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PROCESS OPTIMIZATION OF TOLUIDINE BLUE DYE REMOVAL USING BROWN ALGAE POWDER WITH CENTRAL COMPOSITE DESIGN (CCD)

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ABSTRACT
Discharge of dye-containing effluents from textile and related industries remains a persistent water-quality problem because many synthetic dyes are visible at very low concentrations and resist conventional biological treatment. This study examines the equilibrium removal of Toluidine Blue (TB) from aqueous solution using a low-cost marine algal biosorbent (Sargassum tenerrimum). Batch adsorption experiments were performed to evaluate the effects of solution pH (2–9), initial TB concentration (20–200 mg/L), and biosorbent dosage (10–80 g/L) under fixed mixing conditions and a constant contact period (kept fixed for all experiments). TB removal increased with pH up to an optimum around pH 5, followed by a decline at higher pH. Increasing the initial TB concentration reduced percentage removal but increased the adsorption capacity, consistent with progressive site saturation. Increasing biosorbent dosage improved removal efficiency up to an optimum region, after which the gain became marginal due to reduced effective surface area per unit mass and particle aggregation. Equilibrium data were interpreted using Langmuir, Freundlich, and Temkin isotherms to describe surface coverage and adsorption heterogeneity. Overall, the results support Sargassum tenerrimum as an inexpensive biosorbent for TB removal. Central composite design (CCD) was further applied to optimize the combined effects of pH, initial dye concentration, biosorbent dosage, and temperature on TB removal, predicting an optimum near pH ≈ 5.01, C0 ≈ 20.04 mg/L, w ≈ 30.818 g/L and T ≈ 304.83 K with ~91.66% removal.

Keywords: Toluidine Blue; Biosorption; Sargassum tenerrimum; pH effect; Adsorbent dosage; Adsorption isotherm

INTRODUCTION

Colored effluents from textile dyeing, printing, paper, and leather processing are a common cause of surface-water aesthetic degradation and can also interfere with aquatic photosynthesis by limiting light penetration. Many dyes and their transformation products are persistent and may exhibit toxicity, so regulatory pressure increasingly requires effective decolorization prior to discharge. While coagulation, advanced oxidation, membrane filtration, and biological treatment can remove dyes under certain conditions, these options may be limited by high chemical demand, energy use, sludge generation, or poor biodegradability of aromatic dye structures [1,3]. Adsorption is widely used for color removal because it can treat dilute streams and produce a clear effluent with simple operation [4, 21]. However, commercial activated carbon remains expensive for routine application in small and medium-scale facilities. Biosorption using abundant biomaterials is therefore attractive, especially where the adsorbent is locally available and requires minimal processing [2, 5]. Marine algae-based materials contain functional groups such as hydroxyl, carboxyl, and sulfate (sulfated polysaccharide) that can interact with dye molecules through electrostatic attraction, hydrogen bonding, and surface complexation [19, 20]. Among these, Sargassum species have been widely explored because they are plentiful in coastal regions and can be processed into a stable sorbent [7, 22]. Toluidine Blue (TB) is a cationic thiazine dye used in

biological staining and industrial formulations [9]. Its cationic nature makes solution pH a key variable because pH controls both the dye speciation and the surface charge of the biosorbent. Similarly, initial dye concentration governs the driving force for mass transfer and the extent to which adsorption sites become saturated, while biosorbent dosage influences the number of available sites and the probability of dye–sorbent contact. This paper reports equilibrium adsorption behavior of TB on *Sargassum tenerrimum* and applies response surface methodology using a central composite design (CCD) to optimize TB removal as a function of pH, initial dye concentration, biosorbent dosage, and temperature [13,17]. Detailed kinetic, thermodynamic, and particle-size investigations are not included.

MATERIALS AND METHODS

MATERIALS

Toluidine Blue dye (analytical grade) was used as the adsorbate. Hydrochloric acid (0.1 N) and sodium hydroxide (0.1 N) were used for pH adjustment. Distilled or deionized water was used for all solution preparation and washing steps. The biosorbent was prepared from the marine alga *Sargassum tenerrimum* collected from the coastal region of Visakhapatnam (India).

Biosorbent preparation

Fresh *Sargassum tenerrimum* was rinsed repeatedly with tap water to remove sand, salts, and surface debris, followed by washing with distilled water until the wash water was clear. The material was sun-dried until constant mass, cut into small pieces, ground, and sieved to obtain a uniform powder. The dried powder was stored in airtight containers and used without chemical activation. A single sieve fraction was selected for all experiments to avoid confounding by particle-size effects.

Dye solution preparation

A 1000 mg/L stock solution of TB was prepared by dissolving 1.0 g of dye in 1 L of water. Working solutions (20–200 mg/L) were prepared by dilution. Solution pH was adjusted to the desired value using 0.1 N HCl or 0.1 N NaOH and measured using a calibrated pH meter.

Batch adsorption procedure

Batch adsorption tests were performed in Erlenmeyer flasks containing a fixed volume of TB solution (50 mL) and a measured mass of biosorbent. Flasks were agitated in an orbital shaker at a constant speed (100 rpm). The temperature was maintained at 303 K for all equilibrium tests.

After the fixed contact period of 30 minutes (kept constant for all experiments and selected based on preliminary screening), the biosorbent was separated by filtration.

The residual dye concentration (Ce, mg/L) was measured using a UV–Visible spectrophotometer at the wavelength of maximum absorbance for TB (λmax = 630 nm). Calibration curves were prepared from standards over the working concentration range.

Adsorption capacity at equilibrium (qe, mg/g) and percentage removal were calculated as:

$$qe = (C0 - Ce)\, V / m$$

$$\text{Removal (\%)} = [(C0 - Ce) / C0] \times 100$$

where C0 is the initial dye concentration (mg/L), V is the solution volume (L), and m is the dry mass of biosorbent (g). All measurements were performed in duplicate or triplicate, and average values are reported (± standard deviation where available).

Experimental design for pH, concentration, and dosage

Experiments were organized as one-factor-at-a-time equilibrium tests to isolate the effect of each variable:

Effect of pH: pH was varied from 2 to 9 at fixed initial TB concentration (20 mg/L) and fixed biosorbent dosage (10 g/L).

Effect of initial dye concentration: C0 was varied from 20 to 200 mg/L at pH 5 and fixed biosorbent dosage (10 g/L).

Effect of biosorbent dosage: dosage was varied from 10 to 80 g/L (equivalent to adding 0.5–4.0 g in 50 mL) at pH 5 and fixed initial TB concentration (20 mg/L).

For each run, pH was measured before and after adsorption to confirm stability during the contact period. All other conditions (solution volume, mixing intensity, and contact period) were kept constant across experiments.

Following the single-factor equilibrium screening, response surface methodology (RSM) using a central composite design (CCD) was used to evaluate the combined effects of pH (X1), initial TB concentration (X2), biosorbent dosage (X3), and temperature (X4) on TB removal (%). Based on their significant effects and the known thermodynamic sensitivity of adsorption systems, temperature was additionally incorporated into the response surface optimization using a four-factor central composite design. The factor ranges, CCD matrix, quadratic model development, and statistical analysis are reported in the Optimization section.

RESULTS AND DISCUSSION

Effect of pH

Solution pH strongly influenced TB removal by *Sargassum tenerrimum*. As pH increased from 2 to 5, the percentage removal increased (approximately 65% to about 85% under the tested conditions), indicating improved affinity between the dye and the biosorbent surface. This trend is consistent with changes in biosorbent surface charge and competition with protons [6,8]. At low pH, excess H+ can occupy or neutralize negatively charged functional groups (for example, carboxylate sites), which reduces electrostatic attraction toward the cationic dye and lowers adsorption. As pH approaches the mildly acidic range, deprotonation of surface groups increases the number of negatively charged sites, strengthening

electrostatic attraction and allowing more TB molecules to bind. Above the optimum (around pH 5), the removal declined as pH increased further. A practical explanation is that at higher pH the biosorbent surface may already be largely deprotonated, so additional pH increase does not create many new sites, while other effects (such as increased ionic strength from pH adjustment, changes in dye aggregation, or competition from hydroxide and other anions) can reduce effective binding. Therefore, pH 5 was selected as the working pH for subsequent concentration and dosage experiments. The trend is shown in Figure 01.

