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Original Article

Efficient removal of heavy metals and nutrients from wastewater using dried *Cladophora* biomass: Effect of environmental parameters

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Abstract: This study investigated the efficiency of dried algal biomass for removing selected pollutants from wastewater, including chromium (Cr), nickel (Ni), lead (Pb), nitrate (NO_3), and phosphate (PO_4). The experiments were conducted under different pH levels (5.5, 7, and 10), temperatures (15, 25, and 35°C), and contact times (2, 6, and 24 hours). The results showed that the removal efficiency of heavy metals increased significantly with increasing pH and contact time. The highest removal efficiencies were recorded at pH 10 after 24 hours, reaching 99.56% for Cr, 92.27% for Ni, and 95.72% for Pb. In contrast, NO_3 removal efficiency ranged from −40.21 to 86.83%, while PO_4 removal ranged from −11.57 to 78.50% under different experimental conditions, indicating variable behavior and possible release under certain conditions. The results revealed that dried algal biomass is an effective biosorbent for heavy metal removal, particularly under alkaline conditions, whereas its efficiency for nutrient removal is less stable and highly dependent on environmental factors. These findings highlight the potential of algal biomass for wastewater treatment and emphasize the importance of optimizing operational conditions to improve removal efficiency.

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Introduction

Water pollution by heavy metals is one of the most critical environmental challenges worldwide due to their persistence, toxicity, and potential for bioaccumulation in aquatic ecosystems and living organisms. Industrial activities such as mining, electroplating, tanning, petroleum refining, and chemical manufacturing release significant quantities of toxic metals, particularly chromium (Cr), nickel (Ni), and lead (Pb), into wastewater systems. These metals are non-biodegradable and can accumulate in biological tissues, leading to severe health risks including carcinogenicity, neurotoxicity, and organ damage (He and Chen, 2014; Das et al., 2022).

In addition, excessive nutrient discharge, such as nitrate (NO_3^-) and phosphate (PO_4^{3-}), into water bodies has intensified eutrophication, resulting in oxygen depletion, harmful algal blooms, and the subsequent collapse of aquatic ecosystems (Farahdiba et al., 2020; Asaad and Amer, 2024). Therefore, the simultaneous removal of both heavy metals and nutrients from wastewater has become a priority in modern environmental management strategies.

Conventional wastewater treatment methods, including chemical precipitation, ion exchange, membrane filtration, and electrochemical techniques, are often associated with high operational costs, sludge generation, and limited efficiency at low contaminant concentrations. These limitations have driven increasing interest toward sustainable and cost-effective alternatives, among which biosorption has emerged as a promising green technology (He and Chen, 2014; Gizaw et al., 2021).

Biosorption utilizes biological materials such as algae, bacteria, and fungi to remove contaminants through physicochemical interactions, including ion exchange, complexation, adsorption, and micro-precipitation. Among these biosorbents, macroalgae—particularly species of the genus *Cladophora*—have attracted considerable attention due to their abundance, low cost, and high adsorption capacity (Michalak et al., 2018; Ross et al., 2021). The

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effectiveness of *Cladophora* biomass is primarily attributed to functional groups on the cell wall, such as carboxyl, hydroxyl, and amino groups, which facilitate strong binding to metal ions and other pollutants (Amro and Abhary, 2019; Lee and Chang, 2011). Several studies have demonstrated the high efficiency of *Cladophora* spp. in removing heavy metals from aqueous solutions. For instance, removal efficiencies exceeding 90% for Pb and Cd have been reported under optimized conditions (Abioye et al., 2020), whereas the removal of Cr and other metals is strongly dependent on pH and contact time (Elmaci et al., 2007). Furthermore, modifications to cultivation conditions, particularly nutrient regimes, significantly influence the biochemical composition of the biomass and, consequently, its adsorption performance (Ross et al., 2018; Ross et al., 2021).

In addition to heavy metals, macroalgae have shown potential for removing nutrients such as nitrate and phosphate, although their performance is often more variable due to environmental factors and the anionic nature of these compounds (Farahdiba et al., 2020; Asaad and Amer, 2024). Recent studies have highlighted that parameters such as pH, temperature, and contact time play a crucial role in determining the efficiency and mechanism of biosorption processes (Freitas et al., 2009; Jaafari and Yaghmaeian, 2019).

In Iraq, increasing industrialization and urban expansion have intensified the discharge of untreated or partially treated wastewater into natural water bodies. Previous local studies have confirmed elevated heavy metal concentrations in wastewater systems, underscoring the urgent need for effective and economical treatment technologies (Sulaymon et al., 2013; Bahaa et al., 2019). Despite the availability of algal resources in Iraqi aquatic environments, their application in wastewater treatment remains insufficiently explored.

Accordingly, the present study aims to evaluate the efficiency of dried *Cladophora* biomass as a biosorbent for the removal of selected heavy metals (Cr, Ni, and Pb) and nutrients (NO_3^- and PO_4^{3-}) from wastewater under different environmental conditions. A factorial experimental design was employed to investigate the effects of pH, temperature, and contact time on removal efficiency, providing a comprehensive understanding of the interaction between operational variables and optimizing biosorption performance.

Materials and Methods

Study area and sample collection: Wastewater samples were collected from the final sedimentation basin of the wastewater treatment plant located in Al-Rumaitha city, Al-Muthanna province, Iraq. The collected samples represented the treated effluent discharged into the drainage channel and were transported to the laboratory for further analysis (Fig. 1).

Preparation of algal biomass: The green alga *Cladophora* was collected from local aquatic environments and thoroughly washed several times with distilled water to remove impurities and debris. The biomass was then dried in an oven at 60°C until a constant weight was achieved and subsequently ground into a fine powder for use in the experiments.

Preparation of heavy metal solutions: Stock solutions (1000 mg/L) of Cr, Ni, and Pb were prepared using their respective nitrate salts. Working solutions were prepared by dilution to obtain a final concentration of 5 ppm for each metal in the wastewater samples.

