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Valorization of Canteen Wastewater Through Optimized Spirulina Platensis Cultivation for Enhanced Carotenoid Production and Nutrient Removal

Charith Akalanka Dodangodage [1,*], Geethaka Nethsara Gamage [1], Induwara Arsith Wijesekara [2], Jagath C. Kasturiarachchi [2], Thilini A. Perera [3], Dilan Rajapakshe [4] and Rangika Umesh Halwatura [1]

[1] Department of Civil Engineering, University of Moratuwa, Moratuwa 10400, Sri Lanka; rangika@uom.lk (R.U.H.)
[2] Department of Applied Sciences, Sri Lanka Institute of Information Technology, New Kandy Rd, Malabe 10115, Sri Lanka; jagath.k@sliit.lk (J.C.K.)
[3] Department of Plant Sciences, University of Colombo, Hurstan Rd, Colombo 00300, Sri Lanka
[4] KU Innovation Park, University of Kansas, Lawrence, KS 66047, USA
* Correspondence: dodangodageca.24@uom.lk

Abstract

The valorization of nutrient-rich institutional effluents represents a promising route for sustainable algal biotechnology. This study investigates the potential of canteen wastewater (CW) as an alternative culture medium for Spirulina platensis, integrating wastewater treatment with high-value carotenoid and lipid production. Growth performance, biochemical composition, and nutrient removal efficiencies were systematically evaluated in 2 L photobioreactors under optimized conditions. Spirulina cultured in 75% CW under 180 µmol photons m^{-2} s^{-1} achieved a biomass productivity of 0.071 g L^{-1} day^{-1}, nearly three-fold higher than the synthetic BG-11 control (0.023 g L^{-1} day^{-1}). Nutrient remediation was highly efficient, with 92.12% nitrate and 90.05% phosphate removal, effectively reducing effluent concentrations below discharge limits. Biochemical profiling revealed that wastewater-grown biomass contained 54.3% protein and 7.85% lipids, with a remarkable carotenoid yield of 21.81 mg g^{-1} DW—significantly higher than the control (6.85 mg g^{-1} DW). Mechanistic analysis suggests that the balanced nutrient stoichiometry (C:N:P ≈ 30:4:1) and mixotrophic conditions enhanced biomass quality while mitigating ammonia toxicity. This study demonstrates the first integrated application of canteen wastewater for dual-purpose bioremediation and pigment-rich biomass production, establishing a scalable circular bioeconomy framework for institutional waste management.

Keywords: *Spirulina platensis*; canteen wastewater; phycoremediation; carotenoids; circular bioeconomy

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Academic Editors: Nhuan Nghiem and Tae Hyun Kim

Received: 10 November 2025
Revised: 12 December 2025
Accepted: 23 December 2025
Published: 14 January 2026

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1. Introduction

Global water scarcity has become one of the defining environmental challenges of the twenty-first century. With the global population expected to reach 9.9 billion by 2050, freshwater demand is projected to increase by 20–30% within the next three decades [1,2]. Presently, over two billion people already live in regions experiencing high water stress, a condition that is worsening due to the combined effects of urbanization, industrialization, and climate change [3]. In addition to quantitative depletion, freshwater systems face serious qualitative degradation, manifested not only by nutrient

pollution but also by increased turbidity, pathogen accumulation, and the presence of emerging contaminants [4,5]. These twin crises of scarcity and pollution demand sustainable approaches that conserve water while simultaneously recovering resources from waste streams.

Among the key contributors to nutrient pollution, the food service sector—particularly institutional kitchens, canteens, and restaurants—has expanded rapidly over the past two decades [6]. This has led to a proportional increase in the generation of canteen wastewater (CW), characterized by high concentrations of biodegradable organic matter, fats, oils, and nutrients such as nitrogen (N) and phosphorus (P) [7]. The nutrient and organic loads in CW are often higher than those in domestic sewage, containing proteins, starches, and detergents from dishwashing and food residues [8]. If discharged untreated, CW contributes directly to eutrophication, algal blooms, and oxygen depletion in receiving water bodies [9,10]. In developing regions lacking centralized wastewater treatment infrastructure, direct disposal of canteen effluents is common, causing blockages from fats, oils, and grease (FOG) and aggravating both ecological and public health risks [11,12].

Despite its environmental burden, CW represents an underutilized resource due to its nutrient richness. The high concentrations of N and P make it a potential feedstock for biological valorization, especially through microalgae-based systems. Conventional synthetic culture media such as BG-11 or Zarrouk's medium provide reliable results for laboratory-scale microalgal growth but are prohibitively expensive and resource-intensive for large-scale use [13,14]. Repurposing CW as an algal cultivation substrate can therefore achieve dual benefits—nutrient removal and biomass generation—while advancing the principles of the circular bioeconomy, in which waste is transformed into value-added products [15,16].

Microalgae have long been recognized as efficient agents for wastewater treatment because of their ability to assimilate inorganic nutrients while generating high-value biomass [17,18]. Compared with conventional treatment systems, algal-based processes are more energy-efficient, carbon-sequestering, and capable of producing a wide range of bioproducts such as pigments, lipids, and proteins. Among the various microalgal species, *Spirulina* (genus *Arthrospira*) stands out for its robustness, rapid growth, and exceptional biochemical composition. It can tolerate wide fluctuations in pH, salinity, and ammonia—typically enduring pH levels of 9.0–11.0, salinities up to 30 g L^{-1}, and free ammonia concentrations up to 100 mg L^{-1}—conditions that often limit the growth of other microalgae. *Spirulina* biomass typically contains 60–70% protein by dry weight, along with vitamins, essential amino acids, and bioactive pigments. Of particular interest are carotenoids, lipid-soluble antioxidants with applications in food, pharmaceutical, and cosmetic industries [8,19–26]. Compounds such as β-carotene and zeaxanthin can constitute up to 1–1.5% of dry biomass under optimized conditions and are known for their role in vision health, immune regulation, and oxidative stress mitigation [27]. Growing consumer demand for natural pigments has created a rapidly expanding global carotenoid market [28], reinforcing the economic incentive to develop sustainable algal production systems.

Carotenoid accumulation in *Spirulina* is highly responsive to environmental and nutritional factors, including light intensity, nitrogen and phosphorus availability, and oxidative stress [29,30]. Hence, the nutrient-rich but variable composition of CW offers a unique opportunity to couple bioremediation with carotenoid enrichment through controlled cultivation. Previous studies have reported promising results using various wastewater sources—municipal, aquaculture, piggery, and dairy effluents—for *Spirulina* cultivation [19,28,31,32]. However, systematic investigations of canteen wastewater remain scarce, despite its consistent composition and widespread availability in institutional

settings [7]. This research gap limits the practical deployment of algae-based systems for treating food-service wastewater.

Valorizing canteen wastewater via *Spirulina* cultivation offers distinct environmental and economic advantages. Conventional treatment technologies, including activated sludge and membrane bioreactors, are energy-intensive and often fail to recover valuable by-products [33]. In contrast, algal cultivation can significantly reduce COD, BOD, nitrate, and phosphate concentrations while producing protein- and pigment-rich biomass [7,34]. Furthermore, photosynthetic *Spirulina* growth captures atmospheric CO_2, linking wastewater management with climate mitigation [35,36]. Integrating such systems in institutional facilities—such as university canteens—can transform waste management into a resource recovery process that simultaneously alleviates environmental impacts and generates bioactive compounds for commercial use.

While extensive research exists on *Spirulina* growth in industrial or municipal effluents, the potential of canteen wastewater as a cultivation medium remains underexplored. The predictable nutrient composition of CW and its availability in urban institutions present a consistent substrate for circular biotechnological applications. Moreover, the ability to achieve both nutrient remediation and carotenoid-rich biomass production in a single system has not been previously demonstrated.

Therefore, this study aims to evaluate the feasibility of using diluted canteen wastewater as a growth medium for *Spirulina platensis* and to compare its performance against the conventional BG-11 medium. Specifically, the work quantifies biomass productivity, nutrient removal efficiencies, and carotenoid yield under optimized light and dilution conditions. To the best of our knowledge, this represents the first integrated assessment of canteen wastewater as a dual-purpose substrate for *Spirulina*-based wastewater treatment and carotenoid enrichment, offering a scalable and sustainable model for institutional circular bioeconomy applications.

2. Materials and Methods

2.1. Wastewater Collection and Pre-Treatment

Canteen wastewater (CW) was collected from the dining facility of the University of Moratuwa in sterile polyethylene containers, transported on ice, and processed within 24 h. Suspended solids were removed by filtration through glass microfiber filter papers (Hyundai GF/C, ϕ47 mm, 1.2 µm pore size), and the clarified wastewater was used as the experimental medium. Preliminary screening experiments were conducted to determine optimal dilution and illumination conditions for *Spirulina* sp. cultivation. Based on these results, a 75% (*v*/*v*) dilution of wastewater with sterile distilled water was selected, with pH adjusted to 9.1 using 1 M NaOH (Sigma-Aldrich, St. Louis, MO, USA). The initial chemical oxygen demand (COD) of the diluted wastewater was 342.4 mg L^{-1}. BG11 medium (HiMedia, Mumbai, India) served as the control. Both media were sterilized by autoclaving at 121 °C for 20 min.

