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Batch Study on Rifampicin Removal from Aqueous Solution by Live and Dead Chlorella

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ABSTRACT

Pharmaceutical contamination of aquatic environments poses a significant threat to ecosystems and public health. Rifampicin, a first-line antibiotic used to treat tuberculosis, is often detected in wastewater, contributing to antimicrobial resistance and toxicity to aquatic organisms. In this study, the biosorption potential of live and dead Chlorella for the removal of rifampicin under batch conditions was investigated. Several parameters of dead Chlorella were evaluated to select the optimal conditions for the removal process. The optimal pH was 4, at which the maximum adsorption capacity, q max, was 58 mg/g. The optimum temperature was also tested, and it was found that 25 °C yielded the best result, with a maximum adsorption capacity of q max = 65 mg/g. The contact time, initial rifampicin concentration, and algae biomass dosage were systematically studied to determine their effect on the removal efficiency. The results showed that live Chlorella exhibited much higher adsorption capacity than dead Chlorella. These results suggest that the inactive microalgae biomass could be an effective and environmentally friendly bioadsorbent for treating antibiotic-contaminated wastewater.

Introduction

Pollutants pose a significant threat to the environment and human health [1]. The emergence of these pollutants is due to various factors, including advances in scientific detection methods, increased awareness of environmental issues, and changes in human activities. These pollutants enter the environment through multiple pathways, including industrial waste, agricultural runoff, and biological sedimentation [2]. Recently, the presence of pharmaceutical contaminants in groundwater and surface water has become common. These groups of pollutants are generally referred to as emerging pollutants and are widely distributed in aquatic environments. Among these pollutants are antibiotics, which are commonly used in human and veterinary medicines, and enter water bodies through various human activities. Unfortunately, these antibiotics are found in water bodies in their metabolized and non-metabolized forms even at low concentrations. Environmental health is constantly facing problems due to the persistence of these undesirable pollutants that accumulate in water bodies [1, 3]

Pharmaceuticals are rapidly growing environmental pollutants because they have marked a significant turning point in the development of human sciences, prolonging human lifespans,

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saving lives from fatal diseases, and improving the quality of life, which has significantly increased their prominence in the environment over the past three decades [4].

The presence of antibiotics in the aquatic environment poses a significant environmental risk to living organisms due to their toxicity and long-term persistence in the environment [5] Since the long-term persistence of antibiotics in the environment and their resistance to removal methods pose a threat to human and animal health worldwide, significant measures are needed to reduce the risks posed by antibiotic resistance occurring in the environment [6]. Rifampicin (RIF) is a broad-spectrum antibiotic against bacterial pathogens. It is used as a first-line drug for the treatment of tuberculosis. It is also used to treat other diseases such as cancer, Alzheimer's disease, leprosy, and HIV [7, 8].

Recent studies have shown that RIF exhibits a strong binding affinity with the COVID-19 protease, suggesting that it could be utilized as a treatment for coronavirus and for its prevention [9].

Conventional wastewater treatment plants are insufficient for removing antibiotics [10, 11], and they also cause the release of antibiotics back into the environment, indicating that they are not effective enough [12]. There is an urgent need to protect the ecosystem from these pollutants, which have a profound and harmful impact on the environment, through environmentally friendly technologies [13].

Several methods of technology have been developed, including advanced oxidation, photocatalysis, physical adsorption, bioremediation using activated sludge, and chemical processes, to remove antibiotics [14-16].

Bioremediation is one of the environmentally friendly technologies that can remove pollutants from polluted aquatic and terrestrial environments. It utilizes microorganisms, such as algae, bacteria, fungi, yeast, or reconstituted microbial components, which remove contaminants without causing further pollution [17]. The use of microalgae is one of the most effective and widely employed methods for treating wastewater [18], as it requires minimal cultivation time and a small area with high productivity compared to other biological processes [19].

Although Chlorella has been studied for biosorption of some drugs such as ranitidine [20], its ability to remove rifampicin remains unexplored, despite its persistent toxicity and increasing environmental prevalence. Current research focuses on the use of both dead and live Chlorella biomass and assessing their ability to remove or degrade rifampicin via enzymatic pathways. Furthermore, no previous studies have investigated the effects of critical parameters (e.g., pH, biomass pretreatment) on rifampicin removal, leaving the efficiency and mechanistic insights unclear.

This study aims to compare the ability of live and dead Chlorella to remove rifampicin from aqueous solutions**.**

Material and Methods

Culture Medium

The algal cultures were grown in BG11 medium (HiMedia, M1958), which contains all essential macro- and micronutrients necessary for their growth. Table 1 shows the BG11 composition. The medium was prepared by dissolving 1.627 g of the dehydrated BG11 powder in 1 L of distilled water. The pH was adjusted to 7.1 using 1 M NaOH. The medium was autoclaved at 121°C for 15 minutes and then cooled to room temperature before use [21].

Table 1. Composition of BG11 medium used for algal cultivation

Component	Concentration
$NaNO_3$	1 g/L
$CaCl_2 \cdot 2H_2O$	0.036 g/L
Ferric ammonium citrate	0.012 g/L
$EDTA \cdot Na_2 \cdot 2H_2O$	0.001 g/L
K_2HPO_4	0.04 g/L
$MgSO_4 \cdot 7H_2O$	0.075 g/L
Na_2CO_3	0.02 g/L
Trace metal solution	ML/L1
H_3BO_3	2.86 g/L
$MnCl_2 \cdot 4H_2O$	1.81 g/L
$ZnSO_4$	0.222 g/L
$Na_2MoO_4 \cdot 2H_2O$	0.39 g/L
$CuSO_2 \cdot 5H_2O$	0.079 g/L
$Co(NO_3)_2 \cdot 6H_2O$	0.049 g/L

Culturing the Microalgae Species

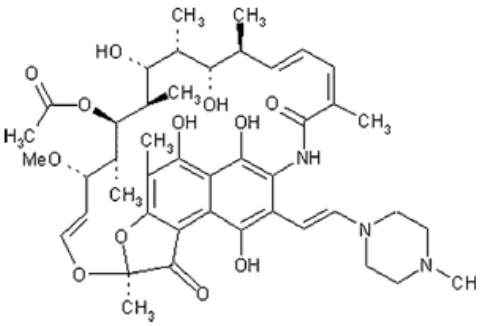
Microalgae *Chlorella sorokiniana sp.MH 923013* was isolated from the Tigris River and registered in the GenBank database (accession number MH 923013) [22]. It was prepared with chlorella in its live form and dead form (powder) for study.

Antibiotic Preparation

Rifampicin was supplied by Samarra Pharmaceutical Industries Company (Samarra, Iraq). Table 2 shows the chemical composition, molecular weight, and pKa values.

A 1000 ppm rifampicin solution was prepared by dissolving 1 g of the drug in 1 liter of distilled deionized water [23].

