

An Investigation of the Biosorption Process of Methylene Blue Dye Using Non-Activated and Activated Cow Hooves as Adsorbents: An Adsorption Isotherm Study

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INTRODUCTION

The pollution of water bodies by harmful industrial waste has emerged as a significant environmental issue in recent years. Among the various pollutants released into aquatic environments, synthetic dyes like methylene blue are particularly worrisome because of their toxicity, resistance to degradation, and long-lasting presence (Tripathy et al., 2025). These dyes are extensively utilized in industries like textiles, paper, and plastics, and their untreated discharge into the environment leads to considerable harm to ecosystems while posing serious health hazards to both humans and aquatic organisms. Tackling this problem demands the development of inventive, effective, and affordable methods for water treatment (Muduli et al., 2023).

Traditional approaches for removing dyes, such as chemical precipitation, membrane filtration, and advanced oxidation techniques, frequently face challenges like expensive operations, the production of secondary pollutants, and intricate technical requirements (Kumari et al., 2024). On the other hand, adsorption processes have emerged as a highly effective and affordable method for eliminating dyes and other pollutants from wastewater. Adsorption is a surface-driven process that utilizes the attractive interactions between pollutants in a solution and adsorbent materials, allowing for the effective removal of harmful substances (Nagendran et al., 2025).

Choosing the right adsorbent materials is essential for ensuring the effectiveness of adsorption systems. Historically, activated carbons have been the preferred option due to their outstanding adsorption characteristics, such as extensive surface area and high porosity (Serafin and Dziejarski, 2024). Nevertheless, the elevated production cost of activated carbon poses a major challenge to its widespread use in developing areas. As a result, there is an increasing focus on discovering affordable and environmentally friendly biosorbents made from biological and agricultural waste (Priyan et al., 2024).

Cow hooves, a by-product of agriculture produced abundantly during cattle processing in abattoirs, hold great potential as a biosorbent material. Cow hooves, abundant in protein and collagen, exhibit distinctive chemical and physical characteristics that render them well-suited for adsorption applications (Suleiman et al., 2021). Nonetheless, studies examining their potential for pollutant removal are scarce, with limited exploration of their applications. Furthermore, the efficiency of raw (non-activated) and chemically treated (activated) cow hooves as adsorbents has not been extensively studied (Nakro et al., 2024).

This research investigates the biosorption of methylene blue dye using both non-activated and activated cow hooves as adsorbents. The aim is to assess and compare the adsorption efficiency of both materials while examining their performance through adsorption isotherm models. Adsorption isotherms are mathematical representations that illustrate how adsorbates are distributed between the solid and liquid phases when equilibrium is reached. They offer important understanding of adsorption mechanisms, capacities, and the interactions occurring on the surface of adsorbent materials (Rajabi et al., 2023).

Two-parameter isotherm models, like the Langmuir and Freundlich models, are commonly utilized because of their straightforward nature and reliability in representing adsorption data (Benjamin et al., 2018). The Langmuir model presumes that adsorption occurs in a single layer on a uniform surface with a limited number of adsorption sites, making it suitable for systems with clearly defined adsorption characteristics. In contrast, the Freundlich model is well-suited for explaining adsorption on uneven surfaces, as it considers the diverse adsorption energies linked to different sites (Amaechi et al., 2024)

Although two-parameter models are widely used, their inability to fully capture the intricacies of complex adsorption systems has driven the creation of three-parameter models, such as the Koble-Corrigan, Vieth-Sladek, and Radke-Prausnitz models. These sophisticated models integrate extra parameters to address aspects like adsorption heterogeneity and multilayer adsorption, offering a deeper and more detailed insight into the adsorption process (Pinto et al., 2024). This research seeks to reveal the adsorption characteristics of methylene blue on cow hooves by utilizing both two- and three-parameter isotherm models, emphasizing the critical factors that affect their effectiveness (Rajabi et al., 2023)

This study adopts a structured method to evaluate the biosorption potential of non-activated and activated cow hooves across different experimental parameters. Critical factors like initial dye concentration, contact duration, pH levels, and temperature are adjusted to maximize adsorption efficiency. The findings are analyzed through adsorption isotherm models to identify the mechanisms driving the process, evaluate the adsorption capacity of the materials, and assess how activation influences their effectiveness (Suleiman et al., 2021)

Beyond contributing to scientific understanding, this research holds significant practical value for promoting environmental sustainability and enhancing resource recovery efforts. Using cow hooves as biosorbents offers an affordable and efficient method for treating wastewater while also promoting waste valorization by transforming agricultural by-products that might otherwise be discarded into valuable resources. The outcomes of this research could act as a foundation for creating comparable biosorption systems utilizing other types of agricultural or biological waste materials (Suleiman et al., 2021)

To conclude, this study aims to tackle the critical problem of dye contamination in water sources by examining the potential of non-activated and activated cow hooves as effective biosorbents. By balancing adsorption efficiency with cost-effectiveness, this research seeks to enhance the understanding of biosorption while offering a sustainable solution for wastewater treatment.

PROCEDURE

Preparation of Adsorbents

Cow hooves were gathered, meticulously washed, dried under the sun for five days, and subsequently dried in an oven at 110°C over a three-day period. The dried hooves were fragmented into smaller pieces, sieved using a 425µm sieve, and the resulting fine particles were stored for later use as Non-Activated Cow Hooves Adsorbent (NACHA). A 50g sample of the material was immersed in a 1M Zinc Chloride solution for 72 hours, washed with distilled water, and subsequently dried in an oven at 110°C for three days. The activated material was allowed to cool and was then secured in an airtight container as Activated Cow Hooves Adsorbent (ACHA) for subsequent experimentation and analysis (Suleiman et al., 2021).