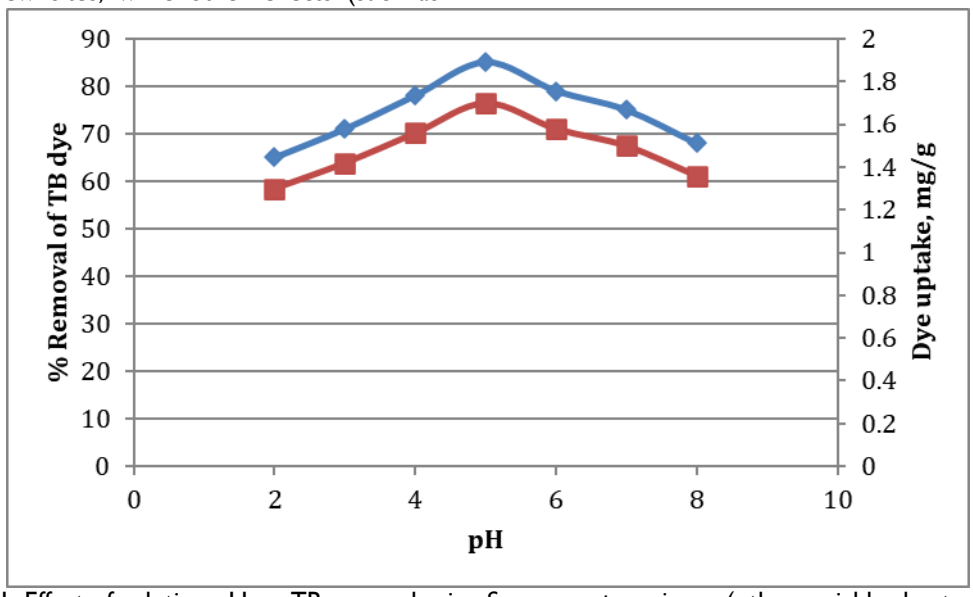

Figure 01: Effect of solution pH on TB removal using *Sargassum tenerrimum* (other variables kept constant).

Effect of initial dye concentration

When the initial TB concentration increased from 20 to 200 mg/L at fixed pH and biosorbent dosage, the percentage removal decreased (about 85% to about 65%), while the equilibrium uptake qe increased. This behavior is typical for adsorption systems with a finite number of sites [10]. At low C0, the number of available adsorption sites is high relative to the number of dye molecules, so a large fraction of TB can be captured. As C0 increases, the driving force for mass transfer (concentration gradient) increases, which promotes higher qe, but the total number of sites becomes insufficient to bind the same fraction of the added dye, so the percentage removal falls.

From an application perspective, this trend implies that a biosorbent dosage optimized for low-strength dye wastewater may not achieve the same removal for higher-strength effluents. In such cases, either a higher dosage, staged adsorption, or integration with a complementary treatment step may be required to meet discharge limits. The trend is shown in Figure 02.

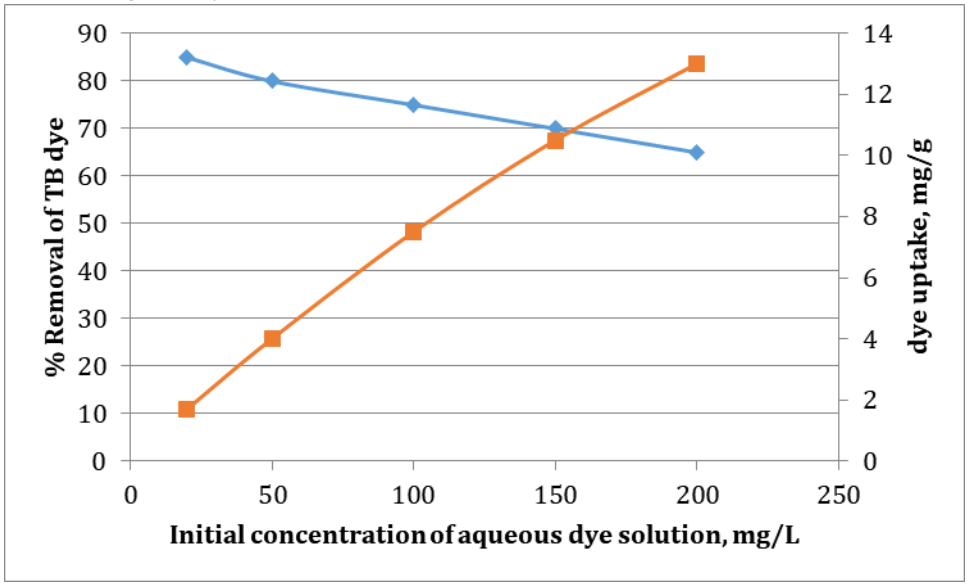

Figure 02: Effect of initial TB concentration on TB removal using *Sargassum tenerrimum* (other variables kept constant).

Effect of biosorbent dosage

Increasing biosorbent dosage generally improved TB removal because a larger mass of sorbent provides more surface area and more functional groups for binding [2]. Under the tested conditions (20 mg/L TB at pH 5), removal increased from about 85% at 10 g/L (0.5 g/50 mL) to approximately 93–94% near an optimum region (around 30 g/L; 1.5 g/50 mL). Beyond this region, the improvement became small. The diminishing benefit at higher dosage can be explained by two common effects. First, at fixed C0 the solution contains a limited number of dye molecules, so once most of them are removed, adding more sorbent cannot increase removal much. Second, at higher solids loading, biosorbent particles can aggregate and overlap, which reduces the effective surface area and can limit access to internal binding sites. For practical design, the optimum dosage is therefore a compromise between maximizing removal and minimizing sorbent consumption.

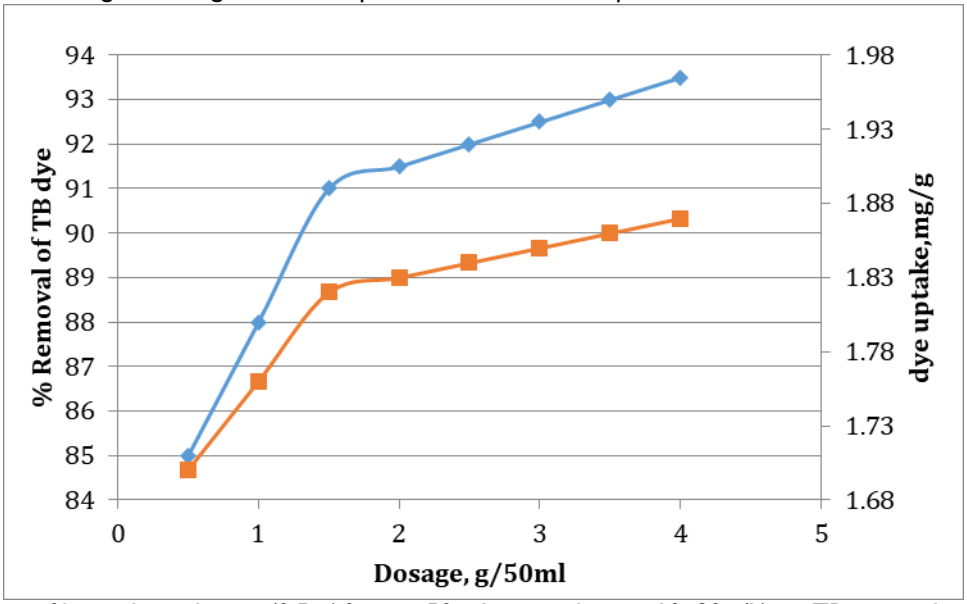

Figure 03: Effect of biosorbent dosage (0.5–4.0 g per 50 mL; equivalent to 10–80 g/L) on TB removal using *Sargassum tenerrimum* (other variables kept constant).

