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# An investigation of the Trypan blue dye's biosorption on fungal biomass

*Tripan mavi boyasının fungal biyokütle ile biyosorpsiyonu hakkında bir çalışma*

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**Abstract**

Trypan blue (TB) is a commonly used hazardous and carcinogenic dye. The aim of this research was to remove TB using heat-inactivated *Aspergilus niveus* biomass. The biosorption of TB dye was studied as a function of pH, the concentration of TB dye, biosorbent amount, and time. In studies, the Langmuir, Freundlich, Redlich-Peterson, and Harkins-Jura isotherm models were evaluated for suitability. The biosorption of the TB dye on the used biomass was found to follow the Langmuir and Redlich-Peterson isotherm models. The pseudo second-order kinetic model was found to be more effective at explaining TB biosorption. The pseudo-second order model's theoretical $Q_e$ value (129.87 mg/g) was found to be close to the experimentally obtained value (128.1 mg/g) at 25 ℃. According to the studies, the highest biosorption capacity was found to be 141.79 mg/g at 45 ℃.

**Keywords**: Adsorption, Biosorption, Fungal biomass, Trypan blue

***Öz***

*Tripan mavisi (TB) yaygın olarak kullanılan tehlikeli ve kanserojen bir boyadır. Bu araştırmanın amacı, ısıyla inaktive edilmiş Aspergilus niveus biyokütlesi kullanarak TB'yi gidermektir. TB boyasının biyosorpsiyonu pH, TB boyası konsantrasyonu, biyosorbent miktarı ve zamanın bir fonksiyonu olarak incelenmiştir. Çalışmalarda Langmuir, Freundlich, Redlich-Peterson ve Harkins-Jura izoterm modelleri uygunluk açısından değerlendirilmiştir. TB boyasının kullanılan biyokütle üzerindeki biyosorpsiyonunun Langmuir ve Redlich-Peterson izoterm modellerini takip ettiği bulunmuştur. Yalancı ikinci dereceden kinetik modelin TB biyosorpsiyonunu açıklamada daha etkili olduğu bulunmuştur. Sözde ikinci dereceden modelin teorik $Q_e$ değerinin (129,87 mg/g) 25 ℃'de deneysel olarak elde edilen değere (128,1 mg/g) yakın olduğu bulunmuştur. Çalışmalara göre, en yüksek biyosorpsiyon kapasitesi 45 ℃'de 141,79 mg/g olarak bulunmuştur.*

***Anahtar kelimeler:*** *Adsorpsiyon, Biyosorpsiyon, Fungal biyokütle, Tripan mavisi*

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## 1. Introduction

One of the greatest environmental hazards we are currently facing is water pollution, which requires urgent attention. Both organic and inorganic substances pollute water resources. Heavy metals, detergents, organochlorines, organochlorines, organophosphorus, polycyclic aromatic compounds,and aromatic compounds derived from petroleum and dyes are examples of chemicals that cause water pollution. (Olakunle et al., 2018). Dyes are a prominent class of organic macromolecules that have widespread applications in various industries, such as textiles, paints, plastics, solar-sensitive devices, optics, sensors, and metal extraction. Classification of dyes is based on various criteria, including their molecular structure, source, colour, and method of application in the colour index (CI). However, a more systematic categorization of dyes can be achieved based on their chromophores. These include quinone-amine dyes, acridine dyes, azo dyes, aryl methane dyes, anthraquinone dyes, and other similar compounds. (Rauf & Salman Ashraf, 2012).

One of the most difficult industrial wastewaters to handle is dye effluent from the fabric and dyestuff industries. This is because that dyes are typically synthetic in origin and contain complex aromatic molecular structures, making them more durable and difficult to biodegrade (Fu & Viraraghavan, 2001).

