PROGRESS IN COLOR, COLORANTS AND COATINGS

available online @ www.pccc.icrc.ac.ir

Prog. Color Colorants Coat. 12 (2019), 39-56

Ministry of Science, Research and Technology
Institute for Color Science and Technology

Application of a New Self-Cleaning Filter for Colored Wastewaters Treatment Using Laccase Enzyme Immobilized on Activated CARBON powder and fiber

M. S. Khazravi[1], M. Bahmaei[1], M. E. Olya[2*], S. M. Etezad[2*]

[1.] Chemistry Department, Tehran North Branch, Islamic Azad University, P. O. Box: 17111-162, Tehran, Iran
[2.] Department of Environmental Research, Institute for Color Science and Technology, P. O. Box: 16765-654, Tehran, Iran

ARTICLE INFO

Article history:
Received: 25 Nov 2018
Final Revised: 15 Jan 2019
Accepted: 15 Jan 2019
Available online: 2 Feb 2019

Keywords:
Activated carbon powder
Activated carbon fibers
Laccase enzyme
Reactive blue 19
immobilized Enzyme

ABSTRACT

The objective of this work is investigation of the adsorption and decomposition of Reactive Blue 19 from industrial wastewaters using laccase enzyme immobilized on activated carbon powder and fiber as adsorbent. Time, pH, temperature, stirring rate, the amount of the adsorbent, dye initial concentration, solution flow rate in the column and column height were studied as key operating parameters to determine the optimal adsorption conditions. The results indicated 80.70% of the adsorption for reactive blue 19 by immobilized laccase enzyme on adsorbent. In addition, kinetic parameters of the enzyme (V_{max} and K_m), optimal temperature effect, optimal pH and thermal stability of the free and stabilized laccase were studied and the results showed 66.66% of laccase enzyme immobilization yield on the adsorbent. The study of adsorption isotherms (Langmuir, Freundlich and Temkin) showed that the process follows a Langmuir model with a correlation coefficient (R^2: 0.9389). Dye removal efficiency and characterization of the intermediate products of removal process were investigated using UV-Vis, TOC and LC/MS methods. Kinetic and thermodynamic models were studied based on the obtained results, the adsorption process follows a pseudo second order kinetic model. Determination of Gibbs free energy, ΔG, enthalpy, ΔH and entropy, ΔS, showed that the reaction is a spontaneous, exothermic process. Prog. Color Colorants Coat. 12 (2019), 39-56© Institute for Color Science and Technology.

1. Introduction

Technological advances have brought about prosperity for industrial and developing countries and have also resulted in many environmental damages. Disturbing the ecosystem balance has destroyed different plant and animal species or placed them on the verge of extinction [1]. All industries require water directly or indirectly. Textile industry, which uses large quantities of water and a wide range of the dyes containing substituted aromatic and heterocyclic compounds, produces a great volume of high temperature wastewaters including chemicals and dyes [2]. Contaminants entering surface and underground waters can be classified into two major groups of biodegradable and non-biodegradable contaminates [3]. Laccase (benzodiol; oxygen oxidoreductase (EC.1.10.3.2)) belongs to a group of enzymes known as aqueous copper oxidase. These enzymes are applied in biological degradation of industrial wastes, bleaching of colored solutions, food industry, cosmetic and health industries, pharmacology, biochemistry, production of biological sensors and biofuel cells [4-5]. Laccase uses oxygen as an electron acceptor to form quinones and is a member of phenol oxidase family. Moderate activity, high solution stability

*Corresponding author: olya-me@icrc.ac.ir;
etezad-ma@icrc.ac.ir

and selectivity of laccase enzyme enable it to oxidize a large number of phenolic, non-phenolic and similar naturally non-degradable compounds along with the reduction of molecular oxygen in water [6-7]. Pollutants entering water generally reduce sunlight penetration into the inner layers of surface waters. In addition, suspended compounds and colloids such as dyes, fat, oil, acids and bases and transition metals destroy the aquatic ecosystem [8-9]. In recent years, activated carbon has been used for removal of dyes and other industrial pollutants from wastewaters due to its structural properties and high density and surface area [10-11].

The application of biological molecules and carbon compounds is a great idea regarding the developments in the areas of biotechnology, material science and sewage treatment industry. One of the most efficient methods for the treatment of industrial sewages is design of a biological system by stabilizing an enzyme on an adsorbent surface for decomposition and biodegradation of aminophenols, methoxyphenols and aromatic amines.

The performance of this biological system depends on the enzyme properties as well as the selection of an appropriate adsorbent bed and immobilized conditions. Chitosan [12], graphite electrodes [13], activated carbon [14], aluminum hydroxide [15], carbon based electrodes [16], silica [17], magnetic carriers [18], carbon-glass electrodes [20], modified chitosan [21], polypropylene membranes [22], carbon nanotube [23], chitosan nanoparticles on glass beads [24] and plasma polymerized allyl amine/carbon electrode [25] are some of the beds used for immobilization of laccase enzyme.

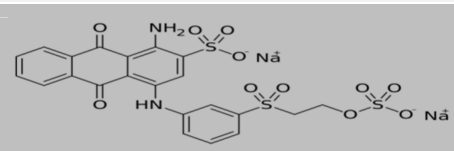
In this work, application of activated carbon powder and fiber as an appropriate adsorbent and bed for immobilized of laccase enzyme and the analysis of the intermediates in the removal process of reactive blue 19 in wastewater has been investigated in batch and fixed bed scales. For the first time a mixing of carbon fiber and powder with immobilized enzyme was used for wastewater treatment processes. According to the presence of the enzymes, this immobilized adsorbent could be a self-cleaning filter. Also, it doesn't need to recovery process and it could be used as a semi-industrial filter for large volume of the wastewaters.

2. Materials and Methods

2.1. Materials

Reactive blue 19 (Table 1) was obtained from Alvan Sabet Co. Laccase enzyme used in this research was a product of Sigma Aldrich Chemical Co. (USA) and activated carbon powder and fiber (10-20 nm diameter) were supplied by Jacobi Co. (Sweden) and NanoPac Persia (Iran) as an adsorbent. Fraction V bovine serum albumin (mw = 66338) was purchased from Fluka Co. (USA). Glutaraldehyde (25%), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 3-aminopropyltrimethoxysilane (97%) were supplied by Sigma Aldrich Chemical Co. Other laboratory grade chemicals including disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium acetate, hydrochloric acid, nitric acid, phosphoric acid, sodium hydroxide, acetone, acetic acid and 96% ethanol were purchased from Sigma Aldrich Chemical Co.

Table 1: Specifications of reactive blue 19 dye.

Parameter	Specification
Name	Reactive blue 19, Remazol Brilliant Blue R (RBBR)
Classification	anthraquinone dye
Symbol	RB19
Type color	Anionic
Molecular Formula:	$C_{22}H_{16}N_2Na_2O_{11}S_3$
Molecular weight (g/mol)	626.54
Wavelength of maximum absorption (nm)	568
Chemical structure of Dye	

2.2. Methods

To measure the adsorption rate of fluids, a JENWAY 6300 Visible Spectrophotometer was used. Surface areas of the adsorbents were measured by a Belsorp mini-II Brunauer-Emmett-Teller (BET) standard analyzer (Japan). A Tescan Mira II field emission scanning electron microscope (FESEM) (USA) and an LC-MS/MS Quattro Micro API micromass Waters 2695 LC/MS instrument was used to study the intermediate compounds. Total organic carbon measurement was performed on a Shimadzu instrument (Japan). A universal 320 R refrigerated centrifuge was used to remove particulate matters and non-interference of suspended particles in optical absorption reading in the tested ranges. A Mettler Toledo pH meter was used to adjust the pH of the solutions.

2.3. Functionalization of adsorbent

Adsorbent was functionalized by chemical activation method using 96% Nitric acid as the strong oxidant under controlled temperature and pressure conditions. According to previous studies, increasing the concentration of activated carbon oxide increases the immobilization of enzyme and improve enzyme stability and activity [26-28]. 0.4 g of adsorbent was treated with 75 mL of 0.3 M nitric acid to oxidize their surfaces to various extents. The obtained mixture was hated at 200 ^{o}C for 4 h in nitrogen pressure of 0.5 MPa. The product was washed with water and dried overnight. The product was coded CA_{ox}.

2.4. Laccase immobilization technique

At first Oxidized activated carbon (1000 mg) were subjected to ultrasonication in 500 mL of 99.9% ethanol for 30 min., followed by addition of 100 µL of (aminopropy) ltrimethoxysilane and stirring using a magnetic stirrer for 72 h. Ultimately, the mixture was placed in an Eppendorf vial and centrifuged at 5000 rpm for 10 min. The precipitate obtained was washed several times with ethanol and dried at ambient temperature.

To immobilize the laccase enzyme, 1000 mg of dry powder prepared in the previous step were added to 500 mL of deionized water. 0.25 mL of gluteraldehyde was added to this and mixture and was stirred for 24 h, followed by transferring into an Eppendorf vial and centrifugation at 5000 rpm for 15 min. The product was washed several times, dried at ambient temperature and named CA_{ox} +GLU). Finally, 1000 mg of powders prepared (CA_{ox}+GLU) and 600 mL of laccase enzyme were stirred for 4 h in 50 mL of sodium acetate buffer (0.1 M, pH=4.5) and recovered by centrifugation.

