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Research Article

Microalgae as a Sustainable Approach for Municipal Wastewater Treatment: Insights into Nutrient Removal, Lipid Accumulation, and Biofuel Potential

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ABSTRACT

Microalgae have recently emerged as promising agents for municipal wastewater treatment due to their rapid growth, efficient nutrient uptake, and simultaneous production of valuable bio-based products. This study evaluated the potential of microalgal strains isolated from freshwater and wastewater habitats in Peshawar, Pakistan, for the removal of total organic carbon (TOC), nitrogen, phosphorus, chemical oxygen demand (COD), and ammonia from concentrated municipal wastewater (CMWW). A total of 21 microalgal strains were successfully cultured in Blue-Green 11 (BG-11) medium. Among these, twelve facultative species viz., *Chlorella vulgaris*, *C. ellipsoidea*, *Chlorococcum humicola*, *Scenedesmus bijuga*, *Botryococcus braunii*, *S. perforatus*, *Spirogyra aequinoctialis*, *S. armatus*, *S. setiformis*, *S. pratensis*, *Oscillatoria chalybea*, and *O. sancta* exhibited stable ecological performance in CMWW with high growth rates and biomass productivity. *C. vulgaris* and *C. ellipsoidea* recorded the highest specific growth rates (0.373 ± 0.001 d^{-1} and 0.362 ± 0.001 d^{-1}, respectively), whereas *O. chalybea* and *O. sancta* showed comparatively lower growth rates. During a six-day batch culture, *C. vulgaris* achieved removal efficiencies of 96% for nitrate, 77.7% for phosphate, 90% for ammonia, 95.5% for TOC, and 95.4% for COD. Gas chromatography-mass spectrometry analysis revealed that *C. vulgaris* had the highest lipid content (28.8%), while *O. chalybea* had the lowest (11.2%). The polyunsaturated fatty acid content in *Chlorella* species and *S. armatus* exceeded 30%, indicating their strong potential for biofuel production. Overall, the study demonstrates that wastewater treatment using microalgae represents an environmentally sustainable approach to waste management while simultaneously generating value-added bioproducts.

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Introduction

Water plays a crucial role in sustaining life within the biosphere. However, rapid industrialization and population growth have led to a decline in the quality of water resources. The discharge of untreated sewage into water reservoirs is a major environmental concern,

contributing to the eutrophication of aquatic ecosystems (Hussain et al., 2012). Variations in pH also exert adverse effects on aquatic organisms. Furthermore, water reservoirs are being depleted due to the continuous disposal of untreated wastewater.

Efficient remediation methods are essential for removing contaminants from wastewater (Lima et al., 2020). Traditional wastewater management techniques, although effective to some extent, are often limited by high operational costs, intensive energy requirements, and secondary waste generation (Miksch et al., 2015). Oswald and Golueke first proposed the use of microalgae for wastewater treatment in the 1950s, and since then, this approach has evolved into a reliable system for secondary and tertiary treatment (Selvaratnam et al., 2015). Through photosynthesis, microalgae generate oxygen, thereby lowering costs and reducing aeration requirements (Robert et al., 2013).

Algae-based treatment systems are increasingly adopted due to their economic feasibility and ecological sustainability. Microalgal species such as *Chlorella*, *Spirogyra*, *Oedogonium*, and *Cladophora* efficiently absorb nutrients from wastewater, removing nitrogen, phosphorus, and other organic compounds. In the process, they also assimilate carbon dioxide, improve water quality, and produce biomass with potential applications in energy and industrial sectors (Kumar et al., 2015). Advanced treatment systems such as Microalgal Activated Sludge (MAAS) integrate photosynthesis and anaerobic digestion to treat wastewater while simultaneously generating bioenergy (Mahdy et al., 2015).

The efficiency of microalgae in wastewater treatment largely depends on the chemical composition of the sewage. Water enriched with magnesium and calcium provides favorable conditions for the growth of green and blue-green microalgae (Pittman et al., 2011). Environmental factors such as light intensity and temperature strongly influence algal growth by regulating photosynthetic and metabolic processes. Moreover, nitrogen and phosphorus concentrations in the culture medium directly affect microalgal biomass accumulation and lipid productivity (Li et al., 2019).

Microalgae also play an important role in climate change mitigation through carbon sequestration, as their photosynthetic efficiency is 40-50% higher than that of terrestrial plants (Chen et al., 2015). Wastewater from domestic, agricultural, commercial, and municipal sources can be utilized for algal biofuel production in a cost-effective and sustainable manner (Bhatt et al., 2014). Co-cultivation of microalgae with wastewater streams provides an eco-friendly approach for bioenergy generation and byproduct recovery. Moreover, microalgae contribute significantly to phycoremediation, offering a natural means of pollutant removal (Rawat et al., 2011).

The advantages of microalgae include their rapid growth rate, adaptability to diverse ecological environments (Nigam and Singh, 2011), and ability to grow without competing for arable land (Alcántara et al., 2015). Microalgae cultivated in wastewater contain valuable compounds such as lipids, amino acids, sugars, and bioactive molecules, which can be utilized for bioenergy production, animal feed, cosmetics, and pharmaceuticals (Abinandan and Shanthakumar, 2015). Furthermore, microalgae serve as a promising alternative feedstock for biodiesel production due to their high content of polyunsaturated fatty acids (Kumar et al., 2019). Microalgal lipids are also valuable precursors for the chemical industry and can be used as edible oils in the food and health sectors (Wang et al., 2016).

In the study area, the sewerage system is largely non-functional, and untreated sewage is discharged directly into water bodies, posing severe risks to both the environment and public health (Bangash, 2001). Algal or other nature-based wastewater treatment systems can mitigate pollution effectively while simultaneously producing microalgal biomass for value-added products.

The objectives of this study are twofold: (i) to achieve effective wastewater treatment using microalgae, and (ii) to enhance biomass production for potential bioenergy and industrial applications. Specifically, it aims to evaluate microalgae as a sustainable solution for municipal wastewater treatment by assessing their nutrient uptake efficiency, lipid yield, and potential for bio-based product development.

Materials and Methods

Collection and identification of microalgae

Microalgae samples were collected from various aquatic sources in Peshawar, including tributaries of the River Kabul and River Shah Alam, as well as ponds and wetlands located along Warsak Road, Bodhni Pull, Gulbahar, and Akbar Poura. The samples were collected in clean plastic jars and transported to the laboratory of Qurtuba University of Science and Information Technology, Peshawar, within 24 h of collection.

The morphological characteristics of the microalgal species were examined under 10x and 40x magnifications using an Olympus microscope (Model No. IFXSZ-107BN, Japan). A total of thirty microalgal species were identified based on morphological features using W. Prescott's Algae of the Western Great Lakes Area (Prescott, 1962).

Isolation and cultivation of unialgal strains

Collected algal suspensions were centrifuged to concentrate the biomass, diluted with sterilized distilled water, and filtered through 60 µm plankton net. The filtrate was subsequently passed through a 0.45 µm glass membrane filter. The retained cells were rinsed with sterile distilled water to minimize bacterial contamination.

Cultivation in BG-11 medium

Approximately 2 g of concentrated algal material from each sample was inoculated into 250 ml Erlenmeyer flasks or sterilized 12-well tissue culture plates containing 100 ml of BG-11 medium, following the method described by Rippka et al. (1979).

The BG-11 medium was prepared 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), $CaCl_2 \cdot 2H_2O$ (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 metals solution (1 ml/L).

To prepare the liquid medium, 900 ml of dH_2O was placed in a beaker, and all nine components were added sequentially with continuous stirring until complete dissolution. The final volume was adjusted to 1 L with dH_2O. The medium was sterilized by autoclaving at 121°C for 15 min, cooled to room temperature, and adjusted to pH 9.1.

After 10 days of incubation, emerging algal colonies were aseptically transferred onto BG-11 agar plates and incubated for two weeks at 25°C on an orbital shaker set at 150 rpm to promote growth. Microscopic examination was performed to confirm the development of unialgal cultures.

Sub-culturing of unialgal strains

For subsequent sub-culturing, distinct green colonies from agar plates were aseptically inoculated into sterile conical flasks containing BG-11 medium. The cultures were maintained at 25 ± 2°C under continuous illumination of 50 µmol m^{-2} s^{-1} and agitated at 150 rpm on an orbital shaker for 10 days. The algal biomass was harvested through a two-step centrifugation process: first at 2000 rpm for 5 min followed by washing with deionized water, and then a second centrifugation to remove residual impurities.

Physical characteristics of municipal wastewater (MWW)

Municipal wastewater was collected from Gulbahar Naher, Peshawar Cantt, Hayatabad Phase 4 Nallah, and Board Bazar Nallah. The high turbidity and suspended solids in the wastewater reduced light penetration, thereby limiting the growth of phototrophic algal cells. The solid particles were agitated prior to sedimentation, followed by filtration and cold settling for 30 min. The final sterilization step involved autoclaving at 121°C for 15 min, as described by Zhou et al. (2011). The clarified supernatant was subsequently used to minimize bacterial contamination during algal growth experiments. Physical and chemical parameters of the wastewater were analyzed at the PCRWR Laboratory, Peshawar.

Isolation of wastewater-adapted strains

A sequential selection approach was employed to isolate facultative algal strains with enhanced tolerance and growth in municipal wastewater. Initial enrichment was carried out on solid BG-11 medium (Rippka et al., 1979) supplemented with glucose, followed by liquid-phase cultivation in orbital shakers. For growth assessment, acclimated unialgal strains were cultured either heterotrophically in BG-11 medium containing glucose (1500 mg L^{-1}, without $NaHCO_3$) under complete darkness or autotrophically under illumination of 50 µmol m^{-2} s^{-1}. Viable strains from both light and dark treatments were then inoculated into clarified municipal wastewater (CMWW) under the same light conditions. Individual cells were microscopically isolated using a micropipette, and growth stability was evaluated by culturing the acclimated strains in CMWW for at least three successive generations. Finally, growth performance was monitored in batch cultures for 4-6 days under continuous illumination of 50 µmol m^{-2} s^{-1}.

Microalgal growth analysis

The biomass concentration of microalgal species was monitored daily by measuring total volatile suspended solids (TVSS), which serve as an indicator of algal growth, following standard methods (APHA, 1995). Each selected microalgal species was cultured in a 250 ml Erlenmeyer flask containing 150 ml of municipal wastewater inoculated with 0.2 g/L of microalgal biomass (per species). The cultures were maintained on a rotary shaker under an illumination intensity of 50 µmol m^{-2} s^{-1}.

Microalgal growth was determined by measuring TVSS. A 5 ml aliquot of the culture suspension was filtered through a microfiber filter (0.45 µm pore size), dried in an oven at 105°C for 10 h, and subsequently ignited at 550°C for 20-30 min. The cell biomass concentration (g/L) was calculated from the difference between the dried filter weight (W_1) and the ignited filter weight (W_2).

