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Optimization of Toluidine Blue Biosorption in Aqueous Solutions Using *Polyporus Squamosus* Fungi as Absorbent by Response Surface Methodology

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Abstract

Textile wastewater including a large number of dyes and heavy metals can have adverse impacts on human health and surface water. In this work, biosorption Toluidine Blue from aqueous media onto natural *Polypourus squamosus* fungi as a low-cost biosorbent was investigated. Central Composite Design (CCD) in Response Surface Methodology (RSM) was successfully applied to optimize the biosorption condition. Medium parameters affected the biosorption of Toluidine Blue were determined to be initial pH, initial Toluidine Blue (Tb) concentration, temperature, and absorbent dosage. All experiments were carried out in a batch system using 250 mL flasks containing 100 mL of Toluidine Blue solution with a temperature-controlled magnetic stirrer. The Tb concentrations remaining in filtration solutions after biosorption were analyzed using UV-Spectro. With the obtained quadratic model, the optimal conditions for maximum biosorbed Toluidine blue were calculated to be 7, 27.5 mg/L, 35°C and 0.05 g for pH, C°, T (°C) and adsorbent dosage, respectively. Furthermore, most known isotherm models such as Langmuir and Freundlich were computed to find the best-fitted model.

Keywords: Biosorption; Toluidine blue; Optimization; *Polyporus squamosus*; Response surface methodology.

1. Introduction

Wastewater contains both organic and inorganic chemicals consisting of dangerous compounds including dyes and surfactants, and also other contaminants such as dissolved and suspended solids, salts, dispersing agents, acids, alkalis, softeners, fixing agents, and other toxic compounds [1]. These dye wastes and contaminants can remain in the environment for a very long period if adequate treatment is not administered, and subsequently, they become oncogenic and mutagenic through compositional changes [2].

Toluidine blue was selected as the model contaminant because it is an important industrial pollutant and has been observed to exert negative effects at very low concentrations. In recent years, response surface methodology (RSM) has been widely used as a statistical method for rational experimental design and process optimization in the absence of mechanistic information, in contrast to traditional methods [3]. The reasons for its popularity are that it does not require additional consumption of chemicals for each parameter, nor is it especially time-consuming, costly, or labor-intensive [4-7].

The other class of technologies is based on the biodegradability of dyes. Biological methods of dye degradation are regarded as the most economically practical and eco-friendly methods. Textile dye biodegradation can be achieved using several microorganisms, which include bacteria and fungi. Literature shows that wide varieties of microorganisms, including bacteria, algae, and fungi, have good sorption ability for a number of pollutants [8]. Among these microorganisms, fungi seem to be a good sorption material, because it can be produced economically using simple fermentation techniques and economic growth media. Fungi are also available as a by-product or waste material from various industrial processes [9]. In the context of this study, *Polyporus squamosus* has selected to investigate the efficacy of fungi in the biosorption of Toluidine blue, which are common, used textile dyes.

Polyporus squamosus is a rather common fungus to find during April and May. Unfortunately, this fungus seems to be more common than morels in some areas, mostly because they are quite a bit larger, up to 2 ft. across sometimes! At least they are much easier to find than morels because they stick out as shelves from the lower portion of dead tree trunks, especially elms. This fungus generally fruits in the spring in most areas, but it is also seen in some places in the fall season. They can grow to be quite large and stick out as shelves from the sides of trees near the base. These shelves have squamules (scales) on the upper surface that give it its species name. The genus name *Polyporus* means "many pores." The pores are lined with basidia that produce basidiospores [10]. *Polyporus Squamosus* commonly referred to as Dryad's Saddle grows in overlapping clusters and tiers on broad-leaved trees.

(A dryad is a mythical wood nymph.) The fruit bodies appear in summer and autumn. Insects quickly devour these large brackets, and in warm weather, they can decay from full splendor to almost nothing in just a few days.

2. Materıals and Methods

2.1. Preparation of Adsorbent

Natural *Polyporus squamosus* fungus was collected from Yüksekova Territory in Hakkari Region by Yuzuncu Yil University, Biology Department, Van, Turkey. Naturally obtained *Polyporus squamosus* fungi was ground with a mill and sieved to obtain the desired particle size (below 150 µm) and then it was stored in desiccators for further utilization after drying oven at 40°C for 24 h.

2.2. Preparation of Stock Solution

A stock solution of Toluidine blue textile dyes was prepared by dissolving a weighed amount of Toluidine blue textile dyes powder in distilled water. The required dilutions were made from the stock solution by using different ppm and pH to prepare solutions in the different weight concentrations.