Table 2. Physicochemical properties and molecular structure of RIF

CAS No.	13292-46-1
Molecular structure	
Molecular formula	$C_{43}H_{58}N_4O_{12}$
Molecular weight (g/mol)	822.953
Log Kow	4.24
pKa	1.70- 7.90
Solubility (H_2O, mg/mL)	1.4

Instruments and Equipment Used in the Study

Table 3 shows the analytical instruments and equipment used in this study. These instruments were essential for characterizing algal biomass, determining rifampicin adsorption (RIF), and assessing biochemical changes. Standard operating protocols and calibration procedures were followed for all measurements to ensure reproducibility of results.

Table 3. Instruments and equipment used

Instrument	Specifications
UV-Vis Spectrophotometer	Generic Model 700-2000 -1800; Range: 320–1100 nm; Resolution: ±2 nm
FT-IR Spectrometer	Shimadzu (Japan) IRAffinity ; Range: 350-7800 cm^{-1}; Resolution: 0.5 cm^{-1}

Experiment on the Removal of Rifampicin Using Dead Algae

A series of experiments was conducted to evaluate the optimal conditions for removing rifampicin using dead Chlorella algae.

Effect of PH

Five experiments were conducted in the pH range (2-6) due to (a) encompassing rifampicin's ionic transition zones near its pKa values, (b) representing common wastewater conditions, and (c) maintaining microalgal viability based on preliminary experiments, for each specific pH (2, 3, 4, 5, 6). The rifampicin solution was prepared at a concentration of 100 ppm in a glass beaker, to which 0.1 g of dry algae mass was added, and the volume was completed to 100 mL with deionized distilled water. Then, the pH was adjusted using hydrochloric acid (HCl) and sodium hydroxide (NaOH) according to the required pH for the experiment. The solution was stirred using a magnetic stirrer (made in China) at a speed of 220 rpm for 40 minutes. After that, the solution was filtered, and the remaining rifampicin concentration was measured using a spectrophotometer. The results showed that pH 4 was the optimal condition in terms of removal efficiency, as indicated by Eq. 1, where the adsorption rate reached a value of q = 58mg/g. The experiment was repeated twice to verify the validity of the results.

$$q = \frac{C_0 - C_f}{w} * \frac{V}{1000} \quad (1)$$

where
q = mg drug ions per gm algae
C_0, C_f = initial and final concentration
V = volume used in ml

Effect of Temperature

Five experiments were conducted at various temperatures to determine the optimal temperature for removing rifampicin. 0.1 g of dead Chlorella was added along with a solution of rifampicin at a concentration of 100 ppm in a glass beaker, and the volume was brought to 100 mL with deionized distilled water. After that, the pH was adjusted to 4. The temperature was set using a magnetic stirrer at temperatures (10, 25, 35, 55, 75) in each experiment at a specific temperature and at a speed of 220 rpm for 40 minutes. After completing the experiments at various temperatures, it was found that a temperature of 25 degrees Celsius yielded the best results in terms of rifampicin removal, with an adsorption rate of q = 65 mg/g. The experiment was repeated twice to verify the validity of the results.

Effect of Algal Weight

Different experiments were conducted with varying amounts of dead Chlorella (0.05, 0.1, 0.15, 0.2, 0.25 g) to determine the effect of increasing or decreasing the algae dose on the adsorption process. 100 ppm solution of rifampicin was added to a glass beaker, and the volume was completed to 100 ml with deionized distilled water. The pH was maintained at 4, and the temperature was set at 25 degrees Celsius. Using a magnetic stirrer, the mixture was stirred at a speed of 220 rpm for 40 minutes. It was observed that as the algae dose increased, the adsorption process also increased due to the increase in the number of active sites. The experiment was repeated twice to verify the validity of the results.

Effect of Contact Time on Removal Efficiency

This experiment was conducted by adding 100 ppm of rifampicin to 0.25 g of dead Chlorella at a temperature of 25°C and pH 4. The mixture was stirred continuously on a magnetic stirrer at a speed of 220 rpm. The sampling process was monitored every two hours for 24 hours**.**

The experiment was repeated twice to verify the validity of the results.

Effect of Concentration of Rifampicin

These experiments were conducted at different concentrations of rifampicin: 10, 20, 40, 60, 80, 100, 150, and 200 ppm. The experiment was repeated twice to verify the validity of the results.

Experiment on the Removal of Rifampicin Using Live Algae

Initially, live Chlorella was cultivated in the previously prepared BG-11 medium, where the process involved inoculating 2 ml of microalgae samples into 250 ml of BG-11 medium. The culture was then incubated under light intensity (168 μE $m^{-2}s^{-1}$) and at a temperature of (25±2) ^{o}C, with pH stabilized between 7.1-8.4. When the algae reached the stationary growth phase, they were harvested for experimentation purposes.

Bioremediation Process

The experiment was conducted at three different concentrations of rifampicin: 50, 100, and 150 ppm, using 0.3g of the harvested microalgae. The volume was then adjusted to 150 ml with BG-11 medium. After that, the growth of the algae was monitored by tracking their optical density, which was measured by determining the absorbance in the spectrophotometer at a wavelength of 540 nm.

The process of removing rifampicin was monitored by measuring the absorbance using a spectrophotometer at a wavelength of 475 nm.

Results and Discussion on Dead Algae

Effect of PH

Fig. 1 illustrates the relationship between pH and maximum adsorption capacity at each pH. It shows that the highest adsorption capacity was at pH 4, reaching 58 mg/g. This is due to the algae surface containing functional groups such as carboxyl (-COOH), hydroxyl (-OH), and amine ($-NH_2$). At pH 4 (in acidic media), these groups are in a protonated state, resulting in a surface that is either positively charged or neutral. Rifampicin, on the other hand, is a complex molecule containing functional groups that can ionize depending on pH. At pH 4, rifampicin is typically in a non-ionized or partially ionized form, facilitating its binding to polar or positively charged groups on the algae surface [24-27].

In contrast, at pH < 4, excess hydrogen ions (H^+) may compete with rifampicin molecules for adsorption sites, reducing efficiency.

In contrast, at pH < 4, excess hydrogen ions (H^+) may compete with rifampicin molecules for adsorption sites, reducing efficiency.

At very low pH (2-3), the surface charge of the algae is strongly positive due to the protonation of functional groups (e.g., -NH_2, -COOH), causing repulsion with cationic molecules (e.g., protonated rifampicin) [28].

