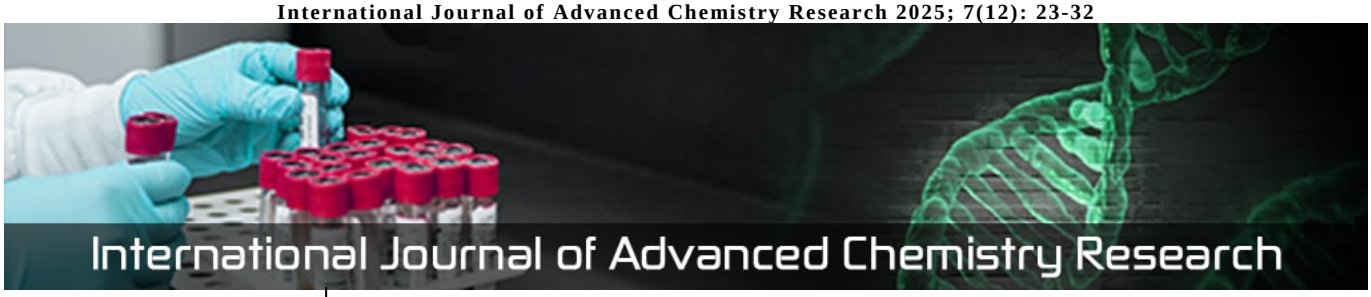

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Adsorptive removal of amoxicillin from aqueous solutions by spirogyra biomass: A kinetic and thermodynamic study

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Abstract
Background & Problem Statement: Emerging contaminants, particularly antibiotics, have emerged as significant environmental pollutants due to their widespread use and persistence in aquatic systems. Amoxicillin, a broad-spectrum β-lactam antibiotic, is frequently detected in wastewater and surface water, posing risks to aquatic ecosystems and human health. Conventional wastewater treatment methods fail to completely remove antibiotics, necessitating alternative approaches such as the adsorption method.
Methodology: This study investigates the adsorption of Amoxicillin onto Spirogyra biomass under various conditions, including pH (3-9), contact time (0-180 minutes), initial Amoxicillin concentration (10-100 mg/L), and temperature (25 °C, 35 °C, 45 °C). Batch adsorption experiments were conducted, and the data were analyzed using kinetic and thermodynamic models. Thermodynamic parameters, including Gibbs free energy (ΔG°), enthalpy (ΔH°), and entropy (ΔS°), were calculated to assess the feasibility of the adsorption process.
Key Findings: The maximum adsorption capacity of Spirogyra biomass for Amoxicillin was 21.68 mg g^{-1}, with optimal adsorption observed at pH 6.0. Adsorption followed the pseudo-second-order kinetic model, indicating chemisorption. The Langmuir model provided the best fit for equilibrium data, confirming monolayer adsorption. Thermodynamic analysis revealed that adsorption was spontaneous, endothermic, and associated with increased randomness at the solid-liquid interface.
Conclusion: Spirogyra biomass proved to be an efficient, eco-friendly biosorbent for Amoxicillin removal from aqueous solutions. The adsorption process was favourable at higher temperatures, suggesting potential applications in wastewater treatment. These findings contribute to the development of sustainable strategies for mitigating antibiotic contamination in water bodies.

Keywords: Amoxicillin, Adsorption, *Spirogyra*, Water, Kinetic study, Thermodynamic study

1. Introduction
The presence of antibiotic residues in the environment is a growing concern due to their continuous discharge into surface water through wastewater. Poorly degradable and highly soluble, these contaminants bypass conventional treatment, infiltrating groundwater and even drinking water, posing risks to aquatic and terrestrial life [1]. Amoxicillin, a broad-spectrum β-lactam antibiotic, is frequently detected in wastewater and surface water [2]. Conventional wastewater treatment technologies are often inadequate for the complete degradation of antibiotics, necessitating the exploration of alternative, eco-friendly, and cost-effective methods [3, 4].
Adsorption is regarded as one of the most efficient techniques for the removal of organic contaminants, owing to its simplicity, low cost, and high efficiency [5, 6]. Recently, the use of algal biomass as biosorbents has gained attention due to their abundance, biodegradability, and high adsorption capacity [7, 8]. In this context, *Spirogyra sp.*, a filamentous green alga, offers a promising biosorbent material due to its rich content of functional groups and surface area [9].
While algal biomass has been widely investigated for the adsorption of dyes, heavy metals, and nutrients, relatively few studies have explored its potential in antibiotic removal. Among antibiotics, amoxicillin is one of the most frequently detected contaminants in aquatic

environments, yet systematic studies on its adsorption using green biosorbents remain scarce. In this context, the present work addresses this gap by evaluating *Spirogyra sp.* biomass as a low-cost and eco-friendly adsorbent for amoxicillin removal, with emphasis on adsorption kinetics, equilibrium isotherms, and thermodynamic behavior.

2. Methodology

2.1 Collection and Preparation of Biosorbent

The biomass of *Spirogyra sp.* was collected from Near Matasyagandha Lake, located in Saharsa, Bihar, India. After thoroughly washing with tap water to remove impurities like dirt and sand. After that double-distilled water (DDW) were utilized to remove rest impurities and then dried in a hot-air oven at 60 °C for 48 hours. The dried algal biomass was ground using a kitchen blender and sieved through a 30-mesh sieve to obtain a uniform particle size of 250-500 µm. The biomass was stored in a plastic container at 40 °C until use. The *spirogyra sp.* biomass was analyzed at the Physical Laboratory (University Department of Chemistry), B.N.M.U. Madhepura, using the standard methods of AOAC [10].

2.2 Characterization of Biosorbent.

The synthesized biosorbents were subjected to characterization through a range of analytical techniques. Fourier Transform Infrared Spectroscopy (FTIR) was utilized to identify the functional groups responsible for adsorption interactions. Scanning Electron Microscopy (SEM) was employed to examine the morphological characteristics of the adsorbents. X-ray Diffraction (XRD) analysis was conducted to ascertain the crystalline structure and phase composition of the adsorbents.

