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Removal of textile dye Reactive Black 5 from aqueous solution by adsorption on laccase-modified silica fume

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ABSTRACT

Textile wastewater with the dye is released in various ways into the environment and it causes significant environmental problems. Due to the stability of modern dyes, conventional biological treatment methods for industrial wastewater are ineffective, resulting in an intensively colored discharge from the treatment facilities. Thus, there is a need for developing more effective treatment methods to eliminate dyes from the waste stream at its source. The most effective methods of dye removal from wastewater are adsorption and the use of enzymes. In this study, the removal of Reactive Black 5 (RB5) from aqueous solutions has been studied using silica fume (SF) waste material after its modification with laccase from Russulaceae (*Lactarius volemus).* Laccase was purified by using saturated precipitate $(NH_4)_2SO_4$, DEAE-cellulose and immobilized on SF. Batch adsorption experiments have been performed as a function of pH, contact time, temperature, and adsorbent dosage. The optimum results were obtained at pH 9, contact time of 60 min, temperature of 30°C, and an adsorbent dosage of 1 mg/mL. The isotherm studies showed that the adsorption experimental data were fitted well by Langmuir isotherm model. The adsorption capacity was found to be 322.58 mg/g. Thermodynamic parameters including the Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS) changes indicated that the adsorption of RB5 onto laccase-modified SF was feasible, spontaneous, and endothermic. ΔH and ΔS were 1,185 kJ/mol and 4.104 J/mol K, respectively. These parameters indicated that the adsorption of RB5 onto laccase-modified SF was feasible, spontaneous, and endothermic. The results show that the laccase-modified SF can be used for the treatment of aqueous solutions as an alternative low-cost adsorbent.

Keywords: Wastewater; Laccase-modified silica fume; Reactive Black 5 removal; Adsorption studies

1. Introduction

Textile industry is rated as one of the most polluting sector among the different human activities due to their high discharge volume and effluent composition. This industry consumes considerable amounts of water during dyeing and finishing operations. Dye-containing effluent can be toxic to the environment since dyes are stable compounds that are not easily biodegradable and they are liable to be

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carcinogenic. The dyes can cause environmental damage to living organisms. The natural growth activity of aquatic life is disturbed due to stop the reoxygenation capacity of water and blocked sunlight by textile dye–polluted wastewater [1–5].

The dyes are classified as acidic, basic, azo, diazo, disperse, metal complex, and antraquinone-based dyes according to their structural varieties and generally considered as a primary contributor for the environmental pollution due to their wide use in many areas especially in textile industry [6,7]. With the increasing production of these dyes, the discharge of dye effluents at high concentration and strong color has caused serious environmental pollution because many of these dyes are toxic and recalcitrant to biodegradation. Due to the chemically and light stable characteristics of azo group, it is not easy to treat such wastewater by biological oxidation. Several physical and chemical methods have been used for the treatment of industrial textile wastewater including microbial biodegradation, membrane filtration, oxidation, and ozonation [8–11]. However, many of these technologies are cost prohibitive, especially when applied for treating large waste streams. Consequently, adsorption techniques seem to have the most potential for future use in industrial wastewater treatment [12] because of their proven efficiency in the removal of organic and mineral pollutants and for economic considerations [13–15]. Activated carbon is the most widely used adsorbent for this purpose because it has a high capacity for the adsorption of color, but its use is limited because of high cost. The removals of dyes from effluent using adsorption process provide an alternative treatment, especially if the adsorbent is inexpensive and readily available [16–18]. An adsorbent can be considered as cheap or low cost if it is abundant in nature, requires little processing and is a byproduct of waste material from waste [19]. Many low-cost adsorbents have been investigated on dye removal from wastewater such as fly ash, red mud, bottom ash, clay, zeolite, calcine alunite, peanut hull, rice husk, and brown seaweed [20–27].

As an alternative low-cost absorbent material, solid wastes are generally used as adsorbent for the remediation of wastewater. One of by-product materials is silica fume (SF). In this work, we attempt to utilize laccase-modified SF, a by-product of the reduction of high-purity quartz with coal in electric furnaces in the production of silicon and ferrosilicon alloys, as an alternative low-cost adsorbent in the removal of Reactive Black 5 (RB5) dye from aqueous solutions. It is a very fine dust of silica from a blast furnace generated during silicon metal production and it has historically been considered a waste product. Although the SF is a waste material of industrial applications, it has become the most valuable by-product among the pozzolanic materials due to its very active and high pozzolanic property [28]. Currently, it is widely used in concrete as an admixture [29] and geotechnical applications to improve the compacted clayey soils [30,31].

Laccase is an enzyme that has potential ability of oxidation. It belongs to those enzymes, which have innate properties of reactive radical production. There are diverse sources of laccase producing organisms like bacteria, fungi, and plants. Laccases use oxygen and produce water as by-product. They can degrade a range of compounds including phenolic and nonphenolic compounds. They also have ability to detoxify a range of environmental pollutants [32]. Laccases are produced by the majority of white-rot fungi described todate as well as by other types of fungi and plants, insects, and some bacteria. Fungal laccases are believed to be involved in the degradation of lignin, the removal of potentially toxic phenols arising during lignin degradation, the fruit body development, pigment production, and antimicrobial activity [33,34].

This paper describes the use of laccase-modified SF for the removal of RB5 dye from aqueous solutions. The adsorption of RB5 dye has been investigated as a function of contact time, pH, temperature, and adsorbent dose. The RB5 dye has been absorbed by laccase-modified SF from RB5 dye–polluted wastewater. Adsorption isotherm, kinetic, and thermodynamic studies have been performed to describe the adsorption process.