2.2. Wastewater Characterization

Initial characterization of the canteen wastewater was performed according to APHA Standard Methods (2007) [37]. All measurements were conducted in triplicate (n = 3). The following parameters were determined:

- pH: Measured using a benchtop pH meter (Mettler Toledo SevenCompact).
- COD: Determined by the open reflux titrimetric method with potassium dichromate digestion.
- Nitrate (NO_3^-–N): UV absorbance at 220 nm with baseline correction at 275 nm.
- Phosphate (PO_4^{3-}–P): Molybdenum blue method, absorbance at 880 nm.

The characterized wastewater parameters were compared against national discharge standards (Central Environmental Authority, Sri Lanka, 2022) [38]. COD levels (420–480 mg L^{-1}) exceeded the permissible discharge limit of 250 mg L^{-1}, while nitrate (8–12 mg L^{-1}) and phosphate (5–7 mg L^{-1}) concentrations also surpassed thresholds for secondary-treated effluent ($NO_3^- \leq 5$ mg L^{-1}; $PO_4^{3-} \leq 2$ mg L^{-1}). These findings confirmed the necessity of nutrient remediation prior to discharge.

2.3. Microalgal Strain and Inoculum Preparation

A pure culture of *Spirulina* sp. was obtained from Progreen Laboratory, University of Moratuwa. Pre-cultures were grown in Zarrouk's medium under controlled conditions (25 ± 2 °C, continuous illumination at 150 µmol photons m^{-2} s^{-1} with cool-white LED lights, and aeration at 0.5 vvm sterile-filtered air) before being transferred to experimental flasks. Stationary-phase pre-cultures were used to provide a physiologically robust inoculum able to withstand rapid changes in osmotic and chemical conditions when transferred to diluted canteen wastewater. Cultures were harvested at stationary phase (defined as the plateau in OD600 of the pre-culture) and standardized to an initial biomass concentration of 0.30 g L^{-1} prior to inoculation [39,40].

2.4. Experimental Setup and Cultivation Conditions

2.4.1. Screening of Wastewater Dilution Factors

Four dilutions of canteen wastewater (25%, 50%, 75%, and 100% *v*/*v*) were prepared using distilled water, with 400 mL working volume in 500 mL Erlenmeyer flasks. Initial pH was adjusted to 9.1 using NaOH. BG11 medium (400 mL) served as the control. Cultures were incubated at 32 °C under a 12:12 h light–dark cycle, aerated continuously, and illuminated at 100 µmol photons m^{-2} s^{-1} (LED, cool-white). Biomass growth was monitored every 48 h by optical density (OD_{600}) and dry weight measurement.

2.4.2. Screening of Light Intensities

The effect of light intensity on biomass production was evaluated using the canteen wastewater medium. Four light intensities (60, 120, 180, and 240 µmol photons m^{-2} s^{-1}, provided by cool-white LEDs) were tested, with BG11 at 100 µmol photons m^{-2} s^{-1} serving as the control. Cultures were incubated under the same conditions as described in Section 2.4.1. Biomass accumulation was monitored every 48 h. The results indicated that 180 µmol photons m^{-2} s^{-1} supported the highest growth and pigment synthesis. Consequently, this intensity was selected for the main experiment, considering practical energy-use implications.

2.4.3. Main Cultivation Experiment

The main batch cultivation was performed in 2 L laboratory glass bottles (GL45) containing 1.5 L of autoclave-sterilized media. The bottles were equipped with 3-port screw caps for aeration and pressure compensation. Aeration ports were fitted with 0.45 µm polytetrafluoroethylene (PTFE) membrane filters for sterile air supply and to minimize evaporative losses. The photobioreactors were illuminated with cool white LED strips at 180 µmol photons m^{-2} s^{-1} under a 12/12 h light/dark cycle [41].

2.5. Analytical Procedures

2.5.1. Biomass Concentration

Microalgal growth was evaluated at 2-day intervals. Optical density (OD_{600}) was measured with a UV–Vis spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan). For dry weight determination, 5 mL culture aliquots were filtered through pre-dried and pre-weighed glass microfiber filter papers (Hyundai GF/C, ϕ47 mm, 1.2 µm

pore size). The filters were oven-dried at 60 °C for 24 h [41]. Biomass concentration was determined using the following equation:

$$Dry\ Weight\ (DW_n) = \frac{W_f - W_i}{V}$$

where DW_n is the biomass concentration (g L^{-1}) on day n, W_f is the final weight of the dried filter with biomass, W_i is the initial weight of the filter, and V is the sample volume.

2.5.2. Nutrient, Phosphate and COD Removal

Nitrate, phosphate, COD concentrations, and pH were determined at 2-day intervals. Culture samples (10 mL) were centrifuged at 4000 rpm for 20 min, and the supernatant was filtered through 0.22 µm nylon syringe filters [41]. Nitrate and phosphate were determined as described in Section 2.2, and COD was determined by the open reflux titrimetric method. Nutrient removal efficiency (*RE*, %) was calculated as:

$$Removal\ Efficiency\ (RE\%) = \frac{C_i - C_f}{C_i} \times 100$$

where (C_i) and (C_f) are initial and final concentrations (mg L^{-1}), respectively. COD removal efficiency was calculated similarly.

2.5.3. Analytical Validation

All analyses were performed in triplicate. UV–Vis measurements were calibrated with standard solutions (potassium nitrate, KH_2PO_4, and potassium hydrogen phthalate). Calibration curves showed excellent linearity ($R^2 \geq 0.996$). Instrumental blanks were run for each batch to eliminate baseline drift.

2.6. Biomass Harvesting and Carotenoid Extraction

At the end of the 20-day cultivation period, biomass was harvested by centrifugation at 5000 rpm for 10 min (Eppendorf 5810R, Eppendorf, Hamburg, Germany), washed twice with sterile distilled water, and oven-dried at 60 °C to constant weight [41–43]. For carotenoid extraction, 100 mg dried biomass was homogenized with 10 mL of analytical grade 95% ethanol (Merck, Darmstadt, Germany), incubated in the dark at room temperature for 1 h, and centrifuged at 5000 rpm for 10 min. Absorbance of the supernatant was measured at 470 nm (Shimadzu UV-1800), and total carotenoid content was calculated using the extinction coefficient ($E^1\%_1$cm = 2500 in ethanol) [44]. Carotenoid yield was expressed as mg g^{-1} dry weight.

$$Chlorophyll\ concentration\ (C) = \frac{A \times 1000}{E \times d}$$

$$Yield\ (Y) = \frac{C}{DW}$$

where A is absorbance at 470 nm, E is extinction coefficient, d is path length (1 cm), and DW is dry biomass weight (g).

2.7. Biochemical Composition

2.7.1. Protein Content

Protein content was determined by the micro-Kjeldahl method [45]. Dried biomass (0.1 g) was digested with concentrated H_2SO_4 and catalyst (K_2SO_4:$CuSO_4$, 10:1), distilled using a Kjeltec™ 8400 Analyzer (Foss, Hillerød, Denmark), and titrated with standardized 0.1 N HCl. Total nitrogen was converted to protein using a factor of 6.25.

2.7.2. Carbohydrate Content

Carbohydrate content was determined using a modified phenol-sulfuric acid method. A 10 mg biomass sample was treated with 0.5 mL acetic acid and heated at 80 °C for 20 min in a water bath (Memmert WNB7, Memmert, Schwabach, Germany). After cooling, pigments were extracted with 10 mL acetone and removed by centrifugation at 3500× *g* for 10 min. The resulting pellet was hydrolyzed with 2.5 mL of 4 M trifluoroacetic acid at 95 °C for 4 h. The hydrolysate was separated by centrifugation at 10,000× *g* for 5 min, and the carbohydrate content in the supernatant was quantified [41,46].

2.7.3. Lipid Content

Lipids were quantified using the Bligh and Dyer method. Dried biomass (0.5 g) was homogenized in chloroform/methanol (2:1, *v*/*v*), phase-separated with saline solution, and the organic phase evaporated to dryness. Lipid content was gravimetrically determined and expressed as % dry weight [47].

2.8. Statistical Analysis

All experiments were performed in triplicate (n = 3). The experimental data are presented as mean ± standard deviation (SD). Statistical differences between the treatment groups (different light intensities) were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using Minitab 17 software.

3. Results

The growth performance of *Spirulina* sp. was evaluated under different wastewater dilutions, light intensities, and optimized culture conditions. Key parameters included optical density (OD_{600}), dry biomass concentration, pH variation, nutrient removal efficiency, biomass productivity, carotenoid yield, and biochemical composition.

3.1. Effect of Wastewater Dilution on Growth

The OD_{600} profiles demonstrated a typical sigmoidal growth pattern across wastewater dilutions (Figure S1, see Supplementary Materials). The 75% wastewater dilution exhibited the most sustained growth, achieving an OD_{600} of 1.09 by day 19. The 100% wastewater condition peaked at 1.03 on day 14 but declined to 0.80 by day 22, indicating possible nutrient or ammonia toxicity.