2.3. Adsorption Study

All experiments were carried out in a batch system using 250 mL flasks containing different mL Toluidine blue textile dyes solution and with a temperature-controlled magnetic stirrer. The Toluidine blue textile dyes concentrations remaining in filtration solutions after biosorption was analyzed by using a UV-VIS spectrophotometric. The adsorbed Toluidine blue textile dyes amount was calculated according to the following equation Eq. 1 [3].

$$q_e = \frac{(Co - Ce) \cdot V}{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where **C◦** and $\mathbf{C_e}$ are the initial and equilibrium concentrations (mg/L) of Toluidine blue textile dyes solution, **m** is the weight (g) use of the biosorbent and **V** is the volume (L).

2.4. Response Surface Methodology

Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables. The main advantage of RSM is the decreased number of experimental trials required to interpret multiple parameters and their interactions. To determine a suitable polynomial equation, which would describe the response surface, Response Surface Methodology (RSM) was employed to optimize the process [11]. Response surface methodology (RSM) is extensively used optimization methods for reasonable experimental design and process optimization in biosorption research. The optimization of the biosorption process goals at finding definite conditions such as environmental design parameters for the best possible response, and efficiency removal.

Generally, biosorption is a property of certain types of inactive, dead, microbial biomaterials to bind and concentrate heavy metals from even very dilute aqueous solutions. Biomass exhibits this property, acting just as a chemical substance, as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi, and bacteria, which was found responsible for this phenomenon. Living as well as dead (metabolically inactive) biological materials have been sought to remove metal ions. It was found that various functional groups present on their cell wall offer certain forces of attractions for the metal ions and provide high efficiency for their removal [12].

For optimization of the adsorption process, an experimental design can be carried out with four independent parameters including the initial concentration (C0), temperature (0C), amount of adsorbent (D), and PH (A). To explore the effect of different variables on the response in the region of investigation, a central composite design (CCD) with three variables at three levels was performed. Initial solution pH, temperature, and initial Tb concentration were evaluated as the most effective independent variables and their levels were determined according to the literature [13].

$$\hat{Y}_n = \beta_0 + \sum_{i=1}^{3} \beta_i\, x_i + \sum_{i=1}^{3} \beta_{\,ij}\, x_i^2 + \sum_{i=1}^{3}\sum_{j=i+1}^{3} \beta_{ij}\, x_i x_j \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where $\hat{y}$ is the response, β_0 is the constant coefficient, X_i (i = 1–4) are non-coded variables, β_i is the linear, and β_{ii} is quadratic, and β_{ij} (i and j = 1–4) is the second-order interaction coefficients.

The variance analysis (ANOVA) data were computed by Design-Expert 6.0 (trial version) to obtain the interaction between the process variables and the response. The quality of the fit of the polynomial model was expressed by the coefficient of determination (R2), and it is statically significance was checked by the F-test in the same program.

3. Results and Dıscussıon

3.1. Fitting of Process Models and Statistical Analysis

Central Composite Design (CCD) assays for optimization of meaningful parameters, such as initial pH, C° (mg/L), T (°C), and amount dosages of the biosorbent with the metal solution (experiment time) (min.), were complete to determine the maximum removal of Toluidine blue by resolution particular 7.0 (test conversion). In CCD, the amounts of the fully considered parameters are shown at three code levels by the platform.

The three coded levels of each parameter were assigned to −1, 0, +1. However the lowest and highest accounts of the parameters were −1 and +1, respectively, the center of the lowest and highest accounts was inspected as the middle mark (0). Both with six responses guide at the middle values to guess the lucid mistake, twenty-four workouts were carried out for optimization.

The statistical meaning of the square model was foredoomed by the analysis of disparity (ANOVA) as shown in **Table 1**. The account of the degree of limitation (R2 =0.7893) announces that 92% of the variability in the reply is cleared by the specimen. The specimen equation for encoded (real) accounts of the equation sample rigging the empirical outcome is shown in bellow:

Biosorbed amount Toluidine blue dye (mg/g) = +7.47288+2.07280 pH+4.51980×E-003 Initial conc.($C_◦$)–0.65385 *T* ($^{\circ}$C)+251.72573 Biosorbent dosage (g)–2.83333E-003 pH·Initial conc.($C_◦$)+0.012750 pH · T ($^{\circ}$C)+0.30556 pH · Biosorbent dosage (g)+2.09259×E-003 Initial conc.($C_◦$) · T ($^{\circ}$C)+3.20370 Initial conc.($C_◦$) · Biosorbent dosage (g)+1.39815 T ($^{\circ}$C) · Biosorbent dosage(g)–0.16803 pH2–7.61941E-004·Initial conc.($C_◦$)2+6.88582E-003 · T ($^{\circ}$C) –2938.61218 · Biosorbent dosage (g).