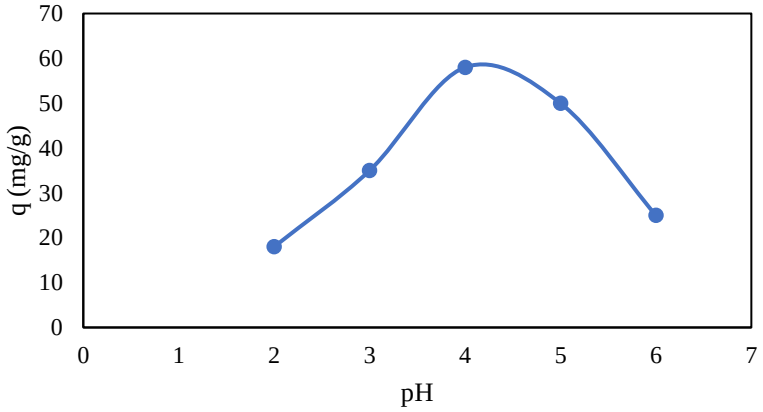


Fig. 1. Effects of pH solution on the RIF biosorption in C. sorokiniana MH923013 at an initial concentration of 100 ppm and room temperature

Effect of Temperature

As shown in Fig. 2**,** at high temperatures (>30 °C) a decline in the adsorption rate occurs due to (a) physical damage to algal cell wall binding sites (e.g., hydrolysis of carboxyl and phosphate groups necessary for the formation of rifampicin complexes, and (b) increased absorption rates as molecular collisions overcome weak interactions (e.g., hydrogen bonds [29, 30]. Therefore, high temperature affects the adsorption process of rifampicin. The higher the temperature, the lower the drug adsorption. The adsorption process is essentially exothermic, meaning it typically operates inefficiently at high temperatures due to the associated high operating costs [31].

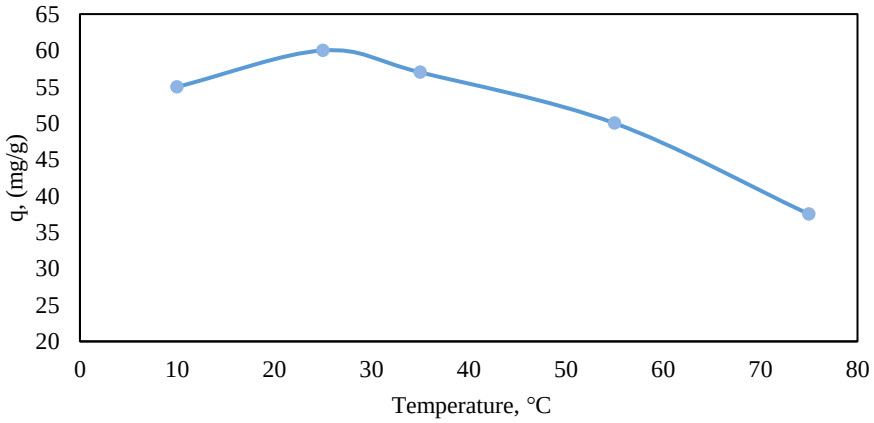


Fig. 2. Effects of temperature on the RIF biosorption in C. sorokiniana MH923013 at an initial concentration of 100 ppm

Effect of Algal Weight

As algal biomass increases, the number of active sites available for rifampicin adsorption increases, resulting in a greater amount of drug being removed from the medium. Adsorption capacity is typically calculated on a per-unit-mass-of-algae basis. As biomass increases, the adsorbed material is distributed across a greater number of sites, resulting in a lower adsorption capacity per unit mass [32, 33].

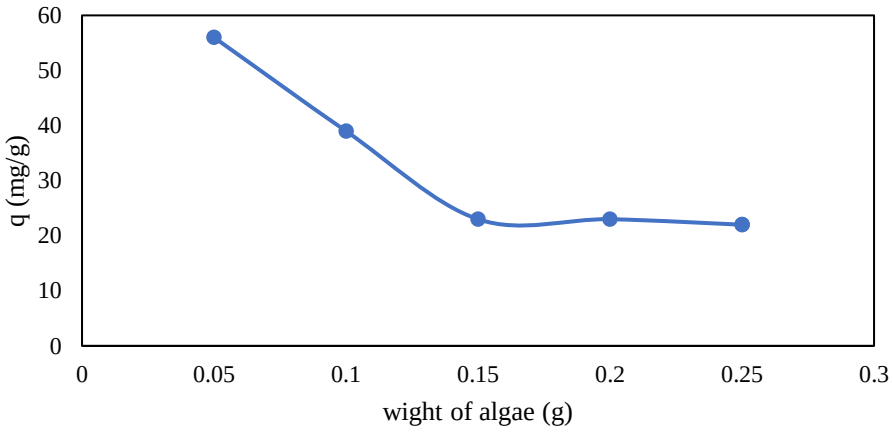


Fig. 3. Effects of the weight of algae on the RIF biosorption in C. sorokiniana MH923013 at an initial concentration of 100 ppm

Effect of Time

Fig. 4 shows the equilibrium time course of RIF uptake at an initial concentration of 100 ppm on C. sorokiniana. The uptake capacity increases rapidly over the first 6 hours to 26 mg/g, then gradually increases to 28 mg/g at 8 hours, after which no significant increase is observed. Equilibrium is reached after 12 hours, reaching a maximum of 28.4 mg/g.

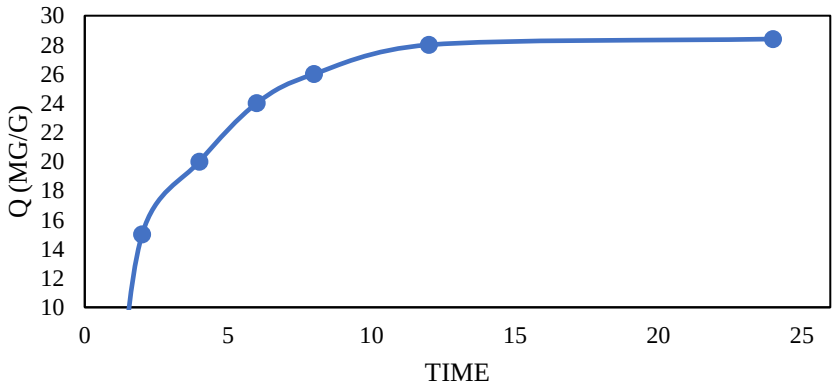


Fig. 4. Effects of time on the RIF biosorption in C. sorokiniana MH923013 at an initial concentration of 100 ppm

Effect of Initial Concentration of RIF

According to Fig. 5, it can be observed that as the initial RIF concentration increases from 20 ppm to 200 ppm, the adsorption capacity increases from 2.8 mg/g to 60.4 mg/g. This increased the difference between the RIF concentration in the solution and the RIF concentration on the microalgae surface, resulting in a greater driving force for mass transfer and thereby enhancing the adsorption process at higher concentrations.

