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Original research article

Application of Scenedesmus Quadricauda Biosorbent for the Biosorption of Heavy Metals in Wastewater

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ABSTRACT

Heavy metal contamination in wastewater is a critical environmental concern, affecting ecosystems, water quality, and food safety. Conventional treatment methods, including precipitation and ion exchange, are often resource-intensive and may inadequately remove heavy metals, allowing them to persist in the environment. This study investigated biosorption as a sustainable alternative, specifically evaluating the green microalga *Scenedesmus quadricauda* for the removal of copper and zinc ions from synthetic wastewater. Batch biosorption experiments were conducted under optimized conditions of pH 6, a contact time of 60 minutes, and a temperature of 28°C. The results revealed high removal efficiencies, with *Scenedesmus quadricauda* achieving 81% removal of copper and 73% of zinc. Further optimization of pH and biosorbent dosage enhanced performance, with copper removal reaching 91% at pH 6 and zinc removal at 82.85% with a biosorbent dose of 0.5 g/L. These findings demonstrate the efficiency of *Scenedesmus quadricauda* as an efficient biosorbent for heavy metal remediation, supporting studies on biosorption as a viable and environmentally friendly approach to wastewater treatment. This study thus highlights biosorption's potential as a long-term solution for mitigating heavy metal pollution in wastewater.

Keywords: Contamination; Heavy metal; Remediation; Scenedesmus quadricauda; Waste water

1. Introduction

Pollution issues caused by wastewater are getting worse due to rising development of industry [1, 2]. Wastes are released into the aquatic environment in both soluble and insoluble forms, thereby rendering wastewater pollution one of the most critical issues faced in today's society. All kinds of life are fatally affected by heavy metal ions, and these ions infiltrate the food chain when wastewater is disposed. [3]. Industrialization and technological development have caused

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an increase in the burden on the environment by releasing large amounts of hazardous waste, heavy metals and organic contaminants that have caused significant harm to the environment [1, 4]. As these metals and metalloids cannot be broken down into non-toxic forms but instead persist in the ecosystem, the buildup of heavy metals and metalloid in waterways continues to pose major risks to global health. Wastewater containing heavy metals has contaminated the environment to an extent that is above the safe level and is harmful to all living things [5-7].

However, due to rising demand and contaminating usage of the remaining supplies, the globe will have a 40% deficit of water in 10 or 20 years [8]. In addition, this wastewater contains several harmful organisms, particularly viruses and protozoan bacteria, which can lead to a number of ailments [9]. It is necessary to find sustainable approaches to wastewater treatment.

With appropriate technology, wastewater may be cleaned to reduce heavy metal pollution and give the water a new use. The challenge is in selecting a method that will effectively solve this issue [10]. The current heavy metal removal techniques, such as chemical precipitation, ion exchange, and others, have a number of disadvantages, such as a large initial investment, continuous maintenance, and operation costs. They also produce a large amount of sludge, which raises the expense of management. Thus, the quest for effective and eco-friendly wastewater treatment technologies has started. In recent years, there has been a lot of interest in biological methods of wastewater treatment [11]. An emerging method known as "biosorption" utilizing microalgae will be evaluated, given its cost-effectiveness, broad accessibility, and high availability [12]. Due to their large surface area, quick development, and ability to bind metals, microalgae have demonstrated potential in the biosorption of heavy metals. Although microalgae have the ability to biosorb heavy metals, there are still a number of limitations [1]. Firstly, it's essential to have sufficient data to choose an appropriate species of algae that has a high affinity for heavy metals and can withstand the wastewater composition. The species-specific absorbing capacity, biomass production, and resistance to unfavorable wastewater conditions all have an impact on how well heavy metals are removed.

