

Original Research

***Vernonia amygdalina*-Based Activated Carbon as a Biosorbent for Wastewater Treatment: Insights into Adsorption Thermodynamics, Isotherms, and Kinetics**Yohannes Shuka Jara ^{1,*}, Mekonnen Abera Anbesa ²

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Abstract

Today, the global community is facing significant environmental challenges due to widespread pollution entering ecosystems, such as toxic dyes in water bodies. This global issue requires environmentally conscious approaches and the development of innovative, eco-friendly and affordable solutions. In this study, activated carbon (AC) derived from *Vernonia amygdalina* stem bark (VA) was prepared by the chemical activation method as an efficient biosorbent for the removal of methylene blue (MB) from the water system. The porosity, structural characteristics, and morphology of the prepared VA biosorbent were investigated using the iodine number, methylene blue (MB) number, pH point of zero charge (pHpzc), Fourier-transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM). The analysis revealed that the biosorbent has higher microporosity with a greater sorption capacity, interactions of functional groups on a slightly acidic surface, and changes in



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morphology before and after adsorption. The maximum removal efficiency of 95% was achieved at optimal conditions, which were determined to be a dosage of 0.25 mg/L, pH 8, 10 mg/L MB concentration, 60 min contact time, and 55°C for the batch studies. Thermodynamic analysis indicated a endothermic character, spontaneous, interfacial randomness and weak physical adsorption process. Adsorption isothermal studies best fit the Langmuir model ($R^2 = 0.99$) with a maximum capacity of 3.94 mg·g⁻¹, and kinetic data followed a pseudo-second-order model ($R^2 = 0.99$). Finally, the results show that activated carbon derived from VA stems bark offers a cost-effective, eco-friendly, and practical solution for removing toxic chemicals from water systems.

Keywords

Adsorption; biosorbent; methylene blue; wastewater treatment; *Vernonia amygdalina*

1. Introduction

Unavailability of acceptable quality water is one of the major problems faced in the 21st century. Due to diverse anthropogenic activities, unplanned urbanization, and increasing industry, the quality of water supplies is deteriorating constantly [1]. The rapid growth of industrial and non-industrial activities leads to significant improper pollutant discharge into ecosystems, presenting a major global challenge. One critical category of pollutants is dyes, which must be effectively removed from wastewater before release into natural ecosystems due to their toxicity and detrimental effects on photosynthetic activity. Dyes are complex aromatic compounds that remain stable in different conditions. Each year, global production of natural and synthetic dyes exceeds 10,000 types, totaling 700,000 to 7 million tons [2-4].

Effluents discharged from various industries contain a range of toxic chemicals, including organic dyes harmful to ecosystems and life. Among these complex organic dyes, Methylene blue (MB) is a commonly used synthetic cationic dye, which is used in many sectors like the textile industry, school labs, and medicine. It is associated with several toxic effects, including adverse reactions in humans, drug interactions, and ecotoxicological impacts on aquatic life [1]. The strongest clinical evidence indicates dose-related toxicity, particularly a specific risk of serotonin toxicity when used in conjunction with selective serotonin reuptake inhibitors (SSRIs) and serotonin reuptake inhibitors (SRIs). Regarding safety limits, the clearest reported thresholds include 1-2 mg/kg for intravenous (IV) treatment of methemoglobinemia, with a maximum cumulative dose of 7 mg/kg for this indication. Additionally, the environmental no-observed-effect concentration (NOEC) is reported to be 4.7 µg/L in *Daphnia magna*, as documented by Li *et al.* [4] and Bužga *et al.* [5]. MB can cause a range of harmful effects, such as skin and mucous membrane irritation, gastrointestinal issues, headaches, and elevated blood pressure. Consequently, there is an urgent need for effective methods to remove MB and other organic dyes from wastewater to mitigate their harmful effects on living organisms [4-6].

Several treatment methods have been employed to eliminate dyes from wastewater, categorized into physical, chemical, and biological approaches [7]. Physico-chemical methods include solvent extraction, membrane filtration, chemical precipitation, photolysis, reduction,

electrochemical treatment, coagulation [8-11], and chemical oxidation with CoMn_2O_4 [12]. However, these techniques often have drawbacks such as high costs, significant energy consumption, and the generation of toxic by-products. Among these methods, adsorption using activated carbon (AC) derived from biomaterials has emerged as one of the most effective and economically viable solutions for dye removal [13]. This process is favored for its simplicity, low operational costs, ease of design, high efficiency, and rapid dye elimination as outlined by Jara [14]. Biomass-based activated carbons improve adsorption because they have many binding sites. During adsorption, dye molecules adhere to the surface of solid adsorbents through physical interactions such as hydrogen bonding and weak van der Waals forces or through chemical bonding, as supported by Yarik *et al.* work [13].

Today, Bio-sorbents are widely used for removing toxic metals and dyes because they have a microporous structure, high adsorption capacity, large surface area, and strong reactivity. Compared to alternative adsorbents, AC offers numerous advantages, including high efficiency in removing odors and tastes, straightforward process design, molecular-level selectivity, low energy requirements, reusability, and durability in harsh environments [13, 15]. Recently, various publications have focused on removing organic dyes, such as methylene blue, from aqueous solutions using natural adsorbents derived from plant parts and waste materials. However, there is still a need for innovative and eco-friendly approaches to address associated challenges such as efficiency, scalability, sustainability, and renewability. To address this, researchers are exploring phytochemical-rich plants as biosorbent sources because their high secondary-metabolite content makes them chemically active. *Vernonia amygdalina* is a well-known medicinal plant and has been extensively studied in pharmacology due to its phytochemical richness [1]; however, its potential in environmental remediation remains unexplored. To the best of our knowledge, the removal of methylene blue from aqueous solutions using VA plant stem bark as a biosorbent has not been reported yet. In order to address this gap, this study aims to prepare a biosorbent known as activated carbon derived from VA stem bark. Utilizing VA plant stem bark as a viable source of activated carbon for removing toxic organic dyes from wastewater offers a cost-effective, environmentally friendly, and practical approach to waste conservation and management. This study will highlight the impact of pollutants, such as dyes discharged from industries, on the ecosystem. It will demonstrate how these pollutants can be effectively treated before being released into the environment by using a simple, synthesized, cost-effective, and eco-friendly VA stem bark-sourced biosorbent, which has high adsorption capacity and ease of sorption. This will enable efficient wastewater treatment to ensure clean water in the future, thereby safeguarding human, animal, and aquatic life from serious exposure to toxic chemicals.

2. Materials and Methods

2.1 Chemicals and Instruments

Sodium thiosulfate (0.1 N $\text{Na}_2\text{S}_2\text{O}_3$), 85% phosphoric acid (H_3PO_4 , Merck), sodium hydroxide (99%, Shraddha Associates, India), potassium iodide (KI), 0.1 M hydrochloric acid (HCl), sodium chloride (AR grade), and methylene blue ($\text{C}_{16}\text{H}_{18}\text{N}_3\text{S}$, NICE) were utilized as received, all being analytical-grade chemicals. Solutions were prepared using distilled water. The biomaterial, VA stem bark, was weighed on a digital analytical balance. The absorbance of methylene blue in water samples was measured using a UV-Vis spectrophotometer equipped with a deuterium background corrector

(CE1021). An electric grinder (National) was employed to grind VA stem bark, while a drying oven (Digit heat, J. P. Selecta, Spain) was used to dry samples in porcelain crucibles. A portable pH meter (Model HI9024, HANNA Instruments) was used to measure pH. Fourier Transform Infrared Spectrometry (Perkin Elmer Spectrum 65 FTIR) and Scanning Electron Microscopy (SEM) were used for the identification of functional groups and the examination of surface morphology of the activated carbon before and after adsorption, respectively. An incubator shaker was used to agitate conical flasks containing the samples.

2.2 Collection of Sample and Preparation of VA Stem Bark Powder

The stem bark of *Vernonia amygdalina* was collected from the Hawassa Tabor Mountain Wildlife Conservation and Repair Park in the Sidama Regional State of Ethiopia, with permission from the Ethiopian Wildlife Conservation Authority (permit number EWCA-H-YS0072/22). The collection was authenticated by Reta Regassa, a botanist at Hawassa Teaching College and the EWCA-Hawassa office. A voucher specimen with the number YS00109/22 was deposited at the HU Herbarium of Hawassa University, as detailed in our previous work on the plant [1]. We thoroughly washed the collected stems with tap and distilled water to remove impurities. The bark was then separated using a knife and air-dried at room temperature (25°C) for seven days. After drying, the bark was crushed into granules, sieved to 250 micrometers, and stored in a tightly sealed plastic container for further analysis, as illustrated in Figure 1.