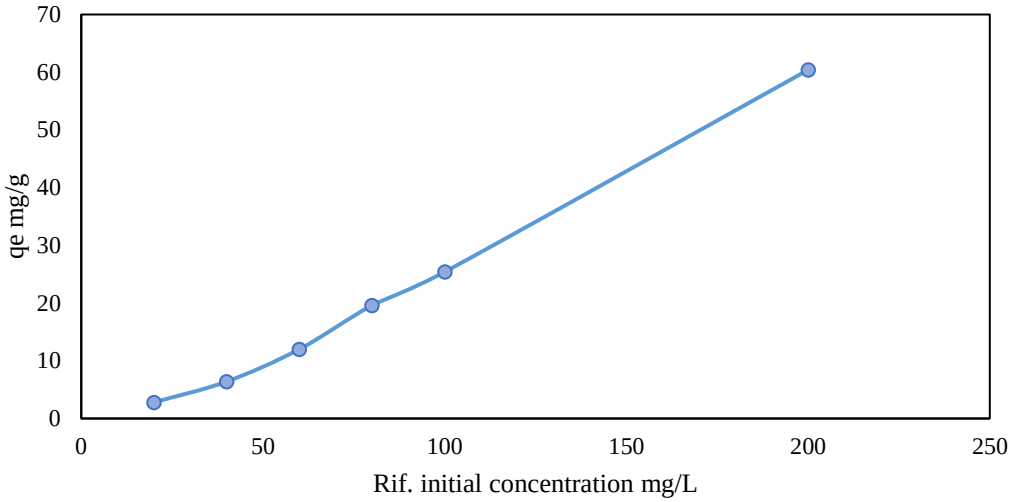


Fig. 5. Effect of initial concentration of RIF on biosorption in C. sorokiniana MH923013 at an initial concentration of 100 ppm

Adsorption Kinetic Modeling

The results showed an apparent discrepancy in the suitability of the proposed kinetic models. While the possibility of the reaction following first-order kinetics was investigated, the model's agreement with the experimental data was poor, with a correlation coefficient (R^2) of 0.8756, indicating its inadequacy in describing the reaction's kinetic mechanism, as shown in Fig. 8.

In contrast, when testing the second-order kinetic model, the analysis revealed a significant improvement in the agreement between theoretical and experimental data, with the correlation coefficient (R^2) increasing to 0.9903, a value close to ideal (1.0), confirming the model's suitability for describing the reaction. This improvement is attributed to the second-order model's ability to explain the dependence of both the adsorbate and adsorbed surface concentrations, as shown in Figs. 6 & 7, which is consistent with mechanisms involving bimolecular interactions or adsorption on heterogeneous surfaces.

The second-order kinetics of the reaction suggest that the adsorbate may be governed by a mechanism governed by factors such as saturation of adsorption sites on the surface or bidirectional interactions between the adsorbate and the surface. These results are consistent with previous studies that have indicated that the adsorption of antibiotics, such as rifampin, on mesoporous surfaces often requires higher-order kinetic models due to the complexity of their surface interactions [34, 35].

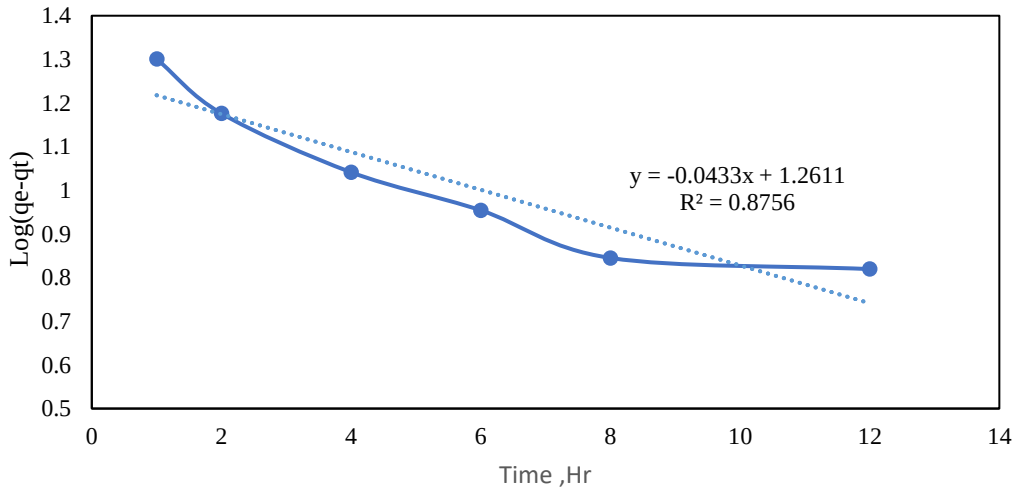


Fig. 6. First-order Kinetic Model

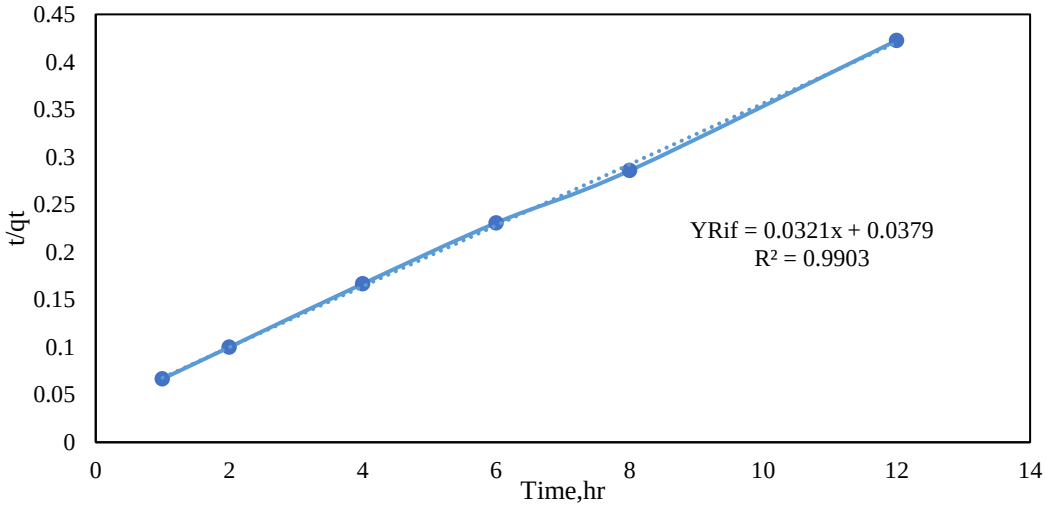


Fig. 7. Second-order Kinetic Model

Characterization Analysis

FTIR Analysis

FTIR (Fourier Transform Infrared Spectroscopy) identifies functional groups in a sample by measuring absorption of infrared light at specific wavelengths (cm^{-1}). The major peaks in the spectra correspond to specific bonds, indicating changes in the chemical structure of algae before and after antibiotic treatment.

Control (Algae Alone)

Fig. 8 reflects the biochemical composition of the algae, including proteins, polysaccharides, lipids, and nucleic acids. The control spectrum represents untreated algae, serving as a baseline for comparison. The key peaks likely correspond to:

O-H stretching (~3200–3600 cm^{-1}): Broad peak due to hydroxyl groups from polysaccharides and proteins [36].