Table-1. CCD results for Toluidine blue dyes adsorption onto *Polyporus squamosus* fungi

Run	Initial pH	Initial TB Conc. C_o/(mg·L^{-1})	*T*/°C	Biosorbent dosage/g	Biosorbed TB /(mg·g^{-1})
1	12	50	20	0.1	10.00
2	12	50	50	0.1	21.344
3	7	27.5	35	0.06	9.49
4	7	27.5	35	0.06	8.18
5	7	27.5	20	0.06	7.80
6	2	50	50	0.1	4.00
7	2	50	20	0.01	15.9294
8	12	27.5	35	0.06	12.08
9	12	50	20	0.01	3.35
10	2	5	20	0.1	0.50
11	12	5	20	0.01	1.75
12	2	50	50	0.01	1.70
13	7	50	35	0.06	1.11
14	7	27.5	35	0.01	2.99
15	7	27.5	35	0.06	9.67
16	7	27.5	35	0.06	9.44
17	12	5	50	0.1	2.50
18	7	27.5	35	0.1	12.08
19	2	27.5	35	0.06	15.9294
20	7	27.5	50	0.06	9.85
21	2	5	50	0.01	0.25
22	2	5	50	0.1	17.701
23	7	50	35	0.06	13.89
24	12	50	50	0.01	21.344
25	12	5	50	0.01	2.50
26	12	5	20	0.1	2.25
27	2	50	20	0.1	15.9294
28	7	27.5	35	0.06	9.17
29	2	5	20	0.01	27.7278
30	7	27.5	35	0.06	9.31

A table showing watched expulsion of Toluidine blue against that acquired is shown in Fig 1. The figure explains that the foretell reply from the experiential specimen is in good accord with the spotted datum. Generally, it is necessary to regulate the fitted pattern to ensure that it expands a good convergent of the true order.

Except the pattern appears a useful befit, emergence with realization and optimization of the fitted response surface want probable grant bad or misguiding outcome. The remaining plays an important function in separate the fill of the sample.

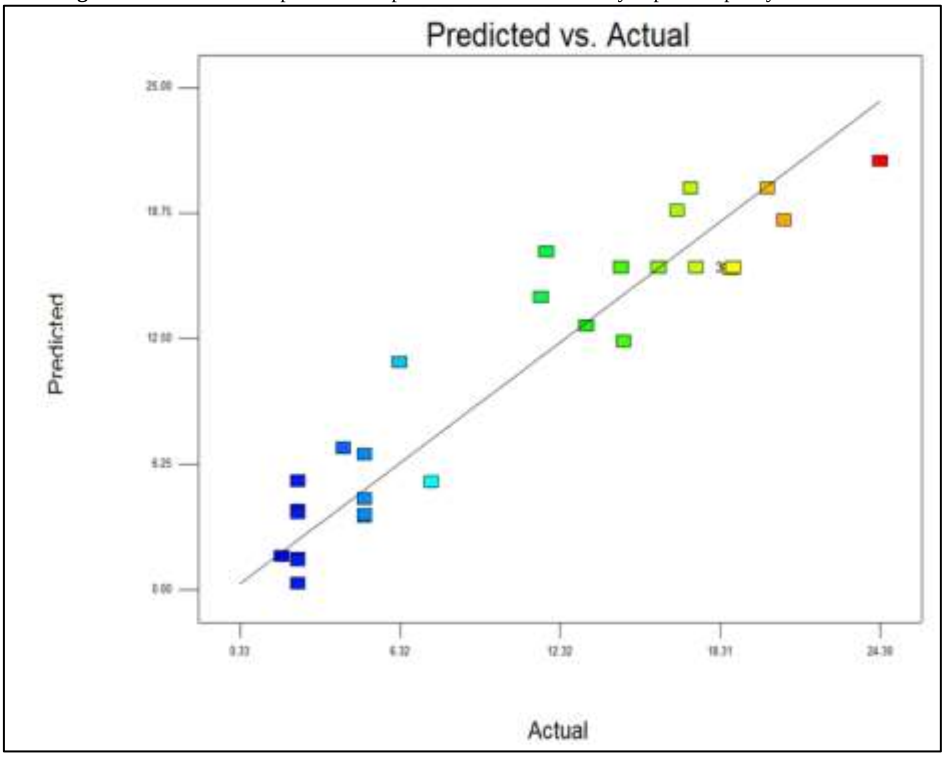
The low p-value appears that the second-order quadratic model for watched outcomes is considerable. The amount of the degree of limitation (R^2=0.9073) announces that 86% of the changeable in the react is cleared by the formal [3].

