Electronic Journal of Biotechnology 46 (2020) 22–29

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research article

Characterization of the biosorption of fast black azo dye K salt by the bacterium *Rhodopseudomonas palustris* 51ATA strain

Ayten Öztürk [a,*], Emel Bayol [b], Meysun I. Abdullah [c]

[a] Department of Biotechnology, Niğde Ömer Halisdemir University, Niğde, Turkey
[b] Department of Chemistry, Niğde Ömer Halisdemir University Niğde, Turkey
[c] Faculty of Pharmacy, Near East University, TRNC, Cyprus

ARTICLE INFO

Article history:
Received 19 December 2018
Accepted 8 May 2020
Available online 14 May 2020

Keywords:
Azo dyes
Biosorption
Dye removal
Fast Black K salt azo dye
Freundlich
Isotherms
Langmuir
Rhodopseudomonas palustris
Scatchard analysis
Temkin
Wastewater

ABSTRACT

Background: Removal of dyes from wastewater by microorganisms through adsorption, degradation, or accumulation has been investigated. Biological methods used for dye treatment are generally always effective and environmentally friendly. In this study, biosorption of the Fast Black K salt azo dye by the bacterium *Rhodopseudomonas palustris* 51ATA was studied spectrophotometrically, at various pH (2–10), temperatures (25°C, 35°C, and 45°C) and dye concentrations (25–400 mg L^{-1}).

Results: The bacterial strain showed extremely good dye-removing potential at various dye concentrations. IR studies at different temperatures showed that the dye was adsorbed on the bacterial surface at lower temperatures. Characteristics of the adsorption process were investigated by Scatchard analysis at 25°C and 35°C. Scatchard analysis of the equilibrium binding data for the dye on this bacterium gave rise to linear plots, indicating that the Langmuir model could be applied. The regression coefficients obtained for the dye from the Freundlich and Langmuir models were significant and divergence from the Scatchard plot was observed.

Conclusion: The adsorption behavior of the dye on this bacterium was expressed by the Langmuir, Freundlich, and Temkin isotherms. The adsorption data with respect to various temperatures provided an excellent fit to the Freundlich isotherm. However, when the Langmuir and Temkin isotherm models were applied to these data, a good fit was only obtained for the dye at lower temperatures, thus indicating that the biosorption ability of *R. palustris* 51ATA is dependent on temperature, pH, and dye concentration.

How to cite: Öztürk A, Bayol E, Abdullah MI. Characterization of the biosorption of fast black azo dye K salt by the bacterium Rhodopseudomonas palustris 51ATA strain. Electron J Biotechnol 2020;46. https://doi.org/10.1016/j.ejbt.2020.05.002.

1. Introduction

The potential of microorganisms in the treatment of wastewater has been widely studied. The removal of water contamination by microorganisms through adsorption, degradation, or accumulation has been investigated [1]. Until now, the use of expensive electrochemical methods in the removal of organic molecules such as herbicides and dyes has been investigated [2]. However, as some purple bacteria such as *Rhodopseudomonas palustris* can grow in the dark aerobically and in the light anaerobically, the phototrophic and heterotrophic characteristics of such bacteria are considered advantageous, which can be employed for their growth in contaminated waste water. The degradation of aromatic rings by some photosynthetic anaerobic bacteria under certain conditions has also been reported [3,4,5,6]. *R. palustris* is a crucial bacterial species, which is capable of degrading the aromatic ring system, hydrogen gas production and nitrogen fixation; therefore, it can be considered as sophisticated eco-friendly bacterium [7].

The color removal of Acid Red B, Reactive Blue GL, Acid Red G, and RBR X-3B azo dyes has been carried out by using *R. palustris* strains. The concentration of the dye, pH, temperature, and the carbon source have been reported to be important factors in such treatments [8]. Bleaching or the removal of the color accomplished by this and electrochemical methods rely on the ability to reduce azo (N═N) group to amine counterparts, thus disrupting the extended conjugation system of dyes. It is reasonable, therefore, to expect that the reduced species could be still environmentally unfriendly with potential carcinogenic activity [9,10,11,12]. Çelik et al. [13] reported that Reactive Red 195 azo dye was used by the photosynthetic *R.*

* Corresponding author.
E-mail address: aozturk@ohu.edu.tr (A. Öztürk).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2020.05.002

palustris 51ATA as a carbon source during its mineralization and degradation under anerobic conditions. However, the effect of bacterial adsorption in the removal of dye should also be investigated. As this method has some advantages over other convenient methods, inactivated biomasses that exist in wastewater or other resources can be obtained easily and as it is inactive, no activation parameters are required [4,14,15]. Therefore, the removal of waste can be achieved in shorter times, while the biomass will not be affected by the toxicity of waste as in the case of active bacteria. Moreover, the saturated surface of the biosorbent and its sensitivity to pH changes could be considered as disadvantageous [5,16,17].