Dry biomass concentrations supported these findings (Figure 1). The 75% wastewater culture reached a maximum of 0.92 g L^{-1} by day 19, significantly higher than the BG11 control (0.71 g L^{-1}, $p < 0.05$). The 100% wastewater treatment reached 0.88 g L^{-1} before declining, while the 25% and 50% dilutions supported only 0.46 and 0.64 g L^{-1}, respectively.

3.2. Effect of Light Intensity on Growth

Light intensity exerted a strong influence on *Spirulina* growth in wastewater (Figure S2, see Supplementary Materials). Cultures grown under 180 µmol m^{-2} s^{-1} attained the highest OD_{600} (1.16 by day 16). No statistically significant difference ($p > 0.05$) was observed between 180 µmol m^{-2} s^{-1} and 240 µmol m^{-2} s^{-1} (1.10). Therefore, 180 µmol m^{-2} s^{-1} was selected as the optimal intensity to minimize energy consumption. Lower light intensities (60 and 100 µmol m^{-2} s^{-1}) produced significantly lower values. Biomass measurements confirmed this trend, with 180 µmol m^{-2} s^{-1} achieving 1.05 g L^{-1} by day 16 (Figure 2).

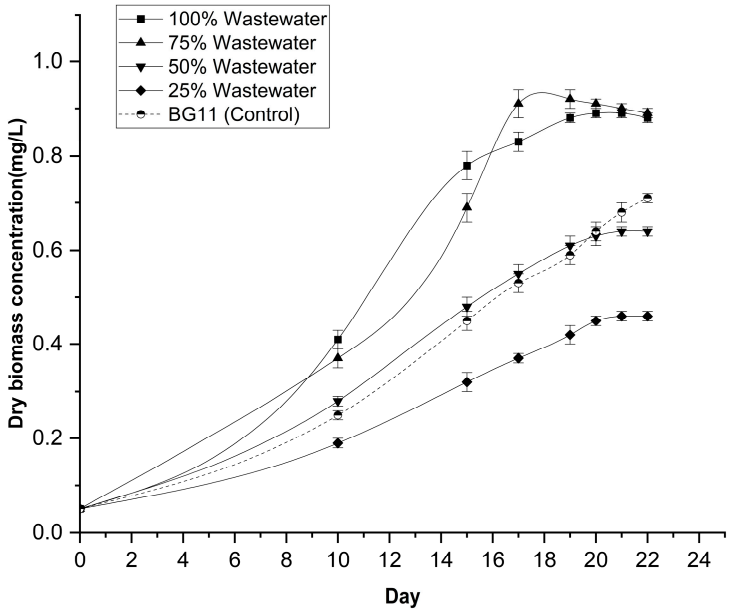

Figure 1. Dry biomass concentration of *Spirulina* sp. cultivated in different canteen wastewater dilutions.

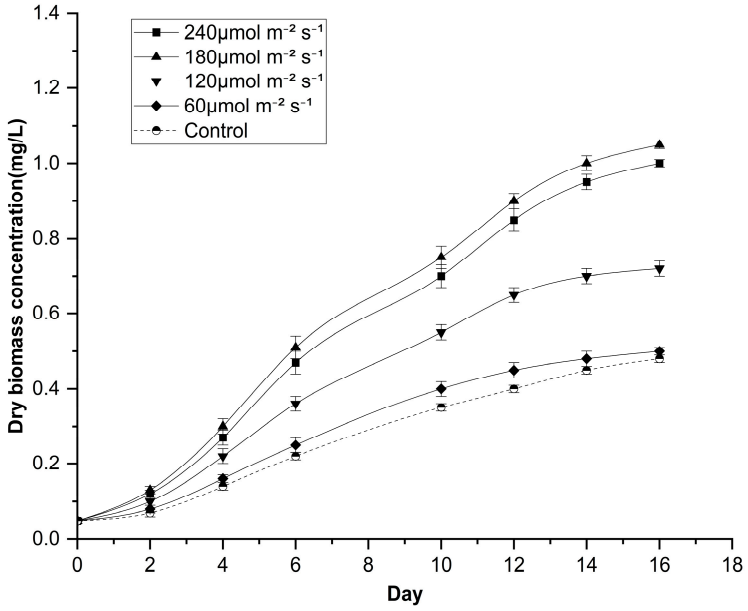

Figure 2. Dry biomass concentration of *Spirulina* sp. under different light intensities.

3.3. Growth Performance in 2 L Bioreactors (Main Experiment)

In the main experiment conducted under optimized conditions (75% CW and 180 μmol m^{-2} s^{-1}), *Spirulina* achieved markedly higher growth compared with BG11. OD_{600} increased from 0.05 ± 0.00 at inoculation to a peak of 1.88 ± 0.01 on day 18 (Table S1). Notably, the biomass accumulation in this phase exceeded that of the screening phase (OD_{600}~1.16), attributable to the superior mixing efficiency and gas exchange provided by the 2 L bioreactors compared to the Erlenmeyer flasks used in screening.

Dry biomass concentrations followed a similar trend (Figure 3 and Table 1), reaching 1.47 ± 0.01 g L^{-1} in wastewater compared with 0.52 ± 0.01 g L^{-1} in BG11 ($p < 0.05$). Color differences were evident, with wastewater cultures developing a dense green pigmentation, while BG11 remained pale.

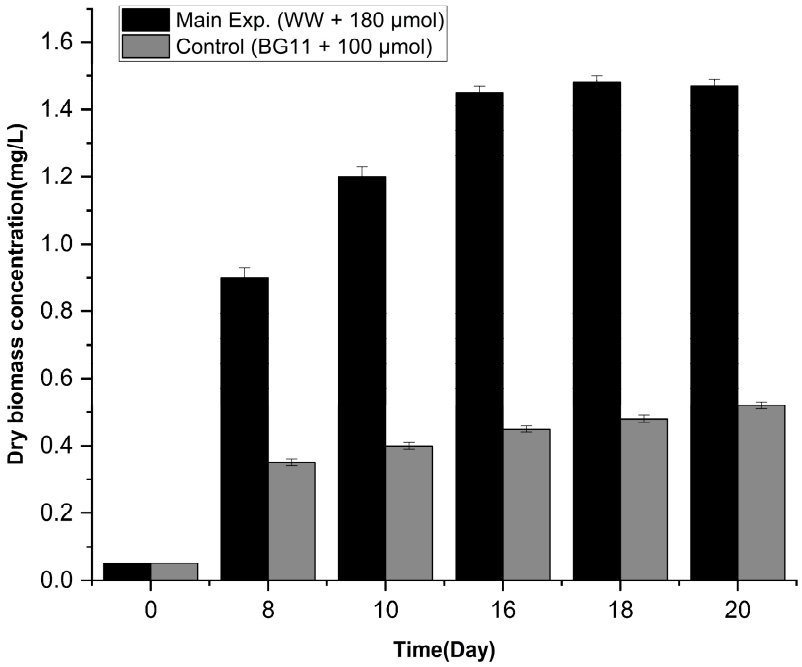

Figure 3. Dry biomass concentration of Spirulina sp. cultivated in Main Experiment (75% CW vs. BG11).

Table 1. Dry biomass concentration of Spirulina sp. cultivated in optimized canteen.

Day	75% Wastewater + 180 µmol (g/L)	Control BG11 (g/L)
0	0.05 ± 0.00^{Ea}	0.05 ± 0.00^{fa}
8	0.90 ± 0.01^{Da}	0.35 ± 0.01^{Eb}
10	1.20 ± 0.01^{ca}	0.40 ± 0.01^{Db}
16	1.45 ± 0.01^{Ba}	0.45 ± 0.01^{cb}
18	1.48 ± 0.01^{Aa}	0.48 ± 0.01^{Bb}
20	1.47 ± 0.01^{ABa}	0.52 ± 0.01^{Ab}

Values that do not share the same lowercase letter (s) within a row and the same uppercase letter(s) within a column are significantly different ($p < 0.05$). Results are mean ± standard deviation of triplicate findings. Lowercase letters indicate statistical differences between treatment; uppercase letters indicate statistical differences between time.

The pH of wastewater-grown cultures rose from 7.51 ± 0.03 on day 0 to 9.73 ± 0.05 by day 16, remaining stable thereafter, while the BG11 control increased only from 7.50 ± 0.02 to 8.60 ± 0.04 (Table 2, Figure S3).

Table 2. pH—Main Experiment (75% CW + 180 µmol).