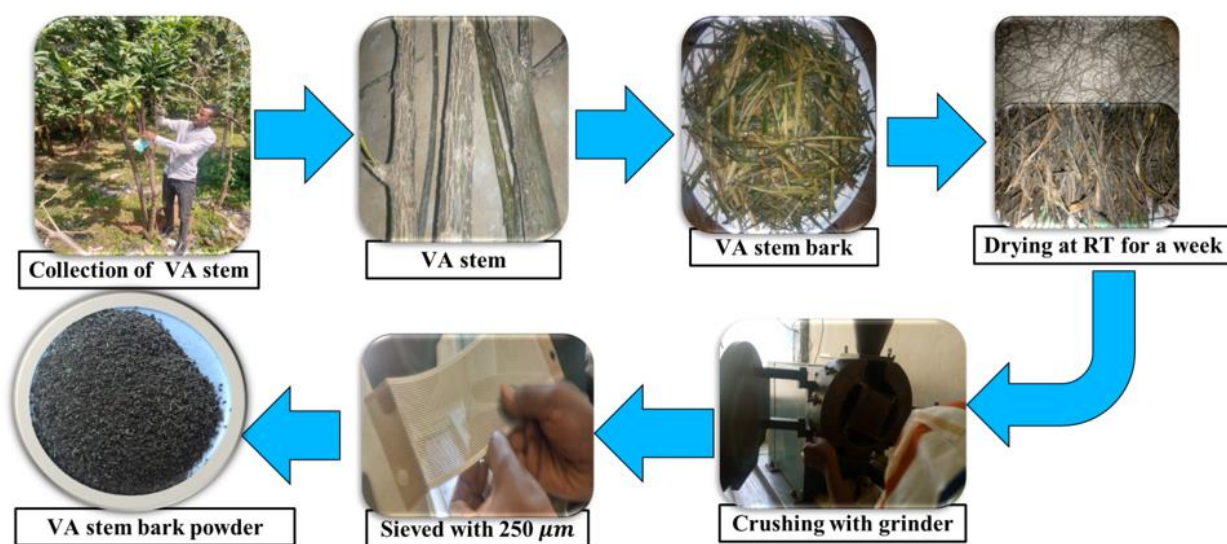


Figure 1 VA stem bark powder preparation.

2.3 Preparation and Optimization of VA Biosorbent

1 g of VA stem bark powder was taken from the previously prepared and was impregnated with 2 mL 85% H_3PO_4 for 24 h. After that, the activated biomaterial was washed with water followed by 0.1 M hydrochloric acid (HCl) to remove the residual chemical agents until the pH value reached 7 and dried at 105°C for 12 hr by using a drying oven. Finally, the dried activated biomaterial was carbonized at 600°C in a muffle furnace for 2 hr. After completing the carbonization process, the prepared biosorbent was cooled at RT, crushed, and sieved to a mesh size of 200 μm to eliminate the effect of sieve size in the study. The same experimental procedure was repeated to optimize the

effect of the key parameters on the porous characteristics of biosorbent AC by varying the impregnation chemicals (H_3PO_4 , NaCl, and NaOH) and carbonization temperatures (400, 500, 600, and 700°C) in air as detailed in the previous report [16, 17]. Finally, the VA biosorbent was re-prepared using the optimized carbonization temperature and chemical activator for the characterization of its porosity, structure, and morphology, as well as for batch adsorption studies.

2.4 Preparation of Dye Solution

A standard methylene blue solution was prepared in the laboratory by using stock Methylene blue stain reagent powder ($C_{16}H_{18}N_3SCl$) and diluting it in series to prepare the desired concentration in mg/L. Briefly, 1 g of MB stain reagent powder was dissolved in 1.0 L of distilled water and a stock solution of 1000 mg/L of MB was prepared. Next, the desired dye concentrations were determined starting from the standard solution of methylene blue (MB). The absorbance of MB solution was measured with a UV-Vis spectrometer (CE1021) at the maximum wavelength (λ_{max}), 665 nm, for the calibration [18].

2.5 Porosity Characterization of the VA Biosorbent

2.5.1 Iodine Number

Iodine number tests were conducted using the ASTM D4607-14 Standard volumetric method, as outlined in EuroLab [19]. Initially, a 0.02 N iodine solution was mixed with the VA biosorbent and shaken occasionally. The solution was then titrated with $Na_2S_2O_3$, yielding values that indicated the amount of iodine adsorbed per weight of carbon, expressed in mg/g [20]. A standard iodine solution was mixed with 0.25 g of the VA biosorbent and treated with 10.0 mL of 5% HCl, then boiled for 30 seconds and cooled. After cooling, add 100 mL of 0.1 N iodine solution and then stirred for another 30 seconds. The mixture was filtered, and 50 mL of the filtrate was titrated with 0.1 N sodium thiosulfate using starch as an indicator. The amount of iodine adsorbed per gram of carbon (X/M) was plotted against the iodine concentration in the filtrate (C) on logarithmic axes, with X/M and C calculated using equations (1) and (2), respectively.

$$\frac{X}{M} \left(\frac{mg}{g} \right) = \frac{(N_1 \times 126.93 \times V_1) - \frac{[V_1 + V_{HCl}]}{V_F} \times (N_{Na_2S_2O_3} \times 126.93) \times V_{Na_2S_2O_3}}{M_C} \quad (1)$$

$$C = N_{Na_2S_2O_3} \times \frac{V_{Na_2S_2O_3}}{V_F} \quad (2)$$

Where: N_1 is the iodine solution normality; V_1 is the added volume of iodine solution; V_{HCl} is the added volume of 5% HCl; V_F is the filtrate volume used in a titration; N is the sodium thiosulfate solution normality; V is the consumed volume of sodium thiosulfate solution; and M is the mass of activated carbon. The iodine number, reported in milligrams, is the amount of iodine adsorbed per gram of carbon at a residual iodine concentration of 0.02 N.

2.5.2 Methylene Blue Number

A total of 10.0 mg of VA biosorbent was placed in contact with 10.0 mL of methylene blue solutions at various concentrations (10, 25, 50, 100, 250, 500, and 1000 mg/L) for a duration of 24 h at 25°C. The remaining concentration of methylene blue in each solution was then analyzed using a UV/Vis spectrophotometer [21]. The amount of methylene blue adsorbed by the biosorbent from each solution was calculated using Equation (3).

$$MBN \left(\frac{mg}{g} \right) = \frac{(C_0 - C_e)}{M} V \quad (3)$$

Where: C_0 is the concentration of the methylene blue solution at time = 0, in mg/L; C_e is the concentration of the methylene blue solution at equilibrium time, in mg/L; V is the volume of the solution treated, in L; and M is the mass of the adsorbent, in g.

2.5.3 Determination of Point of Zero Charge

The salt method was employed for the determination of pH_{pzc} values. A total of 0.25 g of VA biosorbent was added to 30 mL of a 0.01 M NaCl solution. The pH of the solution was adjusted to values ranging from 2 to 10 using HCl (0.1 mol/L) or NaOH (0.1 mol/L), and the initial pH was recorded. After 24 h, the final pH of each sample was measured with a pH meter. The change in pH (Δ pH) values were plotted on the y-axis against the initial pH values (pH_i) on the x-axis. The point where the resulting curve intersects the x-axis indicates the pH_{pzc} [22].

2.6 Instrumental Characterization of the VA Biosorbent

Fourier transform infrared spectroscopy (FT-IR) was used to investigate the various functional groups involved in the adsorption of methylene blue (MB) dye onto the surface of the VA biosorbent. The analysis was conducted at the Central Laboratory of Sciences, Hawassa University, utilizing a PerkinElmer FT-IR BX spectrophotometer. Spectra were recorded in the range of 4000 to 400 cm⁻¹, with samples prepared as KBr pellets. The VA stem bark-derived activated carbon by H₃PO₄ sample (Biosorbent) surface composition, porous structure, and topography were analyzed through scanning electron microscopy (SEM) images at the Laboratory of Applied Biology, Adama Science and Technology University.

2.7 Biosorption of Methylene Blue

Batch adsorption was conducted following the procedure reported by Mesele *et al.* [18], with slight modifications. Briefly, 50 mL of a 15 mg/L methylene blue (MB) solution was placed in a 250 mL conical flask for the batch experiment. Next, 0.1 grams of prepared biosorbent was added. Subsequently, the mixture was stirred slowly and the initial pH of the solution was adjusted to 6 with 1 M HCl. Then, the adsorption was started with a magnetic stirrer at 200 rpm for 75 minutes on a digital hot plate set to 45°C. After the stirring period, the adsorbent was centrifuged. A 2 mL sample of the MB solution after adsorption was taken, and its concentration was determined using a UV-Vis spectrophotometer (CE1021) at 665 nm. Finally, the removal efficiency (%) and adsorption

capacity of the biosorbent before and after dye concentration were calculated using equations (4) and (5), respectively.

$$\text{Removal efficiency (\%)} = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (4)$$

$$\text{Adsorption capacity } (q_e) = \frac{(C_0 - C_e)}{m} V \quad (5)$$

Where: C_0 is the initial solution concentration, C_e is the equilibrium concentration, V is the volume of solution, and m is the mass of adsorbent.