The foundation for the new biosorption technology for metal removal and recovery, which holds promise as a potential economically attractive treatment alternative, is the biosorption of dissolved metals based on the chemical activity of microbial biomass [13]. This technology is particularly suited for a wide variety of industrial metal-bearing point-source effluent discharges. For application as a biosorbent for heavy metals and other toxins, microalgae have undergone substantial research. In many different applications, including food, dietary supplements, medicine, fuel, and wastewater treatment, microalgae have been found to be a beneficial organism [14]. Microalgae are a flexible and affordable choice for treating effluent, and they also produce a useful byproduct. However, their appropriateness to serve these various functions must be evaluated in part according to how well they are able to eliminate heavy metals from effluent [15].

Due to its low nutrient requirements, high biomass production, and lack of harmful byproducts as compared to other biosorbents, this study will focus on using (*Scenedesmus quadricauda*) in the form of biomass as a biosorbent to carry out the biosorption of heavy metals from wastewater [16, 17]. Additionally, this study data will help in the design and improvement of microalgae-based systems for heavy metal remediation by advancing our knowledge of the biosorption mechanisms at play. By investigating the possibility of microalgae as a substitute biosorbent for heavy metal removal, this study seeks to expand the understanding of wastewater treatment. The results of this study can open the door to the creation of environmentally friendly technologies that can reduce the negative impacts of heavy metal contamination while also promoting the effective use of microalgae biomass for resource recovery and environmental protection.

2. Methodology

2.1 Chemicals and reagents

Analytical-grade chemicals were utilized to prepare the heavy metal solution. Sodium hydroxide, hydrochloric acid, and algal biomass are also employed in this investigation. As sources of heavy metal, zinc and copper were utilized. To wash and clean all the plastic and glass objects used in the studies, distilled water was used.

2.2 Preparation of synthetic wastewater containing Zinc and Copper

For this experiment, industrial wastewater was not collected; instead, synthetic wastewater was prepared and utilized in the laboratory. This approach was adopted for several reasons. Firstly, synthetic wastewater enables controlled composition of pollutants, allowing for precise manipulation of variables and facilitating more accurate and reproducible studies. Secondly, the use of synthetic wastewater permits the isolation of specific contaminants, thus simplifying the analytical process. Thirdly, synthetic wastewater facilitates comparison with prior research, as it is frequently standardized in studies to ensure consistency. Lastly, generating synthetic wastewater is more economical than the processes involved in obtaining, processing, and storing industrial effluents. To prepare the synthetic wastewater, stock solutions of Cu (II) and Zn (II) ions were created by dissolving 1 g/L of $CuSO_4$ and $ZnSO_4$ salts in distilled water, followed by stirring with a magnetic stirrer until complete dissolution was achieved. The pH of the solution was adjusted as necessary using 0.1 M hydrochloric acid (HCl) and 0.1 M sodium hydroxide (NaOH). The target concentrations, ranging between 50 and 200 mg/L, were obtained by diluting the stock solution to the desired values. Subsequently, the solution was filtered to remove any residual particulates and stored in a refrigerator until required for experimentation.

2.3 Nutrient medium

The experimental protocol required the use of Bold Basal Medium (BBM) due to its established efficacy and frequent use in algae cultivation. BBM was prepared using broth as a primary component, as it provides a balanced composition of essential minerals and trace elements necessary for optimal algal growth. To prepare the medium, 0.0950 g of broth was dissolved in 50 mL of distilled water. The solution was subsequently sterilized in an autoclave at 121 °C and 15 psi for 20 minutes to ensure aseptic conditions. The micro algal species *Scenedesmus spp.* was chosen for this study, given its rapid growth rate and adaptability to diverse environmental conditions, making it a suitable candidate for laboratory cultivation and experimental analysis.

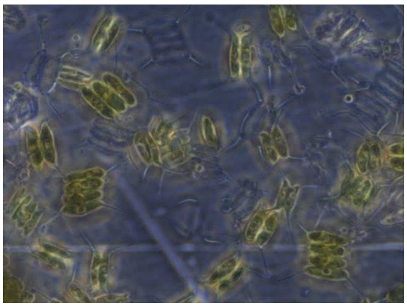

Fig.1. Microscope view of Scenedesmus Quadricauda.