Day	pH—Main Experiment (75% WW + 180 µmol)	pH—Control (BG11)
0	7.51 ± 0.03	7.50 ± 0.02
2	7.82 ± 0.03	7.65 ± 0.03
4	8.30 ± 0.04	7.90 ± 0.03
6	8.55 ± 0.04	8.10 ± 0.03
8	8.68 ± 0.05	8.30 ± 0.04
10	8.85 ± 0.05	8.45 ± 0.04
12	9.20 ± 0.05	8.55 ± 0.04
14	9.72 ± 0.05	8.60 ± 0.04

Table 2. *Cont.*

Day	pH—Main Experiment (75% WW + 180 µmol)	pH—Control (BG11)
16	9.73 ± 0.05	8.60 ± 0.04
18	9.72 ± 0.05	8.58 ± 0.04
20	9.70 ± 0.05	8.55 ± 0.04

3.4. Nutrient Removal Efficiency

The system demonstrated high efficiency in removing inorganic nutrients. As shown in Figures 4 and 5, phosphate and nitrate levels dropped significantly over the cultivation period. The overall nutrient removal efficiencies are summarized in Table 3. In addition to inorganic nutrients, the system effectively reduced organic loading. The Chemical Oxygen Demand (COD) in the 75% wastewater culture decreased from an initial 342.4 ± 5.2 mg L^{-1} to 58.2 ± 3.5 mg L^{-1} by day 20, corresponding to a removal efficiency of 83.00 ± 1.25%. Reductions in COD were negligible in the BG11 control, as the synthetic medium is composed primarily of inorganic salts. The substantial COD reduction in the wastewater culture confirms the mixotrophic capability of *Spirulina* to metabolize organic carbon compounds present in the canteen effluent, which correlates with the observed increase in lipid accumulation.

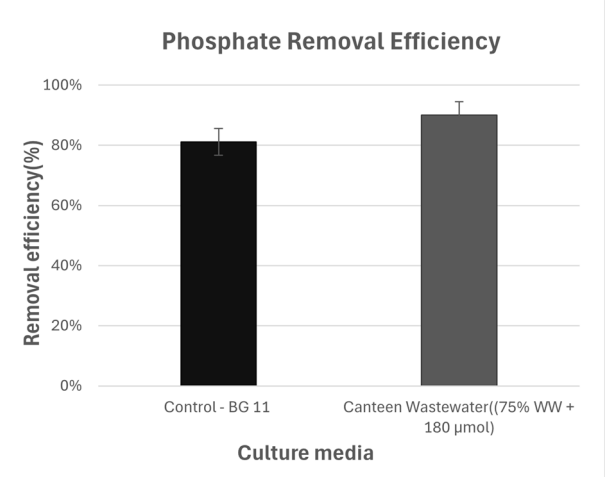

Figure 4. Phosphate removal efficiency.

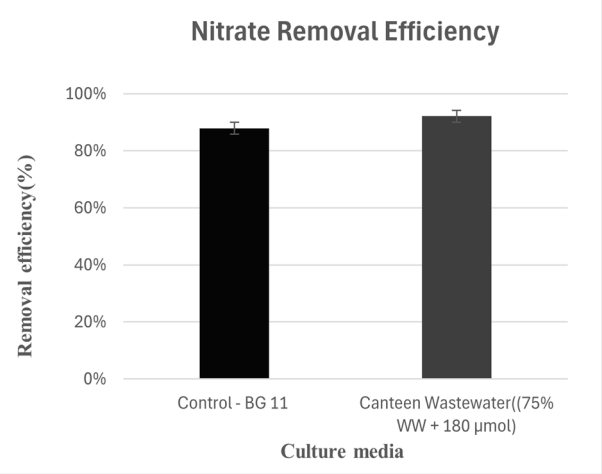

Figure 5. Nitrate removal efficiency.

Table 3. Nutrients removal efficiency (%).

Parameter	BG11 Control	Canteen Wastewater
Phosphate removal efficiency (%)	81.16 ± 2.45	90.05 ± 2.15
Nitrate removal efficiency (%)	87.93 ± 2.62	92.12 ± 2.32
COD removal efficiency (%)	-	83.00 ± 1.25%

3.5. Biomass Productivity and Biochemical Composition

Biomass productivity in wastewater cultures was 0.071 g L^{-1} day^{-1}, nearly three times higher than BG11 at 0.023 g L^{-1} day^{-1} (Table 4). Carotenoid yield under optimized conditions was 21.81 mg g^{-1} DW, significantly higher than the BG11 control (6.85 mg g^{-1} DW).

Table 4. Comparative analysis of biomass productivity and biochemical composition of *Spirulina* sp. grown in optimized Canteen Wastewater (CW) vs. BG11 Control.

Parameter	Canteen Wastewater	BG 11 Control
Biomass Productivity (g L^{-1} day^{-1})	0.071 ± 0.003 [a]	0.023 ± 0.002 [b]
Carotenoid Yield (mg g^{-1} DW)	21.81 ± 1.15 [a]	6.85 ± 0.45 [b]
Protein Content (%)	54.3 ± 2.4 [a]	61.5 ± 2.1 [b]
Carbohydrate Content (%)	13.8 ± 1.2 [a]	15.4 ± 1.2 [a]
Lipid Content (%)	7.85 ± 0.45 [a]	5.90 ± 0.35 [b]

Values are mean ± standard deviation (n = 3). Different lowercase letters within a row indicate statistically significant differences ($p < 0.05$).

The biochemical composition of the wastewater-grown biomass was characterized by 54.3% protein, 13.8% carbohydrates, and 7.85% lipids. While the protein content was slightly lower than the control (61.5%), the lipid content was significantly enhanced in the wastewater culture ($p < 0.05$), likely due to mixotrophic assimilation of organic carbon.

4. Discussion

The present study demonstrates the feasibility of using diluted canteen wastewater (CW) as a nutrient-rich growth medium for Spirulina platensis, achieving enhanced biomass productivity, effective nutrient remediation, and enriched carotenoid accumulation. Optimization of wastewater dilution (75%) and light intensity (180 µmol m^{-2} s^{-1}) produced results superior to those obtained in the standard BG-11 synthetic medium, confirming the dual potential of CW for sustainable wastewater treatment and bioproduct generation.

The screening experiments revealed that the degree of wastewater dilution critically influenced algal growth dynamics. Although undiluted (100%) CW initially supported rapid biomass accumulation, growth declined after day 14, reflecting nutrient and organic overload. This inhibition is attributable to excessive concentrations of free ammonia nitrogen, which is recognized as one of the main limiting factors in Spirulina cultivation. Previous threshold studies have established that concentrations above 1.6 mM (0.027 g L^{-1}) impair growth and that levels above 2 mM (0.034 g L^{-1}) are toxic. Similarly, total ammonia nitrogen above 217–246 mg L^{-1} induces cell death. These thresholds correspond well with the observed growth inhibition in the 100% CW treatment, where nutrient loads likely exceeded tolerance limits [48,49].

High organic loading (COD > 400 mg L^{-1}) also contributes to growth suppression by reducing light penetration and creating oxidative stress conditions unfavorable to photosynthesis. The decline in pigmentation and biomass after day 17 in undiluted CW further

supports this effect. Conversely, the 75% CW dilution provided balanced nutrient availability while maintaining non-inhibitory levels of ammonia and organic matter. Maintaining ammonium below 50% of total nitrogen is reported to sustain stable Spirulina growth, this condition was met in the optimized treatment. Consequently, cultures in 75% CW achieved the highest OD_{600} (1.09) and dry biomass (0.92 g L^{-1}), confirming that moderate dilution ensures sufficient nutrient supply while preventing toxicity [50–53].

Light intensity was another decisive parameter governing biomass yield and metabolite formation. The maximum growth obtained at 180 µmol m^{-2} s^{-1} corresponds to the reported optimal range (150–200 µmol m^{-2} s^{-1}) for photosynthetic efficiency in Spirulina [54,55]. At this irradiance, photosynthetic energy supply and metabolic demand were optimally balanced, maximizing chlorophyll and phycocyanin synthesis without inducing photoinhibition. The slightly reduced productivity at 240 µmol m^{-2} s^{-1} indicates the onset of oxidative stress, corroborated by elevated reactive oxygen species (ROS) and lipid peroxidation under high light exposure [56]. Under such conditions, Spirulina activates antioxidant enzymes—superoxide dismutase, catalase, and peroxidase—yet overall photosynthetic efficiency declines.

In contrast, cultures maintained at lower irradiances (60–100 µmol m^{-2} s^{-1}) exhibited light limitation, where photon flux was insufficient to support optimal metabolic activity despite the organic carbon contribution of CW. These observations confirm that balanced irradiance is critical for achieving high biomass and pigment productivity in nutrient-rich media. The optimized 180 µmol m^{-2} s^{-1} condition thus provides the best trade-off between light energy utilization and oxidative stress control.

This value is comparable to or exceeds yields reported for various food-industry, agro-industrial and other wastewaters (0.06–0.08 g L^{-1} day^{-1}) (Table 5) [51,57–63]. It has been observed that *Spirulina platensis* cultivated in nutrient-rich wastewaters achieved productivities between 0.06 and 0.08 g L^{-1} day^{-1}, depending on light and nitrogen availability. Likewise, previous studies have highlighted that domestic and food-industry effluents offer effective nutrient sources for microalgal biomass production, often surpassing synthetic media due to their organic complexity [61–65].

Table 5. Comparison of biomass productivity in *Spirulina* cultivation using different wastewater sources.