2. Materials and methods

2.1. RB5 textile dye

The wastewater including RB5 textile dye was purchased from Erzurum Industrial Area (Turkey) and used the experimental study. RB5 dye is dissociated in anionic sulfonate in aqueous solution with the molecular structure. It has two sulfonate groups and two sulfatoethylsulfon groups that have negative charges in an aqueous solution [35,36]. The chemical structure and the general characteristics of RB5 are summarized in Fig. 1 and Table 1, respectively.

2.2. SF waste material

The SF, also known as microsilica, is a by-product of the reduction of high-purity quartz with coal in electric furnaces in the production of silicon and ferrosilicon alloys. It is also collected as a by-product in the production of other silicon alloys such as

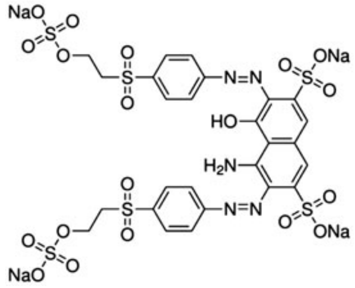

Fig. 1. Chemical structure of RB5 dye.

Table 1
General characteristics of RB5 dye

Chemical formula	Molar mass	Color index number	λ_{max}
$C_{26}H_{21}N_5Na_4O_{19}S_6$	991.82 g/mol	20,505	597 nm

Table 2
Physical property and chemical constituent SF

Parameters	Value
Property	
Density (mg/m^3)	2–2.5
Specific surface area (m^2/g)	3.05
Constituents	
Al_2O_3 (%)	1–3
Fe_2O_3 (%)	0.5–1
CaO (%)	0.8–1.2
MgO (%)	1–2
Ignition (%)	8.44

ferrochromium, ferromanganese, ferromagnesium, and calcium silicon. The SF has been obtained from Ferro–Chromate Factory in Antalya [29,31]. Its physical and chemical properties are summarized in Table 2.

2.3. Purification of laccase enzyme

Lactarius volemus (20 g) were ground in liquid N_2 and then homogenized in a blender with 50 mL of 1 M KCl by shaking and centrifuged at 5.000 rpm for 60 min. The homogenates were centrifuged and precipitates were removed. For the purification of the laccase enzyme, the following procedure was implemented [37]. Laccase was purified from the supernatant in two steps. Firstly, it was partially purified by precipitation in $(NH_4)_2SO_4$. Secondly, ion-exchange chromatography on diethylamino ethyl cellulose (DEAE)-sephadex was used.

The collapse of $(NH_4)_2SO_4$ was done from 0 to 90% in supernatant with the internals of 0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–70, 70–80, and 80–90. Significant activity was not observed below at a range 0–40% $(NH_4)_2SO_4$. The majority of activity was found in the 40–60% precipitate. Solid $(NH_4)_2SO_4$ was added to the supernatant to increase the concentration of $(NH_4)_2SO_4$ from 40% the fraction to 60%. After mixing in an ice-bath for 1 h with magnetic stirring, it was centrifuged (10.000 rpm, 30 min, and 4°C). The supernatant was discarded, and the precipitate was dissolved in 0.01 M acetate buffer (pH 5.0) and dialyzed against the same buffer [37].

2.4. Anion-exchange chromatography

The dialyzed suspension after ammonium sulfate precipitation from the step above was subjected to anion-exchange chromatography on DEAE-sephadex fast flow column (2.5 × 30 cm) pre-equilibrated with 0.02 M acetate buffer pH 5.0. The column was washed thoroughly with the same buffer until no protein was detected in the eluate. The bound proteins were eluted with the same buffer using a linear gradient of NaCl from 0 to 2 M. Fractions of 3 mL volume were collected at a flow rate of 3 mL/min. Protein elution was monitored spectrophotometrically by measuring the absorbance at 280 nm. Activity was measured by using ABTS as the assay substrate. The active fractions from each peak were pooled and stored at 4°C.

Protein concentration: Protein concentration was determined spectrophotometrically (absorbance at 280 nm) as well as by Bradford's method [38], using bovine serum albumin (BSA) as the standard.

Determination of laccase enzyme activity: The reagent 2,2´azino-di-[3-ethyl-benzothiazolin-sulfonate] (ABTS) was used as a substrate for spectrophotometric determination of laccase activity [39,40]. One activity unit (U) was the amount of enzyme that oxidized 1 μmol of ABTS min^{-1} and the activities were expressed in U l^{-1}.

2.5. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was performed after the purification of the enzyme. It was carried out in 3 and 10% acrylamide concentrations for the stacking and running gels, respectively, each of them containing 0.1% SDS [41]. The sample (20 μg) was applied to the electrophoresis medium. Brome tymol blue was used as tracking dye. Gels were stained in 0.1% Coomassie Brilliant Blue R-250 in 50% methanol, 10% acetic acid, and 40% distilled water for

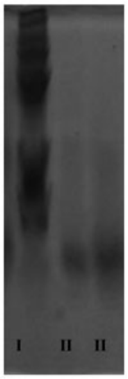

Fig. 2. SDS-PAGE electrophoretic pattern of laccase [homogenate (I); standard protein (BSA, 66 kDa; egg ovalbumin, 45 kDa; pepsin, 34 kDa; trypsinogen, 29 kDa; carbonic anhydrase) (II); purified laccase enzyme from Russulaceae (*L. volemus*) (III)].

1.5 h. It was destained by washing with 50% methanol, 10% acetic acid, and 40% distilled water several times. The electrophoretic pattern was photographed (Fig. 2).

2.6. Molecular weight determination by gel filtration

A column (3 × 70 cm) of Sephadex G100 was prepared. The column was equilibrated with the buffer (0.05 M Na_2HPO_4, 1 mM dithioerythretol, pH 7) until the absorbance was zero at 280 nm. The standard protein solution (bovine serum albumin, 66 kDa; egg ovalbumin, 45 kDa; pepsin, 34 kDa; trypsinogen, 24 kDa; β-lactoglobulin and lysozyme, 14 kDa) was added to the column. The purified laccase enzyme was added into the column separately and then eluted under the same conditions. The flow rate through the column was 20 mL/h. The elution volume was compared with standard proteins [42].

