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# Phytoremediation of Tilapia (*Oreochromis Niloticus*) Aquaculture Wastewater using *Spirulina* sp.

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**ABSTRACT**
Tilapia, *Oreochromis niloticus* (Linnaeus, 1758), aquaculture generates nutrient-rich wastewater containing ammonia, nitrate, and phosphate that may degrade receiving water quality and promote eutrophication. Microalgae-based phytoremediation has emerged as a sustainable alternative for nutrient removal while simultaneously producing valuable biomass. This study aimed to evaluate the effectiveness of Spirulina sp. in treating tilapia aquaculture wastewater and to assess its growth performance under different wastewater concentrations. A laboratory experiment was conducted using five treatments consisting of a negative control (wastewater without Spirulina), a positive control (Spirulina cultured in 100% Zarrouk medium as the optimum growth reference), and wastewater concentrations of 25% (P1), 50% (P2), and 75% (P3). Ammonia, nitrate, and phosphate concentrations were analyzed every two days. At the same time, the relative growth of Spirulina sp. was monitored daily, and chlorophyll content was determined at the end of the cultivation period. The positive control cultivated in 100% Zarrouk medium, representing the optimum growth condition, achieved the highest relative growth of 716.9% on day 9 and the highest chlorophyll content of 0,62 mg/L. In comparison, wastewater treatments resulted in lower physiological performance but demonstrated considerable phytoremediation capacity, with maximum removal efficiencies for ammonia, nitrate, and phosphate of 48.79%, 71.87%, and 55.68%, respectively. Moderate wastewater concentrations produced superior phytoremediation performance compared with higher wastewater loading, indicating that excessive nutrient concentrations reduced nutrient assimilation efficiency. Wastewater concentrations above the optimum level also suppressed the physiological performance of Spirulina sp., as reflected by lower relative growth and chlorophyll content. These findings demonstrate that Spirulina sp. is a promising eco-friendly phytoremediation agent for tilapia aquaculture wastewater and highlight the importance of optimizing wastewater concentration to achieve both high nutrient removal efficiency and sustainable microalgal growth.

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## ■ INTRODUCTION

Aquaculture has become one of the fastest-growing food production sectors worldwide, particularly in the cultivation of tilapia (*Oreochromis niloticus*), due to its high economic value and increasing market demand. However, the rapid intensification of aquaculture practices has led to significant environmental concerns, particularly regarding the generation of nutrient-rich wastewater. Aquaculture effluents commonly contain elevated concentrations of ammonia, nitrate, phosphate, suspended solids, and organic matter derived from unconsumed feed, fish excretion, and metabolic activities (FAO, 2024). Excessive discharge of these nutrients into aquatic environments may stimulate eutrophication, algal blooms, oxygen depletion, and ecological imbalance in receiving waters. Consequently, the development of environmentally sustainable wastewater treatment technologies has become an important issue in modern aquaculture systems.

Tilapia (*Oreochromis niloticus* Linnaeus, 1758) farming is an economically valuable aquaculture activity and plays an important role as a source of animal protein for the Indonesian population. National tilapia production has shown an increasing trend in line with relatively stable domestic and export market demand. However, the intensification of aquaculture practices, particularly in closed-pond and semi-intensive systems, has contributed to the increasing volume of wastewater generated. Such wastewater contains uneaten feed, fish feces, and nitrogen compounds, including ammonia, nitrite, and nitrate, as well as phosphate at relatively high concentrations (Al-Rosyid et al., 2024). The direct discharge of untreated wastewater into receiving water bodies may trigger eutrophication, reduce dissolved oxygen levels, and disrupt the ecological balance of natural aquatic ecosystems.