These hazardous chemicals can cause water pollution, disrupt the environmental balance, and increase chemical and biological oxygen demand (COD and BOD) by altering the pH and chemical content of the water body. As a result, this coloured waste can have severe toxic effects on the aquatic ecosystem (Sarkar et al., 2017).
The dye-containing wastewater, which is given to the receiving environment without treatment, has a negative effect on the photosynthesis process as well as its toxic effects (Gemici & Özden, 2022). Azo dyes are chemical compounds that are made up of a diazotized amine coupled to an amine or a phenol, and they can contain one or more azo linkages. The primary building blocks for azo dyes are aromatic amines(Chung, 2016).Trypan blue is a diazo dye that is used as a direct dye for cotton fabrics and as a vital stain in biosciences to selectively colour dead cells or tissues blue (Priyadarshini et al., 2021) . Trypan blue (TB) is classified as a class-2B carcinogen by the International Agency for Research on Cancer, a class of compounds that can endanger human life. As a result, it is critical that Trypan blue be removed from wastewater (Cai et al., 2020). TB was removed using various adsorbents. Adsorbents such as core-shell activated carbon, orange peel, modified luffa sponge and MgO nanoparticles are examples of these materials (Cai et al., 2020; Eddy et al., 2022; Nadaroglu et al., 2017; Priyadarshini et al., 2021). Aside from adsorption, various techniques have been used to remove TB. These include biodegradation, the photo-Fenton process, and electrochemical oxidation (Britos et al., 2018; Dutta et al., 2019; Ghime & Ghosh, 2020).

The main goal of this study is to examine the removal of Trypan blue, which is a toxic dye, by biosorption by using dead biomass of *Aspergillus niveus (= Fennelia nivea*)

## 2. Materials and methods

### 2.1. Microorganisms and production of biomass

In this investigation, Aspergillus niveus (=Fennelia nivea) biomass—previously isolated from the soil, identified, and kept in the Hacettepe Biology Department stocks (Aracagök et al., 2021)—was utilized as an adsorbent in the removal of Trypan blue. Malt extract broth medium was used to produce fungal biomass to be used as an adsorbent. It was inoculated into Erlenmeyer flasks (1000 ml) with 500 ml malt extract broth and incubated for 5 days at the natural pH of the medium at a shaking speed of 150 rpm at 30°C., When the incubation period is over the media were autoclaved at 110 °C for 25 minutes to obtain dead biomass. The fungal biomass was separated from the liquid medium through the filter paper, the remaining biomass on the filter paper was washed with distilled water. The obtained heat-inactivated A. niveus mass was allowed to dry for two days at 50 °C. The dried dead biomass was pulverized with a mortar and passed through a 0.15 mm diameter sieve.

### 2.2. Dye removal experiments

TB dye removal experiments were performed in 50 ml Falcon tubes with 10 ml solution. Trypan blue powder (Merck, CAS NO: 72-57-1) was used to prepare the dye solution. pH values of dye solutions were adjusted using 0.1-1 N HCl and NaOH solutions.

To examine the impact of pH, from 4 to 9, solutions containing 25 mg/L TB dye and 10 mg of fungal biomass were created. 250 mg/L dye solutions were combined with 10 to 150 mg of dry biomass powder to examine the effects of the fungal adsorbent dosage.

By increasing the concentrations from 30 to 300 mg/L, the impact of the starting TB concentration was examined. As a result, various TB concentrations were examined during the investigation. The biomass used in these trials remained constant at 10 mg. The effects of 250 mg/L TB solutions and 10 mg/g biomass on contact time (5-1.440 min) were studied. The reactors were operated on rotary shakers for 24 hours at 150 rpm.

20 minutes are spent centrifuging the dye and adsorbent mixture at 4000 rpm. When the centrifugation process was complete, the supernatant was examined through a spectrophotometer at a wavelength of 594 nm to determine whether the quantity of TB had changed. Each test was run in triplicate.

The following equations were used to calculate the adsorption capacity ($Q_e$) and percentage of removal rates at equilibrium:

$$Q_e = \frac{(C_i - C_e)}{m} \; x \; V \tag{1}$$

$$\text{removal rate } (\%) = \frac{(C_i - C_e)}{C_i} x100 \tag{2}$$

### 2.3. Isotherms

Freundlich, Langmuir, Harkins-Jura, and Redlich-Peterson isotherm approaches were used to model the TB dye adsorption onto *Aspergillus niveus* biomass.

**Freundlich isotherm:**

This isotherm can be used to multilayer adsorption with non-uniform dispersion of adsorption affinities and heat over the heterogeneous adsorbent surface. Following is how this model is expressed (Foo & Hameed, 2010):

$$\log Q_e = \log K_F + \frac{1}{n} \log C_e \tag{3}$$

The adsorption coefficient is $K_F$, and the adsorption index is 1/n.

