






Article

Production of Carbohydrate-Rich *Chlorella* sp. Biomass Using Clarified Aquaponics Effluent for Bioethanol Feedstock Applications

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Abstract

The integration of microalgal cultivation with wastewater streams offers a promising pathway to enhance resource efficiency within circular bioeconomy frameworks. However, the suitability of clarified aquaponics sedimentation effluent for producing carbohydrate-rich microalgal biomass remains insufficiently evaluated, particularly with respect to nutrient recovery and bioethanol-relevant feedstock potential. In this study, clarified aquaponics sedimentation effluent was assessed as a cultivation medium for *Chlorella* sp. under controlled laboratory conditions. Biomass productivity, nutrient removal performance, and carbohydrate accumulation were systematically evaluated and compared with conventional synthetic medium. *Chlorella* sp. cultivated in clarified aquaponic effluent achieved a maximum biomass concentration of approximately 2.05 g L⁻¹, exceeding that obtained in Bold's Basal Medium. Carbohydrate content exceeded 40% of dry weight, indicating suitability for fermentable sugar production. Nitrate and phosphate removal efficiencies greater than 95% were achieved, with mass balance analysis confirming biological assimilation as the primary removal mechanism (~87.4%). This confirms the dual functionality of the system. The effective nutrient assimilation and confirmed the dual functionality of the system as both a biomass production and nutrient recovery process. Comparable performance under diluted and undiluted effluent conditions further indicated that freshwater dilution is not required following clarification. Light saturation was observed at 180–190 μmol m⁻² s⁻¹, providing guidance for energy-efficient operation. These findings demonstrate that clarified aquaponics effluent can serve as an effective alternative growth medium for producing carbohydrate-rich *Chlorella* sp. biomass while enabling nutrient recovery. The estimated bioethanol potential is theoretical, based on stoichiometric conversion assumptions, and experimental fermentation was not conducted. This work provides quantitative evidence supporting the integration of microalgae into aquaponic systems and establishes a foundation for future pilot-scale, techno-economic, and life-cycle assessments.



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1. Introduction

The world is facing interconnected pressures arising from rapid population growth, increasing urbanisation, rising energy demand, and growing water insecurity. Global primary energy consumption has continued to increase over recent decades and reached approximately 620 Exajoules (EJ) in 2023, with further growth projected as developing economies expand and standards of living improve [1,2]. Simultaneously, freshwater resources are under increasing stress due to climate change, pollution, and inefficient use in agriculture and industry, with the agricultural sector accounting for nearly 72% of global freshwater withdrawals, making water scarcity a critical global risk [3–5]. Addressing these coupled challenges requires integrated, resource-efficient solutions that simultaneously reduce energy dependence, freshwater consumption, and nutrient losses.

Liquid biofuels such as bioethanol have been promoted as renewable alternatives to fossil-derived transport fuels; however, many first- and second-generation bioethanol pathways rely heavily on food crops and resource-intensive agricultural practices. Large-scale ethanol production from corn, sugarcane, and other terrestrial feedstocks competes for arable land, increases freshwater demand, and can contribute to food price volatility and indirect land-use change [5,6]. These limitations have intensified interest in third-generation biofuel feedstocks, which aim to decouple fuel production from food systems by utilising non-food biomass, marginal resources, and low-quality water within circular bioeconomy frameworks [7–9].

Microalgae have emerged as promising third-generation feedstocks due to their rapid growth rates, high areal productivity, and ability to accumulate substantial quantities of carbohydrates under suitable cultivation conditions [7,10]. Recent studies demonstrate that freshwater microalgae cultivated under optimised environmental and nutritional regimes can function as effective bioenergy feedstocks while delivering additional environmental benefits [11]. Among these, *Chlorella* sp. is particularly attractive due to its physiological robustness, tolerance to variable nutrient conditions, and capacity to synthesise carbohydrate-rich biomass that can be hydrolysed into fermentable sugars [12,13]. Beyond biofuel production, microalgal cultivation enables simultaneous nutrient recovery, CO₂ assimilation, and wastewater polishing, thereby improving overall process sustainability [1,14,15].

Despite these advantages, the economic and environmental viability of algal biofuel systems remains strongly dependent on the choice of cultivation medium and the extent to which algal growth can be integrated into existing nutrient and water streams. Wastewater-based cultivation has been widely reported as an effective strategy to reduce nutrient costs, minimise freshwater use, and enhance sustainability through nutrient recycling [16–18]. However, most reported studies focus on municipal wastewater, industrial effluents, or raw aquaculture discharges, often prioritising biomass productivity rather than carbohydrate accumulation relevant to bioethanol production [19–21].

Aquaponics integrates recirculating aquaculture with hydroponic plant cultivation, creating a resource-efficient food production system in which fish waste-derived nutrients support plant growth while plants improve water quality for fish [14,22,23]. To maintain system stability, aquaponic operations employ solids removal and clarification processes, generating a continuous stream of clarified aquaponic sedimentation effluent enriched in dissolved nitrogen and phosphorus but containing relatively low suspended solids

and pathogen loads [14,24]. This clarified fraction represents a distinct and comparatively cleaner nutrient stream than raw aquaculture or municipal wastewaters, yet it has received limited attention as a targeted medium for carbohydrate-oriented microalgal cultivation.

Utilising clarified aquaponic effluent for microalgal cultivation offers several advantages: (i) substitution of freshwater with recycled effluent, reducing water demand; (ii) recovery of dissolved nutrients through conversion into algal biomass; and (iii) enhanced system integration by further polishing aquaponic effluents prior to discharge [25,26]. Nevertheless, critical uncertainties remain regarding whether clarified aquaponic effluents can support not only high biomass productivity, but also elevated intracellular carbohydrate accumulation required for bioethanol-relevant feedstock generation when compared with conventional synthetic media.

Carbohydrate content is a key determinant of microalgal biomass suitability for bioethanol production, as fermentable sugar yield after saccharification directly governs theoretical ethanol output. Although carbohydrate accumulation can be induced through nutrient limitation, light manipulation, or controlled stress, existing evidence is largely derived from synthetic media or well-characterised municipal wastewaters [27–29]. Empirical data linking clarified aquaponic effluent composition to carbohydrate productivity metrics in *Chlorella* sp. remain scarce, particularly with respect to undiluted effluent operation and bioethanol-relevant performance indicators.

Moreover, aquaponic effluent composition varies with fish species, feeding regimes, and solids removal efficiency, potentially influencing algal growth kinetics and biochemical composition. Consequently, experimental evaluation using real clarified aquaponic effluent streams under controlled conditions is required to assess both biomass productivity and carbohydrate accumulation prior to considering scale-up or integrated biofuel applications [18,30].

In this context, the present study investigates the feasibility of producing carbohydrate-rich *Chlorella* sp. biomass using clarified effluent from aquaponic sedimentation tanks. Rather than claiming broad novelty, this work addresses a specific and underexplored gap: the use of clarified aquaponic sedimentation effluent as a targeted cultivation medium for enhancing carbohydrate accumulation relevant to bioethanol feedstock generation. Biomass productivity, carbohydrate content, and theoretical bioethanol potential are quantified as preliminary indicators of feedstock suitability. By focusing on a real aquaponics-derived nutrient stream, this study contributes experimentally grounded insights into resource recovery strategies for integrated food–energy–water systems and provides data essential for future techno-economic and life-cycle assessments.

The specific objectives of this study are to: (1) characterise the nutrient composition of clarified aquaponic sedimentation effluent; (2) evaluate the growth kinetics and biomass productivity of *Chlorella* sp. cultivated in this effluent under controlled laboratory conditions; and (3) quantify carbohydrate accumulation in the harvested biomass and assess its suitability as a fermentable feedstock for theoretical bioethanol production.