Experimental design: The experiment was conducted using a factorial design (3×3×3) to evaluate the effects of pH (5.5, 7, and 10), temperature (15, 25, and 35°C), and contact time (2, 6, and 24 hours) on the removal efficiency of the studied parameters. A total of 27 treatments were performed, each conducted in triplicate. For each experiment, 1 g of dried algal biomass was added to 250 mL of wastewater. The mixtures were incubated under controlled conditions, and samples were analyzed after the specified contact times.

Determination of heavy metals: The concentrations of Cr, Ni, and Pb were measured using an Atomic Absorption Spectrophotometer (AAS) (Model: AA-7000, Shimadzu, Japan) according to standard methods.

Determination of nitrate (NO_3): Nitrate concentration was determined using the UV spectrophotometric method, where the absorbance of the sample was measured at 220 nm and corrected at 275 nm to eliminate organic interference, following standard procedures described by APHA (2017).

Determination of phosphate (PO_4): Phosphate concentration was determined using the ascorbic acid method. In this method, phosphate reacts with ammonium molybdate and potassium antimonyl tartrate under acidic conditions to form a blue-colored complex, which is measured spectrophotometrically at 880 nm according to APHA (2017).

Calculation of removal efficiency: The removal efficiency (R.P) was calculated using the following equation: $R.P = (A - B) / A \times 100\%$, where A represents the initial concentration, and B represents the final concentration after treatment.

Statistical analysis: The data obtained were statistically analyzed using the Statistical Package for the Social Sciences (SPSS). Two-way analysis of variance (ANOVA) was applied to evaluate the effects of pH, temperature, and contact time, as well as their interactions, on the removal efficiency of the studied parameters. The Least Significant Difference (LSD) test was used to determine significant differences among treatments at a significance level of $P<0.05$. All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation.

Results

Chromium removal efficiency: The results showed that Cr removal efficiency increased with increasing pH and contact time. At pH 5.5, the lowest removal efficiency (73.27%) was recorded after 2 hours at 25°C, while the highest value (88.58%) was observed after 24 hours at 15°C (Table 1). At pH 7, the lowest removal efficiency (85.55%) was recorded after 2 hours at 25°C, whereas the highest value (98.98%) was observed after 24 hours at 35°C. At pH 10, the lowest removal efficiency (90.09%) was recorded after 2 hours at 25°C, while the highest value (99.56%) was observed after 24 hours at 35°C. Overall, the lowest removal efficiency (73.27%) was recorded at pH 5.5 after 2 hours, whereas the highest removal efficiency (99.56%) was observed at pH 10 after 24 hours. Removal efficiency increased progressively with increasing contact time and pH, while temperature showed slight variation across the tested conditions. Chromium removal efficiency exhibited a clear upward trend with increasing contact time across all pH levels (Fig. 2). The results showed significant differences among pH levels, temperatures, and contact times.

Nickel removal efficiency: The results revealed that Ni removal efficiency was affected by pH and contact time (Table 2). At pH 5.5, the lowest removal efficiency (40.41%) was recorded after 2 hours at 35°C, while the highest value (54.33%) was observed after 24 hours at 15°C. At pH 7, the lowest removal efficiency (47.03%) was recorded after 2 hours at 35°C, whereas the highest value (79.66%) was observed after 24 hours at 25°C. At pH 10, the lowest removal efficiency (66.55%) was recorded after 2 hours at 25°C, while the highest value (92.27%) was observed after 24 hours at 35°C. Overall, the lowest removal efficiency (40.41%) was recorded at pH 5.5 after 2 hours at 35°C, whereas the highest removal efficiency (92.27%) was observed at pH 10 after 24 hours at 35°C. Removal efficiency increased with increasing contact time and pH, while temperature showed moderate variation across the tested conditions. Nickel removal efficiency increased markedly at higher pH values, particularly at pH 10. The results showed significant differences across pH levels, temperatures, and contact times (Fig. 3).

Lead removal efficiency: The results showed that Pb removal efficiency was influenced by pH and contact time. At pH 5.5, the lowest removal efficiency (46.16%) was recorded after 2 hours at 15°C, while the highest value (89.58%) was observed after 24 hours at 35°C (Table 3). At pH 7, the lowest removal efficiency (61.98%) was recorded after 2 hours at 15°C, whereas the highest value (91.50%) was observed after 24 hours at 35°C. At pH 10, the lowest removal efficiency (85.46%) was recorded after 2 hours at 25°C, while the highest value (95.72%) was observed after 24 hours at 15°C. Overall, the lowest

Table 1. Effect of different pH values, contact times, and temperatures on chromium removal efficiency.

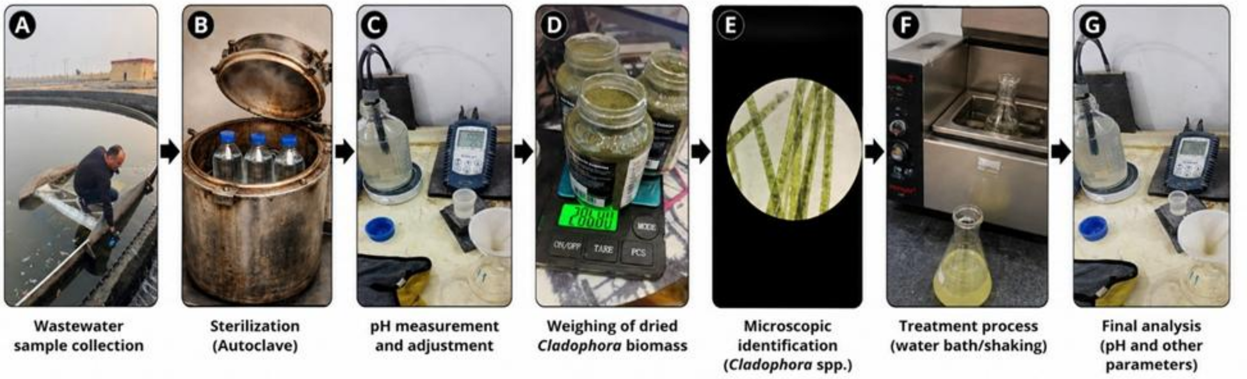
Time Period	pH	Temp			Temp Mean±SD	Time average Mean±SD
		15°C	25°C	35 °C		
2 hours	5.5	80.24	73.27	78.54	77.35±3.63a	86.85±8.44a
	7	87.56	85.55	92.86	88.66±3.77b	
	10	94.30	90.09	99.32	94.57±4.62c	
		87.37±6.14a	82.97±8.9b	90.24±9.21a	86.86±3.66	
	LSD (Temp) = 3.17| **LSD (pH)** = 3.17| **LSD (Interaction)** = 5.50					
6 hours	5.5	86.37	74.71	79.21	80.09±5.88	92.48±8.09b
	7	89.78	91.25	93.90	91.64±2.08	
	10	95.40	94.17	99.38	96.32±2.72	
		90.52±4.04a	86.71±10.1b	90.83±9.03a	89.35±2.29	
	LSD (Temp) = 3.04 | **LSD (pH)** = 3.04 | **LSD (Interaction)** = 5.27					
24 hours	5.5	88.58	81.13	81.92	83.88±4.09a	89.35±7.43ab
	7	92.67	97.64	98.98	96.43±3.32b	
	10	96.59	95.28	99.56	97.14±2.19b	
		92.62±3.59a	91.35±0.42a	93.49±8.68a	92.48±1.07	
	LSD (Temp) = 3.60 | **LSD (pH)** = 3.60 | **LSD (Interaction)** = 6.25					
LSD for time						4.33

