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Research Article

Utilization of Microalgae for the Remediation of Nutrients and Heavy Metals from Industrial Wastewater

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ABSTRACT

Industrial wastewater poses a significant global environmental challenge due to the presence of various organic pollutants and heavy metals at elevated concentrations. This study investigated the potential of microalgal biomass for the treatment of industrial wastewater from the industrial estate in Hayatabad, Peshawar. A total of 25 different microalgal species were collected and morphologically characterized using light microscopy. Out of the 25 microalgal strains, *Chlorella ellipsoidea*, *Oscillatoria curviceps*, *O. rubescens*, *O. tenuis*, *Spirogyra collinsii*, *S. crassa*, *Closterium strigosum*, *C. pritchardianum*, and *C. lunula* successfully proliferated in BG-11 medium. These strains were subsequently transferred to agar media to establish pure cultures, which were then cultivated in both autoclaved and unautoclaved industrial wastewater. Biomass production was evaluated based on Total Volatile Suspended Solids (TVSS). In unautoclaved industrial wastewater, the highest growth rate (0.89 ± 0.03 day^{-1}) and biomass productivity (1.83 ± 0.05 g/L/day) were observed for *C. ellipsoidea*, *S. crassa*, and *O. curviceps*, while *C. strigosum* exhibited the lowest growth rate (0.79 ± 0.05 day^{-1}). *O. rubescens* and *C. strigosum* produced the lowest biomass (1.66 ± 0.10 g/L/day). In the control (autoclaved wastewater), *C. ellipsoidea* again recorded the highest growth rate (0.89 ± 0.05 day^{-1}), while *C. pritchardianum* showed the lowest (0.60 ± 0.07 day^{-1}). Nutrient removal efficiency by *C. ellipsoidea* was notably high, with reductions in COD (90.16%), ammonia (67.26%), and nitrate (94.93%). Moreover, *O. curviceps* was highly effective in removing sulfate and lead. Overall, the study demonstrates that *C. ellipsoidea* and *O. curviceps* are promising candidates for nutrient and pollutant removal from industrial wastewater due to their high biomass productivity and remediation efficiency.

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Introduction

Water is essential for the survival of all living organisms (Latif et al., 2022), and its quality is often more critical than its quantity, particularly for human use (Kabir et al., 2021). Rivers are vital natural resources that provide water for domestic, industrial, agricultural, and recreational purposes, while also supporting diverse freshwater ecosystems. However, increasing anthropogenic activities, such as the discharge of wastewater from industrial, municipal, domestic, and agricultural sources, have led to significant pollution of water bodies (Priya et al., 2022).

Industrial effluents originate from various sources, including marble factories, steel mills, pharmaceutical industries, food processing units, municipal sewage, vehicular emissions such as lead from fuel combustion, and improper disposal of industrial waste (Hasan et al., 2012). These effluents contain a wide range of pollutants, both organic and inorganic in nature (Saxena and Bharagava, 2017). Organic pollutants include biodegradable substances such as fats, proteins, and carbohydrates, while inorganic pollutants comprise nutrients like nitrates and phosphates, as well as toxic heavy metals including copper (Cu), zinc (Zn), lead (Pb), manganese (Mn), and chromium (Cr). These contaminants pose serious threats to aquatic ecosystems and human health.

Microalgae-based treatment systems offer a promising, eco-friendly solution for the removal of such pollutants (Sutherland et al., 2015). Microalgae can grow under extreme conditions, including saline and wastewater environments (Shahid et al., 2020), and are capable of reducing chemical oxygen demand (COD) while simultaneously producing valuable biomass (Whitton et al., 2016). Common microalgal genera employed in wastewater treatment include *Chlorella*, *Chlamydomonas*, *Spirulina*, *Scenedesmus*, *Nostoc*, and *Oscillatoria* (Sharma and Khan, 2013). Among these, *Nostoc* and *Oscillatoria* are particularly effective due to their rapid growth and high nutrient uptake efficiency.

Environmental factors such as light and temperature significantly influence algal growth by affecting photosynthetic activity and metabolic rates. Microalgae also contribute to climate change mitigation through carbon sequestration, as their photosynthetic efficiency is approximately 40-50% higher than that of terrestrial plants (Chen et al., 2015), enabling the fixation of up to 1.83 kg of CO_2 per kilogram of algal biomass produced (Chisti, 2007).

Different microalgal species exhibit varying tolerances to wastewater. *Spirulina* is less tolerant of high-strength wastewater compared to *Chlorella* (Wang et al., 2015). Although *Chlorella* thrives in nutrient-rich effluents, its growth is inhibited when ammonia concentrations exceed 260 mg/L. In contrast, *Spirulina* is better suited for treatment of moderately polluted wastewater, particularly when ammonia levels remain below 40 mg/L (Chang et al., 2013).

This study aims to evaluate the efficiency of selected microalgal species in the bioremediation of industrial wastewater by assessing their biomass productivity and nutrient removal capabilities. It also contributes to the field of phycoremediation by exploring the unique potential of microalgae and cyanobacteria for heavy metal removal, with an emphasis on their scalability and environmentally sustainable applications.