The effects of key parameters on the batch adsorption study were investigated by repeating the same experimental procedure with initial concentration 10, 15, 20, 25, 30 and 35 mg/L temperatures of 25, 35, 45, and 55°C; contact times of 15, 30, 45, 60, 75, and 90 minutes; solution pH values of 2, 4, 6, 8, and 10; and adsorbent dosages of 0.1, 0.15, 0.2, 0.25, 0.3, and 0.35 grams. When one parameter was investigated, the others were kept constant [18].

2.8 Adsorption Thermodynamic, Isotherm and Kinetic Study

We conducted a thermodynamic study based on previously reported work by Mengesha *et al.* [18]. In summary, 50 mL of a 10 mg/L MB solution was combined with 0.25 g of AC derived from VA stem bark biosorbent in 100 mL Erlenmeyer flasks under optimal conditions. The solution was then shaken at 25-55°C at 10°C intervals. After shaking, the concentration of MB in the filtrate was measured using a spectrophotometric method. Finally, the thermodynamic parameters such as enthalpy of adsorption (ΔH°), Gibbs free energy of adsorption (ΔG°), and entropy of adsorption (ΔS°) were calculated as outlined in Eqs. (6) and (7);

$$\ln Kc = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT} \quad (6)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (7)$$

Adsorption isotherm studies were conducted using 0.25 g of VA biosorbent in 50 mL of MB solutions ranging from 10 to 30 mg/L, with 5 mg/L intervals, under optimal conditions. The solution was shaken in a laboratory shaker, then filtered after the reaction time. The concentration of MB in the solution was determined using spectrophotometry. Finally, the adsorption capacity of the adsorbent was calculated, and the experimental data were analyzed by fitting them to the Langmuir and Freundlich isotherm Eq. (8) and (9) [23].

$$\frac{C_e}{q_e} = \frac{1}{q_{max}} C_e + \frac{1}{q_{max}K_L} \quad (8)$$

$$\log q_e = \frac{1}{n} \log C_e + \log K_F \quad (9)$$

Where: q_e - amount adsorbed per unit mass of adsorbent at equilibrium (mg/g); q_{max} - maximum monolayer adsorption capacity; C_e - equilibrium concentration of adsorbate (mg/L); K_L - Langmuir

constant related to adsorption energy; K_F - Freundlich adsorption constant; n = adsorption intensity constant.

Kinetic studies were conducted by adding 0.25 g of VA biosorbent to 50 mL of a 10 mg/L MB solution under optimal conditions for the batch experiment. To monitor the ongoing adsorption process, 5 mL samples of the solution were taken and centrifuged at 5-minute intervals, starting from 30 minutes and continuing until 90 minutes of adsorption time. The concentration of the filtrate was then analyzed using a UV-Vis spectrophotometer. Finally, the experimental data were evaluated against the pseudo-first-order and Ho-McKay pseudo-second-order equations, as described in equations (10) and (11) [24].

$$\log \frac{q_e}{q_e - q_t} = \frac{k_1}{2.303} t \quad (10)$$

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \quad (11)$$

3. Results and Discussion

3.1 Optimization of Carbonization and Impregnation

The adsorption performance of AC derived from VA stems bark was strongly influenced by carbonization temperature, as shown in Figure 2a. Removal efficiency increased gradually from 400 to 550°C, reaching a maximum of 94% at 550°C, which can be attributed to the development of well-structured pores and enhanced surface area at this temperature. However, further increases in the temperature beyond 550°C led to a decline in adsorption capacity, likely due to pore collapse, reduced surface area, and partial degradation of functional groups [25]. Similar research conducted by Karume *et al.* evaluated the impact of carbonization conditions and the nature of the adsorbate on the performance of activated carbon in water treatment and reported findings aligned with this study [26]. Therefore, 550°C was identified as the optimum carbonization temperature for biosorbent preparation and further batch studies, which is strongly recommended by Fonseca *et al.* [27] for biochar and activated carbon production.

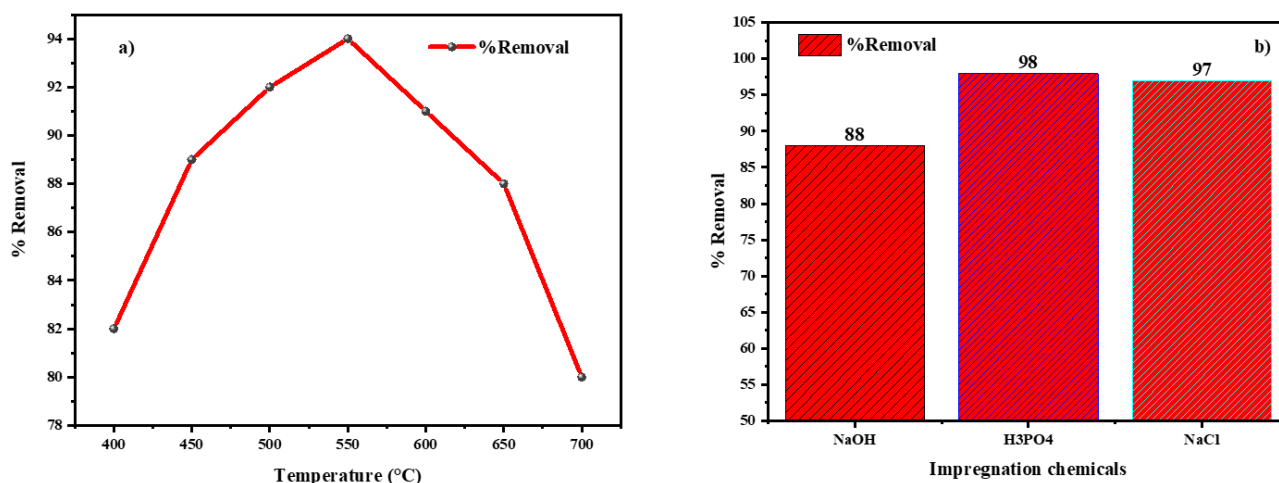


Figure 2 (a) The Effect of carbonization temperature and (b) chemical activators on the MB percentage removal.

The effect of different chemical activation on the microporous structure and adsorption performance of AC derived from VA stems bark for MB removal was systematically evaluated as shown in Figure 2b. Among the tested agents, H_3PO_4 -assisted activation shows the highest adsorption capacity, achieving 98% removal efficiency on the MB, surpassing NaCl (97%) and NaOH (88%). The superior performance of H_3PO_4 can be attributed to its unique chemical activation mechanism. Phosphoric acid promotes the formation of a well-developed microporous and mesoporous network through dehydration and cross-linking reactions during carbonization. Yang *et al.* [28] concluded that Phosphoric acid stabilizes the carbon matrix by forming phosphate esters and polyphosphate linkages with hydroxyl groups in lignocellulosic biomass, which prevents structural collapse and enhances pore development. Furthermore, in another study, Jawad *et al.*, [29] outlined that H_3PO_4 introduces oxygenated and phosphorus-containing functional groups (e.g., $-\text{PO}_4^{3-}$, $-\text{OH}$, $-\text{C-O-P}$), which act as active sites for electrostatic attraction and hydrogen bonding with cationic dyes such as MB [29].

In contrast, NaOH activation relies primarily on alkali-induced etching, which can generate porosity but often leads to partial collapse of micropores at high concentrations. NaCl, while effective in salt-templating pore formation, does not contribute additional functional groups for dye binding, explaining its slightly lower efficiency compared to H_3PO_4 . Prior studies on activated carbons derived from lignin, fruit peels, and agricultural residues utilized H_3PO_4 -assisted activation [2, 28, 29], which is consistent with our findings. This method confirms its ability to simultaneously enhance pore structure and introduce phosphate functional groups, leading to outstanding adsorption efficiency for MB removal compared to NaCl and NaOH. Therefore, we selected phosphoric acid as the optimal activator for biosorbent preparation and batch studies.

3.2 Characterization of the Prepared VA Biosorbent

3.2.1 Iodine Number and Methylene Blue Number

The iodine number and methylene blue number (MBN) are widely recognized as fundamental parameters for characterizing the adsorption performance of activated carbons. The iodine number, defined as the amount of iodine adsorbed per gram of carbon, is directly proportional to the microporous surface area (<2 nm) and is often used as a proxy for total surface area and adsorption capacity [30]. Conversely, the methylene blue number reflects the mesoporous fraction (2-5 nm), providing insight into the ability of activated carbon to adsorb larger organic molecules such as dyes [31, 32].