2.4 Microalgae cultivation

Scenedesmus spp. biomass was obtained from local freshwater and was cultivated in Bold Basal Medium (BBM), selected for its proven suitability in growing various green algal cultures. The initial freshwater microalgae sample exhibited a salinity of 7 ppt, and the stock culture had a cell density of 50.6×10^6 cells per milliliter. Cultivation commenced by inoculating *Scenedesmus spp.* cells at a density of 50.6×10^6 cells into 250 mL Erlenmeyer flasks, achieving a 10% (v/v) inoculum density. Throughout the cultivation period, the pH was maintained at 7, and cultures were exposed to fluorescent white light (Philips) at an intensity of 90 µmol photons for 24-hour intervals. Flasks were agitated using an orbital shaker set at 70 rpm and maintained at 28 °C. Regular inspections of the cultures were performed, with adjustments to nutrient concentrations as needed to ensure optimal growth. Contaminants were systematically removed during cultivation to maintain purity. After 14 days, the cultures reached the desired biomass concentration for harvesting, which was carried out using centrifugation at 4000 rpm for five minutes. The harvested microalgae were then rinsed with distilled water to eliminate residual growth media. The biomass samples were further purified by thoroughly washing with distilled water before moist weight measurements were taken. The biomass was subsequently dried in an oven at 60 °C until complete desiccation was achieved. The dried biomass was then ground and passed through a 2 mm mesh sieve to achieve uniform particle size before being stored in sterile, clean bottles for concentration analysis. No additional modification or treatments were applied to the biomass to maintain its natural structure and functional group. This approach minimizes the use of hardardous chemicals and reduces the environmental impact of the preparation process.

2.5 Batch biosorption experiment

An experimental batch process was used to start the biosorption study. Biological material's capacity to remove pollutants from wastewater or solutions is evaluated using this method. Each conical flask containing a stock solution of 10 mg/L of Cu (II) or Zn (II) received 1.0g of *Scenedesmus spp.* microalgae biomass, respectively. By utilizing 0.1 N NaOH, the pH of the metal solutions was brought to the appropriate level (6). A rotary shaker was used to shake every flask for 60 minutes at 28°C and 120 rpm. Following the sorption process, the mixture was filtered through filter paper to separate the metal-adsorbed from the flocculated biomass, and the number of heavy metals that had been absorbed was measured and evaluated. The percentage removal efficiency (%R) of the heavy metal ions by the biosorbents was calculated using.

$$\%R = [(C_i - C_e) / C_i] \times 100, \qquad (2.1)$$

where C_e is the metal ion concentration in the aqueous phase after contact and C_i is the initial metal concentration before contact. The experimental procedure was optimized for biosorption conditions of pH and biosorbent dosage/concentration.

2.6 Determination of heavy metals concentration using Atomic Absorption Spectrometry (AAS)

After completing the biosorption process, the concentration of copper and zinc in the filtrates was determined using Atomic Absorption Spectrometry (AAS). Standard solutions of copper and zinc were prepared at known concentrations (in mg/L) and used to generate a calibration curve for each metal, with absorbance values measured at 324.8 nm for copper and 213.9 nm for zinc. Filtrate samples were filtered to remove suspended solids and diluted to ensure concentrations fell within the calibration range. Each sample was analyzed in the AAS, with absorbance readings taken at the respective wavelengths for copper and zinc. The calibration curve was then used to convert absorbance into concentration values (mg/L) for each metal.