Materials and Methods

Collection of water samples and algae

Microalgal samples were collected from five sites: Hajizai River, Kangra Ditch, Khayali River, Mamu Khatki, and Shah Alam River. Samples were placed in water-filled bottles and transported to the laboratory at Qurtuba University of Science and Information Technology, Hayatabad, Peshawar. Approximately 25 algal species were examined under a light microscope (Olympus model: NOIF XSZ-107BN) and identified using W. Prescott's Algae of the Western Great Lakes Area (Presscot, 1962).

Moreover, water samples were collected from the industrial estates of Hayatabad, Peshawar, and submitted to the Pakistan Council of Research in Water Resources (PCRWR), Hayatabad, for physicochemical analysis.

Development of Uni-algal cultures

Algal biomass was harvested by centrifugation and diluted with sterile distilled water. Zooplankton were removed using a 60 µm mesh net, followed by filtration through a 0.44 µm glass membrane filter (CN Membrane filter) to reduce bacterial load. Two grams of the algal biomass were inoculated into sterilized 250 ml conical flasks containing 100 ml of BG-11 medium. Cultures were maintained under constant aeration, at 23 ± 1°C, and illuminated with fluorescent light at an intensity of 120 µmol photons/m^2/s. After 10 days, the cells were harvested by centrifugation at 10,000 rpm for 5 min. The biomass was streaked onto 1.5% BG-11 agar plates under sterile conditions and incubated at 25°C for two weeks. Growth and purity were regularly monitored using a light microscope (Ali et al., 2011). A single isolated algal strain was subsequently transferred to liquid BG-11 medium in a sterile flask and incubated on an orbital shaker at 150 rpm for further cultivation.

Characterization of industrial wastewater

Industrial wastewater generally exhibits high turbidity, which reduces light penetration and affects algal growth. To assess the influence of microbial communities, wastewater was divided into two treatments: unautoclaved (natural microbial presence) and autoclaved (sterilized at 121°C for 15 min to eliminate microbial contaminants).

This comparison allowed assessment of algal pollutant removal independent of microbial interference. In both cases, wastewater was pre-filtered using a zooplankton net and centrifuged. The supernatant was vigorously stirred to aid in the coagulation and flocculation of suspended solids and to release trapped air. After 5-10 min of agitation, the samples were allowed to settle for 30 min. The clarified supernatant was filtered through a glass microfiber filter (934-AH, Whatman, USA), autoclaved at 121°C for 15 min, and cooled to room temperature (Zhou et al., 2011).

BG-11 medium preparation

BG-11 medium is a widely used nutrient medium for algal cultivation. It was prepared in the laboratory by dissolving the following components in distilled water (dH_2O): $NaNO_3$ (30 g in 200 ml), K_2HPO_4 (0.8 g in 200 ml), $MgSO_4 \cdot 7H_2O$ (1.5 g in 200 ml), $CaCl_2 \cdot 7H_2O$ (0.72 g in 200 ml), citric acid (0.12 g in 200 ml), ferric ammonium citrate (0.12 g in 200 ml), $Na_2EDTA \cdot 2H_2O$ (0.02 g in 200 ml), Na_2CO_3 (0.4 g in 200 ml), and BG-11 trace metal solution (1 ml/L).

To prepare 1 L of liquid medium, approximately 900 ml of dH_2O was taken, and all nine components were added sequentially with continuous stirring until completely dissolved. The final volume was then adjusted to 1 L with dH_2O. The medium was autoclaved at 121 °C for 15 min. After sterilization, the pH was adjusted to 10.1, and the medium was allowed to cool to refrigeration temperature before use.

Multi-step screening strategy for facultative algal strains

A four-step approach was employed to screen algal strains with high growth potential in industrial wastewater:

Step 1

Algal cultures were initiated in BG-11 medium (Rippka et al., 1979). For green algal strains, BG-11 medium was supplemented with glucose to mimic the composition of industrial wastewater.

Step 2

Uni-algal strains were developed in BG-11 medium under two conditions: (i) autotrophic conditions with light intensity below 50 μmol m^{-2} s^{-1}, and (ii) heterotrophic conditions maintained either under light (50 μmol m^{-2} s^{-1}) or in complete darkness, with high glucose concentrations and without the addition of $NaHCO_3$.

Step 3

Surviving strains were subsequently tested in actual industrial wastewater under light conditions at 50 μmol m^{-2} s^{-1}, as this intensity was reported by Zhou et al. (2011) to be optimal for algal growth and nutrient removal.

Step 4

To assess growth characteristics, selected strains were cultured in industrial effluent for at least three successive generations. This multi-step screening strategy was conducted using both solid media (Petri dishes) and liquid media (batch cultures) incubated on an orbital shaker for 4-6 days under light intensity below 50 μmol m^{-2} s^{-1}.

Determination of algal growth

Algal growth was assessed using Total Volatile Suspended Solids (TVSS) following the method described by APHA (1995). TVSS represents the organic fraction of Total Suspended Solids (TSS) and primarily reflects algal or microbial biomass. For biomass determination, 5 ml of algal culture was filtered through a pre-weighed 0.22 μm membrane filter (47 mm diameter). The filter was then dried at 105°C for 10 h in a calibrated oven. After drying, the filter was ignited at 550°C for 20-30 min. The difference in mass between the dried filter (W_1) and the ignited filter (W_2) was used to calculate the cell biomass (g/L).