In this work, the eco-friendly bacterium, *R. palustris* 51ATA strain, was used in biosorption studies of fast black azo dye, and factors that could affect its removal ability such as temperature and pH were studied. However, the alteration that might have occurred on the bacterial wall surface due to dye adsorption was also investigated by infrared spectroscopy at various temperatures.

2. Materials and methods

2.1. The bacterial growth

R. palustris 51ATA strain, obtained from Lake Akkaya, Nigde, Turkey, which had been previously isolated and identified was used in this study. The bacterium was grown in a liquid-modified AT medium [5]. The bacterial growth was carried out under a 75-Watt light at a distance of 15–25 cm under room temperature conditions.

2.2. Preparation of bacterium for biosorption

At the end of the growth period, cultivations were harvested and centrifuged at 10000 rpm. Bacterial pellets (biomass) were then washed three times with sterilized serum physiologic to remove residues from the media, and were dried at room temperature for 15 days.

2.3. Preparation of dye for biosorption

Fast Black K salt (Sigma-Aldrich) stock solution of 1.0 g L^{-1} was prepared by dissolving an accurately weighed amount of the dye in distilled deionized water. Test solutions of the dye were prepared by diluting the stock solution with distilled deionized water. In this work, the initial dye concentration (C_o) varied from 44 mg L^{-1} to 351 mg L^{-1}, and the pH of each solution was adjusted to the desired value using 0.1 M HCl or NaOH solutions.

2.4. Conditions of biosorption

A 1.0 g L^{-1} bacterium solution was mixed with 100 mL biosorption settings containing dye at the desired pH (2, 4, 6, 8, and 10), temperature (25°C, 35°C, and 45°C), and concentrations (25mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1}, and 400 mg L^{-1}). Experiments were carried out in a water bath with continuous shaking at 10 rpm. The biosorption analysis was conducted under three different physiological conditions (Table 1).

2.5. Biosorption experiments

100 mL dye solution containing dry bacterium (1.0 g L^{-1}) was placed in a 250 mL flask at constant pH, temperature, and concentrations. Samples were taken at 1-, 5-, 15-, 30-, 45-, 60-, 120-, 1080-, 1440-, and 2520-min intervals and were centrifuged for 5 min at 10000 rpm. The supernatant was centrifuged and the UV absorbance measurements were carried out at 457 nm.

Table 1
Physiological conditions of Fast Black K salt dye biosorption by the bacterium.

Biosorption Conditions	Temperatures (°C)		
	25 °C	35°C	45°C
pH	2.0; 4.0; 6.0; 8.0; 10.0	8.0	8.0
C (mg L^{-1})	100	25, 50, 100, 200, 400	25, 50, 100, 200, 400
X (g L^{-1})	1.0	1.0	1.0
Stirring speed (rpm)	10	10	10

C: concentration of dye and X: Concentration of bacterium.

2.6. FTIR studies

Surface characterization of the adsorbent was determined using Fourier transform infrared spectroscopy (FTIR). These measurements were carried out using Thermo Scientific Nicolet iS10 instrument. To identify functional groups of the dye that might have been adsorbed on the surface of bacteria during the biosorption process, FTIR measurements were carried out at three different temperatures (25°C, 35°C, and 45°C). The bacterial mass was centrifuged for 5 min at 10000 rpm, then collected and dried prior to FTIR studies.

3. Results and discussion

3.1. Biosorption studies

Biosorption studies of the Fast Black K salt dye were carried out at five different pH mediums (2, 4, 6, 8, and 10) and five different concentrations of the dye (25, 50, 100, 200, and 400 mg L^{-1}) at three different temperatures (25°C, 35°C, and 45°C). The effect of pH on biosorption was first investigated and the optimum pH (8.0) for dye removal was chosen (Fig. 1). At this pH, the best temperature for biosorption was determined. Previous biosorption studies of toxic hydrocarbons in wastewater have reported that the most important and effective factor was the pH [6,18]. The variation in the biosorption of pollutants by microbial biomass at various pH values could be due to differences in the susceptibility of the bacterial cell wall to pH. For instance, at a low pH, cell wall ligands tightly bind to the hydronium ions H_3O^+, and therefore restrict the approach of the ionizable pollutants through impulsive activity. On the contrary, at higher pH values, ligands such as carboxyl, phosphate,

