

Article

African Silk Biomaterials as Efficient Eco-Friendly Sorbents for Adsorption of Methylene Blue Dye

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Abstract

Methylene blue (MB) dye-contaminated wastewater, particularly from the textile industries, contributes to environmental pollution, posing significant threats to human and aquatic life. Therefore, eco-friendly and low-cost adsorbents for efficient MB removal from contaminated wastewater are needed. This study presents the first comparative investigation of MB removal from water using silk cocoon and silk fibroin (SF) powders from the African wild silkworm *Argema mimosae*. The silk materials were ball milled at different times for up to 150 min and characterized using the XRD, FTIR, TGA, SEM-EDS, zeta potential analysis, and DLS. Batch adsorption experiments were conducted to determine the effects of solution pH, contact time, adsorbent dose, initial dye concentration, and temperature on MB adsorption. The experimental data best fitted the Langmuir and pseudo-second order models for both adsorbents, suggesting monolayer adsorption with a chemisorption rate-limiting step. The maximum adsorption capacities were 78.13 and 81.98 mg g⁻¹ for cocoon and SF powders, respectively. The cocoon and SF powders achieved removal percentages of 86.0% and 93.5%, respectively, within 90 min. Thermodynamic parameters indicated that the adsorption was spontaneous. The findings demonstrate the good potential of *A. mimosae* silk biomaterials as promising, low-cost, eco-friendly, and sustainable adsorbents for the removal of dyes from contaminated water.

Keywords: *Argema mimosae*; cocoon; silk fibroin; adsorbent; methylene blue; adsorption

1. Introduction

Water pollution resulting from the continuous release of dye-contaminated industrial effluents is a growing global concern. Dyes are colored organic compounds with complex molecular structures, which make them relatively non-biodegradable when discharged into water bodies [1]. The compounds are toxic, and their discharge into the aquatic environment poses significant threats to human and aquatic life. As a result, textile effluents have become a subject of environmental concern due to the extensive discharge of dyes into the environment, resulting in considerable environmental pollution [2,3].

Dyes are categorized into natural dyes, such as indigo and cochineal, and synthetic dyes, including azo, direct, acidic and basic dyes [3,4]. Natural dyes derived from plants, animals or minerals have been in use since ancient times; however, the growing demand for dyes necessitated their synthesis from petrochemical compounds [5]. The dye industry has been rapidly expanding, producing nearly 8×10^5 tons of synthetic dyes per year [5].

Azo dyes are the dominant class (above 60%) of synthetic dyes and are the most utilized dyes in textiles [3,6]. Among the azo dyes, methylene blue (MB) is the most used



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synthetic azo dye in the textile industry [7]. MB is reported to induce toxic, carcinogenic and mutagenic effects [8]. In addition, the blue color is visible even at very low levels, reducing sunlight penetration into water, compromising the photosynthesis process in aquatic plants, which in turn affects the food chain [8,9]. Consequently, the dyes form film layers on water surfaces, which decrease dissolved oxygen levels, thereby affecting aquatic fauna [8,9]. Therefore, effective effluent treatment strategies are crucial for removing dyes from wastewater before they are released into the environment. Different technologies have been used to treat MB contaminated wastewater. These include adsorption [10–12], chemical degradation [13,14] and biological treatment [15]. Specifically, adsorption using biosorbents has recently attracted considerable attention. Biobased adsorbents are abundant, inexpensive and environmentally friendly [6]. Low-cost and environmentally friendly sorbents, such as natural clays [16,17] and agricultural waste materials such as nut shells, corncob and rice husks [18–20], have been extensively investigated as a promising approach for removing dyes from wastewater. However, many have shown limitations in both the adsorption rate and capacity [7]. Therefore, there is a need to explore more efficient and eco-friendly adsorbents for wastewater treatment.

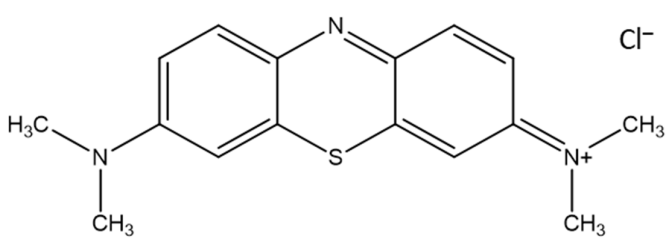
Silkworm silk is among the natural polymers that have attracted significant research interest for water remediation. Silkworm silk is a protein polymer that is produced by the larvae of silk-producing moths when spinning a cocoon. The silkworm cocoon consists of two fibrous proteins: fibroin and sericin. Silk fibroin (SF) contributes to around 70–80% of the overall cocoon weight, whereas sericin, an adhesive protein for binding the fibers in a compact cocoon, forms 20–30%. Silkworm silk is classified into mulberry (domesticated) and non-mulberry (wild) silk. Mulberry silk is mostly produced in China by the domesticated *Bombyx mori* moth species under the family Bombycidae, while non-mulberry silk is mainly produced in India by moths existing in uncontrolled natural environments. The African continent also hosts various species of wild silk moths; however, they remain underexplored [21]. Silk materials from the domesticated *B. mori* have been applied in the treatment of dye-contaminated wastewater, such as removing MB from wastewater, and have proved to be effective adsorbents [7,8,10]. Inspired by such studies and the excellent performance of *B. mori* silk materials in dye adsorption, we hypothesized that African wild silk materials could be an effective and economical low-cost adsorbent to treat dye-containing textile effluents, particularly for African developing countries. Therefore, in this study, we report on the preparation, characterization, and dye removal capacity of ball milled *Argema mimosae* silk cocoon and degummed SF powders. A comparative batch adsorption of MB by cocoon and SF powders was conducted to determine the effects of pH, contact time, adsorbent dose, and initial MB concentration. To our knowledge, this is the first study on the removal of MB dye from wastewater using African wild silk materials.

2. Materials and Methods

2.1. Materials

A. mimosae cocoons were randomly collected from the Kingdom of eSwatini at the Lubuluni area in the Lubombo region. Sodium carbonate ($\text{Na}_2\text{CO}_3 \geq 99.0\%$) and MB dye were obtained from Sigma-Aldrich (Tokyo, Japan), and ultrapure water (18.2 mΩ) produced through a Milli-Q water purification system was used in the study. The structure and physicochemical properties of MB are presented in Table 1.

Table 1. MB physicochemical properties.

Properties	
Structure	
Molecular formula	C ₁₆ H ₁₈ ClN ₃ S
Molecular weight	319.85 g/mol
Log K _{ow}	0.75
pKa	3.8

2.2. Preparation of Adsorbent Materials

The *A. mimosae* cocoons were degummed using a method adapted from Kgomo et al. [21]. Briefly, the procedure involved boiling the cocoons in a sodium carbonate (Na₂CO₃) solution. The silk powders were prepared by cutting 0.5 g of cocoons and degummed silk fibers into small pieces and thereafter loading them separately into milling jars and ball milling using 12 mm stainless steel milling balls at a frequency of 25 Hz at different milling times (30, 60, 90, 120 and 150 min).

2.3. Characterization of Adsorbent Materials

For the materials milled for 30 min, the resultant powder was not sufficient to characterize; hence, they were not included in further studies. The effects of the milling times on the rest of the resultant powders were investigated by utilizing different characterization techniques. The particle size distribution and zeta potential were measured ($n = 3$) by a Zetasizer (Nano-ZS, Malvern Panalytical, Malvern, UK) equipped with a 633 nm He–Ne laser. For the DLS analysis, the powders were suspended in deionized water, while they were suspended in solutions at different pH (2, 4, 6, 8 and 10) for zeta potential. The surface morphology of the powders was observed using a JEOL-JSM-6010 Plus/LA scanning electron microscope (JEOL Ltd., Tokyo, Japan). Samples were fixed onto SEM stubs using a double-sided carbon tape, and each sample was carbon-coated with a sputter coater before analysis. The secondary structure and conformation of the silk powders were investigated using a Shimadzu IR affinity-1 QATR 10 with a single reflection ATR accessory (FTIR-ATR) (Shimadzu Corporation, Kyoto, Japan), while the thermal properties were studied on an SDT Q600 V20.9 (TA Instruments, New Castle, DE, USA). Finally, the crystallinity of the powders was investigated using a Malvern Panalytical Aeris diffractometer (Malvern Panalytical, Almelo, The Netherlands) with a PIXcel detector and fixed slits with Fe-filtered Co-K α radiation. MB dye concentrations were measured by UV-1800 Shimadzu, UV spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

2.4. MB Adsorption Study

A 1000 ppm stock solution of the MB dye was prepared by dissolving it in deionized water. A series of batch adsorption experiments was performed to investigate the adsorption efficiency. In brief, 10 mg silk powders were suspended in 20 mL of 10 ppm MB dye aliquots in Duran 25 mL clear glass bottles and kept in a shaking water bath at 150 rpm for 90 min. To investigate the effects of different adsorption parameters, the experiments were conducted at varying conditions of pH 2–10, adsorbent dose 10–500 mg,

initial concentrations 5–50 ppm, contact times 2–360 min and temperatures 288–318 K. This was followed by filtration through a PVDF syringe filter and spectroscopic analysis. To ensure comparability, all the experiments were carried out under similar conditions.