Microalgal growth equations, expressed in terms of biomass concentration (g/L) derived from TVSS, were calculated as follows:

$$R = \frac{\ln(TVSSt) - \ln(TVSS0)}{t}$$

R' represents the growth rate of microalgae based on TVSS, where TVSSt is the TVSS on day t' and $TVSS_0$ is the TVSS on day 0'. The time interval (t) indicates the number of days between $TVSS_t$ and $TVSS_0$.

The biomass productivity (PBiomass) can be calculated using the following equation (Feng et al., 2011):

$$PBiomass(g\,L - 1\,Day^{-1}) = \frac{DWx - DW1}{TX - T1}$$

DW_x and DW_1 denote the dry biomass concentrations (g L^{-1} Day^{-1}) at T_1 (start of cultivation) and T_X (last day of cultivation) respectively. All the growth measurements were performed in triplicate.

Nutrient removal analysis

To evaluate nutrient reduction, liquid samples were collected and centrifuged at 5000 rpm for 15 min. The resulting supernatants were carefully diluted and analyzed for chemical oxygen demand (COD), total phosphorus (TP), ammonia (NH_3-N), total nitrogen (TN), and total organic carbon (TOC). All analyses were conducted according to the procedures outlined in the Hach DR 5000 Spectrophotometer manual. The removal efficiency (R) was calculated as:

$$R(\%) = \frac{Ci\text{-}Ct}{Ci} \times 100$$

Where R, represented the removal rate, Ci was the initial concentrations of COD, TN TP. NH_4^+- N, and TOC, Ct was the final concentration. All the nutrient reduction values were taken in triplicate.

Lipid extraction

To analyze the lipid content, algal cells were centrifuged at 5000 rpm for 15 min and subsequently freeze-dried (Savant Instruments Inc.). Lipids were extracted from 0.1 g of dried algal powder using a one-step chloroform-methanol (2:1, v/v) extraction method modified from Folch et al. (1957). The extraction was performed in a water bath shaker at 100 rpm for 30 min. The solid residue was separated using a Whatman glass fiber filter (934-AH).

To remove water-soluble impurities, the filtrate was transferred into a 25 ml screw-top glass tube containing 0.9% NaCl solution, resulting in a biphasic system. The lipid-rich lower phase was carefully separated, and the solvent was evaporated under a stream of nitrogen using a Nitrogen Evaporator (NEVAP). This procedure was repeated for all samples.

The lipid content based on dry weight was calculated using the following equation:

$$LW\ (g/g) = (m_2 - m_0) \times V / (3 \times m_1)$$

where:

LW = lipid content (g lipid per g dry weight)

m_1 = weight of algal powder (g)

m_0 = weight of the empty glass tube (g)

m_2 = weight of the tube containing the dried lipids (g)

V = total volume of the lower phase after washing (mL)

The lipid content (LW) obtained on the fourth day was multiplied by TVSS to determine the total lipid concentration (TC) using the formula (Zhou et al., 2012):

$$TC\ (g/L) = LW \times TVSS$$

Fatty acid analysis

The fatty acid composition was determined by converting the lipids into fatty acid methyl esters (FAMEs), followed by GC-MS analysis. FAMEs were prepared using a one-step extraction-transesterification method described by Indarti et al. (2005). Briefly, 100 mg of dried algal biomass was treated with 10 ml of a methanol-sulfuric acid-chloroform mixture (4.25:0.75:5, v/v) at 90°C for 90 min. The chloroform layer containing the FAMEs was collected for GC-MS analysis using a DB-5 MS capillary column.

The oven temperature was initially held at 80°C for 5 min, then increased to 290°C at a rate of 4°C min^{-1}. The injector and detector temperatures were maintained at 250°C and 230°C, respectively. Compounds were identified by comparing their mass spectra with those in the NIST Mass Spectral Database and verified using an external standard (C18:2, Sigma-Aldrich, MO).

Statistical analysis

All experiments were conducted in triplicate. Mean values were calculated to determine various parameters,

and results were expressed as mean ± standard deviation (SD) for n = 3. Data analysis and visualization were performed using Microsoft Excel.

Results

Sampling and isolation of unialgal strains

A total of thirty microalgal strains were collected from various water bodies in Peshawar and identified using a light microscope. Unialgal cultures were established by cultivating the isolates in Blue-Green-11 medium, out of which twenty-one strains exhibited successful growth (Table 1 and Figure 1).

Screening of facultative strains

Twelve facultative strains were isolated from concentrated municipal wastewater through a sequential screening process conducted under both light and dark cultivation conditions. Most of these strains, being natural inhabitants of wastewater, exhibited high growth rates and biomass yields. Under batch culture conditions, the strains remained in the exponential growth phase for 4-6 days before entering the stationary phase. Growth data for these isolates were expressed as total volatile suspended solids (TVSS, g/L), as presented in Table 2.

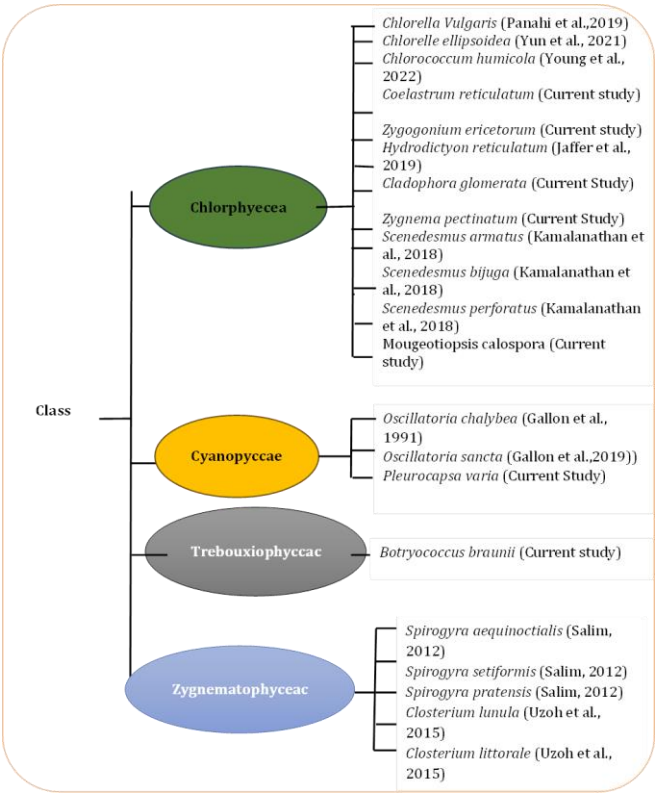


Figure 1. Growth performance of successful microalgal strains on BG-11 medium under specific growth conditions.

Table 1. Microalgal biomass concentration in BG-11 medium over 10-day period at 25±2°C

Microalgal Species	Biomass con. (g/L)	Microalgal Species	Biomass con. (g/L)
Chlorella vulgaris Beyerinch	1.73 ± 0.1	*Chlorococcum humicola* (Neaegeli) Rabenhorst	1.42 ± 0.11
Chlorella elliposoidea Gerneck	1.71± 0.2	*Hydrodictiyon reticulatum*(Linnaeus) Bory	0.35 ± 0.02
Oscillatoria chalybea Mertens	0.45 ± 0.02	*Oscilitoria sancta* (Kuetzing) Gomont	0.49 ± 0.03
Spirogyra aequinoctialis G.S. West	0.48 ± 0.06	*Closterium acerosum* Ehrenberg ex Ralfs	1.43 ± 0.06
Spirogyra pratensis transeau	0.47 ± 0.05	*Closterium littorale* F. Gay	1.41 ± 0.03
Spirogyra setiformis (Roth) Kuetzing	0.46 ± 0.04	*Zygogonium ericetorum* Kuetzing	0.35 ± 0.04
Coelastrum reticulatum (Dangeard) Senn	0.37 ± 0.06	*Cladophora glomerata* (Linnaeus) Kützing	0.25 ± 0.12
Zygnema pectinatum (voucher) C.A Agardh	0.24 ± 0.03	*Botryococcus braunii* Kutzing	0.85 ± 0.14
Mougeotiopsis calospora palla	0.31 ± 0.04	*Scenedesmus armatus* (Chodat)	1.16 ± 0.07
Scenedesmus bijuga var. alternans (Reinsch) Hansgirg	1.15 ± 0.04	*Scenedesmus. Perforate* Lemmermann	1.14 ± 0.08
Pleurocapsa varia (A. Braun) Drouet and Daily	0.41 ± 0.02		

Biomass concentration is denoted in the above table as ± SD, n=3.

Table 2. Comparative assessment of microalgal cell size, growth rate, and biomass yield in municipal wastewater and control conditions.

Species	Cell Size (µm)	CMWW Growth Rate (d^{-1})	CMWW Biomass Productivity ($g\ L^{-1}\ day^{-1}$)	Control Growth Rate (d^{-1})	Control Biomass Productivity ($g\ L^{-1}\ day^{-1}$)
S. aequinoctialis	23–29	0.307±0.025	0.178±0.010	0.313±0.022	0.185±0.007
S. pratensis	17–20	0.302±0.035	0.171±0.008	0.301±0.024	0.169±0.015
S. setiformis	90–95	0.303±0.032	0.173±0.011	0.309±0.017	0.170±0.011
C. vulgaris	6–9	0.373±0.021	0.279±0.009	0.423±0.032	0.390±0.010
C. ellipsoidea	7–8	0.362±0.022	0.262±0.013	0.382±0.026	0.297±0.014
B. braunii	6–10	0.315±0.030	0.188±0.014	0.322±0.040	0.164±0.030
S. armatus	4–7	0.305±0.060	0.174±0.011	0.316±0.025	0.188±0.021
S. bijuga var. alternans	4–8	0.319±0.035	0.194±0.010	0.329±0.030	0.177±0.008
S. perforatus	3–5	0.312±0.031	0.184±0.009	0.328±0.021	0.205±0.008
C. humicola	5–13	0.322±0.042	0.198±0.012	0.329±0.035	0.206±0.009
O. chalybea	6–7	0.261±0.045	0.129±0.015	0.266±0.028	0.136±0.010
O. sancta	6–12	0.243±0.021	0.117±0.012	0.289±0.034	0.156±0.008

Growth rates and biomass productivity

In terms of biomass concentration, microalgal growth rates differed between CMWW and the control during six days of batch cultivation (Figure 2). In CMWW, the highest growth rates were recorded for *C. vulgaris* (0.373 ± 0.021 d^{-1}) and *C. ellipsoidea* (0.362 ± 0.022 d^{-1}), whereas *O. sancta* exhibited the lowest (0.243 ± 0.021 d^{-1}). In the control, *C. vulgaris* (0.423 ± 0.032 d^{-1}) and *C. ellipsoidea* (0.382 ± 0.026 d^{-1}) again showed the highest growth rates, while *O. sancta* (0.289 ± 0.034 d^{-1}) and *O. chalybea* (0.266 ± 0.028 d^{-1}) displayed the lowest.