Different letters within the same column indicate significant differences at ($P<0.05$).

Table 2. Effect of pH, contact time, and temperature on nickel removal efficiency.

Time Period	pH	Temp			Temp Mean±SD	Time average Mean±SD
		15°C	25°C	35 °C		
2 hours	5.5	45.27	42.84	40.41	42.84±2.43a	57.95±18.06a
	7	50.06	51.67	47.03	49.59±2.35b	
	10	87.85	66.55	89.89	81.43±12.9c	
		61.06±20.2a	53.69±12.3b	59.11±23.2a	57.95±3.82	
	LSD (Temp) = 3.17| **LSD (pH)** = 3.17| **LSD (Interaction)** = 5.50					
6 hours	5.5	48.38	46.25	42.05	45.56±3.22a	67.30±18.08a
	7	52.44	56.34	49.03	52.61±3.65b	
	10	88.56	81.21	91.57	87.11±5.33c	
		63.13±19.2a	61.26±15.6b	60.88±23.1b	61.76±1.19	
	LSD (Temp) = 3.04 | **LSD (pH)** = 3.04 | **LSD (Interaction)** = 5.27					
24 hours	5.5	54.33	48.17	48.23	50.25±3.54a	61.75±18.06a
	7	55.06	79.66	53.51	62.74±14.6b	
	10	89.52	84.98	92.27	88.92±3.68c	
		66.30±17.4a	70.94±17.3b	64.67±20.8c	67.30±3.25	
	LSD (Temp) = 1.11 | **LSD (pH)** = 1.11 | **LSD (Interaction)** = 1.93					
LSD for time						10.05

Different letters within the same column indicate significant differences at ($P<0.05$).



Wastewater sample collection
Sterilization (Autoclave)
pH measurement and adjustment
Weighing of dried *Cladophora* biomass
Microscopic identification (*Cladophora* spp.)
Treatment process (water bath/shaking)
Final analysis (pH and other parameters)

Figure 1. Experimental workflow of wastewater treatment using dried *Cladophora* spp. biomass from sample collection to final analysis.

Table 3. Effect of different pH values, contact time, and temperature on Pb removal efficiency.

Time Period	pH	Temp			Temp Mean±SD	Time average Mean±SD
		15°C	25°C	35 °C		
	5.5	46.16	55.03	83.78	61.65±19.6a	
	7	61.98	66.08	84.56	70.87±12.02b	
2 hours	10	93.37	85.46	91.13	89.98±4.07c	72.79±17.1a
		67.17±20.9a	68.86±13.4a	86.49±3.51b	74.17±10.7	
		LSD (Temp) = 7.39 | **LSD (pH)** = 7.39 | **LSD (Interaction)** = 12.81				
	5.5	49.44	58.31	86.87	64.87±19.5a	
	7	74.91	71.97	85.72	77.54±7.24b	
6 hours	10	93.80	92.81	92.91	93.17±0.54c	84.90±16.04b
		72.71±19.3a	74.37±16.8a	88.50±3.36b	78.53±8.67	
		LSD (Temp) = 5.18 | **LSD (pH)** = 5.18 | **LSD (Interaction)** = 8.97				
	5.5	75.68	61.09	89.58	75.45±14.2a	
	7	78.77	84.88	91.50	85.05±6.36b	
24 hours	10	95.72	92.80	94.11	94.21±1.46c	78.52±11.8ab
		83.39±9.5a	79.59±16.4a	91.73±2.05b	84.90±6.21	
		LSD (Temp) = 5.55 | **LSD (pH)** = 5.55 | **LSD (Interaction)** = 9.60				
LSD for time						8.22

Different letters within the same column indicate significant differences at (P<0.05).