The adsorbent materials were also applied to real industrial effluents. In this case, the industrial effluent was spiked with 10 ppm of MB solution and the optimum conditions were applied. The reusability of the silk adsorbents was investigated to evaluate their economic viability. After adsorbing MB dye, the silk adsorbents were agitated for 30 min in 20 mL of 0.1 M HCl and rinsed three times with deionized water to desorb the adsorbed dye. The recovered sorbent was then dried for 30 min at 50 °C and then reused for another adsorption–desorption cycle.

Absorbance measurements were taken at 664 nm maximum wavelength ($\lambda_{\max}$), using a UV-visible spectrophotometer. The percentage removal (% Removal) and the adsorption capacities (q_e , mg g^{−1}) of the silk materials were calculated by Equations (1) and (2), respectively.

$$\% \text{Removal} = \frac{C_o - C_e}{C_o} \times 100 \quad (1)$$

$$q_e = \frac{C_o - C_e}{m} \times V \quad (2)$$

where C_o and C_e (mg L^{−1}) are the initial and equilibrium concentrations of MB dye, respectively, V (L) represents the volume of the MB dye solution, and m (g) represents the mass of the adsorbent.

The effect of contact time on MB dye adsorption was further investigated using kinetic modeling. The adsorption kinetics were studied by fitting the experimental data to the pseudo–first order Equation (3), pseudo–second order (Equation (4)) and Elovich (Equation (6)) kinetic models. Lastly, the Weber–Morris intraparticle diffusion model (Equation (7)) was used to identify a diffusion mechanism.

$$\ln(q_e - q_t) = \ln q_e - \frac{k_f}{2.303} t \quad (3)$$

$$\frac{t}{q_t} = \frac{1}{h} + \frac{1}{q_e} t \quad (4)$$

$$h = k_s q_e^2 \quad (5)$$

$$q_t = \frac{1}{\beta} \ln(\alpha\beta) + \frac{1}{\beta} \ln t \quad (6)$$

$$q_t = k_{id} t^{1/2} + C \quad (7)$$

where q_t (mg g^{−1}) is the adsorption capacity at time t , k_f (min^{−1}) is the pseudo–first order rate constant, k_s (g mg^{−1} min^{−1}) is the pseudo–second order rate constant, and h (mg g^{−1} min^{−1}) is the initial adsorption rate in (Equation (4)). α (mg g^{−1} min^{−1}) is considered the initial adsorption rate, and β (g mg^{−1}) is related to the extent of surface coverage and activation energy for chemisorption. k_{id} (mg g^{−1} min^{−1/2}) is the intra-particle diffusion rate constant, and C (mg g^{−1}) is usually related to the boundary layer thickness.

The linearized forms of the Langmuir (Equation (8)), Freundlich (Equation (10)), and Temkin isotherm models (Equation (11)) were applied to evaluate the adsorption isotherms. Furthermore, a dimensionless constant separation factor (R_L), an important parameter for reflecting the essential characteristics of the Langmuir isotherm and predicting the adsorption strength (favorable or unfavorable), was determined according to (Equation (9)):

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

$$R_L = \frac{1}{1 + K_L C_0} \quad (9)$$

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \quad (10)$$

$$q_e = \frac{RT}{b_T} \ln \alpha + \frac{RT}{b_T} \ln C_e \quad (11)$$

where q_{max} (mg g^{−1}) represents the maximum adsorption capacity, R the gas constant (8.314 J mol^{−1} K^{−1}), and T (K) the temperature; K_f (mg g^{−1}) (L mg^{−1})^{1/*n*} is the Freundlich constant related to the adsorption capacity; n reflects the adsorption intensity; α (L mg^{−1}) is the equilibrium binding constant; and b_T (J mol^{−1}) is the constant related to adsorption heat. The dimensionless constant separation factor (R_L) denotes that the adsorption is irreversible ($R_L = 0$), favorable ($0 < R_L < 1$), linear ($R_L = 1$) and unfavorable ($R_L > 1$).

Thermodynamic parameters such as standard Gibbs free energy change (ΔG°), standard enthalpy change (ΔH°) and standard entropy change (ΔS°) were determined by using the following Van't Hoff (Equation (12)). Subsequently, the ΔG° can be obtained from the following (Equations (13) and (14)).

$$\ln K_C = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT} \quad (12)$$

$$\Delta G^\circ = \Delta H^\circ - \Delta S^\circ T \quad (13)$$

$$\Delta G^\circ = -T \ln K_c \quad (14)$$

$$K_c = \frac{q_e}{C_e} \quad (15)$$

where K_c is the equilibrium constant. The values of ΔH° and ΔS° were calculated from the slope and intercept of the $\ln K_c$ vs. $1/T$ graph, respectively.

3. Results and Discussion

3.1. Characterization of the Adsorbents

3.1.1. DLS Analysis

The particle sizes of the silk powders (60, 90, 120, and 150 min) are depicted in Figure 1. The materials showed small- and large-particle-sized groups. The small particle group for the SF ball milled for 60 min had an average particle size of 43 nm, and increased steadily as the ball milling time increased to 120 min, before decreasing sharply to 50 nm at 150 min. In contrast, the small particle group for the cocoon had an average size of 141 nm after 60 min, decreasing slightly after 90 min and increasing to 220 nm at 120 min and then decreasing to 50 nm after 150 min. The large-sized group for SF after ball milling for 60 min had an average particle size of 531 nm, which increased to 712 nm at 90 min, then a sharp decrease to 342 nm was observed at 150 min. Similarly, the cocoon powders had a large particle size of 825 nm at 60 min, followed by a steady reduction to 458 nm at 150 min. Generally, at the onset of ball milling, silk fibers fracture, resulting in reduced particle sizes. As particles become finer, their fracture resistance increases, while the larger surface area promotes agglomeration via interparticle attraction, forming fibrous aggregates with larger particle sizes. With further milling, collisions between agglomerates and milling balls can break the fibrous aggregates apart, reducing particle size [22]. This explains the trend in average particle size with increasing ball milling time shown in Figure 1. Additionally, across all silk materials, the polydispersity index (PDI) was above

0.5, indicating greater particle size variation, including large aggregates [23–25]. A system with a PDI value > 0.4 indicates a broad particle size distribution and is generally unstable due to Ostwald ripening, in which small particles deposit on larger ones, increasing their size [25]. Similar results were reported by different researchers [22,26–28]. In this study, milling was conducted for up to 150 min to conserve energy, as extended milling is a high-energy process. Moreover, milling for extended periods results in a repeating cycle of agglomeration followed by the breakdown of the fibrous agglomerates, without a significant change in the average particle size [22]. Therefore, for further studies, the cocoon ball milled for 150 min and the SF ball milled for 60 min were selected due to their smaller particle size distributions, which are associated with larger surface areas, which are advantageous for the adsorption of pollutants. A larger surface area provides a larger contact area for MB dye molecules, resulting in higher dye removal efficiencies [8]. Figure 2 shows the particle size distribution of the cocoon (150 min) and SF (60 min) powders, which were used in adsorption experiments.

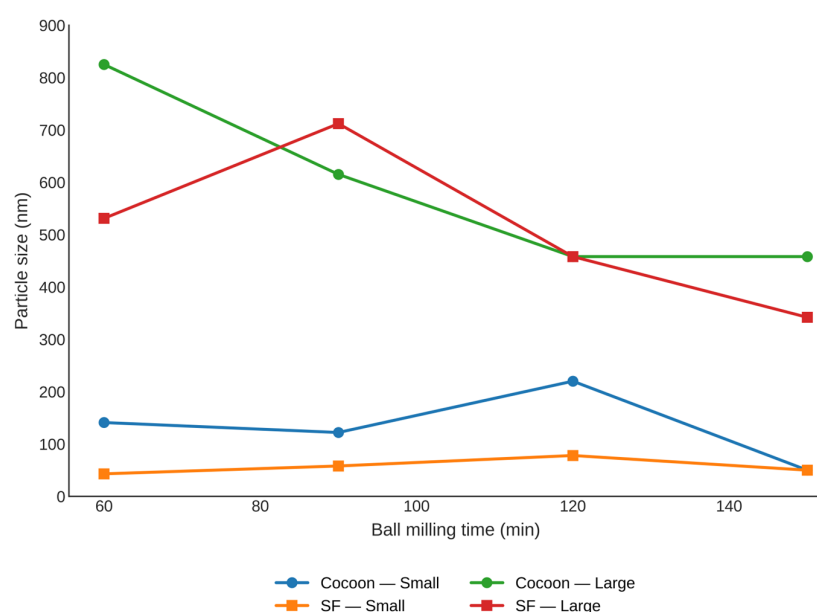


Figure 1. Particle size distribution of cocoon and SF powders.