Scale/System Type	Maximum Productivity (g L^{-1} day^{-1})	System Volume/Area	Key Performance Metrics	Production Conditions	Species/Strain	References
Commercial raceway pond (605 m^2, strain 208)	0.058 (18.7 g m^{-2} day^{-1})	605 m^2 (industrial)	18.7 g m^{-2} day^{-1} DW	Semi-continuous, outdoor	*Spirulina* 208	[61]
Commercial raceway pond (605 m^2, strain 220)	0.041 (13.2 g m^{-2} day^{-1})	605 m^2 (industrial)	13.2 g m^{-2} day^{-1} DW	Semi-continuous, outdoor	*Spirulina* 220	[61]
Indoor raceway pond (4 m^2)	0.045 (44.75 mg L^{-1} day^{-1})	4 m^2	Highest in strain 220	Controlled indoor, pH 9.5	*Spirulina* 220	[61]
Indoor raceway pond (4 m^2)	0.029 (29.20 mg L^{-1} day^{-1})	4 m^2	pH 9.5 optimal	Controlled indoor	*Spirulina* 208	[61]
Pilot-scale cultivation (162 L)	0.12 (0.84 g L^{-1} biomass)	162 L	µ = 0.53 d^{-1} (first 3 days)	Seawater medium	*S. subsalsa*	[62]
Large-scale cultivation (10 L)	0.21 (2.43 g L^{-1} in 10 days)	10 L	2.43 g L^{-1} biomass	Batch, 10 days	*Spirulina* sp.	[59]
Lab-scale cultivation (1 L)	0.23 (2.89 g L^{-1} in 10 days)	1 L	2.89 g L^{-1} biomass	Batch, optimal	*Spirulina* sp.	[59]

Table 5. *Cont.*

Scale/System Type	Maximum Productivity (g L^{-1} day^{-1})	System Volume/Area	Key Performance Metrics	Production Conditions	Species/Strain	References
Outdoor pilot (aquaculture WW)	1.10–3.33 g L^{-1}	240 L (Pilot scale)	Weather-dependent	Outdoor, variable	*Spirulina* LEB 18	[63]
Raceway pond (1400 L)	0.05	1400 L	Modified Zarrouk	Standard operation	*A. platensis*	[61,64,65]
Present study (canteen WW, 75% dilution)	0.071	2 L (lab scale)	Threefold higher than BG-11	180 µmol m^{-2} s^{-1} light	*Spirulina* sp.	–

The productivity achieved in this work positions CW within the lower-to-middle range of industrially relevant substrates. Considering its moderate nutrient load compared with dairy or brewery wastewaters, the result reflects efficient nutrient uptake and favorable light–nutrient synergy. The achieved yield also approaches the median productivity reported for pilot- and industrial-scale systems (0.095–0.161 g L^{-1} day^{-1}), underscoring the scalability of the approach [66–69].

Nutrient removal efficiencies were equally remarkable: 90.05% for phosphate and 92.12% for nitrate. These values are comparable to or exceed removal rates reported for *Spirulina* grown in swine effluent (94% nitrate, 85% phosphate) [19,70] and align with mixed microalgal consortia treating secondary effluents (70–90% nitrate removal) [52]. Additionally, the system achieved a substantial 83% reduction in Chemical Oxygen Demand (COD), lowering concentrations from 342.4 mg L^{-1} to 58.2 mg L^{-1}. This significant organic removal confirms that *Spirulina* actively utilized the available organic carbon via mixotrophic metabolism, a mechanism that not only remediates the effluent but also contributes to the enhanced lipid accumulation observed in the biomass. Such high efficiencies confirm that *Spirulina* can effectively scavenge nutrients even from relatively dilute substrates such as CW.

The observed performance can be mechanistically explained by *Spirulina*'s adaptable nutrient uptake kinetics and stoichiometric flexibility. Phosphate assimilation occurs preferentially and more rapidly than nitrate reduction, with maximum specific uptake rates (q_{max}) of up to 6.5 mg PO_4-P g TSS^{-1} h^{-1} [71,72]. Corresponding uptake constants range from 0.4 to 1.0 d^{-1} for phosphorus and 0.2–1.8 d^{-1} for nitrogen, consistent with the slightly higher phosphate removal observed in this study. Optimal pH (≈9.0) and short adaptation times further enhance uptake efficiency, enabling *Spirulina* to maintain a relatively stable intracellular N:P ratio (10–16:1) even under variable external concentrations [73–77]. These properties make the strain particularly suitable for treating effluents with unbalanced nutrient ratios.

The biochemical composition of CW-grown *Spirulina* reflected nitrogen-replete conditions, with protein content of 54.3% and carbohydrate content of 13.8%. These values are consistent with reports for nutrient-rich wastewater cultures (protein 50–65%; carbohydrate 10–17%) [78,79]. Notably, lipid content increased significantly to 7.85% in the wastewater culture compared to 5.90% in the BG11 control ($p < 0.05$). This accumulation is likely driven by mixotrophic growth, where the organic carbon available in the wastewater is assimilated and diverted toward lipid storage rather than protein synthesis [80]. The high protein fraction indicates potential for use in functional foods, animal feed, or biofertilizer applications. However, it is crucial to note that before any commercial application in food or feed chains, the biomass must undergo rigorous screening for potential contaminants, including heavy metals, pathogens, and cyanotoxins, to ensure safety compliance [81].

Carotenoid accumulation reached 21.81 mg g^{-1} DW under optimized conditions—among the highest values reported for *Spirulina* cultivated in waste-based media. This yield compares favorably with the typical range of 2–20 mg g^{-1} DW [63,82,83] and approaches the upper values achieved under optimized light and nutrient regimes [30]. The enhanced carotenoid synthesis can be attributed to the synergistic effects of moderate irradiance and balanced N:P ratios, which maintain cellular redox homeostasis while activating pigment biosynthesis pathways [76,77]. Adequate nitrogen availability also supports the synthesis of pigment–protein complexes such as phycobiliproteins, crucial for light harvesting and photoprotection [84,85]. Excessive light or nutrient stress may further increase carotenoid accumulation but usually compromises biomass yield, emphasizing the advantage of the balanced conditions identified in this study [30,86].

From a sustainability perspective, integrating *Spirulina* cultivation into institutional wastewater management systems offers significant techno-economic benefits. Canteen wastewater is generated continuously and generally contains a balanced nutrient profile, reducing or eliminating the need for synthetic fertilizers. Utilizing such wastewater in photobioreactors or open raceway ponds can substantially lower medium preparation costs. Additionally, this approach contributes to carbon mitigation by enabling biomass to sequester CO_2, supporting institutional carbon neutrality targets. When combined with on-site renewable energy systems, such as biogas or solar, these phycoremediation frameworks can operate with minimal net energy input. Scaling up this strategy can therefore provide both economic and environmental co-benefits, aligning with circular bioeconomy principles and Sustainable Development Goal 12 (Responsible Consumption and Production) [87,88].

Collectively, these findings establish that diluted canteen wastewater can serve as an effective medium for *Spirulina* cultivation, achieving simultaneous wastewater remediation and high-value biomass production. The process exemplifies a circular bioeconomy approach, converting institutional food-service effluents into carotenoid-rich algal biomass while mitigating environmental pollution. With further pilot-scale validation, techno-economic evaluation, and integration of on-site CO_2 capture, this system can be scaled for institutional or municipal applications, linking wastewater management with nutraceutical and bioproduct manufacturing.

5. Conclusions

This study successfully demonstrated the dual potential of canteen wastewater (CW) as a sustainable medium for *Spirulina platensis* cultivation, simultaneously achieving effective phycoremediation and high-value biomass production. Under optimized conditions (75% CW dilution and 180 µmol m^{-2} s^{-1} light intensity), the system yielded a biomass productivity of 0.071 g L^{-1} day^{-1}, which was three-fold higher than that of the synthetic BG-11 control (0.023 g L^{-1} day^{-1}). The cultivation process effectively remediated the effluent, achieving removal efficiencies of 92.12% for nitrate and 90.05% for phosphate, thereby reducing pollutant loads below standard discharge limits.

Biochemical profiling confirmed that CW-grown biomass is a rich source of valuable metabolites, containing 54.3% protein, 7.85% lipids, and a remarkable carotenoid yield of 21.81 mg g^{-1} DW. The significantly enhanced carotenoid and lipid accumulation compared to the control highlights the ability of *Spirulina* to valorize wastewater stress into commercially relevant bioproducts. These findings validate the proposed circular bioeconomy model, offering a low-cost, energy-efficient alternative to conventional wastewater treatment that aligns with Sustainable Development Goals 6 and 12.

Future research should prioritize scaling up this system to pilot-stage photobioreactors to assess hydraulic stability and techno-economic viability. Furthermore, rigorous analysis of the harvested biomass for potential contaminants, including heavy metals and pathogens,

is essential to ensure safety compliance for application in animal feed or nutraceutical chains. Ultimately, this work provides a scalable framework for transforming institutional liquid waste into renewable biological resources.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/phycology6010015/s1, Figure S1: Optical density (OD 600) profile of *Spirulina* sp. cultivated in different canteen wastewater dilutions; Figure S2: Optical Density of Spirulina sp. under varying light intensities. Figure S3: Detailed variation of pH during *Spirulina* sp. cultivation in 75% Canteen Wastewater compared to BG11 control. Table S1: Optical Density (OD600) of Spirulina sp. cultivated in optimized canteen.