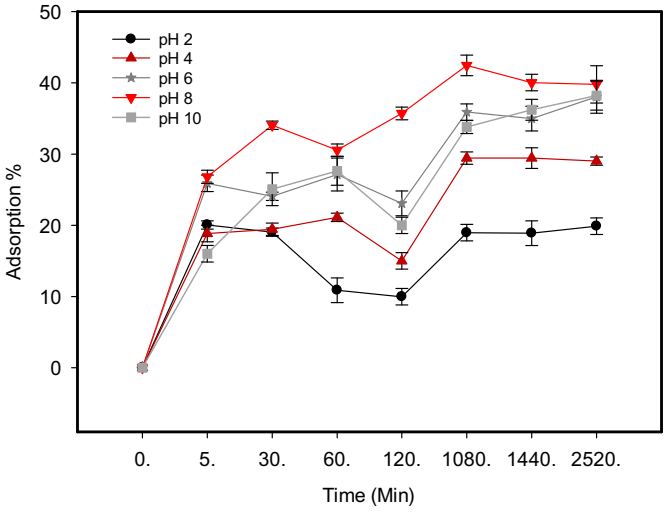

Fig. 1. The effect of pH on the biosorption of Fast Black K salt dye (T: 25°C, X: 1,0 g L^{-1}, and stirring speed: 10 rpm).

imidazole, and amino groups that carry negative charges would be exposed and would have subsequent attraction for pollutants carrying positive charges, which would eventually be adsorbed onto the cell surface [19,20]. Most living microorganisms have been found to adsorb contaminants such as metals and organic pollutants at various pH, because of their physiological properties [21,22,23].

During this study, inactive dry biomass was used. The biosorption of hydrocarbons by inactive mass was reported to be superior to active ones [6,16,23]. Earlier studies have reported that the removal of toxic organic compounds from the environment by bacteria depends on the pH, temperature, concentration of waste material, and other factors such as the presence of salts or other ions in the solution [6,24].

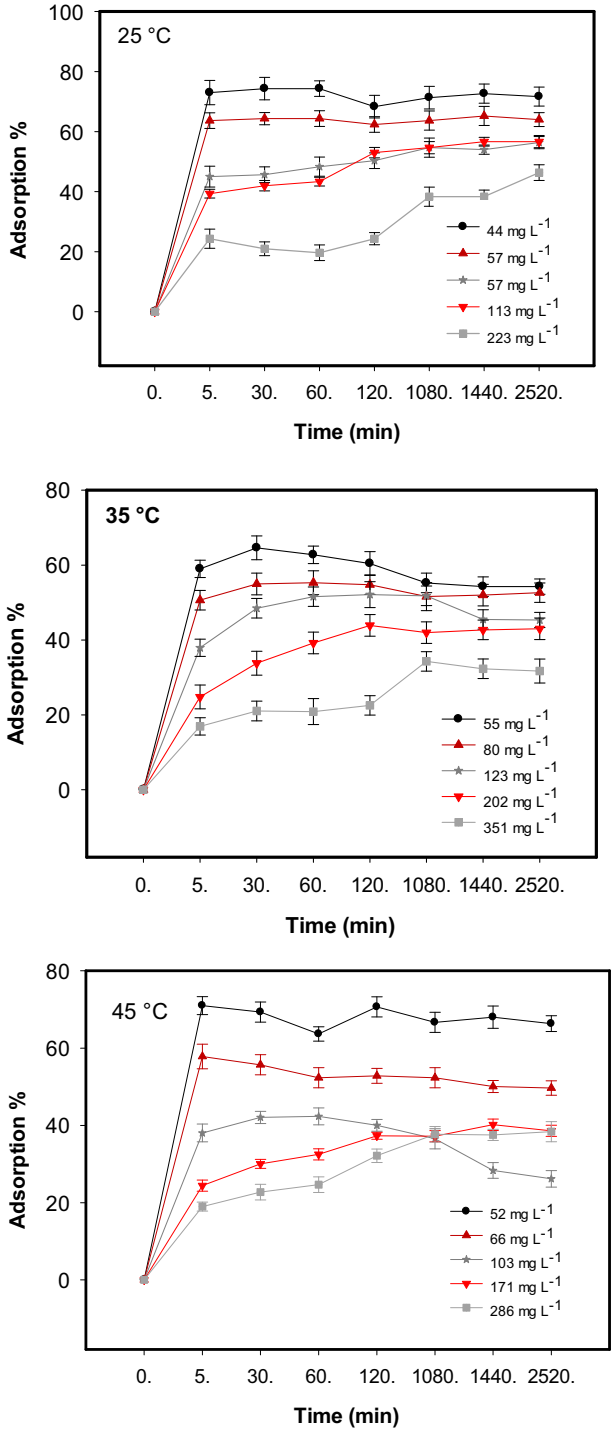

Fig. 2. The effect of temperatures on the biosorption of the dye at pH 8.0.