As seen in Figure 3(a-b) in this study, AC derived from VA stem bark exhibited an iodine number of 615 mg/g, and the methylene blue number reached 524 mg/g, signifying a high degree of microporosity and a value closeness suggesting that the biosorbent contains a significant fraction of mesopores. This balanced pore size distribution enhances adsorption efficiency by accommodating both small molecules (e.g., iodine) and larger dye molecules (e.g., methylene blue). This result aligns with previous reports where iodine numbers above 600 mg/g were associated with highly microporous carbons suitable for water purification and gas adsorption by Itodo *et al.*, [32]. They demonstrated that iodine and methylene blue adsorption tests are reliable indicators of surface area and pore structure in acid- and salt-treated activated carbons. Moreover, Heycarb [30] emphasized that higher iodine numbers correlate with greater sorption capacity, while methylene blue adsorption reflects mesoporous accessibility and functional group availability. However, the

porosity and pore-size characteristics obtained in this study fall within the acceptable ranges and are consistent with previously reported findings. Nevertheless, the absence of advanced characterization techniques, such as BET (Brunauer-Emmett-Teller) surface area analysis, represents a limitation and raises concerns regarding the need for secondary validation of the textural properties. Future studies should incorporate and optimize these advanced characterization methods to provide more reliable verification and facilitate the development of high-performance biosorbents.

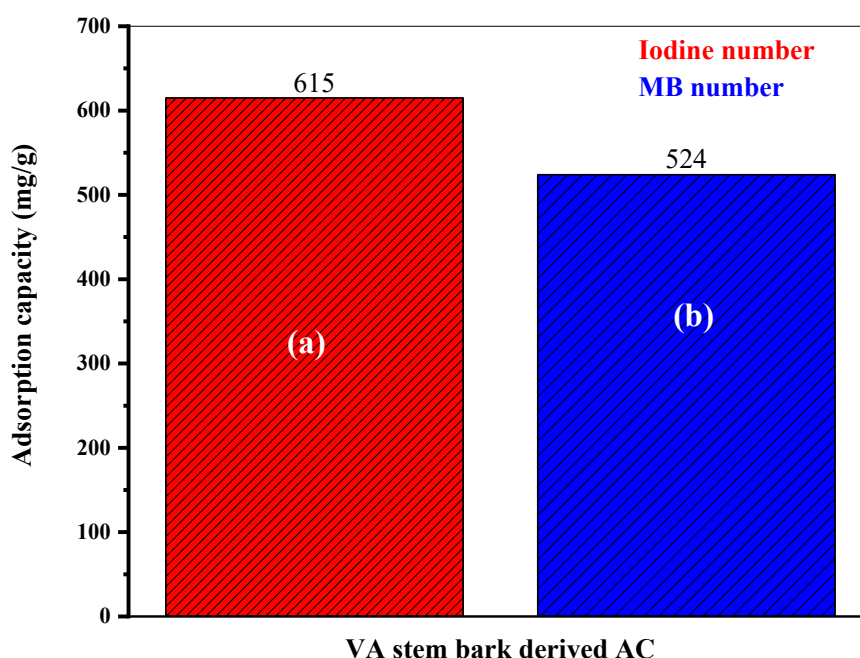


Figure 3 Iodine number (a) and methylene blue number (b).

3.2.2 Determination of pH Point of Zero Charge (pHpzc)

The identification of acidic and basic surface characteristics is a central aspect of surface chemistry because these properties strongly influence adsorption mechanisms, electrostatic interactions, and the overall affinity of adsorbents toward ionic species. The point of zero charge (pHpzc) is particularly important, as it defines the pH at which the net surface charge of an adsorbent becomes zero. As shown in Figure 4, the AC derived from VA stems bark exhibited a pHpzc value of 6, indicating that its surface is dominated by weak acidic functional groups. This interpretation aligns with established findings showing that activated carbons commonly contain acidic oxygenated groups such as carboxylic, lactonic, and phenolic functionalities, which lower the pHpzc and impart acidic character to the surface. Previous studies on activated carbon verified that oxidative processes or naturally oxygen-rich precursors introduce additional acidic groups, thereby decreasing the pHpzc and enhancing surface acidity. For example, oxidation treatments have been shown to significantly reduce pHpzc values by increasing the abundance of carboxylic groups on carbon surfaces, as reported by Uddin *et al.*, [33]. This behavior is consistent with the observed pHpzc of the AC derived from VA stems bark.

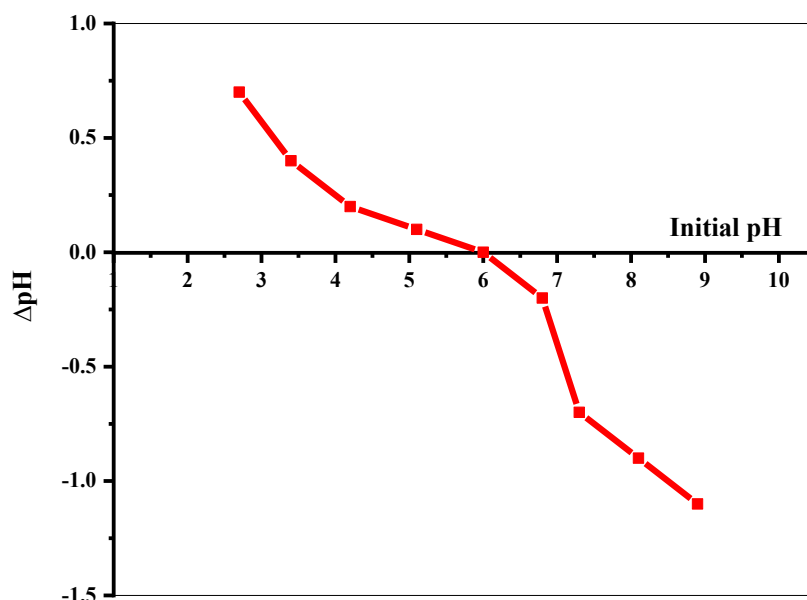


Figure 4 The pH at the point of zero charge.

The relationship between pH and surface charge is well established: when the solution pH is below the pH_{pzc}, surface functional groups tend to be protonated, resulting in a positively charged surface. Conversely, at pH values above the pH_{pzc}, deprotonation occurs, producing a negatively charged surface. This charge reversal phenomenon has been widely documented in adsorption studies involving activated carbons and other porous materials. As a result, at pH < 6, the AC derived from VA stems bark surface becomes positively charged, which enhances its electrostatic attraction toward anionic species. At pH > 6, the surface becomes negatively charged, favoring the adsorption of cationic species. This pH-dependent adsorption behavior is consistent with numerous studies demonstrating that the surface charge of activated carbon plays a decisive role in determining its affinity for ionic contaminants, as highlighted in the state-of-the-art review by Kah *et al.* [34] on activated carbon and other carbonaceous materials.

3.2.3 FT-IR (Fourier Transform Infrared Spectrometry)

The FT-IR spectrum of activated carbon (AC) derived from VA stem bark was scanned over the frequency range of 4000–400 cm⁻¹ to identify distinct types of functional groups on the adsorbent surface before and after adsorption are shown in Figure 5. The increase in intensity and the shift in peak positions to lower frequencies in the AC derived from VA stems bark following methylene blue biosorption validate the participation of different functional groups in the adsorption process. This is confirmed by the shifting of peak positions of the biosorbent before MB adsorption at 3394, 2939, 2859, 1699, 1615, 1417, 1029, and 573 cm⁻¹ to positions after MB adsorption at 3382, 2921, 2844, 1684, 1603, 1406, 1018, and 554 cm⁻¹ [1].

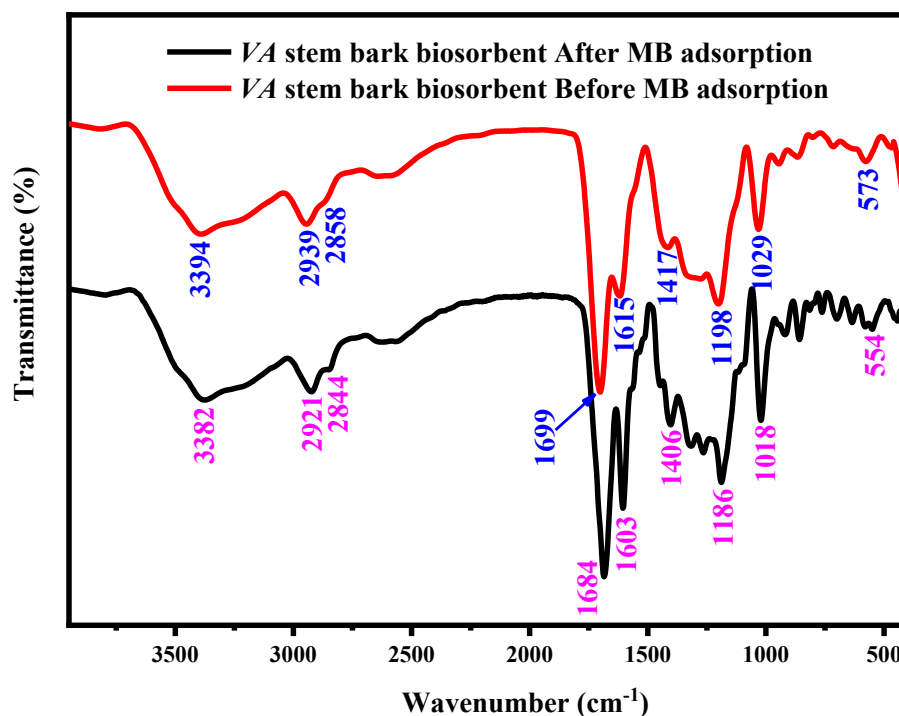


Figure 5 FT-IR spectra of VA stem bark biosorbent (-red) and after MB adsorption (-blue).

