Journal of Academia Vol. 14, Issue 1 (2026) 82 – 91

OPTIMIZATION OF *Spirulina platensis* CULTURE MEDIA USING AQUACULTURE WASTEWATER AS A SUSTAINABLE NUTRIENT SOURCE

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Abstract

Aquaculture effluent, if not utilized, causes environmental impacts due to its high organic matter content. Therefore, nutrients from aquaculture effluent can be used to cultivate *Spirulina platensis*, thereby reducing the cost of synthetic culture media and improving waste management in sustainable aquaculture systems. In this study, tilapia wastewater (TWW), catfish wastewater (CWW), and NPK fertilizer were used as alternative substrates for cultivating *S. platensis*. Cell density was recorded daily for 15 days, and biomass, protein, and chlorophyll content were determined after 15 days of *S. platensis* culture. The culture using TWW produced the highest biomass (1.53 g L^{-1} at 10^{-2} dilution), which was 34% higher than with CWW. In addition, TWW yielded the highest chlorophyll content (0.017 mg mL^{-1} at 10^{-5} dilution), 0.4% higher than CWW, and the maximum protein content (0.587 µg mL^{-1} at 10^{-4} dilution), a 13.5% increase over CWW and comparable to the standard Zarrouk medium. These results support integrating aquaculture waste into Spirulina bioprocessing for environmentally efficient protein production. This indicates that TWW is a consistent and more balanced nutrient source that can support microalgae metabolism. Therefore, environmental sustainability can be enhanced by transforming aquaculture effluents into valuable nutrient sources.

Keywords: Aquaculture waste, protein production, *Spirulina platensis*, sustainable aquaculture, Tilapia wastewater

Article History:- Received: 21 November 2025; Revised: 28 March 2026; Accepted: 15 April 2026; Published: 30 April 2026

Introduction

Spirulina platensis is a valuable microalga due to its high protein content, essential amino acids and fatty acids, micronutrients, and bioactive pigments for the food, chemical, and pharmaceutical industries (Bortolini et al., 2022; Sahil et al., 2024). It is highly digestible (85-95%) because of the absence of a cellulose-rich cell wall, making it suitable as a feed ingredient for zooplankton, fish, crustaceans, mollusks, and shellfish, including indirect delivery via gut-loaded rotifers and Artemia for larval stages (Ansari et al., 2021; Ma & Hu, 2024). For commercial use, protein and chlorophyll content are priorities, and cultivation strategies that increase biomass while optimizing these compounds remain a focus (Bortolini et al., 2022).

Aquaculture effluent is reused as a nutrient source for microalgae cultivation, which can increase biomass yield, reduce environmental impact, and lower production costs (Tom et al., 2021). This effluent contains nitrogen and phosphorus, making it suitable as a Spirulina growth medium (Kunwong et al., 2024). As a result, it can reduce waste and recover resources in line with the principles of the circular bioeconomy (Lim et al., 2021). Although Zarrouk medium remains the standard for Spirulina cultivation due to its alkaline pH and balanced macro- and micronutrient composition, this synthetic medium is expensive for large-scale microalgae cultivation and requires low-cost alternatives such as wastewater-based substrates (Afrin et al., 2025; Ragaza et al., 2020). Therefore, reliance on full-strength synthetic salts can increase operating costs at scale, supporting partial substitution or dilution with organic inputs such as aquaculture wastewater.

Despite growing interest, comparative evidence on the performance of different aquaculture wastewaters from widely farmed species such as tilapia and catfish remains limited. Most studies examine a single effluent type or compare wastewater with purely synthetic media, without controlled, evaluation of organic versus synthetic nutrient sources under matched conditions (Chaudry, 2021). Addressing this gap is important because nutrients such as nitrogen and phosphorus ratios, dissolved organics, trace elements and bioavailability can differ by fish species and production system, potentially affecting Spirulina biomass accumulation and the partitioning of protein and chlorophyll.

In this study, tilapia wastewater (TWW), catfish wastewater (CWW), and NPK fertilizer were compared as supplements to Zarrouk medium, an approach rarely found in literature, enabling species-specific substrate recommendations for microalgae-aquaculture integration. Therefore, the effects of TWW, CWW and NPK fertilizer across graded dilutions on *S. platensis* growth, protein and chlorophyll in modified Zarrouk medium are determined. The efficient use of substrates resulting from this study has the potential to provide cost savings and environmental sustainability in advancing microalgae cultivation integrated with aquaculture operations.