Preparation of adsorbate

The Methylene Blue utilized in this research was provided by BDH Limited. A stock solution of methylene blue dye with a concentration of 1000 mg/L was prepared by dissolving 1.0 g of methylene blue powder in 1000 mL of deionized water. Solutions with varying initial concentrations were prepared by performing a serial dilution of the stock solution in 100 mL of deionized water (Dominguez et al., 2024)

Batch adsorption experiment

A series of Erlenmeyer flasks containing 100 mL of dye solution each were prepared, and 0.10 g of the non-activated and activated cow hooves adsorbent was added individually to them. The Erlenmeyer flasks were tightly sealed and placed in an isothermal water bath shaker set to a constant speed of 250 rpm and a stable temperature of 25°C, where they remained until equilibrium was achieved. The aqueous samples were collected at predetermined time intervals. The concentrations were determined using a UV/Vis spectrophotometer set to a wavelength of 668 nm. In batch and equilibrium studies, the dye uptake at time t (q_t in mg/g) and at equilibrium (q_e in mg/g) are calculated using Equations (1) and (2), while the equilibrium percentage removal is determined using Equation (3).

$$q_t = \frac{(C_0 - C_t)V}{W} \quad \text{..... Equation 1}$$

$$q_e = \frac{(C_0 - C_e)V}{W} \quad \text{..... Equation 2}$$

Percentage of dye removal (%) :

$$\frac{(C_0 - C_t)}{C_0} \times 100 \quad \text{..... Equation 3}$$

Where C_t (mg/L) represents the concentration of methylene blue dye in the liquid phase at time t , C_0 (mg/L) denotes the initial solute concentration, and C_e (mg/L) corresponds to the liquid-phase concentration of MR dye at equilibrium. V is the solution volume in liters (L), while W is the adsorbent mass in grams (g) (Jahannia et al., 2024).

Isotherm Studies

The following two and three parameters isotherm model were considered as listed in Table 1.1 below :

Table 1.1 : Two and Three Isotherm Model

Isotherm	Model	Reference
Langmuir	$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$ $R_L = \frac{1}{1 + K_L C_0}$	Lakherwal (2019)
Freundlich	$q_e = K_F C_e^{1/n}$	Amrutha et al., (2023)
Temnik	$q_e = B \ln(C_e) + B \ln(A)$	Balarak et al., 2017
Dubinin-Radushkevich (D-R)	$q_e = q_m \exp(-\beta \varepsilon^2)$ $\varepsilon = RT \ln(1 + \frac{1}{C_e})$ $E = \frac{1}{\sqrt{2\beta}}$	Amrutha et al., (2023)
Koble-Corrigan	$q_e = \frac{A(C_e)^n}{1 + B(C_e)^n}$	(Kanagalakshmi et al., 2024)

Radke-Prausnitz isotherm model	$q_e = \frac{q_m K C_e}{1 + (K C_e)^\beta}$	(Dhaif-Allah <i>et al.</i> , 2020)
Vieth – Sladek Isotherm model	$q_e = K_{VS} C_e + \frac{Q_{VS} \beta_{VS} C_e}{1 + \beta_{VS} C_e}$	(Li <i>et al.</i> , 2024)

RESULTS AND DISCUSSION

Effect of Some investigated parameter on removal of Methylene Blue dye using NACHA and ACHA

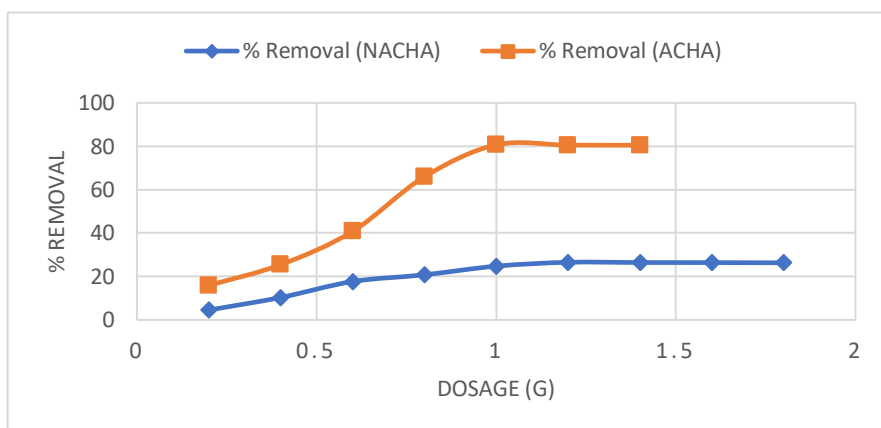


Figure 1 : Effect of NACHA and ACHA dosage on removal of methylene blue

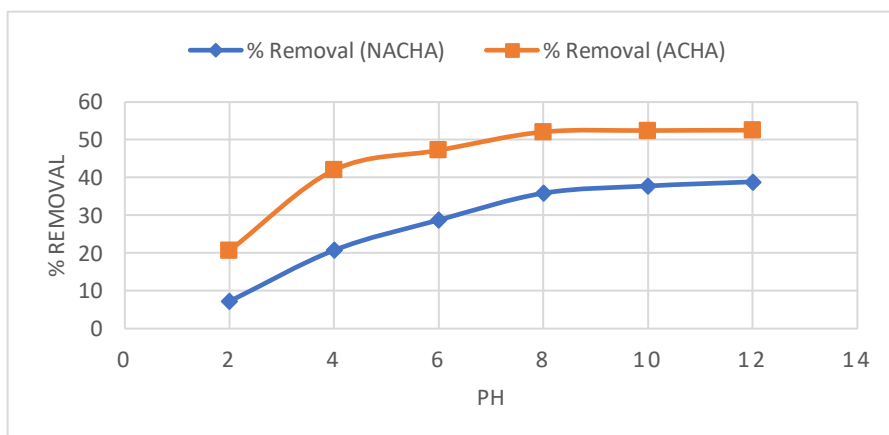


Figure 2 : Effect of pH on removal of methylene blue using NACHA and ACHA

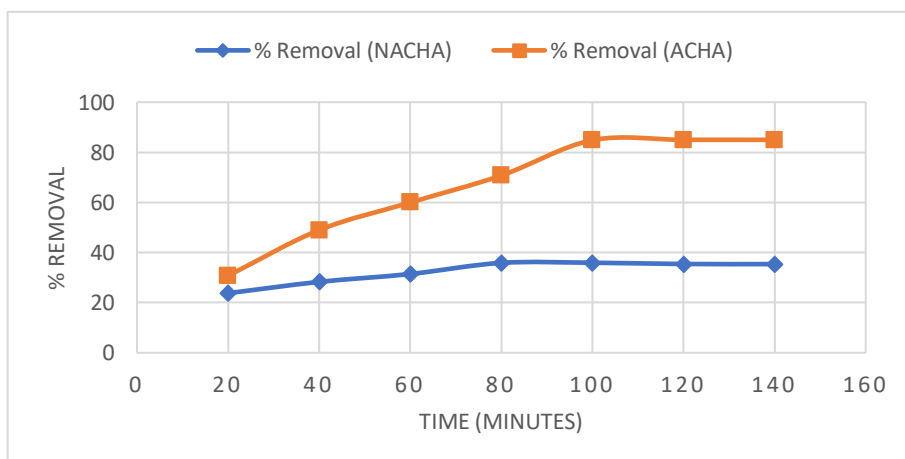


Figure 3: Effect of contact time on removal of methylene blue using NACHA and ACHA