Adsorption isotherm analysis

Equilibrium isotherms relate the amount of dye adsorbed at equilibrium (qe) to the residual concentration in solution (Ce) at a fixed temperature. Isotherm analysis helps compare adsorption capacity, infer surface heterogeneity, and support process design. The experimental equilibrium data were evaluated using Langmuir, Freundlich, and Temkin models [10].

Langmuir isotherm

The Langmuir isotherm postulates monolayer adsorption of solute molecules onto a homogeneous surface with a limited number of identical adsorption sites [12]. Once a site is filled, no further adsorption can occur at that site. The linear equation of the Langmuir isotherm is expressed as:

$Ce/qe = 1/(Qm \cdot b) + Ce/Qm$

The essential feature of the Langmuir isotherm can also be expressed in terms of the dimensionless separation factor RL as:

$RL = 1/(1 + bC_0)$

The Langmuir isotherm was plotted for the present data as shown in Figure 04. The linear regression equation obtained was:

$Ce/qe=0.0521Ce+1.8606$

with a good linearity ($R^2=0.9844$), indicating a strong correlation between experimental data and the Langmuir model. From the slope and intercept of the straight line, the Langmuir constants were calculated as:

- Slope $=1/Qm=0.0521 \Rightarrow Qm=19.19$ mg/g
- Intercept $=1/(Qm \cdot b)=1.8606 \Rightarrow b=0.028$ L/mg

Using the Langmuir constant (b = 0.028 L/mg), the separation factor RL was estimated to check favorability. For C_0 = 20 mg/L, $RL = 1/(1 + bC_0) = 1/(1 + 0.028\times20) = 0.641$, and for C_0 = 200 mg/L, $RL = 1/(1 + 0.028\times200) = 0.152$. Since $0 < RL < 1$ in both cases, the adsorption is favorable over the studied concentration range.

The high correlation coefficient indicates that the adsorption follows the Langmuir model well, suggesting favorable monolayer coverage and appreciable binding affinity under the tested conditions.

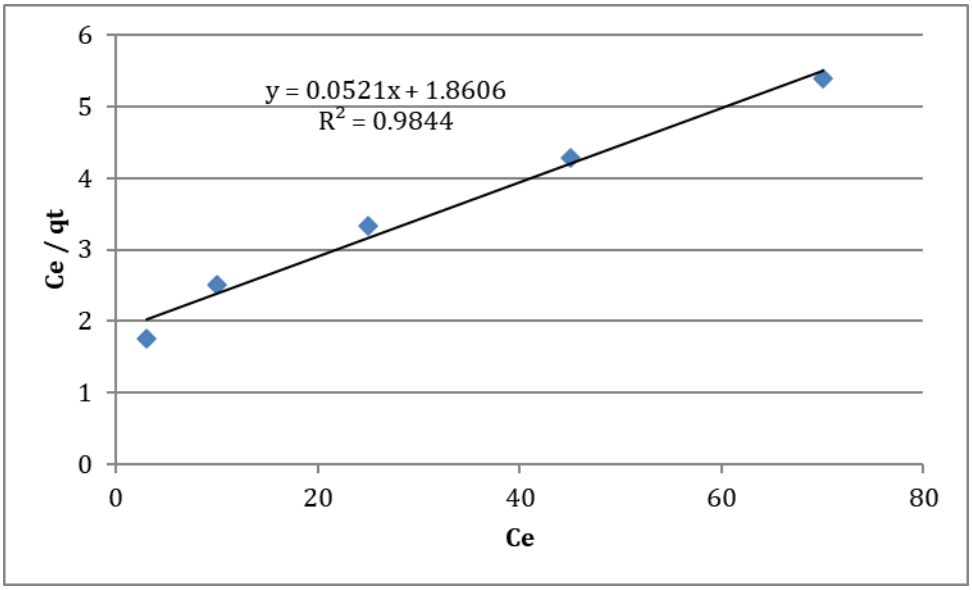

Figure 04: Langmuir isotherm plot for TB adsorption on *Sargassum tenerrimum*.

Freundlich isotherm

The Freundlich isotherm is applied to adsorption on heterogeneous surfaces [10], where the interaction occurs between adsorbed molecules. Unlike the Langmuir model, this equation is empirical and can be used to describe heterogeneous adsorption systems. The Freundlich equation is expressed as:

ln qe = ln Kf + (1/n) ln Ce

If n = 1, adsorption is linear; n > 1 generally indicates favorable adsorption, and 1/n reflects the degree of surface heterogeneity.

The Freundlich isotherm was plotted between ln qe and ln Ce, as shown in Figure 05. The regression equation obtained was:

Ln qe=0.6531ln Ce−0.1471 with a high correlation coefficient (R2=0.9963), showing a good fit of the model to the experimental data.

From the slope and intercept of the straight line:

- Slope = 1/n = 0.6531 ⇒ n = 1.53
- Intercept = ln Kf = −0.1471 ⇒ Kf = 0.863

In this study, the Freundlich fit was strong ($R^2 \approx 0.996$). The fitted parameters were approximately Kf ≈ 0.863 and n ≈ 1.53, indicating favorable uptake on a heterogeneous biosorbent surface. The good fit is consistent with the natural complexity of algal biomass, which contains multiple functional groups and binding environments [19].

binding environments [19].

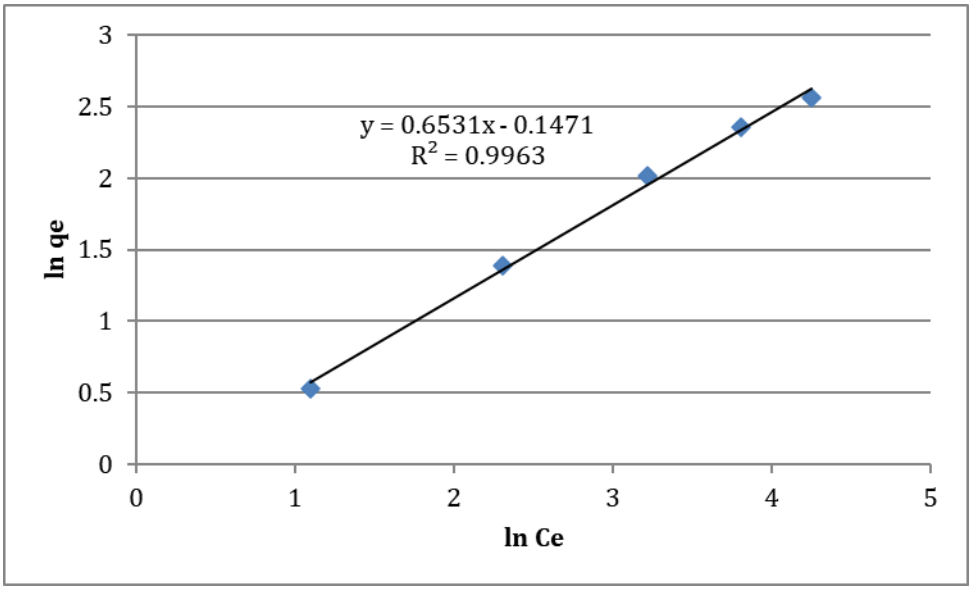

Figure 05: Freundlich isotherm plot for TB adsorption on *Sargassum tenerrimum*.