Similarly, biomass productivity in CMWW was highest for *C. vulgaris* (0.279 ± 0.009 g/L/day) and *C. ellipsoidea* (0.262 ± 0.013 g/L/day) compared to the other tested species. In the control, *C. vulgaris* (0.390 ± 0.010 g/L/day) and *C. ellipsoidea* (0.297 ± 0.014 g/L/day) again exhibited the greatest productivity, maintaining exponential growth throughout the six-day cultivation period. In contrast, *Oscillatoria* species showed the lowest productivity (*O. sancta*: 0.156 ± 0.008 g/L/day; *O. chalybea*: 0.136 ± 0.010 g/L/day) (Figure 3).

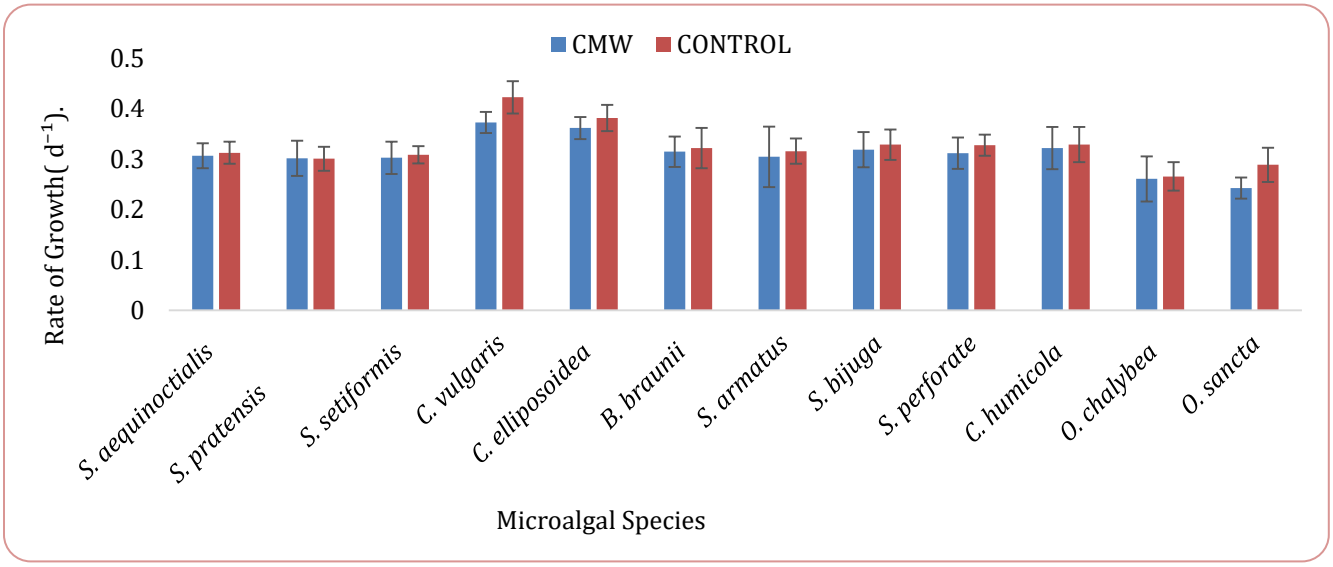


Figure 2. Effects of CMW and control treatments on the growth of microalgal strains. Values are presented as mean ± SD (n = 3).

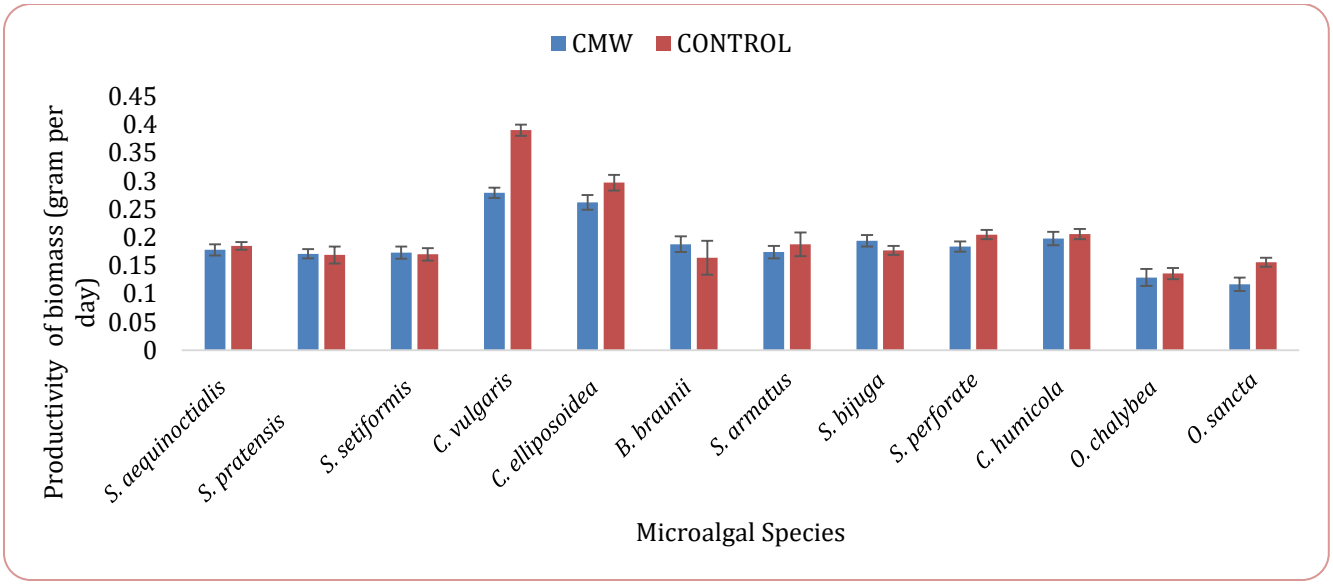


Figure 3: Dry biomass yields of microalgal strains cultivated in CMW and control media.

Physicochemical characteristics of CMWW used in the study

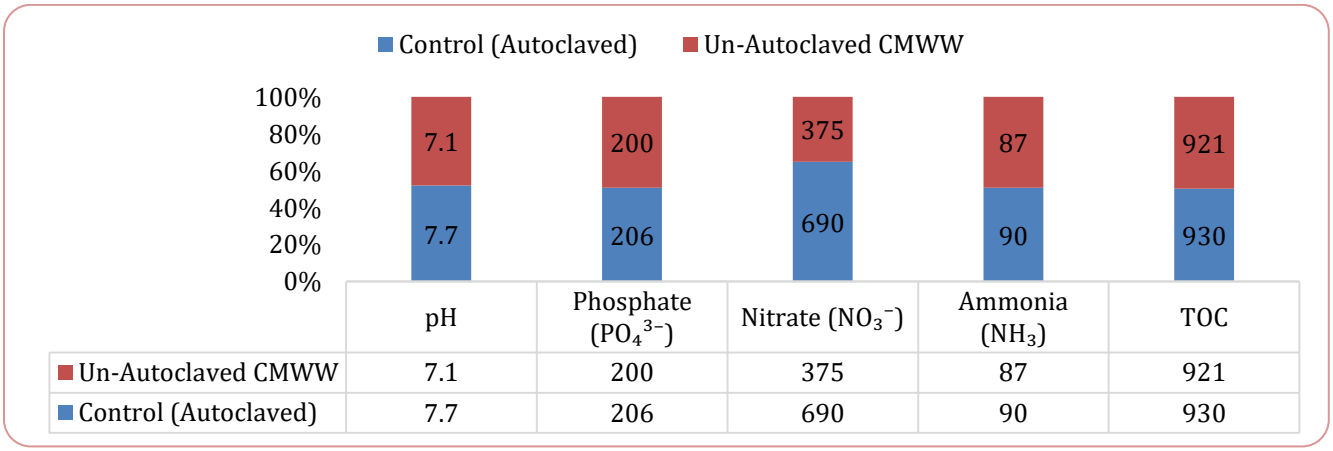
The results indicate that CMWW possessed high nutrient concentrations, including phosphate, nitrate, and ammonia, as well as elevated levels of organic matter (TOC) and COD, suggesting its potential suitability as a medium for microalgal growth. Autoclaving had minimal effects on most nutrient concentrations; however, a noticeable reduction in nitrate levels was observed, likely due to microbial activity in the unautoclaved samples (Figure 4).

Nutrient reduction through microalgal growth

Microalgal species were cultivated in concentrated municipal wastewater (CMWW) to evaluate their nutrient removal efficiency over a six-day batch culture period. *Chlorella* species exhibited the highest growth and nutrient removal efficiency, particularly at pH 7.1-9.1, whereas *O. sancta* showed the lowest reduction rates.

Nitrate removal by microalgal strains

In CMWW, the initial nitrate concentration was 375 mg/L. The highest nitrate reduction was observed in *C. vulgaris* (14.6 ± 3.3 mg/L; 96% removal), while the lowest was recorded in *O. sancta* (192.3 ± 7.69 mg/L; 48.7% removal). In the control, the initial nitrate concentration was 690 mg/L. *C. vulgaris* significantly reduced nitrate levels to 19.9 mg/L (97% removal), whereas *O. sancta* showed the lowest reduction (245.7 ± 4.1 mg/L; 64% removal). Detailed results are presented in Figures 5(a-c).



	pH	Phosphate (PO_4^{3-})	Nitrate (NO_3^-)	Ammonia (NH_3)	TOC
Un-Autoclaved CMWW	7.1	200	375	87	921
Control (Autoclaved)	7.7	206	690	90	930

Figure 4. Physicochemical parameters of municipal wastewater from the study area (mg/L).