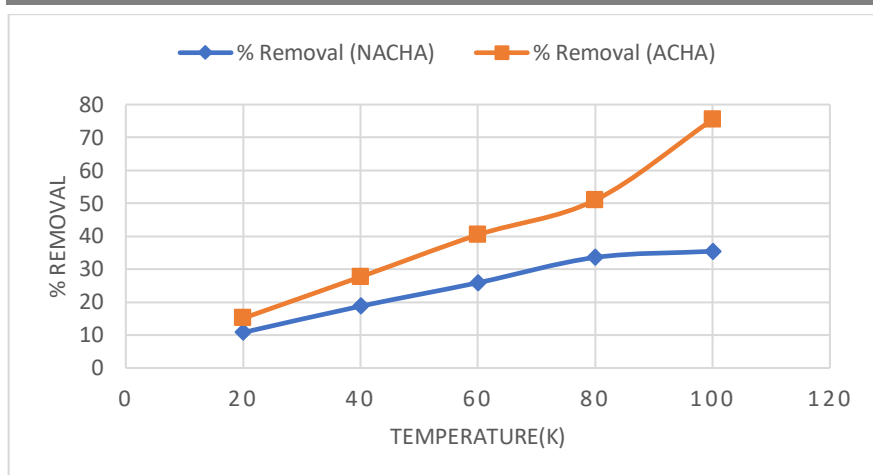


Figure 4 : Effect of temperature on removal of methylene blue using NACHA and ACHA

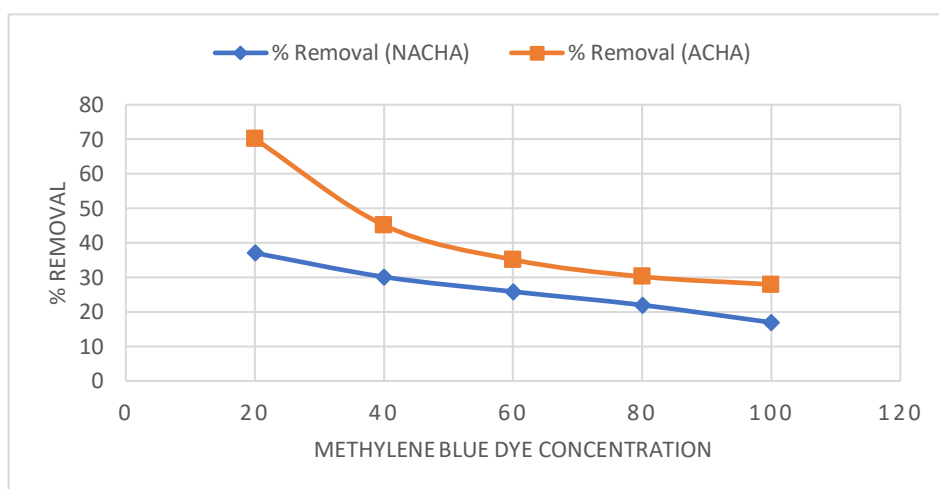


Figure 5 : Effect of methylene dye concentration on removal of methylene blue using NACHA and ACHA

Discussion of Some investigated Parameter on Removal of Methylene Blue dye using NACHA and ACHA

Effect of Dosage

The efficiency of methylene blue removal in adsorption experiments as shown in Figure 1 is largely influenced by the amount of adsorbent used. Higher dosages enhance removal efficiency by providing more active sites, with the improvement being particularly noticeable at lower dosage levels. Nevertheless, the removal efficiency levels off at higher dosages as equilibrium is achieved. The ideal dosage for methylene blue removal was found to be 1.4 g for non-activated cow hooves adsorbent (NACHA), with an efficiency of 25.89%, and 1.0 g for activated cow hooves adsorbent (ACHA), achieving an efficiency of 80.67%, highlighting the superior effectiveness of ACHA (Mousavi et al., 2021).

Effect of pH

The efficiency of methylene blue removal as shown in Figure 2 is affected by pH levels, with activated cow hooves consistently outperforming their non-activated counterparts. At a low pH level, such as pH 2, removal efficiency is significantly reduced due to electrostatic repulsion, with non-activated hooves achieving a removal rate of 7.23% and activated hooves reaching 20.67%. Efficiency increases notably in acidic to neutral pH ranges (pH 4 - 6), with non-activated hooves achieving 28.76% removal and activated hooves reaching 47.22% at pH 6, as reduced surface positivity facilitates better dye adherence. In alkaline environments (pH 8 - 12), removal efficiency levels off as adsorption sites become saturated, with non-

activated hooves achieving 38.88% efficiency and activated hooves reaching 52.54% at pH 12 (Wang et al., 2025).

Effect of Contact Time

The efficiency of methylene blue removal improves over time as shown in Figure 3, as demonstrated by both non-activated and activated cow hooves adsorbents. Activated cow hooves consistently exhibit superior performance compared to non-activated ones, thanks to their increased surface area and enriched functional groups. Efficiency increases quickly at the start, achieving 23.8% for non-activated adsorbents and 30.77% for activated ones within 20 minutes, with further improvements observed at 40, 60, and 80 minutes. At the 100-minute mark, non-activated adsorbents attain a removal efficiency of 35.94%, while activated adsorbents achieve 84.85%. After this point, efficiency stabilizes, signifying that the adsorption sites have reached saturation and equilibrium has been established (Giraldo et al., 2021).