2. Method

2.1. Wastewater Collection and Pre-Treatment

Aquaponics Sedimentation Effluent (ASE) was collected from the Aquaponics facility of the Department of Civil Engineering, University of Moratuwa (6.7950° N, 79.9007° E) in sterile polyethylene containers. Samples were transported on ice and processed within 24 h. Suspended solids were removed by filtration through a 0.45 µm nylon mesh (Millipore, Burlington, MA, USA), and the clarified wastewater was used as the experimental medium. Preliminary screening experiments were conducted to determine optimal dilution and illumination conditions for *Chlorella* sp. cultivation. Based on these results, 100% (*v/v*)

undiluted wastewater was selected, with pH adjusted to 6.8 using 1 M NaOH (Sigma-Aldrich, St. Louis, MO, USA). Bold's Basal Medium (BBM) (HiMedia, Mumbai, India) served as the control. Both media were sterilized by autoclaving at 121 °C for 20 min (Model ST-30C, Hirayama, Saitama, Japan).

2.2. Wastewater Characterization

Initial characterization of Aquaponics Sedimentation Effluent was performed according to APHA Standard Methods [31]. All measurements were conducted in triplicate ($n = 3$). The following parameters were determined:

- pH—Measured using a benchtop pH meter (SevenCompact, Mettler Toledo, Greifensee, Switzerland).
- Nitrate (NO_3^- -N)—UV absorbance at 220 nm with baseline correction at 275 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu Corp., Kyoto, Japan).
- Phosphate (PO_4^{3-} -P)—molybdenum blue method, absorbance at 880 nm.

The initial nitrate and phosphate levels in the raw effluent exceeded the standard tolerance limits for discharge into inland surface waters (Nitrate $< 10 \text{ mg L}^{-1}$, Phosphate $< 2 \text{ mg L}^{-1}$) as stipulated by the Central Environmental Authority (CEA) of Sri Lanka, necessitating treatment prior to release [18].

2.3. Microalgal Strain and Inoculum Preparation

A pure culture of *Chlorella* sp. was obtained from Progreen Laboratory, University of Moratuwa. Pre-cultures were grown in Bold's Basal Medium (BBM) under controlled conditions ($25 \pm 2 \text{ }^\circ\text{C}$, continuous illumination at $150 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ provided by cool-white LED lights, and aeration at 0.5 vvm sterile-filtered air) before being transferred to experimental flasks. Exponential phase cultures were harvested, centrifuged at 4000 rpm for 20 min (Eppendorf 5810R, Hamburg, Germany), and inoculated into experimental media at an initial biomass concentration of 0.3 g L^{-1} . The photobioreactors consisted of 2 L laboratory glass bottles (GL45) containing 1.8 L of autoclave-sterilized media. The bottles were equipped with 3-port screw caps fitted with $0.45 \text{ } \mu\text{m}$ polytetrafluoroethylene (PTFE) membrane filters for sterile aeration and pressure compensation.

2.4. Experimental Setup and Cultivation Conditions

2.4.1. Screening of Wastewater Dilution Factors

Four dilutions of Aquaponics Sedimentation Effluent (25%, 50%, 75%, and 100% v/v) were prepared using distilled water, with 400 mL working volume in 500 mL Erlenmeyer flasks. The initial pH (< 3.9) was adjusted to 6.8 using NaOH. BBM medium (400 mL) served as the control. Cultures were incubated at $25 \pm 2 \text{ }^\circ\text{C}$ under a 12:12 h light–dark cycle, aerated continuously, and illuminated at $100 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (LED, cool-white). Biomass growth was monitored every 48 h by optical density (OD_{680}) and dry weight measurement [32]. Aliquots of 2 mL were transferred to 1 cm path-length quartz cuvettes and measured against a distilled water blank using the UV-1800 spectrophotometer.

2.4.2. Screening of Light Intensities

The effect of light intensity on biomass production was evaluated using the 100% Aquaponics Sedimentation Effluent medium. Four light intensities (90, 140, 185, and $230 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) were tested, with BBM at $100 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ as the control. Light intensity was measured using a quantum sensor (LI-250A, LI-COR Biosciences, Lincoln, NE, USA). Cultures were incubated at $25 \pm 2 \text{ }^\circ\text{C}$ under a 12:12 h light:dark photoperiod [33].

2.4.3. Main Cultivation Experiment

Based on the screening results, the undiluted (100% *v/v*) Aquaponics Sedimentation Effluent was selected for subsequent experiments as it supported high biomass accumulation without freshwater consumption. Similarly, the light intensity screening indicated that 185 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ supported the optimal growth trade-off, and this intensity was selected for the main experiment.

The main batch cultivation was carried out in 2 L sterilized glass vessels with 1.8 L working volume, using the selected undiluted (100% *v/v*) aquaponics sedimentation effluent and BBM control. Cultures were incubated at 25 ± 2 °C under a 12:12 h light–dark photoperiod, aerated with sterile-filtered air pumps (BOYU, Chaozhou, China, 0.45 μm filter), and illuminated with LED lights at 185 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Light intensity was monitored with a quantum sensor (LI-COR LI-250A (Lincoln, NE, USA)). The BBM control was cultivated under the same optimized illumination conditions (185 $\mu\text{mol m}^{-2} \text{s}^{-1}$) to ensure comparability [18].

2.5. Analytical Procedures

2.5.1. Biomass Concentration

Biomass growth was monitored every 48 h. Optical density (OD_{680}) was measured with a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan). Microalgal growth was evaluated at 2-day time intervals by the determination of biomass concentration of cultures. Culture aliquots of 5 mL were filtered through pre-dried and pre-weighed glass microfiber filter papers (Hyundai Micro, Seoul, South Korea, GF/C, $\phi 47$ mm, 1.2 μm pore size) [18]. Thereafter, the glass microfiber filter papers were oven dried at 60 °C for 24 h, and the biomass concentration of cultures was determined as per the following equation (Equation (1));

$$P = (X_f - X_i)/t \quad (1)$$

where DW_n is the biomass concentration (g/L) of microalgae on the *n*th day of cultivation (T_n), W_f is the final weight of the dried filters with microalgal biomass, W_i is the initial weight of the filters and V is the volume of the culture aliquot.

2.5.2. Kinetic Parameter Calculations

The specific growth rate (μ) was calculated during the exponential phase according to Equation (2):

$$\mu = (\ln(X_2) - \ln(X_1))/(t_2 - t_1) \quad (2)$$

where X_2 and X_1 are the biomass concentrations (g L^{-1}) at times t_2 and t_1 , respectively.

The biomass doubling time (T_d) was calculated using Equation (3):

$$T_d = \ln(2)/\mu \quad (3)$$

2.5.3. Nutrient Removal

The nitrate concentration in the media and pH of cultures were determined at 2-day time intervals. Culture samples (5 mL) were centrifuged at $10,000 \times g$ for 5 min and the supernatant was filtered through 0.22 μm nylon syringe filters (Advantec, Tokyo, Japan) [34]. Nitrate and phosphate concentrations were determined as in Section 2.2. Nutrient removal efficiency (RE, %) was calculated as (Equation (4)):

$$\text{RE}(\%) = (C_i - C_f)/C_i \times 100 \quad (4)$$

where (C_i) and (C_f) are initial and final concentrations (mg L^{-1}), respectively.

2.5.4. 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

At the end of the 20-day cultivation period, biomass was harvested by centrifugation at 4000 rpm for 20 min (Eppendorf 5810R), washed twice with sterile distilled water, and oven-dried at 60 °C to constant weight [18].

2.7. Carbohydrate Content

The 10 mg samples were also utilized for the determination of carbohydrate content. 0.5 mL of acetic acid was added to microalgal biomass, and the solution was heated at 80 °C for 20 min in a temperature-controlled water bath (Mettler WNB7, Schwabach, Germany). The solution was cooled, and 10 mL of acetone was added for the extraction of pigments. Thereafter, the samples were centrifuged at $3500 \times g$ for 10 min, and the supernatant was discarded. The pellet was hydrolyzed by incubation with 2.5 mL of 4 M trifluoroacetic acid at 95 °C for 4 h. The hydrolysate was separated from the residual biomass by centrifugation (Merck, Darmstadt, Germany) at $10,000 \times g$ for 5 min. The carbohydrate content was quantified using the phenol-sulfuric acid colorimetric method [35,36]. A standard calibration curve was constructed using D-glucose (Sigma-Aldrich) at concentrations ranging from 0 to 100 mg L⁻¹ ($R^2 > 0.99$). Absorbance was measured at 490 nm against a reagent blank using the UV-1800 spectrophotometer. Total carbohydrate content was calculated based on the linear regression equation obtained from the glucose standard.