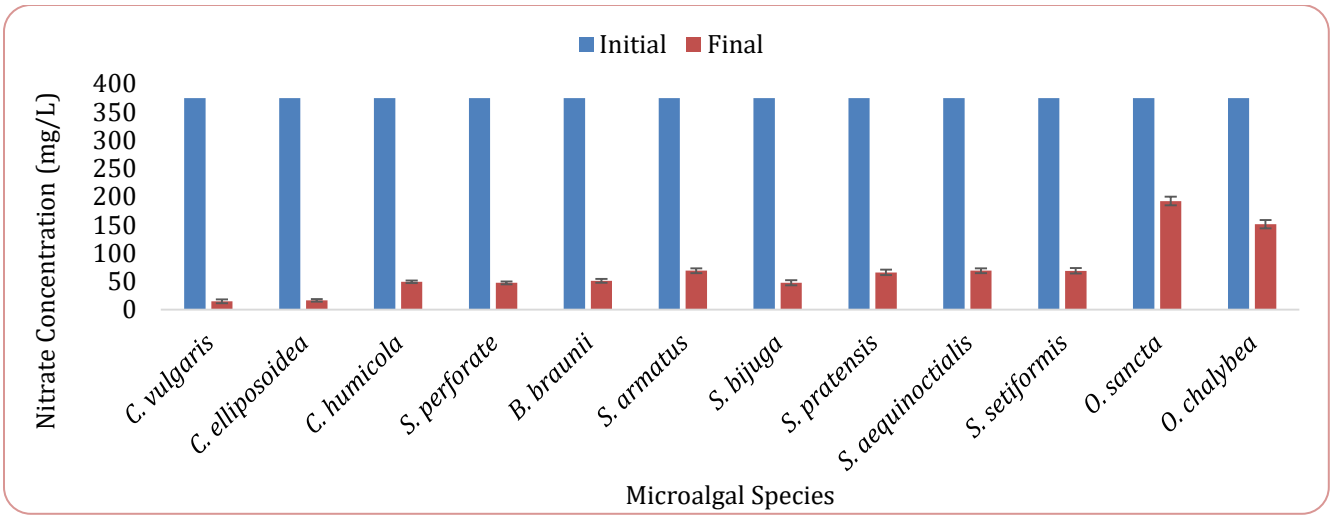


Figure 5a. Initial and final nitrate concentrations (mg/L) in CMWW batch culture.

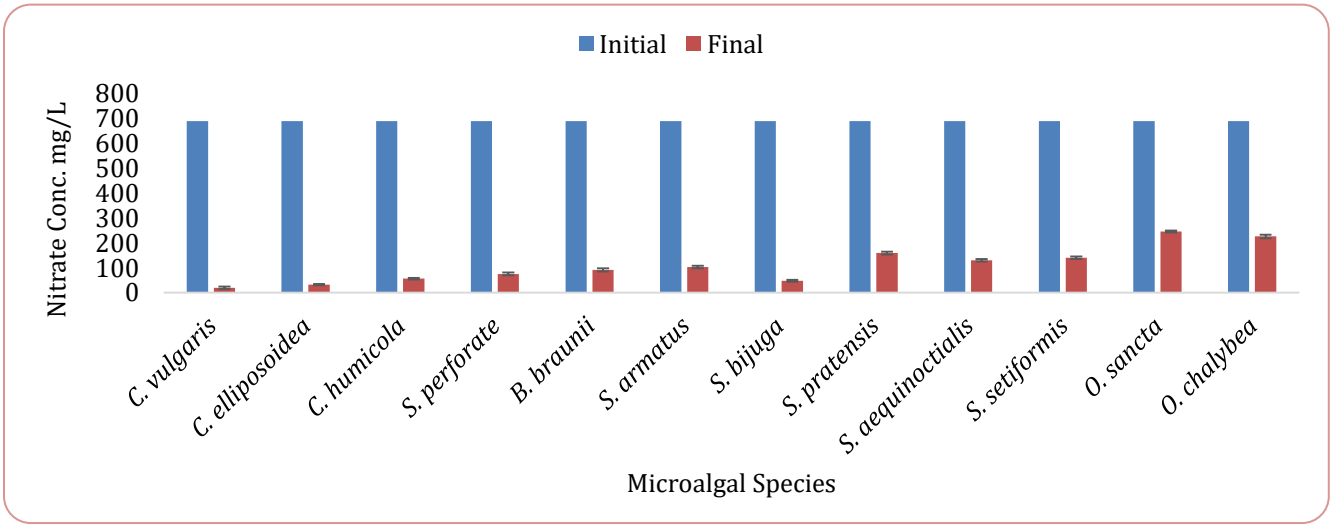


Figure 5b. Initial and final nitrate concentrations (mg/L) in batch culture grown under control conditions.

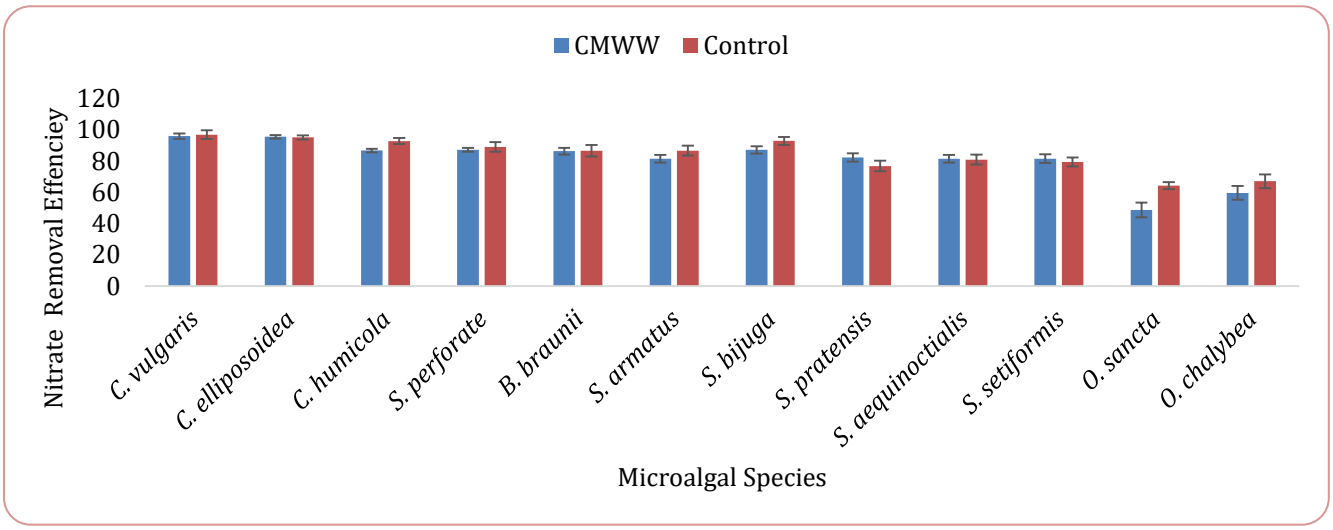


Figure 5c. Nitrate removal efficiency of microalgal species cultivated in CMWW and control media.

Phosphate removal by microalgal strains

In CMWW, the initial phosphate concentration was 200 mg/L. The highest phosphate reduction was observed in *C. vulgaris* (42.2 ± 2.4 mg/L; 77.7% removal), whereas the lowest reduction was recorded in *O. sancta* (105.6 ± 2.6 mg/L; 47% removal). In the control medium, the initial phosphate concentration was 206 mg/L. *C. vulgaris* significantly reduced phosphate to 35.3 ± 3.75 mg/L (82.8% removal), while the lowest reduction was observed in *O. sancta* (101 ± 3.42 mg/L; 54% removal) (Figures 6a-c).

Ammonia removal by microalgal strains

In CMWW, the initial ammonia concentration was 87 mg/L. The greatest reduction in ammonia was observed in *C. vulgaris* (8.62 ± 0.73 mg/L; 90.0% removal), whereas the lowest reduction was recorded in *O. sancta* (36.3 ± 2.9 mg/L; 58% removal). In the control treatment, the initial ammonia concentration was 90 mg/L. *C. vulgaris* significantly reduced ammonia to 7.1 ± 1.09 mg/L (92% removal), while *O. sancta* exhibited the lowest reduction (40.4 ± 4.2 mg/L; 55% removal) (Figures 7a-c).

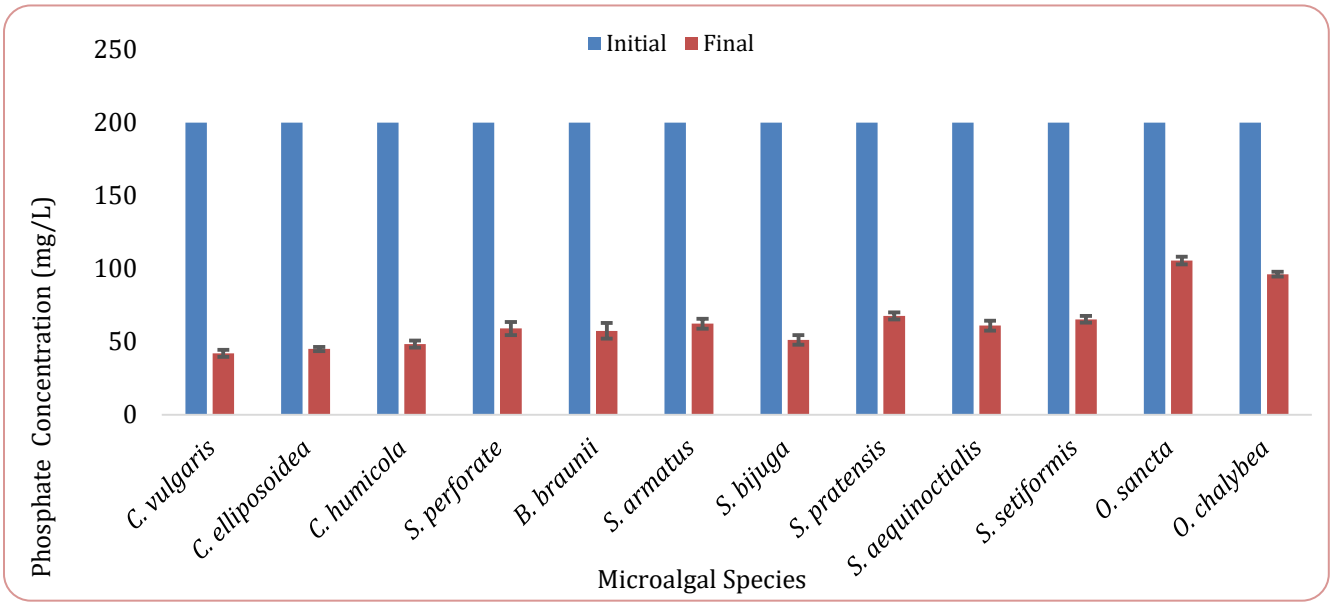


Figure 6a. Initial and final phosphate concentrations (mg/L) in CMWW batch culture.