2.3 Preparation of Amoxicillin Solution

A stock solution of Amoxicillin (250 mg/L) was prepared by dissolving an appropriate amount of Amoxicillin trihydrate in distilled water. Working solutions of desired concentrations (10, 25, 50, 75, and 100 mg/L) were prepared by dilution.

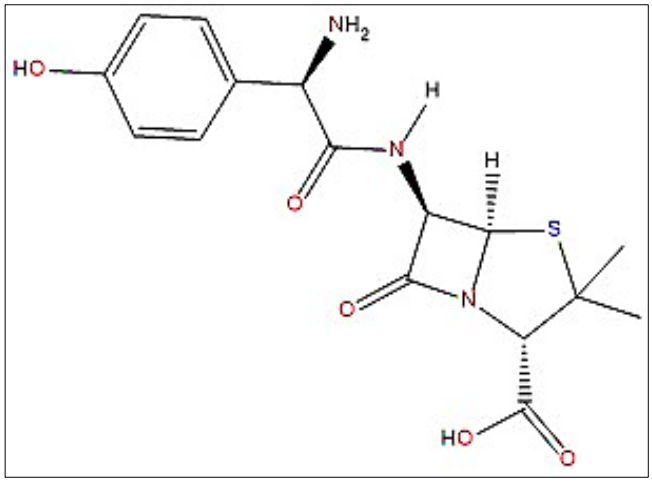

Fig 1: Chemical structure of amoxicillin, a broad-spectrum β-lactam antibiotic. The molecule contains functional groups such as hydroxyl (-OH), amino (-NH_2), and carboxyl (-COOH), which can participate in hydrogen bonding and electrostatic interactions with functional groups on the Spirogyra sp biomass surface during the adsorption process.

2.4 Batch Adsorption Experiments

Batch adsorption experiments were employed to study the effect of various parameters, including pH (3-9), contact time (0-180 minutes), initial Amoxicillin concentration (10-100 mg/L), and temperature (25°C, 35°C, 45°C). These parameter ranges were selected based on their relevance to real-world wastewater conditions and their influence on adsorption efficiency, as reported in previous studies. The experiments were performed by adding an adsorbent dose (2-20 g) of *Spirogyra sp.* biomass to 100 mL of Amoxicillin solution in Erlenmeyer flasks. The mixture was agitated at 150 rpm.

2.5 Analytical Methods

After adsorption, the solution was filtered, and the residual Amoxicillin concentration was determined using a UV-Visible spectrophotometer at 229 nm wavelength. Calibration curves with $R^2 = 0.9992$ were used for accurate quantification.

2.6 Statistical Analysis and Model Selection Criteria

Non-linear/linear regressions were performed for kinetic, isotherm and thermodynamic models. Goodness of fit was evaluated using: coefficient of determination (R^2), residual standard error / residual sum of squares (RSS), visual residual diagnostics (residuals vs. fitted/independent, histogram, and normal Q-Q), and agreement between calculated and experimental capacities ($|q_{e,cal.} - q_{e,exp.}|$). Model selection prioritized (i) random residual pattern with near-zero mean, (ii) lowest RSS, and (iii) q_e agreement; R^2 alone was not decisive.

2.7 Kinetic and Thermodynamic Analysis

Kinetic models, including pseudo-first-order and pseudo-second-order, were applied to analyze the adsorption data. Isotherm models such as Langmuir and Freundlich were used to describe the equilibrium data. Thermodynamic parameters, including Gibbs free energy change (ΔG°), enthalpy change (ΔH°), and entropy change (ΔS°), were calculated to assess the nature of the adsorption process.

3. Results and Discussion

3.1 Composition of Biomass *Spirogyra sp.*

The biochemical composition of *Spirogyra sp.* biomass in the present study was determined to be 61.0% total carbohydrates, 13.6% protein, 1.9% fat, and 12.2% ash, with a moisture content of 11.3%. These values are in close agreement with those reported by Vichit *et al*. (2023), who observed 64.0% carbohydrates, 12.9% protein, 1.6% fat, and 11.4% ash, with a moisture content of 10.0% [11]. When compared with the broader compositional ranges documented by Ge *et al*. (2018) for algal biomass 41.5-55.0% carbohydrates, 16.7-19.5% protein, and 2.8-10.0% fat—the results indicate that Spirogyra sp biomass generally exhibits a higher carbohydrate fraction while containing relatively lower protein and fat contents [12]. Such compositional variations may be attributed to differences in algal species, environmental conditions, and growth stages.

From a biosorption perspective, the high carbohydrate content is particularly advantageous. Carbohydrate polymers, such as cellulose and polysaccharides, are rich in hydroxyl and carboxyl functional groups that can effectively interact with amoxicillin molecules through hydrogen bonding and electrostatic interactions. In contrast, the lower protein and fat fractions may reduce interference from non-polar constituents, thereby enhancing the adsorption efficiency of the biomass.

3.2 Characterization of Biosorbent

The *Spirogyra sp.* biomass was characterized using FTIR, SEM, and XRD techniques to understand its surface properties and adsorption potential.

3.2.1 FTIR Analysis

The FTIR spectrum of Spirogyra sp biomass showed distinct peaks corresponding to functional groups involved in adsorption. A broad band around 3480 cm^{-1} indicated the presence of -OH and -NH groups, while peaks near 2890 cm^{-1} corresponded to C-H stretching. The band at ~1650 cm^{-1} was attributed to C=O stretching of amide groups, and peaks at 1050-1150 cm^{-1} suggested C-O stretching of polysaccharides. FTIR spectra of *Spirogyra sp.* biomass before and after adsorption demonstrated clear changes in functional group vibrations, particularly in the hydroxyl (-OH), carboxyl (-COOH), and amide regions, suggesting active involvement of these groups in amoxicillin binding. These functional groups are responsible for binding amoxicillin molecules through hydrogen bonding and electrostatic interactions.

