

ADSORPTIVE REMOVAL OF CRYSTAL VIOLET AND SUNSET YELLOW FROM WATER USING LABLAB PURPUREAUS HUSK- COST-EFFECTIVE DE-DYING APPROACH

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ABSTRACT

A novel citric acid-activated *Lablab purpureus* husk is proposed to remove crystal violet (CV) and sunset yellow (SY) by column chromatographic elution in a cost-effective way. The adsorbent was characterized by Scanning Electron Microscopy (SEM) and the images supported the adsorptive nature of the material. The experiments were carried out on a chromatographic column by homogenously packing the pre-wetted adsorbent into the column and eluting the dye solution through it. The effects of initial dye concentration, adsorbent dose, pH, contact time, and flow rate were optimized. The efficient removal of up to 300 and 4400 µg/mL of CV and SY, respectively, was observed. The pH range of 4-5 for CV and >4 for SY were found effective. The flow rate was optimized at 1 ml/min. The contact time was found less than 10 minutes. The adsorbent was successfully removed 96.62% and 99.62% of CV and SY, respectively. The Freundlich and Langmuir adsorption isotherm studies were carried out to interpret the interaction between the adsorbate and adsorbent. These models are instrumental in optimizing and scaling up the adsorption process for practical and industrial applications. The experimental data for the adsorption of CV and SY fitted well with the Freundlich isotherm, and the kinetic study of both CV and SY fitted well with the pseudo-second-order reaction.

Keywords: *Lablab Purpureus* Husk, Bio-Adsorbent, Citric Acid, Crystal Violet, Sunset Yellow, Column Chromatography.

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INTRODUCTION

Crystal violet (CV), chemically known as 3,3-bis(p-dimethyl-aminophenyl)-6-dimethylamino-phthalide (Fig.-1a), a cationic dye has been extensively used in dyeing of fabrics in textile industries, staining for slides, leather industries etc. CV even being widely applied in various applications, was not a safe chemical to be released into the environment as it was a potent carcinogen and was able to disrupt the aquatic ecosystem if released into the water bodies without proper treatment.¹ Sunset yellow (SY), chemically known as 6-hydroxy-5-((p-sulfophenyl)azo)-2-naphthalenesulfonic acid, disodium salt (Fig.-1b) has been extensively using in food industries. It was reported to cause histopathological and physiological aberrations in the liver and kidney.²