Effect of Temperature

The efficiency of methylene blue removal improves as the temperature rises as shown in Figure 4 for both non-activated and activated cow hooves, with activated adsorbents consistently delivering better performance than non-activated ones (Thomas et al., 2021). At a temperature of 20 K, removal efficiencies are relatively low, with non-activated adsorbents achieving 10.78% and activated adsorbents reaching 15.11%, though both demonstrate a consistent increase as temperatures rise. At 100 K, removal efficiency reaches its maximum, with non-activated adsorbents achieving 35.45% and activated adsorbents attaining 75.44%. The substantial difference underscores the superior adsorption ability of activated cow hooves, especially at higher temperatures, attributed to their improved functional adsorption sites (Jia et al., 2024).

Effect of Initial dye concentration

The efficiency of methylene blue removal declines as shown in Figure 5 as the initial dye concentration rises for both non-activated and activated cow hooves adsorbents. At the lowest concentration of 20 ppm, the removal efficiencies are at their peak, with non-activated hooves achieving 37.06% and activated hooves attaining 70.09%. As the dye concentration increases to 40 ppm and 60 ppm, adsorption efficiency decreases due to the saturation of adsorption sites. Non-activated hooves exhibit efficiencies of 30.13% and 25.87%, while activated hooves demonstrate higher efficiencies of 45.11% and 35.12%. At elevated concentrations, the pattern persists, with the adsorption efficiency of non-activated hooves declining to 21.98% at 80 ppm and 16.95% at 100 ppm, while activated hooves exhibit efficiencies of 30.21% at 80 ppm and 27.95% at 100 ppm. This suggests a decline in adsorption efficiency as the adsorbents reach their capacity limits ((Parastar et al., 2025)

Two and Three Isotherm Model Plot for the Adsorption of Methylene Blue unto NACHA AND ACHA

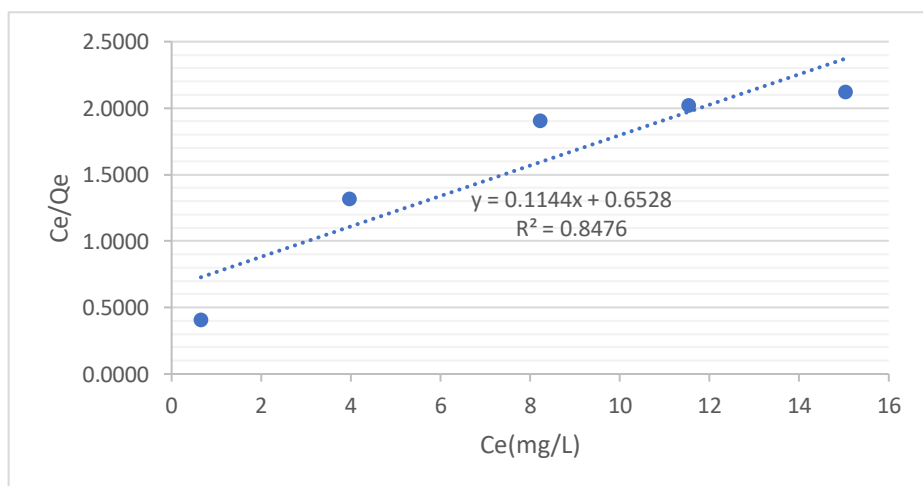


Figure 6 : Langmuir Isotherm model for the adsorption of methylene blue dye unto NACH

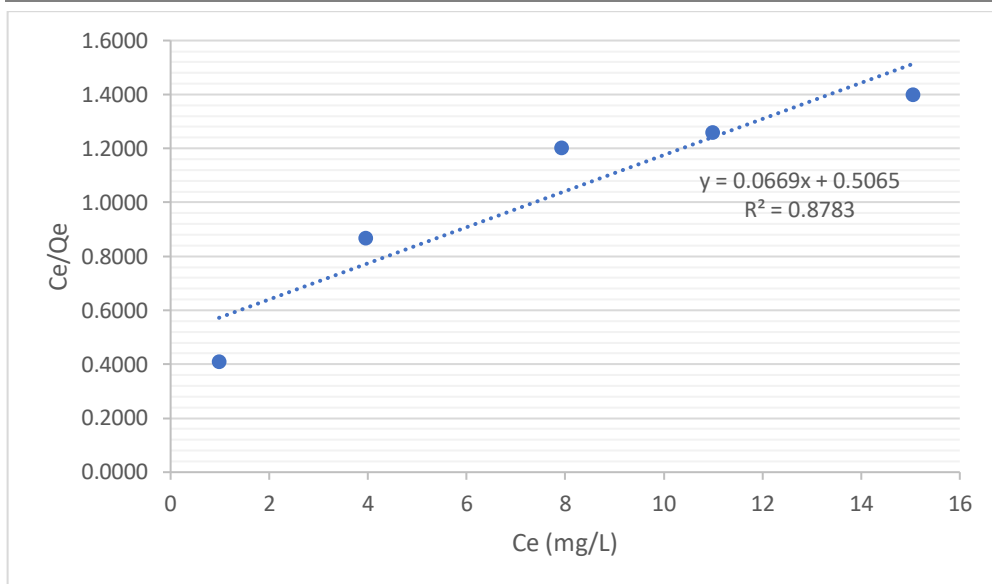


Figure 7 : Langmuir Isotherm model for the adsorption of methylene blue dye unto ACHA

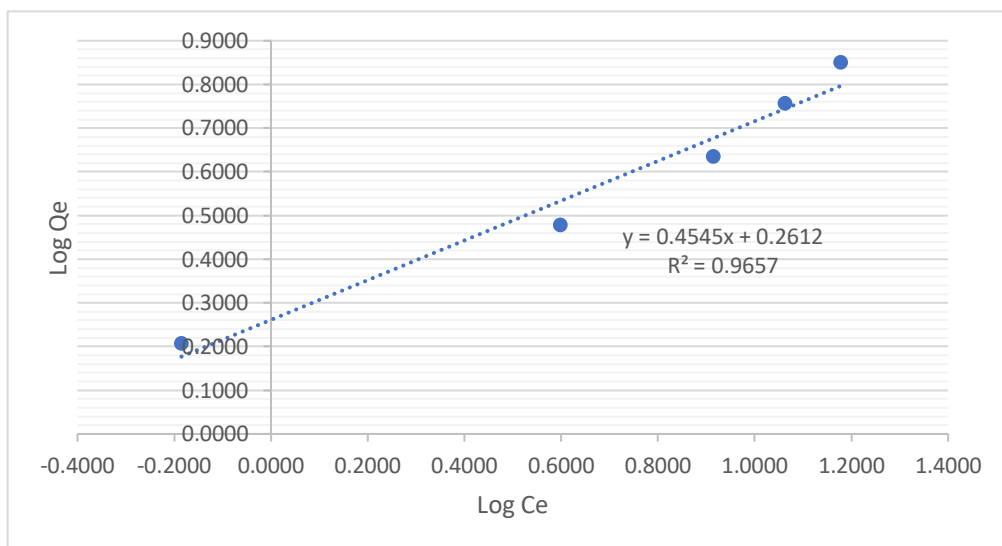


Figure 8 : Freundlich Isotherm model for the adsorption of methylene blue dye unto NACHA