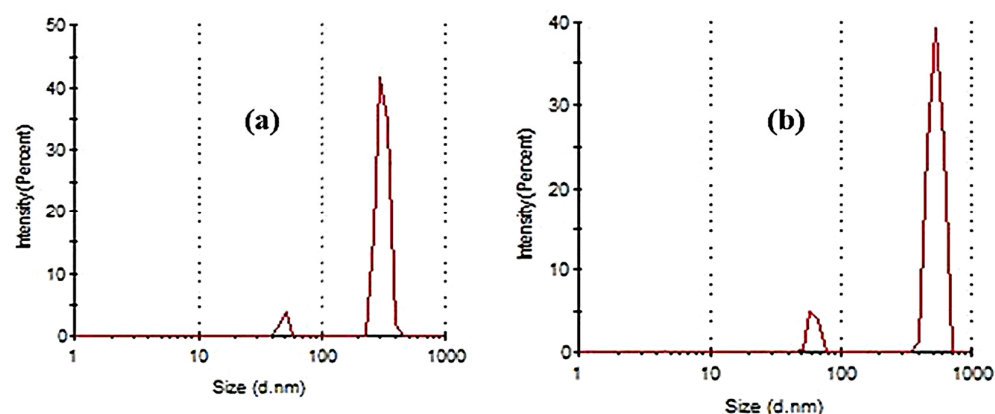


Figure 2. Particle size distribution of: (a) cocoon (150 min) and (b) SF (60 min) powders.

3.1.2. SEM-EDS Analysis

SEM analyses were performed to examine the morphology of the powders. The SEM micrographs of SF and cocoon powders are shown in Figure 3. The SEM images of the powders revealed irregularly shaped and differently sized particles with heterogeneous surfaces. The different-sized particles were also confirmed by the DLS results, which showed smaller and larger particle size groups. Moreover, the images show that the silk powders had aggregates, which were also observed by other researchers on silk particles produced by ball milling [21,22]. The presence of aggregates in both silk powders was also confirmed by the polydispersity index (PDI) above 0.5 for both powders.

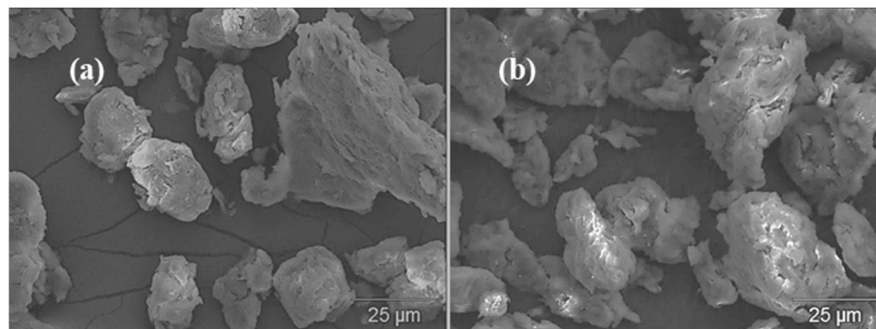


Figure 3. SEM images of (a) cocoon and (b) SF powders.

EDS was used to analyze the elemental composition of silk powder particles. EDS results of SF and cocoon powders are shown in Figure 4. The spectrum shows carbon (C), oxygen (O) and nitrogen (N) peaks. The presence of N assumes the presence of amino acids in both silk materials. The pie charts display the composition of the silk powders with the percentage weights of the elements. An additional peak of sodium (Na) is observed in the SF powder at a very trace amount, likely due to Na_2CO_3 .

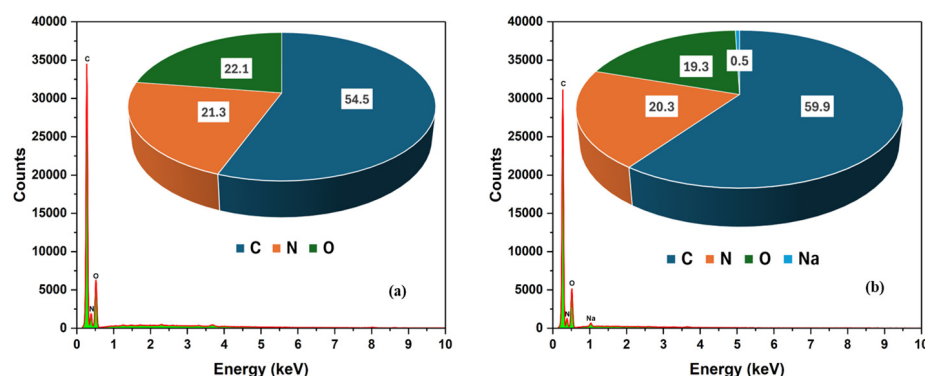


Figure 4. EDS results of (a) cocoon and (b) SF powders.

3.1.3. Zeta Potential Analysis

Zeta potential analysis was carried out to identify the surface charge of the powder particles when suspended in deionized water at different pH conditions (2, 4, 6, 8, 10). Figure 5 shows the average zeta potential ($n = 3$) of cocoon and SF powders at different pH conditions. A positive zeta potential was observed at $\text{pH} \leq 4$, and as pH increased, the zeta potential became negative. This indicates that the surface of particles possesses a positive charge in highly acidic conditions and possesses a negative charge when the pH is increased [29]. It is reported that the isoelectric point of SF is between pH 4–5, meaning that zeta potential values at pH less than the isoelectric point are positive, while the zeta

potential values at pH greater than the isoelectric point are negative, which is consistent with the results obtained in this work [30].

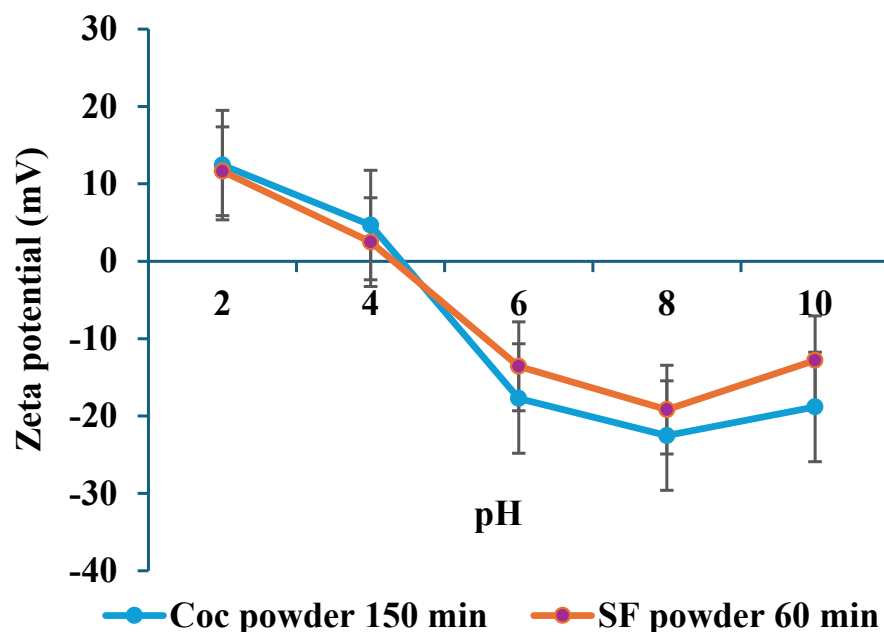


Figure 5. Zeta potential plot for cocoon and SF powders.

The effect of pH on the zeta potential of silk particles is based on the amino acid composition of SF [31]. The zeta potential changes with pH and is positive or negative in acidic or basic pH conditions, respectively [24]. The SF chains contain side chains with -NH_2 groups, and in the presence of H^+ in acidic conditions, the -NH_2 side chains are transformed to -NH_3^+ , which makes the silk materials have a net positive surface charge [32]. In a basic environment, on the other hand, the carboxyl groups (-COOH) are deprotonated into carboxylate ions (-COO^-), thus SF will possess a net negative charge on its surface due to the ionization of carboxyl groups [33].

3.1.4. FTIR Analysis

The FTIR analysis was carried out to obtain information on the conformation of the silk materials from band positions. The conformational structures of the silk materials were determined based on amide band positions. Characteristic bands as a result of amide groups in silk fibroin were observed for Amide I around 1636 cm^{-1} due to C=O stretching, Amide II around 1516 cm^{-1} due to N-H bending and C-H stretching vibrations and Amide III around 1234 cm^{-1} in both powders, which is attributed to the presence of β -sheets crystallites and/or random coils on materials [34–37]. The presence of the β -sheet crystalline structure composed of both poly (Gly-Ala) and poly (Ala) linkages is assumed by a band observed around 1448 cm^{-1} [21,35,36]. Moreover, the band around 1051 cm^{-1} is attributed to the presence of β -sheet conformation composed of (Gly-Ala) sequences common to all silk types, while the band around 964 cm^{-1} is attributed to β -sheets composed of poly (Ala) sequences unique to wild silks [21,35,38]. As shown in Figure 6, the FTIR spectra of SF show minor shifts in the bands compared to the ones observed in cocoons, indicating minimal processing effects on the fibroin β -sheets.