2.7. Preparation of absorbent for experimental study

The SF was thoroughly washed with distilled water until it became neutral. The suspension was wet sieved through a 200-mesh screen. A little amount of the suspension remained on the sieve and was discarded. The solid fraction was washed five times with distilled water following the sequence of mixing, settling, and decanting. The last suspension was filtered, and the residual solid was then dried at 105˚C, ground in a mortar, and sieved through a 200-mesh sieve. Laccase from Russulaceae *(L. volemus)* was purified by using precipitate of saturation $(NH_4)_2SO_4$, DEAE-cellulose and immobilized on SF. One gram of SF sample was shaken with 10 mL (5 mg protein/mL) laccase from *L. volemus* solution for approximately 1 h, and then, the separated particles were stored. The laccase modified-SF was used for the study of RB5 dye removal from aqueous solution.

2.8. Material characterization

The pH values were determined with a pH meter (Thermo scientific Orion 5 star plus multifunction). The scanning electron microscope (SEM) was used to examine the surface of the adsorbent. Images of native adsorbent and metal loaded adsorbent were magnified 5,000 times by SEM-modeled JEOL JSM-6400 SEM. Before SEM examinations, the sample surfaces were coated with a thin layer (20 nm) of gold to obtain a conductive surface and to avoid electrostatic charging during examination. The same machine was also used for the energy dispersive X-ray (EDX) spectra analysis to determine the elemental composition of the SF. In addition, the Fourier transform infrared spectroscopy (FTIR) analyses were carried out to identify functional groups and molecular structure in the bacteria-modified SF and RB5 dye-loaded laccase-modified SF. FTIR spectra were recorded on the on Perkin-a Perkin-Elmer GX2000 FTIR spectrometer. The spectrum of the adsorbent was measured within the range of 4,000–700 cm^{-1} wave number.

2.9. Adsorption procedure

All experiments were performed at laboratory scale. Synthetic wastewater was prepared by dissolving RB5 dye (Fig. 1). A 10 mL sample solution was pipetted into a 100-mL Erlenmeyer flask, and 5 mL 0.001 M dithizone was added as a complexion agent. The pH was adjusted to 7 (optimum value) with 0.001 M NaOH or 0.001 M HCl. The aqueous phase was diluted to 40 mL. The amount of 0.15 g naphthalene was added as a solid solvent. Then, this solution was heated in a water bath to about 85ºC and stirred (800 rpm) for 2 min to dissolve the complex in the molten naphthalene and allowed to stand for 15 min at room temperature to form microcrystalline naphthalene containing RB5 dye complex. After filtration with fritted glass, this microcrystalline naphthalene was washed with deionized water and dissolved in a 2-mL volumetric flask with acetone. The absorbance of the solutions was measured at 228.8 nm with graphite furnace atomic absorption spectrometer. A calibration curve was prepared in the range 0–40 ngmL^{-1} of RB5 dye according to the general procedure. The amounts of the dyes adsorbed onto compost (q_e in mg/g) and the percentages of the dyes removed from the solution (*R* in %) were calculated from the equations:

$$q_e = \frac{(C_o - C_e) \times V}{m} \tag{1}$$

$$R = \frac{(C_o - C_e) \times 100}{C_o} \tag{2}$$

where C_o and C_e are the initial and equilibrium concentrations of copper in solution (mg/L); V is the volume of solution (L) and m is the mass of adsorbent (g).

3. Results and discussion

3.1. Purification and characterization of laccase enzyme from Lactarius volemus

A new laccase from the *L. volemus* was purified and characterized by precipitating in $(NH_4)_2SO_4$ followed by anion-exchange and gel filtration chromatograph. ABTS was used as a substrate in the determination of activity in the protein eluted from the DEAE-sephadex column.

The results pertaining to purification of laccase using all purification techniques are summarized in Table 3. The final purification of 88.1-fold suggested that the laccase is highly abundant in the *L. volemus.*

The purified laccase was examined by SDS electrophoresis. SDS-PAGE revealed a single protein band at 20 kDa (Fig. 2). The molecular weight of the enzyme was determined as 60 kDa by using the gel filtration chromatograph and comparing with known standard proteins. This result shown that purified laccase enzyme has three subunits.

3.2. Characterization study

3.2.1. FTIR

The FTIR can provide very useful information about functional group. The technique can be used to analyze organic materials and some of inorganic materials. The FTIR technique is to measure the absorption of various infrared radiations by the target material, to an IR spectrum that can be used to identify functional groups and molecular structure in the sample. The functional groups and surface properties of the adsorbent before and after adsorption by FTIR spectra were illustrated in Fig. 3(a) and (b), respectively.

Characteristic SF peaks were observed at 477, 618, 804, 1,109, 1,645, and 3,442 cm^{-1} wave numbers. Asymmetric vibrations of Si–O bonds in the SF were seen at the 477, 618, and 804 cm^{-1} wave lengths. Si–O–Si bonds were observed in the form of asymmetric tension vibrations as a large peak at around 1,109 cm^{-1} wave number. Absorbed water from SF in O–H–O molecules at 1,645 cm^{-1} wave number is in the form of bending vibrations (Table 4). Water bonded to the structure with hydrogen bridges was detected at the peaks in 3,442 cm^{-1} region. It is shown that there is no significant change in the functional groups of SF after adsorption of RB5 dye on the SF when RB5 dye were treated with SF. It was concluded that RB5 dye did not damage to functional groups on the adsorbent [43,44].