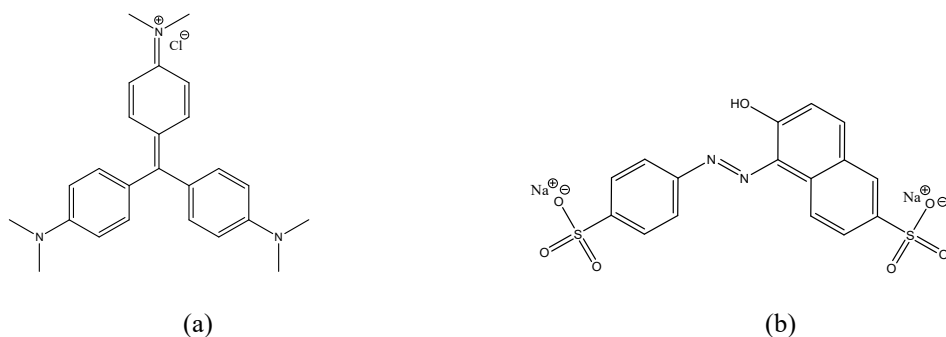


Fig.-1: Chemical Structures of a) CV and b) SY

Industries are using the hazardous dyes in larger amounts and the effluents are discharged into rivers. Many of the dyes including CV and SY are nocuous to the living organisms including humans and aquatics. Thus, it is an innate responsibility to remove such noxious dyes from the water before they are discharged into lakes, rivers, or any water streams. On this background successful attempt to remove two hazardous dyes, namely CV and SY from the effluent using bio-adsorbent obtained from the husk of *Lablab purpureus* was made. Many bio-adsorbents were explored and various methods were reported in the literature for the bio-adsorptive removal of CV and SY. Among them, Hira Urooja *et al.*³ developed a method for bio-adsorptive removal of CV from *Phyllanthus emblica* fruit powder achieving about 84% dye removal at pH 12 using the adsorbent dose of 0.4g. Two bio-adsorbents from the *Tagetes* flower waste were used for the adsorption of CV.⁴ Citric acid-bound red-seaweed biosorbent was reported to remove CV from wastewater.⁵ The maximum CV biosorption efficiency of 93.4% at pH 7. In the report of Onyari *et al.*⁶ the use of a bio adsorbent of dried bark powder of mangrove species *Rhizophora mucronate* for removal of CV dye. At pH 7 and in 60 min the adsorbent removed 99.8% of the dye. It fits well with the pseudo-second-order kinetic model and the Freundlich adsorption isotherm model. Rabia Rehman and Sana Majeed⁷ prepared *Ficus religiosa* leaves and *Ducus carota pomace* biosorbents for the removal of CV dye from an aqueous solution. They reported the adsorbent doses as 0.8 and 1.8 g, contact time of 30 and 25 min, pH of 9 and 3, temperature of 70 °C and 30 °C, and maximum adsorption capacity of 2.4 and 27 mg/g, respectively, for two adsorbents. A biowaste from *Halarrhena antidysenterica* (HA) and *Citrullus colocynthis* (CC) to modified adsorbents are *antidysenterica*-tartaric acid modified (HA-TA) and *colocynthis*-tartaric acid modified (CC-TA) sorbents were also used.⁸ The adsorbents were characterized by Fourier-transform infrared spectroscopy (FT-IR) and SEM. Haddad *et al.*⁹ developed the adsorbent by using Durio seeds (DSs) powder, to remove CV from aqueous medium. The characterization was done by FTIR, TGA-DTG, BET, pH_{PZC}, and SEM-EXD methods. The optimization factors that affect CV adsorption were found as DS dosage of 0.02 – 0.1 g, initial pH of 4–10, temperature of 25–50 °C, and adsorption time of 5–25 min. The highest CV removal was 93.91%. The Kinetics process was well described by the pseudo-second-order model, and Freundlich and Temkin Adsorption isotherms models were fitted well. The hickory wood powder was also used which removes 476.4 mg/g CV dye from aqueous solution.¹⁰ Using fish scales as an adsorbent the CV Dye was removed from the aqueous medium.¹¹ Under the optimized parameters 90% of the dye was found successfully degraded. The other adsorbents such as activated charcoal,¹² citric acid modified acacia leaves,¹³ bay leaves,¹⁴ fermented and un-fermented banana peels,¹⁵ *Urtica dioica* leaf powdered beads,¹⁶ water hyacinth (*Eichhornia crassipes*) powder,¹⁷ cottonwood seeds,¹⁸ coconut shells,¹⁹ olive pomace,²⁰ oak fruit hull (Oak Gal) extract,²¹ mixed fruit peel waste²² were also found reported in the literature for removal of CV from water samples. The other toxic and one of the most used dyes, SY, has been taken as a compound for removal from samples. Several bio-adsorbents including methanol Walnut shell,²³ activated carbon derived from Cassava sieve biomass,²⁴ biopolymers such as chitosan and functionalized chitosans,²⁵ carbon nano biochar,²⁶ chemically modified chitin and chitosan,²⁷ activated carbon of malt bagasse,²⁸ oak fruit hull,²⁹ lady finger stem,³⁰ alligator weed activated carbon³¹ and activated carbon of oak tree wood³² were found reported in the literature. The reported methods for removal of CV and SY have limitations such as the need for maintaining a narrow pH range, higher dose of adsorbent, elevated temperature, etc. In contrast to the works reported, the attempts are put herewith to present two new, novel, eco-friendly, and cost-effective methods to remove CV and SY using *Lablab purpureus* husk, the agriculture/plant waste. For the very first time, column chromatographic elution methodology has been employed for adsorption and succeeded. It is substantial to quote the superiority and the ability of the methods to remove almost 100% dyes efficiently under optimum conditions. The kinetics of adsorption isotherms are well addressed in this paper.

EXPERIMENTAL

Material and Methods

All the spectrophotometric measurements were carried out with an Agilent Cary 60 UV-visible spectrophotometer (Agilent Technologies Ltd, Mumbai, India). All the chemicals used were of analytical grade. Crystal violet (CV) (SD Fine Chem Ltd, Mumbai, India), and Sunset yellow (SY) (Venkateshwara Fine Chem Laboratories, Tumakuru, India) were purchased from local sources. *Lablab purpureus* was purchased from the local market.