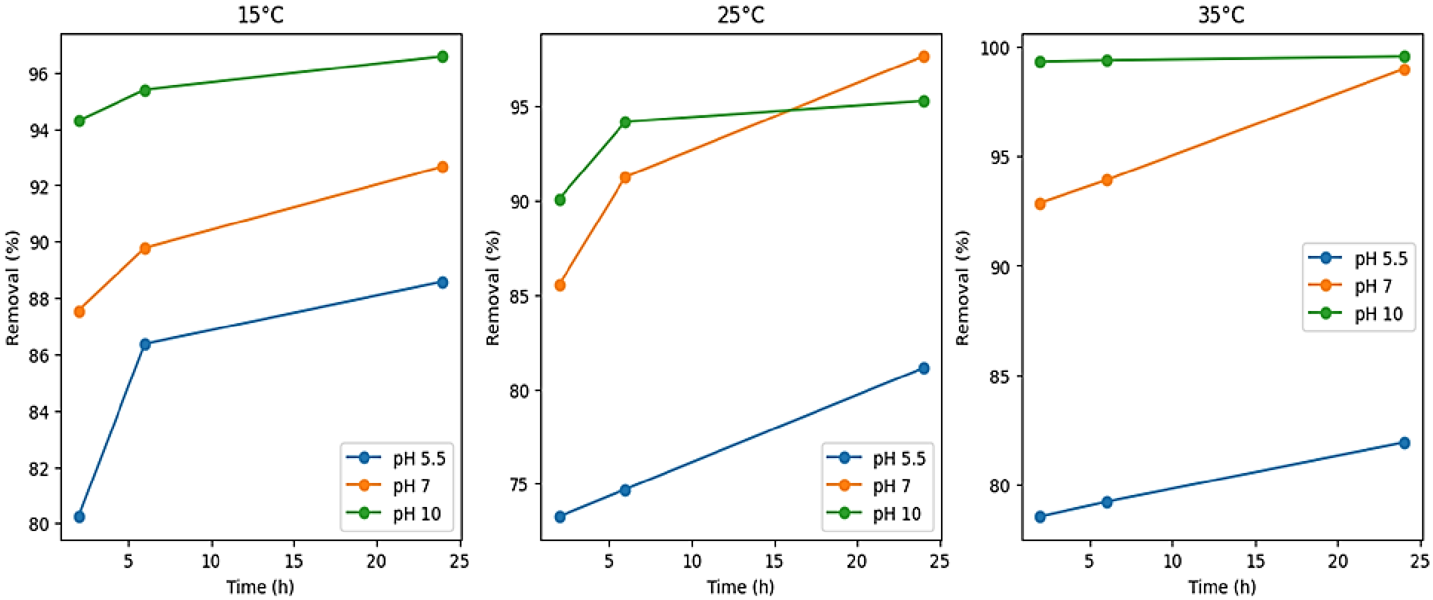


Figure 2. Influence of pH, temperature, and contact time on chromium removal efficiency using dried *Cladophora* spp. biomass.

removal efficiency (46.16%) was recorded at pH 5.5 after 2 hours, whereas the highest removal efficiency (95.72%) was observed at pH 10 after 24 hours. Removal efficiency increased with increasing contact time and pH, while temperature showed noticeable variation across the tested conditions. Lead removal efficiency increased consistently with contact time across all pH levels. The results showed significant differences across pH levels, temperatures, and contact times (Fig. 4).

Nitrate (NO_3) removal efficiency: The results showed that NO_3 removal efficiency varied considerably depending on pH and contact time. At pH 5.5, the lowest removal efficiency (–40.21%) was recorded after 2 hours at 25°C, while the highest value (80.88%) was observed after 24 hours at 15°C (Table 4). At pH 7, the lowest removal efficiency (14.49%) was recorded after 2 hours at 25°C, whereas the highest value (79.74%) was observed after 24 hours at 15°C. At pH 10, the lowest removal efficiency (28.53%) was recorded after 6 hours at 25°C, while the highest value (86.83%) was observed after 24 hours at 15°C. Overall, the lowest removal efficiency (–40.21%) was recorded at pH 5.5 after 2 hours,

Table 4. Effect of different pH values, contact time, and temperature on NO_3^- removal efficiency.

Time Period	pH	Temp			Temp Mean±SD	Time average Mean±SD
		15°C	25°C	35 °C		
	5.5	74.27	-40.21	17.98	17.35±57.2a	
	7	65.40	14.49	18.75	32.88±28.2b	
2 hours	10	73.67	51.59	28.76	51.34±22.4c	27.93±36.9a
		71.11±4.99a	8.62±26.5b	21.83±5.23c	33.85±32.9	
LSD (Temp) = 12.50| **LSD (pH)** = 7.21| **LSD (Interaction)** = 7.21						
	5.5	77.22	-31.88	20.32	21.88±54.5a	
	7	66.69	23.54	20.99	37.07±25.6b	
6 hours	10	81.65	28.53	31.58	47.25±29.8c	51.01±34.6b
		75.19±7.15a	6.73±32.1b	24.30±5.5c	35.40±35.5	
LSD (Temp) = 9.29 | **LSD (pH)** = 9.29 | **LSD (Interaction)** = 16.06						
	5.5	80.88	-7.53	28.07	33.81±44.4a	
	7	79.74	30.71	21.83	44.09±31.1a	
24 hours	10	86.83	63.92	74.67	75.14±11.4b	35.40±34.3ab
		82.48±3.55a	29.03±37.8b	41.52±25b	51.01±27.9	
LSD (Temp) = 14.41 | **LSD (pH)** = 14.41 | **LSD (Interaction)** = 24.97						
LSD for time						19.19

Different letters within the same column indicate significant differences at ($P<0.05$).