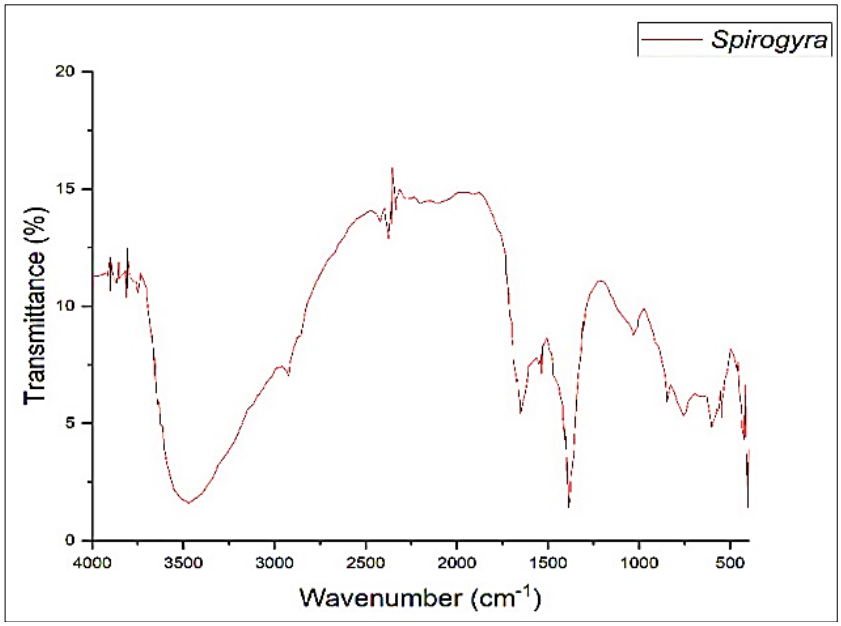

Fig 2: FTIR spectrum of Spirogyra sp biomass, indicating the presence of functional groups such as hydroxyl (-OH), amine (-NH), carbonyl (C=O), and carboxyl (-COOH) groups indicating the functional groups responsible for adsorption of amoxicillin.

3.2.2 SEM Analysis

SEM images revealed a porous and rough surface morphology that provides abundant active sites for adsorption, which became smoother and less porous after adsorption, indicating surface coverage by amoxicillin molecules.

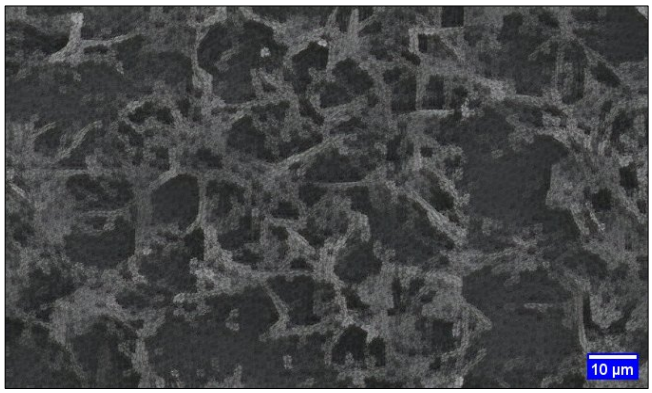

Fig 3: SEM micrograph of Spirogyra sp. biomass at 10 µm scale, showing a porous and fibrous surface morphology with irregular voids and interconnected structures confirming that rough morphology enhances surface adsorption.

3.2.3 XRD Analysis

The XRD pattern of *Spirogyra sp.* biomass exhibited a mostly amorphous structure with a few weak crystalline peaks, indicating the presence of organic biopolymers and minor mineral constituents.

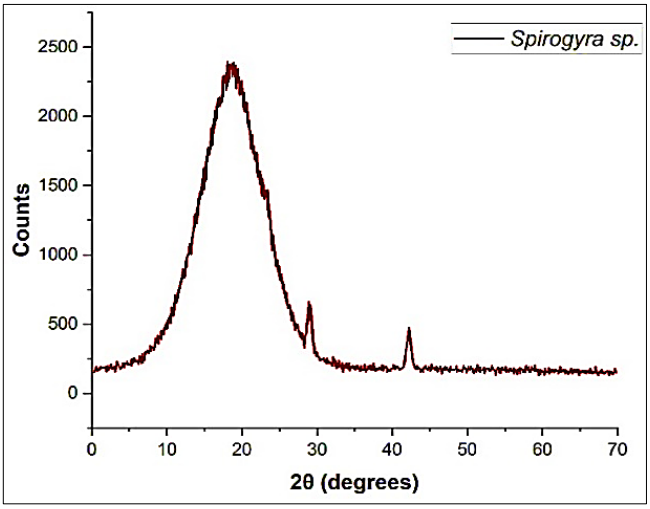

Fig 4: XRD pattern of Spirogyra sp. biomass.

This amorphous nature is favorable for adsorption due to the availability of active binding sites. The broad halo at ~22° (2θ) indicates a predominantly amorphous structure typical of organic biopolymers, while weak reflections at ~26.6°, ~31.9°, and ~44.2° suggest minor mineral phases naturally present in matrix. XRD analysis confirmed the semi-crystalline nature of the biomass, with a noticeable reduction in crystallinity following adsorption, further validating the interaction between the biomass matrix and the antibiotic molecules.

These results collectively confirm that surface functional groups, porosity, and crystallinity of Spirogyra biomass contribute significantly to its adsorption performance.

3.3 Effect of pH

The adsorption capacity of *Spirogyra sp.* biomass for Amoxicillin was significantly influenced by the pH of the solution. Maximum adsorption capacity (q_{max}) of 20.92 ± 0.34 mg g^{-1} was observed at pH 6.0, with a decrease in adsorption capacity to 14.30 ± 0.42 mg g^{-1} at pH 3 and 13.86 ± 0.47 mg g^{-1} at pH 9. This trend can be attributed to the protonation and deprotonation of functional groups on the biomass surface and the speciation of Amoxicillin.

Higher uptake at pH 6 suggests optimal electrostatic/hydrogen-bonding interactions between protonated/neutral amoxicillin moieties and deprotonated polysaccharidic -OH/-COO^- groups on *Spirogyra sp.*; suppression at low pH is attributed to H^+ competition, while decreased uptake at high pH likely arises from surface deprotonation and/or charge repulsion.

3.4 Effect of Contact Time and Initial Concentration

The adsorption capacity increased rapidly during the initial 60 minutes and gradually reached equilibrium at 120 minutes. For an initial concentration of 50 mg/L, the adsorption capacity increased from 10.65 ± 0.25 mg g^{-1} at 30 minutes to 20.72 ± 0.38 mg g^{-1} at 120 minutes. Higher initial Amoxicillin concentrations resulted in increased adsorption capacity due to the greater driving force for mass transfer.