The growth rate based on TVSS was calculated using the following equation:

$$RTVSS = In\ (TVSSt) - In\ (TVSS0)\ /t$$

Where:

RTVSS is the specific growth rate based on TVSS (day^{-1}),

$TVSS_t$ is the TVSS at time t,

$TVSS_0$ is the TVSS at the initial time (day 0),

t is the time interval (in days) between the two measurements.

The algal biomass productivity was calculated using the following equation (Feng et al., 2011):

$$Pbiomass\ (gL^{-1}\ Day^{-1}) = (DW_X - DW_1).\ (Tx-T_1)\ -1$$

Where:

DW_1 and DW_x are the dry biomass concentrations (g/L) at time T_1 (start of cultivation) and T_x (end of cultivation), respectively.

Nutrient assessment

Liquid samples were collected and centrifuged at 5000 rpm for 15 min to analyze nutrient concentrations. The resulting supernatants were retained for further analysis. Parameters including pH, COD, ammonia (NH_3-N), sulfates, nitrates, and heavy metals such as Cd and Pb were measured using the Hach DR 5000 Spectrophotometer in accordance with the Hach manual (Hach, 2008).

Nutrient removal efficiency was calculated by comparing the initial and final concentrations using the formula from Ji et al. (2014):

$$Ri (\%) = (Si_0 - Sit) / Si_0 \times 100$$

Where Si_0 represents the initial concentration (mg/L) of a given parameter (e.g., COD, TP, NH_3-N, or TN), and Sit is its concentration at the end of the cultivation period. The pH was measured using a pH meter (Fisher Scientific, FE150, New Hampshire, USA). All nutrient removal experiments were performed in triplicate.

Statistical analysis

Mean values and standard deviations were calculated using Microsoft Excel. One-way analysis of variance (ANOVA) was performed to determine significant differences between treated and control groups. A p-value ≤ 0.05 was considered statistically significant, and Least Significant Difference (LSD) tests were used to interpret post-hoc differences.

Results

Collection and isolation of microalgal strains

Microalgal samples were collected from ditches, rivers, and wastewater sources, as listed in Table 1. A total of 25 species were initially identified under a light microscope; however, only 9 strains were able to survive and grow in BG-11 medium.

Table 1. Collection sites and seasons for the isolated microalgal strains.

Sr. No.	Name of species	Collection sites	Season
1	*Cylindrospermum licheniforme*	Shahalam River	Early Summer
2	*Mougeotiopsis calospora palla*	Shahalam River	Early Summer
3	*Chlorella ellipsoidea*	Kangra Ditch	Early Winter
4	*Closterium lunula*	Kangra Ditch	Early Winter
5	*C. moniliferum*	Kangra Ditch	Early Winter
6	*C. pritchardianum*	Kangra Ditch	Early Winter
7	*C. strigosum brebison*	Kangra Ditch	Early Winter
8	*Spirogyra collinsii*	Kangra Ditch	Early Winter
9	*S. crassa kuetzing*	Kangra Ditch	Early Winter
10	*Zygnema pectinatum*	Mamu Khatki	Early Winter
11	*Chroococcus turgidus*	Mamu Khatki	Early Winter
12	*C. limneticus*	Mamu Khatki	Early Winter
13	*Cladophora insignis*	Kangra Ditch	Later Winter
14	*Hydrodictiyon reticulatum*	Kangra Ditch	Later Winter
15	*Oscilitoria curviceps*	Naguman River	Later Winter
16	*O. rubescens*	Naguman River	Later Winter
17	*O. tenuis*	Naguman River	Later Winter
18	*Scenedesmus armatus*	Khayali River	Later Winter
19	*Phormidium ambiguum*	Naguman River	Early Autumn
20	*P.incrustatum*	Naguman River	Later Winter
21	*P. inundatum*	Naguman River	Later Winter
22	*P. minnesotense*	Naguman River	Later Winter
23	*P. retzii*	Naguman River	Later Winter
24	*Cosmarium pachydermum*	Naguman River	Early Autumn
25	*Tabellaria fenestrate*	Naguman River	Early Autumn

Determination of algal growth

Algal growth in unautoclaved industrial wastewater

All tested algal strains showed appreciable growth in unautoclaved industrial wastewater over a 6-day cultivation period (Table 2). Initial biomass was low across all strains (0.2 g L^{-1}), but a progressive increase in dry weight was observed, with final values ranging between 1.7 and 1.9 g L^{-1}. The highest biomass productivity (1.83 g L^{-1} day^{-1}) and growth rate (0.89 d^{-1}) were recorded for *C. ellipsoidea*, *S. crassa*, and *O. curviceps*, indicating their superior adaptability and nutrient utilization efficiency under wastewater conditions. These findings suggest that several species, particularly *C. ellipsoidea* and *S. crassa*, hold strong potential for use in wastewater-based algal biomass production.

Algal growth in autoclaved industrial wastewater

All tested algal strains exhibited substantial growth in autoclaved (control) industrial wastewater over the 6-day cultivation period. Initial dry weight was uniformly low (0.2 g L^{-1}), but steady biomass accumulation was observed. Among the strains, *C. ellipsoidea* demonstrated the highest biomass productivity (1.83 g L^{-1} day^{-1}) and growth rate (0.89 d^{-1}), followed closely by *S. crassa* and *O. curviceps* (both 1.76 g L^{-1} day^{-1}). In contrast, *C. pritchardianum* showed the lowest growth rate (0.60 d^{-1}) and productivity (1.36 g L^{-1} day^{-1}) (Table 3).