Methods

Stock culture maintenance

Spirulina platensis culture stocks were maintained at the Aquatic Biotechnology Laboratory, University of Selangor, using Zarrouk medium. The microalgae culture experiments were conducted under controlled conditions with white fluorescent lighting at an intensity of 2-3 kilolux, a 12:12 hour light-dark photoperiod, and a temperature of 28 ± 2°C. These cultures were shaken on a flat orbital shaker (IKA Shaker KS 260) at 150 rpm.

Preparation of Zarrouk medium

Zarrouk medium was prepared using $NaHCO_3$ (carbon source), $NaNO_3$ (nitrogen source), K_2HPO_4 (phosphorus source), $MgSO_4.7H_2O$, $CaCl_2$, $FeSO_4.7H_2O$ and trace elements as described by El-Monem et al. (2021). This medium was used as a standard because its alkaline pH and balanced nutrient profile support Spirulina growth. The chemicals were dissolved in distilled water and sterilized in an autoclave at 121°C for 15 minutes.

Aquaculture wastewater collection and pre-treatment

Wastewater from tilapia (*Oreochromis niloticus*) and catfish (*Clarias macrocephalus*) culture tanks was collected at the Aquaculture Laboratory, Universiti Selangor (UNISEL), Bestari Jaya Campus, Selangor, Malaysia. These tanks were part of a recirculating aquaculture system used for routine fish maintenance and feeding experiments. The wastewater, containing dissolved nutrients from uneaten food, fish excrement, and metabolic waste, was periodically discharged as culture effluent and subsequently reused as a nutrient source for Spirulina cultivation. This wastewater was filtered with a 50 µm plankton net to remove impurities such as uneaten food, feces and suspended solids. No biological or chemical treatment was applied to this water to ensure that the experimental conditions reflected the actual nutrient and organic load of aquaculture effluent.

Preparation of substrate dilutions

Nutrients from TWW, CWW and NPK were tested at concentrations ranging from 10^{-1} to 10^{-6}, to determine the ideal medium for cultivating *S. platensis*. Kunwong et al. (2024) reported that high effluent concentrations contain ammonia toxicity or lack oxygen, which can inhibit microalgae growth. For each dilution, 9 mL of distilled water was added to 1 mL of substrate (treatment) and each was prepared in triplicate.

Inoculation and culture conditions
Each Spirulina culture was prepared with 10 mL of substrate (TWW, CWW, or NPK) at defined dilutions ranging from 10^1 to 10^{-6}, 5 mL of *S. platensis* inoculum (initial optical density approximately OD_{560} = 0.1–0.2), 10 mL of Zarrouk medium, and 100 mL of distilled water. Treatments were compared with a control containing Zarrouk medium without substrate. All experiments were performed in triplicate (n = 3) to ensure statistical reliability. Optical density (OD_{560}) readings were recorded daily to determine the growth pattern. On day 15, cultures were harvested to measure biomass, protein, and chlorophyll content.

Growth monitoring
The cell density of the Spirulina culture was measured with a spectrophotometer (Hitachi, Japan). One milliliter of culture was placed in a cuvette, and the optical density (OD) was measured at 560 nm. This wavelength was chosen based on the absorption peak of chlorophyll A at 560 nm (Yaakob et al., 2021). Growth curves were generated from the OD data to evaluate the effects of substrate type and dilution on cell growth.

Biomass measurement
On day 15, 250 mL of Spirulina culture was centrifuged at 5000 rpm for 10 minutes using a centrifuge (Hettich Asia Pacific, Singapore). The resulting pellet was collected, and the supernatant was discarded. The pellet was washed twice with distilled water to remove residual medium. The suspension was then filtered through Whatman No. 1 filter paper and weighed to determine the biomass. The sample was dried in an oven at 40°C for 24 hours. The dry weight (g/L) was recorded to determine the total biomass.