Table 2
The percentage of Fast Black K salt dye adsorbed at various initial concentrations at equilibrium[a].

t (°C)	C_o	q_{eq}	Ads %
25	44	31.86	72.24
	57	36.14	63.86
	83	42.32	50.97
	113	55.94	49.49
	223	68.18	30.57
35	55	31.98	58.35
	80	42.78	53.24
	123	58.72	47.59
	202	78.18	38.75
	351	89.43	25.46
45	52	35.51	68.10
	66	35.61	53.75
	103	38.21	37.09
	171	58.24	34.12
	286	87.24	30.50

[a] Concentration of bacterium (X) 1.0 g L^{-1}, stirring speed10 rpm, and pH 8.0.

Fig. 2 and Table 2 show that the biosorption at 25°C is maximal (72%). As can be seen in Fig. 2**,** the effect of temperature on biosorption is less important compared to pH. The adsorption percentage at 25°C, 35°C, and 45°C is extremely similar. While the initial concentration (from 44 mg L^{-1} to 223 mg L^{-1}) has increased, the adsorption rate (Ads %) has decreased at 25°C. However, during the dye removal of studies, the increase in temperature led to decrease in the biosorption capacity, which is likely due to changes in elements of the cell wall [25,26].

In previous studies, the biosorption has been reported to be more effective at lower temperatures and the reaction type is exothermic [27]. The mechanism is physical rather than chemical biosorption, and the adsorption of the dye at higher temperatures is reported to be an endothermic process. The physical biosorption is considered to be a reversible process where bonds between the cell surface and toxic compounds are weak and therefore, desorption may occur at higher temperatures [6,16,23,28]. Hence inactive biomasses are considered superior to the active biomasses, because components of the living cell play an important role in the biosorption of toxic compounds and interfere with biological functions of the cell [6,26]. Additionally, certain environmental

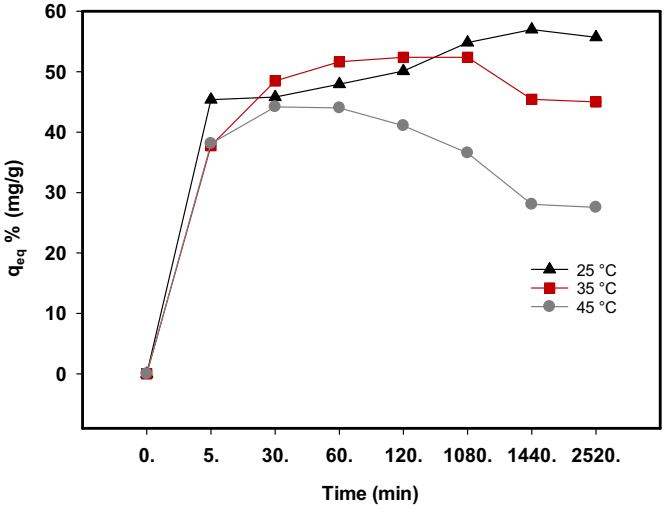

Fig. 3. Time course of biosorption by the bacterium at various temperatures (C_o: 100 mg L^{-1}, X: 1,0 g L^{-1}, stirring speed: 10 rpm, and pH: 8.0).

Table 3
Langmuir, Freundlich, and Temkin isotherm parameters for the dye on the bacterium at pH 8.0 and at various temperatures.

t (°C)	Langmuir			Freundlich			Temkin		
	As	K_b	R^2	K_f	n	R^2	b	A_t	R^2
25	98.040	0.0104	0.9845	5.095	2.053	0.9699	23.389	0.084	0.9750
35	135.14	0.0060	0.9874	3.564	1.767	0.9617	32.312	0.049	0.9889
45	142.86	0.0047	0.7819	3.680	1.841	0.9110	30.070	0.048	0.8713

parameters such as temperature and pH can affect the viscosity, which is highly dependent on temperature and alter the hydrophobicity of microbial components [6,29].

In this work, for each 1.0 g of adsorbent (bacteria) in the solution, the amount of dye adsorbed was calculated from the following equations:

$$\text{Ads}\% = \frac{q_{eq}X}{C_o} \quad \text{[Equation 1]}$$

$$q_{eq} = \frac{C_o - C_{eq}}{X} \quad \text{[Equation 2]}$$

where (q_{eq}: mg g^{-1}) is the amount of adsorbed dye and (C_{eq}: mg L^{-1}) is the amount of non-adsorbed dye. Results are shown in Fig. 3 and Table 1.