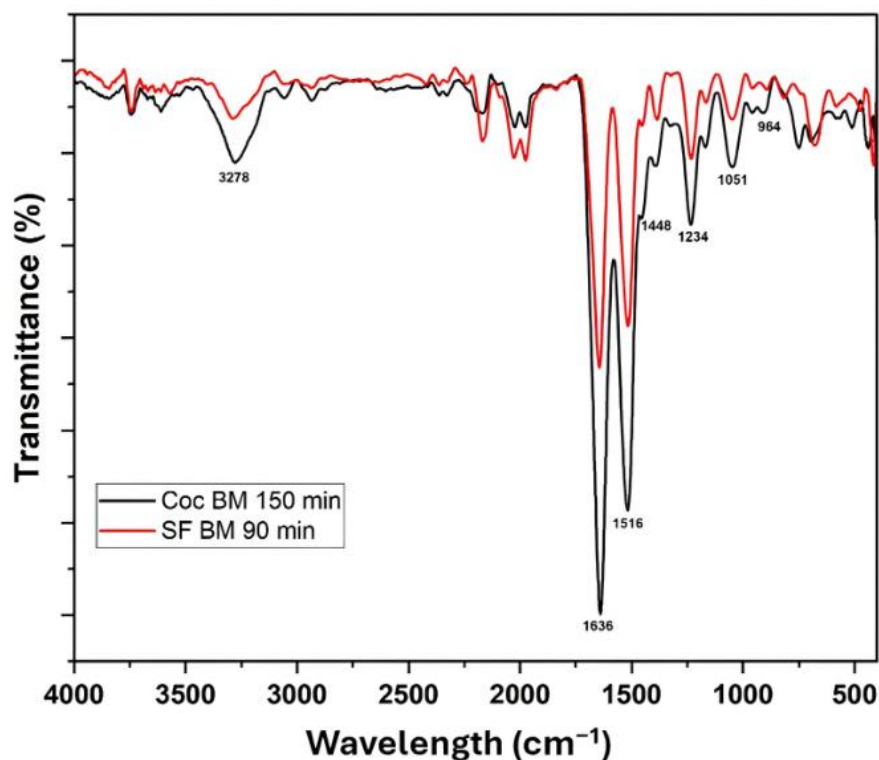


Figure 6. FTIR spectra of cocoon and SF powders.

3.1.5. Thermal Analysis

The thermal stability of the silk powders was investigated using thermogravimetric analysis; the TG curves and derivative TG curves are shown in Figure 7. The weight-loss curves for both silk materials showed four stages. The initial stage corresponds to a weight loss of about 4–10% and occurred at about 55.08 and 44.93 °C for cocoon and SF, respectively, as observed in the derivative thermogravimetric (DTG) curve. A minor decomposition step was observed at about 209.35 and 212.09 °C for cocoon and SF, respectively, corresponding to weight losses of 3–8%. It was followed by two major weight-loss steps, as shown by the DTG curves. The first major step was observed around 344.20 and 332.82 °C for cocoon and SF, respectively, with corresponding weight losses of about 40–50%. The final decomposition step was observed at 557.72 and 536.57 °C for cocoon and SF, respectively, with a corresponding weight loss of about 40%. Zhou and Wang [39] observed the same trend in the thermal decomposition of eri and *B. mori* silk cocoons and degummed silk fibers.

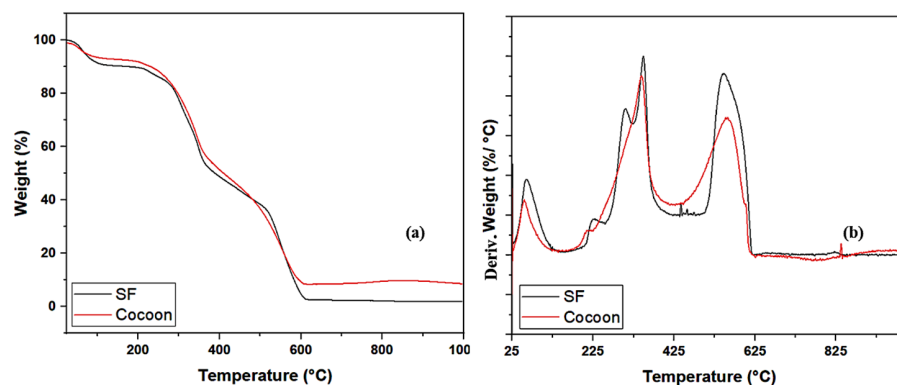


Figure 7. (a) Thermogravimetric (TG) curves and (b) Derivative TG curves of silk powders.

The first weight loss stage has been reported to be due to the loss of moisture [40,41]. The minor decomposition step that follows is attributed to the molecular motion of the fibroin chains due to heating, causing loss of low-temperature volatile compounds [21,42]. The first major weight loss step is due to the breaking of intermolecular side chains and peptide bonds of fibroin [41,43]. The second significant weight loss is attributed to the complete disintegration of the β -sheets of the silk fibroin [21,44]. The results show a decomposition pattern characteristic of silk-based materials [21]. The stability of the materials up to above 300 °C before decomposition takes place indicates the high thermal stability of the silk materials [45,46]. The thermal stability of the adsorbent is a crucial parameter for practical application.

3.1.6. XRD Analysis

The XRD patterns collected as counts per second as a function of 2θ for silk cocoons (Figure 8) show diffraction peaks at around $2\theta = 12.6$ and 23.7° corresponding to crystalline spacings of 7.0 and 3.8 Å, respectively. In SF, similar diffraction peaks are around 12.6 and 23.8° , corresponding to crystalline spacings of 7.0 and 3.7 Å, respectively. The peaks around 12.6° indicate the presence of α -helix structure in all the silk materials. The intense peaks around 23.7 and 23.8° in the XRD curves of the silk materials indicate rich β -sheet crystalline structures [21,43,47,48]. The β -sheets contribute to mechanical properties such as strength and toughness, making the silk adsorbents suitable candidates for water remediation [49]. As the β -sheets of silk contain amino acids, the presence of the β -sheet crystalline structure indicates the presence of amino and carboxyl functional groups, which act as active sites for the removal of dye contaminants from wastewater.

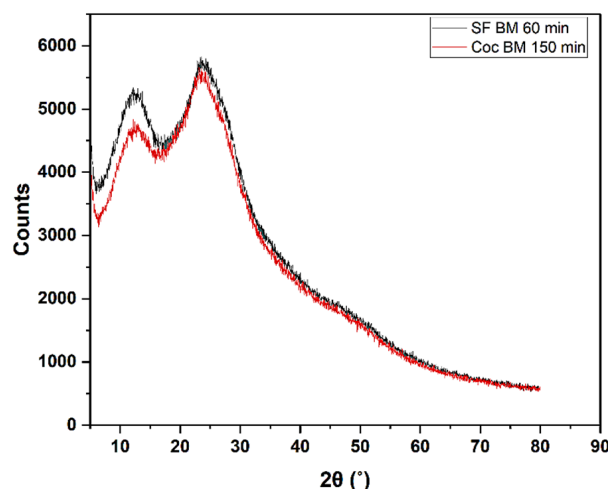


Figure 8. XRD diffractograms of cocoon and SF powders.

3.2. Calibration Standard Curve of MB Dye

The concentrations of MB in the supernatant solutions were calculated using a calibration curve (0.01–10 ppm) with a high regression coefficient ($y = 0.2175x + 0.0032$, $R^2 = 0.9973$), as shown in Figure 9a. The spectrophotometric analysis of MB solutions at 664 nm showed a linear relationship with concentration up to 10 ppm (Figure 9b). It was observed that for concentrations above 10 ppm, there was a deviation from the Beer-Lambert law (Figure 9b inset). This is attributed to the close proximity of the dye molecules preventing the irradiation from passing effectively through the solution [50]. As a result, dilution prior to UV analysis was necessary.

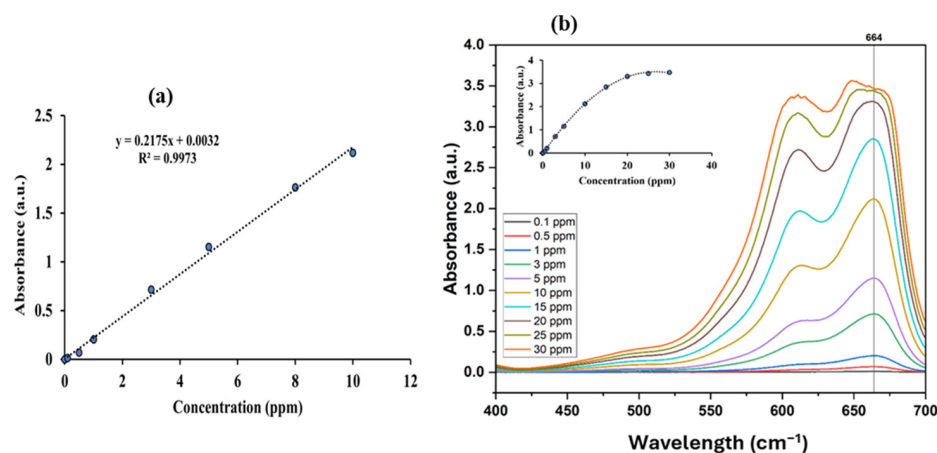


Figure 9. (a) MB calibration curve, (b) Absorbance spectra of standards.