Preparation of Adsorbent

Lablab purpureus husk was collected and dried with the aid of sunlight until the material was ensured to be moisture-free. The dried husk was ground to a fine powder and sieved through meshes of 120-micrometer diameters. The husk powder was subjected to a series of water washes, washed with 0.1M NaOH solution at 500 RPM for 1hr to remove the pigment materials from the husk, washed with water to remove excess alkali, and dried under sunlight. The alkali-washed and dried husk powder was further stirred with 0.5M citric acid solution for 3 hours as it was assumed to enhance the functionality of the adsorbent material, followed by 6-8 times of water wash to remove the surplus unbonded citric acid from the material and dried in the sunlight. The final adsorbent was used for adsorption studies.

Method Development

A chromatographic column was adopted for the experimental convenience. A borosilicate column (25 cm × 20 mm i.d) was packed with the prepared husk adsorbent material with water. It was followed by the removal of almost all the water from the column (except a minimum amount of water that was needed to restrict the column from breaking) and the dye solution was introduced to the column slowly through the walls. The dye solution was eluted and the eluate was analyzed for the presence of dye by measuring the absorbance of the eluate at respective wavelengths of maximum absorption (λ_{max}) of standard aqueous dye solution.

Characterization of Adsorbent

Physiochemical Analysis

Moisture Content (MC)

A 1 g of adsorbent husk (M_0) was taken in a silica crucible, and placed in a preheated hot air oven for about 4 h at 105°C and then weighed (M_1). The MC was calculated by using the below formula.

$$MC = \frac{(M_0 - M_1)}{M_0} \times 100$$

Ash Content (AC)

A 1g of husk adsorbent (M_s) was taken in a crucible and placed in a muffle furnace for about 4 hours at 500°C. Then, cooled to the room temperature, and the ash weight (M_a) was measured. The AC of the adsorbent was calculated using the formula given below.

$$AC = \frac{M_a}{M_s} \times 100$$

Volatile Matter (VM)

One gram of adsorbent husk (M_0) was placed in a preheated muffle furnace for 8 mins at 500°C and then cooled to the room temperature. The weight of the resulting adsorbent was recorded (M_2). The weight loss was measured and the amount of VM in the adsorbent was calculated using the formula:

$$VM = \frac{M_0 - M_2}{M_0} \times 100$$

Fixed Carbon Content (FCC)

The FCC in the adsorbent was calculated by subtracting the Moisture content, Ash content and Volatile matter from 100% using the formula given below.

$$FCC = 100\% - (MC + AC + VM)s$$

SEM Analysis of Adsorbent

The morphological examinations of the alkaline treated, citric acid treated, and dye adsorbed adsorbent were done using SEM and the resulting images were analyzed at a magnification range of 3000X. A clear inspection of the images and comparison were deliberated to fix the compatibility of the adsorbent for the said purpose.

RESULTS AND DISCUSSION

Confirmation of Binding of Citric Acid with Adsorbent by Adsorption of Dye by SEM Analysis

The morphological investigations were carried out for 0.1M NaOH-treated adsorbent material and 0.5M citric acid-activated adsorbent. The surface morphology of the alkaline-treated sample (Fig.-2) and alkaline-treated and citric acid-activated adsorbent confirmed the compact arrangement of constituents and having

tiny holes, rough and uneven areas in which may be dead tissues, and fibrous tissues of the husk and in further magnified view the surface looked like a suitable adsorbent with presence of surface convolutions of different magnitude. Further, the SEM image obtained for dye-adsorbed alkali-treated citric acid-activated husk indicated the closure of the free surface. This confirmed the adsorption of dye on the citric acid-activated husk. Through these observations, one can realize that the surface area provides a fair possibility for the dye to be adsorbed.

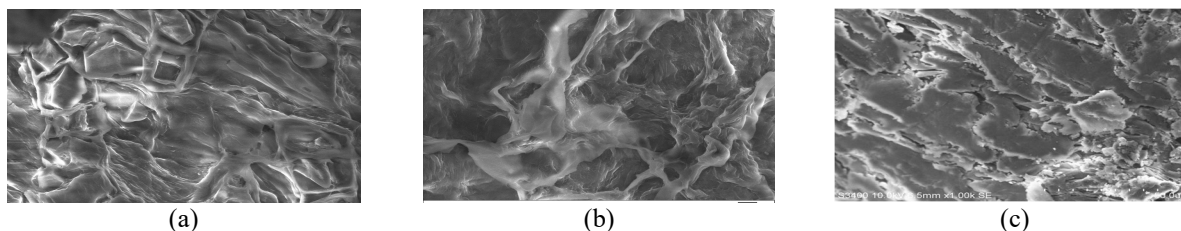


Fig.-2: SEM Images of Adsorbent of: (a) Alkali Treated, (b) Alkali Treated and Citric Acid-Activated, and (c) Alkali Treated, Citric Acid-Activated and 2 (3000X magnification)