This detailed analysis of FT-IR peak shifts and their correlation with the adsorption mechanisms of MB on the VA biosorbent provides a comprehensive understanding of the functional groups involved in the biosorption process. The study identifies several key functional groups and their associated mechanisms. The O-H stretching observed in the range of 3600–3150 cm^{-1} corresponds to hydroxyl groups (-OH), which facilitate MB adsorption through hydrogen bonding with nitrogen atoms in MB, surface complexation via proton-donor interactions, and electrostatic attraction when -OH groups deprotonate at pH levels greater than the pH_{pzc}. Significant peak shifts to lower wavenumbers indicate these interactions. The C-H stretching peaks at 2939 and 2859 cm^{-1} represent aliphatic C-H groups, which engage in hydrophobic interactions with MB's aromatic rings and van der Waals forces. Although these groups are not primary adsorption sites, their shifts suggest interactions with the hydrophobic regions of the carbon matrix.

The C=O stretching at 1699 cm^{-1} is attributed to carboxylic (-COOH) and carbonyl groups, which participate in electrostatic attraction between deprotonated -COO^- groups and MB^+ , surface complexation via lone-pair donation from oxygen to MB, and hydrogen bonding. The significant peak shifts here indicate the active involvement of carboxyl groups in MB binding, consistent with existing literature on dye adsorption. Additionally, the water bending vibration at 1615 cm^{-1} relates to adsorbed water (H-O-H bending), reflecting modifications in hydrogen bonding networks and solvation effects that enhance MB-surface interactions during dye adsorption [32].

The C-O stretching observed at 1198 cm^{-1} corresponds to phenolic, alcoholic, ether, and carboxylate C-O groups, which engage in hydrogen bonding with MB, electrostatic attraction when phenolic groups deprotonate, and surface complexation. Observable FTIR shifts in this region are indicative of known interactions between these functional groups and MB. The C-C/C-H vibrations at 1029 cm^{-1} highlight aromatic ring vibrations, where π - π electron donor-acceptor interactions occur between MB and the aromatic domains of carbon. This π - π stacking mechanism is well

documented and suggests favorable interactions between the conjugated aromatic system of MB and graphitic domains on AC surfaces [28, 35].

Finally, low-frequency peaks at 573 cm^{-1} indicate out-of-plane bending of aromatic C-H or C-C bonds, pointing to structural rearrangements within the carbon matrix upon MB adsorption, as well as weak van der Waals interactions. These shifts suggest that structural changes occur in the carbon matrix as a result of dye adsorption. In general, the specific FT-IR spectral changes relate to the underlying adsorption mechanisms of methylene blue on VA stem bark-derived activated carbon.

3.2.4 Scanning Electron Microscope (SEM)

The SEM micrographs of the AC derived from VA stem bark before and after MB adsorption in Figure 6(a-b) clearly prove the morphological changes associated with the biosorption process. Prior to adsorption, the AC surface exhibits a highly porous structure with irregularly shaped particles and a rough, heterogeneous texture. Such porous architectures are characteristic of lignocellulosic activated carbons and are known to enhance adsorption performance by providing abundant active sites and accessible pore channels for adsorbate diffusion. Similar observations have been reported for activated carbons derived from agricultural biomass, where well-developed pores significantly contribute to dye uptake efficiency in olive-waste AC [35], coffee-grounds AC [36], and papaya-plant AC [37].

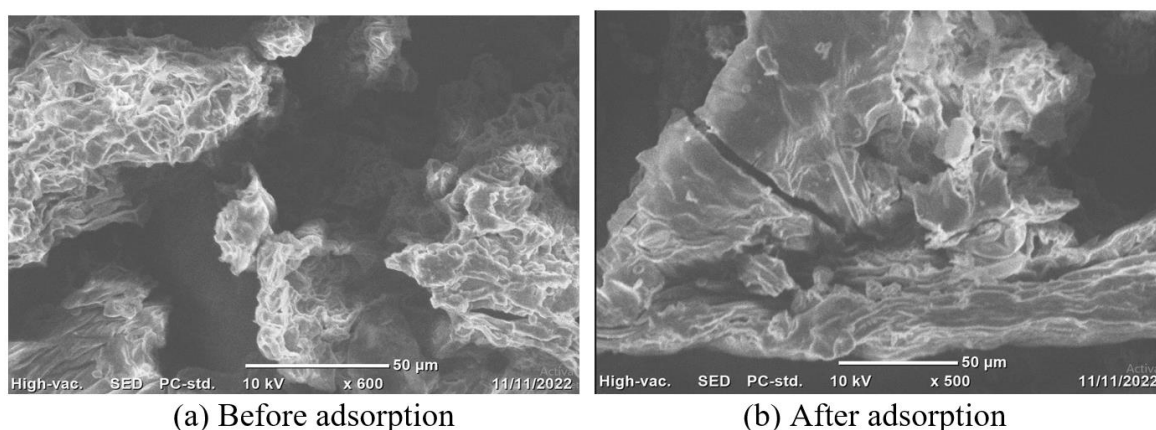


Figure 6 SEM image of VA stem bark biosorbent (a) and after MB adsorption (b).

Following MB adsorption, the SEM image in Figure 6b reveals a noticeable reduction in porosity, along with a smoother and more compact surface morphology. This morphological transformation is consistent with the physical occupation of surface pores by MB molecules, a phenomenon widely documented in dye adsorption studies. For example, Gmati *et al.*, [35] observed that MB adsorption onto olive-waste activated carbon resulted in pore blockage and smoother surface features. Likewise, Araya-Sibaja *et al.*, reported that coffee-grounds activated carbon displayed reduced porosity and more uniform surface coverage after MB uptake [36]. Similar findings were also described by Nady *et al.*, for papaya-plant activated carbon, where dye adsorption led to visible pore filling and surface smoothing [37]. The observed reduction in pore visibility and the more uniform post-adsorption surface in the AC derived from VA stems bark therefore align with established adsorption behavior in the literature. Although the absence of X-ray diffraction (XRD) and energy-dispersive X-ray spectroscopy (EDX) analyses limits discussion of composition and

surface-structure changes, the morphological changes observed in SEM confirm that MB molecules successfully occupy and interact with available pores and surface functional groups. This demonstrates the effectiveness of VA stems derived AC is promising as a biosorbent for methylene blue.

3.3 Batch Adsorption Study

A dosage study is crucial in adsorption research as it assesses the adsorbent's capacity for a specific dye concentration. The effect of varying biosorbent doses (0.1 to 0.35 g) on the removal efficiency of MB dye from 50 mL of 10 mg/L aqueous solution was evaluated using AC derived from VA stem bark. As shown in Figure 7a, the removal efficiency of biosorbent increased from 66% to 92% as the dosage of AC increased from 0.1 to 0.25 g per 50 mL solution, while the adsorption capacity decreased due to the inverse relationship with mass. This increase in efficiency can be attributed to a larger adsorbent surface area and more available adsorption sites [24]. At higher dosages, adsorption reaches equilibrium quickly due to osmotic pressure, leading to efficient use of active sites. Thus, a median dose of 0.25 g was selected as the optimum for future studies.