2.5. Determination of laccase immobilization yield

The percentage of laccase enzyme immobilized on adsorbent and the residue left in the reaction medium as a supernatant is evaluated by bradford method using Bovine serum albumin (BSA) as a standard protein [29].

Enzyme immobilization yield was obtained using equation (1):

$$Y = \frac{C_0 - C_f}{C_0} \times 100 \quad (1)$$

C_0: Total enzyme concentration
C_f: Free enzyme concentration

2.6. Characterization of free and immobilized enzymes

To investigate possible changes in enzyme properties during immobilization, free and immobilized enzyme parameters were determined.

2.6.1. Determination of free and immobilized laccase kinetic parameters (V_{max} and K_m)

To find the kinetic parameters (V_{max} and K_m), the activity of laccase enzyme was determined based on ABTS substrate oxidation rate [30]. The activity of the free and immobilized laccase enzyme was determined using several concentrations of 2.5 M ABTS in sodium acetate 0.1 M (pH=4.5) at 45 ^{o}C. ABTS oxidation rate was measured using UV-Vis spectrophotometer at a wavelength of 420 nm during one minute (ε=36000 M^{-1} cm^{-1}). Enzyme activity unit (U) was determined as the amount of enzyme, which can oxidize 1 micromole of ABTS per minute per unit of solution volume [31]. The activity of free and immobilized laccase enzymes were determined using equation (2). Kinetic parameters of free and immobilized laccase (V_{max} and K_m) were determined using Michaels-Menten plot and prism program.

V_{max} and K_m represent the maximum activity of enzymes in constant concentration . K_m is the substrate concentration when enzyme activity is half of V_{max}

$$U = \frac{abs/min \times f_{dilution} \times 10^6}{\varepsilon} \quad (2)$$

$f_{dilution}$ is the sample dilution factor, 10^6 is the conversion coefficient from mole to micromole per liter, ε is the extinction coefficient (ε=36000 M^{-1} cm^{-1} at 420 nm).

2.6.2. Thermal stability of free and stabilized laccase enzymes

Thermal stability of the enzyme is an important feature, which makes it biocatalytically applicable in the industry [32-33]. To find the thermal stability and resistance of free and immobilized laccase enzymes, they were both incubated at 60 °C. Equal amounts of the enzymes were then removed after different times (0, 5, 15, 30, 45, 60, 90 and 120 min.) and were placed on ice for 30 min and the activity of simples were determined as mentioned in previous section.

2.6.3. Optimal pH

To evaluate the effect of pH on free and immobilized laccase enzymes, the activity of a fixed amount of the enzyme was determined in the presence of 50 µL of 2.5 M ABTS substrate in a mixture of 0.1 M acetate buffer and 100 mmol/L phosphate buffer at different pH values (4, 5, 6, 7, 8, 9 and 10) in the final volume of 2 mL [34-35].

2.6.4. Optimal temperature

To investigate the effect of temperature, the activity of a fixed amount of the enzyme was determined in presence of 50 µL of 2.5 M ABTS substrate in a mixture of 0.1 M acetate buffer at pH=4 at different temperatures (20, 30, 40, 45, 50, 55, 60 and 70 °C) in the final volume of 2 mL.

2.7. Dye removal system

Removal of reactive blue 19 dye by laccase enzyme immobilized on adsorbent was investigated in a batch and a fixed bed systems and influence of key operating parameters such as pH, removal time, initial dye concentration, adsorbent amount, stirring rate, bed height and dye solution flow rate was studied, respectively.

2.7.1. Physical and chemical properties of adsorbent and analytical methods

Characterization of the adsorbent was evaluated by FESEM, FT-IR (450-4000 nm). Also, BET technique was used for measuring of its surface area [36]. The products obtained from decomposition of the dye by immobilized enzyme on the adsorbent were analyzed using an LC-MS/MS Quattro Micro API micro mass Waters 2695 liquid chromatography mass spectrometer**.** An Eclips XDB C-18 capillary column (4.6 m × 150 mm - 5µ) was used for separation of intermediate products at 35 °C. The mobile phase was a mixture of water and acetonitrile (60/40, v/v). The samples were passed through a 0.45 µm filter. Flow rate was 0.5 mL/min and the injection volume was 20 µL. A quadrupole mass analyzer, an electrospray ionization source (ESI) in positive state at a temperature of 120 °C, capillary potential of 4.5 Kv, 35 v CORONA tip voltage, type 5 nebulizer nitrogen gas at a flow rate of 250 L/min and temperature of 380 °C and mass spectrum range of 70-650 were used. Also, measuring the total organic carbon (TOC) was carried out by a Shimadzu TOC analyzer.

2.7.2. Removal efficiency

Decolorization was carried out from 20 mg/L reactive blue 19 synthetic dye using 0.3 g of laccase enzyme immobilized on the adsorbent. Preliminary experiments were performed to obtain optimal conditions in jar test equipment in a period of 20 minutes in a 250 mL beaker at the temperature of 30 °C, initial dye concentration of 20 ppm, stirring rate of 100 rpm, using 0.25 g of the adsorbent.

In addition, to design a continuous form, a steel cylindrical fixed bed reactor with a length of 15 cm and internal diameter of 6 cm was also used to simulate an industrial process.

Laccase enzyme immobilized on adsorbent was placed inside the column. Sampling of reactive blue 19 dye was carried out at specified time intervals and all samples were centrifuged at a rate of 9000 rpm for 120 sec. Finally, adsorption was evaluated using a UV-Vis spectrophotometer at a wavelength of 568 nm. Dye removal efficiency was calculated using equation (3):

$$100 \times R = \frac{A_0 - A_t}{A_0} \quad (3)$$

A_0: Dye adsorption rate (at specific wavelength) at time zero

A_t: Dye adsorption rate after time t

2.8. Adsorption isotherm models

The distribution of the adsorbate between the solid and liquid phases at equilibrium is obtained based on adsorption isotherm models. Adsorption isotherm is an important factor in the design of surface adsorption systems and determination of adsorption capacity. In other words, adsorption isotherm models are mathematical relations, which show the quantitative relation between the equilibrium amount of the adsorbate in state absorption (q_e) and the equilibrium concentration of the adsorbate in liquid phase (C_e) at constant temperature [37]. Langmuir, Freundlich and Temkin adsorption isotherms have been used in this work to interpret the adsorption mechanism based on the correlations between the experimental data and model parameters (Table 2).

2.9. Adsorption kinetic models

Kinetic analysis is necessary in order to study the parameters effective on the process rate and subsequently design the adsorption system. In fact, the chemical kinetic adsorption based on kinetic models defines the reaction paths during times of equilibrium and is greatly connected with the chemical and physical properties of the adsorbent and adsorbate particles and affects the adsorption mechanism [39]. In this work, the mechanism and rate of absorption has been investigated using pseudo first and second order kinetics as well as intrinsic particle infiltration model (Table 3).

Table 2: Adsorption isotherm [38].

Isotherms	Isotherms equation	parameters
Langmuir	$\frac{C_e}{q_e} = \frac{1}{k_L Q_m} + \frac{1}{Q_m} C_e$	q_e= amount of the adsorbate at equilibrium in the solid phase ($mg.g^{-1}$) c_0= initial concentration of the adsorbate in solution ($mg.L^{-1}$) c_e= equilibrium concentration of the adsorbate in solution ($mg.L^{-1}$) Q_m= final capacity of the adsorbent ($mg.g^{-1}$) k_L= Langmuir constant, which is dependent on the bond strength and is a function of system characteristics and time ($mg.g^{-1}$)
Freundlich	$\log q_e = \log K_F + (1/n) \log C_e$	K_F = adsorption capacity of the adsorbent per unit concentration $(mg/g)(L/mg)^{1/n}$ 1/n = adsorption intensity, which is experimentally measured and is sometimes reported as β (dimensionless)
Temkin	$q_e = B_1 \ln K_T + B_1 \ln C_e$	B = constant is associated with the heat of adsorption (J/mol) K_T =Temkin isotherm constant(L/g)

Table 3: Adsorption kinetic models [38].

Kinetic model	Kinetic equation	parameters
Pseudo first orde	$Ln(q_e - q_t) = Ln\, q_e - k_1 t)$	q_e = amount of dye adsorbed at equilibrium (mg/g) q_t = amount of dye adsorbed in time t (mg/g) k_1 = first order kinetic equilibrium rate (L/min)
Pseudo second order	$\frac{t}{q_t} = \frac{1}{k_2 q_{e^2}} + \frac{1}{q_e} t$	K_2= second degree kinetic constant ($g.mg^{-1}.min^{-1}$)
Intrinsic particle infiltration	$q_t = k_p t^{1/2} + I$	kp = intrinsic penetration rate constant($mg / g.min^{-0.5}$) I = boundary layer thickness constant.