Temkin isotherm

The Temkin isotherm considers the indirect interaction between adsorbate molecules and assumes that the heat of adsorption of all molecules in the layer decreases linearly with coverage due to adsorbent–adsorbate interactions [11]. It further assumes that

adsorption is characterized by a uniform distribution of binding energies, up to a maximum binding energy. The Temkin isotherm can be expressed in the following linear form:

qe=Bln A+Bln Ce; Where,

- B=RT/b
- b = Temkin constant related to the heat of adsorption (J/mol),
- A = Temkin isotherm constant (L/g),
- R= universal gas constant (8.314 J/mol·K),
- T = absolute temperature (K).

From the Temkin plot (Figure 06), the fitted regression equation is:

qe = 3.5822 ln(Ce) − 3.1739, (R^2 = 0.9567)

Comparing with the Temkin linear form:

• Slope = B = 3.5822

• Intercept = B ln(A) = −3.1739

Therefore:

ln(A) = (−3.1739) / 3.5822 = −0.8860

A = e^(−0.8860) = 0.4123

Thus, the Temkin parameters are:

• B = 3.5822

• A = 0.4123

• ln(A) = −0.8860

Using b = RT / B and substituting R = 8.314 J mol−1 K−1, T = 303 K, and B = 3.5822:

RT = 8.314 × 303 = 2519.142

b = 2519.142 / 3.5822 = 703.3 J mol−1

So, at 303 K: b = 703.3 J mol−1

The obtained values indicate that the Temkin model provides a reasonable fit to the experimental data. The relatively high correlation coefficient confirms that the adsorption process is influenced by adsorbate–adsorbent interactions and follows the assumptions of the Temkin isotherm [11].

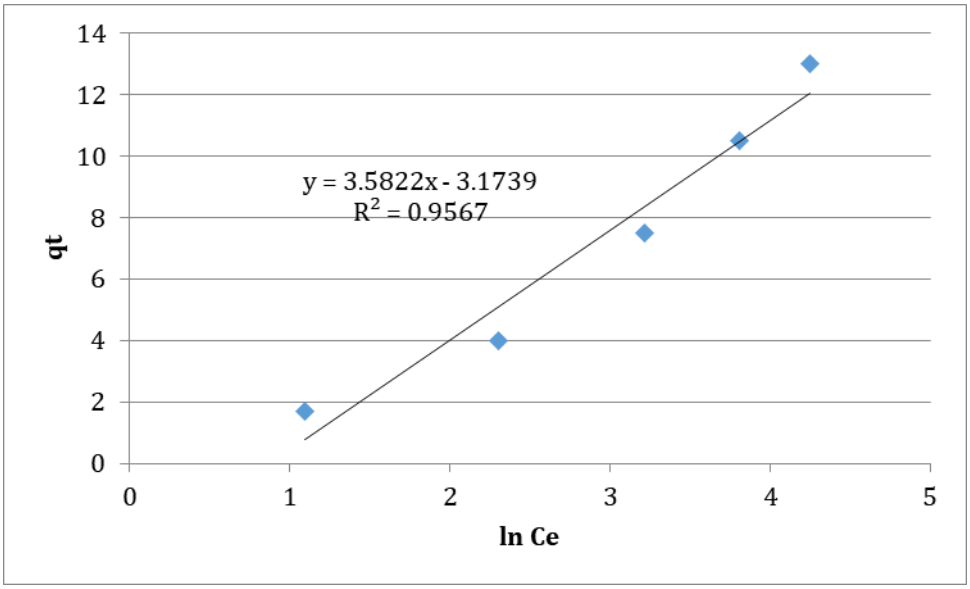

Figure 06: Temkin isotherm plot for TB adsorption on *Sargassum tenerrimum*.

While equilibrium isotherms describe how TB partitions between solution and biosorbent at equilibrium, practical application requires identifying operating conditions that maximize removal under realistic constraints. Therefore, response surface methodology using a central composite design (CCD) was used to quantify the combined effects of pH, initial TB concentration, biosorbent dosage, and temperature on TB removal and to locate an optimum operating window [13,17].

Optimization (Central Composite Design)

Central composite design (CCD) was applied to optimize the operating conditions for the removal of Toluidine Blue (TB) from aqueous solution using the selected brown algal biomass (*Sargassum tenerrimum*). Four independent variables were investigated: solution pH (X1), initial dye concentration (C0, X2), biosorbent dosage (w, X3), and temperature (T, X4). Preliminary single-factor experiments were conducted to evaluate the influence of pH, adsorbent dosage, and initial concentration [15,17].

The response (Y) was defined as the percentage dye removal.

1. Experimental domain and CCD matrix

Each factor was studied at five levels (−2, −1, 0, +1, +2). The investigated ranges were: pH 3–7, C0 10–30 mg/L, w 10–50 g/L (within the screening range of 10–80 g/L), and T 283–323 K (Table 1). A CCD comprising 30 runs (16 factorial points, 8 axial points, and 6 centre-point replicates) was employed. The experimental design matrix, along with the experimental and model-predicted dye removal values, is provided in Table 2 [13,14].

Table 01: Levels of different process variables in coded and uncoded form.

Variable	Name	Range and levels				
		-2	-1	0	1	2
X_1	pH of aqueous solution	3	4	5	6	7
X_2	Initial concentration, C_o, mg/L	10	15	20	25	30
X_3	Biosorbent dosage, w, g/L	10	20	30	40	50
X_4	Temperature, T, K	283	293	303	313	323

Table 02: CCD design matrix and experimental vs predicted dye removal.

Run No.	X_1, pH	X_2, C_o	X_3, w	X_4, T	% Removal of TB	
					Experimental	Predicted
1	-1.00	-1.00	-1.00	-1.00	80.65	80.65
2	-1.00	-1.00	-1.00	1.00	81.98	81.98
3	-1.00	-1.00	1.00	-1.00	80.73	80.73
4	-1.00	-1.00	1.00	1.00	82.57	82.57
5	-1.00	1.00	-1.00	-1.00	82.57	82.57
6	-1.00	1.00	-1.00	1.00	83.40	83.40
7	-1.00	1.00	1.00	-1.00	83.15	83.15
8	-1.00	1.00	1.00	1.00	84.48	84.48
9	1.00	-1.00	-1.00	-1.00	82.57	82.57
10	1.00	-1.00	-1.00	1.00	83.40	83.40
11	1.00	-1.00	1.00	-1.00	83.15	83.15
12	1.00	-1.00	1.00	1.00	84.48	84.48
13	1.00	1.00	-1.00	-1.00	80.98	80.98
14	1.00	1.00	-1.00	1.00	81.32	81.32
15	1.00	1.00	1.00	-1.00	82.07	82.07
16	1.00	1.00	1.00	1.00	82.90	82.90
17	-2.00	0.00	0.00	0.00	81.02	81.02
18	2.00	0.00	0.00	0.00	81.35	81.35
19	0.00	-2.00	0.00	0.00	80.02	80.02
20	0.00	2.00	0.00	0.00	80.35	80.35
21	0.00	0.00	-2.00	0.00	79.35	79.35
22	0.00	0.00	2.00	0.00	81.02	81.02
23	0.00	0.00	0.00	-2.00	77.38	77.38
24	0.00	0.00	0.00	2.00	81.71	81.71
25	0.00	0.00	0.00	0.00	91.54	91.54
26	0.00	0.00	0.00	0.00	91.54	91.54
27	0.00	0.00	0.00	0.00	91.54	91.54
28	0.00	0.00	0.00	0.00	91.54	91.54
29	0.00	0.00	0.00	0.00	91.54	91.54
30	0.00	0.00	0.00	0.00	91.54	91.54

2. Quadratic model development

The experimental data were fitted to a second-order (quadratic) polynomial model to describe the relationship between the response and the four factors. Using multiple regression analysis, the following model was obtained for percentage dye removal (Y). In Eq. (1), X1–X4 represent the actual (uncoded) values of pH, C0 (mg/L), w (g/L), and T (K), respectively.

$$Y = -2891.64 + 36.67X1 - 2.59X1^2 + 6.87X2 - 0.11X2^2 + 0.87X3 - 0.03X3^2 + 18.43X4 - 0.03X4^2 - 0.18X1X2 + 0.01X1X3 - 0.02X1X4 + 0.00X2X3 - 0.00X2X4 + 0.00X3X4 \quad (1)$$

Note: Several interaction coefficients are very small and therefore appear as 0.00 when rounded to two decimal places, as reported by the regression output.