**Langmuir isotherm:**

The Langmuir isotherm makes the assumption, of monolayer adsorption, with adsorption occurring only at a constrained number of specific localized sites that are the identical and equivalent, with no lateral interaction and steric inhibition between the adsorbed species, even on contiguous sites. Following is how this model is expressed (Foo & Hameed, 2010) :

$$\frac{C_e}{Q_e} = \frac{Q_e}{Q_{max}} + \frac{1}{Q_{max} * K_L} \tag{4}$$

Ce (mg/L) denotes the concentration at equilibrium, Qe (mg/g) the adsorption capacity at equilibrium, KL (L/g) the adsorption power, and Qmax (mg/g) the maximal adsorption capacity.

The favourability of the adsorption was assessed using the dimensionless constant separation factor (RL) of the Langmuir model. Adsorption is either linear or nonlinear depending on the dimensionless separation factor values. (RL = 0), favourable (0<RL<1), or unfavourable (1>RL). Following is how this model is expressed (Foo & Hameed, 2010):

$$R_L = \frac{1}{1+K_L C_i} \quad (5)$$

**Harkins-Jura isotherm**:

Multilayer adsorption on the surface of adsorbents with heterogeneous distribution of pores is a possibility according to the Harkin-Jura isotherm model. Following is how this model is expressed (Ayawei et al., 2017):

$$\frac{1}{Q_e^2} = \frac{B}{A} - \frac{1}{A}\ (logCe\ ) \quad (6)$$

B is a model constant, and A is another model constant

**Redlich – Peterson isotherm:**

A hybrid isotherm made out of the Langmuir and Freundlich isotherms is known as the Redlich-Peterson isotherm. Following is how this model is expressed (Wu et al., 2010):

$$\frac{C_e}{Q_e} = \frac{1}{K_R} + \frac{a_R}{K_R} C_e^g \quad (7)$$

aR is the Redlich-Peterson model constant (L/mg), KR is the Redlich-Peterson isotherm constant (L/g), and g is the Redlich-Peterson exponent.

**2. 4. Kinetics**

The adsorption of TB dye on A. niveus biomass was evaluated using the pseudo-first order and pseudo-second-order kinetic models. Linearized first order kinetic model equation (Plazinski et al., 2009) :

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t \quad (8)$$

Another popular kinetic model for adsorption researches is a pseudo-second-order model, which is described as (Ho & McKay, 1998):

$$\frac{t}{q_t} = \frac{1}{q_e^2 K_2} + \frac{t}{q_e} \quad (9)$$

**2.5. Software**

Adsorption isotherms was plotted by R studio (PUPAIM) package. Statistical analysis was performed using Python, Sckit and Scipy libraries.

**3. Results and discussion**

**3.1. Effect of pH**

Heat-inactivated *A. niveus* biomass powder was used to remove TB, and the findings of this investigation are shown in Figure 1. The impact of changing the medium pH on TB removal was investigated at various pH levels with 25 mg/L TB, with a control group for each pH value. The group which pH was adjusted to 6 (Qe: 27.4 mg/g) showed the highest biosorption value at the end of the trial. The Kruskal-Wallis and Dunn's tests were used to determine the statistical significance of the acquired data. Since the p-value of the Kruskal-Wallis test (0.029) is less than 0.05, it can be concluded that there is a difference between the groups with different pH levels. According to Dunn's test (p-value (0.017) < 0.05), the pH 6 and pH 9 groups were the main

contributors to this difference. Based on the obtained data, it can be said that TB removal with A. niveus biomass is more efficient in an acidic medium compared to an alkali medium. In a previous study, both Luffa sponge and modified Luffa sponge effectively removed Trypan blue dye at pH 7 (Nadaroglu et al., 2017). In a study with MgO nanoparticles, the authors observed that Trypan blue adsorption occurs most efficiently at pH 4 (Priyadarshini et al., 2021). In a study with *Trichoderma harzianum*, the maximum removal efficiency for autoclaved and non-autoclaved fungal biomass was reached at pH 8 (Sadhasivam et al., 2005).The adsorbent, which is positively charged with the effect of increasing H+ ion at low pH, interacts more strongly with -SO3- groups in the TB dye (Akkaya et al., 2009). The medium pH value was chosen as 6 for further studies.l, 17-28.