2.10. Thermodynamic parameters of adsorption process

Temperature has a direct effect on the adsorption process. In addition, increasing the mobility of the dye molecules with increased temperature leads to remarkable changes. Considering the optimal values obtained in the previous parts, the effect of temperature on dye removal efficiency was studied and the optimal temperature was obtained. For this purpose, a 150 mL Erlenmeyer flask containing the dye was subjected to experiments designed at temperatures of 25, 35 and 45 ^{o}C. Thermodynamic parameters including Gibbs free energy, ΔG^{o}, enthalpy, ΔH^{o}, and entropy, ΔS^{o}, can determine the direction (spontaneity or non-spontaneity of the reaction) and type (endothermcity, exothermicity and physical or chemical absorption mechanism) of the adsorption process. Equation 4 was used to find these parameters [40].

$$\Delta G^{0} = \Delta H^{0} - T\Delta S^{0} \quad (4)$$

where:
ΔG^{o} = Gibbs free energy changes (Kj.mol^{-1})
ΔH^{o} = enthalpy changes (Kj.mol^{-1})
ΔS^{o} = entropy changes (Kj.mol^{-1})
T = temperature (K)

The direction and type of the reaction can be expressed using Gibbs free energy, enthalpy and entropy changes, as follows [41]:

Thermodynamic properties are obtained using thermal absorption coefficient, K_c, which is found using equation (5) [42]:

$$K_c = \frac{q_e}{C_e} \quad (5)$$

In addition, based on Van't Hoff equation and by plotting $\ln K_c$ vs. 1/T, enthalpy (ΔH^{o}) and entropy changes (ΔS^{o}) are obtained using the slope and intercept, respectively [42].

$$\ln K_c = \frac{\Delta S^{0}}{R} - \frac{\Delta H^{0}}{RT} \quad (6)$$

where R= universal gas constant (J.mol^{-1}.K^{-1})

Furthermore, the Gibbs free energy can be obtained using equation [42]:

$$\Delta G^{0} = -RT \ln K_c \quad (7)$$

3. Results and Discussion

3.1. Immobilization of laccase enzyme on the adsorbent

3.1.1. Measurement of immobilized enzyme amount

The immobilized enzyme concentration was determined using Bradford method. The yield of laccase immobilization on the adsorbent was determined as 66.6%.

3.1.2. Fourier transform (FT-IR) spectroscopy

Figure 1 shows the FT-IR spectra of functionalized adsorbent and its enzyme immobilized spectrum. The essential peaks presented in this analysis were mentioned at 1710.55, 3400, 2840-2950, 1685 and 1614 cm^{-1}. The peak presented at 1710.55 cm^{-1} in (1a) is associated with the carbonyl group (C=O) formed by the functionalization of adsorbent by Nitric acid under laboratory conditions. The FT-IR spectrum of the laccase enzyme immobilized on adsorbent (1b) shows a peak in the 3400-3600 cm^{-1} corresponding to OH vibrations [43] and a peak in the 2840-2950 cm^{-1} range attributed to COOH vibrations. Also, at 1615, 1685 and 1614 cm^{-1} the immobilized of the laccase enzyme on adsorbent could be presented, CHO vibrations associated with the reaction of activated carbon with glutaraldehyde in order to immobilize the laccase enzyme and $CONH_2$ vibrations, respectively. So, these results showed a successful immobilization of the laccase enzyme on the adsorbent surface.

3.1.3. Field-emission Scanning Electron Microscopy (FESEM)

FESEM analysis was carried out to study the surface morphology, determine the shape, porosity and distribution of the particles in order to ensure immobilized of the laccase enzyme on the combined adsorbent. Figure 2 shows the FESEM images adsorbent and immobilized enzyme. Also, these images confirmed that the immobilization process was evaluated successfully.

3.1.4. Brunauer-Emmett-Teller (BET) analysis

Specific surface area, total pore volume, average pore diameter of adsorbent and the enzyme immobilized on adsorbent are shown in Table 4. The results show that the specific surface area of adsorbent has not considerably changed upon immobilized of laccase enzyme and the pores required for the adsorption of reactive blue 19 dye have been supplied.

Table 4: BET test results of the adsorbent.

Absorbent	Special surface area [m^2 g^{-1}]	Total pore volume(p/p0=0.990) [cm^3 g^{-1}]	Average pore diameter [nm]
Adsorbent without enzyme	497.56	0.4028	3.2768
Immobilized laccase enzymes on the adsorbent	470.82	0.3891	3.2037

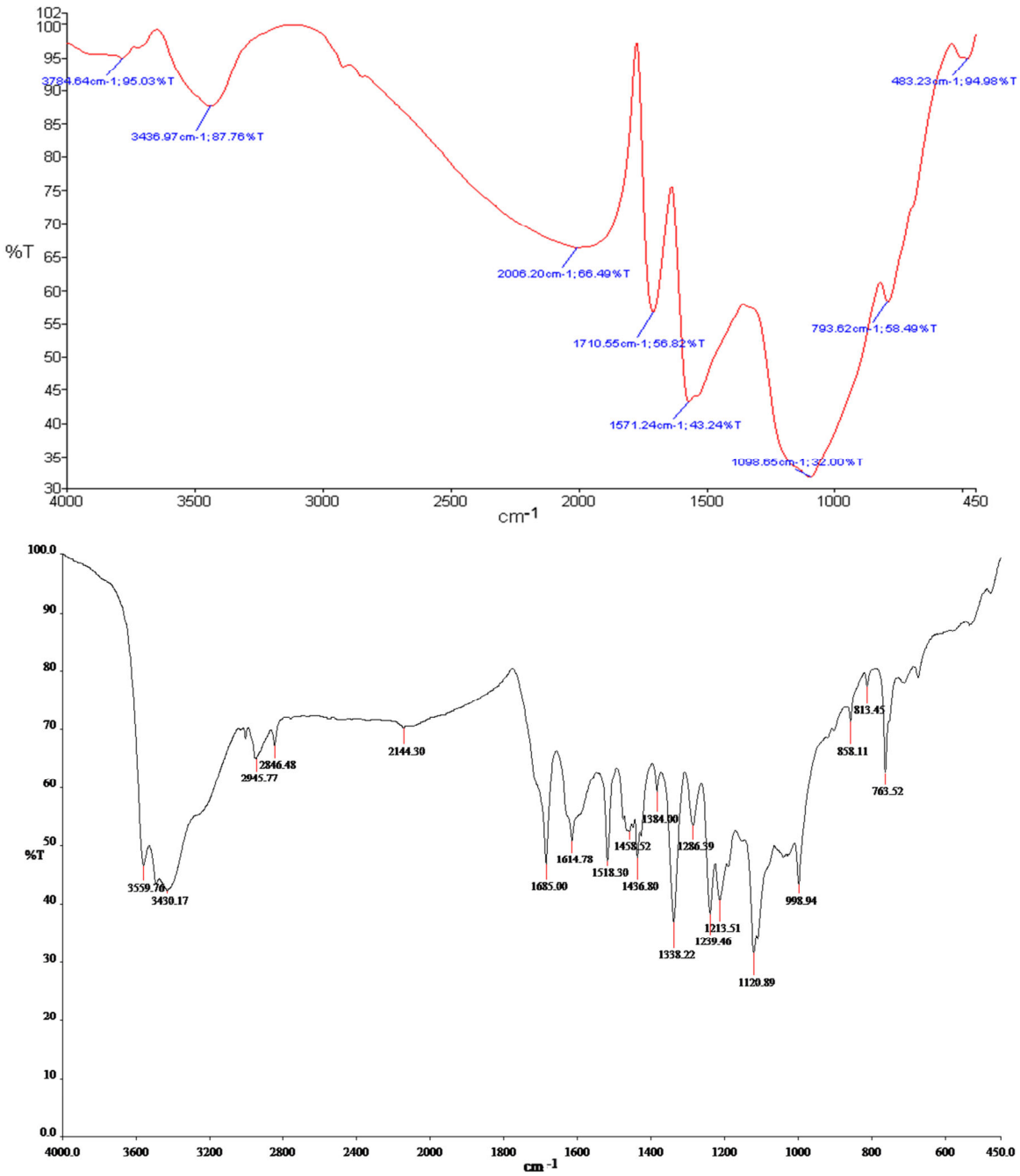
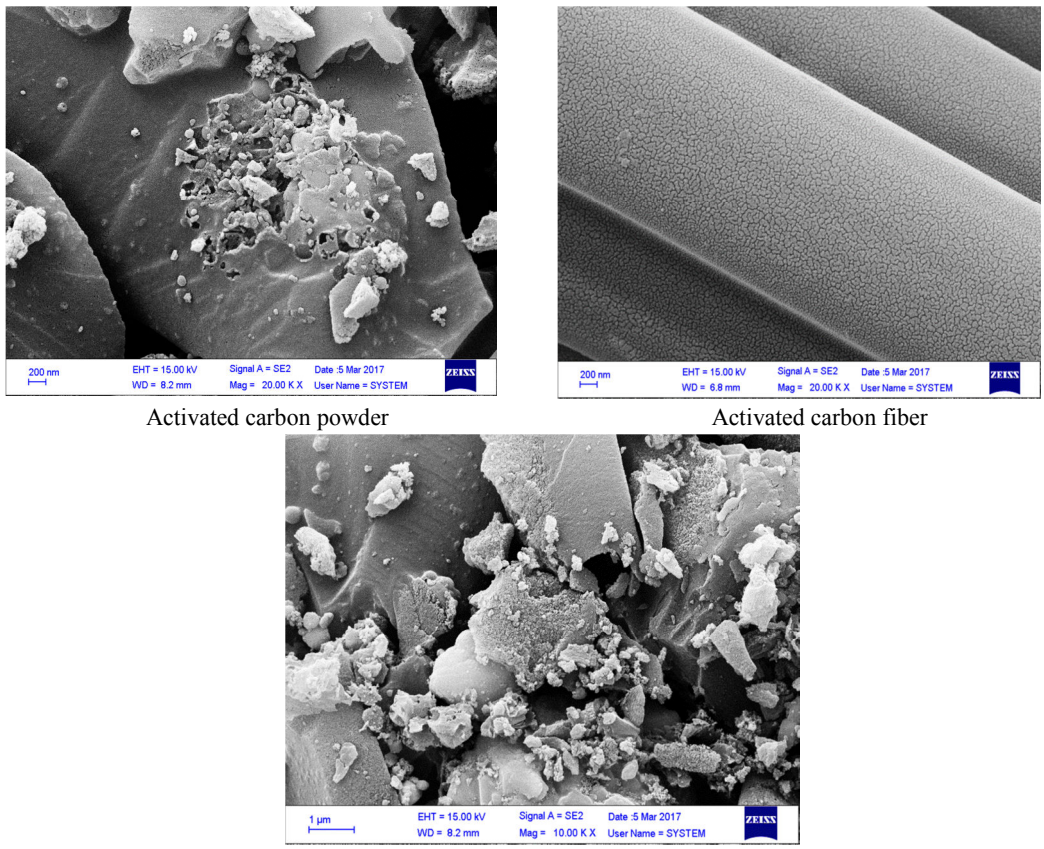


Figure 1: FT-IR spectra of functionalized adsorbent (a) and its immobilized enzyme spectrum (b) .