Author Contributions: Conceptualization, C.A.D.; methodology, C.A.D.; validation, C.A.D.; formal analysis, C.A.D.; investigation, C.A.D.; data curation, C.A.D.; writing—original draft preparation, C.A.D.; writing—review and editing, J.C.K., T.A.P., D.R. and R.U.H.; visualization, C.A.D., I.A.W. and G.N.G.; supervision, J.C.K., T.A.P., D.R. and R.U.H.; project administration, R.U.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available in the article and Supplementary Material.

Acknowledgments: The authors wish to acknowledge the Kalama Mithuro organization for their support during this research. We extend our sincere gratitude to Dhammika Dharmaratne for his valuable support. We also thank the technical officers W. B. U. Rukma, L. M. Dushantha, and D. M. B. Wickramasinghe for their technical assistance, and W. J. Shantha for his support in the laboratory.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zhai, J.; Li, X.; Li, W.; Rahaman, M.H.; Zhao, Y.; Wei, B.; Wei, H. Optimization of biomass production and nutrients removal by Spirulina platensis from municipal wastewater. *Ecol. Eng.* **2017**, *108*, 83–92. [CrossRef]
2. Dodangodage, C.A.; Premarathne, H.; Kasturiarachchi, J.C.; Perera, T.A.; Rajapakshe, D.; Halwatura, R.U. Algae-Based Protective Coatings for Sustainable Infrastructure: A Novel Framework Linking Material Chemistry, Techno-Economics, and Environmental Functionality. *Phycology* **2025**, *5*, 84. [CrossRef]
3. Khan, M.A.; Uddin, I.; Othman, N.S. Factors determining water stress: Do environmental distress, energy consumption and industrialization matter? *Environ. Dev. Sustain.* **2025**, *27*, 25273–25297. [CrossRef]
4. Dodangodage, C.A.; Kasturiarachchi, J.; Perera, T.; Rajapakshe, D.; Halwatura, R. Integrated Microalgal-Aquaponic Systems for Enhanced Water Treatment and Food Security: A Critical Review of Recent Advances in Process Integration and Resource Recovery. *Biol. Biotechnol.* 2025; preprint. [CrossRef]
5. Singh, P.K.; Kumar, U.; Kumar, I.; Dwivedi, A.; Singh, P.; Mishra, S.; Seth, C.S.; Sharma, R.K. Critical review on toxic contaminants in surface water ecosystem: Sources, monitoring, and its impact on human health. *Environ. Sci. Pollut. Res.* **2024**, *31*, 56428–56462. [CrossRef] [PubMed]
6. Ozcicek-Dolekoglu, C.; Var, I. Analysis of food waste in university dining halls: A case study from Turkey. *Fresenius Environ. Bull.* **2019**, *28*, 156–166.
7. Li, H.; Li, X.; Cui, L.; Alam, A.; Lu, W. Coupling nutrient removal and biodiesel production by cultivation of Chlorella sp. in cafeteria wastewater: Assessment of the effect of wastewater disinfection. *Desalination Water Treat.* **2023**, *291*, 101–107. [CrossRef]
8. Ragaza, J.A.; Hossain, S.; Meiler, K.A.; Velasquez, S.F.; Kumar, V. A review on Spirulina: Alternative media for cultivation and nutritive value as an aquafeed. *Rev. Aquac.* **2020**, *12*, 2371–2395. [CrossRef]
9. Chen, Y.; Sun, L.-P.; Liu, Z.-H.; Martin, G.; Sun, Z. Integration of Waste Valorization for Sustainable Production of Chemicals and Materials via Algal Cultivation. *Top. Curr. Chem.* **2017**, *375*, 89. [CrossRef]
10. Ruslinda, Y.; Nur, A.; Komala, P.S.; Leni, L.; Regia, R.A.; Mardatillah, R. Characterization of Wastewater in The University Campus: A Case Study in Universitas Andalas, Indonesia. *Dampak* **2025**, *21*, 18–26. [CrossRef]
11. Gude, V.G. Desalination and water reuse to address global water scarcity. *Rev. Environ. Sci. Biotechnol.* **2017**, *16*, 591–609. [CrossRef]
12. Dada, E.O.; Agu, C.M.; Akinola, M.O. Physicochemical Quality and Genotoxic Potential of Wastewater Generated by Canteen Complex. *ARO-Sci. J. Koya Univ.* **2019**, *7*, 19. [CrossRef]

13. Mohadi, R.; Hermansyah; Mavala, H.; Zulkifli, H. The effect of metal ion Cd(II) concentration on the growth of *Spirulina* sp. cultured on BG-11 medium. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *530*, 012036. [CrossRef]
14. de Morais, E.G.; da Silveira, J.T.; Schüler, L.M.; de Freitas, B.C.B.; Costa, J.A.V.; de Morais, M.G.; Ferrer, I.; Barreira, L. Biomass valorization via pyrolysis in microalgae-based wastewater treatment: Challenges and opportunities for a circular bioeconomy. *J. Appl. Phycol.* **2023**, *35*, 2689–2708. [CrossRef]
15. Gupta, S.; Marchetti, J.M.; Wasewar, K.L. Enhancing nutrient removal, biomass production, and biochemical production by optimizing microalgae cultivation in a mixture of untreated and anaerobically digested dairy wastewater. *J. Water Process Eng.* **2024**, *63*, 105413. [CrossRef]
16. Sarker, N.K.; Kaparaju, P. Microalgal Bioeconomy: A Green Economy Approach Towards Achieving Sustainable Development Goals. *Sustainability* **2024**, *16*, 11218. [CrossRef]
17. Dammak, I.; Fersi, M.; Hachicha, R.; Abdelkafi, S. Current Insights into Growing Microalgae for Municipal Wastewater Treatment and Biomass Generation. *Resources* **2023**, *12*, 119. [CrossRef]
18. Salbitani, G.; Carfagna, S. Ammonium utilization in microalgae: A sustainable method for wastewater treatment. *Sustainability* **2021**, *13*, 956. [CrossRef]
19. Lu, W.; Lin, M.; Guo, X.; Lin, Z. Cultivation of Spirulina platensis using raw piggery wastewater for nutrients bioremediation and biomass production: Effect of ferrous sulfate supplementation. *Desalination Water Treat.* **2020**, *175*, 60–67. [CrossRef]
20. Balan, V.; Pierson, J.; Husain, H.; Kumar, S.; Saffron, C.; Kumar, V. Potential of using microalgae to sequester carbon dioxide and processing to bioproducts. *Green Chem.* **2023**, *25*, 7934–7951. [CrossRef]
21. Kumar, S.; Ghangrekar, M.M. Algae-Based Bioenergy Production as a Carbon Mitigation Technology. In *Algae as a Natural Solution for Challenges in Water-Food-Energy Nexus: Toward Carbon Neutrality*; Kurniawan, T.A., Anouzla, A., Eds.; Springer Nature: Singapore, 2024; pp. 833–850. [CrossRef]
22. Salman, M.; Adeel, S.; Naseer, M.; Haider, M.Z.; Anjum, F. Modern Approach Toward Algal-Based Natural Pigments for Textiles. In *Plant Biomass Derived Materials: Sources, Extractions, and Applications*; John Wiley and Sons: Hoboken, NJ, USA, 2024; pp. 213–241. [CrossRef]
23. Kaur, P. Microalgae as Nutraceutical for Achieving Sustainable Food Solution in Future. In *Microbial Biotechnology: Basic Research and Applications*; Singh, J., Vyas, A., Wang, S., Prasad, R., Eds.; Springer: Singapore, 2020; pp. 91–125. [CrossRef]
24. El-Sayed, A.E.-K.B.; Almutairi, A.W. Nutrient balance for enhanced recovery of stressed Spirulina platensis. *Environ. Sci. Pollut. Res.* **2024**, *31*, 56685–56696. [CrossRef] [PubMed]
25. Marzorati, S.; Schievano, A.; Idà, A.; Verotta, L. Carotenoids, chlorophylls and phycocyanin from Spirulina: Supercritical CO_2 and water extraction methods for added value products cascade. *Green Chem.* **2020**, *22*, 187–196. [CrossRef]
26. Prates, J.A.M. Applications of Bioactive Compounds from Marine Microalgae in Health, Cosmetics, and Functional Foods. *Appl. Sci.* **2025**, *15*, 6144. [CrossRef]
27. Wils, L.; Yagmur, M.; Phelippe, M.; Montigny, B.; Clément-Larosière, B.; Jacquemin, J.; Boudesocque-Delaye, L. Alternative Solvents for the Biorefinery of Spirulina: Impact of Pretreatment on Free Fatty Acids with High Added Value. *Mar. Drugs* **2022**, *20*, 600. [CrossRef]
28. Yaqoob, S.; Riaz, M.; Shabbir, A.; Zia-Ul-Haq, M.; Alwakeel, S.S.; Bin-Jumah, M. Commercialization and Marketing Potential of Carotenoids. In *Carotenoids: Structure and Function in the Human Body*; Zia-Ul-Haq, M., Dewanjee, S., Riaz, M., Eds.; Springer: Berlin/Heidelberg, Germany, 2021; pp. 799–826. [CrossRef]
29. Hamidi, M.; Mohammadi, A.; Mashhadi, H.; Mahmoudnia, F. Evaluation of effective environmental parameters on lipid, protein and beta-carotene production in Spirulina platensis microalga. *Results Eng.* **2023**, *18*, 101102. [CrossRef]
30. Mohammed, I.A.; Ruengjitchatchawalya, M.; Paithoonrangsarid, K. Cultivation manipulating zeaxanthin-carotenoid production in Arthrospira (*Spirulina*) platensis under light and temperature stress. *Algal Res.* **2023**, *76*, 103315. [CrossRef]
31. Vellaiyan, S. Unlocking the potential of Spirulina microalgae: Cultivating in wastewater treatment for sustainable biofuel production. *Sustain. Chem. Pharm.* **2024**, *41*, 101705. [CrossRef]
32. Zhang, F.; Man, Y.B.; Mo, W.Y.; Wong, M.H. Application of Spirulina in aquaculture: A review on wastewater treatment and fish growth. *Rev. Aquac.* **2020**, *12*, 582–599. [CrossRef]
33. Bürck, M.; Fratelli, C.; Campos Assumpção de Amarante, M.; Braga, A.R.C. Unveiling the Potential of Spirulina Biomass—A Glimpse into the Future Circular Economy Using Green and Blue Ingredients. *Biomass* **2024**, *4*, 704–719. [CrossRef]
34. Cardoso, L.G.; Duarte, J.H.; Andrade, B.B.; Lemos, P.V.F.; Costa, J.A.V.; Druzian, J.I.; Chinalia, F.A. *Spirulina* sp. LEB 18 cultivation in outdoor pilot scale using aquaculture wastewater: High biomass, carotenoid, lipid and carbohydrate production. *Aquaculture* **2020**, *525*, 735272. [CrossRef]
35. Saini, J.K.; Gupta, R.; Hemansi; Verma, A.; Gaur, P.; Saini, R.; Shukla, R.; Kuhad, R.C. Integrated Lignocellulosic Biorefinery for Sustainable Bio-Based Economy. In *Sustainable Approaches for Biofuels Production Technologies*; Srivastava, N., Srivastava, M., Mishra, P., Upadhyay, S., Ramteke, P., Gupta, V., Eds.; Springer: Cham, Switzerland, 2019; pp. 25–46. [CrossRef]