2.8. Statistical Analysis

All screening experiments were conducted in duplicate, while the main cultivation experiments and all analytical measurements were performed in triplicate ($n = 3$). Statistical analysis was performed using Minitab 17 software (Minitab Inc., State College, PA, USA; 2014 release, Academic License). Data were analyzed using One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test for pairwise comparisons, with significance set at $p < 0.05$.

2.9. Theoretical Nitrogen Mass Balance Analysis

To distinguish between biological nitrogen assimilation and physicochemical losses (e.g., ammonia volatilization), a theoretical mass balance analysis was performed. The theoretical nitrogen requirement for biomass synthesis (N_{demand}) was estimated based on the standard molecular stoichiometry for microalgal biomass ($\text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P}$). Based on this formula, the theoretical nitrogen fraction (f_N) of the biomass was derived as approximately 6.3% by weight. The theoretical assimilated nitrogen ($N_{\text{assimilated}}$) was calculated using Equation (5):

$$N_{\text{assimilated}} \text{ (mg L}^{-1}\text{)} = \text{Biomass Yield (mg L}^{-1}\text{)} \times f_N \quad (5)$$

This theoretical value was then compared with the experimentally measured Total Dissolved Nitrogen (TDN) removal ($N_{\text{removed}} = N_{\text{initial}} - N_{\text{final}}$) to determine the percent contribution of biological assimilation to total nutrient removal.

3. Results

3.1. Characterization of Aquaponics Wastewater

Prior to inoculation, the physicochemical properties of the sedimentational aquaponics effluent (AE) were analyzed to assess its suitability as a growth medium. The initial composition of the wastewater (Day 0) revealed a nutrient-rich profile characteristic of intensive aquaculture systems.

As summarized in Table 1, the wastewater was slightly acidic to neutral with a pH of 6.81 ± 0.33 , which is within the optimal physiological range for *Chlorella* sp. Notably, the wastewater contained a high concentration of nitrate (153.02 ± 4.26 mg/L), exceeding the nitrate concentration found in the standard Bold's Basal Medium (BBM) control (142.40 ± 2.98 mg/L). However, the phosphate concentration in the wastewater (6.91 ± 0.25 mg/L) was significantly lower than that of the control medium (13.76 ± 0.60 mg/L). This resulted in a high N:P ratio in the wastewater (22:1), suggesting that phosphorus could potentially become the limiting nutrient during prolonged cultivation.

Table 1. Initial physicochemical characteristics of the aquaponics effluent (AE) compared to the standard BBM control (Day 0).

| Parameter | BBM (Control) | ASE (Wastewater) |
|---|-------------------|-------------------|
| pH | 6.80 ± 0.17 | 6.81 ± 0.33 |
| Nitrate (NO_3^-) mg L ⁻¹ | 142.40 ± 2.98 | 153.02 ± 4.26 |
| Phosphate (PO_4^{3-}) mg L ⁻¹ | 13.76 ± 0.60 | 6.91 ± 0.25 |

3.2. Screening of Optimal Aquaponics Effluent (AE) Dilution

The growth of *Chlorella* sp. was evaluated under five different dilution regimes (0% to 100% Aquaponics Effluent AE) to identify the optimal nutrient concentration for biomass production. The growth profiles in terms of optical density (OD₆₈₀) and dry cell weight (DCW) are presented in Figures 1 and 2.

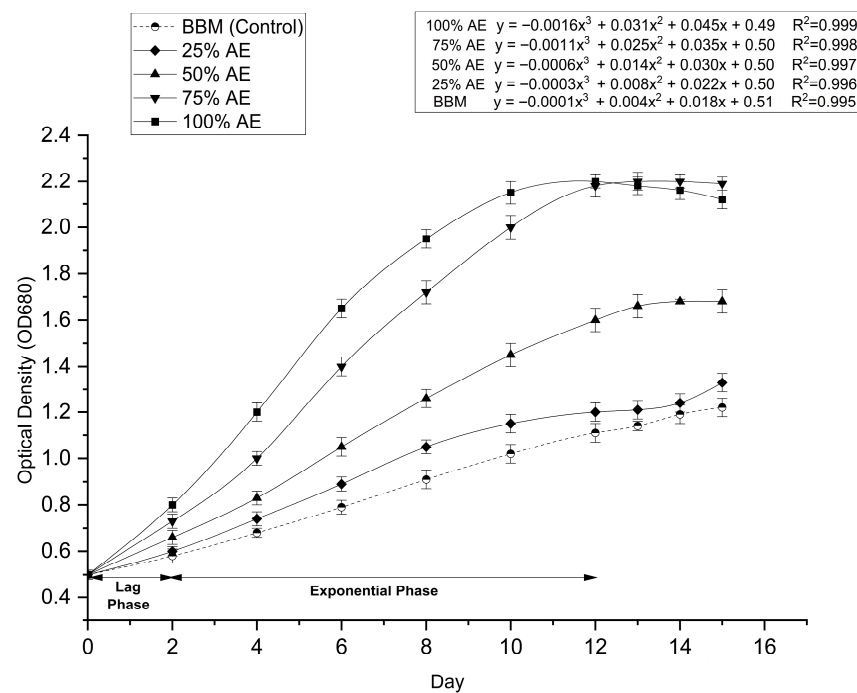


Figure 1. Growth kinetics measured as optical density (OD₆₈₀) of *Chlorella* sp. cultivated in different dilution ratios of aquaponics effluent.

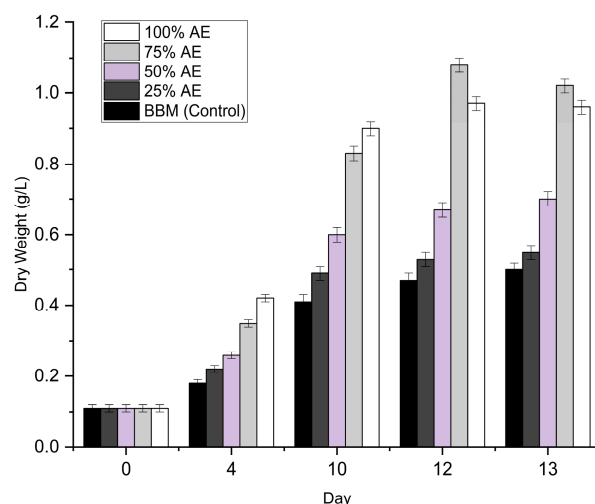


Figure 2. Comparison of dry cell weight (biomass yield) of *Chlorella* sp. grown in various AE concentrations at the stationary phase.

3.2.1. Growth Dynamics (OD₆₈₀)

The cultures grown in higher concentrations of wastewater (75% and 100% AE) exhibited rapid exponential growth compared to the control (BBM) and lower dilutions. A one-way ANOVA analysis confirmed that the dilution factor had a statistically significant effect on growth ($p < 0.001$). By Day 12, the highest optical densities were recorded in 100% AE (2.20 ± 0.03) and 75% AE (2.18 ± 0.05). A Tukey's HSD post hoc test revealed that there was no significant difference ($p > 0.05$) between the 75% and 100% concentrations, though both were significantly superior to the BBM control (1.11 ± 0.04).

The growth curves exhibited characteristic phasic development, aligning with the stages described by [11], with a distinct lag phase (Days 0–2) followed by an exponential log phase (Days 2–10).