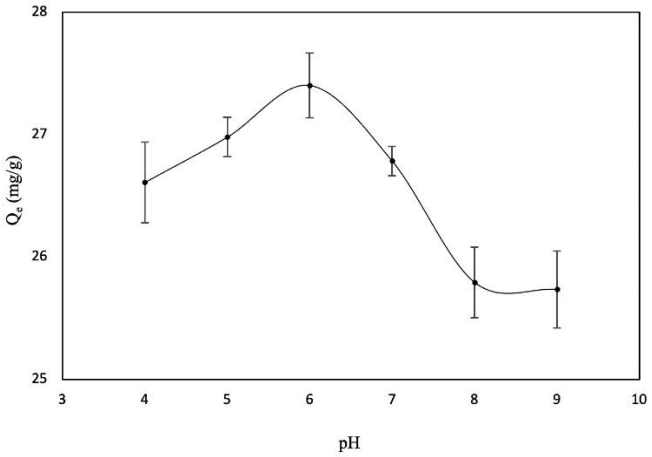


**Figure 1.** Effect of different pH values on trypan blue biosorption using heat-killed A. niveus biomass (10 mg biomass, reaction volume: 10 ml, TB concentration: 25 mg/L, T: 25°C, 150 rpm, each dot is the mean of the three values, and the error bars represent the standard deviation.)

### 3.2. Concentration of Trypan blue and isotherms

The effect of Trypan blue concentration on biosorption and the saturation point were investigated at dye concentrations ranging from 30 mg/L to 350 mg/L. The results showed that the adsorption capacity (126.4 mg/g) had increased to 250 mg/L dye concentration and also that saturation had been reached (Figure 2). In a study with magnesium nanoparticles, it was reported that the maximum TB adsorption capacity was 132 mg/g (Priyadarshini et al., 2021). In different studies using orange peel and avocado seed, TB adsorption capacities were found to be 172.41 and 19.30 mg/g, respectively (Eddy et al., 2022; El-Idreesy et al., 2021). In another study using luffa sponge and modified luffa sponge, TB removal capacities of TB adsorbents were found to be 45.32 and 47.3 mg/g (Nadaroglu et al., 2017).

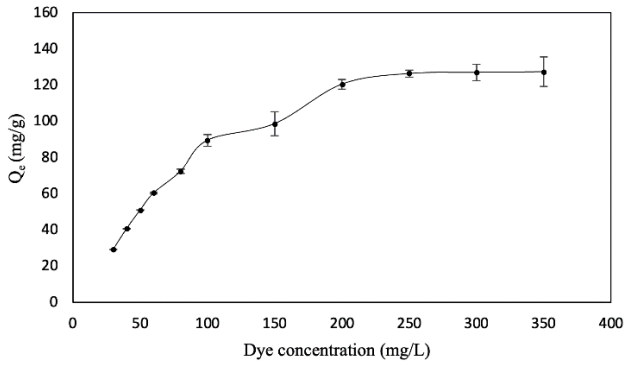


**Figure 2.** Effect of TB concentration on trypan blue biosorption using heat-killed A. niveus biomass (10 mg biomass, reaction volume: 10 ml, pH: 6, T: 25°C, 150 rpm , each dot is the mean of the three values, and the error bars represent the standard deviation.)

Adsorption of TB increases as the concentration of the adsorbate increases. This is due to the fact that as the amount of TB molecules diffused onto the adsorbent surface increases, so does the adsorption rate (Eddy et al., 2022). As the concentration of the dye increases, the adsorption capacity of the adsorbent also increases. This can be attributed to the high driving force for mass transfer that occurs at higher initial dye concentrations (Salleh et al., 2011).

In this study, Langmuir, Freundlich, Redlich-Peterson and Harkins-Jura isotherms were tested to find out which model would better explain the adsorption of TB dye on inactive *A. niveus* biomass (Figure 3).