Table-2. Analyses of difference (ANOVA) for Toluidine blue dye removal using Response Surface Quadratic Model

Origin	**Sum of quadrate**	**Df**	**Mean quadrate**	***F* Account**	**p-account Prob > *F***
type significant	1341.76	14	95.84	8.76	<0.0001
A-Ph	1	5	0.46	0.5087	
B-Initial conc. (C_o)	304.55	1	304.55	27.84	< 0.0001
C-Temperature (°C)	10.89	1	10.89	1.00	0.3343
D-Biosorbent dosage	166.84	1	166.84	15.25	0.0014
AB1.63	1	1.63	0.15	0.7053	
AC14.63	1	14.63	1.34	0.2656	
AD0.076	1	0.076	6.912E-003	0.9348	
BC7.98	1	7.98	0.73	0.4065	
CD14.25	1	14.25	1.30	0.2716	
$A^2$45.15	1	45.15	4.13	0.0603	
$B^2$0.36	1	0.36	0.033	0.8592	
$C^2$6.14	1	6.14	0.56	0.4653	
$D^2$90.61	1	90.61	8.28	0.0115	
Residual	164.11	15	10.94		
Lack of fit significant	144.28	9	16.03	4.85	0.0340
Pure Error	19.83	6	3.30		
Core Total	1505.87	29			

Fig 1. Announces that the objected reply from the experiential specimen is in perfect accord with the observed facts. As the dots on the scheme follow an upright line, it can be finished that the precipitate is naturally assorted and facts conversion is not wanted. Whereupon, it insistent that the potential of the empirical facts acquired from increased square specimens for the adsorption of Toluidine blue dyes by Polyporus squamosus fungi is completely favorable.

Fig-1. The observed TB uptake versus predicted Toluidine blue dye uptake capacity of adsorbent



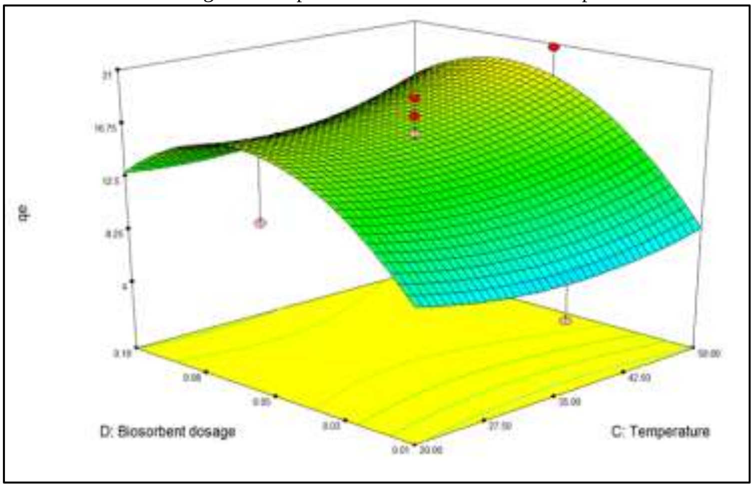
3.2. Effect of Influential Factors

3.2.1 Adsorbent Amount (Dosage) Effect

Fig. 2. offers the synchronous effects of adsorbent dosage and temperature on Toluidine blue dyes waste by *Polyporus squamosus* fungi. The temperature has a positive effect on Toluidine blue dyes adsorption. Waste of Toluidine blue dyes by *Polyporus squamosus* fungi gently increased with temperature and gains its ceiling value of

about 50 ºC. Then waste of Toluidine blue dyes decreased by increasing adsorbent dosage. This is due to the spot that at higher adsorbent dosage, the concentration of the solution drops to a small amount, and the method field balance at a small amount adsorbed per unit weight of adsorbent.

Fig-2. Simultaneous effects of adsorbent dosage and temperature on TB waste at constant pH 7 and initial concentration 27.50 mg/L