Currently, aquaculture wastewater treatment mainly depends on sedimentation or basic filtration techniques, which have limited success in lowering nutrient levels. This condition highlights the need for alternative methods that are more environmentally friendly and sustainable. One promising technology is phytoremediation, which uses photosynthetic organisms, such as aquatic plants and microalgae, to reduce pollutant concentrations in wastewater through natural biological processes (Islam et al., 2023). Conventional wastewater treatment in freshwater aquaculture generally relies on sedimentation tanks, mechanical filtration, and biological filtration to remove suspended solids and organic matter. These technologies are effective in reducing particulate pollutants; however, their performance in removing dissolved inorganic nutrients, particularly ammonia, nitrate, and phosphate, remains limited because these compounds are predominantly present in soluble forms that are not efficiently retained by physical filtration processes. Consequently, nutrient accumulation may still occur in aquaculture effluents discharged into receiving waters, increasing the risk of eutrophication and ecological degradation (Crab et al., 2007; FAO, 2024). Recent studies have therefore focused on integrating biological treatment technologies, including phytoremediation using aquatic plants and microalgae, to improve nutrient removal from aquaculture wastewater. Microalgae-based systems offer several advantages over conventional filtration by simultaneously assimilating dissolved nitrogen and phosphorus into cellular biomass while producing valuable bioproducts. Such systems have demonstrated considerable potential for improving wastewater quality and supporting water reuse in intensive freshwater aquaculture (El-Sheekh et al., 2023; Kumari et al., 2023). Therefore, the application of *Spirulina* sp. represents a promising, environmentally friendly alternative for enhancing nutrient removal beyond the capability of conventional treatment systems.

*Spirulina* sp. is a cyanobacterial microalga known for its high capacity to absorb nutrients, particularly nitrogen and phosphorus, which are the main contributors to pollution in aquaculture waters. In addition to serving as a biological agent for wastewater treatment, *Spirulina* sp. is also capable of producing high-value biomass due to its protein content, natural pigments such as phycocyanin, and essential fatty acids. This biomass has potential applications as a natural feed ingredient, health supplement, and cosmetic raw material (Al-Rosyid et al., 2022). Therefore, the utilization of *Spirulina* sp. in the treatment of tilapia aquaculture wastewater not only contributes to environmental pollution control but also creates opportunities for added economic value through the use of generated by-products.

Phytoremediation has recently gained considerable attention as an eco-friendly and cost-effective approach for treating nutrient-rich wastewater. This technology utilizes photosynthetic organisms, including aquatic plants and microalgae, to absorb, transform, and assimilate pollutants from contaminated water. Among various microalgal species, *Spirulina* sp. has emerged as a promising phytoremediation agent due to its rapid relative growth, high nutrient assimilation capability, tolerance to alkaline conditions, and potential economic value as a biomass source (El-Sheekh et al., 2023). Several studies have demonstrated the effectiveness of *Spirulina* sp. in reducing nitrogen and phosphorus concentrations in domestic and industrial wastewater. For example, Hernández-Pérez et al. (2022) reported that *Spirulina* sp. reduced ammonia concentrations by more than 60% in nutrient-enriched wastewater systems, while Choi et al. (2024) observed significant phosphate uptake by *Spirulina* cultures under controlled laboratory conditions.

Despite these findings, studies specifically investigating the application of *Spirulina* sp. for tilapia aquaculture wastewater treatment remain relatively limited, particularly regarding the relationship between wastewater concentration and the physiological response of *Spirulina* sp. Most previous studies focused primarily on pollutant removal efficiency without comprehensively evaluating the effects of different wastewater concentrations on microalgal growth dynamics and chlorophyll content. In addition, information on the optimum wastewater concentration that simultaneously supports efficient nutrient removal and sustainable *Spirulina* growth remains insufficient. Therefore, further investigation is necessary to determine the tolerance threshold and remediation performance of *Spirulina* sp. under different wastewater loading conditions.

The novelty of this research lies in the integrated evaluation of nutrient removal efficiency, growth performance, and chlorophyll content of Spirulina sp. cultivated in tilapia aquaculture wastewater with different concentration levels. Unlike previous studies that mainly emphasized pollutant reduction, this research simultaneously assessed the physiological responses of Spirulina sp. as indicators of microalgal adaptability and remediation sustainability. Furthermore, this study provides comparative data on the effectiveness of *Spirulina* sp. across multiple wastewater concentrations, which may help optimize microalgae-based phytoremediation systems for aquaculture wastewater management. Through this innovation, the study is expected to provide a practical contribution to the implementation of green technology in the fisheries sector and support the achievement of Sustainable Development Goal (SDG) 6, namely clean water and sanitation. Previous studies have demonstrated that excessively concentrated wastewater may inhibit microalgal growth due to increased ammonia toxicity, osmotic stress, and reduced light penetration caused by elevated turbidity, whereas highly diluted wastewater may not provide sufficient nutrients to sustain optimal biomass production and nutrient assimilation (El-Sheekh et al., 2023; Kumari et al., 2023). Consequently, intermediate wastewater concentrations are expected to provide a more balanced environment by supplying adequate nutrients while minimizing physiological stress. Based on these findings, a wastewater concentration of approximately 50% was hypothesized to provide the optimum condition for both nutrient removal and the physiological performance of Spirulina sp.