Determination of chlorophyll content
A total of 2 mL of Spirulina culture was centrifuged at 2000 x g for 2 minutes, and the resulting pellet was placed in 5 mL of methanol according to the chlorophyll extraction method described by Ansari et al. (2021). The solution was incubated for 5 minutes at 70°C, then cooled and centrifuged again. The supernatant was measured for optical density (OD) at 665 nm and 649 nm. Total chlorophyll (µg/mL) was calculated using Equation 1.

$$\text{Total chlorophyll } (\mu g/mL) = \{[(20.29 \times OD_{665}) + (8.05 \times OD_{649})] \times V \times DF\}/1000 \quad (1)$$

where, V is the volume of methanol (mL) and DF is the dilution factor.

Determination of protein content
The Spirulina culture was centrifuged to 5 mL, and the resulting pellet was dissolved in alkaline buffer to determine the protein content using the Lowry method. The optical density was measured at 750 nm with a spectrophotometer after reaction with the Lowry reagent. The protein content in Spirulina was determined in µg/mL, using Bovine Serum Albumin (BSA) as the standard.

Statistical analysis
One-way analysis of variance was used to assess the effects of different substrates and dilutions on Spirulina cultivation using IBM SPSS Statistics v20. Duncan's post-hoc test was used to determine pairwise variation. Results are presented as mean ± standard deviation (SD), with significance set at $p < 0.05$.

Results and Discussion

Cell density
Figure 1-3 shows the cell concentration of *S. platensis* from the start of culture until day 15 using Zarrouk medium supplemented with aquaculture effluent (TWW and CWW) and synthetic NPK fertilizer at all dilutions (10^1 to 10^{-6}). Figure 1 illustrates the lag phase from day 1 to 5 and the exponential phase on day 6 in Spirulina TWW culture, indicating that cellular adaptation to the new environment occurs over 5 days, followed by cell growth. At a medium dilution of 10^{-4} (optimum OD 0.9), which indicates a good nutrient balance for photosynthesis, higher dilutions of 10^{-5} to 10^{-6}

consistently produced better results than lower dilutions such as 10^{-1}. Liu et al. (2025) stated that lower dilutions experienced nutrient inhibition due to high formation of ammonia and organic compounds. In addition, concentrated wastewater can reduce light penetration and affect carbon fixation efficiency (Delrue et al., 2017; Soni et al., 2019).

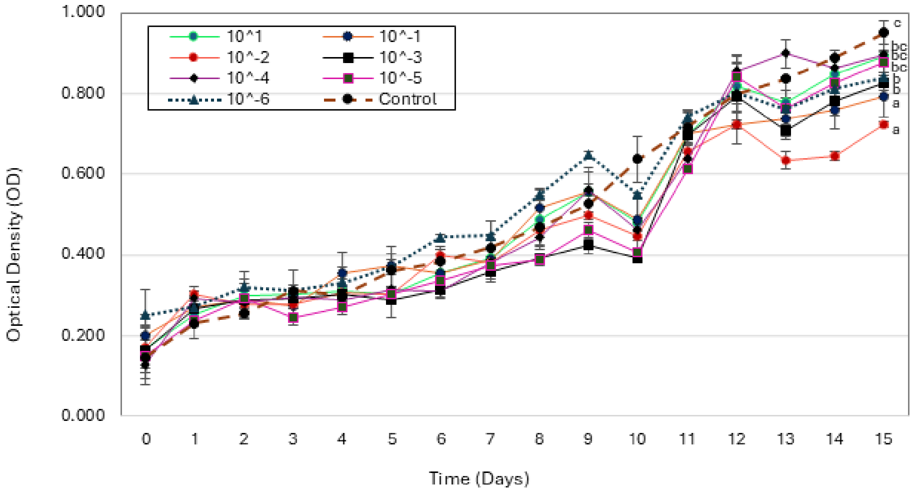

Figure 1. Optical density of *Spirulina platensis* in Zarrouk's medium supplemented with tilapia wastewater substrate for 15 days at all dilutions (10^1 to 10^{-6}). Error bars represent the standard error of triplicate cultures. Different letters at day 15 indicate significant differences between treatments ($p < 0.05$).

Meanwhile, the cell density in Spirulina CWW culture reached a maximum OD of only 0.680 at a 10^{-1} dilution (Figure 2). This is due to the inhibition of photosynthesis caused by bacterial growth, which increases the organic load and oxygen demand (Nogueira et al., 2018; Li et al., 2024). In addition, the nutrient composition of CWW, especially nitrogen and phosphorus, is unbalanced compared to TWW culture.