3. Statistical validation of the model

he adequacy and significance of the fitted quadratic model were evaluated using analysis of variance (ANOVA) (Table 3). The model was statistically significant ($p < 0.0001$), and the coefficient of determination calculated from the ANOVA sums of squares was very high ($R^2 = 0.9999998$; adjusted $R^2 = 0.9999996$). The residual mean square was extremely small, indicating a close agreement between the predicted and experimental TB removal within the studied domain.

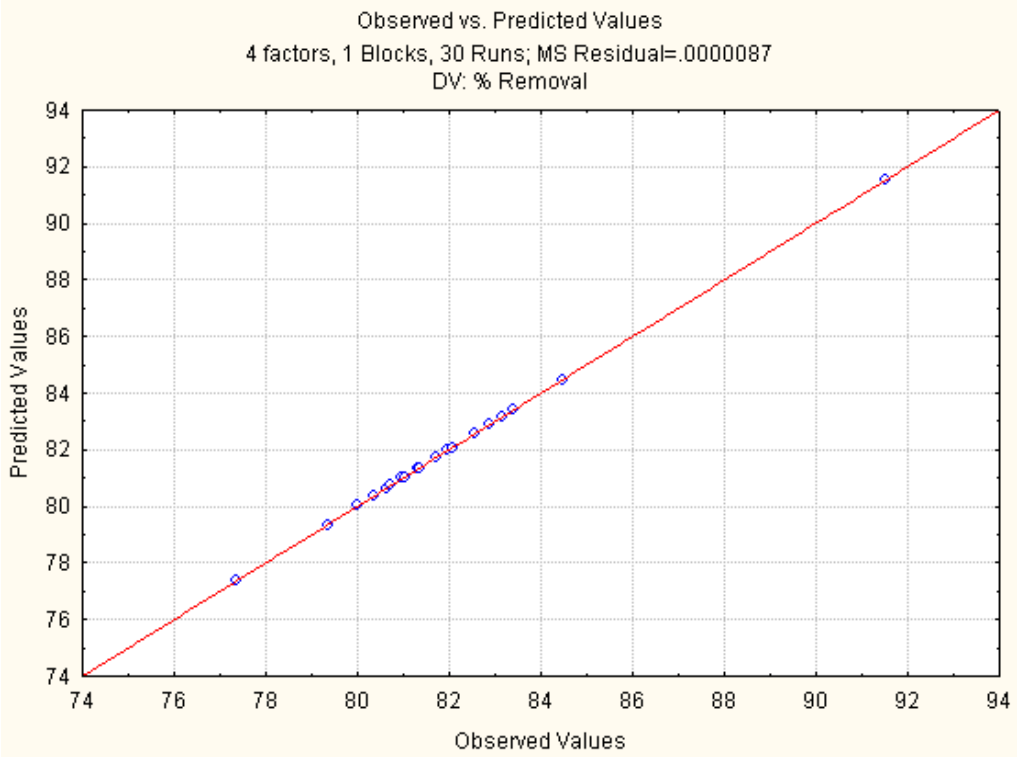

Figure 07: Predicted vs observed.

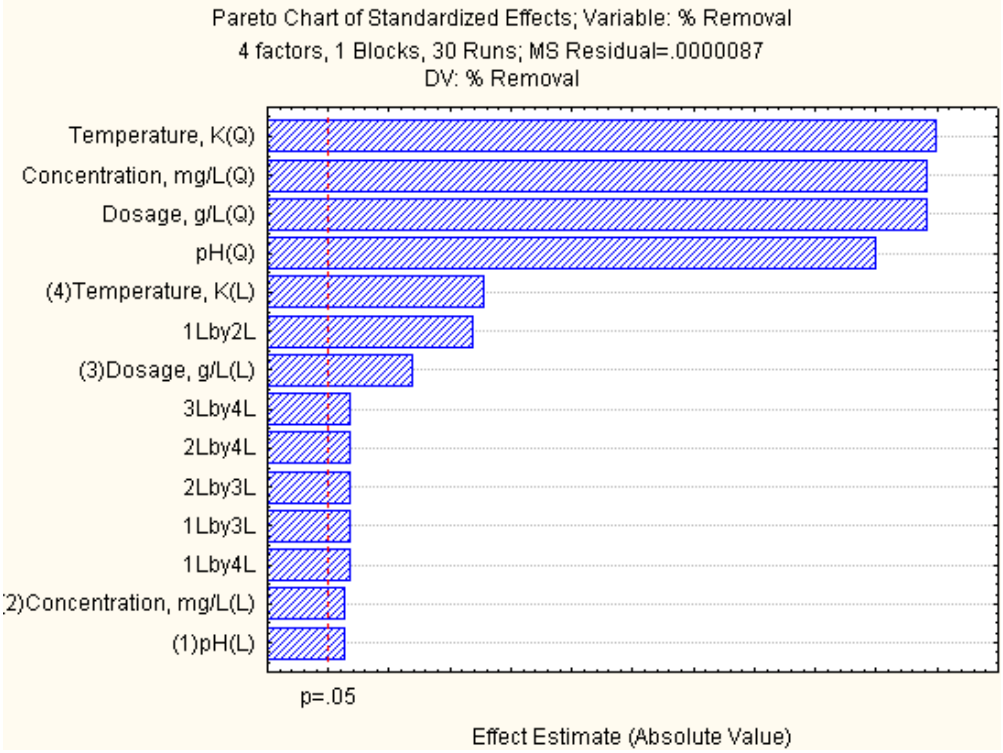

Figure 08: Pareto chart.

Table 03: ANOVA for the fitted quadratic model.

	SS	df	MS	F	p
(1) pH (L)	0.1667	1	0.1667	19178	0.000000
pH (Q)	174.8748	1	174.8748	20122584	0.000000
(2) Concentration, mg/L(L)	0.1667	1	0.1667	19178	0.000000
Concentration, mg/L(Q)	210.2836	1	210.2836	24197014	0.000000
(3) Dosage, g/L(L)	4.1667	1	4.1667	479452	0.000000
Dosage, g/L(Q)	210.2836	1	210.2836	24197014	0.000000
(4) Temperature, K(L)	14.0617	1	14.0617	1618056	0.000000
Temperature, K(Q)	215.8200	1	215.8200	24834087	0.000000
1L by 2L	12.2500	1	12.2500	1409589	0.000000
1L by 3L	0.2500	1	0.2500	28767	0.000000
1L by 4L	0.2500	1	0.2500	28767	0.000000
2L by 3L	0.2500	1	0.2500	28767	0.000000
2L by 4L	0.2500	1	0.2500	28767	0.000000
3L by 4L	0.2500	1	0.2500	28767	0.000000
Error	0.0001	15	0.0000	-	-
Total SS	519.5213	29	-	-	-

Term-wise ANOVA indicates that all linear and quadratic terms as well as the included interaction terms were statistically significant ($P < 0.05$). The largest sums of squares were associated with the curvature (quadratic) terms, especially for temperature and concentration (and dosage), confirming that dye removal is governed by a pronounced optimum region rather than a purely monotonic response.

4. Interpretation of regression coefficients

The sign and magnitude of each coefficient in Eq. (1) indicate the direction and relative strength of its effect on dye removal. Positive linear coefficients for pH, C0, w and T imply that increasing each factor can increase the response in the local region of the design, whereas negative quadratic coefficients ($X1^2$–$X4^2$) indicate diminishing returns followed by a decline at higher levels, i.e., a concave (maximum-type) response.