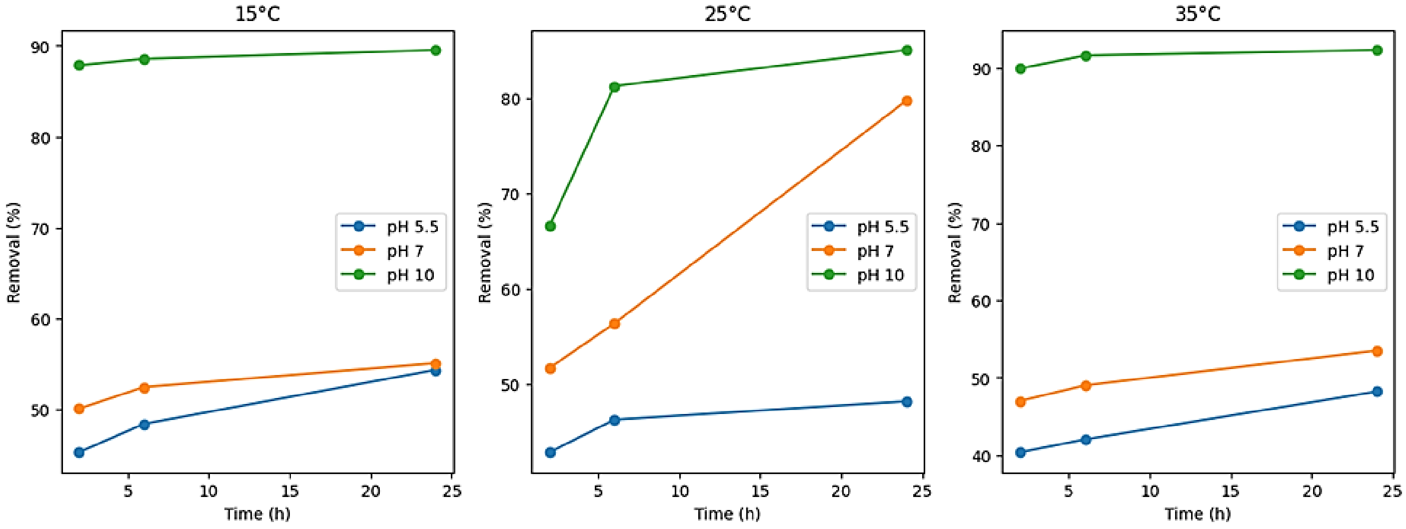


Figure 3. Influence of pH, temperature, and contact time on nickel removal efficiency using dried *Cladophora* spp. biomass.

whereas the highest removal efficiency (86.83%) was observed at pH 10 after 24 hours. Removal efficiency generally increased with increasing contact time, while pH showed variable effects across the tested conditions, and temperature exhibited noticeable variation. Nitrate removal efficiency displayed irregular behavior, with both positive and negative values observed under different conditions (Fig. 5). The results showed significant differences across pH levels, temperatures, and contact times.

Phosphate (PO_4) removal efficiency: The results showed that phosphate (PO_4) removal efficiency varied depending on pH and contact time. At pH 5.5, the lowest removal efficiency (–2.17%) was recorded after 2 hours at 35°C, while the highest value (55.78%) was observed after 24 hours at 15°C (Table 5). At pH 7, the lowest removal efficiency (–11.57%) was recorded after 2 hours at 15°C, whereas the highest value (57.53%) was observed after 24 hours at 35°C. At pH 10, the lowest removal efficiency (–3.77%) was recorded after 2 hours at 25°C, while the highest value (78.50%) was observed after 24 hours at 15°C. Overall, the lowest removal efficiency (–11.57%) was recorded at pH 7 after 2 hours, whereas the highest removal efficiency (78.50%) was observed at pH 10 after 24 hours. Removal efficiency

Table 5. Effect of different pH values, contact time, and temperature on PO_4^{3-} removal efficiency.

Time Period	pH	Temp			Temp Mean±SD	Time average Mean±SD
		15°C	25°C	35 °C		
2 hours	5.5	4.17	11.21	-2.17	4.40±6.69a	8.40±20.3a
	7	-11.57	10.05	9.41	2.63±12.3a	
	10	24.63	-3.77	33.64	18.17±19.5a	
		5.74±16.37a	5.83±27.9a	13.63±15.9a	8.40±4.52	
		LSD (Temp) = 31.44| **LSD (pH)** = 18.15| **LSD (Interaction)** = 18.15				
6 hours	5.5	11.33	14.87	13.34	13.18±1.77a	44.21±28.3b
	7	27.99	23.60	12.49	21.36±7.99ab	
	10	72.51	8.66	49.96	43.71±32.3b	
		37.28±27.7a	15.71±35.1a	25.26±18.5a	26.08±10.8	
		LSD (Temp) = 22.94 | **LSD (pH)** = 22.94 | **LSD (Interaction)** = 39.73				
24 hours	5.5	55.78	25.05	20.17	33.67±19.3a	26.08±26.1c
	7	35.65	26.53	57.53	39.90±15.9a	
	10	78.50	26.95	71.81	59.09±28.03b	
		56.64±18.6a	26.18±27.1b	49.84±23.1a	44.22±15.9	
		LSD (Temp) = 18.3 | **LSD (pH)** = 18.3 | **LSD (Interaction)** = 31.8				
		LSD for time				13.65

Different letters within the same column indicate significant differences at (*P*<0.05).

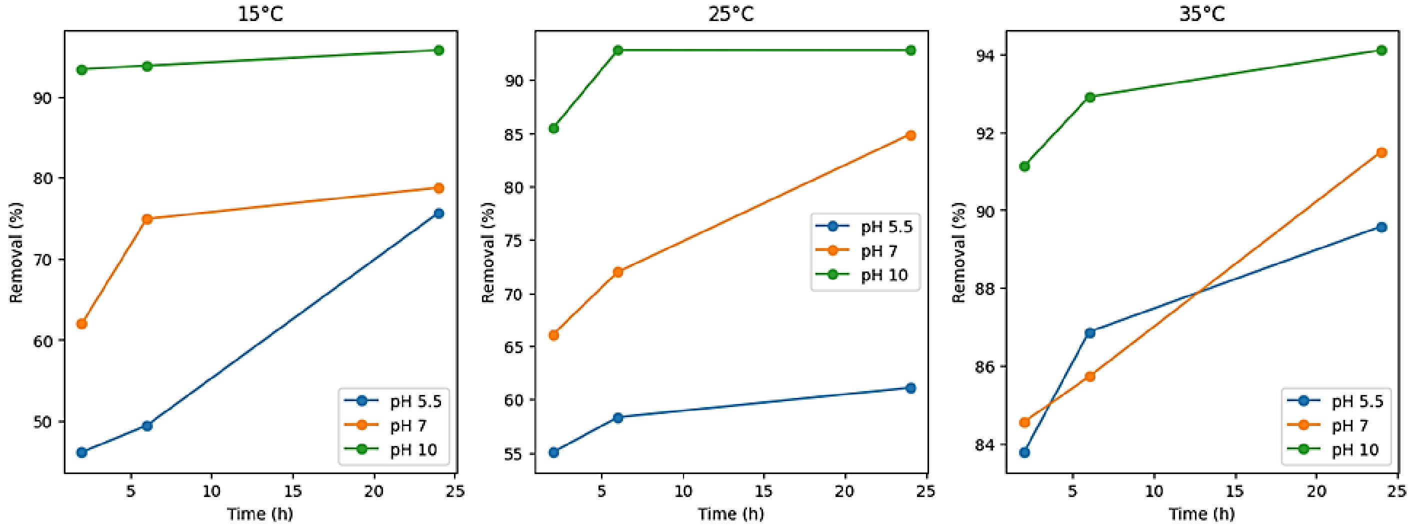


Figure 4. Influence of pH, temperature, and contact time on Pb removal efficiency using dried *Cladophora* spp. biomass.

generally increased with increasing contact time, while pH clearly influenced removal performance, and temperature exhibited variable effects across the tested conditions. Phosphate removal efficiency exhibited fluctuating behavior, including negative values under certain conditions (Fig. 6). The results revealed significant differences across pH levels, temperatures, and contact times.