Table 2. Assessment of algal growth in unautoclaved industrial wastewater.

Species	Cell size (µm)	Dry weight (gL^{-1} day^{-1}) Number of Days				Growth rate (d^{-1})	Biomass productivity (gL^{-1} day^{-1})
		0	2	4	6	6 days	6 days
C. ellipsoidea	7-8	0.2±3.3	0.9±0.15	1.6±0.1	1.9±0.05	0.89±0.03	1.83±0.05
C. lunula	6-9	0.2±3.3	0.9±0.17	1.4±0.1	1.8±0.1	0.85±0.05	1.76±0.1
C. pritchardianum	7-8	0.2±3.3	0.9±0.15	1.6±0.1	1.9±0.1	0.85±0.005	1.76±0.1
C. strigosum	5-9	0.2±3.3	0.7±0.1	1.5±0.15	1.8±0.1	0.79±0.05	1.66±0.1
S. collinsii	15-24	0.2±3.3	0.9±0.05	1.6±0.20	1.9±0.1	0.85±0.05	1.76±0.1
S. crassa	140-160	0.2±3.3	0.9±0.1	1.6±0.05	1.9±0.05	0.89±0.03	1.83±0.05
O. curviceps	10-14	0.2±3.3	0.8±0.05	1.5±0.1	1.9±0.05	0.89±0.03	1.83±0.05
O. rubescens	2-8	0.2±3.3	0.9±0.05	1.4±0.1	1.8±0.1	0.85±0.05	1.66±0.1
O. tenuis	2-8	0.2±3.3	0.9±0.1	1.2±0.1	1.7±0.1	0.85±0.05	1.76±0.1

Data are presented as mean ± standard deviation (SD; n = 3).

Table 3. Assessment of algal growth in autoclaved (control) industrial wastewater.

Species	Cell size (µm)	Dry weight (gL^{-1} day^{-1}) Number of Days				Growth rate (d^{-1})	Biomass productivity (gL^{-1} day^{-1})
		0	2	4	6	6 days	6 days
C. ellipsoidea	7-8	0.2±0.00	0.8±0.0	1.4±0.07	1.8±0.07	0.89±0.005	1.83±0.05
C. lunula	6-9	0.2±3.3	0.6±0.1	1.1±0.05	1.5±0.05	0.69±0.03	1.5±0.05
C. pritchardianum	7-8	0.2±3.3	0.8±0.1	1.3±0.1	1.5±0.1	0.60±0.07	1.36±0.08
C. strigosum	5-9	0.2±3.3	0.6±0.1	1.1±0.1	1.5±0.1	0.73±0.06	1.56±0.1
S. collinsii	15-24	0.2±3.3	0.9±0.15	1.3±0.1	1.8±0.1	0.79±0.005	1.66±0.1
S. crassa	140-160	0.2±3.3	0.8±0.1	1.3±0.1	1.8±0.1	0.85±0.05	1.76±0.1
O. curviceps	10-14	0.2±3.3	0.7±0.1	1.3±0.1	1.7±0.1	0.85±0.05	1.76±0.1
O. rubescens	2-8	0.2±3.4	0.8±0.1	1.1±0.1	1.5±0.1	0.73±0.06	1.66±0.1
O. tenuis	2-8	0.2±3.4	0.8±0.1	1.3±0.1	1.8±0.1	0.79±0.05	1.66±0.1

Data are presented as mean ± standard deviation (SD; n = 3).

Nutrient analysis of industrial wastewater

Effect of algal treatment on pH variation in industrial wastewater

The results presented in Table 4 illustrate the changes in pH levels of industrial wastewater during the phycoremediation process. Initially, both autoclaved and unautoclaved wastewater had a pH of 7.4. By the end of the treatment period, pH levels increased to 8.0, suggesting a reduction in acidifying compounds such as phosphates, nitrates, and nitrites.

Treatment with *C. ellipsoidea* led to a pH increase from 7.4 to 8.0 under both autoclaved and unautoclaved conditions. A similar trend was observed in *Closterium* species: *C. strigosum* increased the pH to 7.7 (unautoclaved) and 7.8 (autoclaved), whereas *C. pritchardianum* raised the pH to 8.3 and 8.1 under the respective conditions.

For *Oscillatoria* species, both *O. curviceps* and *O. rubescens* started at a pH of 7.4. *O. curviceps* increased the pH to 8.1 in unautoclaved and to 7.8 in autoclaved wastewater. In contrast, *O. rubescens* caused no pH change in unautoclaved wastewater (remaining at 7.4), but elevated the pH to 8.1 in the autoclaved treatment, as shown in Table 4.

These results indicate that *C. pritchardianum* and *O. curviceps* induced the most significant pH increases, particularly in unautoclaved wastewater. Their performance was statistically distinct from that of *O. rubescens* and *C. strigosum*, highlighting their potential effectiveness in altering wastewater pH during phycoremediation.

Table 4. Effect of algal treatment on pH variation in industrial wastewater.