Fig. 3 represents the duration of contact with the cell surface (biosorption), which is an important factor that explains the behavior of adsorption within the first hour. As the dye material contains many

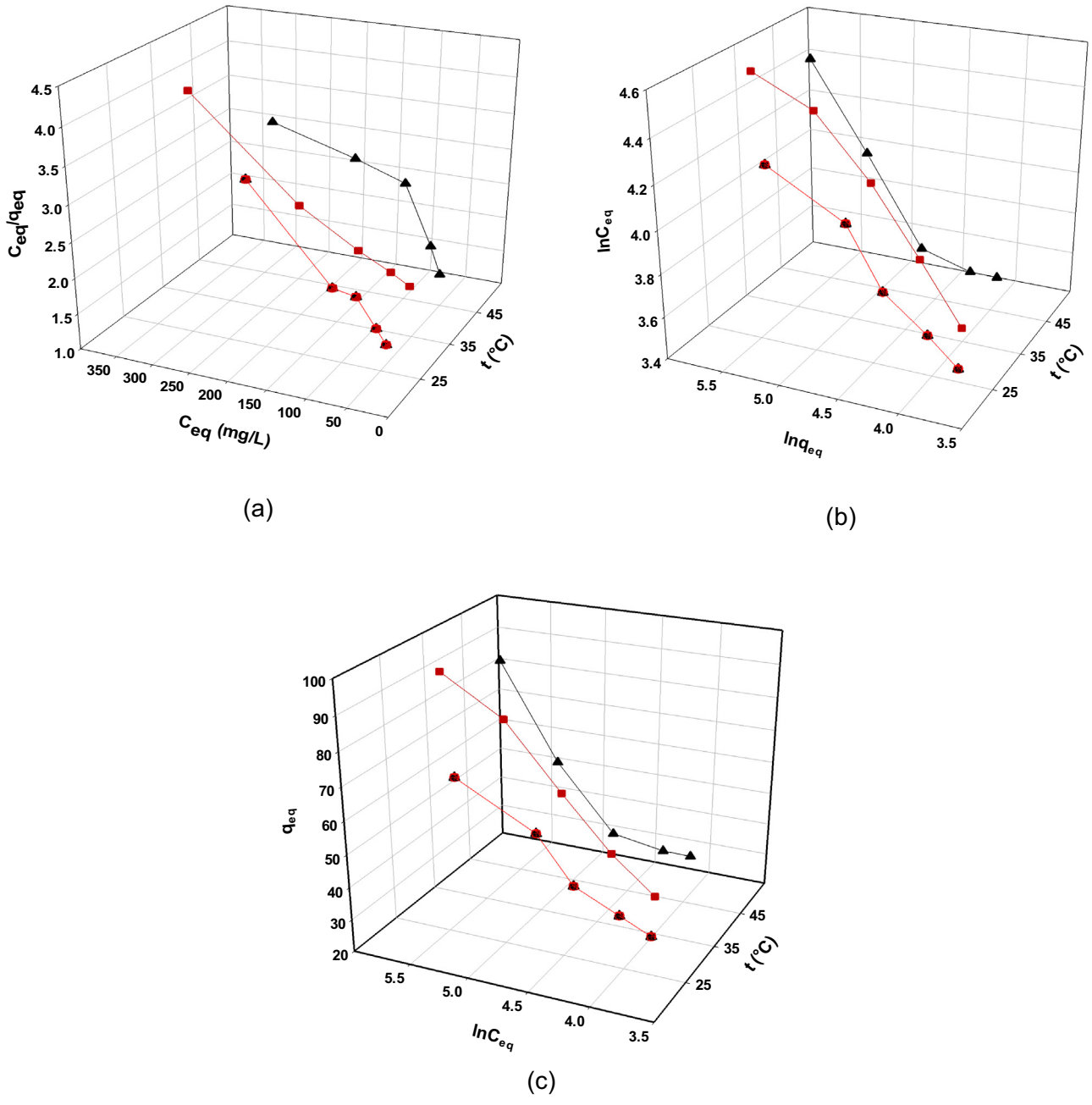

Fig. 4. The linearized (a) Langmuir, (b) Freundlich and (c) Temkin adsorption isotherms for Fast Black K salt.

functional groups, the biosorption process will need a longer time to reach the equilibrium point. Our studies on the duration of contact of compounds with biomass cell walls show that biosorption took place and an equilibrium was reached within the first hour. The nature of the microorganism and the chemical structure of the toxic compound determines the duration of biosorption. The biosorption of organic compounds by inactive biomasses on the cell surface regardless of its metabolism is known to occur through electrostatic attraction, which is a passive type of bonding. However, the duration of biosorption process can last from hours to days depending on environmental factors and the biosorbent used; for example, metals can be adsorbed on the surface enabling an equilibrium to be reached at much faster rates than organic compounds [6,16,18,24].