3.2.2. Biomass Production (Dry Weight)

While the optical density suggested equal performance between the two highest concentrations, the dry weight analysis revealed a significant divergence. As shown in Figure 2, the maximum biomass yield was achieved in 75% AE (1.08 ± 0.02 g/L) on Day 12. Statistical analysis indicated that the biomass yield in 75% AE was significantly higher ($p < 0.05$) than that obtained under the 100% AE condition (0.97 ± 0.02 g L⁻¹). However, the absolute difference in biomass accumulation between the two treatments was marginal (<10%), indicating that *Chlorella* sp. can maintain comparable growth performance even under undiluted aquaponics effluent. Although the 100% AE condition may have introduced mild growth constraints, such as increased self-shading or nutrient imbalance, its ability to support high biomass productivity without the use of freshwater is particularly relevant from a sustainability perspective. Therefore, in alignment with resource-efficiency principles and Sustainable Development Goals related to water conservation and circular resource use, the undiluted (100% v/v) aquaponics effluent was selected as the optimal medium for subsequent light-intensity optimization experiments. Although the 75% dilution yielded marginally higher biomass, the 100% condition eliminates the need for freshwater addition, significantly lowering the operational water footprint. In a circular bioeconomy context, eliminating freshwater input is prioritized over marginal yield gains.

3.3. Optimization of Light Intensity for Biomass Production

Following the determination of the optimal dilution factor, the influence of light intensity on *Chlorella* sp. growth was evaluated under five photosynthetic photon flux

densities (PPFD) ranging from 90 to 230 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The growth dynamics, measured via optical density (OD_{680}), exhibited a significant non-linear response to light availability (Figure 3).

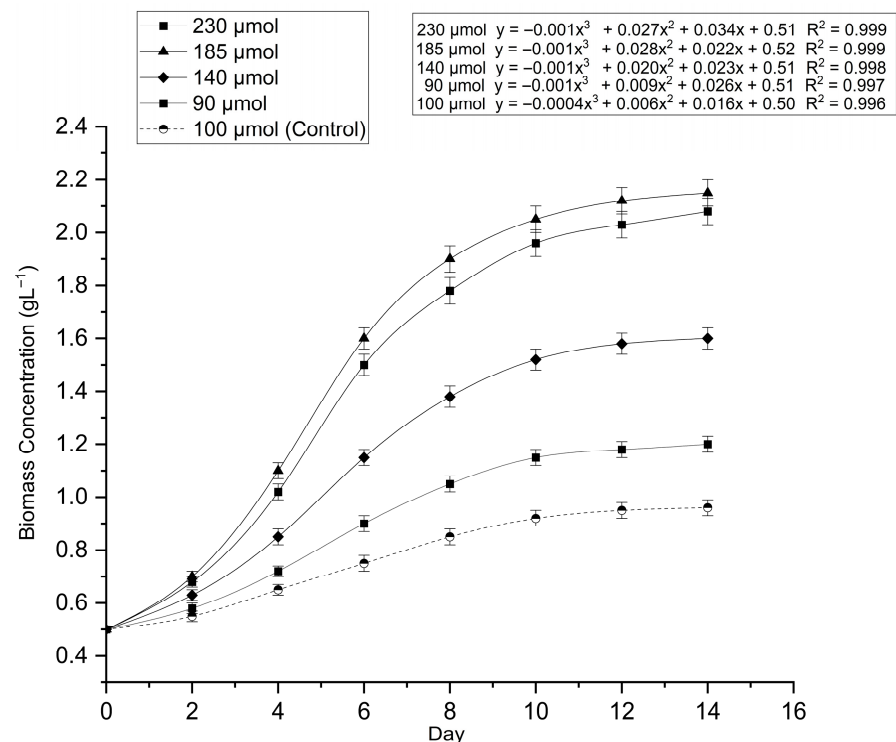


Figure 3. Growth kinetics measured as optical density (OD_{680}) of *Chlorella* sp. cultivated in different light intensities.

A one-way ANOVA confirmed that light intensity had a statistically significant effect on the growth rate ($p < 0.001$). The cultures exposed to higher intensities (185 and 230 $\mu\text{mol m}^{-2} \text{s}^{-1}$) demonstrated rapid exponential growth, achieving the highest final optical densities of 2.15 ± 0.05 and 2.08 ± 0.05 , respectively. Interestingly, the lowest light intensity (90 $\mu\text{mol m}^{-2} \text{s}^{-1}$) recorded a higher optical density (1.20 ± 0.03) compared to the control at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (0.96 ± 0.03), suggesting a distinct photo-acclimatization response, as *Chlorella* sp. is known to alter its light-harvesting complex efficiency under low photon flux densities.

However, the dry cell weight (DCW) analysis provided a more definitive measure of productivity and revealed distinct saturation kinetics (Figure 4). Unlike the optical density results, the biomass yield at 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (0.48 ± 0.02 g/L) was significantly lower than the control (0.55 ± 0.02 g/L; $p < 0.05$), confirming that light limitation constrained actual biomass accumulation despite the higher turbidity. The biomass yield increased consistently with light intensity, peaking at 1.05 ± 0.03 g/L under 185 $\mu\text{mol m}^{-2} \text{s}^{-1}$. A Tukey's HSD post hoc analysis revealed no significant difference ($p > 0.05$) between the yields at 185 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 230 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (1.00 ± 0.03 g/L). This plateau indicates that the culture reached photo-saturation at 185 $\mu\text{mol m}^{-2} \text{s}^{-1}$, where further increases in light energy did not translate into additional biomass. Consequently, 185 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was identified as the optimal illumination intensity for the subsequent main experiment.

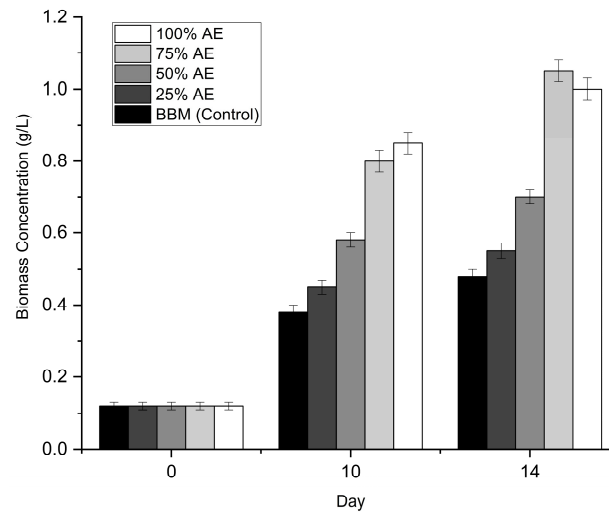


Figure 4. Effect of light intensity on final biomass production.

3.4. Results: Growth Kinetics and Biomass Productivity

The cultivation of *Chlorella* sp. under the optimized conditions (100% Aquaponics Effluent (AE) + $185 \mu\text{mol m}^{-2} \text{s}^{-1}$) was compared against a control group grown in standard BBM. The results demonstrate that the waste-derived medium supported significantly higher growth rates and biomass accumulation than the commercial nutrient solution.

3.4.1. Growth Dynamics

The optical density profile (Figure 5) reveals that the optimized wastewater culture entered the exponential phase earlier and maintained a higher growth trajectory throughout the 14-day period. By Day 14, the optical density of the main experimental group reached 2.94 ± 0.13 , which was significantly higher ($p < 0.001$) than the control group (2.22 ± 0.05). The specific growth rate (μ) during the exponential phase (Days 2–10) was calculated to be 0.130 d^{-1} for the wastewater group, compared to 0.113 d^{-1} for the control. Consequently, the cells in the aquaponics wastewater doubled faster ($T_d = 5.34$ days) than those in the standard medium ($T_d = 6.12$ days).