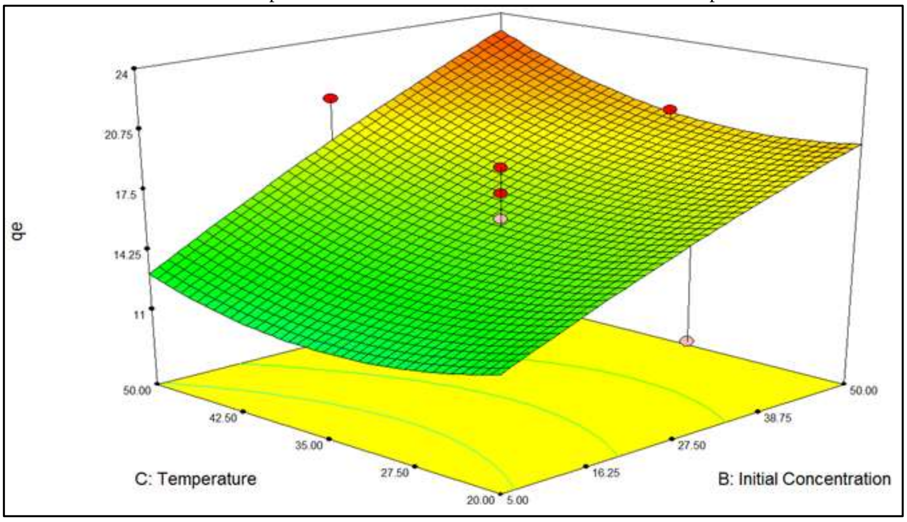


3.2.2. Temperature Effect

Fig. 3. represent the effects of medium-temperature and initial Toluidine blue dyes concentration on dye waste ability of Toluidine blue dyes at constant pH 7 and adsorbent dosage 0.05 g. The waste ability of the Toluidine blue dye increased with initial concentration increasing from 5.00 to 50.00 mg/L.

There are reciprocal effects of temperature and initial Toluidine blue dyes concentration on adsorption ability by *Polyporus squamosus* fungi at a constant pH of 7 temperature showed a considerable effect on the waste of Toluidine blue dyes onto *Polyporus squamosus* fungi.

Fig-3. Simultaneous effects of medium temperature and initial concentration of TB at constant pH 7 and adsorbent dosage 0.05(g)



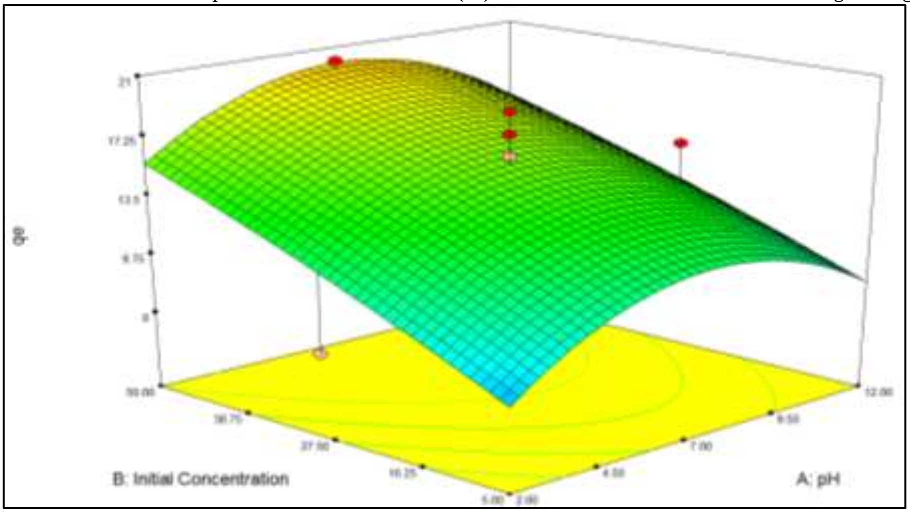
Toluidine blue dyes waste ability for *Polyporus squamosus* fungi softly increased with increasing temperature from 20 to 50°C and almost attained to outside account about 35°C. Then the adsorption ability of Toluidine blue dyes onto *Polyporus squamosus* fungi increase with increasing temperature and this resultant appears adsorption is endothermic in circumference. The temperature of the solution is the main part of adsorption ability. The adsorption is an endothermic process when the adsorption ability increases with increasing temperature were increased with the increase of temperature solution and our mechanisms verified this knowledge as well Dawood, *et al.* [14]. The increase of technique with the increase in adsorption can be due to a purpose for the Toluidine blue dyes to pertain from the solid phase to the block phase when the temperature of the solutions was increased.

3.2.3. pH Effect

In the existing action, the adsorption of Toluidine blue dyes on *Polyporus squamosus* fungi was advised at different pH levels spread from 2.0 to 12.0. At higher pH solution, electrostatic disharmony is necessitated between the dye molecules and the negatively charged surface, so lessening the percentage waste of anionic dyes and adsorption ability [15]. The adsorption ability of dye depends on the pH solution. Mostly, low pH solution outcomes in augmentation in the anionic percentage dye of waste because of the electrostatic gravitation between the positive surface charge of the adsorption and anionic dye. High pH solution consequence in augmentation in the cationic percentage dye waste because the adsorbent exterior shows negatively charged and a positive charge on the solution interface will lower [16].