The morphological investigation is carried out by SEM images of citric acid-activated adsorbent (Fig.-2). It was observed that the surface area in alkali-treated and citric acid-activated adsorbent has more folding-like structures, many tiny holes, and more convolutions, it has many layers, which enhances the surface area of the adsorbent. As a result, adsorption of dye increased as showed in the last image.

Method Development

Optimization of Experimental Parameters

The various experimental parameters like the effect of initial dye concentration, adsorbent dose, contact time, flow rate, and pH on the adsorption of dyes were studied and optimized for the best performance of removing CV and SY.

Effect of Initial Dye Concentration

A 5ml each of CV solutions with different concentrations (200-600 $\mu\text{g/ml}$) with intervals of 50 $\mu\text{g/ml}$ were eluted through a column packed with 1g of adsorbent material and the absorbance of the eluate was measured at 590 nm for the presence of CV. A similar procedure was followed for the SY solutions with different concentrations (2000-6000 $\mu\text{g/ml}$) with an interval of 500 $\mu\text{g/ml}$ and the absorbance of the eluate was measured at 480 nm for the presence of SY. From Fig.-3 and 4, the optimum concentration at which maximum removal of CV and SY was found at 300 $\mu\text{g/ml}$ and 4000 $\mu\text{g/ml}$, respectively, and these concentrations were fixed as upper limit for removal of the dyes.

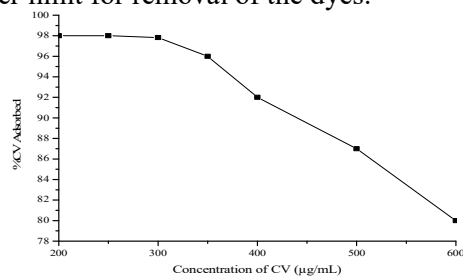


Fig.-3: Effect of Concentration of CV for Removal by Adsorption

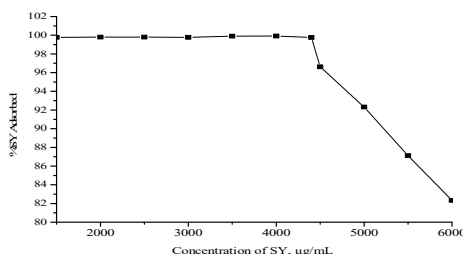


Fig.-4: Effect of Concentration of SY for Removal by Adsorption

Effect of Adsorbent Dose

A 5ml each of 300 µg/ml of CV and 4000 µg/ml of SY were eluted with different amounts of adsorbent (0.25-1g) in separate experiments. The maximum adsorption of CV and SY were found at 1g of adsorbent. Hence, 1g of the adsorbent was used throughout the studies. The results of the effect of varying quantities of adsorbent on adsorption are shown graphically in Fig.-5. It was concluded that 1g of the adsorbent was sufficient to remove about 98.52% CV and 99.62% SY.

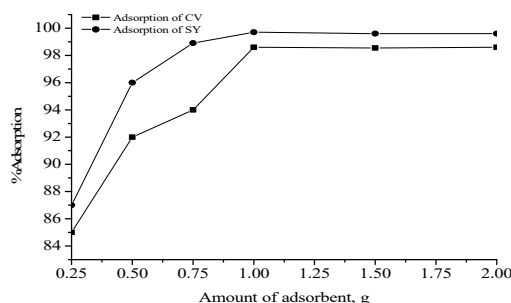


Fig.-5: Effect of Adsorbent Dose on % Removal of CV and SY

Effect of Contact Time

A 5 ml of 300 µg/ml of CV was eluted with a column filled with an adsorbent dose of 1g after successive intervals of time. The same procedure was followed for 5 ml of 4000 µg/ml of SY. It was found that the effect of contact time on the removal of dyes was negligible as there was least significant changes occurred.