Discussions

Chromium removal behavior: The removal behavior of Cr observed in this study shows a strong dependence on both pH and contact time, with removal efficiency increasing progressively under alkaline conditions and with extended exposure periods, ultimately achieving near-complete removal. This trend reflects the fundamental role of surface chemistry and metal speciation in biosorption systems. In aqueous environments, chromium exists primarily as Cr(VI) or Cr(III), and its removal mechanism is often a combination of adsorption and redox transformation. Previous studies have shown that Cr(VI) can be reduced to Cr(III) in the presence of algal biomass, particularly under acidic conditions, after which Cr(III) binds more strongly to functional groups on the cell surface (Konur, 2015). However, the superior performance at higher pH values in the present results suggests that Cr is predominantly

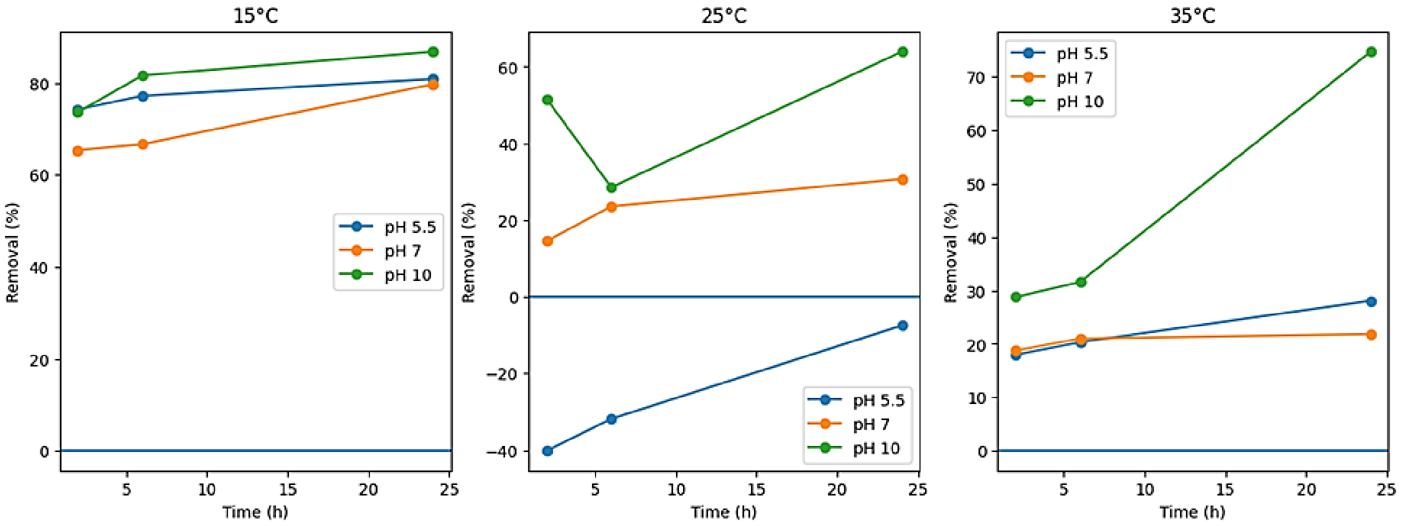


Figure 5. Influence of pH, temperature, and contact time on NO_3^- removal efficiency using dried *Cladophora* spp. biomass.

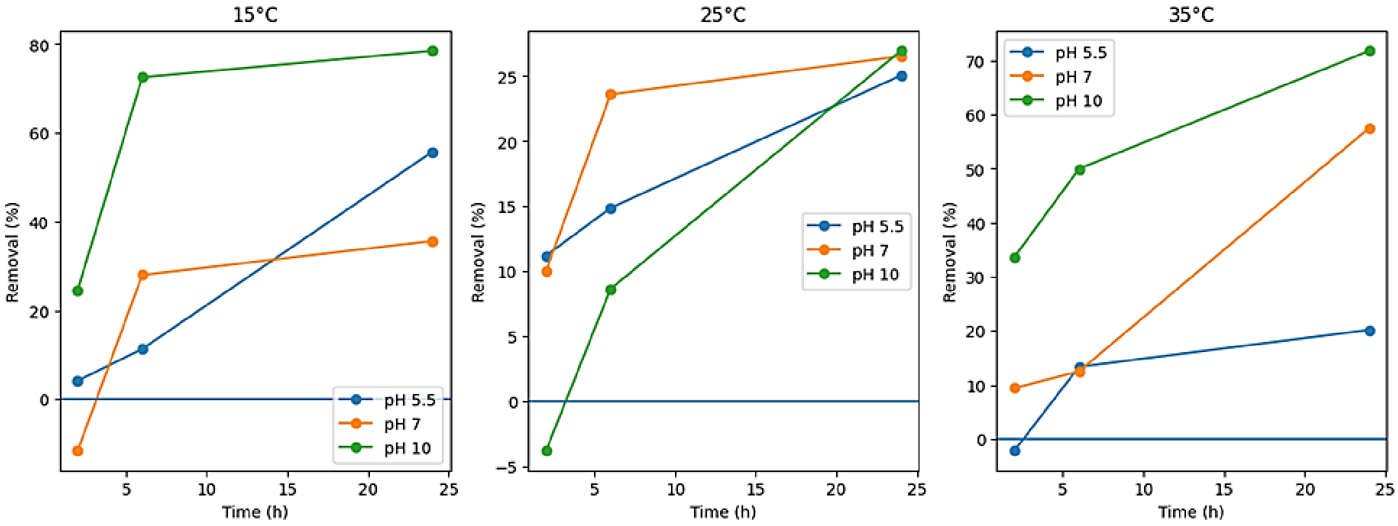


Figure 6. Influence of pH, temperature, and contact time on PO_4^{3-} removal efficiency using dried *Cladophora* spp. biomass.

present in the trivalent form, which exhibits stronger electrostatic attraction toward negatively charged sites formed by deprotonated carboxyl and hydroxyl groups on the *Cladophora* cell wall (Michalak et al., 2018).

