the relation between the concentration of substrate and the rate of dye decolorization is indicated in Fig. 6. The results show that the increasing dye concentration at lower concentration increases the rate of dye decolorization linearly, but from a given dye concentration, increasing dye concentration has not any specific effect on increasing dye decolorization rate (maximum rate of dye decolorization) [21].

Hanes–Woolf plots were made from the initial rates obtained at varying dye concentrations, while the amount of enzyme was held constant (Fig. 7). According to the data in Table 1, the lower $K_m$ value was estimated for the oxidation of AB92 by laccase in single and binary system suggesting that this compound is well susceptible to Denilite II S enzyme attack in comparison with DR31 [21].

### 3.6. Absorbance spectra of enzyme treated and untreated colored solution

UV-vis spectra of dyes during the enzymatic decolorization from single and binary systems are shown in Fig. 8. The results showed a significant decrease of dye absorbance at visible region because of enzymatic decolorization. The increase in the absorbance around 250–300 nm is due to the dark color formation [22–24].

### 3.7. Effect of dye structure on dye decolorization using free laccase enzyme

Laccase can oxidize a wide range of molecules, such as polyamines, aminophenol, lignins, aryl diamine, and some inorganic ions. The nature and position of substituents on the phenolic ring influence the efficiency of oxidation by laccase. Electrophilic functional groups, especially in the ortho-position, negatively affect the substrate's affinity for enzyme. Moreover, steric

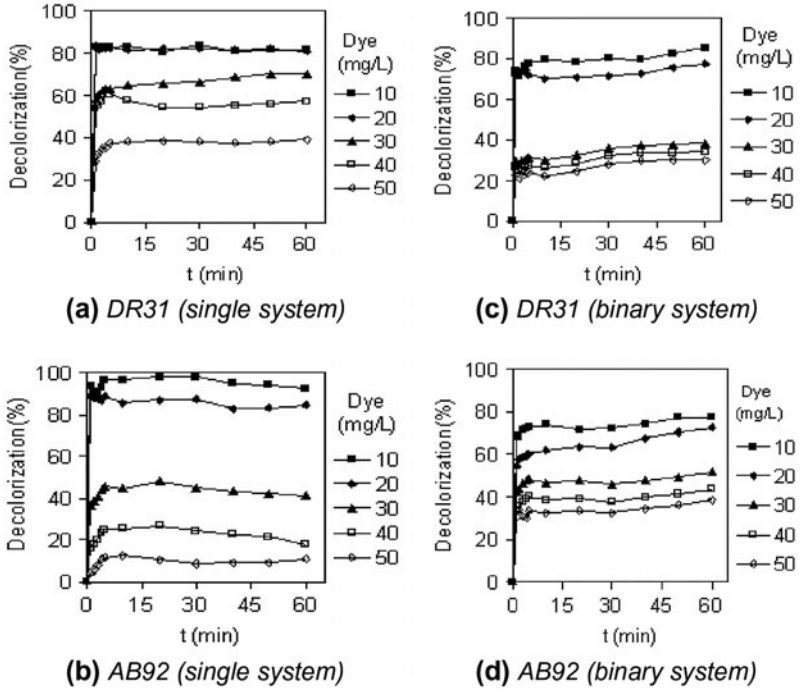



Fig. 5. Effect of dye concentration on enzymatic decolorization of dyes from single and binary systems.

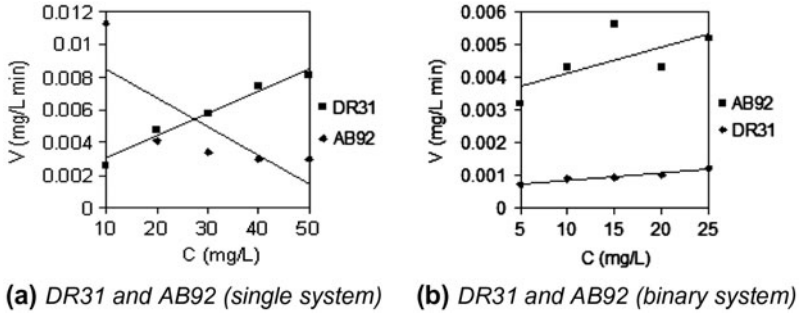



Fig. 6. Saturation curve of the enzyme during the enzymatic decolorization of dyes from single and binary systems.