C-H stretching (~2800–2950 cm^{-1}): Represents aliphatic hydrocarbons.

C=O stretching (~1650–1750 cm^{-1}): Commonly found in carboxyl, amide, or ester groups from proteins and lipids [37].

C-N and C-O stretching (~1000–1300 cm^{-1}): Related to amines and carbohydrates.

P=O (~900–1200 cm^{-1}): Phosphates present in nucleic acids and phospholipids [38].

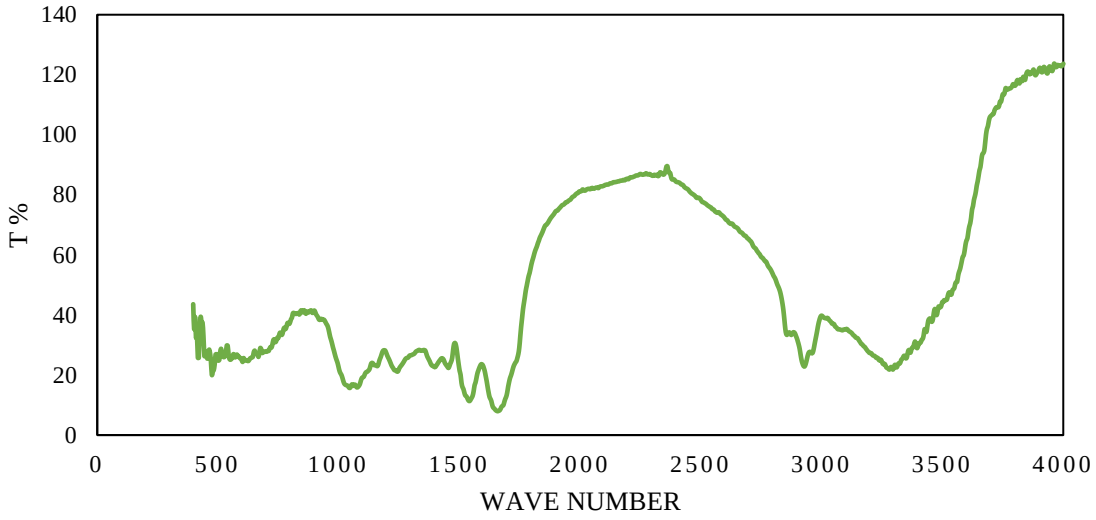


Fig. 8. FTIR for algae C. sorokiniana MH923013

Algae with Rifampicin

Fig. 9 shows Fourier transform infrared (FTIR) analysis of Scenedesmus obliquus before and after RIF exposure. Spectral shifts are noticeable, particularly in the O–H/N–H (3300 cm^{1}), C=O (1650 cm^{1}), and P=O/C–O–P (1000–1250 cm^{1}) regions, providing evidence of RIF binding to proteins, lipids, and nucleic acids/phospholipids. These modifications confirm the biochemical changes induced by RIF, supporting its destructive effects on algal metabolism and cell integrity, with implications for ecotoxicology and bioremediation [36-39].

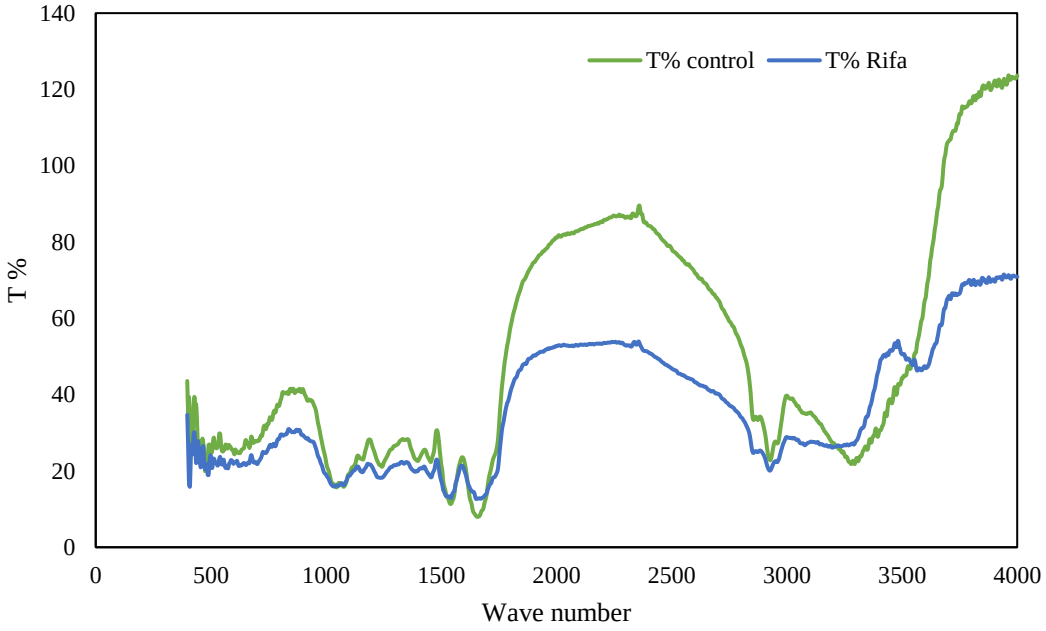


Fig. 9. FTIR for algae C. sorokiniana MH923013 with drug (RIF)

The observed changes suggest that Rifampicin interacts with algae biomolecules, possibly binding to proteins, lipids, or nucleic acids. This may alter the biochemical structure of the algae, affecting their metabolism or cellular composition.

SEM and EDS Analysis

Scanning Electron Microscopy

SEM images revealed significant morphological changes in the algal surface following rifampicin adsorption, as shown in Fig. 10. Before adsorption, the surface exhibited a highly porous structure with a network of nanocavities that provided active sites for adsorption. After the reaction, a relatively homogeneous layer was observed covering most of the pores, with the appearance of fine aggregates of rifampicin (~ 34.51–77.59 nm in diameter). This observation supports the hypothesis that chemisorption occurred via the interaction of the carboxylic groups (-COOH) on the algal surface with the amino groups (-NH2) of rifampicin, as demonstrated by FTIR analysis. Furthermore, the blockage of the pores indicates that part of the adsorption occurred internally (diffusion), consistent with the Freundlich model.