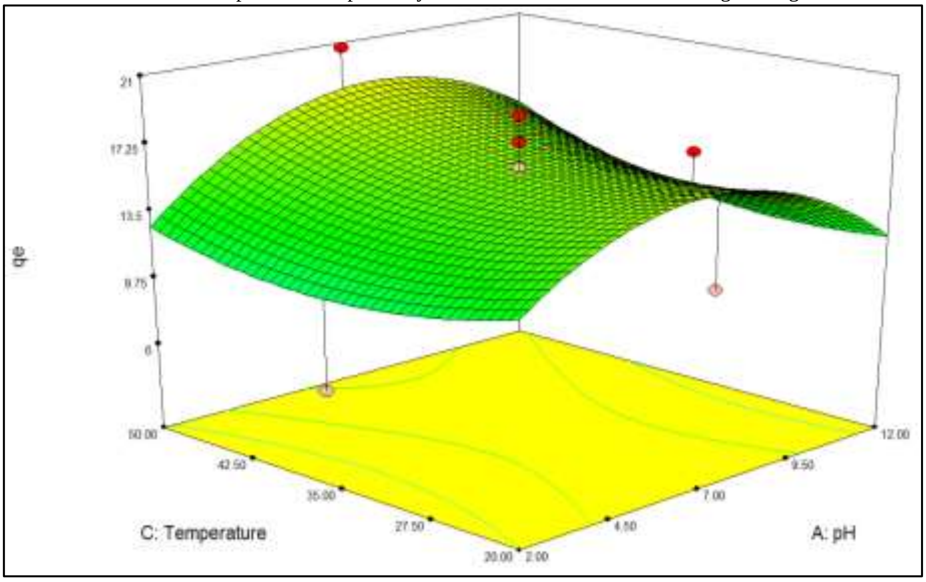
Fig. 4 offer the adsorption ability of *Polyporus squamosus* fungi quickly augment with growing $C_{\circ}$ from 5.00 to 50.00 mg/L and nearly connected a maximum at 50.00 mg/L. This outcome can be clarified because of the react of Toluidine blue dye and *Polyporus squamosus* fungi adsorbent. When $C_{\circ}$ was 27.50 mg/L, the dye understanding change to equilibrium and all positions were satiate with dye. This phase is the systematic adsorption point and the average of addendum of adsorption capacity regularly slows with increasing $C_{\circ}$, at last, the dye uptake ranges balance. At the lower Toluidine blue dye concentration, the proportion of number of moles of dye waste to the present adsorption point is low, and thus the quantity adsorbed per unit adsorbent augment slowly [17].

Fig-4. Simultaneous effects of and pH and initial concentration ($C_{\circ}$) on TB waste at constant adsorbent dosage 0.05 g and 35°C



The Toluidine blue dye waste ability of biosorbent is unexpectedly excessed when the pH of the solution excessed from 2 to 12 as shown in Fig. 5. The outcome showed that the utmost waste of dye was done at pH 7. Toluidine blue dyes waste ability for *Polyporus squamosus* fungi gently excessed with excessing temperature from 20 to 50°C and about arrived at a maximum value about 35°C Synchronous impacts of pH and temperature on the waste ability of Toluidine blue dye for *Polyporus squamosus* fungi at a constant initial concentration of 27.50 mg/L are explained in Fig. 5.

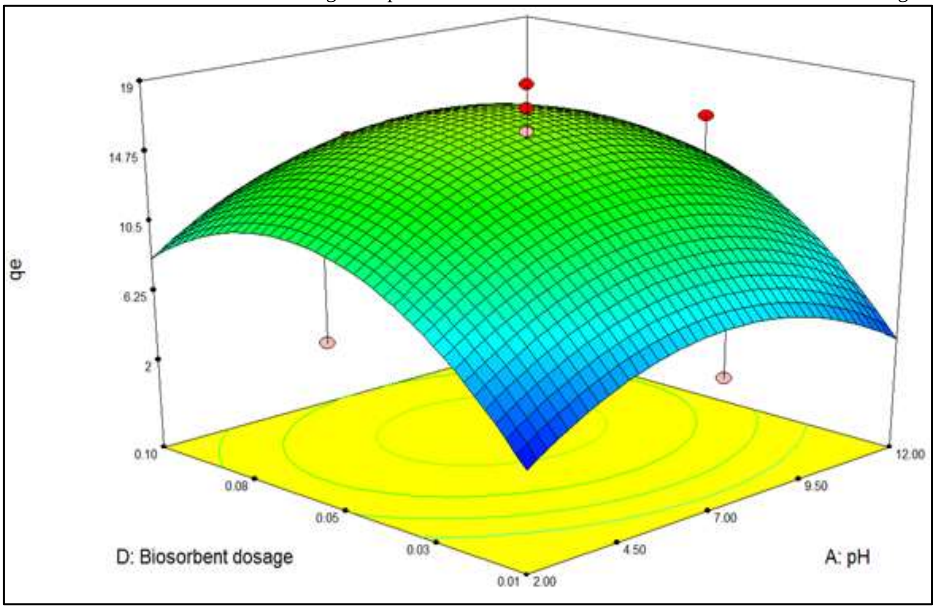
Fig-5. Simultaneous effects of temperature and pH on dye waste at constant adsorbent dosage 0.05 g and $C_{\circ}$ of 27.50 mg/L



Temperature showed a large impact on the waste of Toluidine blue dyes onto *Polyporus squamosus* fungi. Toluidine blue dyes removal disposition for *Polyporus squamosus* fungi genially excessed with excessing temperature from 20 to 50°C and about arrived at a maximum value about 35°C Synchronous impacts of pH and temperature on the waste ability of Toluidine blue dye for *Polyporus squamosus* fungi at a constant initial concentration of 27.50 mg/L are shown in Fig. 5.