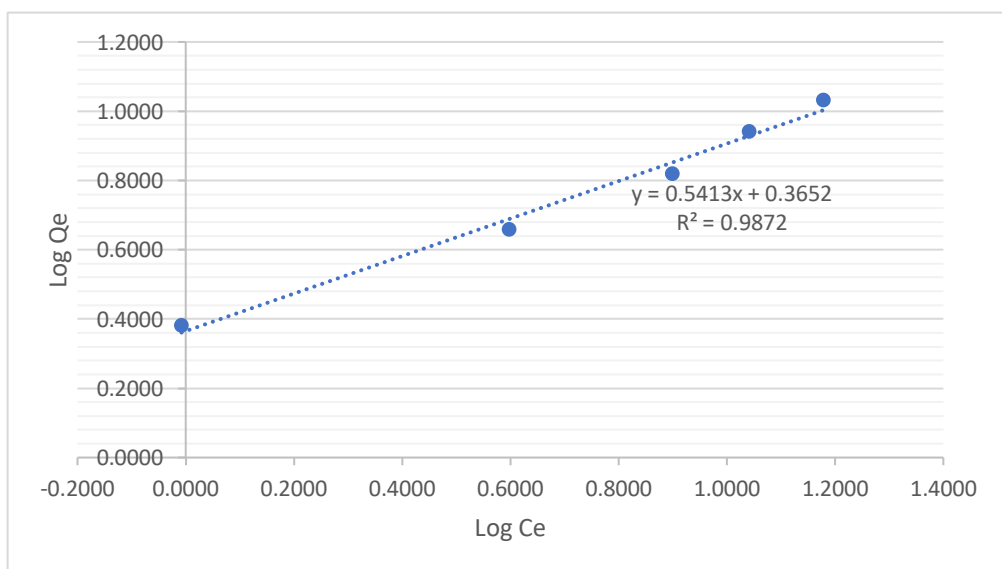


Figure 9 : Freundlich Isotherm model for the adsorption of methylene blue dye unto ACHA

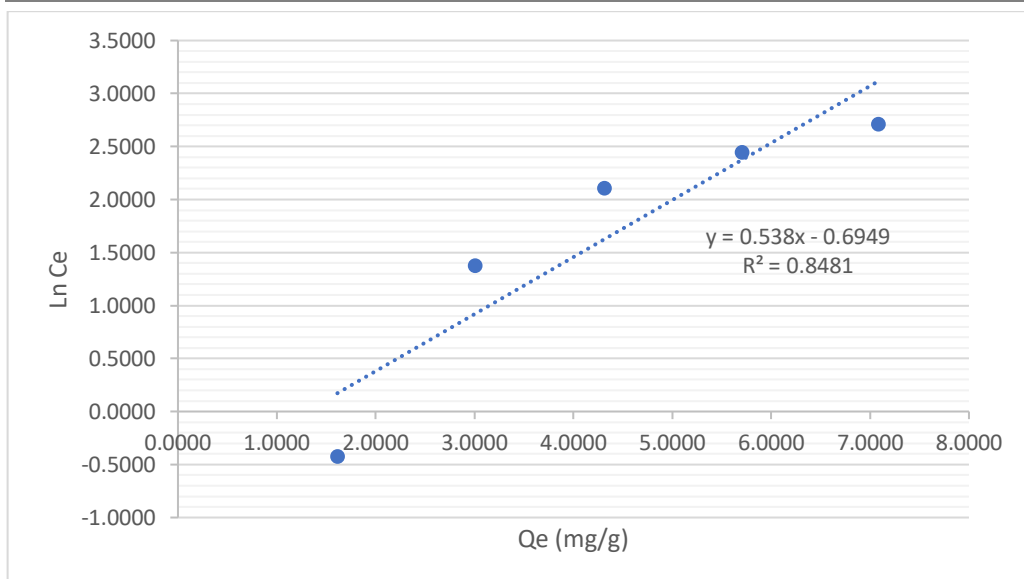


Figure 10 : Temnik Isotherm model for the adsorption of methylene blue dye unto NACHA

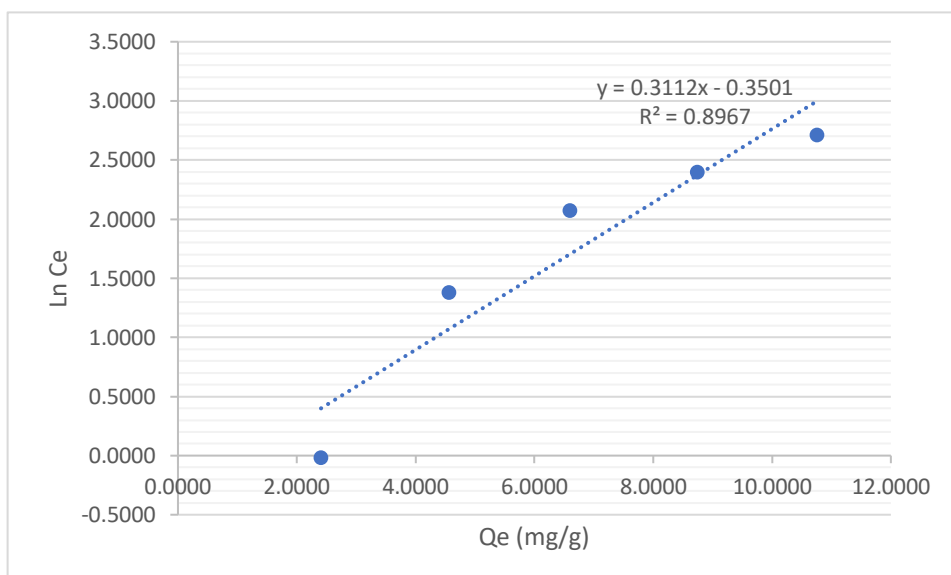


Figure 11 : Temnik Isotherm model for the adsorption of methylene blue dye unto AACHA