2.7 Biomass regeneration and heavy metals recovery

After completing the initial biosorption cycle, the *Scenedesmus quadricauda* biomass was subjected to a regeneration process to desorb adsorbed zinc and copper ions. This process enabled the recovery of heavy metals without reusing the biomass in further biosorption cycles. Instead, the regenerated biomass was stored for potential future applications. The procedure was as follows:

2.7.1 Acid desorption process

The metal-laden *Scenedesmus quadricauda* biomass was immersed in a 0.1 M hydrochloric acid (HCl) solution, using a biomass-to-solution ratio of 1:20 (w/v) to ensure effective desorption of metal ions. The mixture was agitated on an orbital shaker at 100 rpm for 30 minutes to facilitate metal release.

2.7.2 Rinsing and neutralization

Following the desorption process, the biomass was filtered and rinsed with distilled water to neutralize any residual HCl. The rinsed biomass was then air-dried at 60°C for 12 hours before being stored in sterile containers.

2.7.3 Electrochemical recovery of metals

The acidic desorption solution containing released metal ions was processed through an electrochemical cell with inert electrodes to recover the metals. A controlled electric current (2 volts) was applied for 2 hours, enabling the selective deposition of metals onto the cathode. The recovered metals were then collected for potential reuse.

2.7.4 Biomass storage

The regenerated *Scenedesmus quadricauda* biomass, having undergone regeneration and metal recovery, was stored in sterile containers for potential future studies or applications. No disposal or incineration was necessary since the biomass was preserved after completing its designated experimental roles. This storage ensured it could be analyzed for future potential applications or alternative studies.

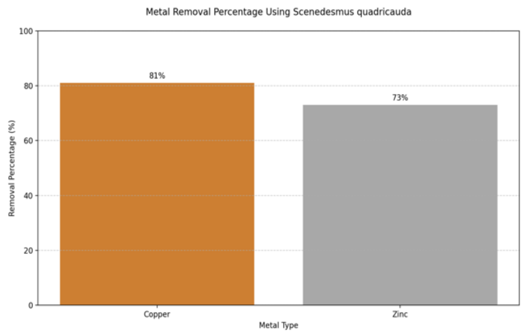

Fig. 2. Initial Batch biosorption experiment. The percentage of copper and zinc removal from wastewater using *Scenedesmus quadricauda* as biosorbents.

Table 1. Batch biosorption experiment result using *Scenedesmus quadricauda* spp *as biosorbent f*or Copper and Zinc removal.

HEAVY METAL	INITIAL CONCENTRATION	FINAL CONCENTRATION	% OF HEAVY METAL REMOVAL
COPPER	10mg/L	1.9mg/L	81%
ZINC	10mg/L	2.7mg/L	73%

3. Results and Discussion

3.1 Initial Batch Biosorption experiment: *Scenedesmus quadricauda* spp as biosorbent for Copper and Zinc removal

The batch biosorption experiment demonstrated the effectiveness of *Scenedesmus quadricauda* spp. in removing copper (Cu) and zinc (Zn) from synthetic wastewater under controlled conditions. Following biosorption, the concentration of Cu in solution decreased from an initial 10 mg/L to 1.9 mg/L, corresponding to a removal efficiency of 81% (Fig. 2 and Table 1). Similarly, Zn concentrations dropped from 10 mg/L to 2.7 mg/L, achieving a removal rate of 73%. These results show the significant capacity of *Scenedesmus quadricauda* as a biosorbent for Cu and Zn, with particularly high effectiveness for Cu ion removal. The enhanced biosorption of Cu relative to Zn is attributable to the specific functional groups on the algal cell surface, including carboxyl, hydroxyl, and amine groups [18 - 20], which exhibit strong affinity for Cu ions through chelation and electrostatic interactions. These groups are protonated at lower pH levels but become deprotonated at pH 6, increasing their negative charge and promoting interactions with positively charged Cu ions. This mechanism is supported by the observed optimal removal rates at pH 6, where the biosorbents surface charge favors Cu adsorption due to the larger ionic radius and lower hydration energy of Cu ions compared to Zn ions, which typically exhibit weaker interactions with the same functional groups. The comparatively lower adsorption affinity for Zn may also be attributed to competitive ion dynamics within the solution. In wastewater systems, Zn ions often compete with coexisting ions, such as chloride and other cations, for binding sites on the biosorbent surface. This competition can reduce Zn adsorption efficiency, as seen in previous studies, including those by Omar [17], which also noted a preferential uptake of Cu over Zn by *Scenedesmus quadricauda* spp. Additionally, Zn ions tend to interact less strongly with the algal cell surface functional groups due to their smaller ionic radius and different electronic configuration, which may limit their binding strength relative to Cu ions. The high affinity for Cu suggests that *Scenedesmus quadricauda* could be advantageous in industrial settings where copper contamination is prevalent.