Species	Concentration (ppm) of un-autoclaved industrial wastewater			Concentration (ppm) of autoclaved industrial wastewater		
	Initial Concentration	Final Concentration	Removal %age	Initial Concentration	Final Concentration	Removal %age
C. ellipsoidea	7.4	8.0	8.0	7.4	8.0	8.0
O. curviceps	7.4	8.1	8.1	7.4	7.8	7.8
O. rubescens	7.4	7.4	7.4	7.4	8.1	8.1
C. strigosum	7.4	7.7	7.7	7.4	7.8	7.8
C. pritchardianum	7.4	8.3	8.3	7.4	8.1	8.1

Reduction in COD by algal strains

Table 5 present the reduction in COD in industrial wastewater treated with various algal strains. The initial COD concentration of untreated wastewater was 610 mg L^{-1}, which was significantly reduced to 60 mg L^{-1} by *C. ellipsoidea*, corresponding to a removal efficiency of 90.16%. *O. curviceps* and *O. rubescens* reduced COD levels to 139 mg L^{-1} (77.21%) and 186 mg L^{-1} (69.50%), respectively. Moderate reductions were observed for *C. strigosum* (307 mg L^{-1}, 49.67%) and *C. pritchardianum* (319 mg L^{-1}, 47.70%).

In the control treatment (autoclaved wastewater), the initial COD concentration was 355 mg L^{-1}. *C. ellipsoidea* again showed the highest removal, lowering COD to 30 mg L^{-1} (91.54%). *O. curviceps* and *O. rubescens* reduced COD to 71.2 mg L^{-1} (79.94%) and 57 mg L^{-1} (83.94%), respectively. In contrast, *C. strigosum* and *C. pritchardianum* showed lower removal efficiencies, with final COD concentrations of 153 mg L^{-1} (56.90%) and 175 mg L^{-1} (50.70%), respectively.

Overall, *C. ellipsoidea*, *O. curviceps*, and *O. rubescens* consistently exhibited higher COD removal efficiencies compared to *C. strigosum* and *C. pritchardianum* under both treatment conditions. These differences were statistically significant, confirming the superior performance of the former strains in reducing organic pollutants from industrial wastewater.

Table 5. Effect of different algal strains on COD levels in industrial wastewater.

Species	Concentration of COD (mg/L) in un-autoclaved industrial wastewater			Concentration of COD (mg/L) in autoclaved industrial wastewater		
	Initial Concentration	Final Concentration	Removal %age	Initial Concentration	Final Concentration	Removal %age
C. ellipsoidea	610	60	90.16%	355	30	91.54 %
O. curviceps	610	139	77.21%	355	71.2	79.94%
O. rubescens	610	186	69.50%	355	57	83.94%
C. strigosum	610	307	49.67%	355	153	56.90%
C. pritchardianum	610	319	47.70%	355	175	50.70%

Ammonia removal efficiency of algal strains in industrial wastewater

Ammonia reduction in industrial wastewater is presented in Table 6. The initial ammonia concentration in untreated industrial wastewater was 23.52 mg/L. After treatment with *C. ellipsoidea*, the concentration decreased to 7.7 mg/L, corresponding to 67.26% removal efficiency. *O. curviceps* and *O. rubescens* reduced the concentration to 12.23 mg/L (48.00%) and 16.08 mg/L (31.63%), respectively. Similarly, *C. strigosum* and *C. pritchardianum* lowered ammonia levels to 13.9 mg/L (40.90%) and 15.1 mg/L (35.79%).

In the control (autoclaved industrial wastewater), the initial ammonia concentration was 8.51 mg/L. After algal treatment, *C. ellipsoidea* reduced it to 1.88 mg/L (77.90%), *O. curviceps* to 2.80 mg/L (67.09%), and *O. rubescens* to 0.85 mg/L (90.01%). *C. strigosum* and *C. pritchardianum* lowered the ammonia levels to 3.80 mg/L (55.34%) and 4.90 mg/L (42.42%), respectively.

Overall, *O. rubescens*, *C. ellipsoidea*, and *O. curviceps* demonstrated superior ammonia removal efficiency compared to the control. In contrast, *C. strigosum* and *C. pritchardianum* showed no statistically significant improvement over the control group.

Table 6. Effect of different algal strains on ammonia concentration in industrial wastewater.

Species	Concentration of ammonia (mg/L) in un-autoclaved industrial wastewater			Concentration of ammonia (mg/L) in autoclaved industrial wastewater		
	Initial Concentration	Final Concentration	Removal %age	Initial Concentration	Final Concentration	Removal %age
C. ellipsoidea	23.52	7.7	67.26%	8.51	1.88	77.90%
O. curviceps	23.52	12.23	48.00%	8.51	2.8	67.09%
O. rubescens	23.52	16.08	31.63%	8.51	0.85	90.01%
C. strigosum	23.52	13.9	40.90%	8.51	3.8	55.34%
C. pritchardianum	23.52	15.1	35.79 %	8.51	4.9	42.42%

Sulfate removal efficiency of algal strains in industrial wastewater

In industrial wastewater, the initial sulfate concentration was 52 mg/L. *C. ellipsoidea* reduced it to 30 mg/L (42.30%), while *O. curviceps* and *O. rubescens* decreased it to 17 mg/L (67%) and 25.01 mg/L (51.93%), respectively. *C. strigosum* and *C. pritchardianum* reduced the concentration to 30.1 mg/L (42.11%) and 34.1 mg/L (34.42%), respectively.