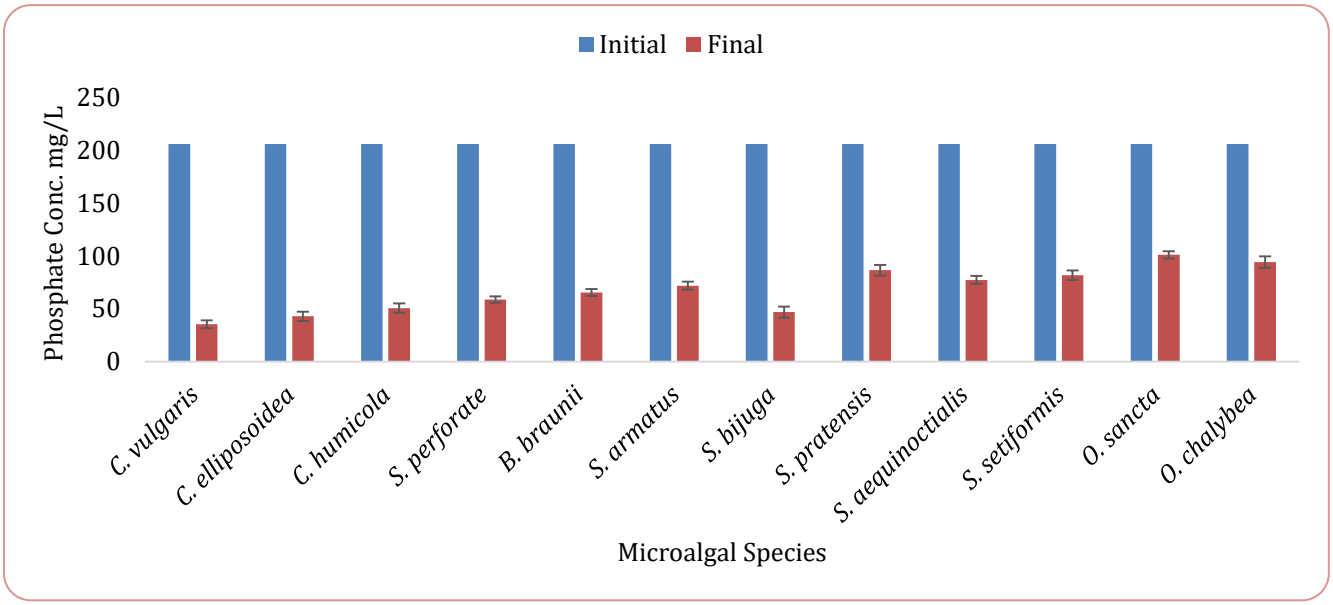


Figure 6b. Initial and final phosphate concentrations (mg/L) in batch culture grown under control conditions.

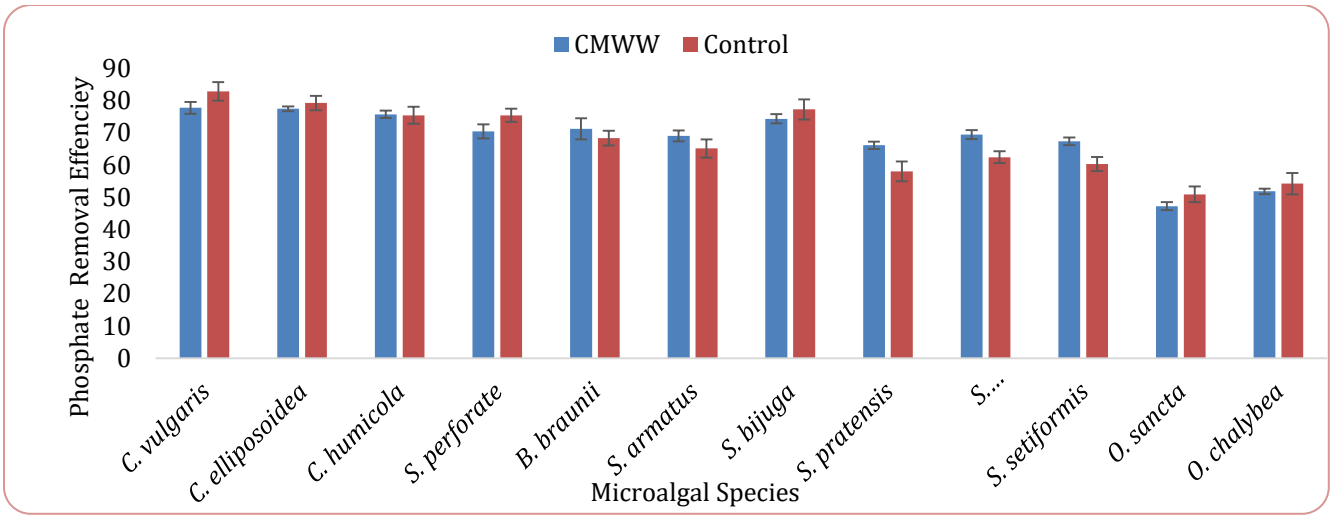


Figure 6c. Phosphate removal efficiency of microalgal species grown in CMWW and control media.

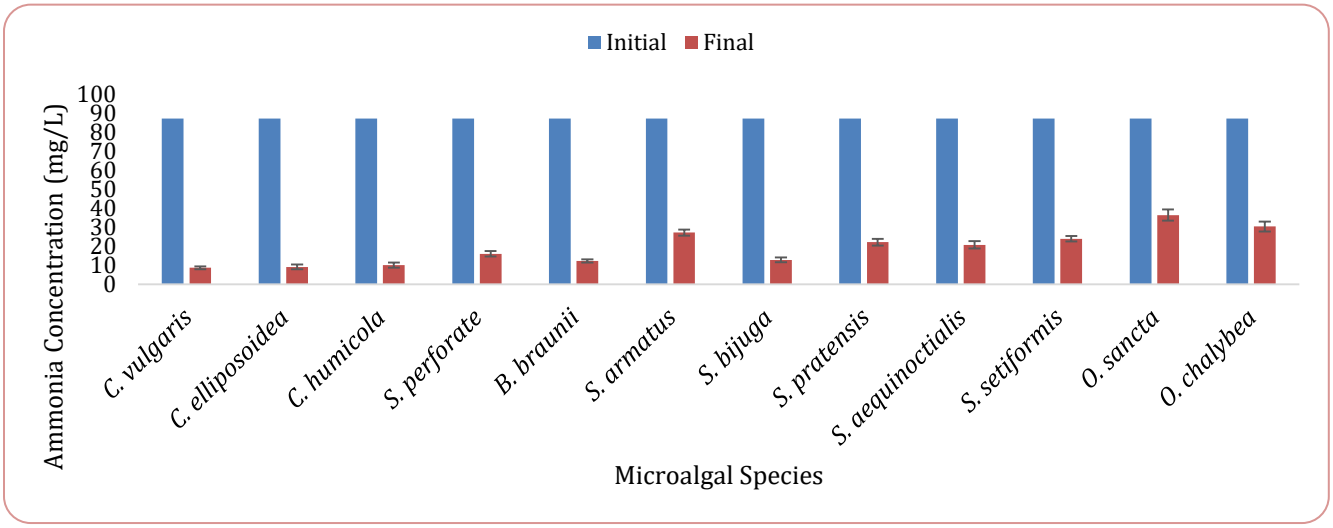


Figure 7a. Initial and final ammonia concentrations (mg/L) in CMWW batch culture.

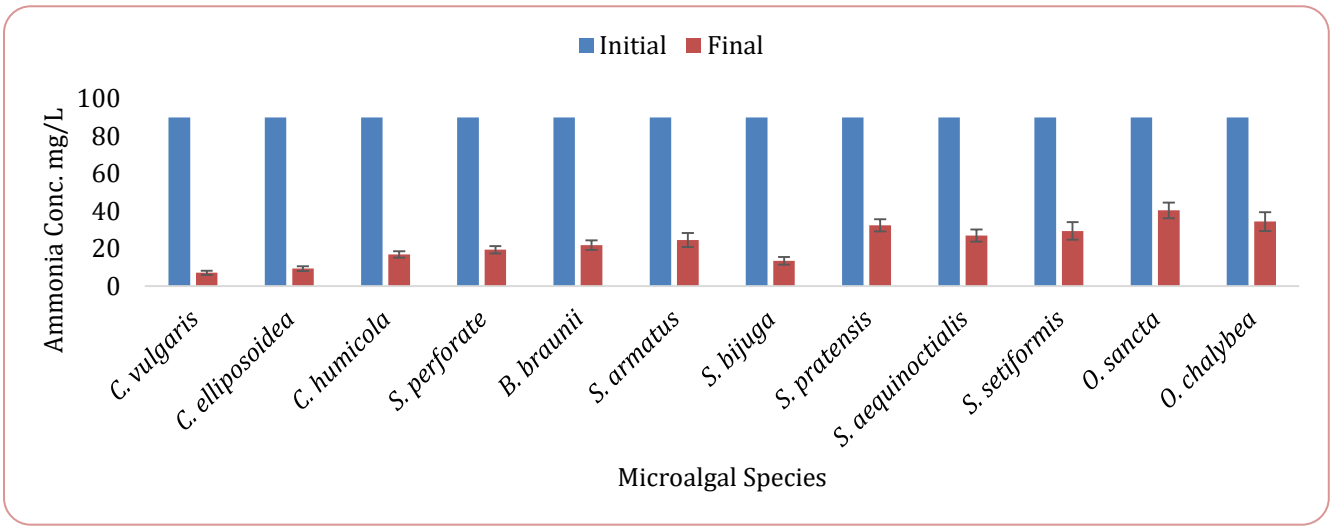


Figure 7b. Initial and final ammonia concentrations (mg/L) in batch culture grown under control conditions.

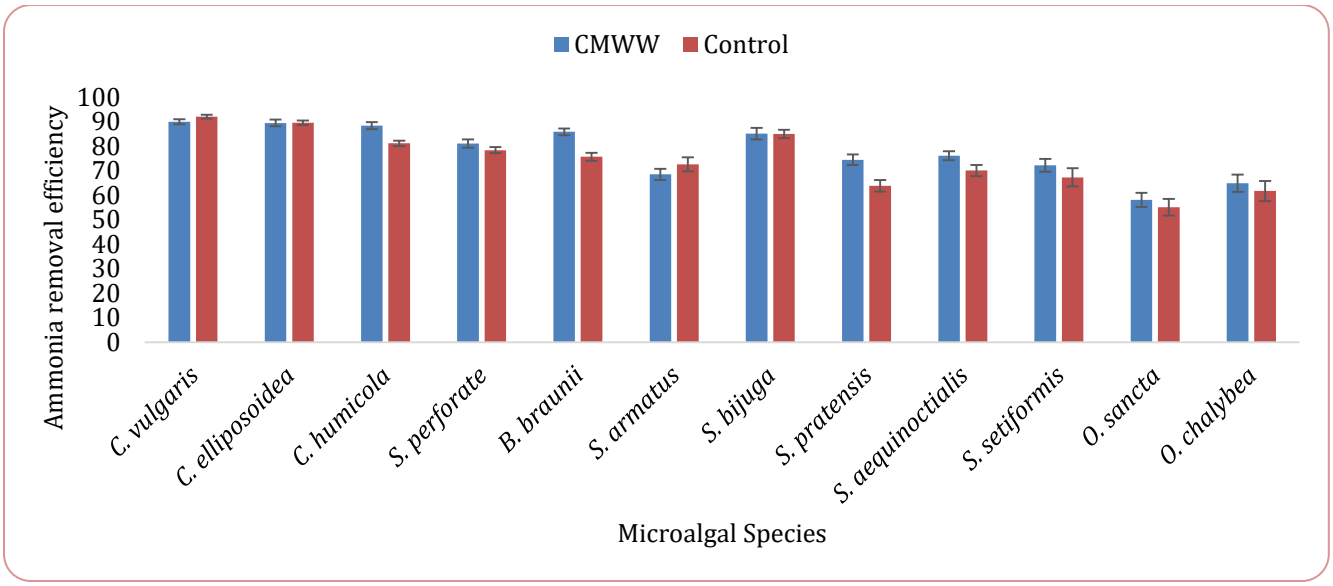


Figure 7c. Ammonia removal efficiency of microalgal species cultivated in CMWW and control media.