Activated carbon powder

Activated carbon fiber

g Immobilized enzyme on the adsorbent

Figure 2: FESEM images of activated carbon powder, fiber and immobilized enzyme.

3.2. Investigation of the effective parameters on enzyme immobilized process

3.2.1. Determination of the activity of the free and immobilized enzymes and V_{max} and K_m kinetic parameters

The activity of the free and immobilized enzymes were determined in several concentration of substrate (ABTS) and kinetic parameters were calculated using prism program (Figure 3). V_{max} and K_m values for the free enzyme are 0.02655U and 14.3 µM, respectively, and the corresponding values for the immobilized enzyme are 0.00380U and 200 µM, respectively. K_m for immobilized laccase is 14 times more than the free enzyme, which is probably due to the constraints of mass transfer and the internal penetration into the immobilized enzyme [44]. A unit (U) is a portion of the enzyme, which can oxidize a micromole of ABTS per minute

3.2.2. Determination of optimal pH

The activity of free and immobilized enzymes at different pH values (4, 5, 6, 7, 8, 9 and 10) was studied. As observed in Figure 4, the optimal pH values for the immobilized and free enzymes were found as 5 and 4, respectively. In addition, the increase of NH_2 group in enzyme active sites can increase the activity of the immobilized enzyme compared with the free enzyme at higher pHs [45]. The change in the optimal pH of the enzyme can be attributed to the changes in the charges of the amino acids surrounding enzyme activated sites. Similar results have been reported for immobilized Panus conchatus laccase enzyme [46].

3.2.3. Determination of optimal temperature

Investigation of the activity of free and immobilized enzymes at temperatures of 20, 30, 40, 45, 50, 55, 60

and 70 ^{o}C showed that the activity of the immobilized laccase enzyme has decreased at certain temperatures compared with the free enzyme. However, the optimal temperature of the immobilized form has less variation in the 40-70 ^{o}C range, making it more readily applicable in the industry. As observed, the optimal temperatures for the free and immobilized enzyme are 45 and 50 ^{o}C, respectively. Figure 5 shows that the decreased activity of the immobilized enzyme at higher temperatures is less than that of the free enzyme, which indicates the enhanced stability of the immobilized enzyme. The main reason for the reduced enzyme activity of the immobilized enzyme compared with the free enzyme seems to be the occupation of a number of enzyme activated sites and the limitation of the movement of the bonds inside the enzyme [48].

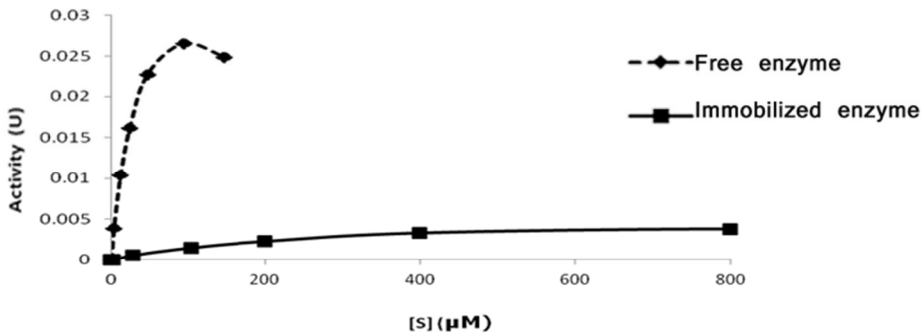


Figure 3: Michaelis–Menten plot for free and immobilized enzyme.

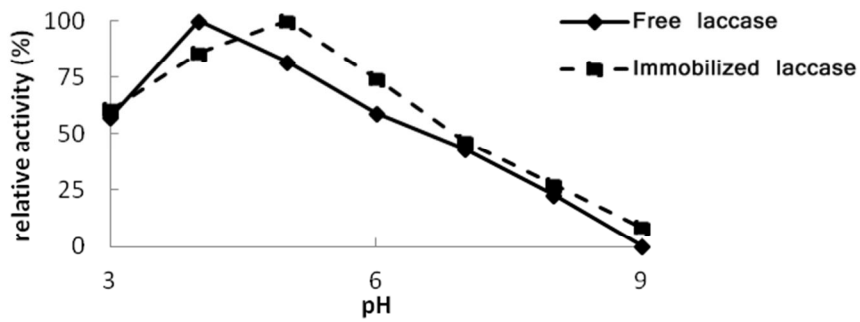


Figure 4: Determination of optimal pH values for free and immobilized laccase enzyme.

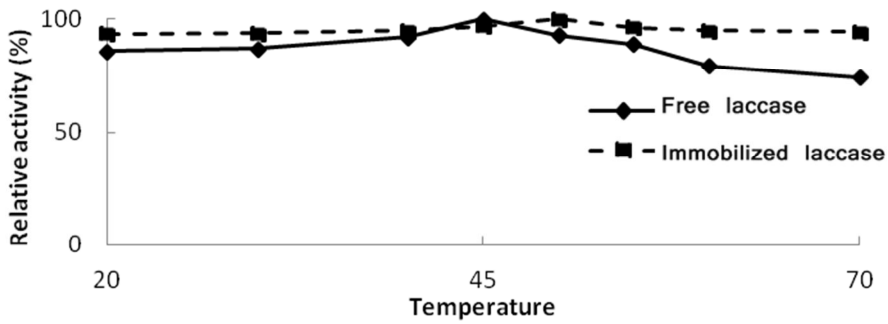


Figure 5: Optimal temperature for free and immobilized laccase enzyme.

3.2.4. Investigation of thermal stability of the free and immobilized enzymes

Thermal stability protects the enzyme against thermal denaturation as well as other types of denaturation. The data obtained in thermal stability experiments on free and immobilized enzyme following incubation at 60 ^{o}C showed (Figure 6) that the relative activity of the immobilized enzyme is more than that of free enzyme. So immobilized enzyme is more stable than free enzyme. This indicates the successful formation of covalent bonds and appropriate spatial arrangement of the laccase enzyme immobilized on the bed [49]. Similar results have been reported concerning the enhancement of enzyme strength and thermal stability due to covalent bond formation [49]. Some covalent bonds are formed during the enzyme immobilized process. The formation of these bonds has a great influence on limiting the conformational changes of the enzymes. According to the investigations carried out, stabilized enzymes are more resistant to environmental changes and less activated in comparison with free enzymes [50-51].

3.3. Investigation of the effect of different parameters on removal efficiency

Influence of key operating parameters such as contact time, pH of solution, stirring rate, adsorbent amount, and initial dye concentration was studied in a batch system. Also, effect of bed height and flow rate of the solution was studied in fixed bed system.

In order to study the effect of adsorbent contact time on the removal of reactive blue 19 dye by immobilized enzyme on adsorbent, at first, adsorption takes place slowly since there are always Van der Waals forces involved in adsorption. Furthermore, since the number of active sites for the adsorption of reactive blue 19 dye is constant, maximum adsorption is achieved after a short time and no considerable change is observed in the dye removal percent with time. This indicates that equilibrium has been established between the adsorbent and the dye [52]. According to the results, maximum removal efficiency was obtained as 80.70% after 12 min.

Also, pH has an important role in adsorption systems [53]. Because of the production of H^+ in acidic medium it can makes the surface charge more positive and increase the adsorption rate of the dye molecules on the surface. According to pH studies, optimum value of removal was obtained in acidic condition (pH=4.5).

Increasing the stirring rate increases the formation of Van der Waals forces and removal efficiency up to an optimum value and after it desorption is observed since the contact forces are stronger than van der Waals attractions. Adsorption process was investigated in 30, 60, 150 and 200 rpm and the optimum stirring rate was 60 rpm.

Increasing the adsorbent dosage provides increased accessibility of adsorption sites and thus increases the dye removal efficiency. The results studied with 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35 and 0.4 g of the adsorbent indicated that the minimum and maximum adsorptions of the dye immobilized on adsorbent are 48.62 and 80.70%, respectively, for adsorptions of 0.05 and 0.35 g.