Effect of pH

The effect of pH on adsorption was studied by varying the pH of the CV solution from pH 1-10. For SY solution pH 3-6 was the range of study of effect. From Fig.-6 the maximum adsorption of CV was observed at a pH range of 4-5. The removal of SY was efficient at pH beyond 4.

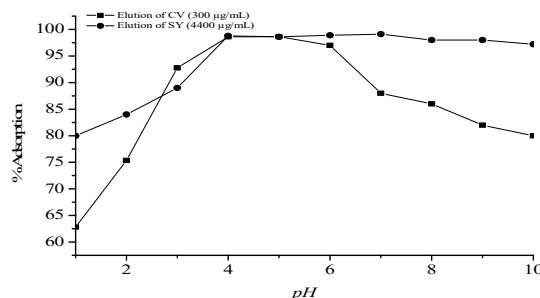


Fig.-6: Effect of pH on Removal of CV and SY using the Adsorbent

Effect of Flow Rate

The effect of flow rate on adsorption was studied by varying the flow rate of the elution (1-5ml/min) with an interval of 1ml/min for the elution of CV and SY. Satisfactory and maximum dye removal was achieved at a flow rate of 1ml/min for both CV and SY. Thus, the same was recommended as the optimum flow rate for elution.

Adsorption Isotherm Studies

Freundlich and Langmuir adsorption isotherms were used to understand the nature of adsorption.^{20, 22} Freundlich isotherm was used to describe the adsorption onto heterogeneous surface as it assumes that different sites on the adsorbent have different adsorption energies were involved in adsorption and have empirical relation between them. On the contrary Langmuir isotherm assumes the formation of a mono adsorptive layer on the surface of the adsorbent as all the adsorptive sites have equal affinity for the adsorbate as in a homogeneous surface. The linear expression for Freundlich adsorption isotherm is given by:

$$\log q_e = \log K_f + \frac{\log C_e}{n}$$

Where q_e is the amount of solute adsorbed per unit weight of adsorbent (mmol/g of adsorbate), and C_e is adsorbate concentration at equilibrium. K_f and n are empirical constants enclosing all the experimental parameters affecting the adsorption process.²² In the present study the adsorption of crystal violet has n value less than 1 and it was greater than 1 for the adsorption of sunset yellow. The adsorption parameters are summarised in Table-1.

The linear expression for Langmuir adsorption isotherm is given by:

$$\frac{C_e}{q_e} = \frac{1}{bq_m} + \frac{C_e}{q_m}$$

Where C_e is the equilibrium concentration of solute (mmol/l), q_e is the amount of solute adsorbed per unit weight of adsorbent (mmol/g of adsorbate), q_m is the adsorption capacity (mmol/g), or monolayer capacity, and b is a constant (l/mmol). Isotherm parameters b and q_m were determined from the intercept and the slope of the C_e/q_e versus C_e graph, respectively. The results are given in Table-2. The Langmuir isotherm characteristics were effectively expressed by a dimensionless constant called separation factor (R_L) that can be evaluated by relationship as given below:

$$R_L = \frac{1}{1 + bC_0}$$

where b is the Langmuir constant and C_0 is the initial dye concentration. The values of R_L at different dye concentrations are presented in Table-1.

Table-1: Separation Constant (R_L) Values for CV and SY Adsorption Values at Different Initial Concentrations

Crystal violet		Sunset Yellow	
Concentration in $\mu\text{g/ml}$	R_L values	Concentration in $\mu\text{g/ml}$	R_L values
100	0.0030	100	0.0153
150	0.0020	200	0.0077
200	0.0015	300	0.0051
250	0.0012	400	0.0038
300	0.0010	500	0.0031

The adsorption isotherm parameters for both the dyes by alkali and acid-modified adsorbent of *Lablab purpureus* husk are presented in Table-2. The R^2 values of Freundlich isotherms were greater than the Langmuir isotherms for both the dyes. Hence, it was concluded that the adsorption process of both dyes was well fitted with Freundlinch adsorption isotherm.