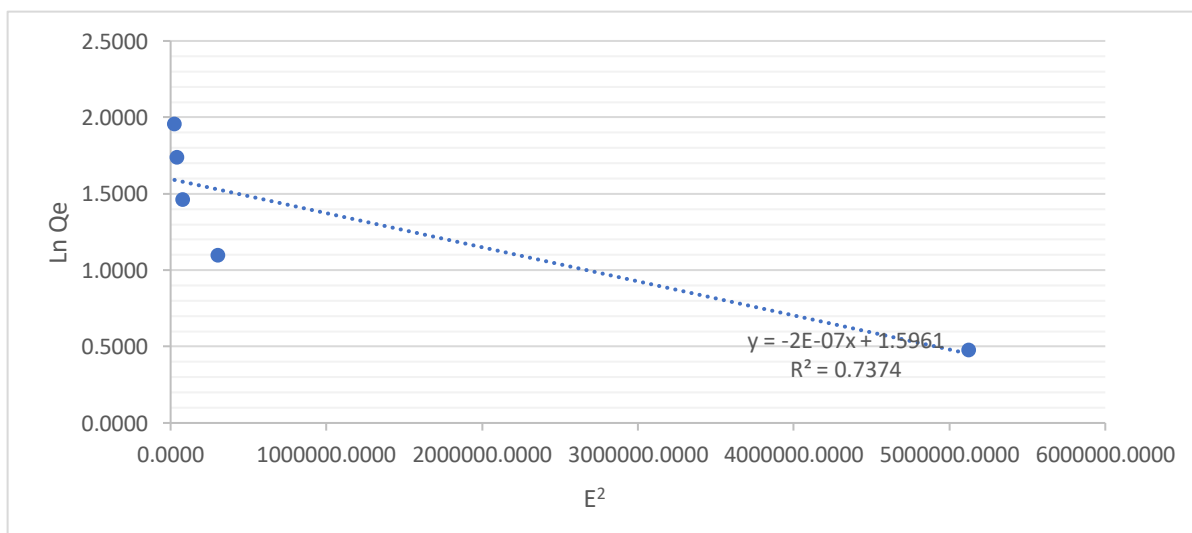


Figure 12 : D -R Isotherm model for the adsorption of methylene blue dye unto NACHA

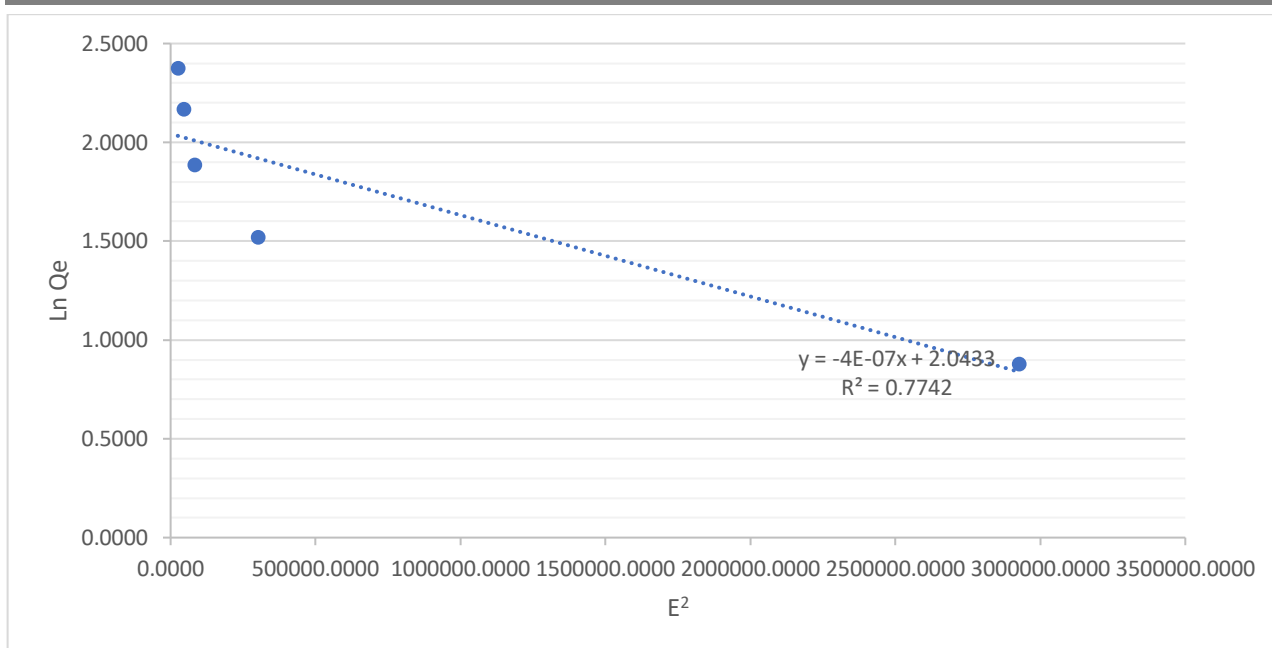


Figure 13 : D -R Isotherm model for the adsorption of methylene blue dye unto ACHA

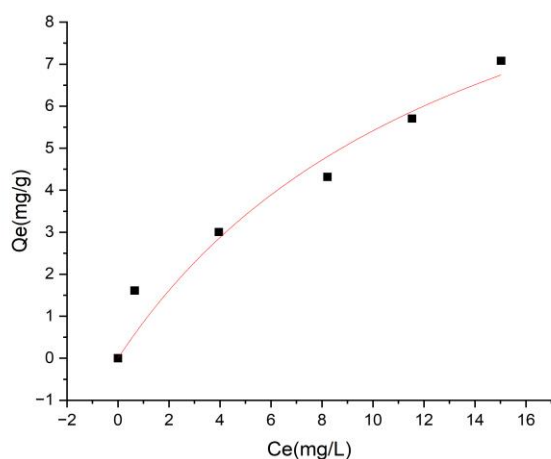


Figure 14 : K - C Isotherm model for the adsorption of methylene blue dye unto NACHA