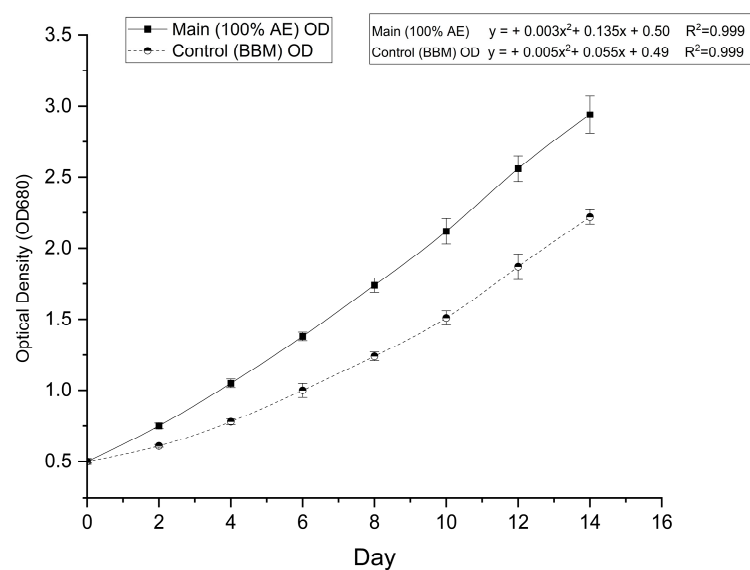


Figure 5. Comparative growth curves (OD_{680}) of *Chlorella* sp. cultivated under optimized conditions versus the standard Bold's Basal Medium (Control).

3.4.2. Biomass Productivity

The superiority of the aquaponics effluent (AE) was most evident in the dry biomass yield (Figure 6). While both cultures started with an initial biomass of 0.12 ± 0.01 g/L, the optimized group diverged sharply after Day 10. By the end of the cultivation period (Day 15), the main experiment achieved a maximum biomass of 2.05 ± 0.03 g/L, representing a 75% increase over the control (1.20 ± 0.03 g/L, $p < 0.001$).

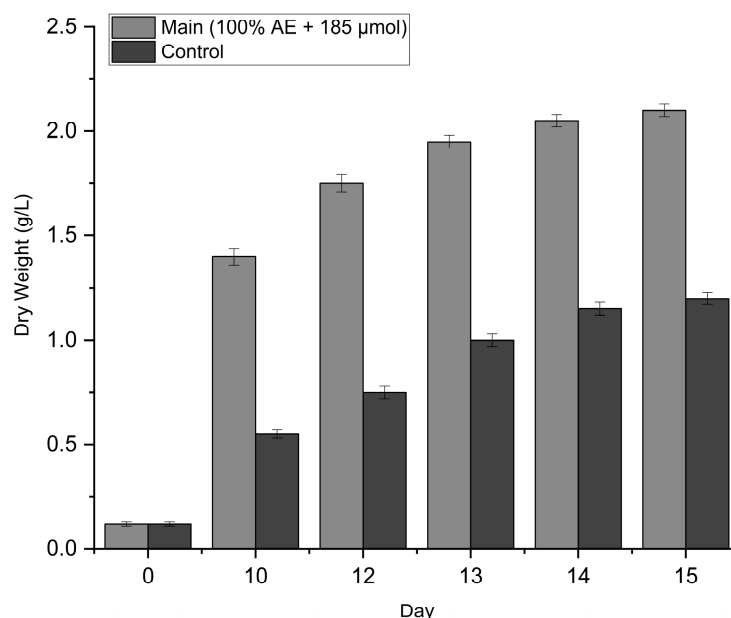


Figure 6. Temporal biomass accumulation (dry cell weight) in aquaponics wastewater compared to the BBM control.

When it comes to productivity (P_{max}) [37], the overall biomass productivity for the optimized system was 0.132 g/L/day, nearly double that of the control (0.072 g/L/day). This confirms that aquaponics wastewater, when paired with optimized lighting, provides a nutrient-dense environment that outperforms standard inorganic media.

3.5. Results: Nutrient Removal Efficiency

To evaluate the bioremediation potential of the system, the depletion of nitrate (NO_3^-) and phosphate (PO_4^{3-}) was monitored throughout the cultivation period. The results confirm that *Chlorella* sp. is highly effective at recovering nutrients from aquaponics wastewater.

3.5.1. Nitrate Removal

The system demonstrated high nitrate removal kinetics (Figure 7). The aquaponics wastewater initially contained higher nitrate loads (153.02 ± 4.26 mg/L) compared to the BBM control (142.40 ± 2.98 mg/L). Despite this higher initial burden, the optimized culture achieved a significantly faster removal rate of 10.56 mg/L/day, compared to 9.09 mg/L/day in the control. By Day 14, the nitrate concentration in the AE was reduced to just 5.20 ± 0.22 mg/L, corresponding to a removal efficiency of 96.60%. This was significantly higher ($p < 0.001$) than the 89.39% efficiency observed in the control group.

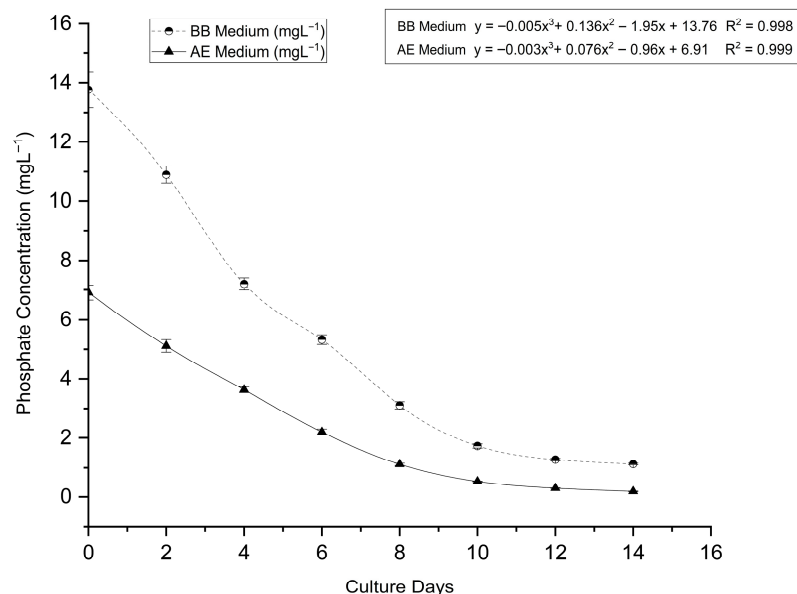


Figure 7. Kinetics of nitrate (NO₃⁻) depletion in AE and BBM control medium.

3.5.2. Phosphate Removal

Phosphate removal followed a similar trend of rapid depletion (Figure 8). Although the initial phosphate concentration in the wastewater (6.91 ± 0.25 mg/L) was approximately half of the BBM control (13.76 ± 0.60 mg/L), the culture scavenged the available phosphorus to near-exhaustion. By Day 14, the residual phosphate in the wastewater dropped to 0.19 ± 0.01 mg/L, representing a 97.25% removal efficiency. This complete depletion suggests that the system is capable of polishing wastewater effluent to meet stringent discharge standards.

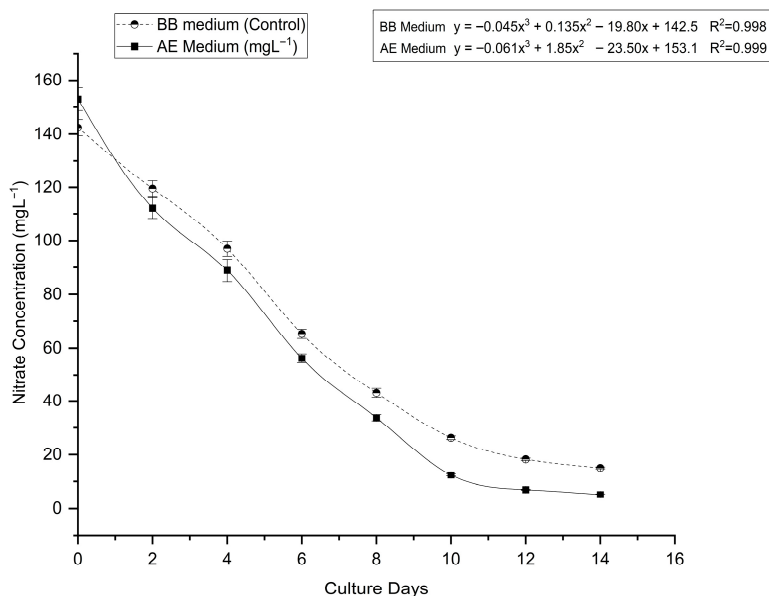


Figure 8. Phosphate (PO₄³⁻) removal profile of *Chlorella* sp., highlighting the near-complete depletion of phosphorus in the AE.