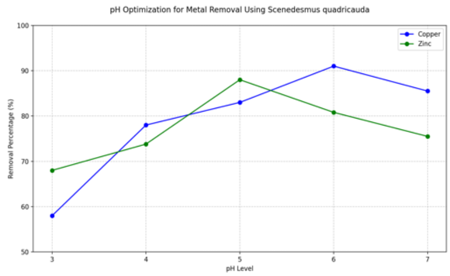

Fig. 3. A line plot showing the observed trends via removal efficiencies of zinc and copper across different pH for copper and zinc, identifying the optimal pH for maximum removal efficiency.

Table 2. Optimization of pH *via* Batch biosorption experiment for removal of copper.

pH	INITIAL CONCENTRATION	FINAL CONCENTRATION	% OF HEAVY METAL REMOVAL
3	10 mg/L	4.2 mg/L	58%
4	10 mg/L	2.2 mg/L	78%
5	10 mg/L	1.7 mg/L	83%
6	10 mg/L	0.9 mg/L	91%
7	10 mg/L	1.45 mg/L	85.5%

3.2 pH process optimization

The sorption of heavy metals can be strongly impacted by a number of important factors, one of which is the pH of a solution. The effect of pH on copper (Cu) biosorption by *Scenedesmus quadricauda* was evaluated under controlled conditions: 1 hour of contact time, a biosorbent dose of 0.5 g, and a constant temperature of 28 °C. pH values were adjusted from 3-7. Initially, Cu was present at a concentration of 10 mg/L, and after biosorption, the following concentrations were recorded at different pH levels: pH 3: 4.2 mg/L (58% removal), pH 4: 2.2 mg/L (78% removal), pH 5: 1.7 mg/L (83% removal), pH 6: 0.9 mg/L (91% removal), pH 7: 1.45 mg/L (85.5% removal). The findings demonstrate that as pH rose from 3 to 6, Cu biosorption increased also as seen in Fig. 3 and Table 2. The efficiency of biosorption by *Scenedesmus quadricauda* is influenced by the pH of the solution, primarily due to its effects on surface charge [19]. At lower pH levels, functional groups on the microalga's cell surface such as carboxyl, hydroxyl, and amine groups are protonated, reducing their availability for metal ion binding [20]. As the pH increases, these groups deprotonate, allowing for increased electrostatic attraction between the negatively charged biosorbent surface and the positively charged heavy metal ions [21]. This mechanism aligns with the observed increase in biosorption efficiency at pH 6, where copper removal peaked at 91%. In addition to surface charge effects, pH may also influence other biological processes or physical properties of a biosorbent that can affect biosorption efficiency [22]. Several studies indicate that pH changes can alter cellular structure and metabolic processes in microalgae, potentially impacting the biosorption mechanism [22-24]. For instance, optimal pH levels can maintain cell wall integrity and enhance the presence of active sites, while suboptimal pH levels may lead to cell damage, reducing the capacity for metal binding [25]. Additionally, pH fluctuations can influence the solubility of metal ions, as well as the formation of metal complexes, which can either enhance or inhibit the uptake process [26]. This impact of pH on both surface charge and cellular or structural stability as seen in previous studies suggests that pH control is critical for maximizing biosorption potential in *Scenedesmus quadricauda*. As a result, there is a general tendency for accumulation to increase with pH. At higher pH levels, the buildup typically starts to decline once more. Since the cell surface is more positively charged at lower pH levels, there is less interaction between metal ions and functional groups on the cell wall [17]. Therefore, 6 is found to be the ideal pH for the biosorption of Cu utilizing *Scenedesmus quadricauda* spp.