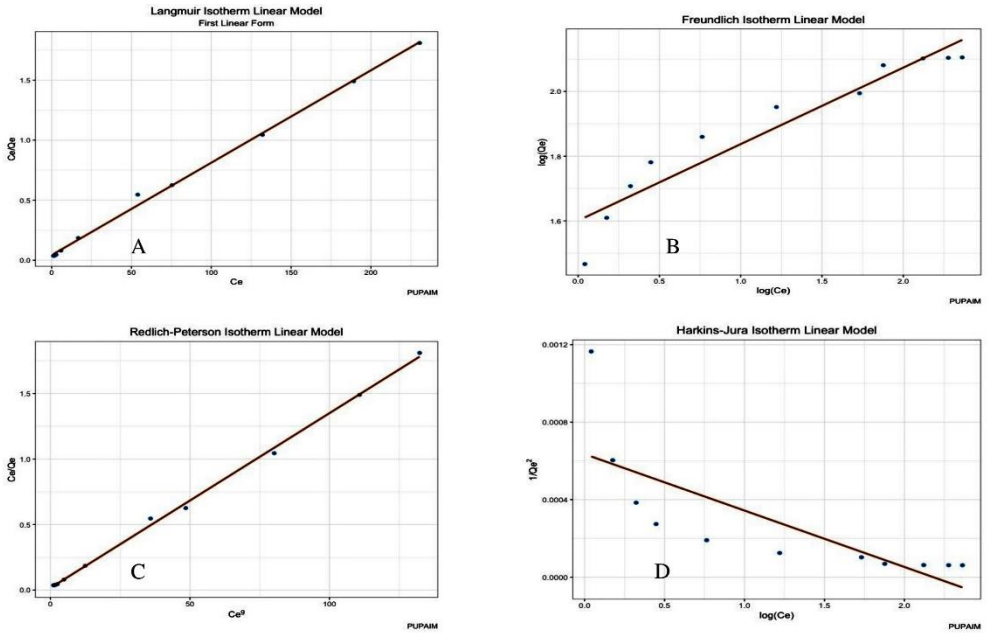


**Figure 3** Plots of Langmuir (A), Freundlich (B), Redlich-Peterson (C) and Harkins- Jura isotherms (D)

Redlich-Peterson and Langmuir isotherms have the highest R-squared value among the graphed isotherms (Table 1). The Freundlich and Langmuir isotherms are combined in the Redlich-Peterson isotherm model (Ayawei et al., 2017). Exponent **g** for the Redlich-Peterson isotherm can range from 1 to 0. The model is referred to as being Langmuir isotherm fitted if this number is close to 1 (Vijayaraghavan et al., 2006). It can be said that the biosorption of TB on the fungal mass follows the Langmuir model because the $R^2$ (0.9977) value obtained from the Langmuir isotherm is high, the g value (0.89) obtained from the Redlich-Peterson isotherm is close to unity. The monolayer and homogeneous Badsorption constrained sorption sites on the adsorbent were described using the Langmuir isotherm model. Adsorbate covers sorption sites once, and there will be no more adsorption after that (Hussain et al., 2021).

**Table 1**. Isotherm parameters

| **Isotherm models** | | |
|---|---|---|
| Langmuir | $Q_{max}$ | 130.02 |
| | $K_L$ | 0.1780 |
| | $R^2$ | 0.9977 |
| Freundlich | $K_F$ | 39.89 |
| | n | 4.25 |
| | $R^2$ | 0.9104 |
| Redlich-Peterson | $K_R$ | 60.25 |
| | $a_R$ | 0.8 |
| | g | 0.89 |
| | $R^2$ | 0.9984 |
| Harkins-Jura | A | 3434 |
| | B | 2.18 |
| | $R^2$ | 0.59 |

The Freundlich model was found to be more accurate in explaining TB adsorption with MgO nanoparticles (Priyadarshini et al., 2021). According to a study that used nano-Zn modified Luffa sponge for TB adsorption, the Langmuir isotherm model best explained the adsorption process (Nadaroglu et al., 2017). A study of TB adsorption with adsorbent from orange peel revealed that the adsorption followed the Langmuir isotherm model (Eddy et al., 2022). Due to the $R_L$ values being smaller than 1, which were computed using the original dye concentration employed, TB biosorption is favourable (Table 2).

**Table 2**. $R_L$ values for various experimental initial dye concentrations ($C_i$) at 25°C.

| $C_i$ (mg/L) | $R_L$ |
|---|---|
| 30 | 0.15 |
| 42 | 0.117 |
| 53 | 0.095 |
| 63 | 0.081 |
| 78 | 0.066 |
| 106 | 0.050 |
| 152 | 0.035 |
| 195 | 0.027 |
| 233 | 0.023 |
| 298 | 0.018 |
| 357 | 0.015 |

### 3.3. Effect of *A. niveus* biomass amount on Trypan blue removal

To find the biosorbent dose that resulted in the most effective TB removal, solutions containing 250 mg/L of TB dye and *A. niveus* mass in the range of 1000–15,000 mg/L were added. As anticipated, the percentage of removal rose as the biomass increased, yielding 94.2% Trypan blue elimination at 5000 mg/L biomass. (Figure 4). The number of binding sites for the dye in the solution will rise as the amount of biomass rises (Bayramoglu & Yilmaz, 2018).