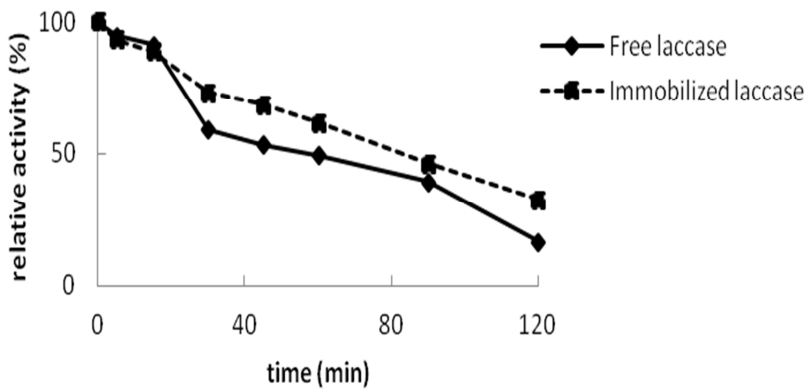


Figure 6: Thermal stability of the free and immobilized enzymes.

Effect of bed height dye removal efficiency by laccase enzyme immobilized on adsorbent was studied in 30, 70 and 100 cm of bed height. The results show that the dye removal efficiency is directly proportional to bed height in fixed bed systems. Higher bed height causes formation of more sites with the adsorbent and the dye spends more time for physical contact with the adsorbent, and the number of Van der Waals interactions increase, leading to increased removal efficiency and ultimately lower concentration of the output. In addition, increased height lowers the slope of the plot, broadening in the mass transfer area and increases the chance of linking between the adsorbate and adsorbent [54].

One of the effective parameters on the adsorption of the dye in fixed bed system is the optimization of dye solution flow rate. As observed in present research in different flow rate (10, 20 and 30 ml/min), at lower dye flow rate, the dye has more time to saturate the adsorbent, leading to the removal of more reactivated 19 blue dye [55]. As the flow rate increases, there is less effective balance between the adsorbent and the dye and the penetration of the dye into the adsorbent pores decreases [56].

3.4. Measurement of Total Organic Carbons (TOC)

One of the important indicators in the study of the qualitative properties of the effluent in the process of degradation of the dye by the laccase enzyme immobilized on adsorbent is the measurement of the total amount of organic carbons. As observed in Figure 7, degradation percentage of reactive blue 19 dye in 20 minutes follows an increasing trend, which indicates the oxidation of dyes by laccase enzyme immobilized on adsorbent and production of intermediates.

3.5. Identification of intermediate products

LC/MS analysis was carried out for this purpose in order to investigate the intermediate changes and reaction products of reactive blue 19 dye with laccase enzyme. The information in Figure 8, corresponding to the peaks before and after the decomposition process, shows that the abundance of some of the peaks has lowered and they have finally disappeared and some intermediates have thus been identified. Moreover, the color removal during the reaction indicates the degradation of chromophore groups. A route for decomposition of the dye by laccase enzyme has been suggested based on these observations (Figure 9).

Due to structure of anthraquinone dyes, they are considered one of the most efficient dyes. Due to the weakness of π-π bonds, these dyes are converted into wastes, which still have high molecular weights. As the process of decomposition continues, aromatic rings are converted into linear organic compounds and less environmentally harmful inorganic compounds eventually.

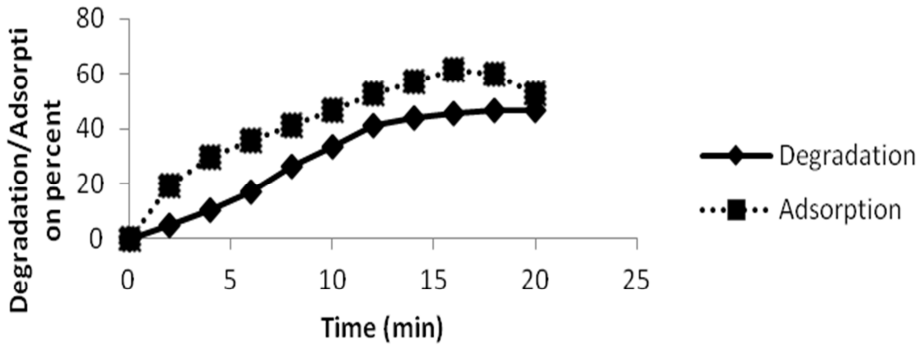


Figure 7: Degradation percentage and adsorption of reactive blue 19 dye by laccase enzyme immobilized on the adsorbent.

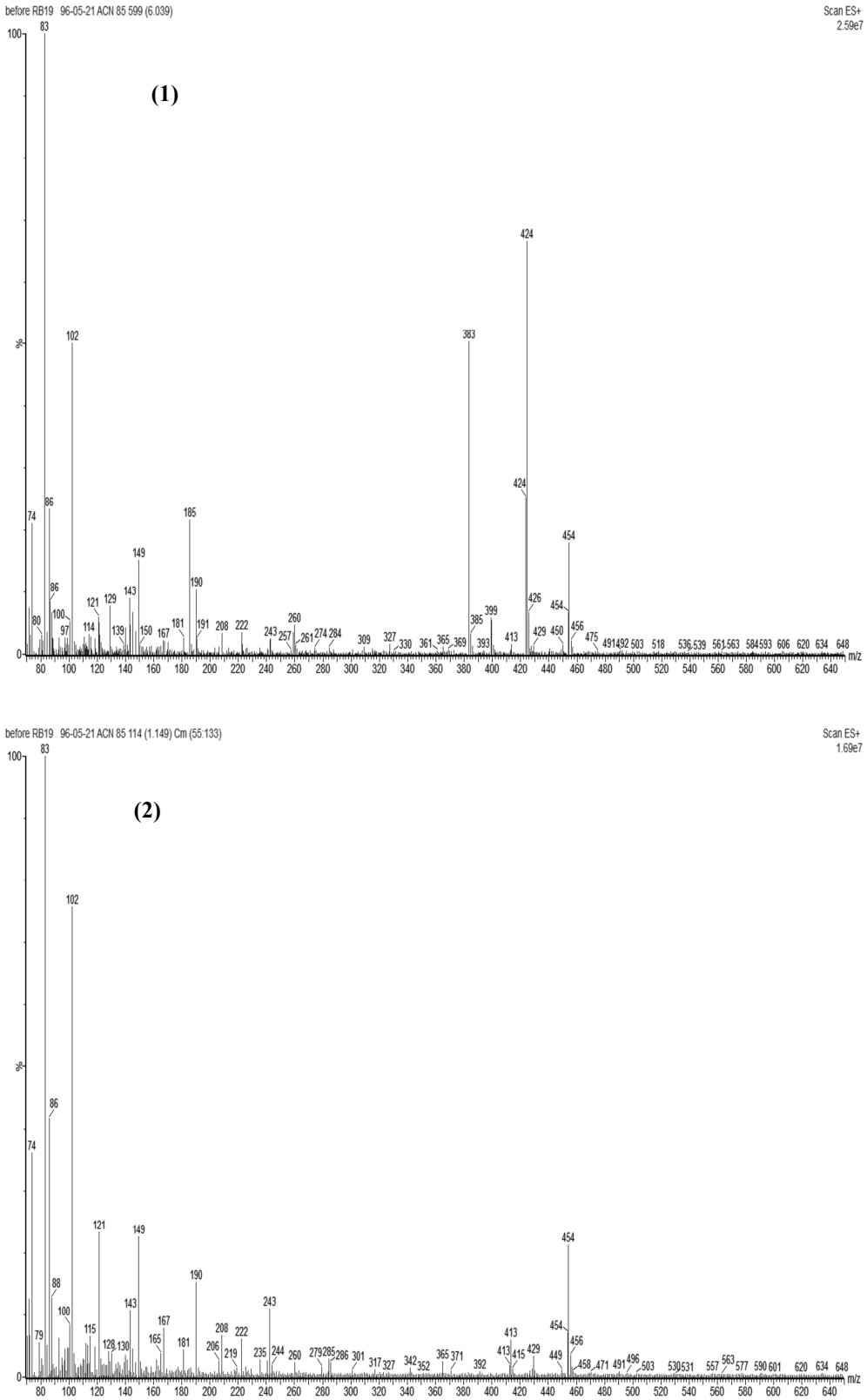


Figure 8: LC/MS graphs under optimal conditions during the degradation process by immobilized enzyme.