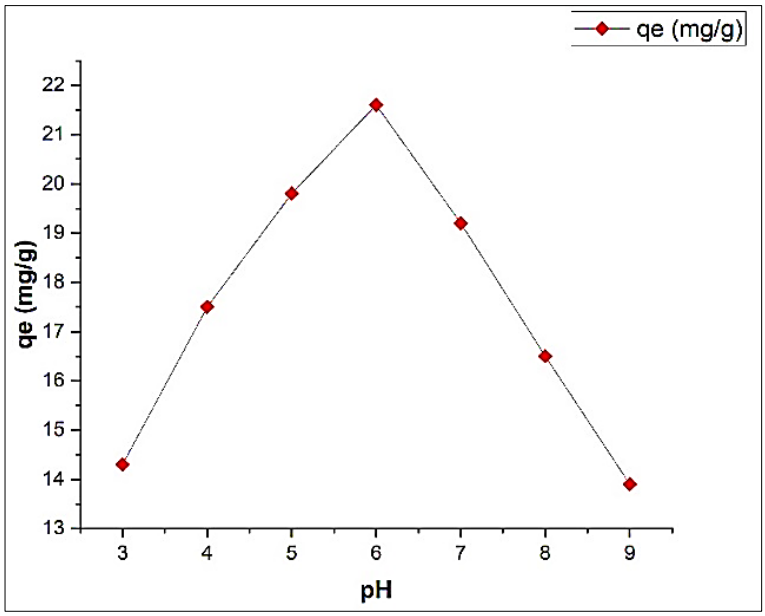

Fig 5: Effect of pH on $\mathbf{q_e}$ (3-9); optimum at pH 6; decline at ≤3 and ≥8.

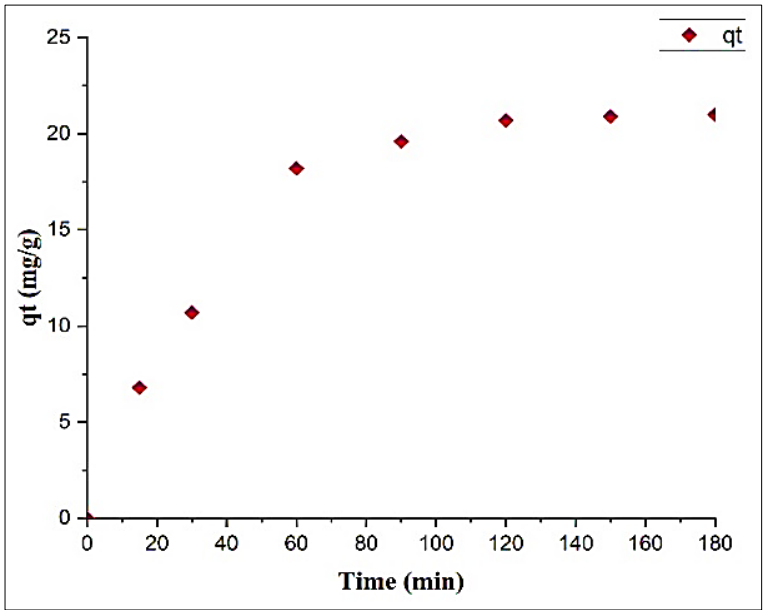

Fig 6: The Adsorption kinetics: q_t vs. contact time (0-180 min) at representative concentration. Rapid uptake occurs within 30-60 min followed by a plateau; equilibrium at ~120 min.

The rapid initial uptake reflects abundant vacant sites and film diffusion, followed by intraparticle diffusion and eventual site saturation.

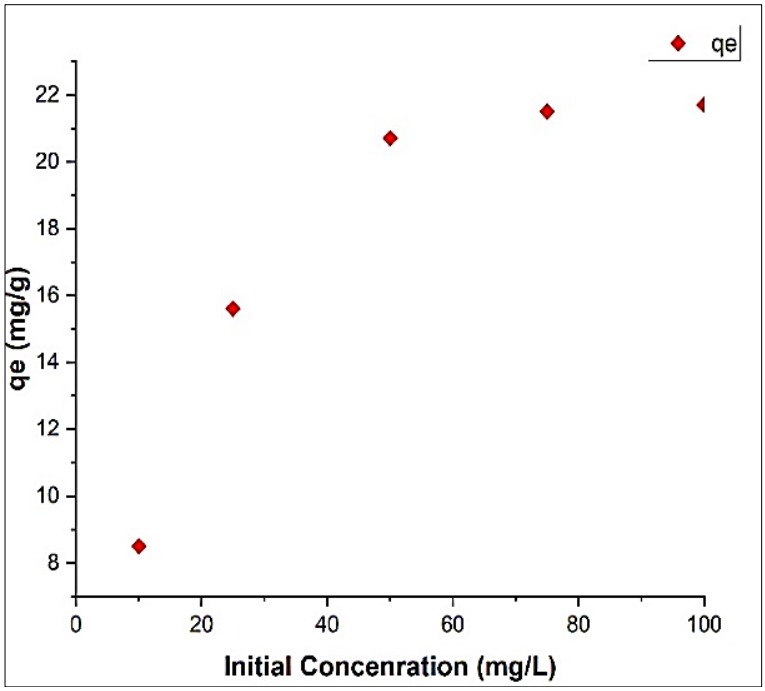

Fig 7: The Dependence of q_e on initial amoxicillin concentration (10-100 mg L^{-1}); q_e increases with concentration and approaches a plateau at higher C_0 due to site saturation.