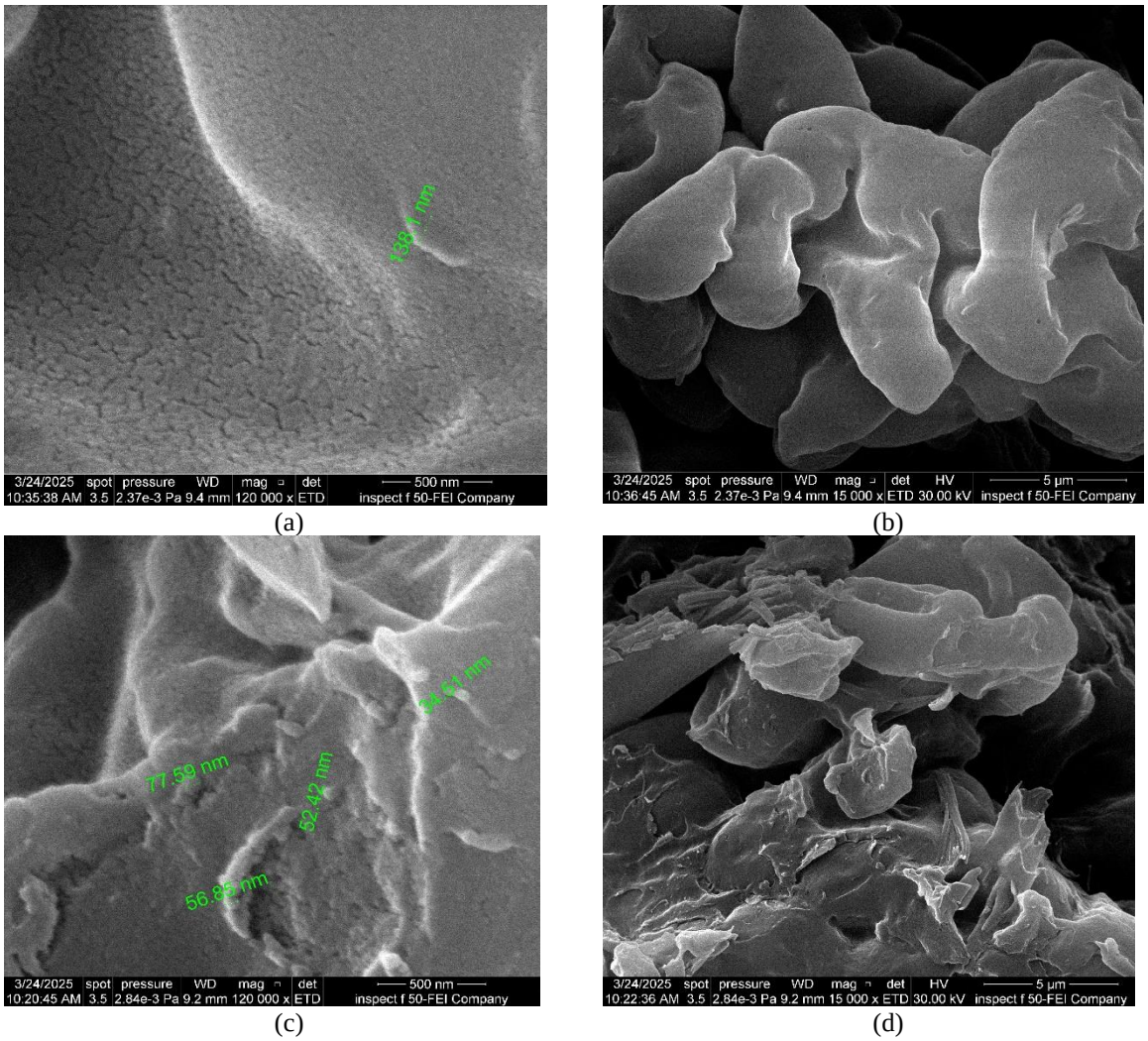


Fig. 10. SEM image of C. sorokiniana MH923013, before (a, b), and after (c, d) adsorption

Energy Dispersive X-ray Spectroscopy

EDS Before Adsorption of Rifampin

EDS analysis of the C. sorokiniana MH923013 biomass before adsorption, Figs. 11 & 12, and Table 4 show the presence of significant elements such as carbon and oxygen, which are mainly attributed to the organic components of the algae (such as sugars, lipids, and proteins). Small proportions of mineral elements such as potassium and copper were also observed, which may have originated from the environment in which the algae grew.

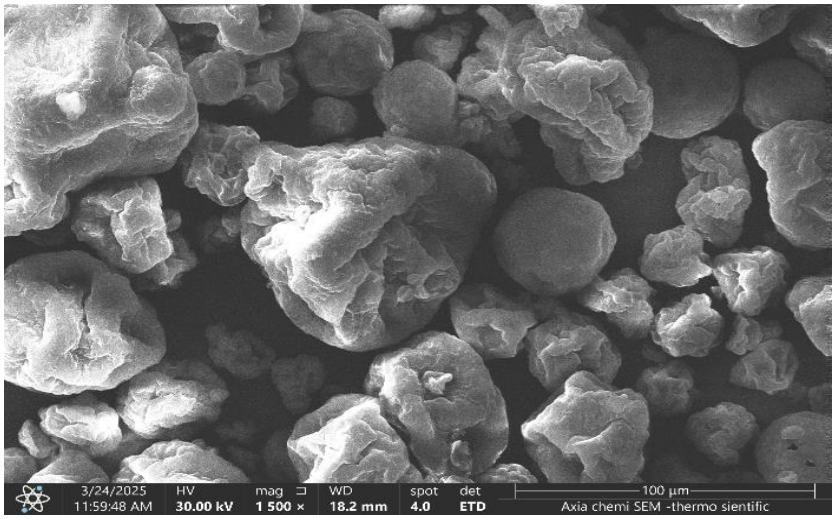


Fig. 11. SEM image of sorokiniana MH923013 before antibiotic exposure, showing smooth surface morphology

Table 4. EDS elemental composition of sorokiniana MH923013 before antibiotic exposure, showing mainly carbon and oxygen

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	64.8	0.5	56.4	0.4
O	33.7	0.6	39.0	0.7
Mg	0.4	0.0	0.7	0.1
K	0.7	0.0	1.9	0.0
Ni	0.2	0.0	0.7	0.1
Cu	0.3	0.0	1.4	0.2

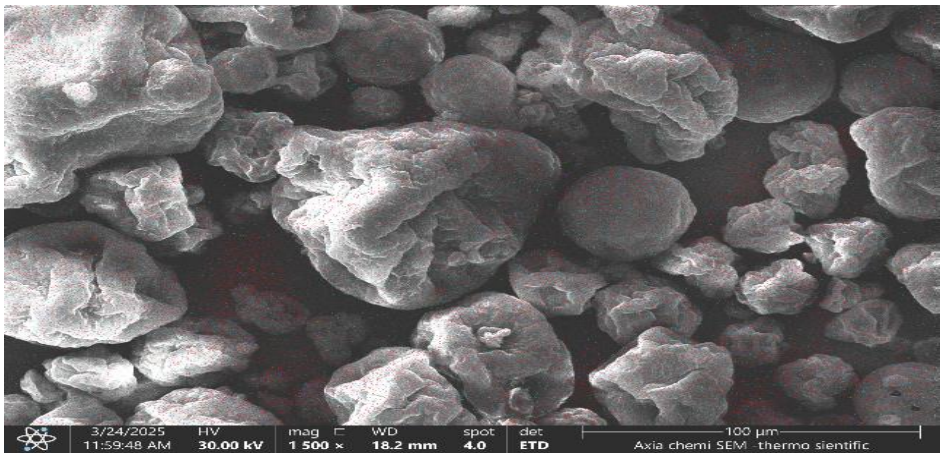


Fig. 12. SEM image of sorokiniana MH923013 after rifampin adsorption, showing surface changes indicating adsorption

After the Adsorption of Rifampin

Figs. 13 & 14, along with Table 5, show that the EDS analysis revealed the appearance of new peaks for elements such as iron, nitrogen, and silica, indicating the presence of rifampin on the surface.