3.2.2. SEM study

SEM has been a primary tool for characterizing the surface morphology and fundamental physical properties of the adsorbent surface. It is useful for determining the particle shape, porosity, and appropriate size distribution of the adsorbent [45,46]. SEM of SF and RB5 dye adsorbed by the laccase modified-SF are shown in Fig. 4. It is clear that, SF has considerable numbers of pores where, there is a good possibility for dyes to be trapped and adsorbed into these pores. Based on analysis of the images taken by SEM before and after the dye adsorption process, highly heterogeneous pores within SF particles were observed. After RB5 dye adsorption, the pores were packed with dyes.

3.2.3. EDX study

The EDX measurements were recorded for qualitative analysis of the element constitution of the adsorbents and the EDX spectra of native adsorbent

Table 3
The purification process of laccase from Russulaceae (*L. volemus*)

Enzyme fraction	Volume	Activity	Total activity		Protein	Specific Activity	Purification
	(mL)	(U/mL)	U	%	(mg/ml)	(U/mg)	fold
Crude extract	100	135.2 ± 3.1	1.35×10^4	100	196.5 ± 1.3	0.69	–
$(NH_4)_2SO_4$	80	82.3 ± 2.2	6.5×10^3	60.3	44.5 ± 2.5	1.85	2.7
DEAE-sephadex	50	75.3 ± 1.5	3.77×10^3	30.0	4.83 ± 2.3	15.59	22.6
Sephacryl S 200	30	68.7 ± 1.6	1.87×10^3	15.5	1.13 ± 1.1	60.80	88.1

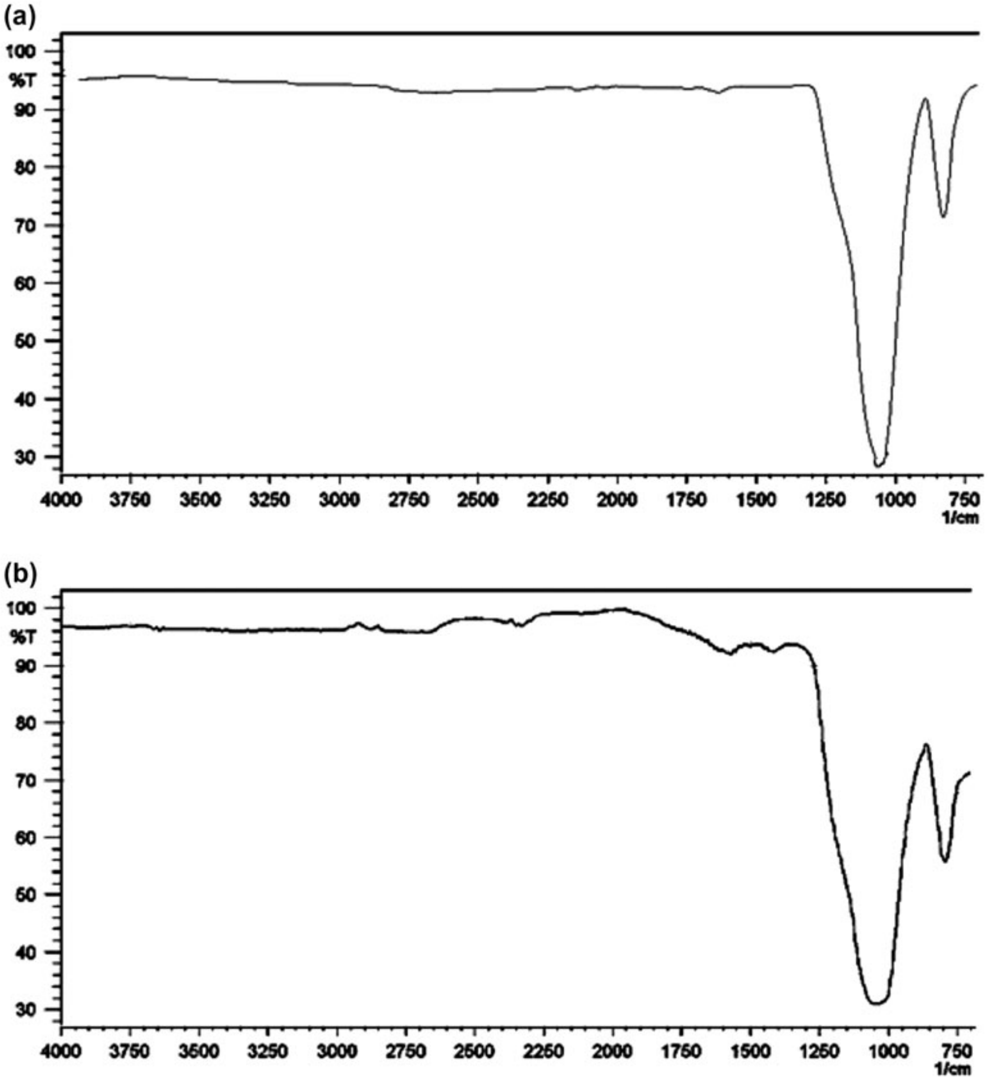

Fig. 3. FTIR spectrum of adsorbent (a) and adsorbent loaded with copper ions (b).

Table 4
Main FTIR vibration bands of Si–O species

Wave number (cm^{-1})	Type	Bound
477	Asymmetric stretching	Si–OH
618	Asymmetric stretching	Si–OH
804	Asymmetric stretching	Si–OH
1,109	Asymmetric stretching	Si–O–Si
1,645	Bending vibrations	O–H–O

and RB5 dye–loaded adsorbent were illustrated in the Fig. 5(a) and (b). From the EDX spectra, the RB5 dye ions were adsorbed onto the laccase-modified SF adsorbent. It is shown from EDX spectra that after RB5 dye adsorption, element concentrations increased in the RB5 dye-loaded adsorbent (Table 5).