The effect of the initial dye concentration on biosorption and the concentration at equilibrium (q_{eq}) as well as the percentage of adsorption are summarized in Table 2. As can be seen in Table 2, when the concentration of the dye was increased, the adsorption capacity (q_{eq}) of the biomass also increased. The adsorption percentage was found to be higher when both the concentration of the dye and the temperature were low (25°C). It is well known that biosorption could be either chemical or physical, but when the pollutant is an organic compound, then the adsorption is considered physical [6,23,24].

3.2. Isotherms

To understand and explain the pattern of the biosorption of the dye by the cell wall, mathematical models (Langmuir, Freundlich, and Temkin) have been used. The Langmuir, Freundlich, and Temkin isotherms of Fast Black K dye biosorption by the biomass of *R. palustris* strain 51ATA are presented in Table 3. The best fit of the curve that was generated in accordance with these models are given in Fig. 4a, b, and c.

Considering the fact that the rate of absorption is a function of the Langmuir isotherm and K_b is a Langmuir constant related to the energy of sorption. If the K_b value is high then the affinity of biosorbent is enhanced for the dye [30,31]. In this study, the Langmuir constant K_b value was highest at 25°C. The Langmuir model is given by [Equation 3]]:

$$\frac{C_{eq}}{q_{eq}} = \frac{1}{K_b A_s} + \frac{C_{eq}}{A_s} \qquad \text{[Equation 3]}$$

The Freundlich isotherm ensures heterogeneous energetic distribution of active sites on the surface of the biosorbent, which is a reversible binding interaction type [32]. The following linear form of the Freundlich, [Equation 4]] and [Equation 5]], can explain this isotherm:

$$q_{eq} = K_f C_{eq}^{\frac{1}{n}} \qquad \text{[Equation 4]}$$

$$\ln q_{eq} = \ln K_f + {}^{1}/_{n} \log C_{eq} \qquad \text{[Equation 5]}$$

The adsorption partition constant of dye was further determined by the Freundlich isotherm, where, K_f is the Freundlich adsorption constant of dye related to the adsorption capacity and (n) is the adsorption intensity of an adsorbent. The constants K_f and n were determined by the linear regression from the plot of $\ln q_{eq}$ against $\ln C$. Therefore, when the K_f value is low it indicates minimal adsorption of dye, whereas a higher K_f value suggests greater sorption ability. In this study, the K_f value was higher at 25°C (5,095) and lower at both 35°C and 45°C (3,564 and 3680), thus indicating a favorable adsorption. The values of n were low at 35°C and 45°C (1,767 and 1841) but were higher at 25°C (2,053), suggesting maximum biosorption of the dye, as shown in Table 3.

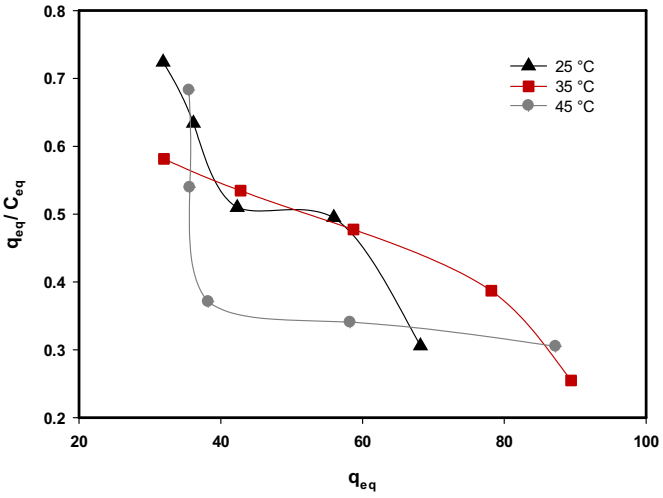

Fig. 5. Scatchard plots for Fast Black K salt adsorption.