3.3. MB Dye Adsorption Studies

3.3.1. Effect of pH on MB Adsorption

The solution pH has been observed to influence the MB adsorption on the silk adsorbents. Poor removal percentages were observed under acidic conditions (pH 2–4), with significant improvement as pH increased from 6 to 10 (Figure 10). Variation in solution pH has been reported to affect the degree of ionization of the MB molecules and the silk adsorbent surface functional groups, thereby facilitating electrostatic interactions between the charged adsorbate molecules and the charged adsorbent surface functional groups [2,8]. MB has a pKa value of 3.8, corresponding to the demethylation of the dimethylamino group. The MB structure (Table 1) constitutes a permanently charged quaternary nitrogen within the central heterocyclic ring system. The positive charge does not change with pH alterations [8]. Therefore, at pH > 3.8, the MB side groups are deprotonated, while the core structure remains positively charged due to the quaternary nitrogen, making MB cationic even under acidic conditions [8]. As observed from the zeta potential results, in acidic conditions, the silk adsorbents exhibit a positive surface charge due to the protonation of surface amino groups. As a result, in acidic conditions, charge repulsion between the positive charges reduces MB removal. Around pH 6 and beyond, a significant improvement in the removal of the dye was observed, owing to the negatively charged silk surface resulting from the deprotonation of carboxylic surface groups, which led to electrostatic attraction with the cationic MB dye. This was consistent with the results of other researchers [8,10]. Therefore, pH 8 was chosen for further experiments to avoid working at extremely basic pH conditions.

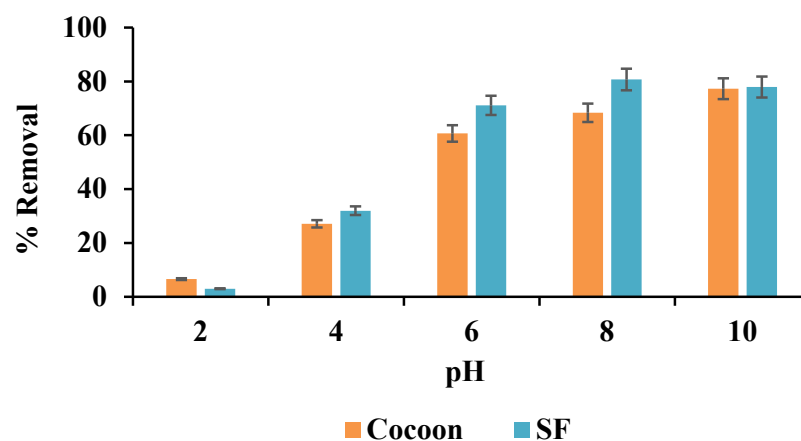


Figure 10. Effect of pH on the removal of MB by silk powders (adsorbent dose = 10 mg, contact time = 30, [MB] = 10 ppm and temperature = 25 °C).

3.3.2. Adsorption Kinetics

The interaction between the silk adsorbents and the MB dye as a function of contact time is a crucial parameter for understanding MB dye removal processes and determining the optimal contact time for maximum adsorption. As shown in Figure 11, the removal of MB dye using cocoon and SF powders increased with contact time up to 90 min, after which no significant change occurred. Therefore, 90 min was chosen as the optimum time for both powders. The cocoon and SF powders achieved removal percentages of 86.0 and 93.5%, respectively. The adsorption of MB is believed to be due to the abundant vacant sites at the initial stages of the adsorption process. However, with time, the vacant sites become saturated with the MB dye, eventually reaching an equilibrium [9,10,20].

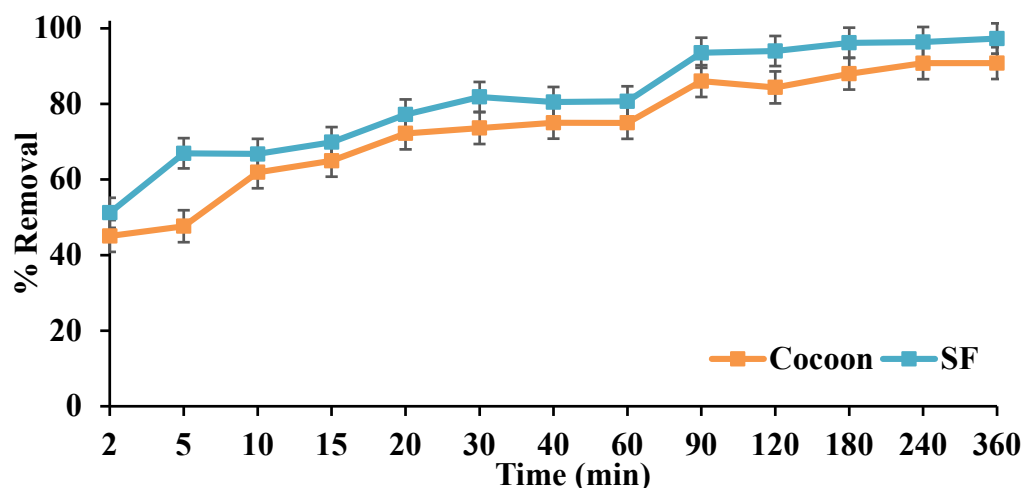


Figure 11. Effect of contact time on the removal of MB by silk powders (pH = 8, adsorbent dose = 10 mg, [MB] = 10 ppm and temperature = 25 °C).

The adsorption kinetics were studied by modeling the experimental data using the pseudo-first order, pseudo-second order and Elovich kinetic models. The results in Figure 12 and Table 2 show that the experimental data best fit the pseudo-second order model, thus being the most suitable model to describe the adsorption process, with a higher R^2 value of 0.9992 and 0.9991 for cocoon and SF, respectively. Moreover, the experimental q_e values were 15.70 and 17.06 for cocoon and SF, respectively. These values were closer to the pseudo-second order calculated q_e values, suggesting that the pseudo-second order model is the rate-limiting step. This indicates that the adsorption of MB on the surface of silk adsorbents was through chemisorption [7,8]. Furthermore, the slope of the equations for both cocoon and SF was quite small, suggesting an extremely high adsorption rate on the material's surface [8]. The pseudo-second order rate constants (k_s) were almost the same for both adsorbents, which were 0.010 and 0.012 for cocoon and SF, respectively. However, the slightly higher value observed for SF suggests a faster adsorption rate and potentially more accessible active sites on SF compared to the cocoon powder. From the Elovich equation, the values of α for MB adsorption by cocoon and SF were 135.7 and 390.8 $\text{mg g}^{-1} \text{min}^{-1}$, respectively. This indicates that the SF powder had more than double the active sites for adsorption compared to the cocoon powder [51].

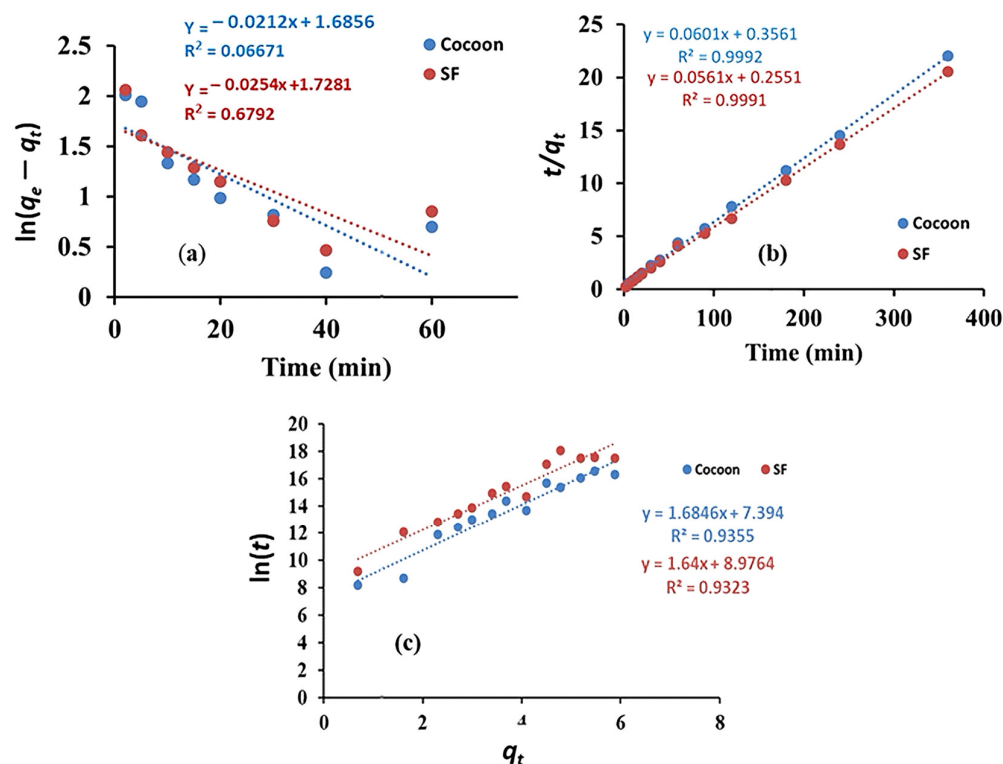


Figure 12. Adsorption kinetic curves of (a) pseudo–first order model, (b) pseudo–second order model and (c) Elovich model.