3.3. *Adsorption study*

3.3.1. *Effect of pH*

One of the most important factors affecting the capacity of adsorbents in wastewater treatment is pH [47]. The pH of the solution affects the surface charge of the adsorbents as well as the degree of ionization of different pollutants. The hydrogen ion and hydroxyl ions are adsorbed quite strongly, and therefore, the adsorption of other ions is affected by the pH of the solution. Change of pH affects the adsorptive process through dissociation of functional groups on the adsorbent surface active sites [48]. The effect of the initial pH of the dye solution toward the adsorption of RB5 by laccase-modified SF in dye solution is shown in Fig. 6. In order to evaluate the influence of

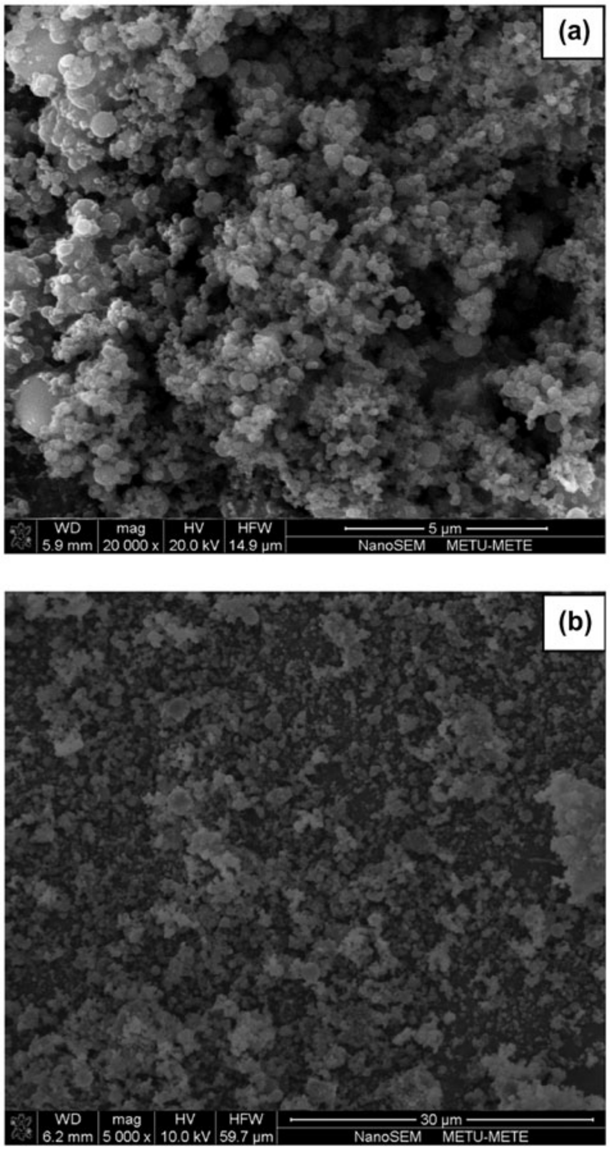

Fig. 4. SEM images of native SF (a) and RB5 dye loaded laccase modified-SF (b).

pH on the adsorption, the experiments were carried out at different initial pH values ranging from 3 to 9. The optimum pH, at which the maximum removal occurred, was obtained as 9. At lower pH values, the amount of dye adsorbed decreased due to repulsive force between positively charged surface and the positively charged dyes molecules [49]. At lower pH, more protons will be available, thereby increasing electrostatic attractions between negatively charged dye anions and positively charged adsorption sites and causing an increase in dye adsorption. When the pH of the solution is increased, the positive charge on the oxide or solution interface decreases and the adsorbent surface becomes negatively charged. The adsorbent surface metal binding sites as well as dye chemistry in solution were influenced by solution pH. It is expected that with increase in pH values, more and more ligands having negative charge will be exposed which result in increase in attraction of positively charged dye ions.

3.3.2. Effect of contact time

The contact time is inevitably a fundamental parameter in all transfer phenomena such as adsorption [50]. Therefore, it is important to study its effect on the capacity of retention of RB5 dye by laccasa-modified SF. The effect of contact time on removal of the dye is shown in Fig. 7. The removal increased quickly within the initial 25 min and remained almost unchanged after 60 min, indicating reaching an apparent equilibrium. Rapid absorption and equilibrium in a short period of time is related to the efficacy of the adsorbent, especially for wastewater treatment [51–53]. The amount of the removal increased with the increase in the contact time and reached a

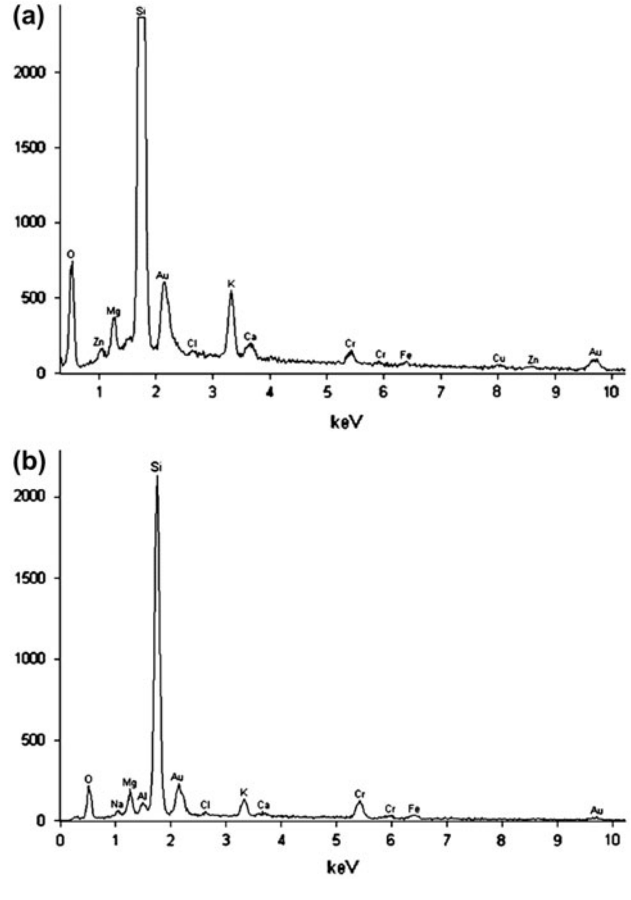

Fig. 5. EDX spectra of native SF (a) and RB5 dye loaded laccase modified-SF (b).