Similarly, the pH of 5 produced the highest percentage of zinc elimination, at a high percentage of 88%. This pH value is in the range of slightly acidic. Compared to zinc ions, this demonstrates that *Scenedesmus quadricauda* spp. removes a higher percentage of copper ions. Numerous factors, including *Scenedesmus quadricauda* spp.'s surface charge and the solution's ionic form of zinc ions being in a favorable alignment, can be ascribed to pH 5's maximum performance. This causes a stronger electrostatic attraction and more zinc ions to attach to the surface of the biosorbent. Additionally, there can be competing processes that diminish the biosorption capability at pH values noticeably lower or higher than 5. For instance, the biosorbent surface may be protonated at lower pH levels, which would result in fewer zinc ion binding sites. At higher pH levels, on the other hand, precipitation or the creation of insoluble metal hydroxides may take place, limiting the number of soluble zinc ions that are available for biosorption. The discovery that pH 5 is the ideal level for removing zinc has use for the treatment of wastewater. It gives users and researchers who use *Scenedesmus quadricauda* spp. as a biosorbent for zinc removal a clear set of instructions. The effectiveness of heavy metal removal can be maximized in wastewater treatment procedures by maintaining a pH between 5 and 6 (Fig. 3, Tables 2-3), potentially eliminating the need for additional chemical treatments.

Table 3. Optimization of pH *via* Batch biosorption experiment for removal of zinc.

pH	INITIAL CONCENTRATION	FINAL CONCENTRATION	% OF HEAVY METAL REMOVAL
3	10mg/L	3.2mg/L	68%
4	10mg/L	2.62mg/L	73.8%
5	10mg/L	1.2mg/L	88%
6	10mg/L	1.92mg/L	80.8%
7	10mg/L	2.45mg/L	75.5%

3.3 Optimization of biosorbent dosage using *Scenedesmus quadricauda* spp as biosorbent for the removal of Copper and Zinc from wastewater

For dosage optimization, 0.25g - 1g of biosorbent was used. Other conditions were maintained (using the optimized pH value from zinc and copper experiments, temperature of 28 °C and a contact time of 1hour. The maximum removal of zinc was achieved using a biosorbent dosage of 0.5 g/L, resulting in a removal efficiency of 82.85% (Tables 4-5 and Fig. 4). This high removal efficiency can be attributed to the large surface area and high density of functional groups on the surface of the *Scenedesmus quadricauda* cells. These functional groups, such as carboxyl, hydroxyl, and amino groups, can bind to zinc ions through a variety of mechanisms, including ion exchange, chelation, and electrostatic attraction. The removal efficiency of zinc remained relatively constant when the biosorbent dosage was increased to 0.75 g/L and 1.0 g/L (Table 4). This suggests that the biosorbent became saturated with zinc ions at a dosage of 0.5 g/L. Beyond this point, additional biosorbent did not provide any additional binding sites for zinc ions, and the removal efficiency remained constant. The optimal biosorbent dosage for zinc removal is dependent on a number of factors, including the initial zinc concentration, the pH of the wastewater, and the contact time between the biosorbent and the wastewater. However, our findings suggest that a biosorbent dosage of 0.5 g/L is sufficient for removing a significant amount of zinc from synthetic wastewater.

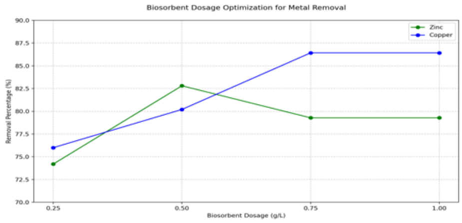

Fig. 4. A line plot showing the observed trends via removal efficiencies of zinc and copper across different biosorbent dosage.