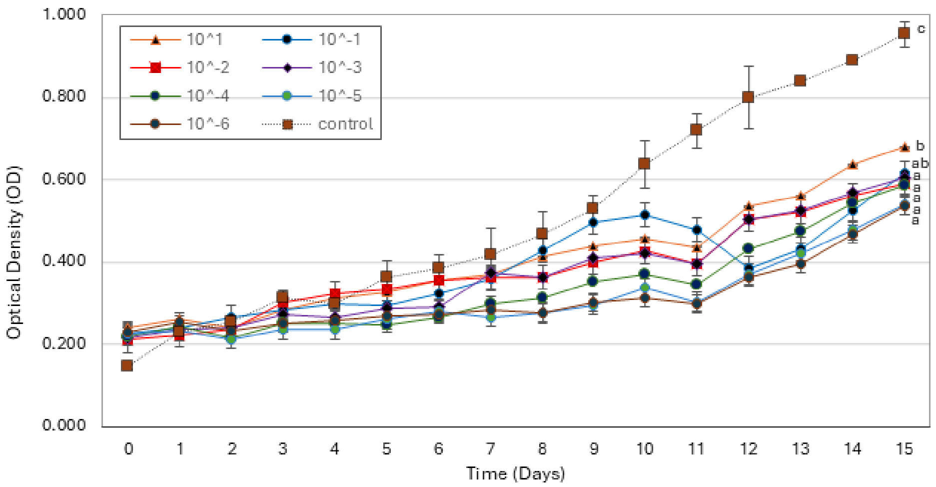

Figure 2. Optical density of *Spirulina platensis* in Zarrouk's medium supplemented with catfish wastewater substrate for 15 days at all dilutions (10^1 to 10^{-6}). The error bars represent the standard error of triplicate cultures. Different letters at day 15 indicate significant differences between treatments ($p < 0.05$).

Figure 3 shows that Spirulina NPK culture has a lower cell density than TWW and CWW. Although Spirulina NPK culture contains the macronutrients nitrogen, phosphorus, and potassium, it lacks essential micronutrients that serve as organic cofactors for metabolism and chlorophyll synthesis in microalgae (Lathifah et al., 2021; Zhang et al., 2020). Osmotic stress from concentrated fertilizer solutions, as well as high salt and ammonia levels, can reduce microalgae growth performance (Wang

& Zhang, 2022). Therefore, aquaculture effluent substrates provide a nearly complete nutrient profile that is more compatible with microalgae physiology than synthetic media.

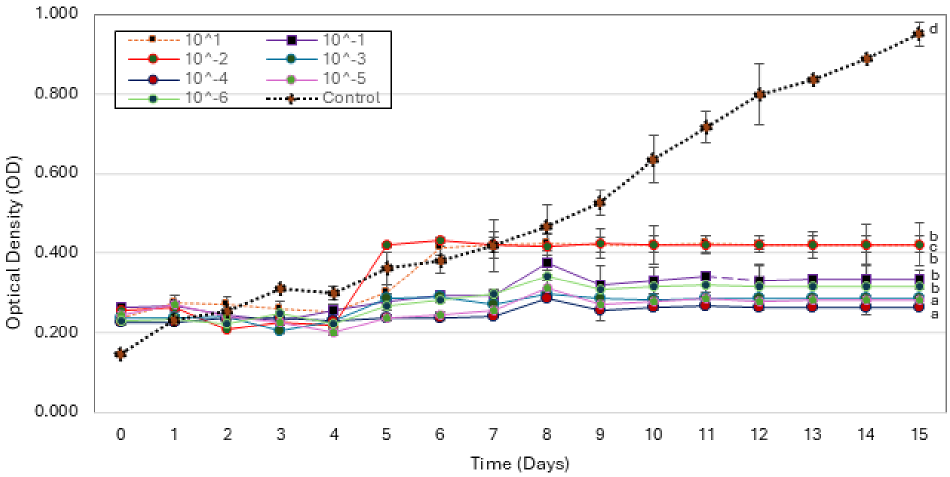

Figure 3. Optical density of *Spirulina platensis* in Zarrouk's medium supplemented with NPK fertilizer substrate for 15 days in all dilutions (10^{1} to 10^{-6}). Error bars represent the standard error of triplicate cultures. Different letters at day 15 indicate significant differences between treatments ($p < 0.05$).