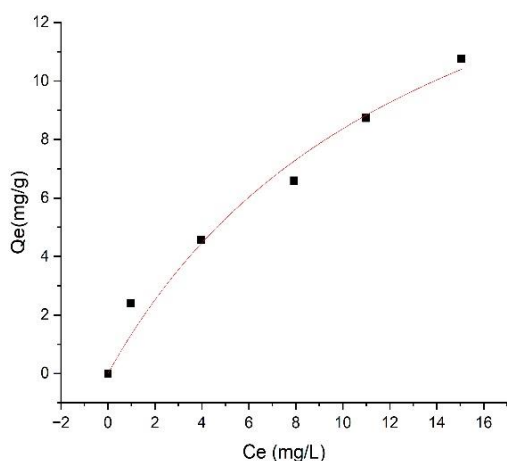


Figure 15 : K -C Isotherm model for the adsorption of methylene blue dye unto ACHA

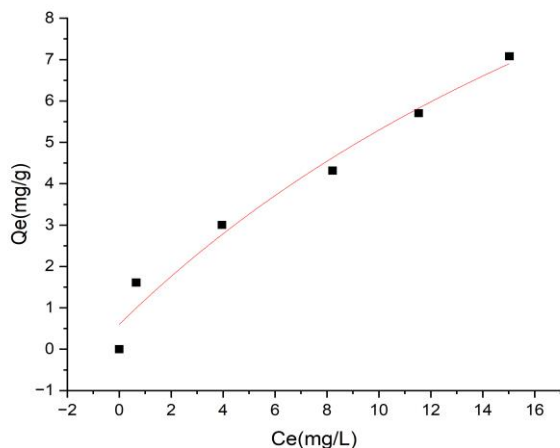


Figure 16 : Vieth Sladek Isotherm model for the adsorption of methylene blue dye unto NACHA

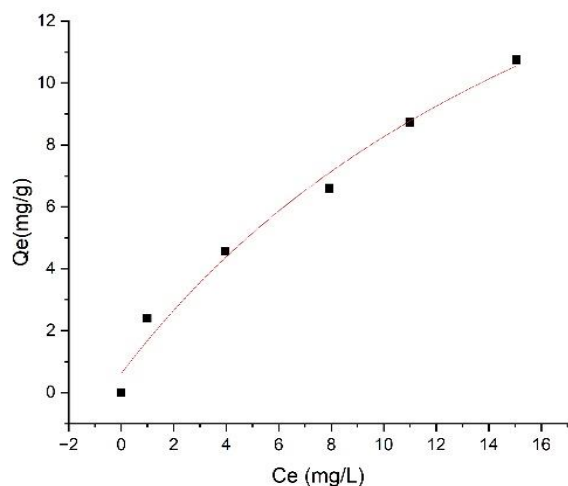


Figure 17 : Vieth Sladek Isotherm model for the adsorption of methylene blue dye unto ACHA

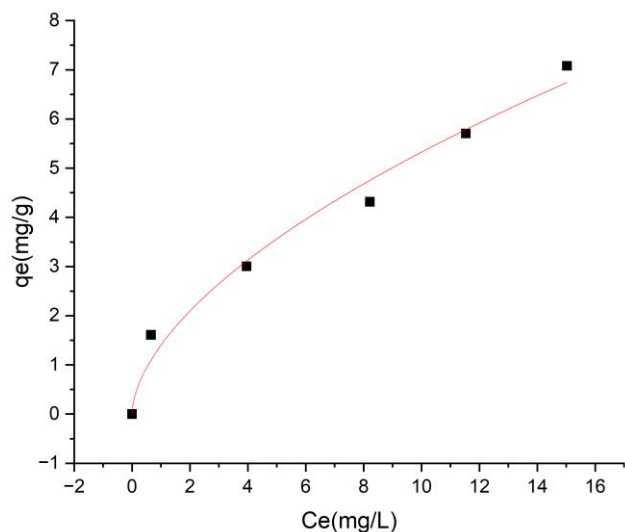


Figure 18 : Radke Prausnitz Isotherm model for the adsorption of methylene blue dye unto NACHA

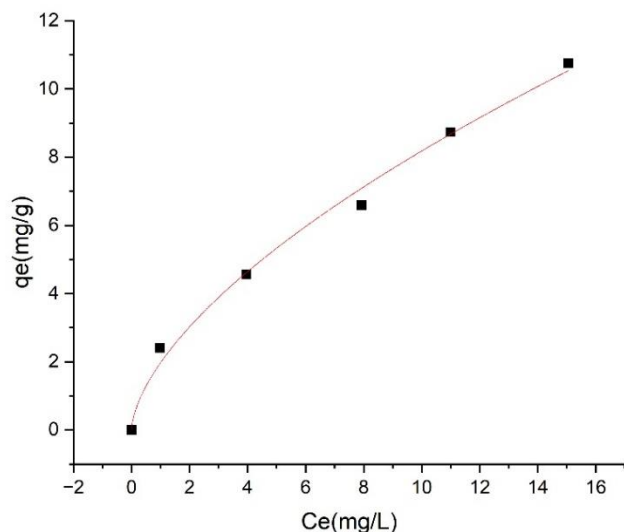


Figure 19 : Radke Prausnitz Isotherm model for the adsorption of methylene blue dye unto ACHA

Table 1.2 : Parameters of Two and Three Isotherms Model for Adsorbing Methylene Blue Dye onto Non-Activated Cow Hooves Adsorbent (NACHA) and Activated Cow Hooves Adsorbent (ACHA)