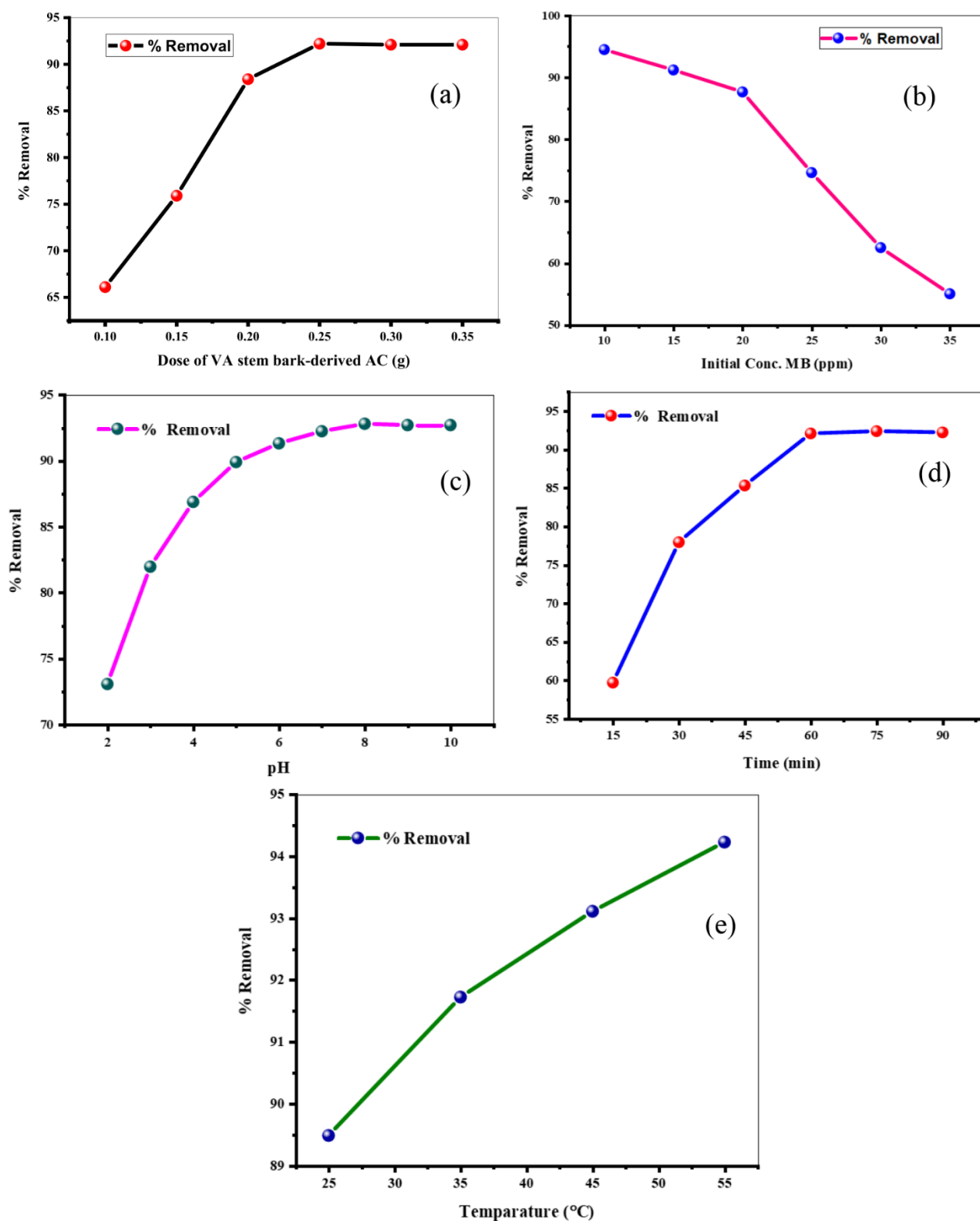


Figure 7 (a) Effect of adsorbent dose (Adsorption conditions: Volume of the MB aqueous solution 50 ml, initial concentration of MB: 10 mg/L, pH: 8, temperature: 45°C, and contact time: 60 min). (b) Effect of initial dye concentration (Adsorption conditions: Volume of the MB aqueous solution 50 ml, pH: 8, biosorbent dose: 0.25 g, temperature: 45°C, and contact time: 60 min). (c) Effect of solution pH (Adsorption conditions: Volume of the MB aqueous solution 50 ml, initial concentration of MB: 10 mg/L, biosorbent dose:

0.25 g, temperature: 45°C, and contact time: 60 min). (d) Effect of contact time (Adsorption conditions: Volume of the MB aqueous solution 50 ml, initial concentration of MB: 10 mg/L, biosorbent dose: 0.25 g, pH: 8, and temperature: 45°C). (e) Effect of temperature (Adsorption conditions: Volume of the MB aqueous solution 50 ml, initial concentration of MB: 10 mg/L, biosorbent dose: 0.25 g, contact time: 60 min and pH: 8).

The influence of initial MB concentration on removal efficiency was assessed by preparing 50 ml solutions with varying concentrations from 10 to 35 mg/L. The presented result in Figure 7b shows a drastic decrease from 93.8 to 54.6%, in removal efficiency as the initial concentration of MB is varied from 10 mg/L to 35 mg/L. This can be ascribed to a limited biosorbent surface area and the availability of fewer active sites due to a fixed dosage [38]. Besides, the higher adsorption capacity was obtained at the higher initial dye concentration [39]. Therefore, the optimum initial concentration of MB removal is 10 mg/L.

Solution pH is a critical factor governing dye adsorption because it directly affects adsorbent-adsorbate interactions. For MB, adsorption typically increases with rising pH because the adsorbent surface progressively deprotonates, enhancing electrostatic attraction between negatively charged functional groups and positively charged dye molecules. Conversely, at low pH, surface protonation generates a net positive charge, resulting in electrostatic repulsion and reduced uptake. This behavior is widely reported for carbonaceous and lignocellulosic adsorbents [40, 41]. In the present study, MB removal increased markedly from 71% at pH 2 to a maximum of 93% at pH 8, beyond which no significant improvement was observed, as shown in Figure 7c. Enhanced adsorption at alkaline pH is attributed to the negatively charged surface of the AC derived from VA stem bark. Since the pH_{pzc} is 6, pH values above this point favor strong electrostatic attraction with cationic MB. Conversely, when the solution pH is below the pH_{pzc} (acidic region), removal efficiency is very low due to charge repulsion; the surface is positively charged because functional groups tend to be protonated, as discussed in the pH_{pzc} section. As pH rises to approach or exceed the pH_{pzc}, surface charge dynamics shift, maximizing adsorption at pH 8 due to electrostatic attraction. Beyond this point, adsorption plateaus as binding sites become saturated. Therefore, pH 8 was identified as the optimal condition for MB adsorption and was employed in subsequent experiments.

The adsorption kinetics of MB onto AC derived from VA stems bark show a time-dependent uptake profile, with equilibrium attained at 60 minutes and a maximum removal efficiency of 90%, as seen in Figure 7d. This trend reflects typical adsorption behavior where initial rapid uptake is driven by abundant active sites and strong concentration gradients, followed by a plateau as saturation approaches and diffusion limitations dominate. The increase in removal efficiency from 15 to 60 minutes aligns with the intraparticle diffusion model, where dye molecules initially adsorb onto the external surface and subsequently diffuse into internal pores [1]. The rapid phase is attributed to external mass transfer and surface adsorption, while the slower phase reflects pore diffusion and site saturation [34]. Equilibrium at 60 minutes suggests that the biosorbent possesses a high affinity and sufficient surface area for MB, consistent with activated carbon materials derived from lignocellulosic biomass [42]. The plateau beyond 60 minutes indicates that most accessible binding sites are occupied, and further contact time does not significantly enhance adsorption, a behavior typical of pseudo-second-order kinetics where chemisorption is rate-limiting. Thus, the optimal contact time of 60 minutes ensures maximal utilization of the biosorbent's capacity without unnecessary extension, supporting efficient process design for MB removal in aqueous systems.

Temperature is also the main factor affecting the adsorption process and its effect was measured by varying the temperature from 25 to 55°C, with 10°C intervals, under optimized biosorbent conditions. Figure 7e illustrates that increasing the temperature results in a corresponding rise in both adsorption efficiency and adsorption capacity, showing more than 94% removal efficiency at 55°C. This trend indicates that the adsorption of methylene blue onto the AC derived from VA stems bark is an endothermic process. As temperature increases, the adsorption equilibrium shifts in favor of greater uptake, which is consistent with endothermic behavior where higher thermal energy enhances the interaction between adsorbate and adsorbent. Elevated temperatures also increase the mobility and kinetic energy of dye molecules, reducing mass-transfer resistance and enabling more effective penetration into the adsorbent's pores [43]. Consequently, the enhanced molecular motion at higher temperatures promotes greater adsorption, leading to improved efficiency and capacity [44]. The maximum removal efficiency obtained was 94.23% at 55°C, which can be considered optimal (~95%) in terms of energy consumption, even though no clear decline point was observed. Moreover, higher temperatures may lead to the decomposition of biomaterials, as reported in previous studies [39-43].