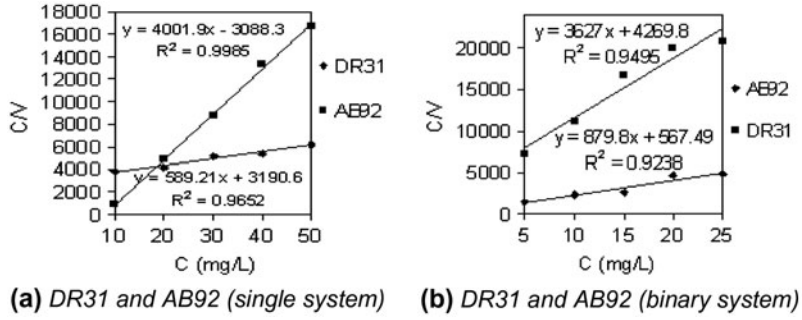
Table 2
Kinetic constants of enzymatic decolorization of dyes from single and binary systems using laccase

| Substrate | $K_m$ (μM) | $V_{max}$ (mg/ L min) |
|---|---|---|
| DR31 in single system | 5.415 | $1.697 \times 10^{-3}$ |
| DR31 in binary system | 1.177 | $2.757 \times 10^{-4}$ |
| AB92 in single system | 0.7717 | $2.498 \times 10^{-4}$ |
| AB92 in binary system | 0.645 | $1.136 \times 10^{-3}$ |

overcrowding and high redox potential negatively affect the laccase oxidation rates [25]. According to the dye structure of DR31 and AB92, Laccase has more effect on decolorization of AB92 because of lower spatial hindrance.

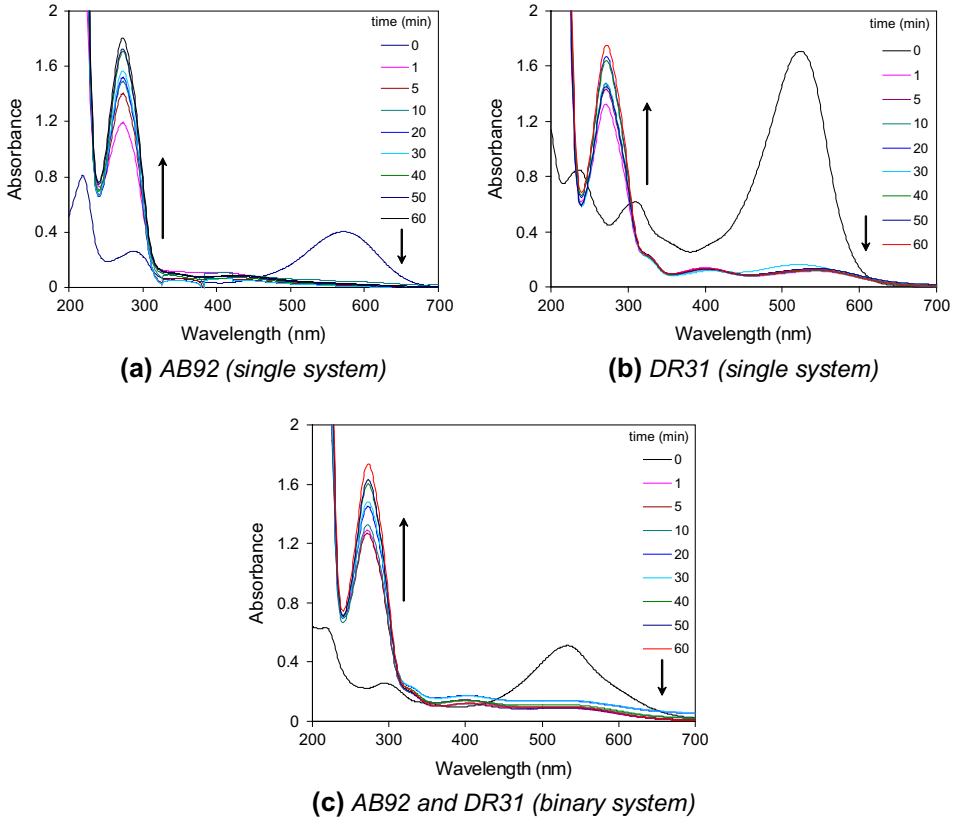
## 4. Conclusion

Laccase enzyme has a significant effect on decolorizing of DR31 and AB92 from single and binary systems. Optimized enzyme concentration, pH, and time for decolorization of DR31 and AB92 are 500 mg/L, 5, and 10 min, respectively. The addition of sodium chloride to the dye solution significantly decreases the decolorization rate of dyes because of its inhibitory effect on enzymatic processes and inactivation of enzyme. The enzymatic decolorization of DR31 and AB92 is followed by Michaelis–Menten kinetic model in single and binary systems. Generally, different factors (temperature, pH,




**(a)** *DR31 and AB92 (single system)* **(b)** *DR31 and AB92 (binary system)*

Fig. 7. Linearization plots: A Hanes–Woolf plot of enzymatic decolorization of dyes from single and binary systems.




**(a)** *AB92 (single system)* **(b)** *DR31 (single system)*

**(c)** *AB92 and DR31 (binary system)*

Fig. 8. UV–vis spectra of dyes during enzymatic dye degradation in single and binary systems.

and enzyme concentration, etc.) have direct effect on dye decolorization in single and binary systems. Moreover, molecular interaction between dyes can have a significant effect on dye decolorization using Denilite II S enzyme. The results showed that the decolorization efficiency of Denilite II S enzyme in single system is more than in the binary system (especially in DR31**)**.

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