Biomass yield

Figure 4 shows the biomass yield of *S. platensis* after a 15-day cultivation period in Zarrouk medium supplemented with different nutrient substrates, TWW, CWW, and NPK fertilizers at six dilution levels (10^{-1} to 10^{-6}). The highest biomass yield was observed in TWW at a 10^{-2} dilution (1.53 g/L), followed by moderate yields in CWW (1.19 g/L at 10^{-3}) and the lowest in cultures supplemented with NPK (below 0.6 g/L across all dilutions). This indicates that TWW effluent can provide a balanced nutrient composition, with essential macronutrients (nitrogen, phosphorus, potassium) and trace elements available for the growth of *S. platensis*, compared to CWW and synthetic NPK fertilizers. At a moderate dilution of 10^{-2}, optimal growth and cell proliferation of this microalga are observed. However, at higher effluent concentrations (10^{-1}), ammonia toxicity limits cell growth efficiency. In contrast, high dilutions (10^{-5}–10^{-6}) result in limited nutrient availability, which interferes with the growth of this microalga.

An ideal effluent concentration with balanced nutrients is essential for establishing Spirulina biomass cells. Previous studies indicate that photosynthetic efficiency and nitrogen uptake are influenced by the optimal effluent range (Kim et al., 2018; Liu et al., 2025). Biomass enhancement in *Spirulina subsalsa* was achieved using monosodium glutamate wastewater at an intermediate dilution (Zhang et al., 2020). In contrast, in CWW effluent, the potential for oxygen depletion due to high microbial activity limits microalgal photosynthesis and carbon assimilation (Böpple et al., 2024). Therefore, at moderate dilution, TWW can provide a sustainable nutrient source for *S. platensis* cell growth and support the production of high-value biomass.

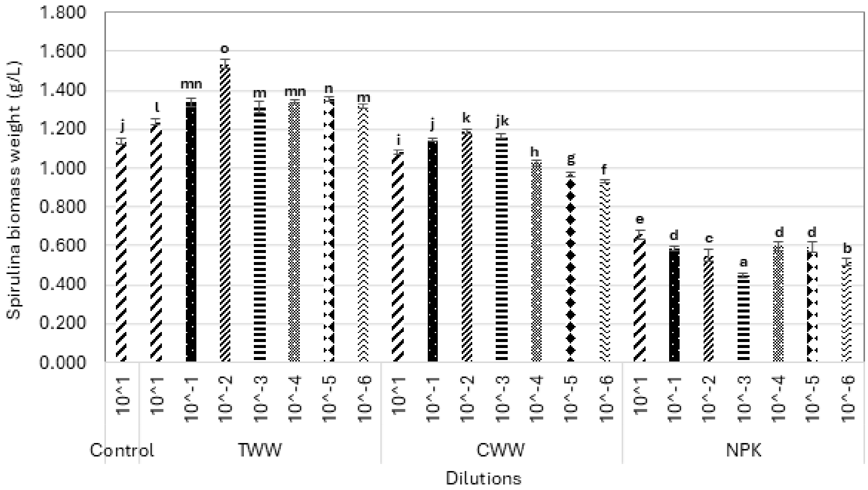

Figure 4. Biomass of each dilution after a 15-day growth cycle of Spirulina in Zarrouk's medium supplemented with various substrates and dilutions. Error bars indicate the standard deviation and statistically significant differences between treatments are indicated by different letters ($p < 0.05$).

Protein content

The protein concentration in *S. platensis* on day 15 exceeded 0.5 µg/mL when using TWW and CWW effluents. TWW effluent at a 10^{-4} dilution produced the highest protein concentration (0.587 µg/mL), comparable to Zarrouk medium (Figure 5). This indicates that TWW effluent can provide a nutrient composition similar to Zarrouk medium, supporting amino acid synthesis and protein formation in this microalga. At this dilution, it also reduces the toxicity of ammonia and organic compounds while supplying sufficient nutrients for protein biosynthesis. In contrast, NPK synthetic fertilizer resulted in the lowest protein concentration at a 10^{-4} dilution (0.252 µg/mL), likely due to its unbalanced nutrient composition, which limits protein synthesis.