As represented in Fig. 6. while the waste of Toluidine blue dyes excessed pointedly when pH rose from 2 to 12, waste ability reduce over this pH. Maximum waste was observed at pH 7. The pH demand of dye understanding is related to both the dye chemistry in solution and the functional groups on the adsorbent surface, which impacts the surface freightage of the adsorbent and the degree of ionization of the adsorbate. Surface charge intensity is regarding media pH.

Fig-6. Simultaneous effects of adsorbent dosage and pH on TB waste at constant initial concentration of 27.50 mg/L at 35 °C

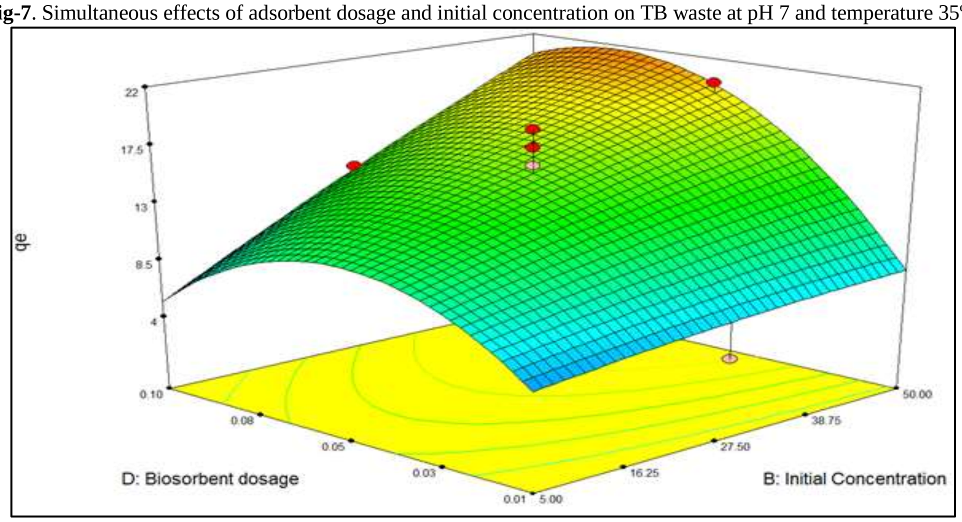


The waste ability of *Polyporus squamosus* fungi quickly excessed when the adsorbent dosage excessed from 0.01 g to 0.1 g and almost attain a maximum at 0.1 g. The excess in waste of Toluidine blue dye with excessing adsorbent dosage can be depicted by the excess in energetic places in the antecedent amount of adsorbent, accordingly fixed that easier penetration of dye waste to active sites. Then waste of Toluidine blue dyes reduced by excessing adsorbent dosage from 0.01 to 0.1 g. For each unit weight of adsorbent this is due to the reality that at higher adsorbent dosage, the initial concentration of the solution breakdown to a lower value and the order connect balance at lower values adsorbed for each unit weight of adsorbent, display that the adsorption sites stay unsaturated [18].

Fig. 7 displays the adsorption ability of *Polyporus squamosus* fungi quickly excessed with excessing C0 from 5.00 to 50.00 mg/L and about attained a maximum at 27.50 mg/L. This outcome can be clarified because of the interaction of Toluidine blue dye and *Polyporus squamosus* fungi adsorbent. At the lower Toluidine blue dye concentration, the proportion of the number of moles of dye waste to the presenting adsorption place is low, and wherefore the amount adsorbed per unit adsorbent excesses tardily.

The waste ability from *Polyporus squamosus* fungi quickly excessed and about attained a maximum at 0.1 g. The excesses in waste of Toluidine blue dye with excessing adsorbent dosage can be clarified by the excesses in strong sites in the forward amount of adsorbent, accordingly fixed that easier penetration of dye waste to active sites [19].