In a study in which Pb (II) and Cr (III) biosorption was performed, it was reported that the heavy metal removal rate increased with increasing biomass.

In the same study, it was shown that the removal efficiency decreased due to the aggregates formed at a biomass concentration above 1 g/L (Bueno et al., 2008).

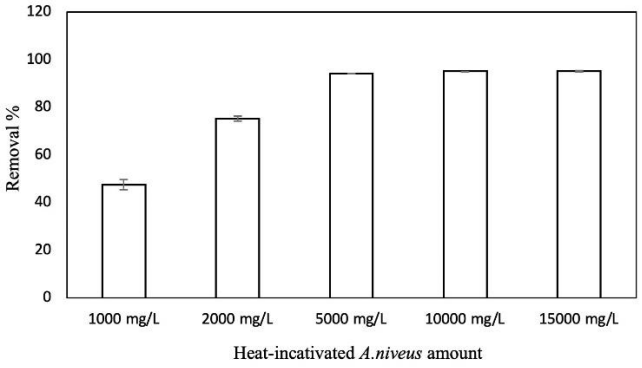


**Figure 4.** Effect of heat- killed *A. niveus* biomass amount on trypan blue removal percentage (reaction volume: 10 ml, pH:6, T: 25°C, 150 rpm, the error bars represent the standard deviation.)

### 3.4. Contact time and kinetics

Figure 5 shows the biosorption of TB dye onto biomass as a function of contact time. According to the findings, the dye was rapidly removed within the first five minutes. The dye biosorption process, which took place

rapidly in the first 60 minutes, continued more slowly in the following minutes and reached equilibrium at 180. minute (128.1 mg/g).

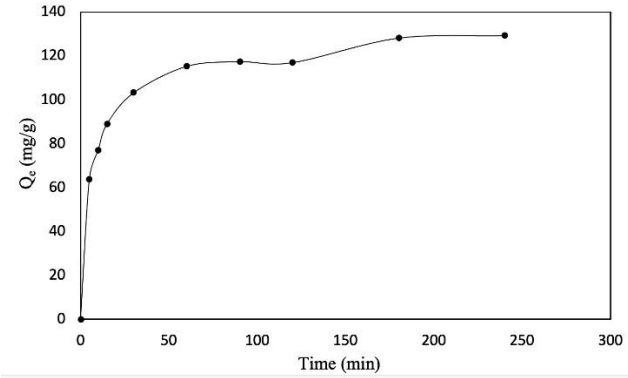


**Figure 5** The influence of contact time on biosorption of trypan blue (10 mg biomass, reaction volume: 10 ml, Dye concentration: 250 mg/L, pH: 6, T: 25°C, 150 rpm)

In the study with luffa sponge, the authors reported that equilibrium was reached within 30 minutes (Nadaroglu et al., 2017).

The Largergren model, also known as the pseudo-first-order model, is based on the proportionality between the number of filled sites versus the available sites. According to this hypothesis, physical interactions only occur between the adsorbate and the adsorbent's surface at one specific active site. The pseudo-second-order kinetic model assumes that the adsorption follows the square of the difference between the number of available and filled adsorption regions and that the adsorbate could bind to 2 existing sites with various binding energies. (Galloni et al., 2022). Table 3 depicts the assumed pseudo-second order model for TB dye biosorption on fungal biomass, with $R^2$ as the coefficient of determination. The slope of a linear line ($t/q_t$ vs. t) was used to calculate the theoretical amount of Trypan blue dye that fungal biomass can adsorb, $Q_e$ (129.87 mg/g). This value was found experimentally to be 128.1 mg/g.