Similar results were reported in previous studies, which found that optimal protein accumulation and nitrogen assimilation efficiency in microalgae are achieved with moderate nutrient concentrations (Li et al., 2022; Liu et al., 2025). In contrast, effluents with excess nutrients cause osmotic stress and oxygen deficiency, subsequently inhibiting protein synthesis and microalgal cell proliferation (Soni et al., 2019; Srimongkol et al., 2022). However, CWW effluent results in moderate protein synthesis, likely due to competition between microbes and organic matter in the culture (Nogueira et al., 2018). Additionally, imbalanced nutrient content and the absence of trace nutrients also limit protein synthesis, as observed in cultures using synthetic NPK fertilizers (Cepoi et al., 2018; Zhang et al., 2020). Moreover, microalgae cultured in organic effluents with superior nutrients produce higher-quality secondary metabolites and micronutrients due to enhanced metabolic performance (Wang & Zhang, 2022). This process can also support a circular bioeconomy by recycling resources and reducing fertilizer dependency (Abdelfattah et al., 2023; Jasni et al., 2021). In summary, the different nutrient profiles in TWW and CWW effluents result in varying protein contents, with tilapia effluent generally providing a better N:P ratio and less inhibitory organic matter.

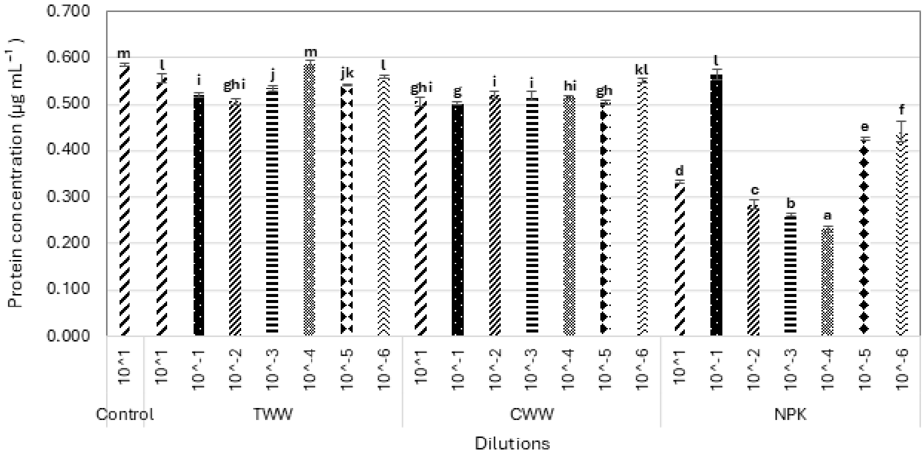

Figure 5. Protein content of each dilution after a 15-day growth cycle of Spirulina in Zarrouk's medium supplemented with various substrates and dilutions. Error bars indicate the standard deviation and statistically significant differences between treatments are labeled with different letters ($p < 0.05$).

Chlorophyll content

The chlorophyll concentration in *S. platensis* after 15 days of culture was highest in TWW effluent at a 10^{-5} dilution (0.017 mg/mL). Spirulina cultured in Zarrouk medium had a chlorophyll content intermediate between TWW and CWW (Figure 6). These results suggest that TWW provided a more favorable growth environment for chlorophyll biosynthesis than CWW and NPK under the conditions tested. Notably, optimal chlorophyll accumulation was observed at dilutions of 10^{-4} to 10^{-5}. At these dilutions, light penetration increased, while nutrient availability remained sufficient to support pigment synthesis. Additionally, inhibitory effects from concentrated nutrient inputs or salinity-related stress have been reported in microalgal cultivation systems, including those using fertilizer-based or wastewater-based media (Lathifah et al., 2021; Zhang et al., 2020).