Interaction terms describe how the influence of one factor changes with the level of another. The negative X1X2 term (pH×C0) indicates that the positive effect of increasing pH becomes less pronounced as the initial dye concentration increases. In contrast, the positive X1X3 term (pH×dosage) suggests a synergistic combined effect of pH and biosorbent dosage within the investigated ranges. The remaining interaction terms are comparatively small in magnitude (rounded as 0.00), yet still statistically significant due to the low experimental error.

Table 04: Estimated regression coefficients for the fitted quadratic model [Regression. Coefficients; Var.:% Removal; R-sqr=1.; Adj:1. 4 factors, 1 Blocks, 30 Runs; MS Residual=.0000087; DV: % Removal).

	Regression coefficient	Standard Error	t (15)	p
Mean/Intercept	-2891.64	0.643482	-4493.73	0.000000
(1) pH (L)	36.67	0.045187	811.50	0.000000
pH (Q)	-2.59	0.000577	-4485.82	0.000000
(2) Concentration, mg/L(L)	6.87	0.009022	761.87	0.000000
Concentration, mg/L(Q)	-0.11	0.000023	-4919.04	0.000000
(3) Dosage, g/L(L)	0.87	0.004505	194.20	0.000000
Dosage, g/L(Q)	-0.03	0.000006	-4919.04	0.000000
(4) Temperature, K(L)	18.43	0.003794	4858.38	0.000000
Temperature, K(Q)	-0.03	0.000006	-4983.38	0.000000
1L by 2L	-0.18	0.000147	-1187.26	0.000000
1L by 3L	0.01	0.000074	169.61	0.000000
1L by 4L	-0.02	0.000147	-169.61	0.000000
2L by 3L	0.00	0.000015	169.61	0.000000
2L by 4L	-0.00	0.000029	-169.61	0.000000
3L by 4L	0.00	0.000015	169.61	0.000000

5. Optimum conditions and model prediction

The optimum operating conditions were identified by maximizing the fitted response surface within the CCD factor ranges. In the experimental CCD runs, the highest dye removal was obtained at the centre-point conditions (coded levels 0, 0, 0, 0), corresponding to pH = 5, C0 = 20 mg/L, w = 30 g/L and T = 303 K. Under these conditions, the experimental dye removal was 91.54%, which was also matched by the model prediction (Table 02). Numerical optimization of the quadratic model predicted a closely similar optimum at pH = 5.008, C0 = 20.036 mg/L, w = 30.818 g/L and T = 304.833 K, with a predicted dye removal of 91.66% [16].

6. Interaction effects and response surface analysis

The combined effects of the four variables on dye removal can be visualized using three-dimensional response surface and corresponding contour plots. Each plot is typically generated by varying two factors while holding the remaining two at their centre levels. Consistent with Eq. (1), the response surfaces are expected to exhibit clear curvature for each factor (negative quadratic coefficients) and modest tilting/warping where interaction effects are present [17].

Figure 09(a–f): Response surface/contour plots for pairwise interactions.

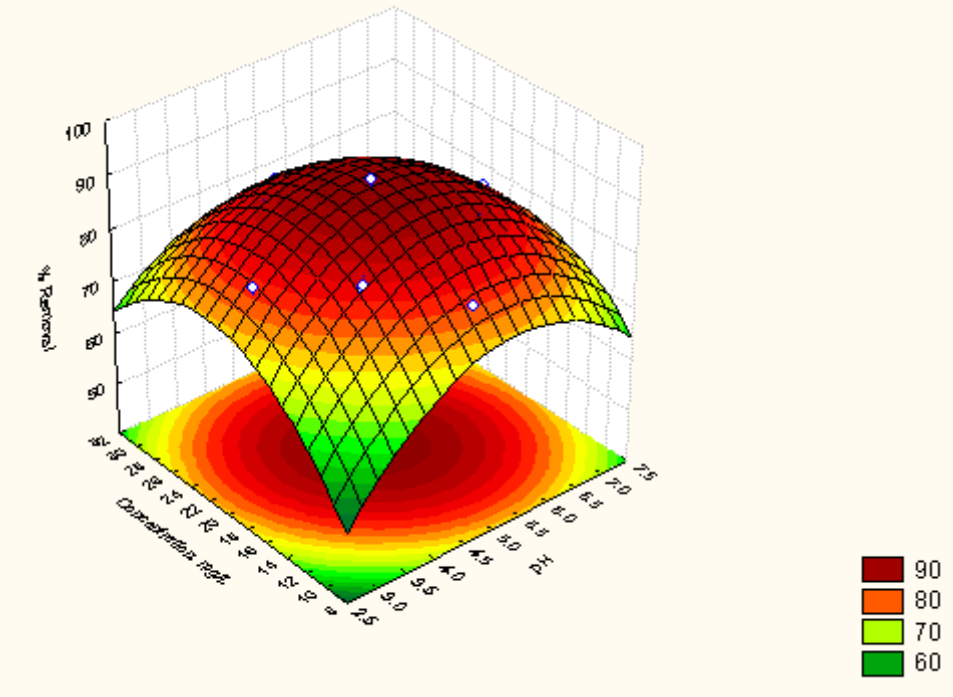

Figure 09a: Surface contour plot for the effects of initial concentration and pH on TB % removal.

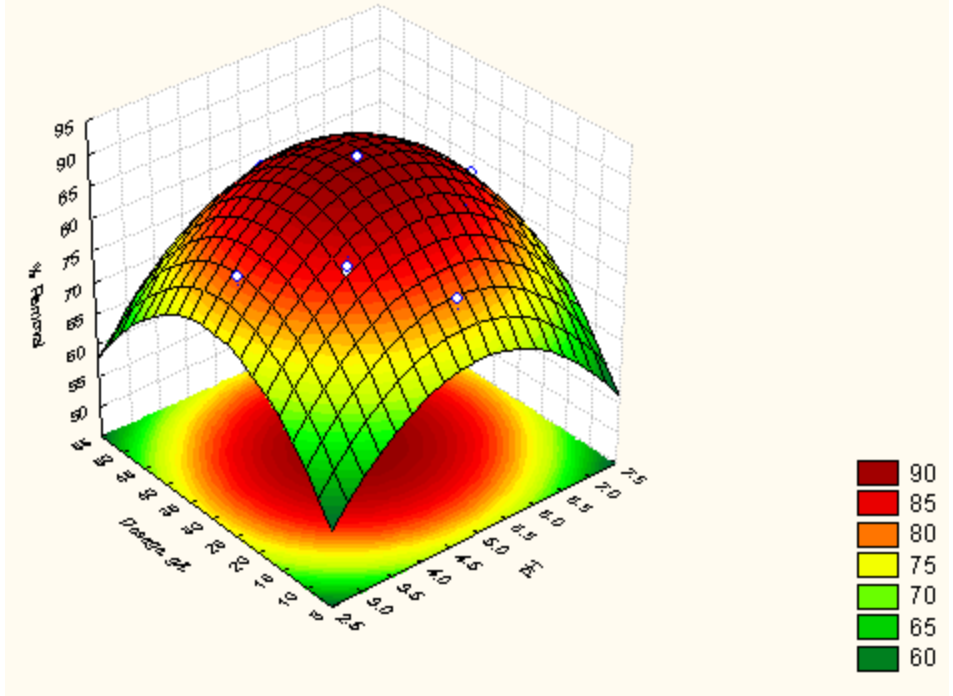

Figure 09b: Surface contour plot for the effects of dosage and pH on TB % removal.