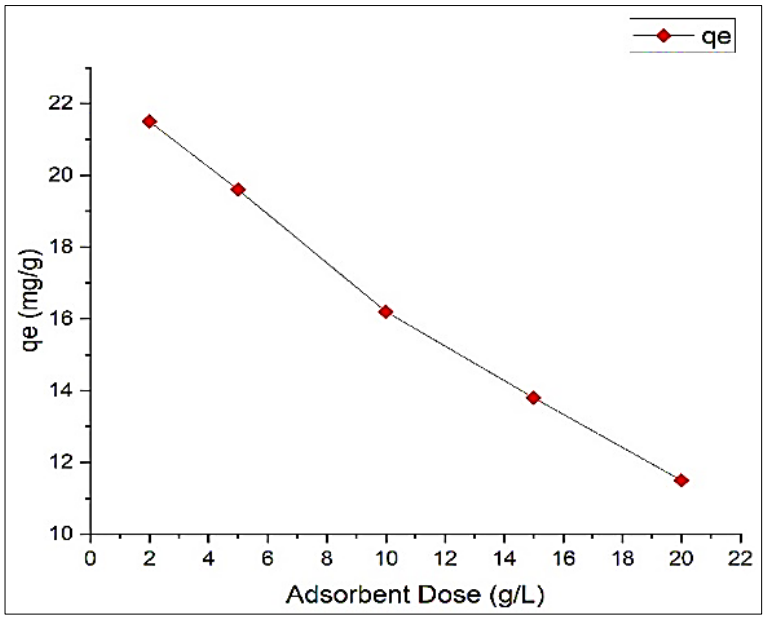

Fig 8: Effect of adsorbent dose (2-20 g L^{-1}) on q_e. Capacity per gram decreases with dose due to site aggregation/overlap; nevertheless, total removal (%) typically increases.

3.5 Kinetic Studies

The adsorption kinetics followed the pseudo-second-order model with a high correlation coefficient ($R^2 > 0.99$), suggesting chemisorption as the rate-limiting step. The calculated equilibrium adsorption capacity (q_e) was consistent with experimental data, with a q_e value of 20.85 mg g^{-1} obtained from the pseudo-second-order kinetic model closely matching the experimental value of 21.10± 0.37 mg g^{-1}, thereby validating the reliability of the kinetic analysis.

The maximum adsorption capacity of *Spirogyra sp.* biomass obtained in this study (21.68 mg g^{-1}, Langmuir isotherm) is comparable to or higher than many reported biosorbents. For instance, *Chlorella sp.* biomass exhibited 18.2 mg g^{-1} for cephalexin (Angulo *et al.*, 2018). This comparison highlights that *Spirogyra* biomass is a competitive and sustainable adsorbent for antibiotic removal, validating its practical potential in wastewater treatment.

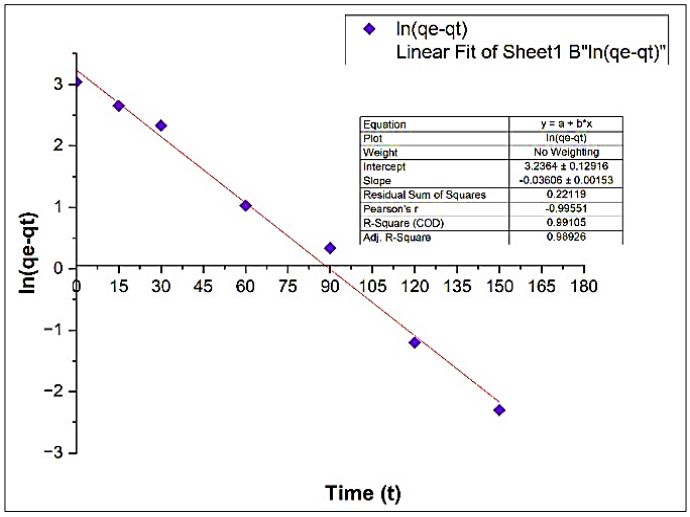

Fig 9: The PFO linear plot, $\ln(q_e - q_t)$ vs. t; slope = $-k_1$, intercept = $\ln q_e$.

Kinetic data were fitted to pseudo-first-order (PFO) and pseudo-second-order (PSO) models. While the linear fit of PFO gave a high R^2 (≈0.991), PSO provided closer agreement between $q_{e,cal.}$ and $q_{e,exp.}$ and a lower residual scatter (see residual diagnostics below). Accordingly, adsorption is best described by the PSO model, indicating that chemisorption governs the rate via electron sharing/exchange with surface functional groups. The PSO-derived q_e (≈20.8 mg g^{-1}) is consistent with the experimental value (≈21.1 mg g^{-1}).

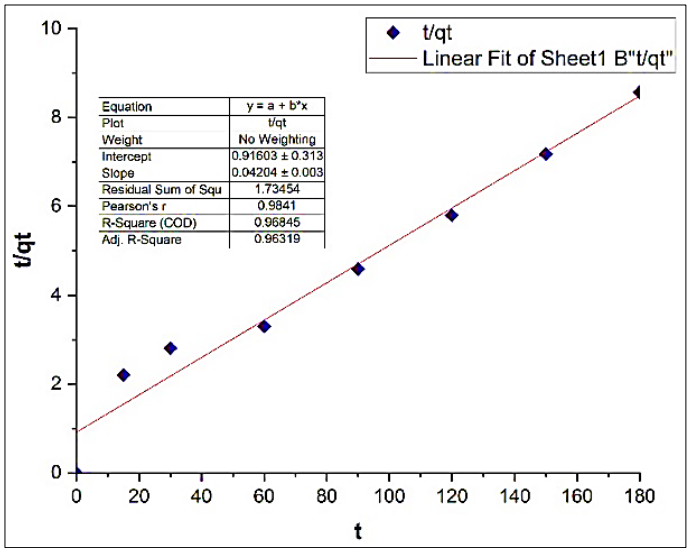

Fig 10: The PSO linear plot, t/qt vs. t; slope = 1/qe, intercept = $1/(k_2 q_e^2)$.