In the control treatments, sulfate levels decreased from 41.0 mg/L to 14.78 mg/L (63.95%) by *C. ellipsoidea*, and to 9.1 mg/L (77.80%) and 5.0 mg/L (87.80%) by *O. curviceps* and *O. rubescens*, respectively. Further reductions to 22.3 mg/L (45.60%) and 25.4 mg/L (38.04%) were observed for *C. strigosum* and *C. pritchardianum*, respectively (Table 7).

These results indicate that *O. curviceps* and *O. rubescens* exhibited significantly higher sulfate removal efficiencies compared to the other strains, demonstrating statistically significant differences.

Table 7. Effect of different algal strains on sulfate concentration.

Species	Concentration of sulfate (mg/L) in un-autoclaved industrial wastewater			Concentration of sulfate (mg/L) in autoclaved industrial wastewater		
	Initial Concentrati	Final Concentration	Initial Concentration	Final Concentration	Initial Concentration	Final Concentration
C. ellipsoidea	52	30	42.30%	41.0	14.78	63.95%
O. curviceps	52	17	67.30%	41.0	9.1	77.80%
O. rubescens	52	25.01	51.93%	41.0	5.0	87.80%
C. strigosum	52	30.1	42.11%	41.0	22.3	45.60%
C. pritchardianum	52	34.1	34.42%	41.0	25.4	38.04%

Nitrate removal efficiency of algal strains in industrial wastewater

In unautoclaved industrial wastewater, the initial nitrate concentration was 6.12 mg/L. *C. ellipsoidea* reduced this concentration to 0.31 mg/L, corresponding to a removal efficiency of 94.93% (Table 8). Similarly, *O curviceps* and *O. rubescens* decreased the nitrate concentration from 6.12 mg/L to 2.12 mg/L and 3.10 mg/L, achieving removal efficiencies of 65.35% and 49.34%, respectively. Similarly, *C. strigosum* and *C. pritchardianum* reduced nitrate levels to 3.2 mg/L and 3.8 mg/L, corresponding to removal rates of 47.71% and 37.90%, respectively. In a separate control condition (initial concentration: 5.11 mg/L), *C. ellipsoidea* achieved a 96.08% reduction, lowering nitrate to 0.2 mg/L. Under the same conditions, *O. curviceps* and *O. rubescens* further reduced nitrate to 1.1 mg/L and 0.6 mg/L, with removal efficiencies of 78.47% and 88.25%, respectively. *C. strigosum* and *C. pritchardianum* achieved lower reductions to 2.3 mg/L and 3.1 mg/L, with removal rates of 54.99% and 39.33% (Table 8).

Generally, the data indicate that *C. ellipsoidea*, *O. curviceps*, and *O. rubescens* consistently demonstrated superior nitrate removal efficiency compared to the other tested strains under both treatment conditions, with statistically significant differences in performance.

Table 8. Effect of different algal strains on nitrate levels.

Species	Concentration of nitrate (mg/L) in un-autoclaved industrial wastewater			Concentration of nitrate (mg/L) in autoclaved industrial wastewater		
	Initial Concentration	Final Concentration	Initial Concentration	Final Concentration	Initial Concentration	Final Concentration
C. ellipsoidea	6.12	0.31	94.93%	5.11	0.2	96.08%
O. curviceps	6.12	2.12	65.35%	5.11	1.1	78.47%
O. rubescens	6.12	3.1	49.34%	5.11	0.6	88.25%
C. strigosum	6.12	3.2	47.71%	5.11	2.3	54.99%
C. pritchardianum	6.12	3.8	37.90%	5.11	3.1	39.33%

Cd removal efficiency of algal strains in industrial wastewater

Table 9 illustrates the Cd removal efficiency of different algal strains in industrial wastewater. *C. ellipsoidea* significantly reduced the initial Cd concentration from 0.64 mg/L to 0.15 mg/L, achieving a removal rate of 76.56%. *O. curviceps* and *O. rubescens* reduced Cd levels to 0.55 mg/L and 0.57 mg/L, corresponding to removal rates of 14.06% and 10.93%, respectively.

Likewise, *C. strigosum* and *C. pritchardianum* decreased Cd concentrations to 0.43 mg/L and 0.44 mg/L, with removal rates of 32.81% and 31.25%, respectively.

In the control group, Cd concentrations decreased from 0.52 mg/L to 0.10 mg/L by *C. ellipsoidea*, indicating a removal rate of 80.76%. The control also showed reductions to 0.40 mg/L and 0.28 mg/L by *O. curviceps* and *O. rubescens*, with removal rates of 23.07% and 46.15%, respectively. Furthermore, *C. strigosum* and *C. pritchardianum* reduced Cd levels from 0.52 mg/L to 0.33 mg/L and 0.34 mg/L, achieving removal efficiencies of 36.53% and 34.61%. On the whole, *C. ellipsoidea* demonstrated the highest cadmium removal efficiency among all tested strains, with statistically significant differences compared to other treatments.

Table 9. Effect of different algal strains on Cd levels.