Based on the background described above, this study addressed the following research questions: (1) How effective is *Spirulina* sp. in reducing ammonia, nitrate, and phosphate concentrations in tilapia aquaculture wastewater? (2) How do different wastewater concentrations affect the relative growth and chlorophyll content of *Spirulina* sp.? and (3) What is the optimum wastewater concentration for microalgal growth and phytoremediation performance?. Accordingly, the research hypothesis proposed that wastewater concentration significantly influences the nutrient removal efficiency, relative growth, and chlorophyll content of *Spirulina* sp., with the 50% wastewater concentration expected to provide the optimum balance between nutrient availability and physiological tolerance, thereby resulting in the highest phytoremediation performance.

## ■ METHOD

### Materials

The materials used in this study included: Tilapia aquaculture wastewater containing residual feed and feces; a culture of *Spirulina* sp. as the phytoremediating microalga; and Zarrouk medium as the initial growth medium for *Spirulina* sp. The equipment used included glass reactors (5 L capacity), aerators, a pH meter, a dissolved oxygen (DO) meter, and laboratory chemical analysis instruments in accordance with APHA standard methods (Srimongkol et al., 2022). The positive control treatment consisted of a pure *Spirulina* sp. culture cultivated in 100% Zarrouk medium without the addition of tilapia aquaculture wastewater. This treatment was established to represent the optimum growth condition of *Spirulina* sp. under standard culture conditions and served as a reference for evaluating the physiological response and remediation performance of *Spirulina* sp. in wastewater treatments. The phytoremediation experiment was carried out for 10 days, during which Spirulina sp. was cultivated under controlled laboratory conditions. Water samples were collected at 2-day intervals (days 2, 4, 6, 8, and 10) to evaluate changes in ammonia, nitrate, and phosphate concentrations. Each treatment was conducted in triplicate (three replications) to ensure data validity (Osti et al., 2020). The analyzed parameters included ammonia ($NH_3$-N), nitrate ($NO_3^-$), and phosphate ($PO_4^{3-}$). Measurements were carried out before and after treatment. Ammonia concentration was determined using the Nessler method, which measures Total Ammonia Nitrogen (TAN), including both ionized ammonium ($NH_4^+$) and un-ionized ammonia ($NH_3$). Because the toxic effects of ammonia on microalgae are primarily associated with the $NH_3$ fraction, the proportion of un-ionized ammonia was estimated based on the measured pH and temperature using the equilibrium relationship proposed by Emerson et al. (1975).

$$\mathrm{f}NH_3 = \frac{1}{1+10^{(pKa-pH)}}$$

$$\mathrm{pK}a = 0{,}09018 + \frac{12729{,}92}{\mathrm{T}}$$

$$\mathrm{NH_3} = TAN\ x\ \mathrm{f}NH_3$$

### Equipment and Parameters

*Spirulina* sp. was cultivated using Zarrouk medium under optimum conditions, namely pH 9–10, temperature 28–32°C, and light intensity of approximately 3000 lux, until the culture reached the exponential growth phase (Agarwal & Rani, 2022). During the cultivation period, *Spirulina* sp. cultures were maintained under a controlled light–dark (L:D) photoperiod of 12:12 h to support optimal photosynthetic activity and cellular metabolism. Light intensity was set at approximately 3000 lux at the surface of the glass reactor using fluorescent lamps under a 12:12 h light–dark photoperiod. However, the actual light intensity inside the culture medium was not directly measured in this study. This condition is acknowledged as a methodological limitation because tilapia aquaculture wastewater contains suspended solids and organic matter that may reduce light penetration and affect the photosynthetic activity of *Spirulina* sp. Therefore, future studies should measure internal irradiance at different depths within the reactor to determine the actual photon availability received by microalgal cells during phytoremediation.