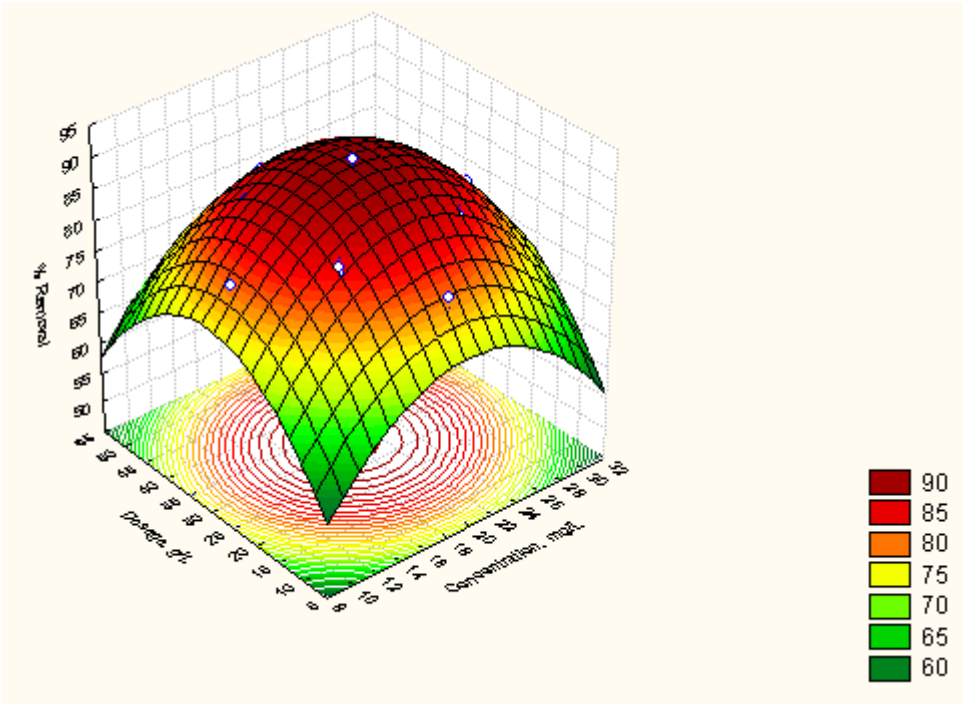

Figure 09c: Surface contour plot for the effects of dosage and initial concentration on TB % removal.

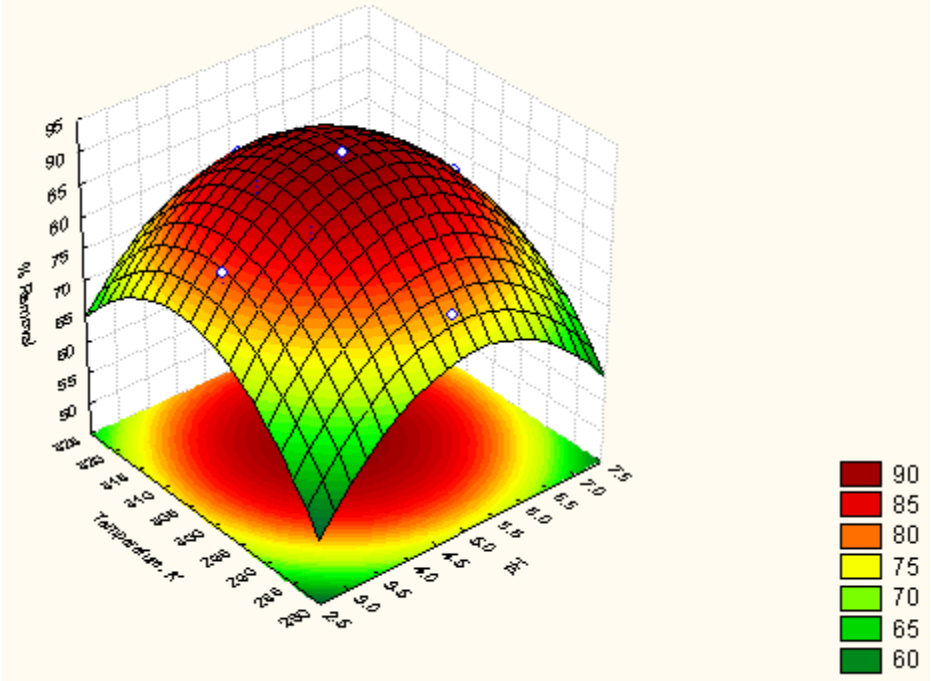

Figure 09d: Surface contour plot for the effects of temperature and pH on TB % removal.

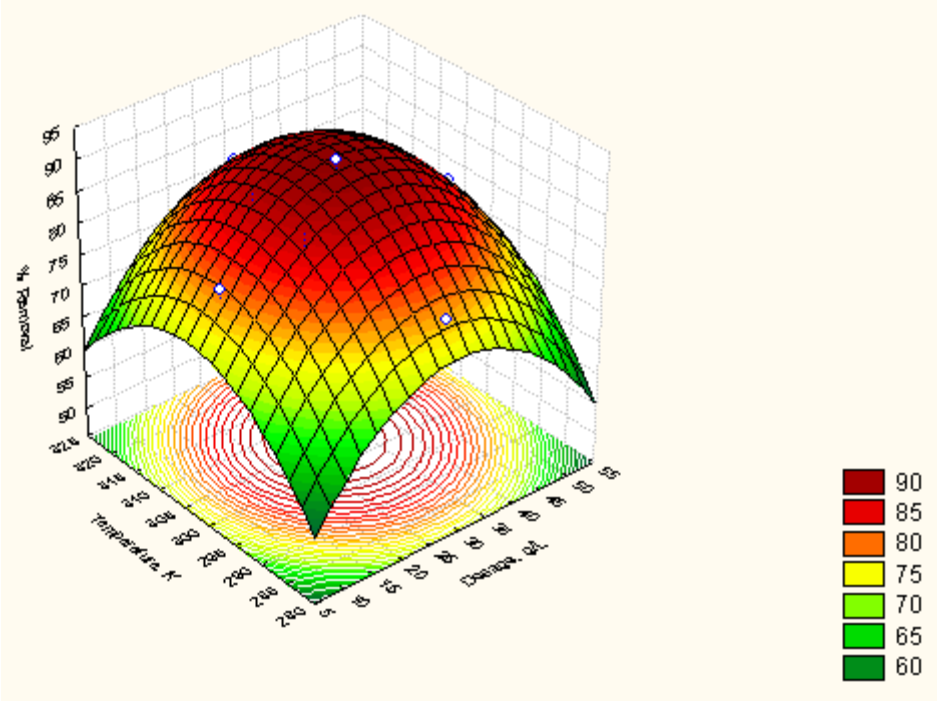

Figure 09e: Surface contour plot for the effects of temperature and dosage on TB % removal.

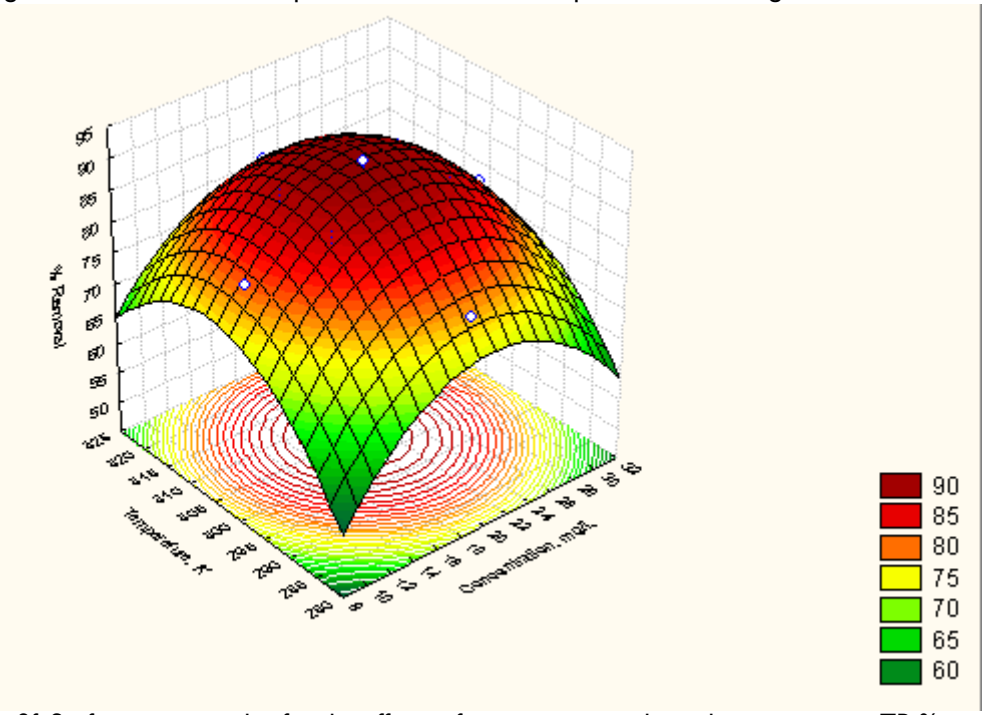

Figure 9f: Surface contour plot for the effects of temperature and initial concentration TB % removal.