3.5.3. pH Evolution

The pH profile (Figure 9) provided insight into the photosynthetic activity of the cultures. Both groups exhibited a pH increase over time, indicative of dissolved CO₂ consumption during photosynthesis.

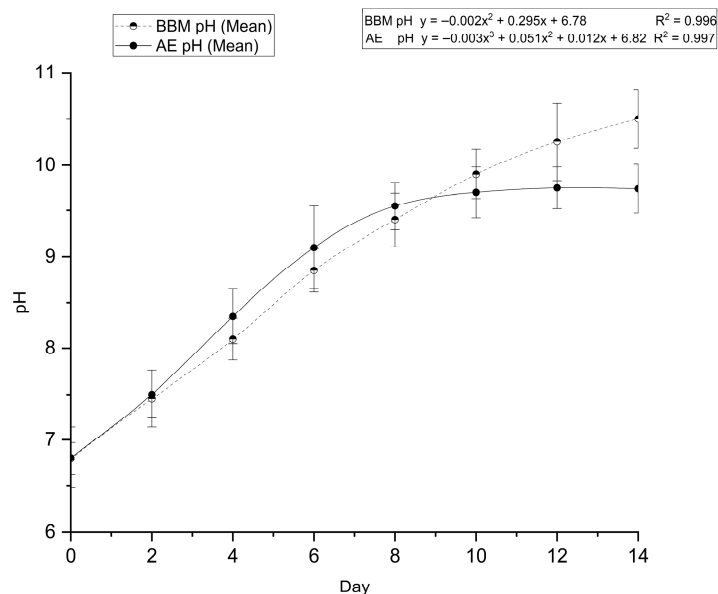


Figure 9. Evolution of culture pH during the cultivation period.

Control (BBM), the pH rose continuously, reaching a highly alkaline peak of 10.50 by Day 14. The pH of the AE rose initially but stabilized at approximately 9.75 after Day 10. This stabilization in the wastewater group is advantageous for large-scale cultivation, as extreme alkalinity (pH > 10) can sometimes precipitate essential micronutrients or inhibit growth. The natural buffering capacity of the aquaponics effluent likely contributed to maintaining this more favorable range.

3.6. Nitrogen Mass Balance Verification

The mass balance analysis confirmed that biological assimilation was the primary driver of nitrogen removal. In the undiluted AE condition, the total observed nitrate removal was 147.81 mg L⁻¹ (Table 2). Based on the final dry biomass concentration of 2.05 g L⁻¹ (Table 3) and the stoichiometric nitrogen fraction (6.3%), the theoretical nitrogen assimilated into the biomass was calculated to be 129.15 mg L⁻¹. This stoichiometric uptake accounts for 87.4% of the total nitrogen removed from the medium. The remaining difference suggests minor physicochemical losses or variations in intracellular nitrogen storage, but confirms that the vast majority of removal is biologically driven rather than due to volatilization.

Table 2. Nitrate and phosphate levels at the beginning and end of the main experiment, along with their respective consumption efficiency.

| Medium | Parameter | Initial (mg/L) | Final (mg/L) | Efficiency (%) |
|---------------------|---------------------------------|----------------|--------------|----------------|
| BB medium (Control) | NO ₃ ⁻ | 142.08 ± 2.90 | 14.21 ± 1.10 | 90.00 ± 0.80 |
| BB medium (Control) | P-PO ₄ ³⁻ | 13.76 ± 0.60 | 1.10 ± 0.10 | 92.01 ± 0.81 |
| AE medium | NO ₃ ⁻ | 153.00 ± 4.20 | 5.21 ± 0.20 | 96.59 ± 0.16 |
| AE medium | P-PO ₄ ³⁻ | 6.91 ± 0.25 | 0.19 ± 0.01 | 97.25 ± 0.17 |

Consequently, the total nutrient removal efficiency was calculated to quantify the treatment performance (Table 2). The aquaponics wastewater medium demonstrated superior bioremediation capacity, achieving significantly higher removal rates (*p* < 0.05) for both nitrate (96.59%) and phosphate (97.25%) compared to the control. This high efficiency suggests that the system effectively mitigates the risk of eutrophication while generating biomass.

Table 3. Comparison of biomass production, carbohydrate accumulation, and theoretical bioethanol potential of *Chlorella* sp. cultivated in BBM (Control) and aquaponics effluent (AE).

| Parameter | Control (BBM) | AE (Optimized) | Increase over Control (%) |
|--------------------------------|---------------|----------------|---------------------------|
| Dry Cell Weight (g/L) | 1.15 ± 0.03 | 2.05 ± 0.03 | +78.3% |
| Carbohydrate Content (%) | 23.54 ± 2.24 | 40.71 ± 2.14 | +72.9% |
| Total Carbohydrate Yield (g/L) | 0.271 ± 0.02 | 0.835 ± 0.02 | +208.1% |
| Theoretical Bioethanol (g/L) | 0.138 | 0.427 | +209.4% |

3.7. Biomass Accumulation and Carbohydrate Productivity

To assess the potential of the produced biomass as a feedstock for bioethanol, the dry cell weight (DCW), carbohydrate content (%), and total carbohydrate yield were analyzed after 14 days of cultivation.

3.7.1. Enhanced Biomass and Carbohydrate Profile

The biochemical analysis (Table 3) confirms that aquaponics effluent (AE) significantly enhances both cell growth and energy storage compared to the standard BBM control. The biomass consistent with the growth kinetics observed in Section 3.4, the final dry cell weight reached 2.05 ± 0.03 g/L, representing a 78.3% increase over the control yield of 1.15 ± 0.03 g/L. This superior growth is attributed to the organic carbon and micronutrients present in the fish effluent, which facilitate mixotrophic growth [38].

Decisively, the algal cells grown in wastewater accumulated significantly higher intracellular carbohydrates. The carbohydrate content in the AE group reached $40.71 \pm 2.14\%$ of dry weight, a 72.9% increase compared to the control ($23.54 \pm 2.24\%$). This accumulation is a stress response to the nitrogen depletion observed towards the end of the cultivation period, a known trigger for starch synthesis in *Chlorella* sp.

3.7.2. Theoretical Bioethanol Potential

The combination of high biomass and high carbohydrate content resulted in a significant increase in total feedstock availability. The total carbohydrate yield in the ASE medium was calculated to be 0.835 g L^{-1} , which corresponds to a 208% enhancement compared to the control (0.271 g L^{-1}). Based on the standard stoichiometric conversion factor of $0.511 \text{ g ethanol/g hexose}$, the theoretical bioethanol potential of the ASE-grown biomass is approximately 0.427 g L^{-1} , compared to just 0.138 g L^{-1} for the control. These findings confirm that aquaponics wastewater is a highly feasible, low-cost medium for producing carbohydrate-rich feedstock for biofuel applications [39,40].

4. Discussion

The present study demonstrates that clarified aquaponics effluent (AE) is a highly effective cultivation medium for *Chlorella* sp., yielding significantly higher biomass productivity than conventional Bold's Basal Medium (BBM). When benchmarked against other wastewater-derived media, the observed biomass concentration (2.05 g L^{-1}) is at the upper end of values reported for *Chlorella* cultivated in aquaculture effluents ($0.9\text{--}1.6 \text{ g L}^{-1}$) and municipal wastewaters ($0.6\text{--}1.8 \text{ g L}^{-1}$) [41–43]. This performance suggests that clarified AE provides a nutritionally favorable yet non-inhibitory environment, distinguishing it from untreated municipal or industrial wastewaters, where growth is often constrained by toxic compounds, high turbidity, or ammonia toxicity.