$$\text{Relative Growth (\%)} = \left(\frac{Xt - Xo}{Xo}\right) \text{x } 100$$

**Phytoremediation Process**

A total of 4 L of wastewater was introduced into each glass reactor, followed by the addition of *Spirulina* sp. inoculum at 10% of the reactor volume. During the phytoremediation process, continuous aeration was supplied to each reactor using an aquarium aerator at an airflow rate of approximately 1.0 L/min (equivalent to approximately 0.25 vvm). Aeration was maintained uniformly across all treatments to ensure adequate mixing, prevent sedimentation of microalgal biomass, and maintain dissolved oxygen availability throughout the experimental period. The selected aeration rate was considered sufficient to support the growth and metabolic activity of *Spirulina* sp. without causing excessive turbulence that could disrupt cell integrity (Kumari et al., 2023). Water samples were collected at the end of each contact time period for water quality analysis. Prior to the dilution process, raw tilapia aquaculture wastewater (100% concentration) was characterized to determine the baseline concentrations of the main pollutants, including ammonia, nitrate, and phosphate. Initial measurements were conducted using APHA standard methods to ensure the accuracy and reproducibility of the experimental procedures. Raw wastewater was analyzed prior to dilution to determine baseline nutrient concentrations. Wastewater treatments were subsequently prepared volumetrically using distilled water to obtain 25%, 50%, and 75% wastewater concentrations. Therefore, the initial nutrient loads in each treatment were proportional to the dilution ratio and are presented in Table X as estimated initial concentrations derived from measured raw wastewater values. Wastewater dilution treatments were subsequently prepared using distilled water to obtain concentrations of 25% (P1), 50% (P2), and 75% (P3), while the untreated wastewater served as the reference concentration. The dilution process was performed volumetrically using calibrated measuring cylinders to ensure consistency among treatments. Standardization of initial pollutant concentrations before treatment was performed to validate the comparative effectiveness of *Spirulina* sp. in reducing nutrient concentrations across different wastewater loading conditions.

**Laboratory Analysis**

This research employed a laboratory experimental method with a quantitative approach to evaluate the effectiveness of *Spirulina* sp. in reducing pollutant concentrations in tilapia aquaculture wastewater. The system used was a batch phytoremediation system, namely a closed, non-flowing system in which *Spirulina* sp. was cultivated in simple reactors containing aquaculture wastewater under different contact times (Enochs et al., 2023). Laboratory analysis was conducted in accordance with APHA standards, including ammonia determination by the Nessler method, nitrate determination by titration, phosphate determination by the ascorbic acid method, and direct measurements of dissolved oxygen (DO) and pH using digital meters (Srimongkol et al., 2022).

**Data Analysis**

The data obtained were analyzed using a quantitative descriptive approach, presenting graphs of pollutant concentration dynamics over time. Furthermore, a one-way analysis of variance (ANOVA) was performed to evaluate the effect of variation in contact time on the removal efficiency of each observed parameter (Li et al., 2019). Prior to conducting one-way analysis of variance (ANOVA), assumption tests were performed to ensure that the data met the requirements for parametric statistical analysis. Data normality was evaluated using the Shapiro–Wilk test, while homogeneity of variance among treatments was assessed using Levene's test. The results of these preliminary analyses indicated that the data were normally distributed and exhibited homogeneous variances ($p$ > 0.05), thereby fulfilling the assumptions required for ANOVA. Subsequently, a one-way ANOVA was

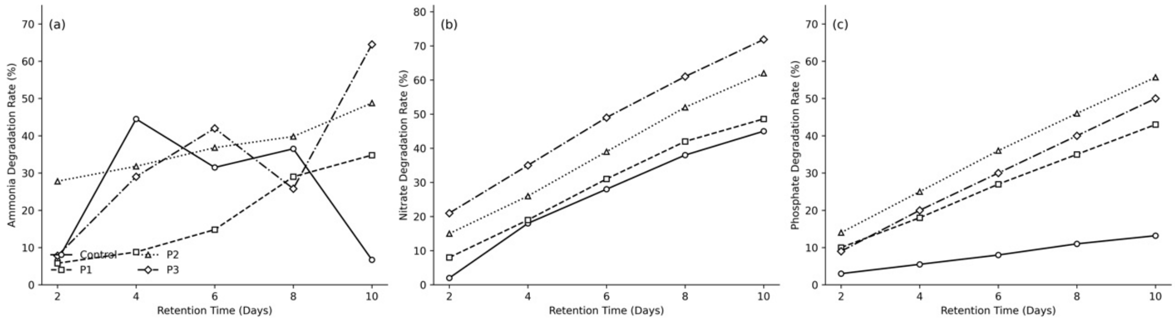


**Figure 1.** Degradation rate of (a) ammonia, (b) nitrate, and (c) phosphate

conducted to assess the effects of different wastewater concentrations on nutrient removal efficiency, relative growth, and chlorophyll content in *Spirulina* sp. When significant differences among treatments were detected, further comparisons were conducted using Tukey's Honestly Significant Difference (HSD) post hoc test at a 95% confidence level.