Total organic carbon removal by microalgal strains

In CMWW, the initial TOC concentration was 921 mg/L. The highest TOC reduction was observed in *C. vulgaris* (41.1 ± 6.3 mg/L; 95.5% removal), while the lowest reduction was recorded in *O. sancta* (423 ± 11.21 mg/L; 54% removal). In the control, the initial TOC concentration was 930 mg/L. *C. vulgaris* significantly reduced TOC to 29.5 ± 6.35 mg/L (96.8% removal), whereas *O. sancta* exhibited the lowest reduction (313.3 ± 8.84 mg/L; 66% removal) (Figures 8a-c).

COD removal by microalgal strains

The reduction of COD in CMWW by 12 microalgal species was evaluated under batch cultivation. COD removal efficiency varied significantly among the tested species, ranging from 95.4% in *C. vulgaris* to 52.3% in *O. sancta*. *C. vulgaris* exhibited the highest reduction, decreasing COD from 227 mg/L to 10.4 ± 4.26 mg/L, whereas *O. sancta* reduced COD to 108.1 ± 4.70 mg/L. In the control treatment, COD decreased from 230 mg/L to 7.4 ± 2.29 mg/L. Overall, COD removal efficiencies ranged from 96.7% for *C. vulgaris* to 51.2% for *O. sancta* (Figure 9a-c).

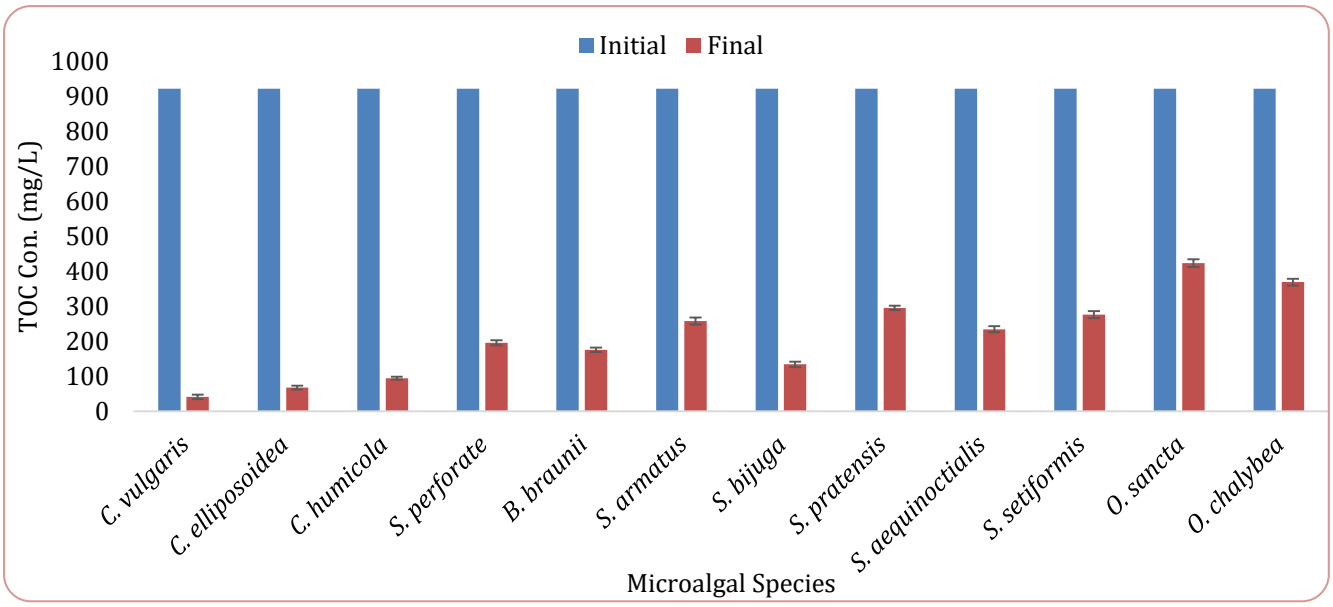


Figure 8a. Initial and final TOC concentrations (mg/L) in CMWW batch culture.

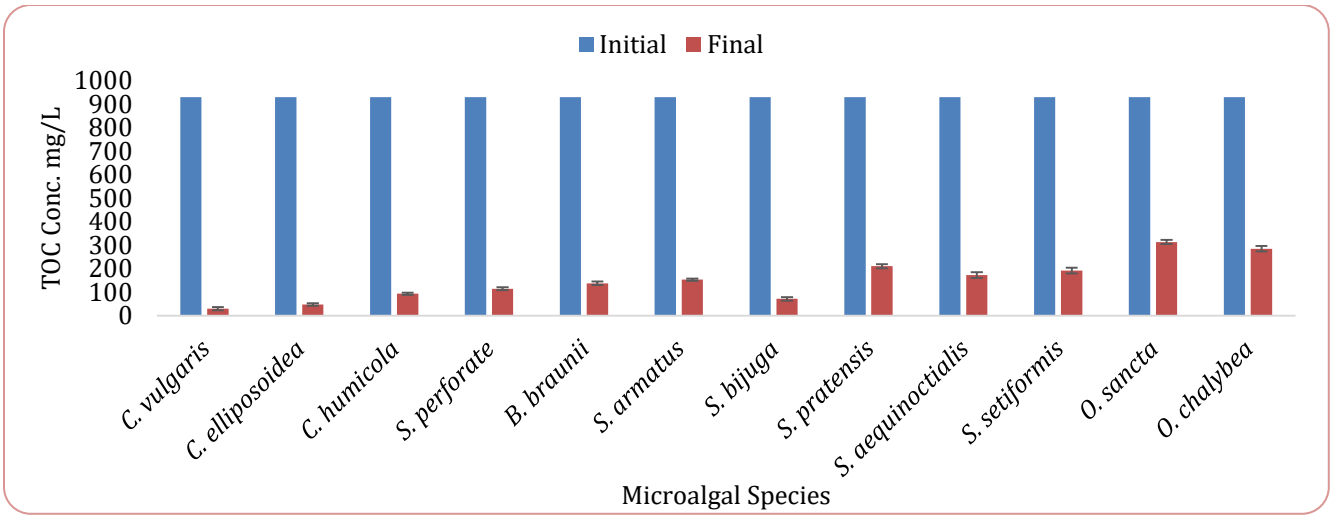


Figure 8b. Initial and final TOC concentrations (mg/L) in batch cultures grown under control conditions.

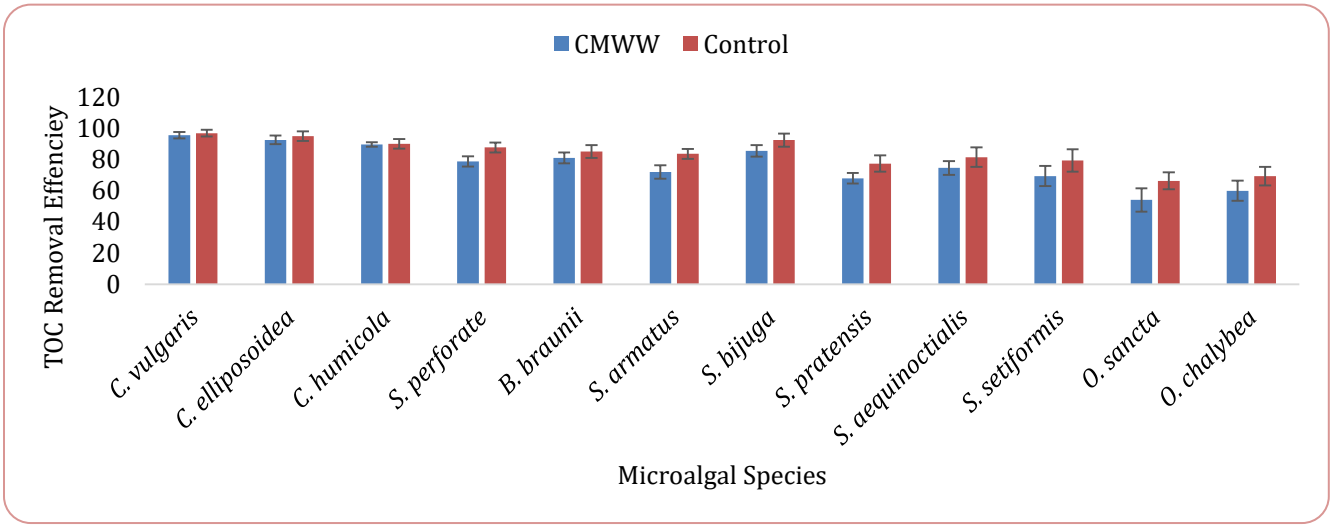


Figure 8c. TOC removal efficiency of microalgal species grown in CMWW and control conditions.