Table 5
Results of EDX spectrum

Elements	Native adsorbent		RB5 dye loaded adsorbent	
	Weight (%)	Atom (%)	Weight (%)	Atom (%)
Mg	2.11	2.62	4.76	6.03
Si	77.65	83.68	67.10	73.64
K	9.21	7.13	6.06	4.77
Ca	2.19	1.66	1.16	0.89
Cr	3.11	1.81	12.81	7.59
Fe	1.17	0.64	3.61	1.99
Cu	1.88	0.89	–	–
Zn	1.82	0.84	–	
Na	–	–	1.61	1.01
Al	–	–	1.47	1.68
Cl	–	–	1.43	1.24

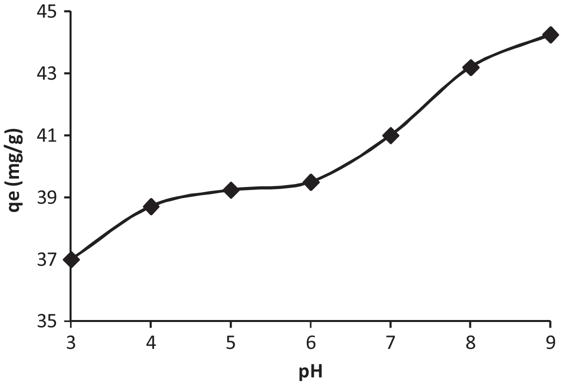

Fig. 6. Effect of pH on the removal of RB5 dye by laccase modified-SF.

constant value. This may be due to the attainment of equilibrium condition at 30 min of contact time, which is fixed as the optimum contact time. At the initial stage, the rate of the removal of RB5 was higher, due to the availability of more than required number of active sites on the surface of adsorbent. The rate of the removal became slower at the later stages of contact time, due to the decreased or lesser number of active sites [54,55].

3.3.3. Effect of temperature

Temperature known to have a profound effect on various chemical processes is one of the most important controlling parameter in adsorption. It affects the adsorption rate by altering molecular interactions and solubility of adsorbate [56–58]. The effect of temperature was investigated in the temperature range 20–80˚C (Fig. 8). It was observed that the removal of RB5 dye increased from 29.96 to 34.96 mg/g by increasing the temperature from 20 to 30˚C. This increase in adsorption is mainly due to increase in number of adsorption sites caused by breaking of some of the internal bonds near the edge of the active surface sites of the adsorbents [57]. This phenomenon also leads to an increment in the availability of active surface sites, increased porosity and in the total pore volume of the adsorbent. However, the adsorption capacity slightly decreased from 34.96 to 29.22 mg/g with increasing the temperature from 30 to 80˚C. This may be attributed by the fact that the mobility of the dye molecule increases with increasing the temperature, which may be responsible for the decrease in adsorption capacity of adsorbent. The similar results were reported in the previous studies [59,60].

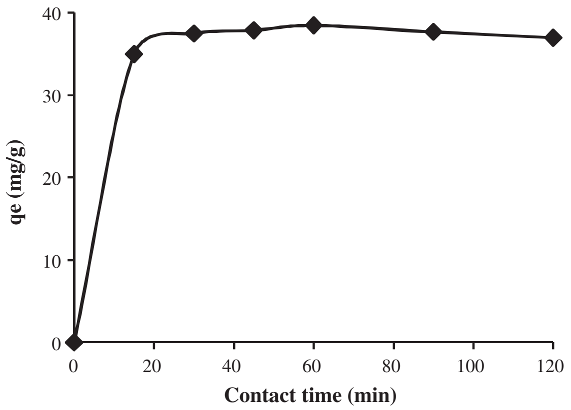

Fig. 7. Effect of contact time on the removal of RB5 by laccase modified-SF.

3.3.4. Effect of adsorbent dosage

Dosage study is an important parameter in adsorption studies because it determines the capacity of adsorbent for a given initial concentration of dye solution [55]. The effect of the adsorbent dosage was studied by varying the adsorbent amounts from 0.125 to 2.0 mg/mL. The effect of laccase-modified SF dosage on amount of RB5 dye adsorbed was shown in Fig. 9. A trend of increase in adsorption capacity with increase in adsorbent dosage was observed from 0.125 to 1 mg/mL. Any further addition of the adsorbent beyond this did not cause any significant changes in the adsorption. The amount of maximum RB5 dye removal was 35.18 mg/g at 1 mg/mL of adsorbent dose. Increase in adsorbent dosage increased the percent removal of dye, which is due to the increase in absorbent surface area of the adsorbent [61].

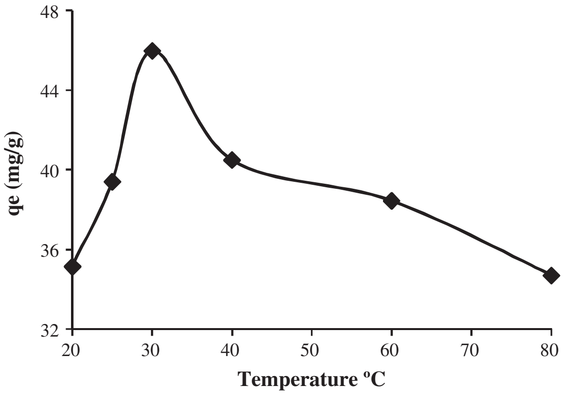

Fig. 8. Effect of temperature on the removal of RB5 dye by laccase modified-SF.