Scenedesmus quadricauda spp. was also tested with varying dosage for copper removal from wastewater. The study examined how different biosorbent dosages influenced the removal of copper ions. It was observed that two specific dosages, 0.75g/L and 1.0g/L (Table 5), both yielded the maximum removal percentage of 86.42%. This indicates that the optimal dosage range for copper removal lies between 0.75g/L and 1.0g/L (Table 5). Additionally, a closer analysis of the dosage impact revealed patterns. As the biosorbent dosage increased to 0.75g/L (Fig. 4), there was a noticeable improvement in the removal percentage. However, this percentage remained constant at 86.42% when the dosage was further increased to 1.0g/L. These findings point to a key principle in biosorption: the removal of heavy metals can increase with higher biosorbent dosage up to a certain threshold, which signifies the biosorbent's capacity to adsorb heavy metals effectively. Beyond this point, the removal percentage reaches a plateau, signifying that the biosorbent is saturated with heavy metals. This saturation implies that there's a limit to the amount of heavy metals the biosorbent can efficiently adsorb. Hence, the ideal biosorbent dosage depends on the specific characteristics of the biosorbent and the nature of the heavy metal being targeted.

Table 4. Optimization of biosorbent dosage *via* Batch biosorption experiment for removal of zinc.

BIOSORBENT DOSAGE	INITIAL CONCENTRATION	FINAL CONCENTRATION	% OF HEAVY METAL REMOVAL
0.25 g/L	7mg/L	1.8mg/L	74.2%
0.5 g/L	7mg/L	1.2mg/L	82.85%
0.75 g/L	7mg/L	1.45mg/L	79.28%
1.0 g/L	7mg/L	1.45mg/L	79.28%

Table 5. Optimization of biosorbent dosage *via* Batch biosorption experiment for removal of copper.

BIOSORBENT DOSAGE	INITIAL CONCENTRATION	FINAL CONCENTRATION	% OF HEAVY METAL REMOVAL
0.25 g/L	7mg/L	1.68mg/L	76%
0.5 g/L	7mg/L	1.38mg/L	80.2%
0.75 g/L	7mg/L	0.95mg/L	86.42%
1.0 g/L	7mg/L	0.95mg/L	86.42%

4. Conclusion

This study demonstrates the efficiency of the microalgal species *Scenedesmus quadricauda* as a biosorbent for the removal of heavy metals, specifically copper (Cu) and zinc (Zn), from wastewater. Batch experiments confirmed that *Scenedesmus quadricauda* exhibits strong biosorption capabilities, achieving removal rates of 81% for Cu and 73% for Zn under initial experimental conditions. Through further optimization of pH and biosorbent dosage, the biosorption performance was enhanced, with Cu removal reaching 91% at pH 6 and Zn removal achieving 82.85% at a dosage of 0.5 g/L.

These findings highlight the potential of *Scenedesmus quadricauda* as an effective and sustainable biosorbent for heavy metal remediation in wastewater treatment. Our findings suggest the species is particularly well-suited for synthetic wastewater types with pH conditions close to neutral (around pH 5-6) and for moderate concentrations of Cu and Zn, where competition from other ions is minimal. This suggests that *Scenedesmus quadricauda* could be effectively applied in industrial effluents or other wastewater types that meet these specific conditions, thereby enhancing the selectivity and efficiency of the biosorption process.

The promising results presented here support the viability of microalgae-based biosorption as a long-term, eco-friendly approach to addressing heavy metal contamination in wastewater. However, to fully assess the applicability of *Scenedesmus quadricauda* for large-scale use, further research involving continuous flow systems and real wastewater samples is recommended. These additional investigations would provide critical understanding into the operational feasibility, resilience under variable wastewater compositions, and potential for large-scale deployment of this biosorbent technology.

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