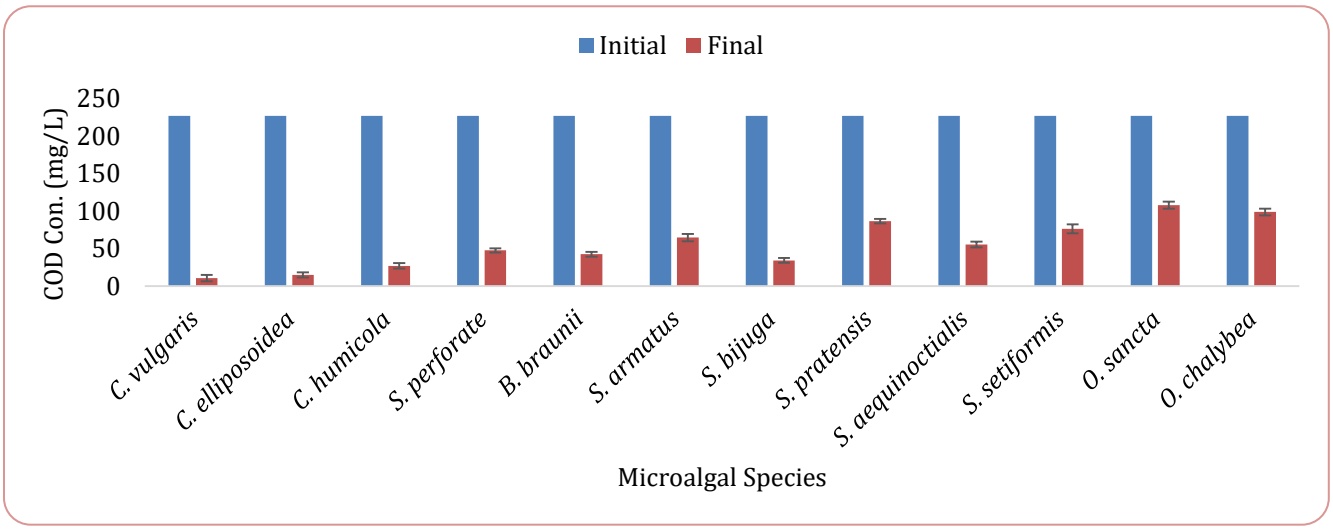


Figure 9a. Initial and final COD concentrations (mg/L) in CMWW batch culture.

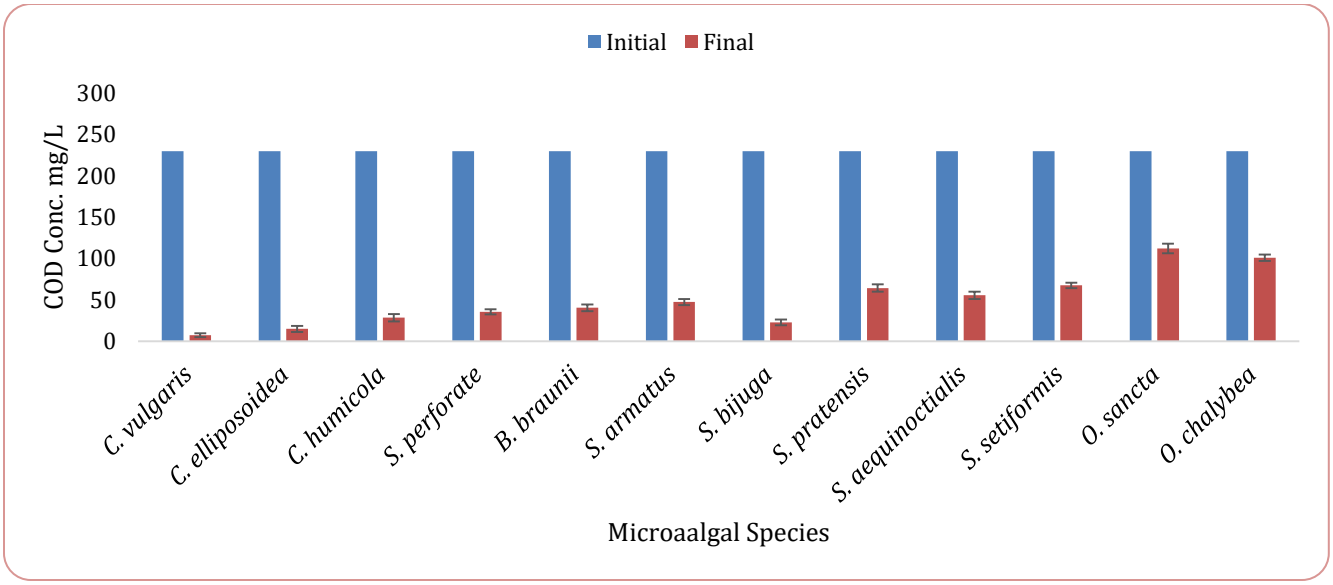


Figure 9b. Initial and final COD concentrations (mg/L) in batch culture grown under control conditions.

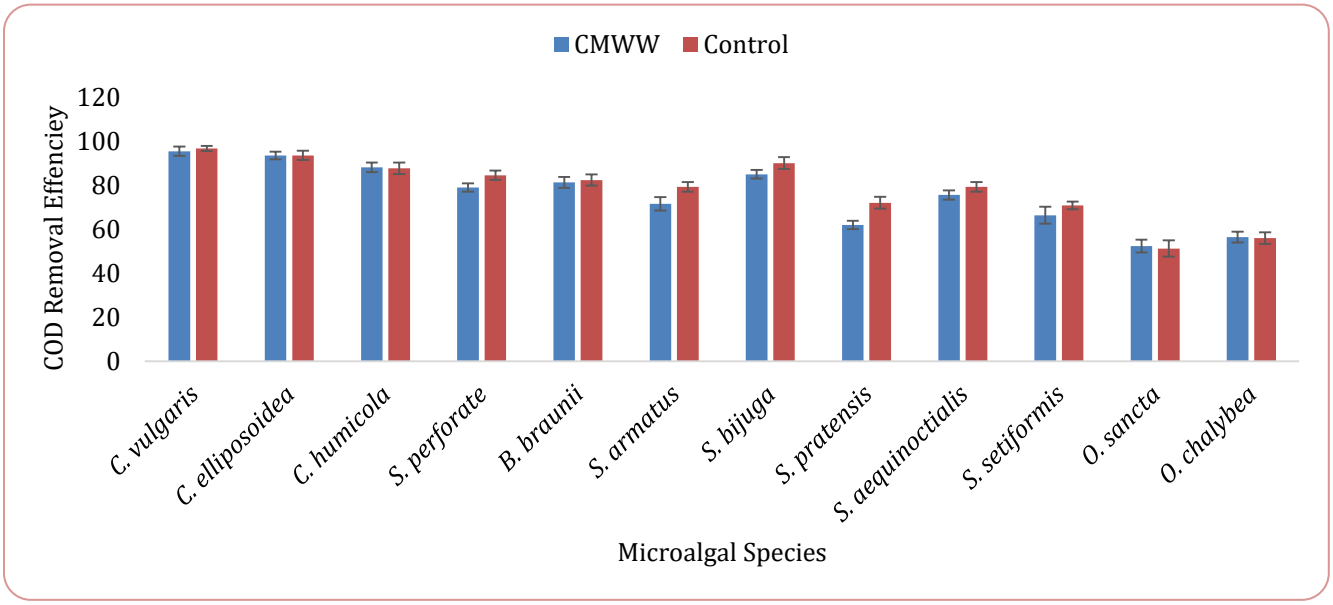


Figure 9c. COD removal efficiency of microalgal species grown in CMWW and control media.

Assessment of lipid yield and lipid concentration in microalgae cultivated in CMWW

The study evaluated the lipid yield and total lipid content in microalgal biomass harvested after 6 days of cultivation in CMWW. The total lipid content among the twelve microalgal species ranged from 11.2 ± 0.8% to 28.8 ± 0.6%, with *C. vulgaris* exhibiting the highest lipid productivity (0.0803 ± 0.0014 g L^{-1}). Moreover, the microalgal isolates significantly reduced the total organic carbon in the wastewater, achieving removal efficiencies ranging from 41.2% to 78.5% (Figures 10a and 10b).

Evaluation of fatty acid composition

The study investigated the fatty acid profiles of microalgae cultivated in CMWW, focusing on both saturated and unsaturated fatty acids. After 6 days of cultivation, *C. vulgaris* and *S. setiformis* exhibited higher total fatty acid contents compared to *O. sancta* and *O. chalybea*. GC-MS analysis revealed that the lipid fractions of all strains were predominantly composed of C16 and C18 fatty acids. The proportion of saturated fatty acids ranged from 26% to 76%, while unsaturated fatty acids accounted for 24% to 74% (Table 3).

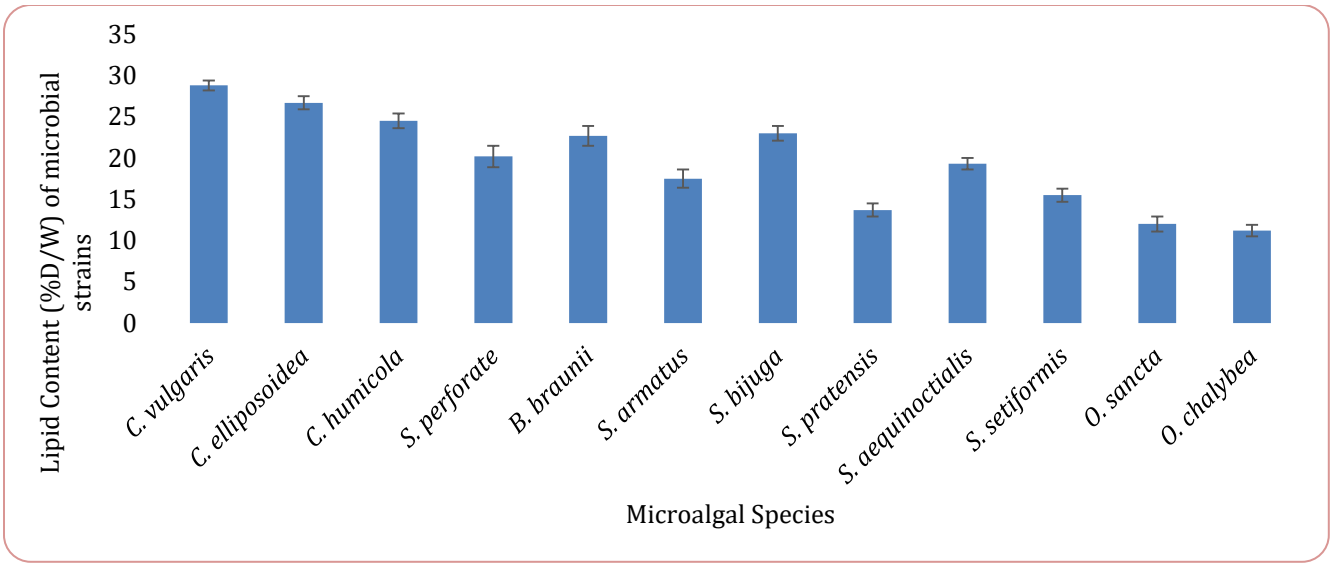


Figure 10a. Lipid content (% DW) of microalgal strains grown in CMWW.