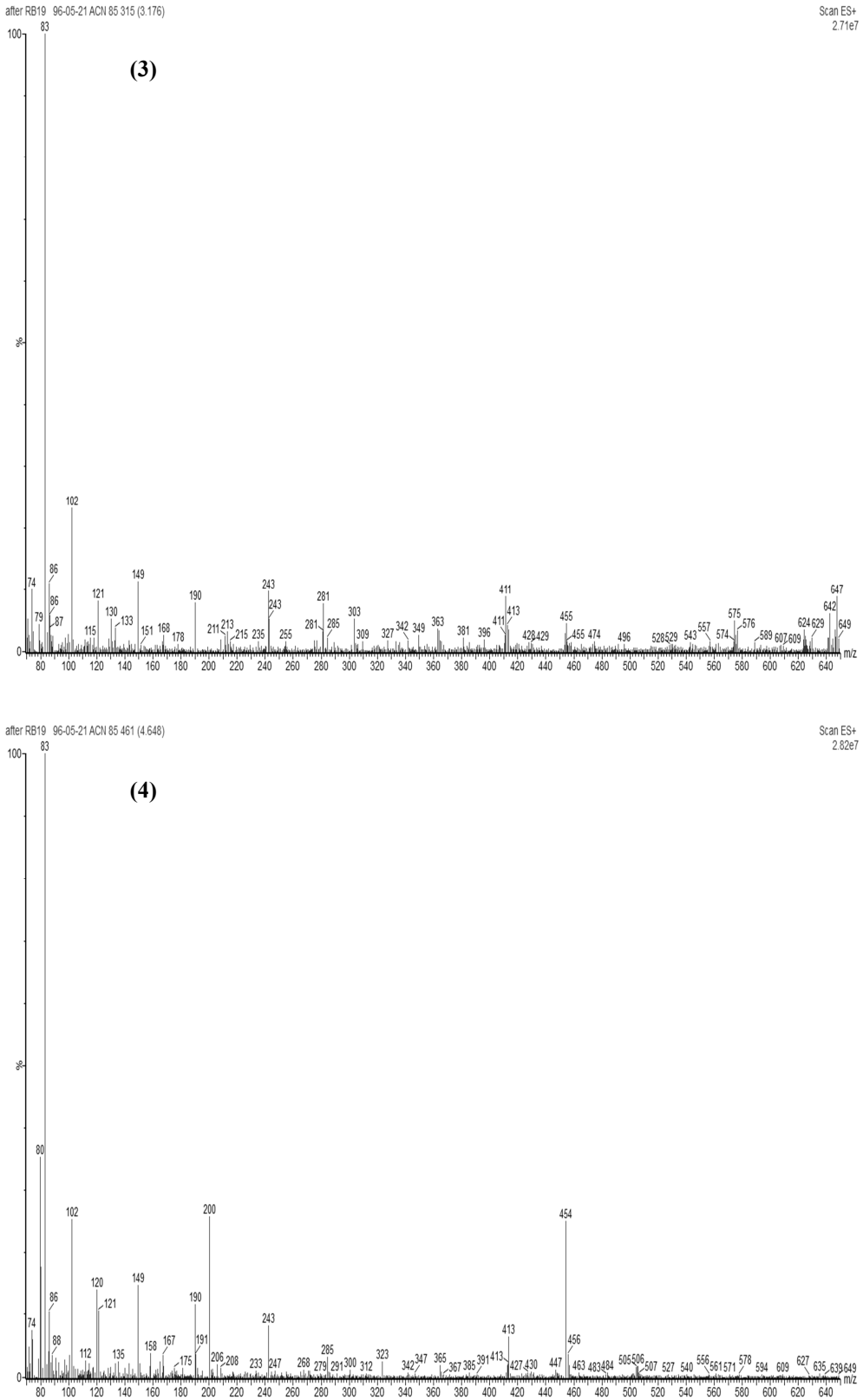


Figure 8: Continue.

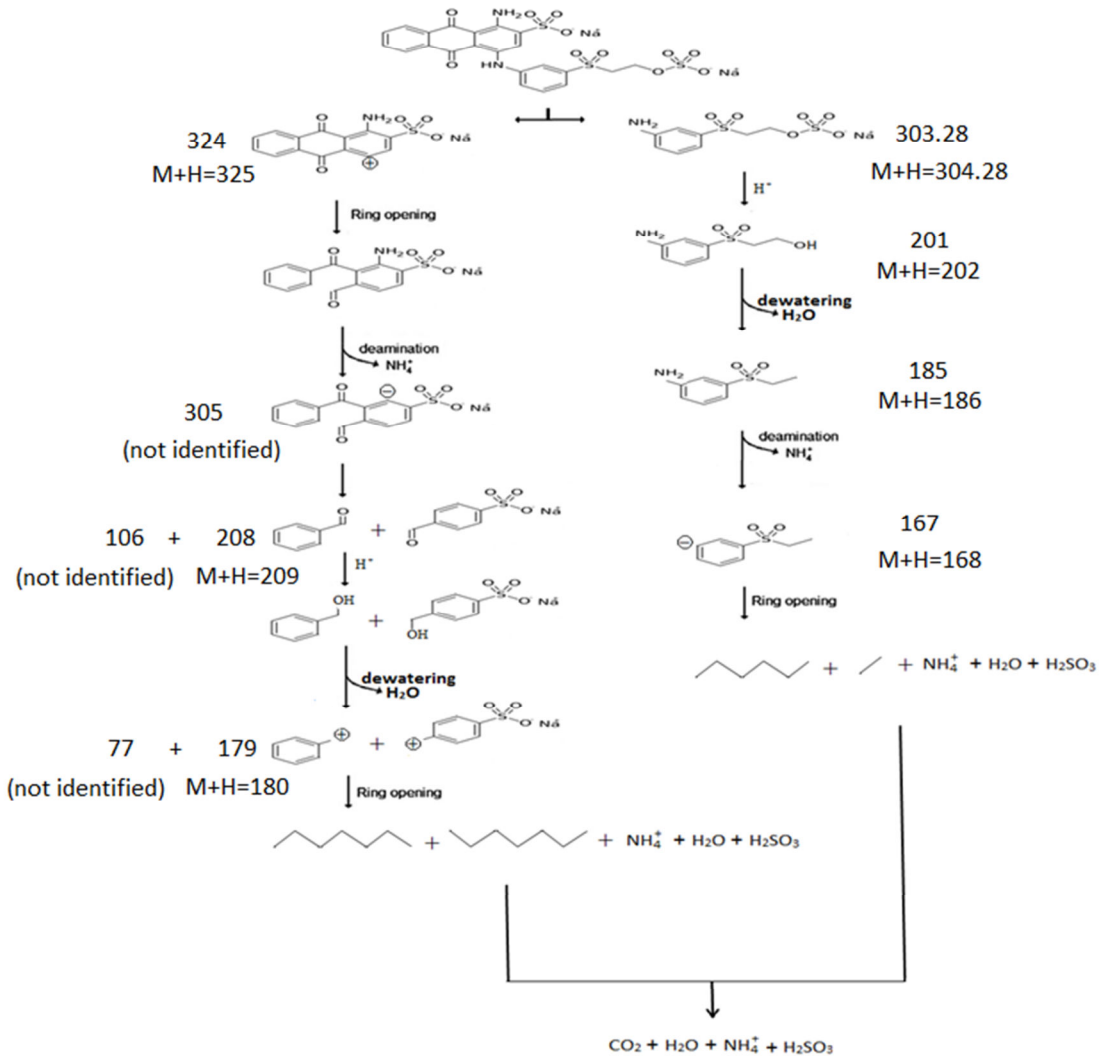


Figure 9: Proposed route for degradation of reactive blue 19 dye by laccase enzyme.

3.6. Investigation of surface isotherms

Adsorption isotherms can be used to determine the absorbance value as a function of the equilibrium concentration of the adsorbent. Isotherms study and the results shown in Table 5. It can be stated that the dominant isotherm in dye removal through adsorption by adsorbent follows Langmuir isotherm since this isotherm has the highest correlation coefficient (R^2=0.9383) in comparison with other isotherms.

Table 5: Langmuir, Temkin and Freundlich isotherm constants for dye removal by adsorbent.

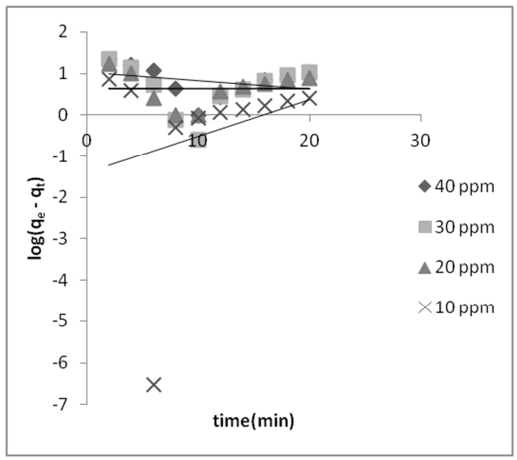
Adsorbents	Langmuir			Freundlich			Temkin		
	Q_0 (mg/g)	K_L (L/mg)	R_2	K_F (L/mg)	n	R_2	K_T (L/mg)	B_1	R_2
Immobilized laccase enzymes on the adsorbent	42.9389	0.1600	0.9389	136.8674	2.1404	0.7375	1.1598	12.152	0.7277

3.7. Investigation of adsorption kinetics

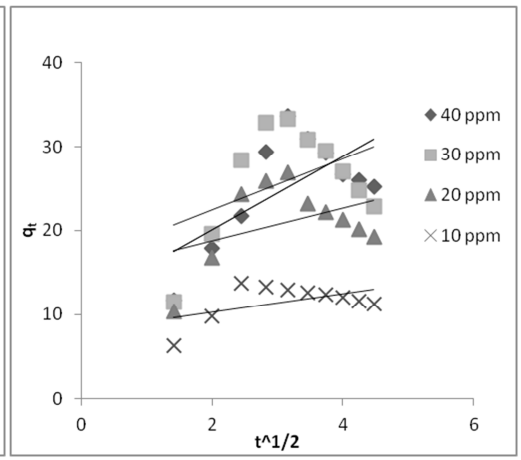
Table 6 shows the parameters obtained from pseudo first and second order kinetic models and intrinsic particle infiltration model using different dye concentration. Considering the results obtained and comparison of correlation coefficient (R^2) with those of other kinetic models show that the process of adsorption of the dye by laccase enzyme immobilized on adsorbent follows a pseudo second order kinetics. As observed in Figure 10, the slope and intercept of the plot of t/q variations vs. t gives K_2 and Cal values (q_e), respectively. The results showed that the highest correlation coefficient (R^2= 0.9935) was that of the pseudo second order kinetics.

Table 6: Kinetic coefficients for dye removal by laccase enzyme immobilized on adsorbent.