Table 2. Kinetic parameters for the adsorption of MB onto adsorbents.

Model	Parameters	Cocoon	Silk Fibroin
Pseudo–first order	q_e (mg g ^{−1}) _{cal}	5.63	5.40
	k_f (min ^{−1})	0.06	0.05
	R^2	0.6792	0.6671
Pseudo–second order	q_e (mg g ^{−1}) _{cal}	16.64	17.83
	k_s (g mg ^{−1} min ^{−1})	0.010	0.012
	h (mg g ^{−1} min ^{−1})	2.81	3.92
	R^2	0.9992	0.9991
Intraparticle diffusion	C_1 (mg g ^{−1})	6.74	8.74
	K_{id1} (mg g ^{−1} min ^{−1/2})	1.30	1.14
	R^2	0.90	0.91
	C_2 (mg g ^{−1})	13.06	14.92
	K_{id2} (mg g ^{−1} min ^{−1/2})	0.20	0.17
	R^2	0.6456	0.34
Elovich	α (mg g ^{−1} min ^{−1})	135.73	390.77
	β (g mg ^{−1})	0.59	0.61
	R^2	0.94	0.93

The intraparticle diffusion plot of q_t vs. $t^{1/2}$ usually displays multilinearity, which indicates a multistep adsorption process attributed to surface adsorption and intraparticle diffusion [20]. The intraparticle diffusion plot indicates that the MB adsorption on the silk powders consisted of two stages (Figure 13). The first stage reflects rapid diffusion of MB from the solution to the sorbent surface, driven by a large concentration gradient and more unoccupied active sites. The second stage reflects gradual intraparticle diffusion resulting from small residual MB in the aqueous solution.

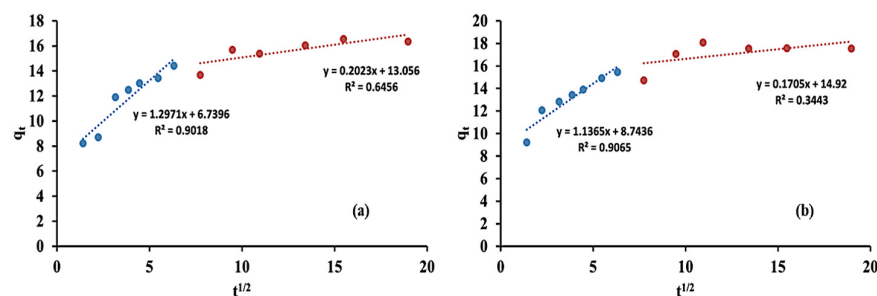


Figure 13. Adsorption kinetic curves of intraparticle diffusion for (a) cocoon and (b) SF powder.

3.3.3. Effect of Adsorbent Dose

Optimization of adsorbent dosage is crucial in adsorption to ensure effective and economical dye removal from aqueous solutions. Figure 14 shows that MB percentage removal increases with increasing adsorbent dosage for both materials. As expected, the abundance of active sites with increased adsorbent dose resulted in increased dye removal. However, it is observed that increasing the adsorbent dose significantly decreases the adsorption capacity, as indicated by the line graph in Figure 14. This observation suggests that the presence of excessive binding sites results in insufficient MB molecules to occupy adsorption sites. It has been reported that using a smaller adsorbent dose allows rapid saturation of active sites, making it suitable for practical applications and ensuring economic efficiency [8]. Accordingly, the ideal dose for all the adsorbents was 10 mg to fully utilize the material's adsorption capability.

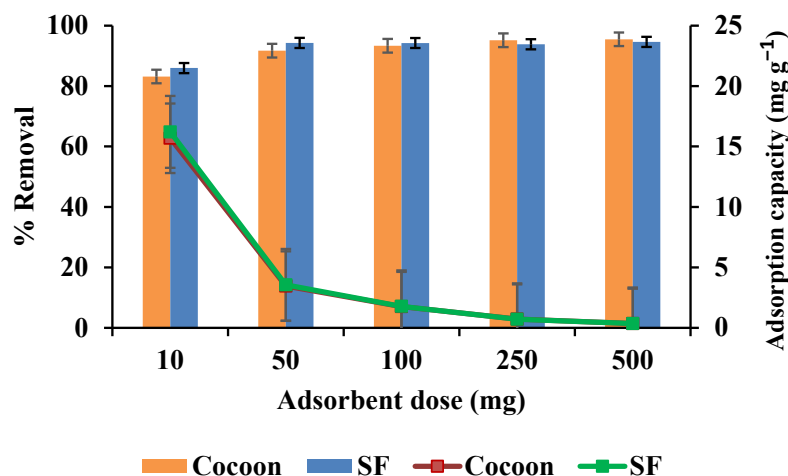


Figure 14. Effect of adsorbent dose on the removal of MB by silk powders (pH = 8, contact time = 90 min, [MB] = 10 ppm and temperature = 25 °C).

3.3.4. Effect of Initial Dye Concentration

The effect of initial concentration on the percentage removal of MB is shown in Figure 15. The percentage removal was steady from 5 to 30 ppm and decreased at 50 ppm. This suggests that adsorption occurs up to a certain concentration, and beyond that concentration, further adsorption is hindered by the saturation of available active sites. The q_e values corresponding to the concentrations from 5 to 50 ppm were 8.38 to 57.3 mg g⁻¹ and 10.10 to 84.00 mg g⁻¹ for cocoon and SF, respectively. The adsorption capacity increased with increasing initial concentration from 5 to 50 ppm. The higher adsorption capacities at higher concentrations are attributed to the higher concentration gradient of MB molecules, which is the driving force for mass transfer. The material has shown superior performance in removing MB from aqueous solutions, achieving high removal percentages even at higher dye concentrations, making it a potential candidate for treating dye-contaminated wastewater.

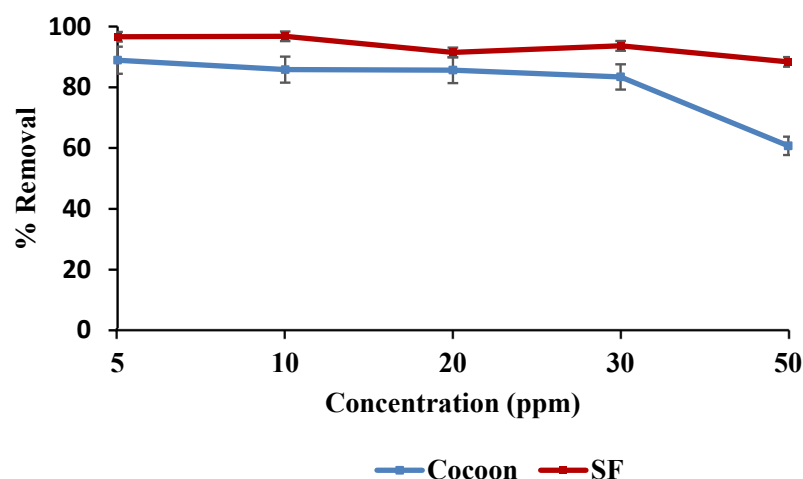


Figure 15. Effect of initial concentration on the removal of MB by silk powders (pH = 8, contact time = 90 min, adsorbent dose = 10 mg and temperature = 25 °C).

3.3.5. Adsorption Isotherms

Adsorption isotherms are useful tools for understanding the distribution of MB between the solid and liquid phases and for evaluating the maximum adsorption capacity. The results of the plotted isotherms are depicted in Figure 16 and Table 3, which show that the experimental data best fit the Langmuir model for both adsorbent materials. The results suggest that both the cocoon and SF adsorbents exhibit homogeneous, monolayer adsorption. The q_{max} of the cocoon and SF were 78.13 and 81.98 mg g⁻¹, respectively, indicating comparable performance to *B. mori* adsorbent materials. Different studies obtained maximum adsorption capacities of *B. mori* cocoons to be 3.8, 86.2 and 137.08 mg g⁻¹, whereas maximum adsorption capacities of 3.8, 20.58 and 179.54 mg g⁻¹ were reported for degummed SF [7,8,10]. In this study, the R_L values were in the range of 0.09–0.48 and 0.02–0.19 for cocoon and SF powders, respectively. These values indicated that the adsorption systems were favorable. From the Freundlich model, the K_f values of the sorbents were 14.65 and 31.48 for cocoon and SF powders, respectively. The values indicate that the SF adsorbent performed better than the cocoon adsorbent in dye adsorption. The $1/n$ values obtained from the Freundlich isotherm plots were 0.56 and 0.59 for cocoon and SF, respectively. Since the values fall between 0 and 1, they further confirm that the adsorption was favorable for both adsorbents [52]. From the Temkin model, the b_T values were greater than zero for both sorbent materials, indicative of an exothermic adsorption process; however, the model mostly suits the cocoon adsorbent.