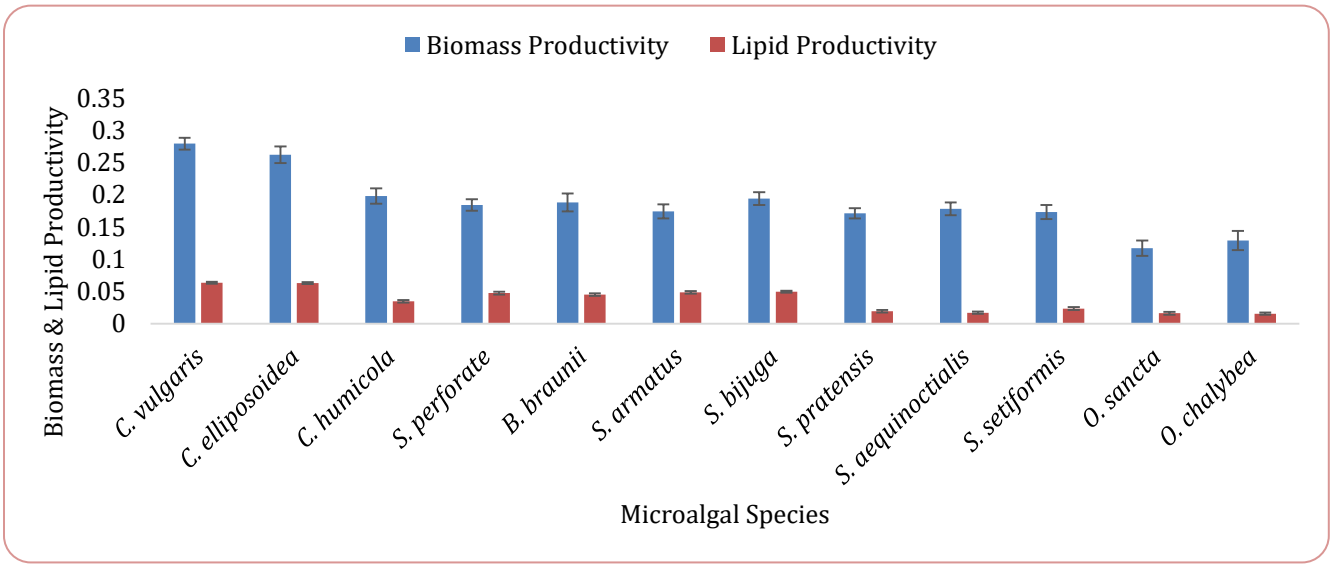


Figure 10b. Biomass and lipid productivity of microalgal strains cultivated in CMWW.

Table 3. Major fatty acid composition and total lipid content of screened microalgal strains.

Fatty Acids	*C. vulgaris*	*C. ellipsoidea*	*C. humicola*	*S. Perforate*	*B. braunii*	*S. armatu*	*S. bijuga*	*S. pratensis*	*S. aequinoctialis*	*S. setiformis*	*O. sancta*	*O. chalybea*
Saturated	30	32	70.5	35.6	26	40.6	42.5	76	73	75.5	65.3	57
Unsaturated	70	68	29.5	64.4	74	59.4	57.5	24	27	24.5	34.7	43
Mono	39.2	37.6	17.5	37.6	46.3	27.2	20.1	16	18.5	15	16.3	20.2
Poly	30.8	30.4	12	26.8	27.7	32.2	27.4	8	8.5	9.5	17.8	22.8
C16-C18	90.5	92	85.6	86	88.2	75.4	83.6	93.5	92	92.6	56.7	66.5
Total Lipids	28.8 ± 0.6	26.7 ± 0.8	24.5 ± 0.9	20.2 ± 1.3	22.7 ± 1.2	17.5 ± 1.1	23 ± 0.9	13.7 ± 0.8	19.3 ± 0.7	15.5 ±0.8	12 ± 0.9	11.2 ± 0.7

Discussion

The present study demonstrated that microalgae can effectively treat municipal wastewater through the isolation and selection of suitable strains for nutrient removal and biomass production. These strains also hold potential for biofuel and other industrial applications. A total of thirty microalgal species were collected from various water bodies in Peshawar, out of which 21 strains successfully grew in BG-11 medium. The initial algal biomass nearly doubled after 10 days of cultivation, confirming the suitability of BG-11 medium. This finding is consistent with that of Chinnasamy et al. (2010), who reported that most microalgae, particularly green and blue-green species, exhibit robust growth in BG-11 medium. Similarly, Bajwa et al. (2017) demonstrated that BG-11 was the most effective medium for microalgal strains, promoting enhanced synthesis of proteins, total carbohydrates, and overall biomass accumulation.

Of the twenty-one isolates grown in BG-11 medium, twelve facultative strains were selected for cultivation in un-autoclaved CMWW for 6 days, with autoclaved CMWW serving as the control. *C. vulgaris* exhibited the highest growth in both CMWW and the control medium. Notably, microalgal strains such as *Chlorella*, *Chlorococcum*, *Scenedesmus*, *Botryococcus*, and *Spirogyra* demonstrated superior growth efficiency and biomass accumulation. These results are consistent with those of Kabir et al. (2018), who reported that microalgal species inhabiting wastewater can adapt to growth conditions resembling their natural habitats. In contrast, *Oscillatoria* species (*O. chalybea* and *O. sancta*) showed the slowest growth rates, which aligns with the findings of Cheng et al. (2018), who suggested that factors such as organic carbon depletion, nutrient limitation, and pH fluctuations can restrict the growth of certain microalgal species.

Growth performance was influenced by wastewater characteristics, species type, and cultivation conditions, including temperature, pH, and light. *Chlorella*, *Chlorococcum*, *Scenedesmus*, *Botryococcus*, and *Spirogyra* exhibited the highest biomass productivity. Under photoheterotrophic conditions with glucose as a carbon source, *Chlorella* achieved greater dry weight and productivity, supporting previous findings that sugar supplementation enhances microalgal growth and lipid accumulation (Ji et al., 2013; Kong et al., 2020).

All microalgal isolates in this study demonstrated the ability to reduce nutrients while accumulating biomass. This observation agrees with Wang et al. (2010), who reported that nutrient uptake efficiency directly influences algal growth. During the experiment, the culture pH gradually increased from 7.1 to 9.1, consistent with Gonzalez et al. (2008), who noted that pH variation is affected by microalgal growth, nitrification, metabolite release, and organic matter decomposition. All tested species reduced nitrate, phosphate, ammonia, TOC, and COD, with *C. vulgaris* and *C. ellipsoidea* exhibiting the highest removal efficiencies. These findings corroborate those of Rasheedy et al. (2017), who reported that *C. vulgaris* removed 100% of NO_2^-, 98% of NH_3, and 84.7% of total phosphorus from municipal wastewater after 6 days of treatment. Conversely, *Oscillatoria* species showed slower nutrient reduction and biomass decline, consistent with Ge et al. (2018), who attributed biomass loss after 144 h to nutrient depletion.

The study further revealed that *C. vulgaris* accumulated higher lipid content during the stationary phase, consistent with Dean et al. (2010), who reported that algae reduce cell division under stress and efficiently convert carbon into high-energy lipids to cope with nutrient deficiency. Ten species exhibited high proportions of C16-C18 fatty acids, with *C. vulgaris* and *C. ellipsoidea* exceeding 90%. Since C16-C18 fatty acids are ideal for biodiesel production, these strains are strong candidates for biofuel generation, consistent with the findings of Xu et al. (2006) and Miao and Wu (2006).

In this study, nitrogen and phosphorus deficiency increased monounsaturated fatty acids (MUFAs), reaching 46.3% in *B. braunii*, 37.6% in *S. perforata*, and over 37% in *C. vulgaris* and *C. ellipsoidea*. Previous studies have also reported that nitrogen and phosphorus stress elevate oleic acid content in algal cells, ranging from 0.4-12.1% in *D. tertiolecta*, 16-31% in *C. vulgaris*, and 4.1-16.4% in *Nannochloropsis* sp. (Ho et al., 2010; Msanne et al., 2012). The high MUFA levels observed in this study may result from alterations in photosynthetic carbon assimilation in most of the tested microalgal strains. Moreover, most strains contained more than 10% polyunsaturated fatty acids (PUFAs; omega-3), which are associated with significant health benefits, including reduced body fat, improved fetal development, enhanced cardiac function, and lower risks of diabetes, heart disease, Alzheimer's disease, and other disorders (Smedman and Vessby, 2001).

Despite the promising potential of microalgae for wastewater remediation and biomass accumulation,

several limitations remain in scaling up from laboratory to industrial systems. In laboratory experiments, environmental variables such as temperature, light, and CO_2 levels are controlled; however, these parameters fluctuate in large-scale systems. This study focused on lipid content and fatty acid composition but did not assess biodiesel quality, economic feasibility, or life-cycle impacts. Furthermore, the effects of heavy metals and other wastewater contaminants on algal growth were not fully examined. Future research should therefore include pilot-scale operations, diverse wastewater types, and comprehensive evaluations to assess the scalability and commercial viability of microalgae for wastewater treatment and biofuel production.

Conclusion

This study demonstrates the potential of microalgae for the treatment of municipal wastewater by isolating and selecting stable strains capable of efficiently removing key contaminants such as nitrogen, phosphorus, ammonia, TOC, and COD. Among the 12 microalgal strains identified, *C. vulgaris* and *C. ellipsoidea* exhibited superior nutrient removal efficiency and biomass productivity. These strains also produced valuable by-products, particularly lipids, with several exhibiting high concentrations of polyunsaturated fatty acids suitable for biofuel production.

Microalgae-based wastewater treatment offers a sustainable and dual-benefit approach, enhancing water quality while generating biomass for use in biofuels, pharmaceuticals, and animal feed. The findings highlight the potential for integrating microalgal systems into municipal wastewater treatment frameworks to achieve both environmental sustainability and economic gains. Continued research and technological advancements will further optimize their efficiency and large-scale applicability.

Recommendations

Based on the present study, specific microalgal species exhibit strong phytoremediation potential. It is therefore suggested to integrate microalgal systems with conventional treatment methods to enhance overall efficiency. Pilot-scale testing of microalgae-based treatment systems should be implemented for real-world evaluation. Furthermore, strategies should be developed to commercialize high-value products such as bioplastics and pharmaceuticals derived from microalgal biomass, while utilizing the residual biomass as biofertilizer or animal feed to promote a circular economy.

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Authors' Contributions

SJ, FMS, FH, FM, KUR, and FS jointly conceived and designed the research study; SJ was responsible for data collection, while SJ, FM, GMS, MF, and FS contributed to data processing, statistical analysis, and interpretation of results; SJ, FS, and FMS prepared the initial draft of the manuscript; All authors critically reviewed, edited, and approved the final version of the manuscript for publication.

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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 7: Affordable and Clean Energy
SDG 13: Climate Action

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