The significant decrease in the proportions and intensity of elements in the EDS spectrum of Chlorella algae after treatment with rifampicin can be explained by two mechanisms. The first is attributed to the occurrence of surface masking, which results from the accumulation of adsorbed molecules on the surface, thereby hindering the detection process of the algae.

Second, the adsorption process may lead to "leaching or ion exchange" of weakly bound surface elements, resulting in their partial removal or replacement. Together, these two mechanisms contribute to the apparent decrease in elemental composition signals observed after adsorption [40].

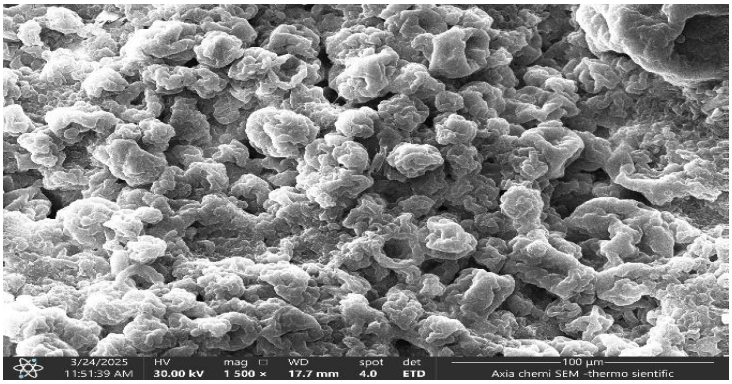


Fig. 13. SEM image of sorokiniana MH923013 after treatment, showing increased surface roughness and aggregation

Table 5. EDS data of sorokiniana after rifampin adsorption, showing the appearance of new elements indicating antibiotic binding

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	58.3	0.4	50.9	0.3
N	9.6	0.9	9.8	0.9
O	31.3	0.5	36.4	0.6
Si	0.4	0.0	0.8	0.0
Fe	0.2	0.0	0.6	0.0
Cu	0.3	0.0	1.5	0.1

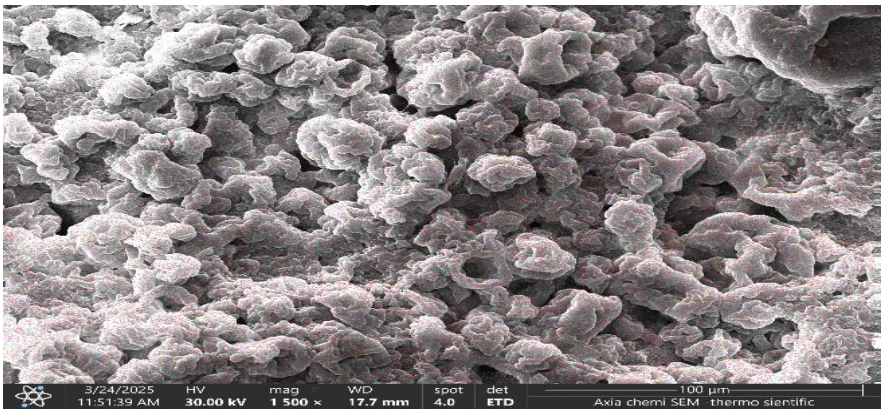


Fig. 14. SEM image of sorokiniana MH923013 after exposure to rifampin, showing aggregated and rougher surface morphology

Results and Discussion Live Algae

According to Figs. 15 & 16. The study results showed that the removal efficiency of rifampicin by green algae (Chlorella) was significantly affected by the initial concentration of the drug. At the lowest concentration (50 ppm), the highest removal efficiency (100%) was observed on the fifth day, while maintaining stable algal growth. The increase in optical density (OD) was associated with increased biomass as the algal concentration increased from 2 × 10^6 to 3.5 × 10^8. Conversely, higher concentrations (100 and 150 ppm) resulted in a gradual decrease in removal efficiency (85% and 78%, respectively), with signs of algal growth inhibition, indicating potential toxic effects of rifampicin at higher concentrations.

These results demonstrate that the interaction between rifampicin and the algae is not static, but rather depends on a delicate balance between the algae's adsorption/biodegradation capacity and its tolerance to toxicity. It also confirms that lower concentrations (≤50 ppm) may be more suitable for sustainable bioremediation applications, as they achieve optimal removal efficiency while conserving biomass. The enhanced rifampicin adsorption by live Chlorella can be attributed to three key mechanisms: Active uptake in live cells internalizes rifampicin via membrane transporters (e.g., ABC transporters) and passive diffusion, as demonstrated in *Chlorella* for other antibiotics [41]. While metabolic degradation in live algae degrades rifampicin using phase I (e.g., cytochrome P450) and phase II (e.g., glutathione S-transferases) enzymes [42]. Also, dynamic biosorption in live cells maintains functional groups (carboxyl/phosphate) on their cell walls and regenerates binding sites through growth [43]. It is worth noting that the main removal mechanisms may include surface adsorption, bioaccumulation, or enzyme catalysis, which require further molecular studies to clarify. These results provide valuable insights for designing innovative algal biomass-based water treatment systems, taking into account both environmental and economic factors. Fig. 15 illustrates the changes that occurred to the algae and the drug during the experiment. Fig. 16 represents the time dynamics of algal growth.

In the initial phase (days 1-3), there is a rapid interaction between the drug and the functional groups on the algal surface (physical or chemical adsorption). On days 5-7, the adsorption peaks indicate that the active sites are optimally utilized, resulting in peak algal growth. On days 8-12, surface saturation decreases, or the mechanism shifts to slower biodegradation. On days 14 and onward, the algae's adsorption capacity is exhausted.

After completing 14 days, an analysis of the protein, carbohydrate, and lipid content is carried out. Table 4 shows the percentages before and after adsorption.