Increase in adsorption with increase in adsorbent dosage attributed to the increase in active sites for the adsorption of dye molecules with increasing adsorbent dosage. At very low adsorbent concentration, the adsorbent surface becomes saturated with the dye and the residual dye concentration in the solution to large [55].

3.4. Equilibrium adsorption isotherms

The relationship between the amount of a substance adsorbed at constant temperature and its concentration in the equilibrium solution is called the adsorption isotherm. The adsorbent isotherm is important from both theoretical and practical point of view [62]. In order to optimize the design of an adsorption system to remove dye from solutions, it is important to establish the most appropriate correlation for the equilibrium curve. Therefore, the two well-known and widely applied isotherm equations, namely the Langmuir and Freundlich models, were applied to determine the experimental data. There are different equation parameters and the underlying thermodynamic presuppositions of these models often provide insight into both adsorption mechanism and surface properties of the adsorbents [35,63,64].

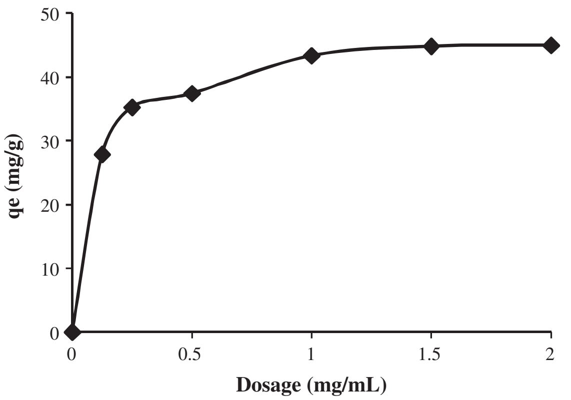

Fig. 9. Effect of adsorbent dosage on removal of RB5 by laccase modified-SF.

The suitability of the Langmuir and Freundlich adsorption isotherm models to the equilibrium data was investigated and the Langmuir and Freundlich isotherm constants fort the RB5 adsorption on the laccase-modified SF were determined.

The Langmuir adsorption isotherm is often used to describe the maximum adsorption capacity of an adsorbent and it is given as:

$$q_{\mathrm{e}} = \frac{q_{\max} \times b \times C_{\mathrm{e}}}{1 + b \times C_{\mathrm{e}}} \tag{3}$$

where $q_{\max}$ (mg/g) and b (L/mg) are Langmuir constants that are indicators of the maximum adsorption capacity and the affinity of the binding sites, respectively. They can be determined from a linear form of Eq. (2) (by plotting the $C_{\mathrm{e}}/q_{\mathrm{e}}$) versus C_{e}, represented by calculated with the following equation:

$$\frac{C_{\mathrm{e}}}{q_{\mathrm{e}}} = \frac{1}{b \times q_{\max}} + \frac{C_{\mathrm{e}}}{q_{\max}} \tag{4}$$

The values of $q_{\max}$ and b were calculated from the slope and intercept of the Langmuir plot of C_{e} versus $C_{\mathrm{e}}/q_{\mathrm{e}}$ from Fig. 10, the empirical constants $q_{\max}$ and b were found to be 322.58 mg/g and 106.52 L/mg (Table 6), respectively. The applicability of the linear form of Langmuir model to laccase-modified SF was proved by the high correlation coefficient R^2 (0.9924) > 0.95. This suggests that the Langmuir isotherm provides a good model of the sorption system.

The Freundlich isotherm model is an empirical relationship describing the adsorption of solutes from a liquid to a solid surface and assumes that different sites with several adsorption energies are involved [65]. It is described by the following equation:

$$q_{\max} = K_F C_{\mathrm{e}}^{1/n} \tag{5}$$

where K_F and n are the Freundlich constants related to the sorption capacity of the adsorbent (mg/g) and the energy of adsorption, respectively. They can be calculated in the following linear form:

$$\log q_{\mathrm{e}} = \log K_F + \frac{1}{n} \log C_{\mathrm{e}} \tag{6}$$

K_F and n values were calculated from the intercept and slope of the plot (Fig. 11). In the literature, it is

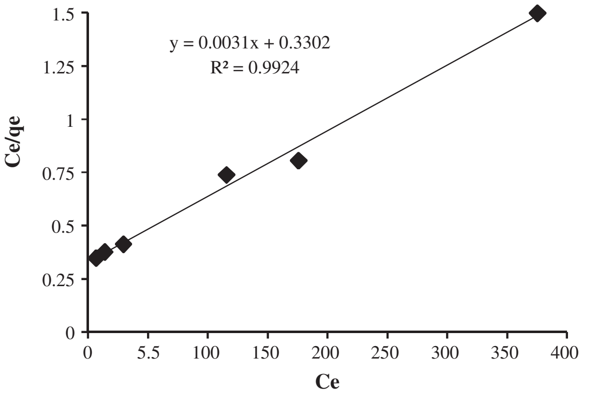

Fig. 10. Langmuir adsorption isotherm.