The superior growth observed in AE relative to BBM can be mechanistically attributed to nitrogen speciation and trace nutrient bioavailability. Unlike BBM, which supplies nitrogen predominantly as nitrate, aquaponic effluents contain a mixture of nitrate, ammonium,

and organic nitrogen species. Ammonium assimilation requires lower energetic investment than nitrate reduction, thereby enhancing growth efficiency when present at non-toxic concentrations, a phenomenon widely reported for *Chlorella* spp. in aquaculture-derived waters [44,45]. Furthermore, AE provides balanced C:N:P ratios and optimal P/N stoichiometry derived from fish waste, which facilitates efficient macromolecular synthesis and higher biomass accumulation compared to the rigid stoichiometry of synthetic media [46]. Moreover, the non-sterile nature of AE fosters a beneficial phycosphere microbiome; unlike sterile BBM, these bacteria release growth-promoting compounds, organic acids, and essential vitamins (e.g., B12), establishing a mutualistic interaction that significantly boosts biomass yields [47,48]. Additionally, AE provides chelated micronutrients and dissolved organic carbon and serves as a potential source of bioactive compounds, such as phytohormones (e.g., auxins, gibberellins) originating from the hydroponic plant component or fish metabolites, which are known to stimulate cell division and stress tolerance [49]. Finally, the presence of inorganic carbon species (HCO_3^- , CO_3^{2-}) in AE provides a natural buffering system that maintains pH within the physiological range (9–10), preventing the sharp alkaline shifts and CO_2 limitation often observed in unbuffered BBM cultures [50,51], enabling partial mixotrophic growth that is absent in strictly inorganic media.

The dilution-dependent response further highlights the robustness of *Chlorella* sp. in AE. While many wastewater studies report sharp declines in productivity at high effluent concentrations due to turbidity or inhibitory compounds, the performance of AE contrasts sharply with that of other waste streams. For instance, urban and municipal wastewaters typically require extensive dilution (10–70%) to avoid biomass losses of 50–80% caused by high turbidity and excessive nitrogen loads [52,53]. Similarly, piggery digestates and food processing effluents often necessitate 50–90% dilution to mitigate severe ammonium toxicity ($>1000 \text{ mg L}^{-1}$) and N/P imbalances, which otherwise inhibit growth by over 50% [54–58]. In contrast, the results of this study align with emerging evidence from aquaculture-based systems, where pre-treatment and strain selection facilitate undiluted operation. Recent studies have demonstrated that nutrient-deprived *Chlorella* sp. can effectively polish moderate-strength aquaculture effluents ($>90\%$ removal) in semi-continuous undiluted modes [57,59], while *Chlorella vulgaris* cultivated in recirculating aquaculture system (RAS) effluents maintained sustained growth without heavy dilution following clarification [58,60]. Furthermore, undiluted co-cultivation of *Chlorella pyrenoidosa* in shrimp aquaculture water has been shown to maintain toxic ammonia ($<1.0 \text{ mg L}^{-1}$) and nitrite ($<0.1 \text{ mg L}^{-1}$) levels below inhibitory thresholds, boosting survival without the dilution typically required in untreated systems [61]. Consequently, the minimal ($<10\%$) difference between 75% and 100% AE in the present study indicates that clarification of aquaponics sedimentation effluent effectively removes optical and particulate constraints, enabling undiluted operation without productivity penalties common in anaerobic or industrial digestates [48]. From a system-level perspective, this is a critical advantage, as undiluted operation eliminates freshwater demand and simplifies integration into existing aquaponic infrastructure.

Light optimization revealed a clear photosaturation threshold at approximately $180\text{--}190 \mu\text{mol m}^{-2} \text{ s}^{-1}$. This saturation point is slightly lower than values reported for *Chlorella* cultivated in synthetic media ($200\text{--}300+ \mu\text{mol m}^{-2} \text{ s}^{-1}$), reflecting the additional light attenuation imposed by dissolved organic matter and particulate scattering in AE [44]. This phenomenon aligns with observations in other wastewater streams, such as diluted centrate, where *Chlorella* strains exhibit reduced Photosystem II efficiency (F_v/F_m dropping to 0.15) and growth inhibition at higher effluent loads due to effective light limitation [62]. Importantly, this finding has direct engineering implications: increasing irradiance beyond this range would increase energy consumption without proportional

biomass gains, thereby reducing overall system efficiency. At lower irradiance, the observed increase in optical density without corresponding biomass accumulation indicates pigment-level photoacclimation, a common adaptive response in wastewater-grown microalgae. Studies confirm that under sub-saturating light or nutrient stress in real effluent, *Chlorella* significantly upregulates total chlorophyll (Chl a + b) synthesis to compensate for attenuation, thereby increasing optical turbidity even when biomass carbon accumulation is limited [62–64].

Beyond biomass production, nutrient removal performance is a defining strength of the AE-based system. The near-complete removal of nitrate and phosphate (>95%) compares favorably with reported removal efficiencies for *Chlorella* and *Scenedesmus* cultivated in aquaculture and municipal wastewaters, which typically range from 70–90% for nitrate and 75–95% for phosphate [41–43,65,66]. These results align with performance metrics from advanced High-Rate Algal Ponds (HRAPs) and microalgae-bacteria consortia, which achieve comparable 90–98% ammonium and 62–95% total phosphorus removal, often outperforming conventional primary and secondary treatments [67,68]. Notably, the removal efficiencies achieved in the present study exceed those commonly reported for synthetic media-based cultivation (often 50–80%), where nutrient uptake is suboptimal and not a design objective. This underscores the dual functionality of AE cultivation as both a biomass production and nutrient recovery process, mirroring the symbiotic efficiency observed in co-culture systems where simultaneous biomass generation (up to 4.9 g VSS m⁻² d⁻¹) and nutrient polishing occur in a single operational step [66,69].

A mass-balance-based assessment further indicates that approximately 87.4% of nitrate removal was attributable to biological assimilation into algal biomass rather than physico-chemical losses. This contrasts with municipal wastewater systems, where denitrification, volatilization, or sedimentation can contribute substantially to apparent nutrient removal, complicating nutrient recovery accounting. The direct coupling between nutrient uptake and biomass formation observed here strengthens the case for AE-based algal cultivation as a true circular economy intervention rather than a passive polishing step [70,71].

The stabilization of pH observed in AE cultures, in contrast to the progressive alkalization observed in BBM, reflects the intrinsic buffering capacity of aquaponic effluents. Similar buffering behavior has been reported in integrated aquaculture–algae systems and hydroponic biofilters, where bicarbonate alkalinity and mixed nitrogen species interactions effectively maintain pH balance and prevent the sharp alkalization associated with rapid CO₂ depletion [44,72,73]. This buffering is further reinforced by the distinct microbial dynamics of aquaponic systems; unlike unbuffered synthetic media, the presence of efficient nitrifying communities (e.g., *Nitrospira* spp.) enhances pH homeostasis even under varying nutrient loads [74,75]. From a scale-up perspective, this pH stability is particularly advantageous, as excessive alkalinity in synthetic media systems can impair micronutrient solubility and compromise long-term operational stability by triggering precipitation events that are naturally mitigated in the buffered aquaponic matrix [75].

Carbohydrate accumulation represents a central outcome of this study. The carbohydrate content exceeding 40% dry weight is comparable to or higher than values reported for carbohydrate-enriched *Chlorella* grown under nitrogen limitation in municipal wastewater (30–42%) and aquaculture effluents (32–45%) [43–45]. These findings also align with recent benchmarks for glucose- or acetate-supplemented wastewater cultures, which report carbohydrate ranges of 18–57% depending on the organic load [76,77]. The concurrence of high biomass productivity and elevated carbohydrate fraction is particularly noteworthy, as many wastewater systems exhibit a trade-off where full nitrogen availability restricts carbohydrate content to <20%, whereas severe nitrogen limitation (1/4–1/2 dosing) enhances storage to 57–70% but suppresses growth [78]. This balance indicates that AE provides

sufficient nutrients to sustain growth while still enabling progressive nitrogen depletion to trigger carbon storage pathways, effectively mimicking a two-stage cultivation process. This dual performance is likely supported by mixotrophic mechanisms, where dissolved organics sustain initial biomass accumulation rates (e.g., 55–63 mg L⁻¹ d⁻¹) before nutrient depletion shifts metabolism toward storage, avoiding the growth limitations of pure photoautotrophy [77–79]. A comprehensive comparison of these results with recent literature across various wastewater streams is presented in Table 4. As shown, the biomass yield (2.05 g L⁻¹) achieved in this study outperforms systems utilizing municipal, swine, and dairy wastewaters, and is comparable to high-performing integrated aquaculture systems. This confirms that clarified aquaponics effluent serves as a superior, nutrient-balanced matrix for high-density carbohydrate production.