Species	Concentration of Cd (mg/L) in un-autoclaved industrial wastewater			Concentration of Cd (mg/L) in autoclaved industrial wastewater		
	Initial Concentration	Final Concentration	Initial Concentration	Final Concentration	Initial Concentration	Final Concentration
C. ellipsoidea	0.64	0.15	76.56%	0.52	0.1	80.76%
O. curviceps	0.64	0.55	14.06%	0.52	0.4	23.07%
O. rubescens	0.64	0.57	10.93%	0.52	0.28	46.15%
C. strigosum	0.64	0.43	32.81%	0.52	0.33	36.53%
C. pritchardianum	0.64	0.44	31.25 %	0.52	0.34	34.61%

Pb removal efficiency of algal strains in industrial wastewater

The initial concentration of Pb in unautoclaved industrial wastewater was 25.3 mg/L. After treatment with *C. ellipsoidea*, the concentration decreased to 15.4 mg/L, corresponding to a removal efficiency of 38.97%. *O. curviceps* and *O. rubescens* reduced the concentration to 15.01 mg/L and 17.8 mg/L, with removal rates of 40.67% and 32.49%, respectively. Similarly, *C. strigosum* and *C. pritchardianum* lowered the concentration to 17.1 mg/L and 17.8 mg/L, corresponding to removal efficiencies of 32.41% and 29.64% (Table 10). In the control treatment, the Pb concentration declined from 22.4 mg/L to 11.85 mg/L, showing a higher removal rate of 47.09%. *C. ellipsoidea* also exhibited a reduction, but *O. curviceps* and *O. rubescens* achieved further reductions to 13.2 mg/L and 9.8 mg/L, yielding removal rates of 41.07% and 56.25%, respectively. *C. strigosum* and *C. pritchardianum* reduced lead levels to 13.2 mg/L and 14.0 mg/L, with corresponding removal efficiencies of 41.07% and 37.5% (Table 10). Among all tested algal strains, *O. rubescens* was the most effective in lead removal under both treatment conditions, indicating a statistically significant difference compared to other strains.

Table 10. Effects of different algal strains on Pb levels.

Species	Concentration of Pb (mg/L) in un-autoclaved industrial wastewater			Concentration of Pb (mg/L) in autoclaved industrial wastewater		
	Initial Concentration	Final Concentration	Initial Concentration	Final Concentration	Initial Concentration	Final Concentration
C. ellipsoidea	25.3	15.4	38.97%	22.4	11.85	47.09%
O. curviceps	25.3	15.01	40.67%	22.4	13.2	41.07%
O. rubescens	25.3	17.08	32.49%	22.4	9.8	56.25%
C. strigosum	25.3	17.1	32.41%	22.4	13.2	41.07%
C. pritchardianum	25.3	17.8	29.64 %	22.4	14.0	37.5%

Discussion

The current study assessed the growth performance and biomass productivity of various microalgal strains cultivated in both autoclaved (control) and unautoclaved industrial wastewater. Out of 25 initially screened strains, only nine demonstrated robust adaptability and sustained growth in batch culture over a six-day period, indicating their potential suitability for nutrient removal in wastewater treatment applications.

Industrial wastewater provides a nutrient-rich medium conducive to microalgal cultivation, supplying both macro- and micronutrients essential for cellular growth, photosynthesis, and division. Among the strains evaluated, *Chlorella* sp. exhibited significant adaptability and vigorous growth in BG-11 medium, particularly under aerated conditions, optimal temperature (24-28°C), and pH 7.5. These findings align with those of Munir et al. (2015) and Mohammadi et al. (2019). Notably, the growth rate of *Chlorella* sp. was higher in autoclaved wastewater compared to raw centrate, corroborating the results of Li et al. (2011), who suggested that autoclaving reduces microbial competition and enhances nutrient bioavailability.

Spirogyra sp. also demonstrated marked biomass accumulation within seven days of cultivation in BG-11 medium, consistent with observations by Henderson et al. (2008), who reported that *Spirogyra* proliferated well under controlled light conditions with optimized scattering and fluorescence. Likewise, *Oscillatoria* sp. exhibited a rapid increase in population density, from 1208 to 1599 cells/L between days 4 and 7, confirming its ability to thrive under optimized aeration and light intensity (Angreni et al., 2017; Setiawan et al., 2020).

Closterium species developed well in BG-11 medium within a pH range of 7.7-8.8 and under favorable temperature conditions, surviving through the batch culture and reaching the stationary phase by day 10. Regisata et al. (2017) emphasized the critical role of light intensity in facilitating photosynthesis in microalgae. In line with Abioye et al. (2020), the observed discoloration from green to brown among some strains indicates sensitivity to prolonged exposure or elevated heavy metal concentrations, commonly associated with nutrient depletion, self-shading, and environmental stressors such as pH fluctuations and reduced CO_2 availability.

Biomass productivity varied across strains. *C. ellipsoidea*, *S. crassa*, and *O. curviceps* exhibited the highest productivity, followed by *C. strigosum* and *O. rubescens*.

In contrast, *C. pritchardianum* displayed comparatively lower productivity. These results support Huber and Dale (2009), who reported rapid biomass doubling in some microalgae, occurring within as little as 5 h.

pH was also a critical factor influencing growth. Most strains grew optimally at near-neutral pH (7.1-8.0), consistent with reports by Zhu and Zhang (2010) and Garcia et al. (2000), while values exceeding pH 8 negatively impacted growth. As microalgae photosynthetically assimilate CO_2, pH may increase, particularly with *C. vulgaris* cultures (Kshirsagar, 2013). According to Zhou et al. (2011), *C. vulgaris* demonstrated superior biomass productivity over nine days, outperforming *C. humicola* and *Selenastrum* sp., attributed to its higher acclimation capacity, making it a promising candidate for wastewater treatment and biomass recovery.