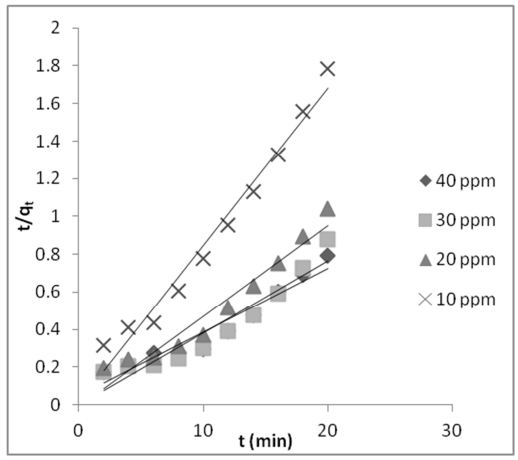
Adsorbents	dye concentration (ppm)	(q_e)Exp	Pseudo-first order			Pseudo-second order			Intraparticle diffusion		
			(q_e)Cal	K_1	R^2	(q_e)Cal	K_2	R^2	I	K_P	R^2
Immobilized enzymes on adsorbent	10	13.7279	24.9977	0.2033	0.0609	12.0048	0.4956	0.9754	8.1232	1.0848	0.2551
	20	26.9005	4.2884	0.0004	8E-06	20.79002	0.2461	0.9469	14.774	1.968	0.1658
	30	33.3403	4.4565	0.0029	0.0002	26.3157	0.4512	0.914	16.369	3.0481	0.2078
	40	33.6601	10.4496	0.0462	0.0965	29.4985	0.0250	0.9313	11.345	4.3728	0.4412



Pseudo-first order



Intraparticle diffusion



Pseudo-second order

Figure 10: Kinetic models for adsorption of reactive blue 19 dye by laccase enzyme immobilized on adsorbent.

Table 7: Thermodynamic parameters.

Adsorbents	Temperature (K)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (kJ/mol)	R^2
Immobilized laccase enzyme on the adsorbent	298	-3.54478	11.8379	0.05156	0.9993
	303	-3.8773			
	318	-4.2903			

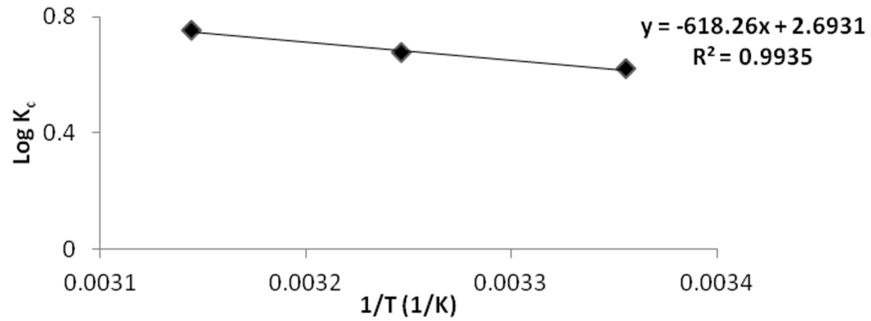


Figure 11: Thermodynamic effect of the dye adsorption by laccase enzyme immobilized on the adsorbent.

3.8. Investigation of adsorption thermodynamics

The study of thermodynamic parameters such as Gibbs free energy (ΔG^0), enthalpy (ΔH^0) and entropy changes (ΔS^0) gave comprehensive information on temperature effect on adoption. Therefore, the thermodynamic parameters were investigated at temperatures of 298, 308 and 318 K. As observed in the plot of $\ln K_c$ vs. 1/T, (Figure 10), increasing the temperature from 25 to 45 ^oC increases the free Gibbs energy from -3.62 to -5.88, which indicates the spontaneity of the adsorption process. In addition, the efficiency of the adsorption of reactive blue 19 dye by laccase enzyme immobilized on the adsorbent changed from 81.20 to 91.47, which indicates the increased interaction with the sites on the adsorbent surface [57]. The slope and intercept of Van't Hoff plot can easily give ΔS and ΔH values. As observed in Table 5, positive ΔH shows the endothermic nature of the adsorption process such that increasing the temperature increases the adsorption of reactive blue 19 dye. Positive ΔS indicates increased disorder in the confrontation of solid/solution phases during the adsorption process [42].

4. Conclusion

In the present research, laccase enzyme has been immobilized on the mixed powder and fiber activated carbon as an adsorbent for dye removal of colored solutions. The enzyme activity and influence of different key operating parameters were studied and the optimum condition of the process was obtained. Also, degradation of the organic pollutants and production of intermediate compounds was investigated by LC/MS method. According to the kinetic, thermodynamic models and determination of Gibbs free energy and enthalpy, the adsorption process was an exothermic process that follows a pseudo second order kinetic model. The results indicated an effective adsorption and degradation of the organic pollutants using the immobilized adsorbent used in the present study. So, this adsorbent is capable to adsorb the pollutions and the adsorbent surface could be treated by immobilized enzyme and it could be used as a self-cleaning filter for wastewater treatment processes.

Aknowlegment

Authors would like to show their gratitude to Dr. N.M. Mahmoodi for his help to purchase the chemical material.

5. References

1. M. Zaharia, A. Pătras, M. R. Gogonea, A. Tănăsescu, C. A. Popescu, Cluster design on the influence of energy taxation in shaping the new EU-28 economic paradigm. Energies, 10(2017), 257-264.
2. Z. K. Feng, W. J. Niu, J. Z. Zhou, C. T. Cheng, H. Qin, Z. Q. Jiang, Parallel multi-objective genetic algorithm for short-term economic environmental hydrothermal Scheduling. Energies, 10(2017), 163-171.

3. P. A. Soares; R. Souza, J. Soler, T. F. C. V. Silva, S. M. A. G. U. Souza, R. A. R. Boaventura, V. J. P. Vilar, Remediation of a synthetic textile wastewater from polyester-cotton dyeing combining biological and photochemical oxidation processes. *Sep. Purif. Technol.* 172(2017), 450–462.
4. A. I. El-Batal, N. M. ElKenawy, A. S. Yassin, M. A. Amin, Laccase production by Pleurotus ostreatus and its application in synthesis of gold nanoparticles. *Biotechnol. Rep*. 5(2015), 31–39.
5. P. Giardina, V. Faraco, C. Pezzella, A. Piscitelli, S. Vanhulle, and G. Sannia, Laccases: a never-ending story, *Cell Mol Life Sci,* 67(2010), 369–385.
6. L. Mendoza, M. Jonstrup, R. Hatti-Kaul, B. Mattiasson. Azo dye decolorization by a laccase/mediator system in a membrane reactor: Enzyme. Mediator reusability, 49 (2011), 478 – 484
7. M. B. Kaczmarek, N. Kwiatos, M.Szczęsna-Antczak, S. Bielecki, Laccases – enzymes with an unlimited potential, *Biotechnol Food Sci*, 81(2017), 41-70
8. S. Papic, N. Koprivanac, A.L. Bozic, A. Metes, Removal of some reactivated dyes from synthetic wastewater by combined Al(III) coagulation/carbon adsorption process, *Dyes Pigm*. 62 (2004), 291–208.
9. W. P. Cunningham, B. W. Siago, Environ. Sci. Global concern. McGraw Hill, New York. 2001, 267-269.
10. A. A. Peláez-Cid, A. M. Herrera-González, M. Salazar-Villanueva, A. Bautista-Hernández, Elimination of textile dyes using activated carbons prepared from vegetable residues and their characterization. *J. Environ. Manag*. 181(2016), 269–278.
11. K. D. Belaid, S. Kacha, M. Kameche, Z. Derriche, Adsorption kinetics of some textile dyes onto granular activated carbon. *J. Environ. Chem. Eng.* 1(2013), 496–503.
12. Fei Zheng, Bao-Kai Cui a, 1 , Xue-Jun Wu a , Ge Meng a , Hong-Xia Liu b , Jing Si Immobilization of laccase onto chitosan beads to enhance its capability to degrade synthetic dyes, *Int Biodeterior Biodegradation*, 110(2016), 69-78
13. C. Sarika, M. S. Shivakumar, L. Devil, K. Rekha, B. Narasimhamurthy, S. Thomas, N. Kalarikkal, I. C.. Lekshmi1, A comparative study on NiO nanocrystal modified graphite and Au electrode matrices as immobilization supports for laccase enzyme in amperometric biosensing for catechol detection, *Adv.Mater. Proc.*, 3(2018), 304-311.
14. C. Zhang, L. Gong, Q. Mao, P. Han, X. Lua, J. Qub, Laccase immobilization and surface modification of activated carbon fibers by bio-inspired polydopamine, RSC Adv. 8(2018), 14414–14421
15. Gh. Ghorbani , M. Ebrahimi , N. Farhadyar ,The immobilization of laccase enzyme from Trametes versicolor on the surface of porous zinc oxide nanoparticles and studying features of the immobilized enzyme, *Int. J. Bio-Inorg. Hybr. Nanomater.* 7(2018), 21-28.
16. S. Rubenwolf, O. Strohmeier, A. Kloke, S. Kerzenmacher, R. Zengerle, F. Vonstetten, Carbon electrodes for direct electron transfer type laccase cathodes investigated by current density cathode potential behavior, *Biosens. Bioelectron.* 26(2010), 841-845.
17. L. F. Bautista, G. Morales, R. Sanz, Immobilized strategies for laccase from Trametes versicolor on meso structured silica materials and the application to the degradation of naphthalene. *Bioresour. Technol*. 101(2010), 8541-8548.
18. G. Bayramoglu, M. Yilmaz, M. Y. Arica, Reversible immobilized of laccase to poly (4-vinyl pyridine) grafted and Cu (II) chelated magnetic beads, Biodegradation of reactivated dyes, *Bioresour. Technol.* 101(2010), 6615-6621.
19. J. M. Ortiz, R. Flores, R. V. Duhalt, Molecular design of laccase cathode for direct electron transfer in a biofuel cell. *Biosens. Bioelectron*. 26(2011), 2626-2631.
20. T. Beneyton, A. El. Harrak, A. D. Griffiths, P. Hellwig, V. Taly, Immobilized of CotA, an extremophilic laccase from Bacillus subtilis, on glassy carbon electrodes for biofuel cell applications. *Electrochem. Commun*. 13(2011), 24-27.
21. H. Cabana, A. Ahamed, R. Leduc, Conjugation of laccase from the white rot fungus Trametes versicolor to chitosan and its utilization for the elimination of triclosan. *Bioresour. Technol*. 102(2011), 1656-1662.
22. S. Georgieva, T. Godjevargova, D. G. Mita, N. Diano, C. Menale, C. Nicolucci, Non isothermal bio-remediation of waters polluted by phenol and some of its derivatives by laccase covalently immobilized on polypropylene membranes. *J. Mol. Catal. B*. 66(2010), 210-218.
23. Y. Zhang, D. Rochefort, Comparison of emulsion and vibration nozzle methods for micro encapsulation of laccase and glucose oxidase by interfacial reticulation of polyethylene imine, *J. Microencapsulation*. 27(2010), 703-713.
24. A. Sadighi, M. A. Faramarzi, Congo red decolorization by immobilized laccase through chitosan nanoparticles on the glass beads, *J Taiwan Inst Chem E*. 44(2013), 156-162.
25. M. Ardhaoui, S. Bhatt, M. Zheng, D. Dowling, C. Jolivalt, F. A. Khonsari, Biosensor based on laccase immobilized on plasma polymerized allylamine/carbon electrode. *Mater. Sci. Eng. C*. 33(2013), 3197-3205.
26. J. Zhang, J. Zhang, F. Zhang, H. Yang, X. Huang, H. Liu, S. Guo, Graphene oxideas a matrix for enzyme immobilized, *Langmuir*. 26(2010), 6083–6085.
27. S. Stankovich, D. A. Dikin, R. D. Piner, K. A. Kohlhaas, A. Kleinhammes, Y. Jia, Y.Wu, S. T. Nguyen, R. S. Ruoff, Synthesis of graphene-based nanosheets viachemical reduction of exfoliated graphite oxide, Carbon N. Y. *J. Carbon*, 45(2007), 1558–1565,
28. D. Li, M.B. Müller, S. Gilje, R.B. Kaner, G. G. Wallace, Processable aqueousdispersions of graphene nanosheets, *Nat. Nanotechnol*. 3 (2008), 101–105
29. W. Tischer, Wedekind F., Immobilized enzymes: medthods and bio-catalysis from discovery to applications. *Top Che,* 200(1999), 95-125.