36. Ijaz, S.; Ahmed, M. Harnessing Microalgae for Wastewater Treatment: Optimizing Cultivation, Pollutant Removal, and Photobioreactor Technologies for a Sustainable Future. In *Green Technologies for Wastewater Treatment and Bioenergy Production*; CRC Press: Boca Raton, FL, USA, 2025; pp. 64–109.
37. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 1926; Volume 6.
38. Indrani, M.W.; Mafasia, M.B.F.; Gunawardana, T.; Sriyani, G.T.W.; Gunarathne, K. The Role of Central Environmental Authority in Managing Solid Waste in Sri Lanka. *J. Account. Financ.* **2024**, *11*, 1–24. [CrossRef]
39. Mattos, E.R.; Singh, M.; Cabrera, M.L.; Das, K.C. Biorefining and Carbon Cycling Program. Effects of Inoculum Physiological Stage on the Growth Characteristics of Chlorella sorokiniana Cultivated Under Different CO_2 Concentrations. *Appl. Biochem. Biotechnol.* **2012**, *168*, 519–530. [CrossRef] [PubMed]
40. Nicodemou, A.; Konstantinou, D.; Koutinas, M. Enhanced biomass and lipid production from olive processing wastewater using Scenedesmus obliquus in a two-stage cultivation strategy under salt stress. *Biochem. Eng. J.* **2024**, *205*, 109290. [CrossRef]
41. Premaratne, M.; Liyanaarachchi, V.C.; Nishshanka, G.K.S.H.; Nimarshana, P.H.V.; Ariyadasa, T.U. Nitrogen-limited cultivation of locally isolated *Desmodesmus* sp. For sequestration of CO_2 from simulated cement flue gas and generation of feedstock for biofuel production. *J. Environ. Chem. Eng.* **2021**, *9*, 105765. [CrossRef]
42. Richmond, A. *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*; Blackwell Science Ltd.: Oxford, UK, 2004.
43. Potts, M. *Nostoc. The Ecology of Cyanobacteria*; Whitton, B.A., Potts, M., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000.
44. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In *Methods in Enzymology*; Elsevier: New York, NY, USA, 1987; Volume 148, pp. 350–382.
45. Michałowski, T.; Navas, M.J.; Asuero, A.G.; Wybraniec, S. An Overview of the Kjeldahl Method of Nitrogen Determination. Part I. Early History, Chemistry of the Procedure, and Titrimetric Finish. *Crit. Rev. Anal. Chem.* **2013**, *43*, 178–223. [CrossRef]
46. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Roberts, P.A.; Smith, F. Phenol sulphuric acid method for carbohydrate determination. *Ann. Chem.* **1956**, *28*, 350–359. [CrossRef]
47. Pérez-Palacios, T.; Ruiz, J.; Martín, D.; Muriel, E.; Antequera, T. Comparison of different methods for total lipid quantification in meat and meat products. *Food Chem.* **2008**, *110*, 1025–1029. [CrossRef]
48. Karimi, S.; Shaygannejad, V.; Mohammadalipour, A.; Feizi, A.; Hooshmand, S.; Kafeshani, M. Effects of spirulina (*Arthrospira*) platensis supplementation on inflammation, physical and mental quality of life, and anthropometric measures in patients with relapsing-remitting multiple sclerosis (RRMS): A triple-blinded, randomized, placebo-controlled trial. *Nutr. J.* **2025**, *24*, 132. [CrossRef]
49. Mallamaci, R.; Storelli, M.M.; Barbarossa, A.; Messina, G.; Valenzano, A.; Meleleo, D. Potential Protective Effects of Spirulina (Spirulina platensis) against In Vitro Toxicity Induced by Heavy Metals (Cadmium, Mercury, and Lead) on SH-SY5Y Neuroblastoma Cells. *Int. J. Mol. Sci.* **2023**, *24*, 17076. [CrossRef]
50. Yuan, X.; Kumar, A.; Sahu, A.K.; Ergas, S.J. Impact of ammonia concentration on Spirulina platensis growth in an airlift photobioreactor. *Bioresour. Technol.* **2011**, *102*, 3234–3239. [CrossRef]
51. Mirhosseini, N.; Davarnejad, R.; Hallajisani, A.; Cano-Europa, E.; Tavakoli, O.; Franco-Colín, M.; Blas-Valdivia, V. Cultivations of Arthrospira maxima (Spirulina) using ammonium sulfate and sodium nitrate as an alternative nitrogen sources. *Iran. J. Fish. Sci.* **2021**, *20*, 475–489. [CrossRef]
52. Attene, L.; Deiana, A.; Carucci, A.; De Gioannis, G.; Asunis, F.; Ledda, C. Efficient Nitrogen Recovery from Agro-Energy Effluents for Cyanobacteria Cultivation (Spirulina). *Sustainability* **2023**, *15*, 675. [CrossRef]
53. Converti, A.; Scapazzoni, S.; Lodi, A.; Carvalho, J.C.M. Ammonium and urea removal by Spirulina platensis. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 8–16. [CrossRef] [PubMed]
54. Kim, S.; Moon, M.; Kwak, M.; Lee, B.; Chang, Y.K. Statistical optimization of light intensity and CO_2 concentration for lipid production derived from attached cultivation of green microalga *Ettlia* sp. *Sci. Rep.* **2018**, *8*, 15390. [CrossRef] [PubMed]
55. Shaikh, S.; McKay, G.; Mackey, H.R. Light intensity effects on bioproduct recovery from fuel synthesis wastewater using purple phototrophic bacteria in a hybrid biofilm-suspended growth system. *Biotechnol. Rep.* **2024**, *44*, e00863. [CrossRef] [PubMed]
56. Marie, M.; Bigot, K.; Angebault, C.; Barrau, C.; Gondouin, P.; Pagan, D.; Fouquet, S.; Villette, T.; Sahel, J.-A.; Lenaers, G.; et al. Light action spectrum on oxidative stress and mitochondrial damage in A2E-loaded retinal pigment epithelium cells. *Cell Death Dis.* **2018**, *9*, 287. [CrossRef]
57. Prakash Pandey, J.; Tiwari, A. Optimization of Biomass Production of *Spirulina maxima*. *J. Algal Biomass Utln* **2010**, *1*, 20–32.
58. Lao, I.K.M.; Edullantes, B. Growth, Productivity, and Size Structure of Spirulina Strain Under Different Salinity Levels: Implications for Cultivation Optimization. *Phycology* **2025**, *5*, 31. [CrossRef]
59. Lim, H.R.; Khoo, K.S.; Show, P.L. Impact of nutrient deficiency and harvesting strategy on biomass and phycocyanin production in Spirulina cultures. *Front. Bioeng. Biotechnol.* **2025**, *13*, 1546801. [CrossRef]