The Temkin isotherm is usually used for heterogeneous surface energy systems (nonuniform distribution of sorption heat). This isotherm contains a factor that takes into account the adsorbent–adsorbate interactions. By ignoring extremely low and large concentration values, the model assumes that the heat of adsorption of all molecules in the layer would decrease linearly rather than logarithmically [33]. As implied in the equation, its derivation is characterized by a uniform distribution of binding energies that was obtained by plotting the quantity-sorbed q_e against $\ln C_e$ and constants were determined from the slope and intercept [33]. The model is given by [Equation 6]].

$$q_e = \frac{RT}{b} \ln A_T + \frac{RT}{b} \ln C_e \qquad \text{[Equation 6]}$$

where, A_T is the Temkin isotherm equilibrium binding constant (L/g), b is the constant related to the heat of adsorption (J/mol), R is the universal gas constant (8.314 J/mol K), and T is temperature.

The value of b derived from the Temkin plot (Fig. 4c) was equal to 32.312 at 35°C, which represents the heat of adsorption and indicates a physical adsorption process. The best fit was obtained from the experimental data at 35°C (R^2 = 0.9889). The process was endothermic as indicated by the positive energy value.

The dye adsorption constant (b) is directly related to the dye coated onto bacteria (adsorbent-adsorbate interaction). The value of b varied as the temperature increased, indicating that the adsorption of dye onto the surface of bacterium was unstable throughout the temperature increase (Table 3). This is probably due to changes on the bacterial cell wall surface that might have occurred as a result of the temperature increase as reported in the literature [34,35]. The change in the cell wall structure allows bacterial adsorption to some pollutants or other materials by modifying their cell surfaces according to their hydrophobicity to permit direct hydrophobic-hydrophobic interactions with the contaminants/materials or vice versa. However, the hydrophobicity/hydrophilicity of dye and cell structures determine their characteristics for bonding to each other,

Table 4
Scatchard analysis parameters for the dye.

t (°C)	K_d	q_m	R^2
25	99.010	99.570	0.9063
35	188.68	144.49	0.9495
45	200.00	140.48	0.4933

such as through hydrophobic interactions. A general overview of the molecular binding mechanisms between dyes and cell structures as well as bonding parameters have been given extensively by other researchers [36,37]. In this study, the change in temperatures might have resulted in alterations of the permeability of bacterial membranes and cell walls. As a result, the maximum adsorption potential of bacteria should be measured within the respective strain-specific optimal ranges of temperature and pH.