3.4 Adsorption Thermodynamic Study

Thermodynamic parameters provide essential insight into the fundamental nature of MB adsorption onto AC derived from VA stem bark. The key parameters evaluated in this study include the standard Gibbs free energy change (ΔG°), the standard enthalpy change (ΔH°), and the standard entropy change (ΔS°). These parameters are interrelated through the classical thermodynamic expressions shown in Equations (6) and (7) [45]. The values of ΔH° and ΔS° were obtained from the slope and intercept of the Van't Hoff plot of $\ln K_c$ versus $1/T$ and the plot exhibited good linearity, with a correlation coefficient, R^2 value of 0.93 as seen in Figure 8, and the calculated thermodynamic parameters are summarized in Table 1. The thermodynamic analysis demonstrated that MB adsorption onto AC derived from VA stem bark is thermodynamically favorable over the investigated temperature range. The negative values of ΔG° at 298, 308, 318, and 328 K (-1.37, -1.89, -2.41, and -2.93 kJ mol⁻¹, respectively) confirm the spontaneous nature of MB uptake, with the increasingly negative ΔG° indicating that the adsorption becomes progressively more favorable with increasing temperature. This behavior is consistent with the positive enthalpy change ($\Delta H^\circ = +14.16$ kJ mol⁻¹), which confirms the endothermic character of the adsorption process and explains the enhanced adsorption affinity at elevated temperature. The positive entropy change ($\Delta S^\circ = +52.09$ J mol⁻¹ K⁻¹) suggests an increase in randomness at the solid-solution interface during adsorption, which may arise from the displacement and release of water molecules and other species previously associated with the porous carbon surface as MB molecules are adsorbed. The thermodynamic findings are further supported by the equilibrium and kinetic results and demonstrate the potential of VA stem bark-derived AC as an effective biomass-based adsorbent for MB removal from aqueous systems. These findings are broadly consistent with previous reports on MB adsorption using activated carbons derived from agricultural and plant wastes, including coffee grounds, papaya-derived materials, and olive wastes [34-37].

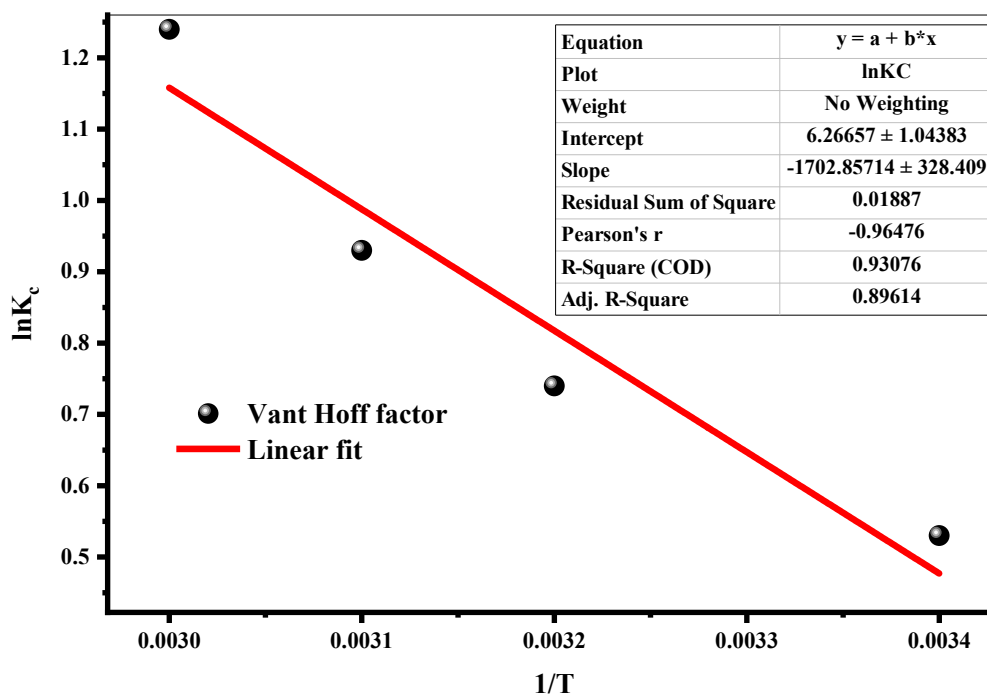


Figure 8 Van't Hoff plot for thermodynamic parameter.

Table 1 Thermodynamics parameters for the adsorption.

Temperature		ΔG° (kJ/mol)	ΔS° (J/mol·K)	ΔH° (kJ/mol)
°C	(K)			
25	298	-1.37	+52.09	+14.16
35	308	-1.89		
45	318	-2.41		
55	328	-2.93		

3.5 Adsorption Isothermal Study

The Langmuir model posits that adsorption takes place on a uniform surface in a monolayer without interactions among adsorbed molecules. Its linear form is represented by Equation (8) and a plot of C_e/q_e versus C_e gives a straight line with slope ($q_{\max}$) and intercept (K_L), as shown in Figure 9a. Similarly, another equilibrium study model is known as the Freundlich model, which describes adsorption occurring on a heterogeneous surface through multilayer formation. The Freundlich model is expressed with linearized Equation (Eq. (9)), which gives a straight line with slope and intercept to calculate the values of K_F and n , via plotting $\log q_e$ versus $\log C_e$, as shown in Figure 9b.

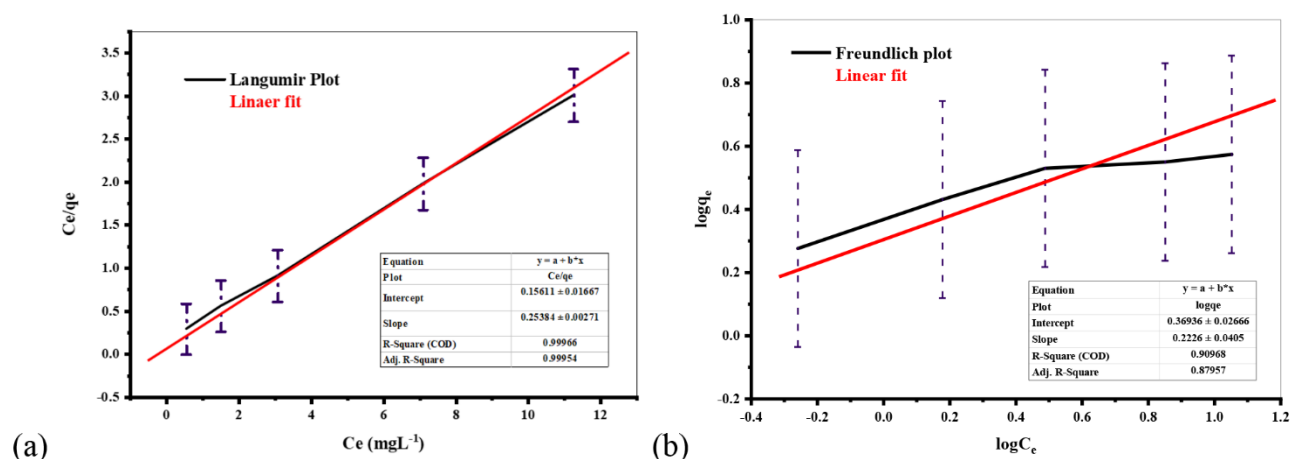


Figure 9 Langmuir (a) and Freundlich (b) isotherm adsorption for VA Biosorbent.

All model-derived parameters and constants are calculated and summarized in Table 2, based on the linear equations obtained from the plots of the two adsorption models, which are presented in Figure 9. As shown in Table 2, the Langmuir model exhibited an excellent fit to the experimental data, with a correlation coefficient, R^2 of 0.999, which is closest to 1 and significantly higher than that obtained for the Freundlich model ($R^2 = 0.909$). This result indicates that the adsorption of MB onto the VA biosorbent is predominantly governed by monolayer adsorption on a relatively homogeneous surface containing energetically equivalent adsorption sites, rather than alignment with a heterogeneous multilayer adsorption mechanism. Similarly, Patawat *et al.* [44], Tran *et al.* [45], and Makhwedzha *et al.* [2] reported Langmuir-dominant behavior in their studies of methylene blue removal on biomass-derived *Dipterocarpus alatus* fruit carbon, coffee husk hydrochar, and orange-lemon peel carbon, respectively. These findings confirm our results and provide evidence of uniform adsorption energies and monolayer uptake. The maximum monolayer adsorption capacity, $q_{\max}$ obtained from the Langmuir model was 3.94 mg/g, indicating significant adsorption, particularly at high initial dye concentrations and low doses. This capacity can be attributed to the presence of abundant pore structures and surface functional groups that enhance electrostatic attractions and π - π interactions between the cationic dye molecules and the biosorbent surface. The microporous nature of the adsorbent and its porosity were verified by the previous iodine number test in section 3.2.1 and aligned with the obtained results. The Langmuir constant, K_L of 1.60 L/mg suggests a strong affinity between MB molecules and the adsorbent surface, characteristic of favorable adsorption systems. In comparison, the adsorption capacity of this material is relatively low when contrasted with higher-performing activated carbons, such as 83.3 mg/g for bamboo-derived activated carbon reported by Bakara *et al.* [46] and 149.3 mg/g for jackfruit-peel AC studied by Aziz *et al.* [47], but it is necessary to consider this result at high initial dye concentration and low dose. Although the high K_F value (2.34) and $n < 10$ adsorption intensity which is only considered as indicators of adsorption favorability and strong adsorbent-adsorbate affinity systems rather than evidence of the adsorption mechanism [48], they further confirm the favorable adsorption [18]. The previously done work by Makhwedzha *et al.* [2] and Misran *et al.*, [49] reported higher n values for methylene blue removal on orange-lemon peel AC, and banana-stem AC, all of which were taken as evidence of favorable adsorption intensity even when Freundlich was not the best-fitting model. These findings suggest that AC derived from VA stem bark is an economically feasible biosorbent for the removal of MB from water systems. This analysis

underscores the effectiveness of the studied adsorbent while highlighting areas for potential improvement in adsorption performance [2, 44, 49].