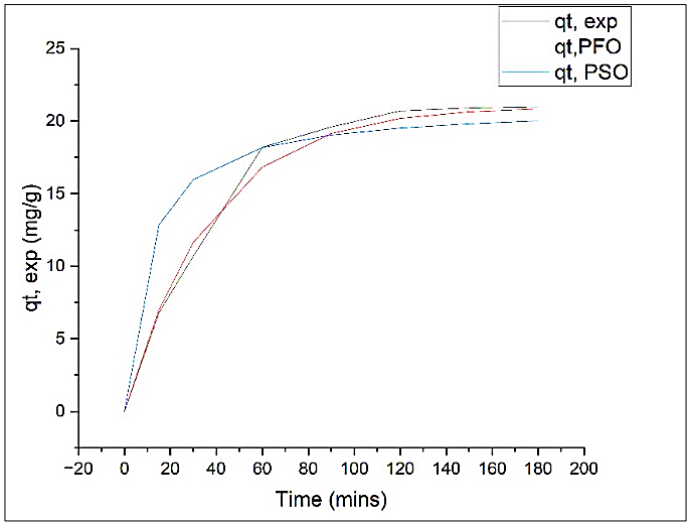

Fig 11: Experimental qt vs. time overlaid with PFO and PSO predictions. PSO traces the experimental curve more closely, especially near equilibrium.

3.5.1 Residual Diagnostics for Kinetic Models

Residual analysis corroborated model selection. For PFO, residuals display larger magnitude and mild structure across time and fitted values, indicating model inadequacy at longer times. PSO residuals are more randomly distributed about zero with lower spread, consistent with superior descriptive power for the rate process. Normal Q-Q plots show PSO residuals closer to the reference line than PFO. (Figures 12-13).

Kinetic modeling demonstrated that the pseudo-second-order (PSO) model provided the best fit to the experimental data, with qe,calc values closely matching qe,exp (21.1 vs 21.0 mg g^{-1}) and the highest R^2 values. Residual diagnostics further validated this observation, as the residuals for the PSO model were randomly distributed around zero, while those of the pseudo-first-order (PFO) model showed systematic deviation. This strongly supports the PSO model as the most reliable representation of amoxicillin adsorption onto *Spirogyra* biomass, indicating that chemisorption involving valence forces and functional group interactions is the dominant rate-controlling mechanism.

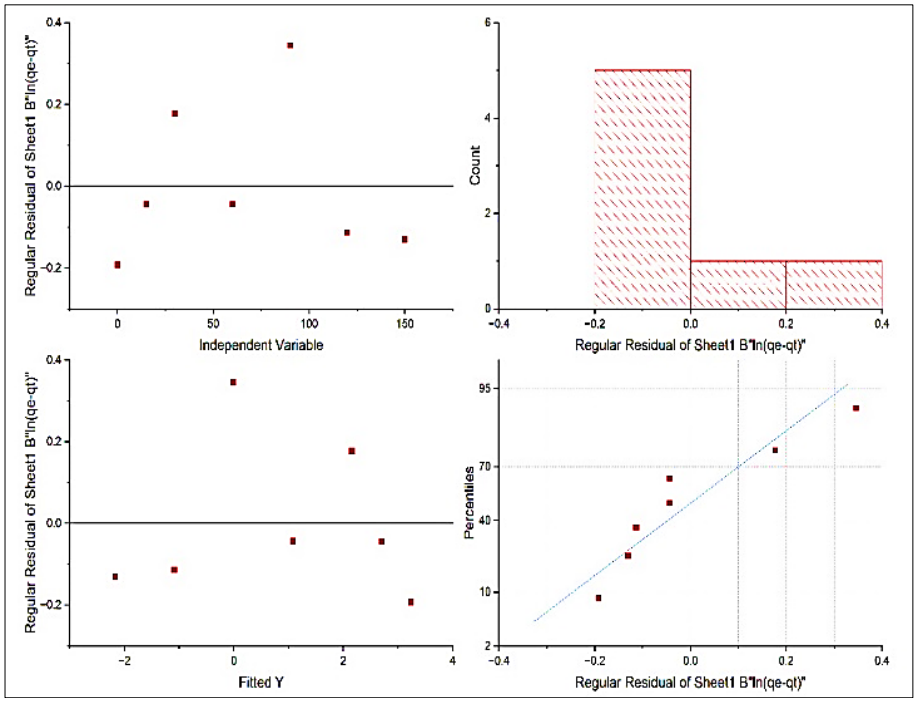

Fig 12: The PFO residual panels: (a) residuals vs. time; (b) histogram; (c) residuals vs. fitted; (d) normal Q-Q. Patterned spread and off-center distribution indicate poorer fit.

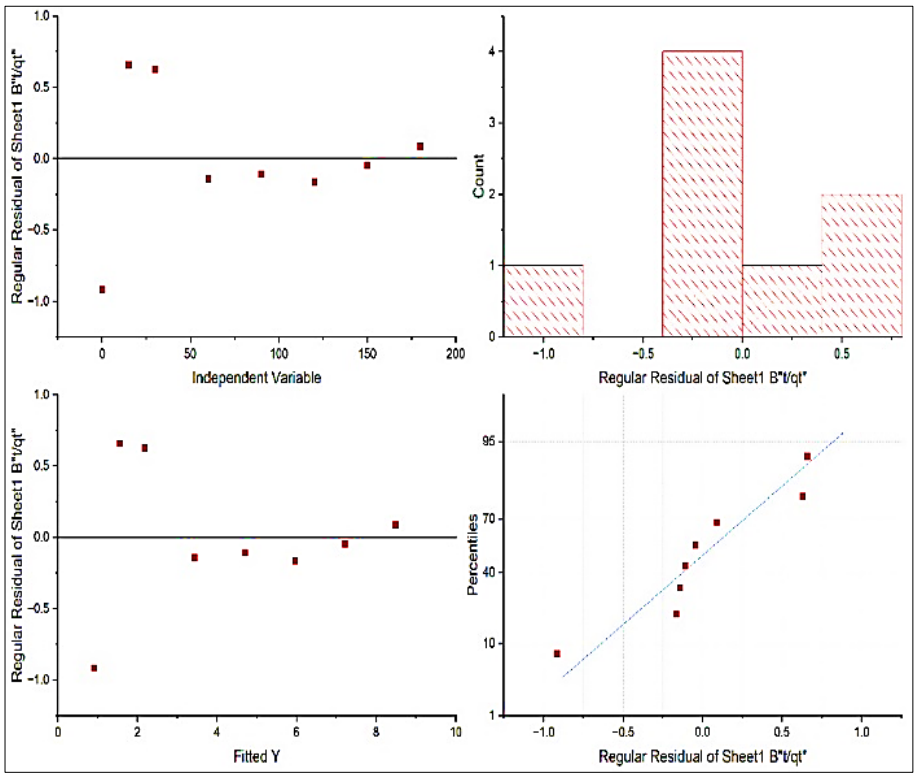

Fig 13: The PSO residual panels: analogous plots; residuals cluster around zero with reduced structure, supporting PSO.