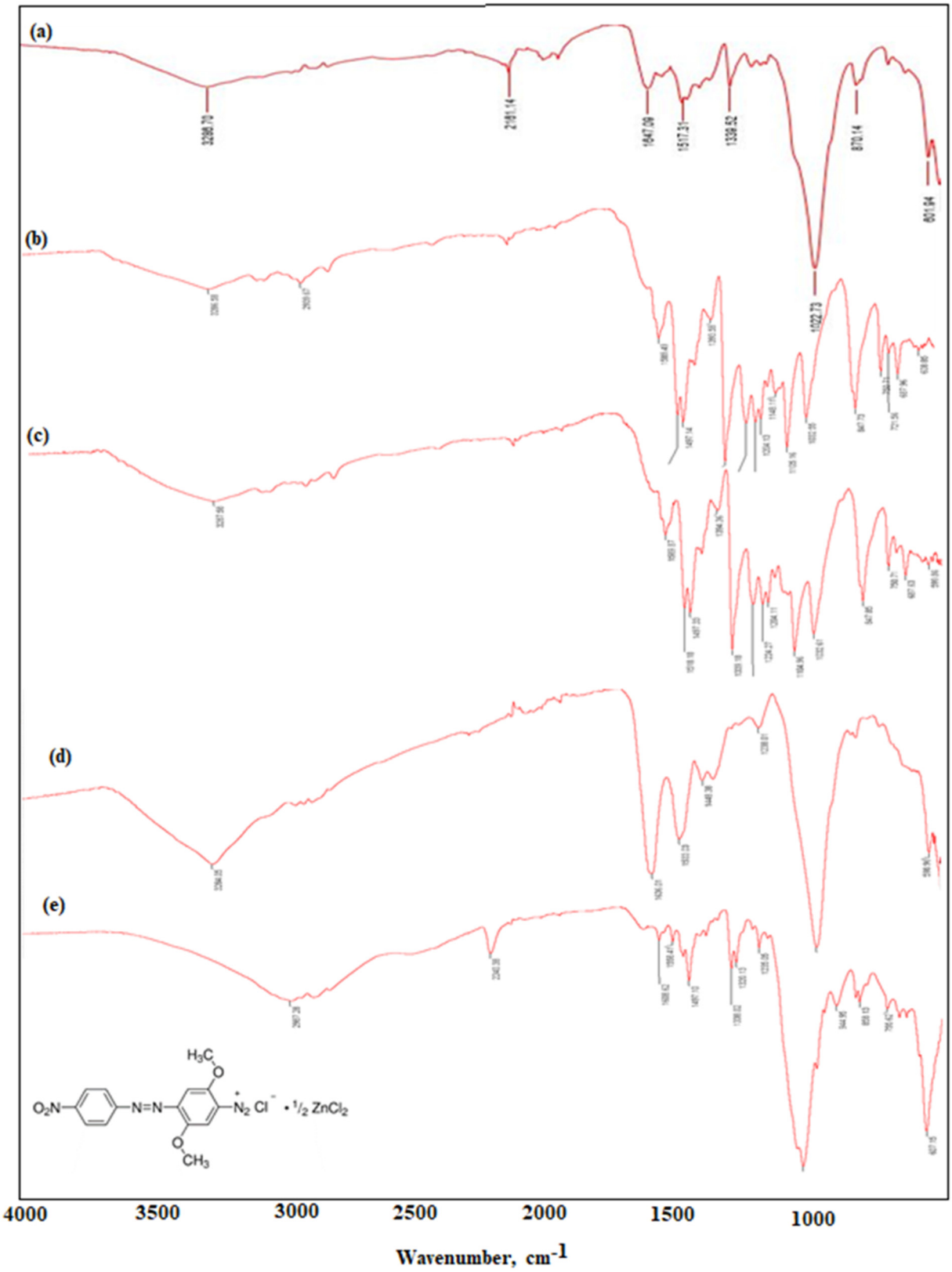

Fig. 6. FTIR spectra of Fast Black K salt biosorbed by the bacterium, a) 25°C, b) 35°C, c) 45°C, d) The bacterium *R. palustris* 51ATA and e) Fast Black K salt.

3.3. Scatchard analysis

When the Langmuir isotherm model and Scatchard analysis were applied to the experimental data, a good fit for the dye adsorption was also obtained. To evaluate saturation capacities of the cell toward the dye, the adsorption isotherms were applied and analyzed by the Scatchard equation, which was not only used to determine adjustable parameters [38], but also to estimate the number of site types and their relative affinity for the dye (Fig. 5 and Table 4). The presence of more than one inflection point on a plot based on Scatchard analysis usually indicates the presence of more than one type of binding site on the cell. The Scatchard analysis plot was drawn from q_{e}/C_{eq} versus q_{eq} using [Equation 7]].

$$\frac{q_{eq}}{C_{eq}} = K_b \left(q_m - q_{eq}\right)s \qquad \text{[Equation 7]}$$

However, when the Scatchard plot showed deviation from linearity, the Freundlich model was used to construct the adsorption isotherms of ligands at particular concentrations in solutions. At 25°C and 35°C, equilibrium binding data for the dye gave rise to a linear plot, indicating that the Langmuir model could be applied for the adsorption process.

3.4. FTIR studies

The FTIR of the dye, bacteria, and the biosorbed dye were studied at 25°C, 35°C, and 45°C. From the infrared spectra, it was concluded that the bacteria adsorbed the dye molecule more efficiently at low temperature. Most of the functional groups of the dye were adsorbed by the bacteria much more than at higher temperatures. However, in the IR spectra obtained at 35°C and 45°C, peaks representing N═N absorption (1558), OCH_3 (2841), Ar-CN (2240), Ar-NO_2 (1608), and N—O (1497) stretching were not present. Instead, at higher temperatures N—O, C—N, C—O, C—Cl, and aromatic ortho and para substitution peaks were observed. This suggests that at lower temperatures, nitrogen and other aromatic substituents are easily adsorbed and consumed by bacteria at room temperature (Fig. 6a, b, c, d, and e).

4. Conclusion

The present study showed that the cell wall of the bacterial strain *R. palustris* is capable of adsorbing Fast Black K salt at various temperatures. The potential of this strain is its ability to remove the dye at pH 8.0 and within a wide range of temperatures, at the initial concentration of the dye. FT-IR results showed that most of the Fast Black K salt dye was adsorbed by the *R. palustris* 51ATA strain. Therefore, this bacterium is a highly promising bacterial species and can be used for the treatment of textile industry effluents.

Mathematical models, namely Freundlich, Langmuir, and Temkin have provided excellent information on biosorption mechanisms and surface behavior of the biosorbent. The adsorption behavior of the dye onto the cell surface and its removal from aqueous solutions has been described by various equations. These equations took into account the quantity, intensity, and capacity factors that are important in estimating the amount of the dye for removal. Freundlich model-based adsorption isotherms explained the sorption data at various temperatures more effectively than the other adsorption isotherms.

Financial support

Authors are grateful to the Niğde Ömer Halisdemir University for its financial support (Project No FEB 2013/13).

Conflict of interest

The authors declare that they have no conflict of interest.

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