60. Villaró, S.; Morillas-España, A.; Acién, G.; Lafarga, T. Optimisation of Operational Conditions during the Production of Arthrospira platensis Using Pilot-Scale Raceway Reactors, Protein Extraction, and Assessment of their Techno-Functional Properties. *Foods* **2022**, *11*, 2341. [CrossRef]
61. Zhu, B.; Shen, H.; Li, Y.; Liu, Q.; Jin, G.; Han, J.; Zhao, Y.; Pan, K. Large-Scale Cultivation of Spirulina for Biological CO_2 Mitigation in Open Raceway Ponds Using Purified CO_2 From a Coal Chemical Flue Gas. *Front. Bioeng. Biotechnol.* **2020**, *7*, 441. [CrossRef] [PubMed]
62. Liu, M.; Jiang, L.; Yu, Z.; Ma, M.; Chen, H.; Pei, H. Cultivation strategy optimization and pilot-scale production of Spirulina subsalsa grown in seawater and monosodium glutamate wastewater. *Bioresour. Bioprocess.* **2025**, *12*, 83. [CrossRef] [PubMed]
63. Cardoso, L.G.; Lombardi, A.T.; de Jesus Silva, J.S.; Lemos, P.V.F.; Costa, J.A.V.; de Souza, C.O.; Druzian, J.I.; Chinalia, F.A. Scaling-up production of *Spirulina* sp. LEB18 grown in aquaculture wastewater. *Aquaculture* **2021**, *544*, 737045. [CrossRef]
64. Hoeniges, J.; Welch, W.; Pruvost, J.; Pilon, L. A novel external reflecting raceway pond design for improved biomass productivity. *Algal Res.* **2022**, *65*, 102742. [CrossRef]
65. Rayen, F.; Behnam, T.; Dominique, P. Optimization of a raceway pond system for wastewater treatment: A review. *Crit. Rev. Biotechnol.* **2019**, *39*, 422–435. [CrossRef]
66. Baraldi, L.; Usai, L.; Torre, S.; Fais, G.; Casula, M.; Dessi, D.; Nieri, P.; Concas, A.; Lutzu, G.A. Dairy Wastewaters to Promote Mixotrophic Metabolism in Limnospira (Spirulina) platensis: Effect on Biomass Composition, Phycocyanin Content, and Fatty Acid Methyl Ester Profile. *Life* **2025**, *15*, 184. [CrossRef]
67. Ngerem, E.C.; Sanusi, I.A.; Kana, G.E.B.; Olaniran, A.O. Optimization of co-valorisation techniques for dairy and paper pulp wastewater in the cultivation of Chlorococcum sp. with a focus on mixture design, microwave-assisted pretreatment, and bioethanol production. *Heliyon* **2025**, *11*, e42531. [CrossRef]
68. Usai, G.; Cordara, A.; Mazzocchi, E.; Re, A.; Fino, D.; Pirri, C.F.; Menin, B. Coupling dairy wastewaters for nutritional balancing and water recycling: Sustainable heterologous 2-phenylethanol production by engineered cyanobacteria. *Front. Bioeng. Biotechnol.* **2024**, *12*, 1359032. [CrossRef]
69. AlFadhly, N.K.Z.; Alhelfi, N.; Altemimi, A.B.; Verma, D.K.; Cacciola, F. Tendencies Affecting the Growth and Cultivation of Genus Spirulina: An Investigative Review on Current Trends. *Plants* **2022**, *11*, 3063. [CrossRef]
70. Hajri, A.K.; Alsharif, I.; Albalawi, M.A.; Alshareef, S.A.; Albalawi, R.K.; Jamoussi, B. Utilizing Mixed Cultures of Microalgae to Up-Cycle and Remove Nutrients from Dairy Wastewater. *Biology* **2024**, *13*, 591. [CrossRef]
71. Lochab, S.; Oberoi, H.S.; Gothwal, M.; Abbey, D.; Raghuram, N. Nitrate assimilatory enzymes of Spirulina (*Arthospira*) platensis are more thermotolerant than those of rice. *Physiol. Mol. Biol. Plants* **2009**, *15*, 277–280. [CrossRef] [PubMed]
72. Ali, A.; Jha, P.; Sandhu, K.S.; Raghuram, N. Spirulina nitrate-assimilating enzymes (NR, NiR, GS) have higher specific activities and are more stable than those of rice. *Physiol. Mol. Biol. Plants* **2008**, *14*, 179–182. [CrossRef] [PubMed]
73. Aizpuru, A.; González-Sánchez, A. Traditional and new trend strategies to enhance pigment contents in microalgae. *World J. Microbiol. Biotechnol.* **2024**, *40*, 272. [CrossRef] [PubMed]
74. Bossa, R.; Di Colandrea, M.; Salbitani, G.; Carfagna, S. Phosphorous Utilization in Microalgae: Physiological Aspects and Applied Implications. *Plants* **2024**, *13*, 2127. [CrossRef]
75. Sočo, E.; Domoń, A.; Azizi, M.; Pająk, D.; Cieniek, B.; Michel, M.M.; Papciak, D. Efficiency of *Spirulina* sp. in the Treatment of Model Wastewater Containing Ni(II) and Pb(II). *Materials* **2025**, *18*, 3639. [CrossRef]
76. Iba, W.; Akib, N.I.; Jumardin, L.O.M.; Arif, B.; Yosalina, S.; Andas, J.A. *Organic Culture Media for Sustainable Carotenoid Production From Microalgae*; IntechOpen: London, UK, 2023.
77. Guedes, A.C.; Amaro, H.M.; Malcata, F.X. Microalgae as sources of carotenoids. *Mar. Drugs* **2011**, *9*, 625–644. [CrossRef]
78. Lim, H.R.; Khoo, K.S.; Chew, K.W.; Chang, C.K.; Munawaroh, H.S.H.; Kumar, P.S.; Huy, N.D.; Show, P.L. Perspective of Spirulina culture with wastewater into a sustainable circular bioeconomy. *Environ. Pollut.* **2021**, *284*, 117492. [CrossRef]
79. Podgórska-Kryszczuk, I. Spirulina—An Invaluable Source of Macro- and Micronutrients with Broad Biological Activity and Application Potential. *Molecules* **2024**, *29*, 5387. [CrossRef]
80. Peng, Y.; Gao, F.; Hang, W.W.; Yang, H.; Jin, W.; Li, C. Effects of organic matters in domestic wastewater on lipid/carbohydrate production and nutrient removal of Chlorella vulgaris cultivated under mixotrophic growth conditions. *J. Chem. Technol. Biotechnol.* **2019**, *94*, 3578–3584. [CrossRef]
81. Nynas, A.-L.; Berndtsson, E.; Newson, W.R.; Hovmalm, H.P.; Johansson, E. Protein Fractionation of Leafy Green Biomass at the Pilot Scale: Partitioning and Type of Nitrogen in the Fractions and Their Usefulness for Food and Feed. *ACS Food Sci. Technol.* **2024**, *4*, 126–138. [CrossRef]
82. Park, W.S.; Kim, H.J.; Li, M.; Lim, D.H.; Kim, J.; Kwak, S.S.; Kang, C.M.; Ferruzzi, M.G.; Ahn, M.J. Two classes of pigments, carotenoids and c-phycocyanin, in spirulina powder and their antioxidant activities. *Molecules* **2018**, *23*, 2065. [CrossRef]
83. Ismaiel, M.M.S.; El-Ayouty, Y.M.; Piercey-Normore, M. Role of pH on antioxidants production by Spirulina (*Arthrospira*) platensis. *Braz. J. Microbiol.* **2016**, *47*, 298–304. [CrossRef]

84. Murton, J.; Nagarajan, A.; Nguyen, A.Y.; Liberton, M.; Hancock, H.A.; Pakrasi, H.B.; Timlin, J.A. Population-level coordination of pigment response in individual cyanobacterial cells under altered nitrogen levels. *Photosynth. Res.* **2017**, *134*, 165–174. [CrossRef]
85. Kumar, D.; Kannaujiya, V.K.; Richa; Pathak, J.; Sundaram, S.; Sinha, R.P. Composition and functional property of photosynthetic pigments under circadian rhythm in the cyanobacterium Spirulina platensis. *Protoplasma* **2018**, *255*, 885–898. [CrossRef]
86. Fu, W.; Guomundsson, Ó.; Paglia, G.; Herjólfsson, G.; Andrésson Ó, S.; Palsson, B.O.; Brynjólfsson, S. Enhancement of carotenoid biosynthesis in the green microalga Dunaliella salina with light-emitting diodes and adaptive laboratory evolution. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 2395–2403. [CrossRef]
87. Bazyar, P.; Sheidaee, E. Algae-Based Carbon Sequestration: Optimizing Renewable Energy and Climate Strategy. In *Advances in AI for Simulation and Optimization of Energy SystemsI*; CRC Press: Boca Raton, FL, USA, 2025; pp. 83–108.
88. Bedru, T.K.; Garuma, W.B.; Baskaran, R.; Jayakumar, M.; Selvaganapathy, T. Road Map from Microalgae Biomass to Biorefinery Prospects and Challenges. In *Algal Biorefineries in Circular Economy for Climate Change Resilience*; CRC Press: Boca Raton, FL, USA, 2025; pp. 57–80.

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