Regarding nutrient removal, *Chlorella* sp., *Scenedesmus* sp., and *Oscillatoria* sp. effectively assimilated nitrogen compounds. Talaiekhozani and Rezania (2020) reported complete ammonia removal by *Oscillatoria* sp., while Kshirsagar (2013) documented nitrate reductions of 78% and 70% by *Scenedesmus* and *Chlorella*, respectively. Sulfate removal was strain-dependent; Mohammadi et al. (2018) recorded removal rates of 36%, 35%, and 27% for *S. obliquus*, *C. vulgaris*, and *O. minuta*, respectively. Subramanian et al. (2017) also reported up to 89% sulfate removal by *Oscillatoria* from pharmaceutical effluents. However, high sulfate concentrations (>14.2 mg/L) can inhibit biomass accumulation due to ionic interference (Lv et al., 2017).

Heavy metal removal results were promising. *C. ellipsoidea* achieved the highest Cd removal under both autoclaved (76.56%) and unautoclaved (80.76%) conditions. These findings align with Duque et al. (2019), who reported 8.6% Cd removal by *Chlorella* sp. at 7 mg/L, while *Scenedesmus* sp., being more tolerant, removed up to 32.7%. Similarly, Setiawan et al. (2020) observed 24.05% Cd removal by *Oscillatoria* at 1 mg/L, with declining efficiency at higher concentrations. *C. calcitrans* removed up to 92% of lead from wastewater, as reported by Soeprobowati and Hariyati (2014), underscoring the phycoremediation potential of microalgae.

Despite the demonstrated efficacy of microalgae in wastewater treatment and biomass production, challenges persist in scaling up these systems. Laboratory successes do not guarantee commercial viability due to several limitations: (a) biomass harvesting is energy-intensive due to the dilute nature of algal cultures; (b) open pond systems are typically limited to low-value products; (c) high-yield monocultures require sterile, closed photobioreactors, which are costly and technically demanding; and (d) heterotrophic algal cultures generally grow more slowly than phototrophic cultures. Addressing these limitations requires optimized cultivation systems, low-cost harvesting strategies, and genetic or metabolic improvements in algal strains.

Conclusion

Although there is growing interest in the use of microalgae for wastewater treatment, limited data exist on the performance of indigenous strains in actual industrial effluents under both autoclaved and unautoclaved conditions. This study bridges that gap by assessing the growth and pollutant-removal capacities of locally sourced microalgae in real industrial wastewater.

Microalgae offer an eco-friendly, sustainable method for treating wastewater by effectively removing nutrients and heavy metals while simultaneously producing biomass. In this study, 25 algal species were collected, identified, and cultured in BG-11 medium. Following screening, nine species survived in the medium, with five (*Chlorella ellipsoidea*, *Oscillatoria curviceps*, *Oscillatoria rubescens*, *Closterium strigosum*, and *Closterium pritchardianum*) thriving in industrial wastewater and acting as effective phycoremediators.

Among these, *C. ellipsoidea* exhibited the highest removal efficiency for COD (90.16%), ammonia (67.26%), and nitrate (94.93%), while *O. curviceps* was most effective in sulfate and lead removal. Both autoclaved and unautoclaved centrate supported algal growth, though autoclaved wastewater yielded slightly higher removal efficiencies. Nevertheless, practical implementation remains constrained by strain selection, adaptability to wastewater variability, and cost-effective scalability. Overcoming these barriers will require innovations in reactor design, integration with existing treatment infrastructure, and thorough economic feasibility assessments.

Recommendations

Select microalgal species that demonstrate high efficiency in pollutant removal from wastewater. Conduct preliminary investigations to identify species capable of thriving in specific types of industrial wastewater. Enhancing microalgal growth and nutrient removal efficiency may require the supplementation of essential nutrients to the industrial effluent.

Maintaining optimal conditions is critical; therefore, parameters such as pH should be regularly monitored and adjusted. Evaluate a range of growth conditions, including pH, light intensity, temperature, nutrient concentration, and mixing techniques, to optimize contaminant removal and biomass productivity.

Ensure sustained performance by maintaining these parameters within optimal ranges. Prior to full-scale implementation, conduct pilot-scale studies to assess the feasibility and effectiveness of microalgal treatment under the specific industrial conditions.

Explore cost-effective algal biomass harvesting methods, such as flocculation and filtration, to enhance efficiency and minimize operational costs. Future research should emphasize the scale-up of treatment systems, process optimization, and comprehensive techno-economic analyses to establish microalgae-based wastewater treatment as a viable and sustainable alternative to conventional methods.

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Authors' Contributions

FM, FMS, FH, FS, and KUR designed the research work; FM and FS collected the data; FM, SI, and FS contributed to the data analysis; FM and KUR contributed to the write-up; All authors reviewed the final version of the manuscript and gave their approval.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 6: Clean Water and Sanitation

SDG 12: Responsible Consumption and Production

SDG 13: Climate Action

SDG 14: Life below Water

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