## ■ RESULTS AND DISCUSSION

### Ammonia, Nitrate, and Phosphate Removal Rates

Ammonia in aquatic environments is generally non-toxic when present as the ionized form, ammonium ($NH_4^+$), which is relatively safe for aquatic organisms such as fish and shrimp. In contrast, un-ionized ammonia ($NH_3$) is toxic at elevated concentrations (Mangkoedihardjo & Al-Rosyid, 2023). The results showed that the ammonia removal rate tended to increase over time in all treatments, except in the negative control. On day 10, the highest ammonia removal efficiency was observed in treatment P2, reaching 48.79%, with a final ammonia concentration of 0,012 mg/L. Meanwhile, the lowest removal rate was observed in the negative control (P0), at 6.68%, although the final ammonia concentration remained unchanged at 0.012 mg/L (Figure 1a). The similarity in final ammonia concentrations between P0 and P2 was attributable to differences in initial ammonia concentrations among treatments following dilution. Each treatment contained varying proportions of aquaculture wastewater, resulting in unequal initial nutrient loads prior to the phytoremediation process. Treatment P2 initially had a substantially higher ammonia concentration than P0; therefore, despite achieving a higher removal efficiency, the residual ammonia concentration at the end of the experiment was comparable to that of P0. In contrast, the negative control exhibited a lower removal percentage because its initial ammonia concentration was already relatively low, leading to a smaller measurable decrease during the observation period. This result indicates that removal efficiency percentages should be interpreted alongside initial pollutant concentrations, as identical final concentrations may result from different initial nutrient loads and remediation capacities. Statistical analysis indicated a significant difference in ammonia removal efficiency between the negative control and the three other treatments ($p < 0.05$).

On day 10, treatment P3 showed the highest nitrate removal efficiency, reaching 71.87%, with a final nitrate concentration of 5.31 mg/L. In contrast, the lowest nitrate removal efficiency was recorded in treatment P1 at 48.60%, with a final concentration of 5.39 mg/L (Figure 1b). Although descriptive differences were observed, statistical analysis indicated that nitrate removal rates among treatments were not significantly different ($p >$ 0,05). Data are presented as mean ± standard deviation (SD) (n = 3). Error bars represent the standard deviation of replicate measurements. The results of this study demonstrated that treatment P2 (50% wastewater concentration) generally exhibited higher nutrient removal performance compared to treatment P3 (75% wastewater concentration). This finding indicates that moderate wastewater concentrations provided more favorable environmental conditions for the metabolic activity and nutrient assimilation processes of *Spirulina* sp. In contrast, excessively high wastewater concentrations may impose physiological stress on microalgal cells and reduce phytoremediation efficiency.

At moderate nutrient concentrations, *Spirulina* sp. can effectively utilize nitrogen and phosphorus compounds for cellular metabolism, protein synthesis, and photosynthetic activity. Nitrogen is an essential component in amino acid, nucleic acid, and chlorophyll formation, while phosphate plays a crucial role in energy transfer and ATP synthesis. Adequate nutrient availability therefore supports optimal biomass production and enhances nutrient uptake efficiency (El-Sheekh et al., 2023). However, when nutrient concentrations exceed the tolerance threshold of the microalgal cells, the physiological balance of *Spirulina* sp. may be disrupted.

In addition to ammonia toxicity, high organic and nutrient loading in treatment P3 may increase turbidity and reduce light penetration into the culture medium. Reduced light availability limits photosynthetic activity and reduces *Spirulina* sp.'s capacity to assimilate nutrients efficiently. According to Srimongkol et al. (2022), excessive nutrient concentrations in wastewater systems may suppress microalgal productivity due to the combined effects of osmotic stress, reduced light penetration, and metabolic imbalance. Consequently, although higher wastewater concentrations contain greater nutrient availability, the physiological stress experienced by *Spirulina* sp. may reduce its remediation performance. These findings suggest that wastewater concentration plays an important role in determining the balance between nutrient availability and *Spirulina* sp.'s physiological tolerance. Moderate wastewater concentrations appear to provide optimum conditions for nutrient assimilation and microalgal growth, whereas excessive nutrient loading may inhibit metabolic activity and reduce phytoremediation efficiency.