Adsorbent	Langmuir	Freudlinch	Temnik	Dubinin Raduhkevich	Koble Corrigan	Radke Prausnitz	Vieth Sladek
NACHA	qm = 8.77192 KL = 0.17463 R ² = 0.8476	k _f = 1.82473 n = 2.20022 R ² = 0.9657	B = 0.538 A = 0.2748 R ² = 0.8481	q _m = 4.93375 E = 14.97088 β = 2.2301E-07 R ² = 0.7374	A _{kC} = 2.103 B _{kc} = 0.1597 n = 0.43709 R ² = 0.9568	q _{mRP} = 1.4019 k _{RP} = 2.45E45 m _{RP} = 0.4204 R ² = 0.98276	q _m = 19.75088 k _{VS} = 0.59982 B _{VS} = 0.03119 R ² = 0.97256
ACHA	qm = 14.9476 KL = 0.13208 R ² = 0.8783	k _f = 2.31846 n = 1.84740 R ² = 0.9872	B = 0.3112 A = 0.32465 R ² = 0.8967	q _m = 7.71603 E = 11.02183 β = 4.116E-07 R ² = 0.7742	A _{kC} = 2.1957 B _{kc} = 0.10965 n = 0.65455 R ² = 0.9776	q _{mRP} = 1.9716 k _{RP} = 2.47E45 m _{RP} = 0.3820 R ² = 0.9936	q _m = 24.39136 k _{VS} = 0.61627 B _{VS} = 0.04567 R ² = 0.984

Discussions on the Isotherm Model for the removal of Methylene Blue using NACHA and Acha

The Langmuir model is based on the premise that adsorption occurs as a single layer on a uniform surface. The maximum adsorption capacity of NACHA was determined to be 8.77192 mg/g, accompanied by a Langmuir constant of 0.17463 and a correlation coefficient of 0.8476, indicating a moderate fit to the model. ACHA demonstrated an increased adsorption capacity (qm = 14.9476 mg/g) alongside a marginally reduced Langmuir constant (KL = 0.13208), achieving a stronger correlation coefficient (R² = 0.8783). This indicates that ACHA demonstrates greater affinity and aligns more effectively with the model than NACHA (Wilkins and Brandani, 2021).

The Freundlich model explains adsorption occurring in multiple layers on a surface that is not uniform. NACHA demonstrated a Freundlich constant of kf = 1.82473 and an intensity factor of n = 2.20022, along with a robust correlation coefficient of R² = 0.9657. ACHA exhibited greater constants, with kf = 2.31846 and n = 1.84740, alongside an improved correlation coefficient of R² = 0.9872, demonstrating a stronger fit. The findings suggest that both NACHA and ACHA undergo adsorption on heterogeneous surfaces, with ACHA exhibiting superior performance (Vigdorowitsch et al., 2021).

The Temkin model accounts for interactions between adsorbate molecules and presumes a gradual linear reduction in adsorption energy. NACHA had an adsorption constant of 0.538 and an equilibrium binding constant of 0.2748, with a correlation coefficient of R² = 0.8481, indicating a moderate fit to the model. ACHA exhibited a smaller value for B (0.3112) and a greater value for A (0.32465), achieving enhanced

alignment with the model, as indicated by $R^2 = 0.8967$. This indicates that ACHA exhibited more favorable molecular interactions and energy distribution (Zhou et al., 2022).

The D-R model assesses adsorption on microporous materials and determines the average adsorption energy (E). For NACHA, the maximum adsorption capacity was 4.93375 mg/g, with a mean adsorption energy of 14.97088 kJ/mol and a low correlation coefficient of $R^2 = 0.7374$, indicating a weak fit to the model. ACHA demonstrated a greater adsorption capacity ($q_m = 7.71603$ mg/g), reduced energy ($E = 11.02183$ kJ/mol), and improved alignment with the model, as reflected by $R^2 = 0.7742$. The elevated energy value for NACHA signifies more intense adsorption forces in comparison to ACHA (Sivanandan et al., 2018).

The Koble-Corrigan model integrates the principles of both Langmuir and Freundlich models to describe adsorption in heterogeneous systems. NACHA exhibited adsorption constants of $AkC = 2.103$ and $Bkc = 0.1597$, along with a correlation coefficient of $R^2 = 0.9568$, indicating a strong fit to the model. ACHA demonstrated elevated constants, with $AkC = 2.1957$ and $Bkc = 0.10965$, along with an improved correlation coefficient of $R^2 = 0.9776$, highlighting superior adsorption on heterogeneous surfaces for ACHA (Song et al., 2022).

The Vieth-Sladek model emphasizes the energy interactions between the adsorbate and the adsorbent. NACHA demonstrated an adsorption capacity of $q_m = 19.75088$ mg/g, with constants $k_{VS} = 0.59982$ and $B_{VS} = 0.03119$, achieving a robust correlation coefficient of $R^2 = 0.97256$, indicating a strong model fit. ACHA exhibited a greater adsorption capacity ($q_m = 24.39136$ mg/g) and constants ($k_{VS} = 0.61627$, $B_{VS} = 0.04567$), with exceptional alignment to the model ($R^2 = 0.984$), reflecting improved interactions between the adsorbate and adsorbent (Ramsenthil et al., 2022).

The Best Fit : The Freundlich Isotherm proved to be the most accurate for both NACHA ($R^2 = 0.9657$) and ACHA ($R^2 = 0.9872$), indicating that multilayer adsorption on non-uniform surfaces is the primary mechanism for methylene blue adsorption (Foo and Hameed, 2010).

CONCLUSION

In Conclusion, the adsorption characteristics of methylene blue on NACHA and ACHA were examined using multiple isotherm models, highlighting significant variations in adsorption capacity and interaction dynamics. Although the Langmuir model exhibited a moderate fit, suggesting single-layer adsorption, the Freundlich model displayed the highest correlation for both adsorbents, validating that multilayer adsorption on non-uniform surfaces is the primary mechanism.

ACHA demonstrated superior performance compared to NACHA across all models, displaying greater adsorption capacity and a stronger fit, indicating enhanced efficiency resulting from activation. The Temkin and Vieth-Sladek models provided additional confirmation of ACHA's enhanced molecular interactions and optimized adsorption energy distribution, further strengthening its efficiency. The results underscore the crucial role of surface heterogeneity in adsorption processes, stressing the importance of customized adsorbent modifications to enhance pollutant removal efficiency. This research offers important knowledge for the development of advanced biosorption systems, fostering progress toward sustainable water treatment approaches.

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