Table 3. Isotherm parameters and correlation coefficients were calculated for the adsorption of MB onto adsorbents.

Isotherm	Parameters	Cocoon	Silk Fibroin
Langmuir	q_{max} (mg g ⁻¹)	78.13	81.98
	K_L (L mg ⁻¹)	0.23	0.82
	R^2	0.9906	0.9761
Freundlich	N	1.78	1.70
	K_f (mg g ⁻¹) (L mg ⁻¹) ^{1/n}	14.65	31.48
	R^2	0.8977	0.9614
Temkin	α (L mg ⁻¹)	3.14	7.60
	b_T (J mol ⁻¹)	14.97	20.01
	R^2	0.9461	0.8992

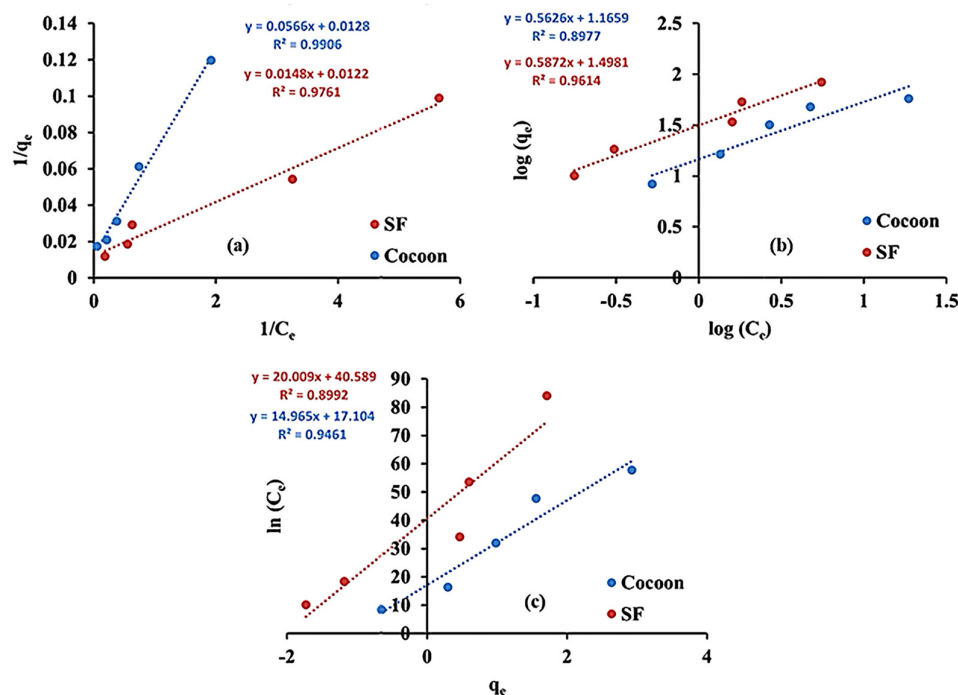


Figure 16. Adsorption isotherm curves corresponding to (a) Langmuir, (b) Freundlich and (c) Temkin.

3.3.6. Thermodynamics of Adsorption

The variations in ΔG° , ΔH° , and ΔS° with temperature help in understanding the thermodynamic driving forces governing adsorption, energetics and feasibility of the adsorption process at different temperatures [9]. A slight improvement in the removal percentage was observed from 288 to 298 K for both adsorbent materials. A slight decline in the removal percentage was observed up to 318 K for both cocoon and SF adsorbents (Figure 17). The results show a slight effect of temperature on MB removal using the silk powders, indicating that our materials can work even in ambient conditions.

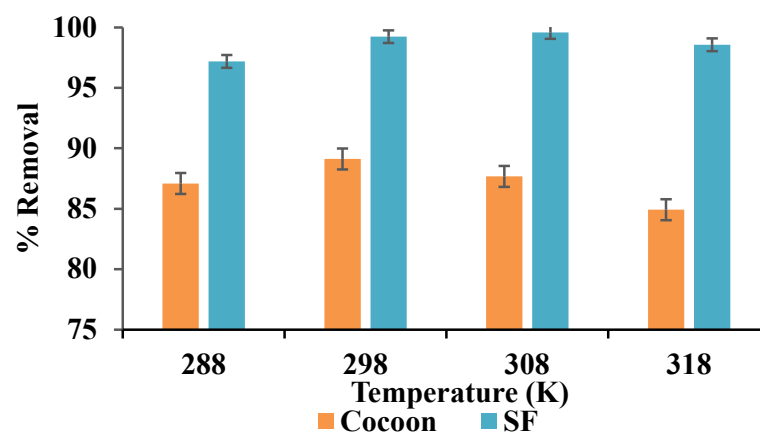


Figure 17. Effect of temperature on the removal of MB by silk powders (pH = 8, contact time = 90 min, adsorbent dose = 10 mg and [MB] = 10 ppm).

Thermodynamic parameters (Table 4) were calculated from the Van't Hoff plot of $\log K_c$ and $1/T$ (Figure 18). The negative ΔG° values indicate a spontaneous adsorption process for both adsorbents. It is observed that the ΔG° values increase with increasing temperature, indicating a decrease in the spontaneity of the adsorption process at higher temperatures [21]. This indicates that MB adsorption was less favorable at increased temperatures. ΔH° is used to describe whether an adsorption process is exothermic ($\Delta H^\circ < 0$)

or endothermic ($\Delta H^\circ > 0$). In this study, a negative ΔH° value was obtained for cocoon powder, indicating that the adsorption process was exothermic, whereas it was endothermic in the SF. The ΔH° values are also important in determining the adsorption mechanism of MB. ΔH° values greater than 40 kJ mol^{-1} depict chemisorption, whereas ΔH° values less than 40 kJ mol^{-1} indicate physisorption. In this study, the ΔH° values of both cocoon and SF were less than 40 kJ mol^{-1} (Table 4). These results assume physisorption for both the cocoon and SF adsorbent materials, which contradicts the isotherm and kinetic model results; however, the experimental data showed a poor fit on the Van't Hoff plot for both adsorbent materials. This was evident in the R^2 values of 0.2985 and 0.1958 for cocoon and SF adsorbents, respectively. ΔS° gave positive values, indicating the randomness and disorder of the adsorption of the dye on both adsorbents [53]. However, a bigger value was observed in SF, suggesting a high disorder in the system [53,54].

Table 4. Thermodynamic parameters for the adsorption of MB on the adsorbents.

Adsorbent	ΔG°				ΔH° (J mol^{-1})	ΔS° (J mol^{-1})
	288 K	298 K	308 K	318 K		
SF	−1219.97	−1655.61	−1898.94	−1565.92	21,613.37	114.89
Cocoon	−749.46	−833.12	−817.67	−770.06	−4982.58	5.30

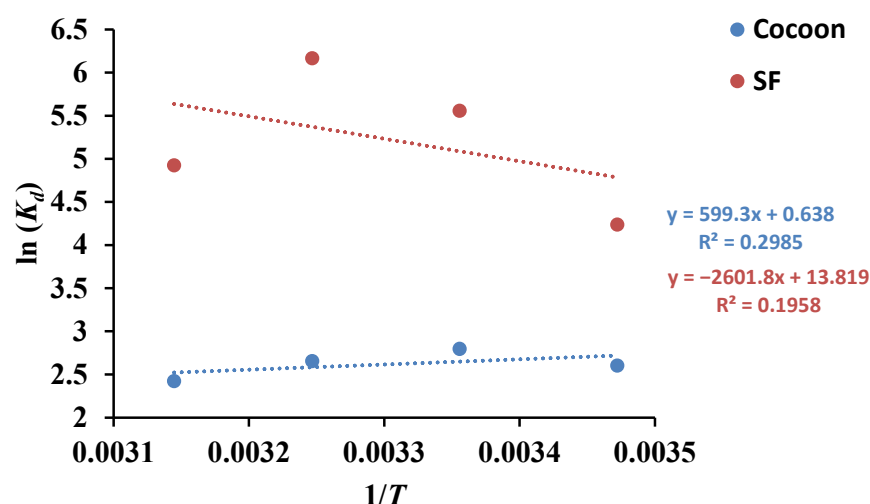


Figure 18. Van't Hoff plot for both silk powders.

3.4. Reusability Study

The adsorption–desorption cycles were repeated twice, and the removal efficiency of both adsorbents declined in the consecutive cycles, as observed in Figure 19. The silk cocoon removal percentages were 86.26%, 40.69%, and 25.53% for the first, second, and third cycles, respectively. The SF removal percentages were 86.55%, 62.94%, and 27.89% for the first, second, and third cycles, respectively. The reduced removal efficiency after each cycle can be attributed to the weakened silk adsorbent properties of the desorbed samples. Also, since the adsorption process involves the formation of chemical bonds between MB and silk, it impedes regeneration because the MB molecules are strongly bound to the adsorbent surface. Additionally, some of the powder was lost during the washing step; thus, using a dosage less than 10 mg in the next cycle may have contributed to the lower removal percentages. To mitigate loss of adsorbent material, in future studies, silk-magnetic composites can be used so that the material can be separated from the solution using a magnet. However, these results show that the adsorbent material can be reused for multiple cycles.