Table-2: Isotherm Parameters for the Adsorption of CV and SY

Crystal violet	Isotherms	R^2	Parameters	
	Freundlich	0.9906	$n = 0.3120$	$K_f = 5.4265 \text{ mg/g}$
	Langmuir	0.9497	$b = 1.1695 \text{ l/mg}$	$q_m = 0.0167 \text{ mg/g}$
Sunset yellow	Freundlich	0.6828	$n = 4.1753$	$K_f = 2.4868 \text{ mg/g}$
	Langmuir	0.1011	$b = 0.6405 \text{ l/mg}$	$q_m = 7.6107 \text{ mg/g}$

The results above reflect that *Lablab purpureus* husk, being a natural material, has a heterogeneous surface with various functional groups and adsorption sites, making the Freundlich isotherm model suitable. The Freundlich model accounts for multilayer adsorption, which can occur due to the complex surface of the husk. Different sites on the husk may have different adsorption energies, which the Freundlich model effectively describes. Langmuir Adsorption Isotherm revealed that even though the surface is heterogeneous, parts of the *Lablab purpureus* husk may exhibit properties consistent with monolayer adsorption, especially at lower adsorbate concentrations. The Langmuir model's assumption of a finite number of identical sites can be approximated in certain ranges of adsorbate concentrations and on specific sections of the husk surface. This model helps in predicting the maximum adsorption capacity, which is crucial for practical applications of adsorption processes. The two isotherm methods when applied to the column elution approach, the models help understand how adsorption progresses under dynamic flow conditions, providing insights into the efficiency and capacity of *Lablab purpureus* husk as an adsorbent. Understanding the adsorption characteristics through these models aids in designing larger-scale adsorption

columns, and optimizing parameters such as contact time, flow rate, and column dimensions. These isotherm studies allow for assessing the performance and potential regeneration or reuse of the adsorbent material, making the process economically and environmentally viable.

Kinetic Study

The results presented in Tables 3 to 6 reflect the experimental data of the study of kinetics of the degradation reactions associated with CV and SY. The graphical models were also used to ascertain the probable kinetics.

Table-3: Results of Pseudo First-Order Model for SY Adsorption on Proposed Citric Acid-Activated Adsorbent

Concentration (µg/ml)	q_e exp.(mg/g)	K_1 (min ⁻¹)	recall. (mg/g)	R^2
600	2.5801	-0.001026	0.33149	0.290025

Table-4: Results of Pseudo-Second Order Model for SY Adsorption on Proposed Citric Acid Activated Adsorbent

Adsorbent	Concentration (µg/ml)	q_e exp.(µg/ml)	K_2 (min ⁻¹)	q_e calc. (µg/ml)	R^2
Citric acid blended husk	600	0.5599	0.004039	0.40783	0.9918

Table-5: Results of Pseudo First Order Reaction for Adsorption of CV on Proposed Adsorbent

Concentration(µg/ml)	q_e exp.(µg/ml)	K_1 (min ⁻¹)	q_e calc. (µg/ml)	R^2
300	9.38702	0.0006977	0.40783	0.212738

Table-6: Results of Pseudo-Second Order Reaction for Adsorption of CV on Proposed Adsorbent

Concentration(µg/ml)	q_e exp.(mg/g)	K_2 (min ⁻¹)	q_e calc. (mg/g)	R^2
300	0.5599	0.004039	0.40783	0.9918

The results of data in Tables 3-6 and their severe analysis promptly gave the inference that CV and SY dyes adsorb well on the proposed adsorbent. Further, the given data of q_e and R^2 concludes that the t/q_t v/s time graph was found to fit well with the Pseudo second-order reaction.

Study of Physicochemical Characteristics of Citric Acid-Activated Adsorbent

The experimental investigations for physicochemical characteristics such as MC, AC, VM, and FCC of the proposed citric acid-activated adsorbent were found to yield satisfactory results to consider the sorption property. The results of a study of physicochemical characteristics are presented in Table-7.

Table-7: Results of a Study of Physicochemical Characteristics of Citric Acid Activated Adsorbent (n = 3)

MC±SD, %	AC±SD, %	VM±SD, %	FCC±SD, %
2±0.015	1.27±0.005	93.93±1.09	1.8±0.006

CONCLUSION

A novel, eco-friendly column chromatographic method has been developed to remove hazardous dyes, crystal violet (CV) and sunset yellow (SY), using adsorbent material made from *Lablab purpureus* husk. This approach achieved over 98% dye removal, with SEM images showing that activation with citric acid enhanced the adsorbent's surface area and capacity. Key parameters like initial dye concentration, adsorbent dose, contact time, and pH were optimized. The method is promising for treating industrial effluents. The adsorption followed both Freundlich and Langmuir isotherms, indicating multilayer and monolayer adsorption, respectively. The high adsorption capacity confirmed the effectiveness of *Lablab purpureus* husk in environmental remediation.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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