Combining statistical fit and residual behavior, PSO is selected as the governing kinetic model for amoxicillin uptake by *Spirogyra sp.* biomass.

3.6 Isotherm Studies

The equilibrium data were best fitted to the Langmuir isotherm model, indicating monolayer adsorption on a homogenous surface. The maximum adsorption capacity (q_{max}) was determined to be 21.68 mg g^{-1} with a Langmuir constant (K_L) of 0.248 L/mg. The Freundlich model also showed a reasonable fit, with a Freundlich constant (K_F) of 3.41 mg g^{-1} $(L/mg)^{1/n}$ and a heterogeneity factor (n) of 2.36, indicating favorable adsorption.

The linear regression shows a good fit (R^2=0.950), confirming that the Freundlich model can describe the adsorption process. The slope (1/n = 0.244) corresponds to n ≈ 4.09, which indicates favorable adsorption (n>1). The intercept ($\ln K_F$=2.11) gives K_F≈8.25, reflecting the adsorption capacity of the biomass on a heterogeneous surface.

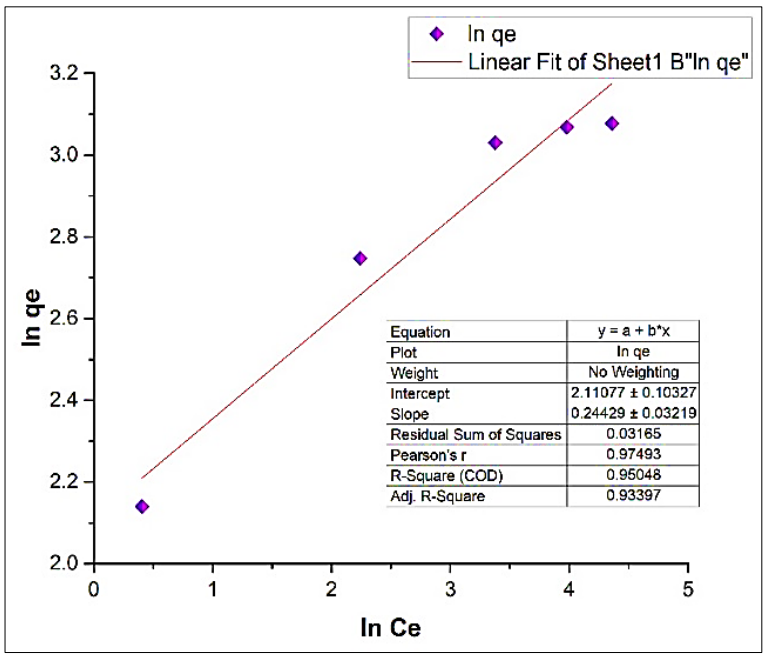

Fig 14: Freundlich adsorption isotherm for amoxicillin uptake onto Spirogyra sp biomass.

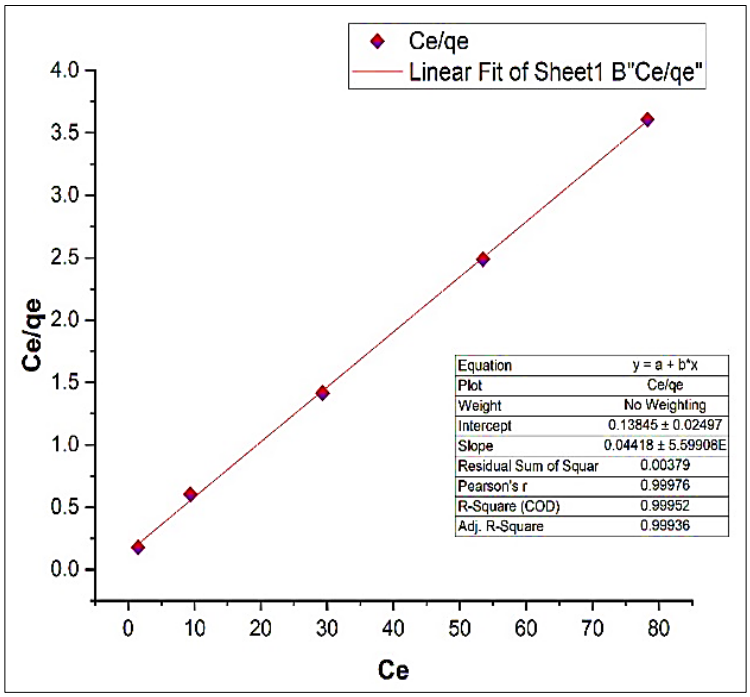

Fig 15: The Langmuir adsorption isotherm for amoxicillin uptake onto Spirogyra sp biomass.

The Langmuir plot demonstrates an excellent linear fit (R^2=0.9995), suggesting that adsorption follows a monolayer coverage model on a homogeneous surface with finite identical sites. From the slope (0.04418) and intercept (0.13845), the calculated maximum adsorption capacity is q_{max} = 1/slope ≈ 22.64 mg g^{-1}, and the Langmuir constant is K_L= slope/intercept ≈ 0.319 L/mg.

Between the two models, both Freundlich and Langmuir describe the adsorption process well, but the higher R^2 and consistency with $q_{e,exp}$ suggest that the Langmuir model provides a superior fit, indicating that amoxicillin adsorption onto *Spirogyra sp.* biomass occurs predominantly as a monolayer on homogeneous sites.

3.7 Thermodynamic Studies

The standard thermodynamic parameters were evaluated from the van't Hoff relation, $\ln K_c = \frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R}$. The negative slope of $\ln K_c$ vs. $\frac{1}{T}$ yielded $\Delta H^\circ \approx +22.5$ kJ·mol^{-1}, confirming an endothermic process, while the positive intercept produced $\Delta S^\circ \approx +91.7$ J·mol^{-1}·K^{-1}, indicating increased interfacial randomness. The resulting ΔG° values (−4.8,−5.7,−6.6 kJ·mol^{-1} at 298-318 K) confirm spontaneity that improves with temperature. These thermodynamic parameters indicate that higher temperatures favour the adsorption process.