The progressive increase in removal efficiency with time further indicates that Cr biosorption follows a multi-stage mechanism. Initially, rapid uptake occurs due to the availability of abundant active sites on the biomass surface, followed by a slower phase controlled by intraparticle diffusion and gradual occupation of internal binding sites. This interpretation is consistent with kinetic studies that report pseudo-second-order behavior for Cr adsorption onto algal biomass, suggesting that chemisorption involving electron sharing or exchange is the dominant mechanism (Lee and Chang, 2011; Abbas et al., 2014). Moreover, similar time-dependent trends have been observed in other algal systems, where equilibrium is not achieved immediately but requires sufficient contact time for diffusion into micro-porous structures (Guarín-Romero et al., 2019). Comparatively, the high removal efficiencies achieved in this study exceed those reported in several global investigations, which may be attributed to differences in biomass characteristics, surface functional group density, and experimental conditions such as pH and contact time (Freitas et al., 2009). These findings are further supported by regional studies demonstrating that macroalgae collected from Iraqi environments can achieve removal efficiencies ranging between 80–100% under optimized conditions (Bahaa et al., 2019). Overall, Cr removal in this system is governed by a combination of electrostatic attraction, chemical complexation, and diffusion processes.

Nickel removal behavior: The removal behavior of Ni in this study exhibited a distinct pattern compared to Cr, characterized by relatively low removal efficiency under acidic conditions and a gradual but significant improvement as pH and contact time increased. This trend can be attributed to the intrinsic physicochemical properties of Ni^{2+}, particularly its high hydration energy, which forms a stable hydration shell that limits its interaction with active binding sites at low pH. As the pH increases, deprotonation of functional groups enhances the negative surface charge of *Cladophora* biomass, thereby facilitating stronger electrostatic attraction and complexation with Ni^{2+} ions (Elmaci et al., 2007).

The time-dependent increase in removal efficiency suggests that nickel biosorption is governed not only by surface adsorption but also by slower diffusion processes. Unlike Cr, nickel requires longer contact time to penetrate internal binding sites within the biomass matrix, reflecting diffusion-controlled kinetics. This behavior has been consistently reported in biosorption studies, where nickel adsorption follows pseudo-second-order kinetics and is influenced by intraparticle diffusion mechanisms (Abbas et al., 2014; Guarín-Romero et al., 2019).

Comparative analyses indicate that Ni exhibits a lower affinity for algal biomass than other heavy metals, which explains its relatively lower removal efficiency under similar conditions. This behavior has been consistently reported in biosorption studies, where Ni^{2+} exhibited a weaker adsorption capacity than Pb^{2+} and Cr^{3+} due to differences in ionic radius, hydration energy, and binding affinity toward functional groups (Elmaci et al., 2007; Mohammed et al., 2019). Furthermore, experimental and environmental studies have demonstrated that nickel removal is more sensitive to variations in operational parameters such as pH and contact time, reflecting its dependence on surface charge conditions and diffusion-controlled mechanisms (Guarín-Romero et al., 2019). In addition, thermodynamic investigations have shown that nickel biosorption is generally a spontaneous process with a moderate temperature dependence, in which increased temperature may enhance ion mobility but does not significantly alter adsorption capacity, relative to the dominant influence of pH (Suganya et al., 2016). These findings collectively support the observed behavior in the present study and confirm that nickel removal is governed by weaker interactions and greater sensitivity to environmental conditions than those of other heavy metals.

Lead removal behavior: Pb exhibited consistently high removal efficiency across all experimental conditions, with a marked increase under alkaline pH and extended contact time. Notably, Pb removal was significantly higher than that of Ni under similar conditions, indicating a stronger affinity of Pb^{2+} ions toward the biomass surface. This behavior is primarily attributed to the larger ionic radius and higher polarizability of lead ions, which enhance their ability to form stable complexes with functional groups such as carboxyl, hydroxyl, and amine groups present on *Cladophora* biomass (Michalak et al., 2018; Amro, 2019). The rapid increase in removal efficiency at early contact times suggests that Pb adsorption is dominated by fast surface interactions, particularly ion exchange and complexation mechanisms. Lead ions can readily replace lighter cations such as Na^+, K^+, and Ca^{2+} within the biomass structure, resulting in rapid uptake and high removal efficiency even at relatively short contact times (Sulaymon et al., 2013).

Furthermore, the high efficiency observed in this study aligns with previous findings demonstrating near-complete removal of lead using algal biomass under optimized conditions, with removal efficiencies exceeding 95-100% reported for *Cladophora* and similar macroalgal systems (Amro, 2019; Bahaa et al., 2019). These findings confirm that lead exhibits the highest adsorption affinity among the metals studied, primarily due to its strong interactions with functional groups and its ability to form stable surface complexes. The removal mechanism is therefore dominated by electrostatic attraction, ion exchange, and chemical complexation, which have consistently been identified as the main pathways governing Pb^{2+} biosorption in algal systems (Sulaymon et al., 2013; Michalak et al., 2018).