Previous studies have reported that aquaculture effluent can increase light utilization and nutrient uptake efficiency in Spirulina cultures at moderate dilutions (Kunwong et al., 2024; Wang & Zhang, 2022). Abdelfattah et al. (2023) and Koutra et al. (2022) also stated that organic wastewater substrates provide trace metals that stimulate growth and pigment synthesis. Additionally, the organic carbon and microbial metabolites present in wastewater likely enhance pigment stability and energy conversion efficiency (Mohd Syahril et al., 2012; Wang & Zhang, 2022). In this study, TWW consistently outperformed CWW and NPK fertilizers, demonstrating that TWW contained a more suitable effluent composition. The differences in metabolism, gut, and nutritional properties of aquaculture species such as tilapia and catfish influence nutrient release patterns in wastewater. The consistent results for chlorophyll, protein, and biomass content in *S. platensis* grown in TWW effluent indicate that chlorophyll is a sensitive biological marker for assessing cultures' health in terms of nutrient efficiency and metabolic status. To further enhance protein and chlorophyll synthesis, the synergistic interactions between nutrients and microalgae should be studied in detail. This knowledge can then be used to optimize the biochemical output of high-quality Spirulina cells for large-scale cultivation.

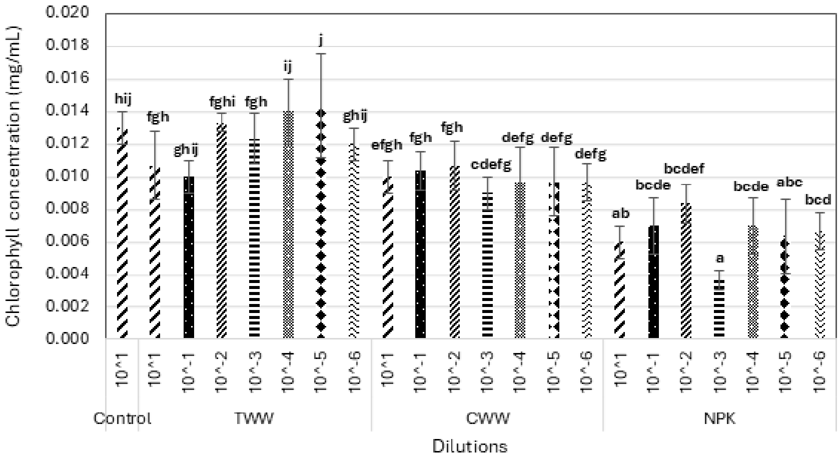

Figure 6. Chlorophyll content of each dilution after a 15-day growth cycle of Spirulina in Zarrouk's medium supplemented with different substrates and dilutions. Error bars indicate standard deviation, and statistically significant differences between treatments are indicated by different letters ($p < 0.05$).

Conclusion

Nutrient sources from aquaculture effluent can be used to culture *S. platensis*. However, the nutrient profile of the effluent depends on the species and the dilution required to achieve optimal results. This study shows that TWW effluent provides the best performance in biomass, protein, and chlorophyll production. Additionally, cultivating Spirulina with aquaculture effluent can reduce the cost of culture media and promote environmental sustainability. However, the use of this effluent needs to be studied in detail on a large scale because it involves variability in wastewater composition. In conclusion, cultivating Spirulina using aquaculture effluent offers a promising approach for food and bioresource sustainability and environmental protection.

Acknowledgement/Funding

The authors express their deep appreciation and gratitude to ARC Berkat Agro Food Sdn Bhd and Universiti Teknologi MARA, Malaysia for their support and funding of this study. This study is funded by the Ministry of Higher Education under the FRGS for research support (FRGS/1/2023/WAB04/UNISEL/02/1).

Author Contribution

R Othman - conceptualization, validation, resources, supervision, project administration, funding acquisition. AIA Zulkurnain - methodology, data curation, writing-original draft preparation. NA Suliman - methodology, resources, writing-review and editing. FN Mansor - investigation (data collection), writing-review and editing. EF Hashim - resources, data curation. MS M Zan - formal analysis, resources, writing-review and editing, funding acquisition. SH Mohtar - writing-review and editing, funding acquisition. IF A Ghani - validation, writing-review and editing, funding acquisition.

Conflict of Interest

The authors declare that they have no competing financial, personal or institutional interests that may have influenced the work reported in this study.

Declaration on the Use of Generative AI

The authors acknowledge the use of OpenAI's ChatGPT (GPT-5, 2025 version) exclusively for language refinement, grammar correction and stylistic enhancement of the manuscript. The AI tool was not used for data analysis, interpretation of results, generation of scientific content or drawing conclusions. All intellectual and scientific contributions, including conceptualization, experimental design, data collection, analysis and interpretation, were performed by the authors.

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