Fig-7. Simultaneous effects of adsorbent dosage and initial concentration on TB waste at pH 7 and temperature 35ºC



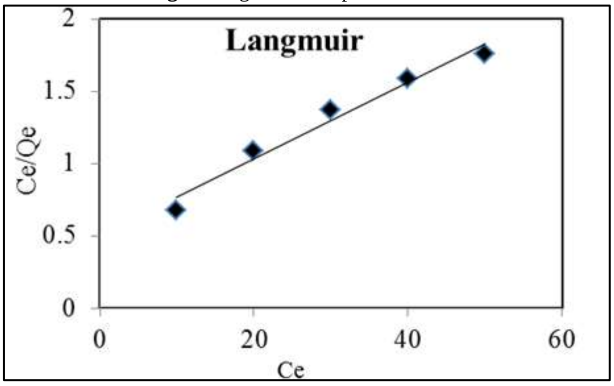
3.3. Biosorption Isotherm Studies

Several isotherm models were always utilized for analysis of the experimental result to inspect the association between the adsorbent and adsorbate amount at equilibrium. In order to explain the experimental data, the Langmuir and Freundlich kinetic models were investigated. The main Langmuir assumption is the monolayer formation (homogenous) of adsorbate species on the surface of adsorbent in the solution at equilibrium without interaction between the solute molecules [20].

A linear Langmuir adsorption isotherm is given in Fig. 8. The values of **QM** and **KL** of the linear term of Langmuir adsorption isotherm have studied the slopes and object of the **Ce/QE** with **Ce** according to Eq. (3).

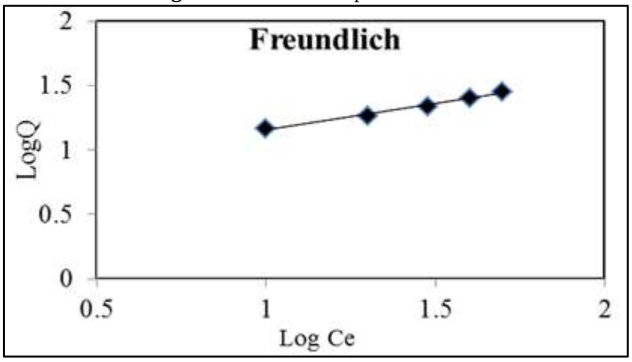
$$\frac{C_e}{q_e} = \frac{1}{q_{max} K_L} + \frac{1}{q_{max}} C_e \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Fig-8. Langmuir adsorption isotherm



The slope and the object agree to 1/n and k_f relatively. It was detected that the plot of ln qe and ln Ce crop a reversible line. In the sitting research, the value of the connection coefficient (R^2=0.9702) is higher than the Langmuir isotherm value, but the slope is reversible.

Fig-9. Freundlich adsorption isotherm



Freundlich isotherm model however Langmuir isotherm suppose that enthalpy of adsorption is separate from the amount adsorbed, the experimental Freundlich equation, founded on sorption on the heterogeneous surface, can be extract supposition a logarithmic reducing in the enthalpy of adsorption with the excess in the part of taken sites. The Freundlich equation is simply experimental based sorption on the heterogeneous surface.

The slope and the object agree to (1/n) and kf, relatively. It was detected that the plot of log qe and log Ce yields a reversible line (Fig. 9). In the sitting research, the value of the attachment coefficient (R^2 = 0.9878) is higher than the Langmuir isotherm value, but the slope is reversible, which means it isn't Freundlich isotherm model.

Sitting a comparison between the Toluidine blue biosorption ability of *Polyporus squamosus* and some biosorbents used in the literature. *Polyporus squamosus* is a cheap biosorbent and is plenty in nature. We can infer that *Polyporus squamosus* has a higher biosorption ability for Toluidine blue than some other natural biosorbents. Due to these properties, *Polyporus squamosus* has a large possibility for the waste of Toluidine blue from aqueous media.

4 Conclusion

Studies on the waste of organic pollutants by biosorbent such as fungi are exclusive. In increment *Polyporus, the squamous* fungus is an inbred substance that does not make toxic dangerous to have possible for use in, unlike place. Biosorption of Toluidine blue from watery surroundings by a new biosorbent, *Polyporus Squamosus*, will fabricate an important contribution to the literature because it is a novel adsorbent-adsorbate gathering.

The Central Composite Design (CCD) in Response Surface Methodology (RSM) was successfully used to optimize the biosorption adverb for Toluidine blue biosorption onto *Polyporus squamosus* fungus. A quadratic formal was average in words of initial pH, initial concentration C°, temperature T (°C), and time to explain the maximum biosorbed Toluidine blue. With the acquired quadratic formal, the better status for maximum biosorbed Toluidine blue was studied to be 7, 27.5 mg/L, 35°C, and 0.05 g for pH, C, T(°C) and adsorbent dosage, respectively. Beneath the resolved optimal circumstance, the outside amount of biosorbed Toluidine blue and waste yield were studied using the quadratic model to be 22.8 mg/g and 30.22%, respectively. *Polyporus squamosus*, which an active biosorbent for the waste of organic pollutants such as Toluidine blue**.**

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