From an application standpoint, it is critical to emphasize that the bioethanol yields discussed herein are theoretical values derived from stoichiometric conversion assumptions. Standalone bioethanol production from microalgae remains economically challenging, with reported production costs exceeding \$90 L⁻¹ when biomass cultivation and chemical hydrolysis are considered in isolation [80]. However, within a biorefinery cascading framework, the economic relevance of carbohydrate-rich biomass changes fundamentally. When wastewater treatment offsets nutrient costs and lipids are first extracted for biodiesel, fermentation of residual carbohydrates becomes economically viable, with reported bioethanol costs as low as \$0.73 L⁻¹ [80,81]. This cascading model is further validated by large-scale initiatives such as the MIRACLES project, which demonstrated that sequential extraction—recovering high-value lipids for biodiesel prior to carbohydrate fermentation—combined with waste heat reuse and medium recycling, is essential for achieving commercial viability [82,83].

The integration of *Chlorella* cultivation into aquaponic systems therefore represents a multifunctional strategy that simultaneously addresses nutrient recovery, biomass production, and bioenergy feedstock generation. Parallel studies confirm that *Chlorella* sp. cultivated in hydroponic or aquaculture effluents can achieve >85% nutrient removal while producing polyunsaturated fatty acid (PUFA)-rich biomass, effectively reducing synthetic fertilizer requirements by 50–90% compared to standalone mixotrophic cultivation [84–86]. Life-cycle assessments (LCAs) consistently demonstrate that coupling algal cultivation with wastewater treatment significantly reduces freshwater demand (up to 90%), fertilizer inputs, and eutrophication potential compared with standalone algal biofuel systems [15,87,88]. Reviews of second- and third-generation bioethanol pathways indicate that such hybrid systems represent the optimal balance between productivity and environmental sustainability [44,45,89,90].

Proposed Continuous Operation Strategy based on the kinetic parameters determined in batch mode, a theoretical model for continuous operation was established. The maximum specific growth rate (μ_{\max}) observed in the 100% AE medium was 0.42 d⁻¹ (Figure 3). According to Chemostat theory, the critical dilution rate (D_{crit}) corresponds to μ_{\max} , beyond which cell washout occurs. To ensure stable operation and maximum biomass productivity (P_{\max}), the operating dilution rate (D_{op}) is recommended to be set at approximately 75% of μ_{\max} ($D_{\text{op}} = 0.31 \text{ d}^{-1}$). This corresponds to a Hydraulic Retention Time (HRT) of 3.2 days ($\tau = 1/D$). Operating at this HRT in a continuous stirred-tank reactor (CSTR) would theoretically maintain the culture in the late-log phase, preventing nutrient exhaustion while maximizing the volumetric production of carbohydrate-rich biomass.

Despite the strong biological and environmental performance demonstrated, several scale-up challenges remain, including light distribution in dense cultures, hydraulic retention time optimization, biofouling, and seasonal variability in effluent composition. Addressing these constraints will require pilot-scale continuous operation and integration

with downstream processing, which form the logical next steps for advancing this system toward industrial relevance.

Table 4. Comparison of microalgal biomass yield and carbohydrate content across different wastewater types.

| Wastewater Type | Microalgal Species | Biomass Yield (g L ⁻¹) | Carbohydrate Content (% DW) | Key Outcome | Study/Source |
|--------------------------------------|-------------------------------|------------------------------------|-----------------------------|--|--------------|
| Fish farm wastewater | <i>Chlorella vulgaris</i> | 0.8–1.4 | 28–38 | Effective nutrient removal with moderate carbohydrate accumulation | [44] |
| Aquaculture effluent | <i>Chlorella sorokiniana</i> | 0.9–1.6 | 30–40 | Improved growth under diluted effluent conditions | [42] |
| Integrated aquaculture–algae systems | Mixed <i>Chlorella</i> spp. | 1.0–2.0 | 35–45 | Enhanced biofuel feedstock potential through wastewater valorization | [41] |
| Wastewater-grown <i>Chlorella</i> | <i>Chlorella vulgaris</i> | 1.2–2.1 | 32–44 | High carbohydrate productivity coupled with nutrient recovery | [45] |
| Wastewater-grown | <i>Chlorella vulgaris</i> | 1.2–2.1 | 32–44 | High productivity; >90% nutrient recovery | [91] |
| Municipal wastewater | <i>Scenedesmus obliquus</i> | 0.7–1.5 | 25–35 | >95% NH ₄ ⁺ removal; biogas synergy | [66,92,93] |
| Swine wastewater | <i>Chlorella zofingiensis</i> | 1.1–1.9 | 30–42 | 85–98% COD reduction; lipid co-production | [94,95] |
| Dairy wastewater | <i>Chlorella pyrenoidosa</i> | 0.9–1.7 | 28–40 | P removal >90%; feed valorization | [96,97] |
| Distillery effluent | <i>Chlorella vulgaris</i> | 0.6–1.3 | 26–38 | Synergy with ethanol byproducts | [79,98] |
| Food industry effluent | <i>A. protothecoides</i> | 1.0–1.8 | 33–45 | Heterotrophic growth; 92% TP removal | [96] |
| Agroindustrial waste | Mixed consortia | 0.8–1.6 | 29–41 | Reduced eutrophication potential (80%) | [94] |
| Clarified Aquaponics | <i>Chlorella</i> sp. | 2.05 | 40.70 | High biomass & bioethanol potential | This study |

5. Conclusions

This study established the feasibility of using clarified aquaponics sedimentation effluent (AE) as a cultivation medium for producing carbohydrate-rich *Chlorella* sp. biomass, with the dual objective of nutrient recovery and bioethanol-relevant feedstock generation. The results demonstrate that clarified AE can effectively replace synthetic media while maintaining high microalgal productivity under controlled laboratory conditions.

The optimal conditions determined for *Chlorella* sp. cultivation in this system were 100% Clarified AE, an irradiance of 185 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and a photoperiod of 12:12 h. Under these parameters, the culture achieved a maximum biomass concentration of 2.05 g L⁻¹ with a carbohydrate content exceeding 40% of dry weight—values competitive with those reported for *Chlorella* grown in aquaculture and municipal wastewaters. Nitrate and phosphate removal efficiencies exceeding 95% were observed, with stoichiometric analysis

attributing approximately 87.4% of nitrogen removal to direct biomass assimilation. This supports the role of microalgae as an active nutrient recovery mechanism rather than a passive polishing step.

The comparable performance observed between diluted and undiluted effluent highlights the effectiveness of the clarification process and indicates that undiluted operation is feasible, thereby eliminating freshwater demand and simplifying integration into existing aquaponic infrastructures. Furthermore, the identification of a specific light saturation threshold at $185 \mu\text{mol m}^{-2} \text{s}^{-1}$ provides practical design guidance for energy-efficient reactor operation in wastewater-based cultivation systems.

From an application perspective, the carbohydrate-rich biomass produced in this study represents a theoretical feedstock for bioethanol production. While stoichiometric conversions indicate promising ethanol potential, realized yields will depend on downstream hydrolysis and fermentation efficiencies that were not evaluated in this work. Accordingly, the present findings should be interpreted as a biological and resource-recovery assessment rather than a demonstration of commercial bioethanol production.

Several limitations should be acknowledged. The experiments were conducted at laboratory scale under batch conditions, and the temporal variability of aquaponic effluent composition and microbial community dynamics was not assessed. These factors may influence performance under continuous or pilot-scale operation. Future work should therefore prioritize continuous cultivation strategies, experimental validation of carbohydrate conversion through enzymatic hydrolysis and fermentation, and pilot-scale integration within aquaponic systems. Coupling such studies with techno-economic and life-cycle assessments will be essential to determine the realistic industrial and environmental potential of algae-based resource recovery within integrated food–energy–water systems.

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