30. Lineweaver, H., Burk, D., 1934. The Determination of Enzyme Dissociation Constants. J. *Am. Chem. Soc*. 56, 658–666
31. P. Ander, K. Messner, *Biotechnol. Tech*. 12 (1998) 191–195.
32. J. F. Osma, J. L. Toca-Herrera, S. Rodrı´guez-Couto, Biodegradation of a simulated textile effluent by immobilisedcoated laccase in laboratory-scale reactors. *Appl. Catal. A. Gen*. 373(2010), 147–153
33. B. Karagoz, G. Bayramoglu, B. Altintas, N. Bicak, Y. M. Arica, *Bioresour. Technol*. 102(2011), 6783–6790
34. H. Lineweaver, D. Burk, The determination of enzyme dissociation constants. *J. Am. Chem. Soc*. 56(1934), 658–666.
35. D. Yinghui, W. Qiuling, F. Shiyu, Laccase stabilization by covalent binding immobilized on activated polyvinyl alcohol carrier. *Lett. Appl. Microbiol.* 35(2002), 451–456.
36. X. Song, C. Wang, D. Zhang, Surface structure and adsorption properties of ultrafine porous carbon fibers. *Appl. Surf. Sci*. 255(2009), 4159-4163.
37. S. Netpradit, P. Thiravetyan, S. Towprayoon, Application of 'waste'metal hydroxide sludge for adsorption of azo reactivated dyes, *Water Res*. 37(2003), 763-772.
38. M. Asif Tahir, Haq Nawaz Bhatti and Munawar Iqbal, Solar Red and Brittle Blue direct dyes adsorption onto Eucalyptus angophoroides bark: Equilibrium, kinetics and thermodynamic studies, *JECE*. 4 (2016) 2431-2439
39. N. Bakhtiari, S. Azizian, Adsorption of copper ion from aqueous solution by nanoporous MOF-5: A kinetic and equilibrium study. *J. Mol. Liq*. 206(2015), 114-118.
40. R. I. Yousef, B. El-Eswed, H. Ala'a, Adsorption characteristics of natural zeolites as solid adsorbents for phenol removal from aqueous solutions: kinetics, mechanism, and thermodynamics studies, *Chem. Eng. J.* 171 (2011), 1143–1149.
41. M. Greluk, Z. Hubicki, Kinetics, isotherm and thermodynamic studies of Reactivated Black 5 removal by acid acrylic resins. *Chem. Eng. J*. 162(2010), 919-926.
42. L. Wang, Application of activated carbon derived from 'waste'bamboo culms for the adsorption of azo disperse dye: Kinetic, equilibrium and thermodynamic studies. *J. Environ. Manage*.102(2012), 79-87.
43. M. E. Olya, M. Vafaee, M. Jahangiri, Modeling of acid dye decolorization by TiO_2–Ag_2O nano-photocatalytic process using response surface methodology, *J. Saudi Chem. Soc*. 21(2017), 633-642.
44. I. Ardao, G. Alvaro, M. D. Benaiges, *Biochem. Eng. J.* 56 (2011), 190–197.
45. A. Leonowicz, J. M. Sarkar, J. M. Bollag, Improvement in stability of an immobilized fungal laccase. *Appl. Microbiol. Biotechnol*. 299(1988), 129–135.
46. D. Yinghui, W. Qiuling, F. Shiyu, Laccase stabilization by covalent binding immobilized on activated polyvinyl alcohol carrier. *Lett. Appl. Microbiol.* 35(2002), 451–456.
47. M. Asgher, S. Kamal, H. M. Nasir Iqbal, Improvement of catalytic efficiency., thermo-stability and dye decolorization capability of pleurotus ostreatus IBL-02 laccase by hydrophobic sol gel entrapment. *Chem. Cent. J.* 6(2012), 110.
48. H. Sun, H. Yang, W. Huang, S. Zhang, Immobilized of laccase in a sponge-likehydrogel for enhanced durability in enzymatic degradation of dye pollutants. *J. Colloid Interf. Sci*. 450(2015), 353–360.
49. A. P. Tavares, C. G. Silva, G. Dražić, A. M. Silva, J. M. Loureiro, J. L. Faria, Laccase immobilized over multi-walled carbon nanotubes, Kinetic., thermodynamic and stability studies. *J. Colloid Interf. Sci.* 15 (2015), 52–60.
50. N. Sri Kumaran, G. Dharani: Decolorization of textile dyes by white rot fungi phanero cheate chrysos porium and pleurotus sajor-caju. *JATES*, 1(2011), 361-370.
51. Q. Husain: Potential applications of the oxidoreductive enzymes in the decolorization and detoxification of textile and other synthetic dyes from polluted water, *Crit. Rev. Biotechnol*, 4(2006), 201-221.
52. A. Ahmad, M. Rafatullah, M. H. Ibrahim, R. Hashim Scavenging behavior of meranti sawdust in removal of Methylene Blue from aqueous solution, *J. Hazard. Mater*. 170(2009), 357
53. M. Wawrzkiewicz, Z. HubickiRemoval of Tartrazine from aqueous solutions by strongly basic polystyrene anion exchange resins, *J. Hazard. Mater*. 164(2009), 502
54. Z. Zulfadhly, M. D. Mashitah, S. Bhatia, Heavy metals removal in fixed-bed column by the macro fungus Pycnoporus sanguineus, *Environ. Pollut*. 112(2001), 463–470.
55. W. H. A. Al-Taliby, Evaluation of Methylene Blue Removal from Wastewater by Adsorption onto DifferentTypes of Adsorbent Beds. M.Sc. Thesis, University of Babylon, College of Engineering, Dept. of Civil Engineering, 2009.
56. A. A. Hassan, Removal of Blue Dye from Wastewater by Adsorption on Activated Carbon. M.Sc. Thesis, University of Babylon, College of Engineering, Dept. of Civil Engineering, 2005.
57. K. Chinoune, K. Bentaleb, Z. Bouberka, A. Nadim and U. Maschke, *Appl. Clay Sci*. 123, 64 (2016).

How to cite this article:
M. S. Khazravi, M. Bahmaei, M. E. Olya, S. M. Etezad, Application of a New Self-Cleaning Filter for Colored Wastewaters Treatment Using Laccase Enzyme Immobilized on Activated CARBON powder and fiber. Prog. Color Colorants Coat., 12 (2019), 39-56.