Nitrate and phosphate removal behavior: In contrast to heavy metals, nitrate and phosphate exhibited highly variable behavior, including negative removal efficiencies, indicating that desorption and release processes initially dominated over adsorption. These negative values indicate the release of internally bound nitrogen and phosphorus compounds from *Cladophora* biomass into the surrounding solution, particularly under acidic conditions and during short contact times. Similar observations have been widely reported in biosorption studies, where untreated or dried biomass releases soluble intracellular components during the early stages of interaction (Volesky, 2001; Konur, 2015; Gizaw et al., 2021). From a mechanistic perspective, this behavior is strongly influenced by pH. Under acidic conditions, protonation of functional groups reduces the negative surface charge, weakening adsorption forces and promoting the release of previously bound anions. Additionally, partial degradation or destabilization of organic components within the biomass may contribute to nutrient release, further explaining the observed negative values (Davis et al., 2003).

Phosphate showed a relatively higher removal efficiency than nitrate, which can be attributed to its higher charge (−3) and stronger interaction potential with available binding sites. However, both nutrients exhibited lower removal efficiency than heavy metals due to electrostatic repulsion between negatively charged ions and the biomass surface, as well as the absence of metabolic uptake mechanisms in dried biomass (Foo and Hameed, 2010).

As contact time increased, removal efficiency improved, indicating a transition from initial desorption to gradual re-adsorption and equilibrium-controlled uptake. This behavior reflects a dynamic system governed by competing processes of release, adsorption, and redistribution, which are strongly influenced by environmental conditions such as pH and ion competition (Babel and Kurniawan, 2003).

Integrated comparative interpretation: The overall removal performance observed in this study clearly demonstrates a pronounced superiority of heavy metal removal over nutrient removal, with removal efficiencies consistently following the order Cr > Pb > Ni >>> PO_4^{3-} > NO_3^-. This hierarchy is not merely a reflection of experimental outcomes but rather represents a fundamental distinction in the physicochemical interactions that govern biosorption processes. The significantly higher removal efficiencies for chromium, lead, and nickel than for nitrate and phosphate highlight the inherent selectivity of *Cladophora* biomass toward cationic pollutants.

This preferential behavior can be primarily attributed to the surface characteristics of the algal biomass, which is rich in negatively charged functional groups such as carboxyl, hydroxyl, and amine groups. These functional groups play a critical role in facilitating strong electrostatic attraction and chemical complexation with positively charged metal ions. As a result, metals such as Pb^{2+} and Cr^{3+} exhibit high binding affinity and rapid adsorption kinetics, leading to efficient removal even under varying operational conditions. Similar observations have been widely reported in biosorption studies, in which macroalgal biomass consistently exhibits greater affinity for heavy metals than for other classes of contaminants (Michalak et al., 2018). In addition to electrostatic attraction, ion exchange mechanisms further enhance the removal efficiency of heavy metals. The presence of intrinsic cations such as Ca^{2+}, K^+, and Na^+ within the biomass matrix enables their replacement by metal ions in solution, thereby promoting rapid and effective adsorption. This multi-mechanistic process, which also includes surface complexation and intraparticle diffusion, provides a synergistic effect that significantly improves overall heavy metal removal performance. Furthermore, the gradual increase in removal efficiency with contact time observed in this study indicates that diffusion into internal binding sites plays an important role, particularly for metals such as nickel, which exhibit slower adsorption kinetics.

In contrast, the removal of anionic species such as nitrate and phosphate is governed by fundamentally different and less favorable mechanisms. Due to their negative charge, these ions experience electrostatic repulsion from the negatively charged biomass surface, particularly at neutral and alkaline pH levels. This repulsion significantly limits their adsorption capacity and results in lower, more variable removal efficiencies than those observed for heavy metals. Moreover, the absence of strong chemical interactions comparable to those observed for metal ions further reduces their binding potential. Previous studies have confirmed that anion adsorption onto biosorbents is generally weaker and more sensitive to environmental conditions, particularly pH and ionic strength (Foo and Hameed, 2010).

Another critical factor contributing to the disparity in removal performance is the absence of biological assimilation mechanisms in dried biomass systems. While living algal systems can actively uptake nutrients such as nitrate and phosphate through metabolic processes, dried biomass relies solely on physicochemical interactions. This limitation not only reduces nutrient removal efficiency but also increases the likelihood of desorption or release phenomena, as observed in the present study. Consequently, nutrient removal is governed by a dynamic equilibrium between release, adsorption, and redistribution processes, rather than a unidirectional uptake mechanism.

Furthermore, the variability observed in nutrient removal highlights the sensitivity of these processes to operational parameters such as pH, contact time, and the presence of competing ions. Small changes in these parameters can shift the balance between adsorption and desorption, leading to significant fluctuations in removal efficiency. This contrasts with heavy metal removal, which tends to be more stable and predictable due to stronger binding interactions and well-defined adsorption mechanisms.

Overall, the marked difference between heavy metal and nutrient removal in this study confirms that *Cladophora* biomass exhibits high selectivity for cationic contaminants, making it particularly suitable for heavy metal remediation. At the same time, the relatively low and variable nutrient removal underscores the need for optimized operational conditions or complementary treatment strategies to achieve efficient nutrient removal. These findings are consistent with previous research emphasizing the importance of tailoring biosorption systems based on the type of pollutant and the desired treatment outcome.

Limitations of the study: This study was limited to batch experimental conditions using dried algal biomass. The adsorption kinetics and isotherm models were not investigated, and the variability of real wastewater was not fully addressed. Further research is needed to evaluate large-scale applications and continuous treatment systems.

Conclusion

The present study demonstrated that dried algal biomass is an effective, low-cost biosorbent for removing heavy metals from wastewater. The removal efficiencies of Cr, Ni, and Pb increased significantly with increasing pH and contact time, reaching the highest values under alkaline conditions (pH 10) after 24 hours. In contrast, NO_3 and PO_4 exhibited variable removal behavior, including negative removal values under certain conditions, indicating possible release from the algal biomass and lower adsorption affinity for anionic species. The results also indicated that pH was the most influential factor affecting removal efficiency, followed by contact time, while temperature showed a comparatively smaller effect.

Overall, the findings confirm the potential of dried algal biomass for heavy metal removal in wastewater treatment. However, further optimization is required to improve the removal of nutrients such as nitrate and phosphate. Future studies should focus on improving nutrient removal efficiency through biomass modification and optimization of operational conditions.

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