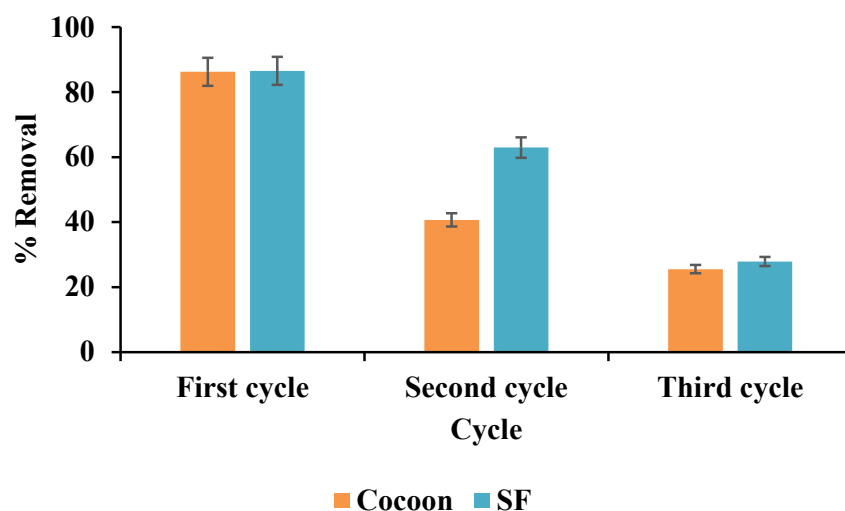


Figure 19. Repeatability study of silk powders for removing MB dye (pH = 8, contact time = 90 min, adsorbent dose = 10 mg [MB] = 10 ppm and temperature = 25 °C).

The performance of the silk adsorbents was compared with other adsorbents reported in the literature (Table 5). The adsorption capacities of the silk adsorbents in the current study were comparable to those of *B. mori* adsorbents used for the same purpose. This shows that the adsorbents demonstrated good potential for the removal of MB dye from contaminated wastewater, thus can be considered for the treatment of dye-contaminated wastewater in developing countries, usually in Africa, as *A. mimosae* silk is naturally available.

Table 5. Adsorption capacities of MB dye with *Bombyx mori* silk adsorbents.

Adsorbent	Adsorption Capacity (mg g ⁻¹)	Optimum Conditions	Ref.
SF powder	20.58	2 mg mL ⁻¹ , 20 ppm, 60 min and pH = 7.5	[10]
Cocoon powder	137.08	100 mg dose, 30 ppm, 24 h and pH = 9	[7]
SF powder	179.54		
Cocoon powder	3.8	15 min, 0.1 g, pH 8 and 30 ppm	[8]
SF powder	3.8		
Cocoon powder	78.13	10 mg, 90 min, pH 8, 10 ppm and 298 K	This study
SF powder	81.98		

3.5. Application in Industrial Effluent

The performance of silk adsorbents was further checked in real wastewater to study matrix effects on MB dye removal. Spectrophotometric analysis of the real samples showed no peak at 664 nm, showing the absence of MB species. Therefore, industrial effluent was spiked with 10 ppm of MB solution. It was found that the cocoon powder achieved 57.13% removal efficiency, while the SF powder achieved 54.94%. The decline in adsorption efficiency may be attributed to the more complex matrix of real wastewater, which contains organic and inorganic pollutants that may compete with MB molecules for the active sites of the adsorbents, thereby hindering MB adsorption. Based on these results, it can be concluded that the silk adsorbents are potential eco-friendly materials for use in treating complex matrices, such as industrial effluents.

3.6. Possible MB Adsorption Mechanisms

The presence of β -sheet crystallites on the silk adsorbent indicates the presence of amino acids that contain functional groups capable of interacting with the dye molecules, thereby enhancing their removal from water. Silk possesses functional groups, such as amino and carboxyl groups, which serve as active sites for MB adsorption. These functional groups form hydrogen bonds and electrostatic interactions with MB, resulting in the adsorption of the dye molecules onto the surface of the silk adsorbents, as illustrated in Figure 20. The experimental data best fit the Langmuir model, suggesting monolayer adsorption. Additionally, the MB adsorption occurred via chemisorption, as evidenced by the best fits of the experimental data to the pseudo-second order model. This implies a strong attraction between MB molecules and the functional groups of the silk adsorbents, resulting in irreversible adsorption via chemical bonding. Moreover, the intraparticle plot displayed that the adsorption process was characterized by a rapid adsorption of MB on the adsorbent surface and gradual intraparticle diffusion.

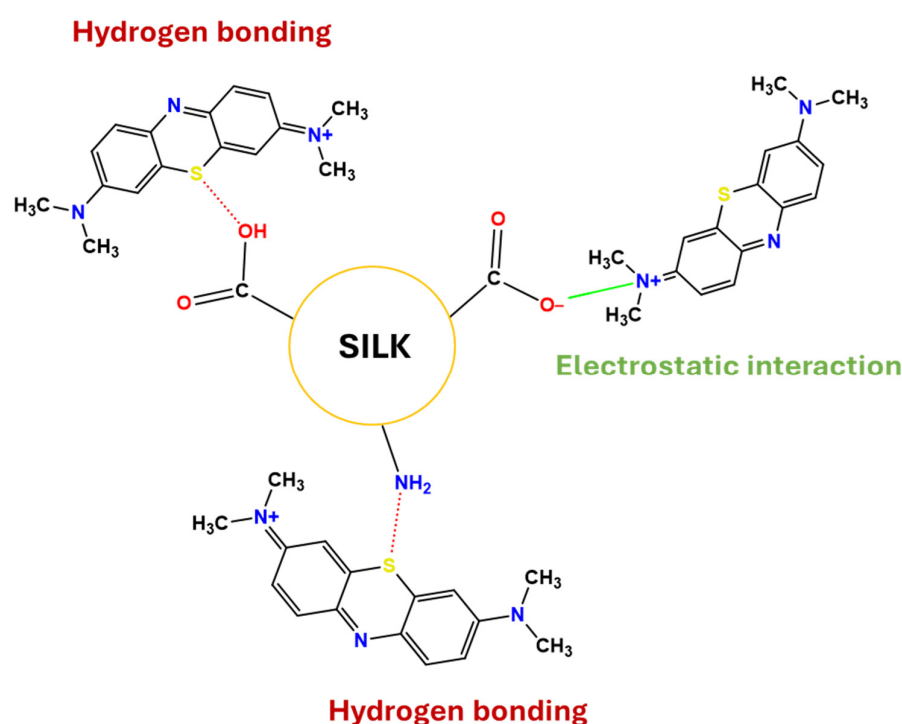


Figure 20. Possible mechanisms for MB adsorption on the surface of silk adsorbents.

4. Conclusions

Argema mimosae silk cocoon and SF powder adsorbents were successfully ball milled and investigated as potential eco-friendly adsorbents for the removal of MB dye from water. The ball milled powders revealed that the powders from both materials possessed both small and large particle size groups. The polydispersity index (PDI) for the adsorbents was above 0.5, indicating greater particle-size variation, confirming that ball milling effectively reduced particle size but also led to aggregation. Milling times of 150 min and 60 min were selected for silk cocoons and SF, respectively, to prepare powders used in adsorption studies. This was further supported by SEM images, which revealed that the powders consisted of irregularly shaped powder particles with rough surfaces that were agglomerated. The XRD and FTIR results showed that the silk materials were rich in β -sheets, corresponding to amino and carboxyl functional groups, which act as active sites for the adsorption of the dye. The variation in solution pH facilitated the ionization of silk surface functional groups, which resulted in electrostatic interactions with the charged MB dye molecules.

The experimental data fitted to the Langmuir and pseudo-second order kinetic models, assuming a monolayer adsorption via chemisorption mechanisms, indicate a strong interaction between silk adsorbents and the dye. The maximum adsorption capacity for MB dye was estimated to be 78.13 and 81.98 mg g⁻¹ for the cocoon and SF powders, respectively. Dye removal up to 86.0% and 93.5% for cocoon and SF powder, respectively, was achieved within 90 min of contact time. The materials were used for 2 cycles, and a significant decrease in the removal efficiency was observed for the second cycle. Therefore, future studies focusing on improving the reusability of the materials and developing composite forms to minimize adsorbent loss during regeneration, such as silk-magnetic composites, should be considered. Overall, this work highlights the potential of African silk materials as eco-friendly, low-cost alternative adsorbents for water remediation, particularly for the removal of MB dye. This study demonstrates the potential of silk materials for removing textile dyes and warrants further research on the fabrication and investigation of innovative silk-based materials for their potential application in the industrial treatment of textile effluents. Particularly wild silks, which remain underutilized.

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