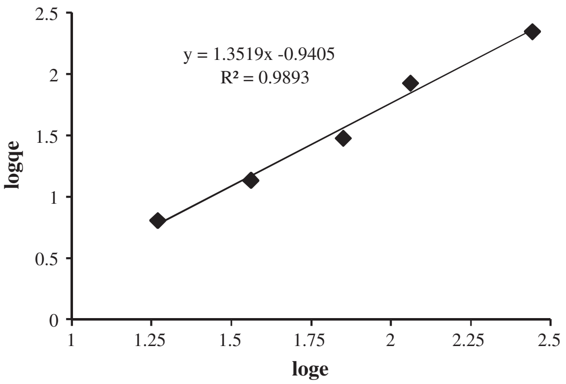

Fig. 11. Freundlich adsorption isotherm.

Table 6
Values of the Langmuir and Freundlich adsorption isotherms

Adsorption isotherm	Value
Langmuir constants	
$q_{\max}$ (mg/g)	322,58
b (L/mg)	106,52
R^2	0.9924
Freundlich constants	
K_F	0.110
N	0.740
R^2	0.9892

pointed out that the parameters, K_F and n affect the adsorption isotherm. The larger K_F and n values indicate the higher the adsorption capacity. The magnitude of exponent n gives an indication of the favorability of the adsorption. The n value is 0.74 (Table 5) and it is good adsorption characteristic [66].

Based on the high correlation coefficient R^2 (0.9892) > 0.95, it has been deduced that Freundlich model better fitted to the experimental data (Table 6). The high correlation coefficient showed that both adsorption isotherm models are suitable for describing the adsorption equilibrium of RB5 dye.

3.5. Adsorption kinetics

In environmental engineering practice, both energy and entropy consideration must be taken into account in order to determine what processes will occur spontaneously [67,68]. The aim of thermodynamic study is to establish the thermodynamic parameters that can characterize the adsorption process of RB5 dye onto laccase-modified SF. The adsorption capacity of laccase-modified SF adsorbent increased with increase in the temperature of the system from 293 to 303 K. Thermodynamic parameters such as ΔG^o, ΔH^o, and ΔS^o were determined using the following equations [69]:

$$K_L = \frac{C_s}{C_e} \tag{7}$$

$$\Delta G^\circ = -RT \ln K_L \tag{8}$$

$$\ln K_L = \left(\frac{\Delta S^\circ}{R}\right) - \left(\frac{\Delta H^\circ}{RT}\right) \tag{9}$$

where K_L is the equilibrium constant, C_s is the solid phase concentration at equilibrium (mg/L), C_e is the liquid phase concentration at equilibrium (mg/L), T is the temperature in Kelvin, and R is the gas constant.

The ΔH^o and ΔS^o values are obtained from the slope and intercept of plot ln K_o against $1/T$ [70]. These parameters were 1,185 kJ/mol and 4.104 J/mol K, respectively. Von't Hoff plot of effect of temperature on the adsorption of RB5 on laccase-modified SF was illustrated on Fig. 12, and the observed thermodynamic values were summarized in Table 7.

The ΔG^o is −60.55 kJ/mol (313 K), and this negative value of ΔG^o indicates the adsorption is favorable and spontaneous. The ΔG° values decrease with an increase in temperature, indicating an increased trend in the degree of spontaneity and feasibility of RB5 dye adsorption. The negative values of ΔH° further confirm the exotermic nature of adsorption process. Hence, the adsorption of RB5 dye on laccase-modified SF is chemical in nature. The positive values of ΔS° indicate the increased disorder and randomness at the solid solution interface of RB5 dye with the adsorbent.

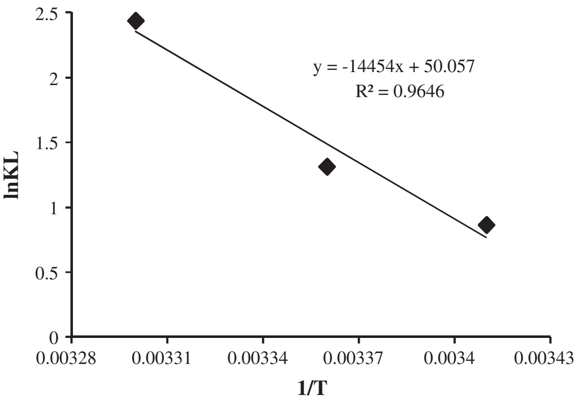

Fig. 12. Influence of temperature on the thermodynamic behavior of RB5 dye.

Table 7
Thermodynamic parameters for the RB5 dye adsorption

Temperature (K)	Thermodynamic parameters		
	ΔG^o (kJ/mol)	ΔH^o (kJ/mol)	ΔS^o (J/mol K)
298	−20.73	−1,185	4.104
303	−32.11		
313	−60.55		

4. Conclusion

In this study, SF modified using laccase from Russulaceae (*L. volemus)* was converted into an adsorbent, and the suitability of the activated SF for the adsorption of RB5 dye from textile wastewater was investigated. The results indicate that laccase-modified SF, which is a cheap and abundant material, can be successfully used as an effective adsorbent for the removal of RB5 dye from textile-polluted wastewater. The maximum removal of RB5 dye by adsorbent was at pH 9. The removal of RB5 dye increases with increasing time in the first 60 min. It is observed that the degree of adsorption increases with increasing temperature and maximum adsorption of RB5 dye are obtained at 30°C, which is the temperature of the solution. The total amount of RB5 dye removed from the solutions increases by the amount of adsorbent. The adsorbent concentration increases when the number of adsorbent particles surrounding the RB5 dye increases. Based on the correlation coefficient values, it has been deduced that the Langmuir model is better fitted to the experimental data. The high correlation coefficient showed that both adsorption isotherm models are suitable for describing the adsorption equilibrium of RB5 dye. Thermodynamic parameters such as ΔG^o, ΔH^o, and ΔS^o indicate that the adsorption of RB5 onto laccase modified-SF adsorbent is feasible, spontaneous and endothermic. Based on the results, laccase-modified SF can be used as a relatively efficient and low-cost absorbent for the removal of RB5 dye from textile wastewater.

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