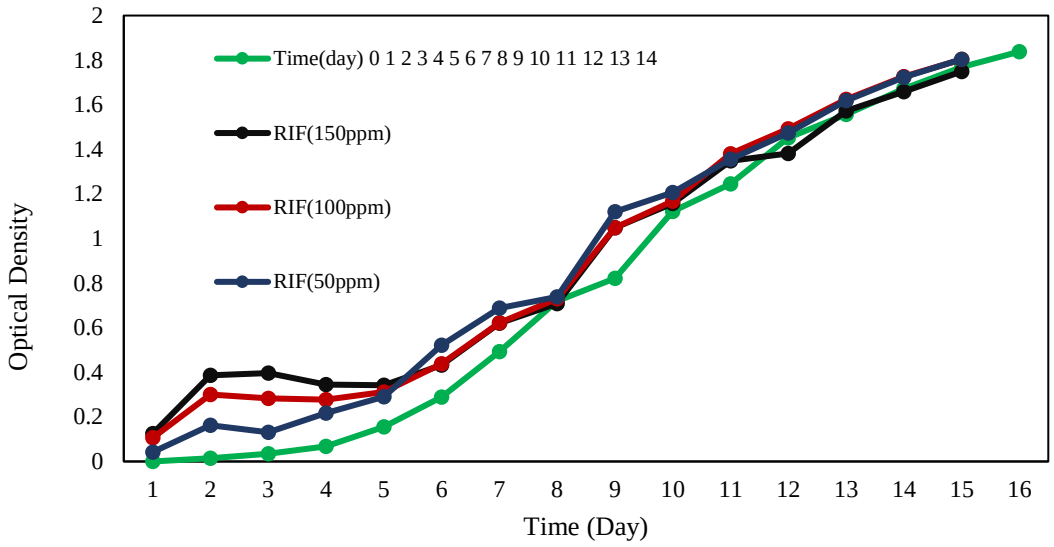


Fig. 15. Growth curve of Chlorella algae with rifampicin at concentrations of 50, 100, and 150

After completing 14 days, an analysis of the protein, carbohydrate, and lipid content is carried out. Table 6 shows the percentages before and after adsorption.

Table 6. Protein, lipid, and carbohydrate before and after adsorption

Name	Protein %	Lipid %	CHO %
Control	56.98	13.65	16.08
RIF	35.95	9.08	12.44

Rifampicin may reduce algal growth rates, leading to :Reduced biomass accumulation (and thus reduced protein and lipid content and CHO.Reduced synthesis of new cellular [44]. Table 7 shows a comparison of the removal percentage between live and dead algae.

Table 7. Comparison Between Dead and Live Algae after 24 hours

Biomass Type	Initial Conce. (ppm)	Final Concentration (mg/L)	Removal Efficiency %
Dead	100	28	72 %
Live	100	20	80 %

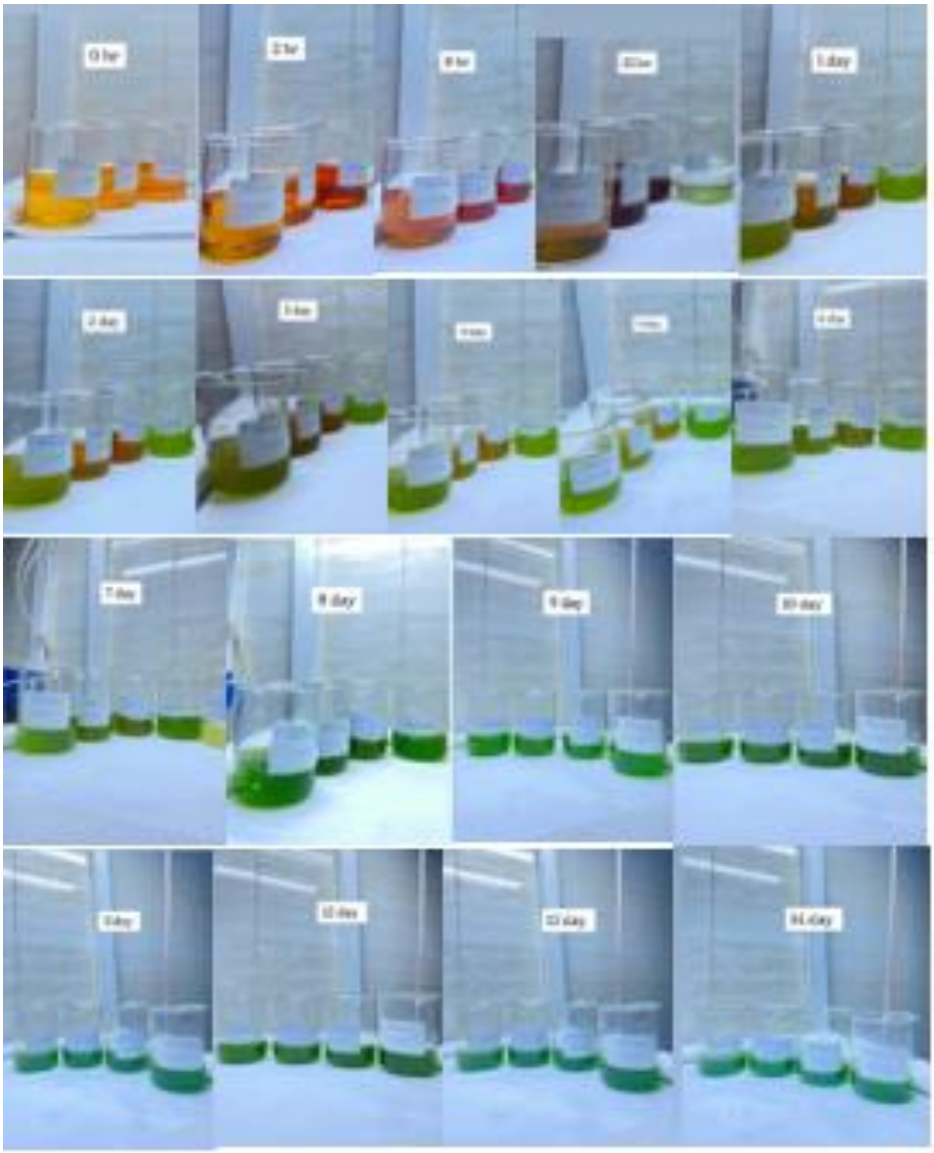


Fig. 16. The changes that occurred in Algae and RIF over 14 days

Conclusion

This study demonstrates the potential of Chlorella as a sustainable, dual-action solution (biosorption and biodegradation) for removing rifampicin, addressing a critical gap in pharmaceutical wastewater treatment. With high removal efficiency under optimal conditions (pH 5, 25 °C) and low energy consumption, our system offers a cost-effective alternative to conventional methods (such as activated carbon or ozone), which entail high operational costs. The removal of rifampicin from aqueous solutions was investigated using *Chlorella sorokiniana MH923013* as an adsorbent. Both dead and live *Chlorella sorokiniana MH923013* were used. The effects of several parameters on the removal of dead Chlorella sorokiniana MH923013, including pH, temperature, algae weight, contact time, and initial rifampicin concentration, were investigated. The kinetic data for rifampicin adsorption showed a good fit with second-order kinetic models, as indicated by R^2 values and a comparison of experimental and calculated qe values. In the live *Chlorella sorokiniana MH923013* growth and removal were tracked for 14 days.

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