Table 2 Adsorption isotherm parameters and kinetics study for MB adsorption.

Isothermals							
Models	Langmuir				Freundlich		
Parameters	K_L (L/mg)	q_{max} (mg/g)	R^2	R_L	K_F (mg/g)(mg/L) ^{1/n}	N	R^2
Results	1.60	3.94	0.99966	0.058 to 0.017	2.34	4.49	0.90968
Kinetics							
Models	Pseudo First-order			Pseudo second-order			
Parameters	q_e	K_1	R^2	q_e	K_2	R^2	
Results	0.855	3.66×10^{-3}	0.906	2.05	5.6×10^{-3}	0.998	

3.6 Adsorption Kinetics Study

To predict the adsorption rate and step-determined mechanism during the adsorption of MB dye on the surface of AC derived from VA stem bark [50], the kinetic models stated in equations (10) and (11) were used, respectively. Pseudo-kinetic experiments for MB adsorption on AC derived from VA stem bark were conducted at various reaction times (30, 45, 60, 75, and 90 minutes) under optimal conditions. The PFO kinetics were analyzed by plotting $\log(q_e - q_t)$ against time (t), yielding a straight line with slope $(-k/2.303)$ and intercept $\log(q_e)$, as shown in Figure 10a. Similarly, for PSO kinetics, t/q_t was plotted against time (t), which gives a straight line with a slope of $1/q_e$ and an intercept of $1/K_2q_e^2$ as seen in Figure 10b.

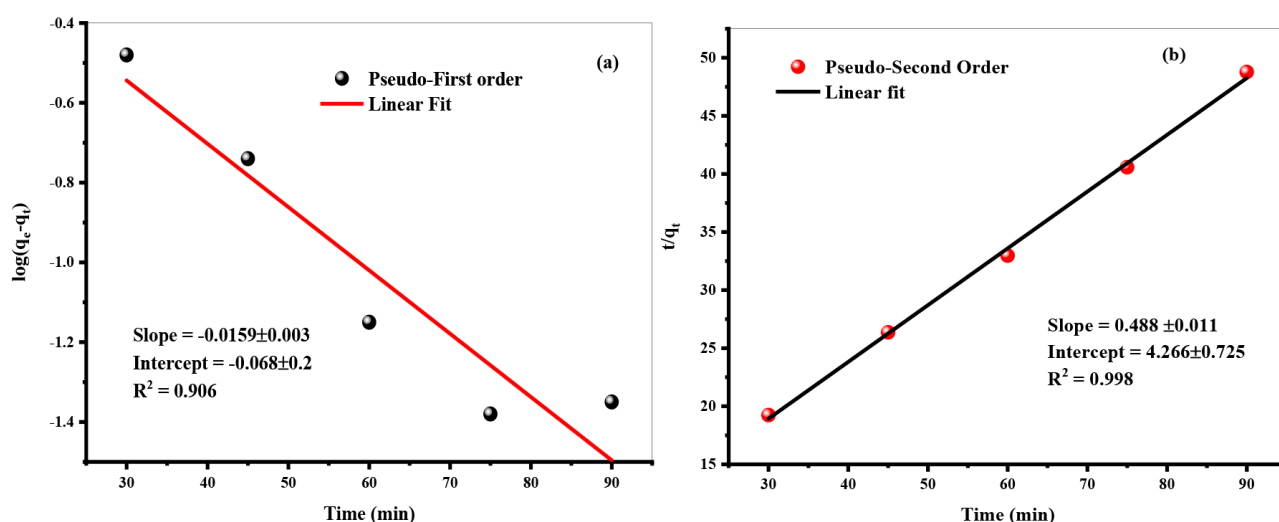


Figure 10 (a) Pseudo First-order and (b) Pseudo Second-order for the adsorption.

The kinetic parameters governing the biosorption of methylene blue onto VA stem bark biosorbent, including correlation coefficients (R^2), equilibrium rate constants (k_1 and k_2), and equilibrium adsorption capacity (q_e), were evaluated for both kinetic models and are presented in Table 2. The PFO model typically reflects adsorption processes controlled by the availability of

sorption sites and solid-phase capacitance. In contrast, the PSO model is widely recognized for describing systems where the dominant rate-limiting step involves surface-reaction-limited systems, diffusion-limited systems, and even Langmuir-type adsorption under restricted conditions. As illustrated in Figure 10(a-b) and summarized in Table 2, the PSO model provides a superior fit for MB adsorption onto the adsorbent AC derived from VA stems bark, yielding an R^2 value of 0.998 substantially higher than that obtained from the PFO model. This strong agreement with the PSO model is consistent with studies reporting dye adsorption onto lignocellulosic and carbonaceous materials [51, 52].

3.7 Adsorption Mechanism

The adsorption mechanism of MB on the surface of VA-based biosorbent is mainly governed by electrostatic attraction between the MB^+ and $R-COO^-$ present on the AC surface. This interaction serves as the primary driving force for dye uptake due to the strong Coulombic attraction between the oppositely charged species. In addition, the aromatic rings of MB interact with the graphitic domains of the AC through π - π electron donor-acceptor interactions, which further stabilize the adsorbed molecules. The highly porous structure of the biosorbent material also promotes micropore filling, allowing MB molecules to diffuse into and occupy the internal pore network, thereby increasing the adsorption capacity. Furthermore, hydrogen bonding between the nitrogen-containing groups of MB and surface hydroxyl ($-OH$) or carbonyl ($C=O$) groups contributes to adsorption, although its role is considered secondary compared with electrostatic and π - π interactions.

The thermodynamic analysis supports this proposed mechanism by demonstrating that the adsorption process is spontaneous, endothermic, and accompanied by a positive entropy change, indicating increased randomness at the solid-solution interface during adsorption. Equilibrium data are well described by the Langmuir isotherm model ($R^2 = 0.99$), suggesting that MB molecules form a monolayer on a relatively homogeneous surface with energetically equivalent adsorption sites. This finding is consistent with the uniform distribution of active sites depicted in the adsorption mechanism. Moreover, the adsorption kinetics follow the PSO model, indicating that surface interactions and intraparticle diffusion primarily control the adsorption rate. Initially, MB molecules are transported from the bulk solution to the activated carbon surface via film diffusion, then diffuse into the porous structure and adsorb onto available active sites until monolayer coverage is achieved.

4. Conclusion

In conclusion, activated carbon derived from VA stem bark was successfully prepared as a biosorbent through chemical activation. Characterization techniques including iodine number, MB number, pHpzc, FT-IR, and SEM revealed that the biosorbent possesses high microporosity and strong sorption capacity, which was further supported by its maximum removal efficiency ($\sim 95\%$) for 10 mg/L MB using 0.25 g of biosorbent within 1 hour under optimal conditions. The adsorption data fitted the Langmuir isotherm model best ($R^2 = 0.999$), with a maximum monolayer capacity of 3.94 mg/g, and followed pseudo-second-order kinetics ($R^2 = 0.999$). These findings indicate that VA stem bark-derived AC is a promising, economically viable biosorbent material for removing MB and potentially other dyes from aqueous solutions, as a preliminary report. Future research should

utilize BET and EDX to analyze the surface and elemental composition of VA stem bark-derived AC, while MS-GC could be used to analyze degradation products, and its effectiveness against mixed pollutants and potential integration with nanomaterials for enhanced wastewater treatment.

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Author Contributions

Yohannes Shuka: Conceptualization, writing - original draft, Methodology, formal analysis, Software, Performed the experiments; Analyzed and interpreted the data, writing - review and editing, Supervise overall work. Mekonnen Abera: Validation, formal analysis, Analyzed and interpreted the data, writing - review & Editing. All authors have read and approved the published version of the manuscript.

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Competing Interests

There is no conflict of interest.

Data Availability Statement

The data supporting the findings of this study are fully included within this paper, and raw data are available upon request from the corresponding author. Additional it was attached as Supplementary File.

AI-Assisted Technologies Statement

Artificial intelligence (AI) assisted-tools were used solely for basic grammar correction and language refinement in the preparation of this manuscript. Specifically, Grammarly was employed to improve the readability and linguistic clarity of the English text. All scientific content, data interpretation, and conclusions were developed independently by the author. The authors have thoroughly reviewed and edited the AI-assisted text to ensure its accuracy and accept full responsibility for the content of the manuscript.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Supplementary File: Raw Experimental Data on the Adsorption Study.

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