**Table 3.** Kinetic parameters

| **Pseudo-first order** | | | **Pseudo-second order** | | |
|---|---|---|---|---|---|
| **$Q_e$ (mg/g)** | $k_1$ ($min^{-1}$) | $R^2$ | $Q_e$ (mg/g) | $k_2$ (g/mg/min) | $R^2$ |
| **61.16** | 0.00011 | 0.914 | 129.87 | 0.00102 | 0.997 |

Adsorption of Trypan blue onto chitin fits pseudo-second order rather than pseudo-fist order kinetic model (Akkaya et al., 2009). Another research showed that the adsorption of trypan blue dye on core-shell granular activated carbon follows the pseudo-second order kinetic model(Cai et al., 2020).

### 3.5. Temperature effect

The effect of temperature on the adsorption TB was investigated using 10 millilitres of solution containing 250 mg per liter of the dye, which was contact in with 10 milligrams of biomass at pH 6. The studies were conducted at three different temperatures: 4°C,25°C, and 45°C.

As can be seen in Figure 6, which shows the change in TB adsorption capacity with temperature change, the adsorption capacity increased with increasing temperature. The adsorption efficiency of TB with fungal biomass increased from 72 mg/g at 4°C to 149.79 mg/g at 45°C with increasing temperature. A study with Luffa sponge reported that the maximum removal of TB by adsorption occurred at 20°C (Nadaroglu et al., 2017) In a study with MgO nanoparticles, it was reported that TB removal decreased with increasing temperature (Priyadarshini et al., 2021).

It was reported that the adsorption of Reactive Yellow 86, an azo dye, with free and immobilised fungal biomass increased with increasing temperature (Bayramoglu & Yilmaz, 2018).

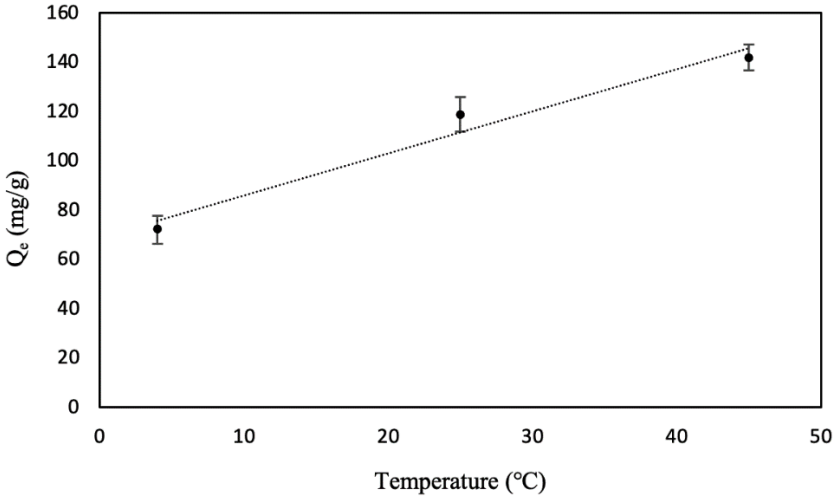


**Figure 6**. Effect of temperature on TB adsorption(10 mg biomass, reaction volume: 10 ml, Dye concentration: 250 mg/L, pH: 6, 150 rpm, each dot is the mean of the three values, and the error bars represent the standard deviation.)

## 4. Conclusion

The efficacy of heat-treated *Aspergillus niveus* biomass for the removal of the toxic and carcinogenic dye Trypan blue was examined, with the pH of the medium considered a crucial parameter due to its impact on both the adsorbent and adsorbate charge. The experimental results indicated that the highest biosorption efficiency was achieved at a pH of 6, with significantly higher biosorption values observed at lower pH levels than at higher pH levels. Additionally, the highest biosorption capacity was observed at a Trypan blue concentration of 250 mg/L, and the biosorption process followed both Langmuir and Redlich-Peterson isotherms. Notably, the Redlich-Peterson exponent, g, is closely approximated 1, suggesting conformity to the Langmuir model. The biosorption of Trypan blue by *A. niveus* was found to be rapid, with saturation of the adsorbent occurring at the 180th minute. Further analysis revealed that the pseudo-second order kinetic model provided a better fit for the biosorption of Trypan blue by *A. niveus* as compared to the pseudo-first order model.

## Author contribution

Experimental design and writing of the article were done by Y. Doruk ARACAGÖK

## Declaration of ethical code

The author of this publication affirms that there is no need for a legal permit or the approval of an ethical committee for the materials and procedures utilized in this investigation.

## Conflicts of interest

The author declares that there is no conflict of interest.

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