The positive enthalpy change ($\Delta H^\circ = +22.5$ kJ·mol^{-1}) confirms the endothermic nature of the adsorption process, suggesting that higher temperatures favor adsorption. This may be explained by the disruption of structured water molecules surrounding amoxicillin, facilitating interaction with the biomass surface. The positive entropy change ($\Delta S^\circ = +91.68$ J·mol^{-1}·K^{-1}) indicates increased randomness at the solid-solution interface, likely due to the release of water molecules during adsorption. The negative Gibbs free energy values ($\Delta G^\circ = -4.8$ to -6.6 kJ·mol^{-1}) across all temperatures confirm the spontaneity of the process. Taken together, these results suggest that adsorption is primarily governed by chemisorption involving hydroxyl and carboxyl groups, supported by physical interactions such as hydrogen bonding and electrostatic attraction.

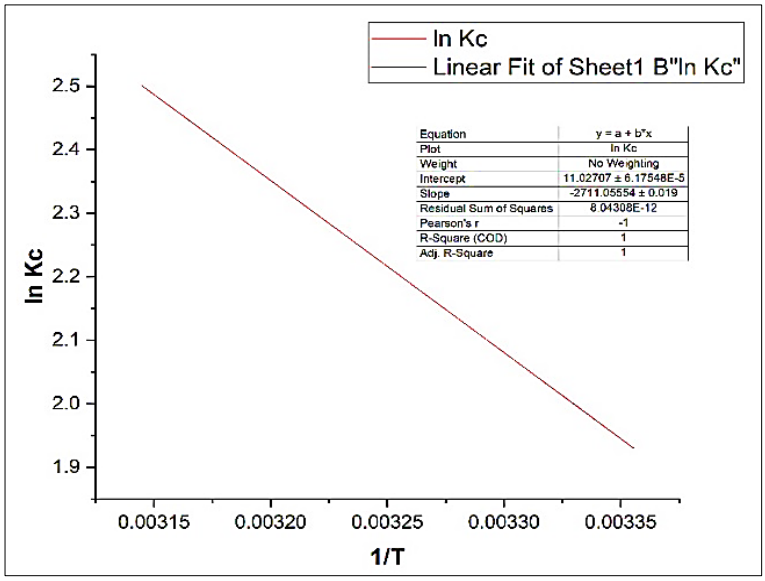

Fig 16: The van't Hoff plot.

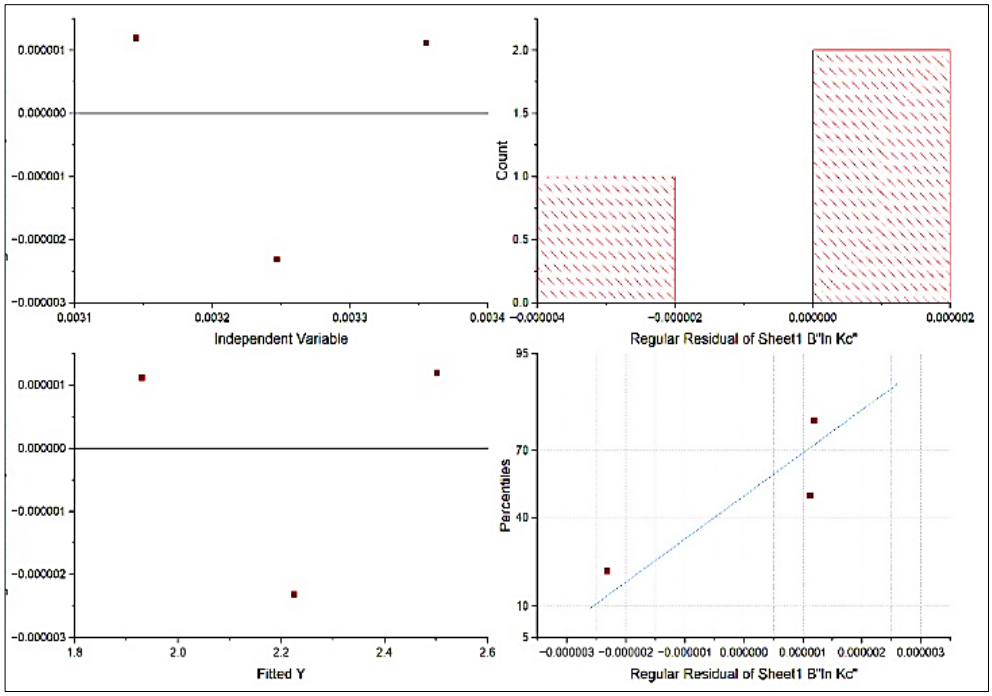

Fig 17: The Residual panels for the van't Hoff fit showing very small, symmetrically distributed residuals and an approximately normal Q-Q pattern evidence of a robust linear relation and trustworthy ΔH∘ and ΔS∘ estimates.

4. Conclusion

This study demonstrated that *Spirogyra sp.* biomass is an effective biosorbent for the removal of Amoxicillin from aqueous solutions. The adsorption process was influenced by pH, contact time, initial concentration, and temperature. Kinetic studies revealed that the adsorption followed pseudo-second-order kinetics, while isotherm analysis indicated monolayer adsorption as per the Langmuir model. Thermodynamic parameters confirmed the endothermic and spontaneous nature of the adsorption process.

Model-selection takeaway

Although the PFO linear regression returned a slightly higher R^2 on one linearization, the pseudo-second-order model was chosen on the basis of

1. closer agreement between qe,cal and qe,exp,
2. lower residual scatter with random structure, and
3. better reproduction of the full qt-t trajectory near equilibrium. Together with the Langmuir isotherm and favorable thermodynamic parameters, these results indicate chemisorption-dominated, monolayer uptake that is spontaneous and endothermic for amoxicillin on *Spirogyra sp.* Biomass.

The findings suggest that *Spirogyra sp.* biomass can be used as an eco-friendly, cost-effective material for removing antibiotics from contaminated water.

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