Overall, CCD provided a statistically robust framework to quantify main, quadratic and interaction effects, and to identify the operating window that maximizes dye removal using the brown algal biosorbent.

CONCLUSION

This work investigated equilibrium biosorption of Toluidine Blue onto *Sargassum tenerrimum* and applied CCD-based optimization considering pH, initial dye concentration, biosorbent dosage, and temperature. TB removal increased with pH in the acidic range and reached an optimum around pH 5, consistent with improved electrostatic attraction as surface functional groups become deprotonated. Increasing initial dye concentration reduced percentage removal but increased adsorption capacity, reflecting saturation of finite adsorption sites at higher loading. Increasing biosorbent dosage enhanced removal up to an optimum region, after which the benefit became marginal, likely due to limited remaining dye in solution and particle aggregation.

Isotherm analysis showed that both Langmuir and Freundlich models described the equilibrium data well, indicating favorable uptake with measurable monolayer capacity ($Q_m \approx 19.19$ mg/g) and surface heterogeneity ($n \approx 1.53$). The Temkin model further supported that adsorption energy changes with coverage, which is expected for biomass-based sorbents. Overall, *Sargassum tenerrimum* appears to be a low-cost biosorbent for TB removal and provides a foundation for future multi-factor optimization and scale-up. CCD results indicated that the optimum region lies close to the center-point conditions, with the highest experimental removal of 91.54% at pH = 5, C0 = 20 mg/L, w = 30 g/L and T = 303 K. Numerical optimization predicted a closely similar optimum at pH = 5.008, C0 = 20.036 mg/L, w = 30.818 g/L, and T = 304.833 K with a predicted removal of 91.66%.

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AUTHOR CONTRIBUTIONS

CH. Asha Immanuel Raju contributed to the conception and design of the study, supervision, data analysis, and manuscript preparation. Randrianoelivony Andriniaina Mahefa, Fazul Abdou Said, Nasrin Nahar Fima, and Prionti Pramanik contributed to the experimental work, data collection, data analysis, and manuscript preparation. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

INSTITUTIONAL REVIEW BOARD STATEMENT

Institutional Review Board approval was not required because this study did not involve human participants, human subjects, or human-derived data.

ETHICAL APPROVAL

Ethical approval was not required because the study involved laboratory-based adsorption experiments using *Sargassum tenerrimum* biomass and Toluidine Blue dye solutions and did not involve human participants or animals.

INFORMED CONSENT STATEMENT

Informed consent was not applicable because this study did not involve human participants or personal data.

These statements are consistent with the manuscript's methodology, which describes laboratory adsorption experiments using *Sargassum tenerrimum*, Toluidine Blue solutions, and response surface methodology.

REFERENCES

1. Robinson T, McMullan G, Marchant R, Nigam P. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresource Technology. 2001 May 1;77(3):247-55.
2. Crini G. Non-conventional low-cost adsorbents for dye removal: a review. Bioresource Technology. 2006 Jun 1;97(9):1061-85.
3. Gupta VK, Suhas. Application of low-cost adsorbents for dye removal–a review. Journal of Environmental Management. 2009 Jun 1;90(8):2313-42.
4. Sánchez-Sánchez Á, Suárez-García F, Martínez-Alonso A, Tascón JM. Synthesis, characterization and dye removal capacities of N-doped mesoporous carbons. Journal of Colloid and Interface Science. 2015 Jul 15;450:91-100.
5. Crini G, Badot PM. Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: A review of recent literature. Progress in Polymer Science. 2008 Apr 1;33(4):399-447.
6. Arumugam N, Chelliapan S, Kamyab H, Thirugnana S, Othman N, Nasri NS. Treatment of wastewater using seaweed: A review. International Journal of Environmental Research and Public Health. 2018 Dec;15(12):2851.
7. Saldarriaga-Hernandez S, Nájera-Martínez EF, Martínez-Prado MA, Melchor-Martínez EM. Sargassum-based potential biosorbent to tackle pollution in aqueous ecosystems–An overview. Case Studies in Chemical and Environmental Engineering. 2020 Sep 1;2:100032.
8. Kim N, Lee JW, Jang T, Yoon S, Choi JH, Hong E, Park JA. Adsorption of cationic dyes using *Sargassum horneri* and *Ulva australis* biosorbent. Desalination and Water Treatment. 2025 Jul 25:101351.
9. Sridharan G, Shankar AA. Toluidine blue: A review of its chemistry and clinical utility. Journal of Oral and Maxillofacial Pathology: JOMFP. 2012 May;16(2):251-255.
10. Foo KY, Hameed BH. Insights into the modeling of adsorption isotherm systems. Chemical Engineering Journal. 2010 Jan 1;156(1):2-10.
11. Ayawei N, Ebelegi AN, Wankasi D. Modelling and interpretation of adsorption isotherms. Journal of Chemistry. 2017;2017(1):3039817.
12. Langmuir I. The adsorption of gases on plane surfaces of glass, mica and platinum. Journal of the American Chemical Society. 1918 Sep;40(9):1361-403.
13. Box GEP, Wilson KB. On the experimental attainment of optimum conditions. In: Kotz S, Johnson NL, editors. Breakthroughs in statistics: methodology and distribution. New York (NY): Springer; 1992. p. 270–310.

14. Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta. 2008 Sep 15;76(5):965-77.
15. Baş D, Boyacı İH. Modeling and optimization I: Usability of response surface methodology. Journal of Food Engineering. 2007 Feb 1;78(3):836-45.
16. Derringer G, Suich R. Simultaneous optimization of several response variables. Journal of Quality Technology. 1980 Oct 1;12(4):214-9.
17. Khuri AI, Mukhopadhyay S. Response surface methodology. Wiley interdisciplinary reviews: Computational Statistics. 2010 Mar;2(2):128-49.
18. Ho YS, McKay G. Pseudo-second order model for sorption processes. Process Biochemistry. 1999 Jul 1;34(5):451-65.
19. Davis TA, Volesky B, Mucci A. A review of the biochemistry of heavy metal biosorption by brown algae. Water Research. 2003 Nov 1;37(18):4311-30.
20. Wang J, Chen C. Biosorbents for heavy metals removal and their future. Biotechnology Advances. 2009 Mar 1;27(2):195-226.
21. Yagub MT, Sen TK, Afroze S, Ang HM. Dye and its removal from aqueous solution by adsorption: a review. Advances in Colloid and Interface Science. 2014 Jul 1;209:172-84.
22. Moghazy RM, Labena A, Husien S. Eco-friendly complementary biosorption process of methylene blue using micro-sized dried biosorbents of two macro-algal species (*Ulva fasciata* and *Sargassum dentifolium*): Full factorial design, equilibrium, and kinetic studies. International Journal of Biological Macromolecules. 2019 Aug 1;134:330-43.