Although ammonia concentration in this study was determined as Total Ammonia Nitrogen (TAN) using the Nessler method, the potential toxicity to *Spirulina* sp. is primarily associated with the un-ionized ammonia ($NH_3$) fraction rather than total ammonia. The proportion of $NH_3$ increases with increasing pH and temperature due to the equilibrium between $NH_4^+$ and $NH_3$. Considering that the experimental pH ranged from approximately 8.6 to 9.1 and the temperature remained within the optimum cultivation range, a greater fraction of TAN was expected to occur as $NH_3$ compared with neutral conditions. Therefore, higher wastewater concentrations may have exposed *Spirulina* sp. to increased $NH_3$ levels, contributing to physiological stress, reduced photosynthetic efficiency, and lower nutrient assimilation at the highest wastewater concentration (P3). This interpretation is consistent with the known dependence of ammonia speciation on pH and temperature (Emerson et al., 1975; US EPA, 2013).

Phosphate removal generally increased over the observation period, except in the control treatment. On day 10, treatment P2 achieved the highest phosphate removal efficiency of 55,68%, with a final phosphate concentration of 2.18 mg/L. Conversely, the lowest removal rate was found in the negative control (P0), reaching only 13.18%, with a final phosphate concentration of 2.30 mg/L (Figure 1c). Statistical analysis demonstrated a significant difference between the negative control and the three other treatments ($p < 0.05$).

Phosphate removal by *Spirulina sp.* is closely associated with active phosphate transport systems and intracellular phosphorus assimilation pathways in cyanobacteria. Under nutrient-enriched conditions, phosphate ions are transported across the cell membrane through high-affinity phosphate transporters and subsequently incorporated into essential biomolecules, including nucleic acids, phospholipids, ATP, and phosphorylated metabolites involved in cellular metabolism and photosynthesis. Cyanobacteria are also capable of accumulating excess phosphorus intracellularly in the form of polyphosphate granules, which function as phosphorus reserves under fluctuating environmental conditions. This physiological adaptation contributes to the high phosphate removal capacity frequently observed in *Spirulina*-based phytoremediation systems (Plouviez & Brown, 2024; Li & Dittrich, 2019; Grillo & Gibson, 1979; Sarkar & Sharma, 2024).

During the experiment, water quality parameters including pH, salinity, and dissolved oxygen (DO) were also monitored. Values are presented as the midpoint of the observed range ± half-range deviation obtained during the experimental period. Table 1 presents the average values of water quality parameters observed during the experimental period, including pH, dissolved oxygen (DO),

**Table 1.** Water quality parameter values

| Parameter | Unit | Treatment | | | |
|---|---|---|---|---|---|
| | | **P0** | **P1** | **P2** | **P3** |
| pH | - | 8.7 ± 0,7 | 9.05 ± 0.25 | 8.6 ± 0.7 | 8.75 ± 0.55 |
| Dissolved oxygen | mg/L | 5.73 ± 0.71 | 5.67 ± 0.59 | 5.77 ± 0.44 | 5.72 ± 0.68 |
| Salinity | PPT | 0 - 2 | 0 - 2 | 0 - 2 | 0 - 2 |

and salinity across all treatments. Overall, the environmental conditions remained relatively stable and within ranges suitable for the growth and metabolic activity of *Spirulina* sp. throughout the phytoremediation process.

The pH values across all treatments ranged from slightly alkaline to alkaline, with average values between 8.60 ± 0.70 and 9.05 ± 0.25. The highest average pH was observed in treatment P1, while the lowest was recorded in treatment P2. These alkaline conditions are considered favorable for the cultivation of *Spirulina* sp., as this microalga generally exhibits optimal growth in pH ranges between 8 and 10. Stable alkaline conditions may also support nutrient assimilation processes and reduce the toxicity of certain nitrogen compounds in the culture medium.

Dissolved oxygen concentrations remained relatively consistent among treatments, ranging from 5.67 ± 0.59 mg/L to 5.77 ± 0.44 mg/L. The highest average dissolved oxygen concentration was observed in treatment P2, whereas the lowest was found in treatment P1. The observed DO concentrations indicated sufficient oxygen availability to support microalgal metabolic activity and maintain aerobic conditions throughout the experiment. Adequate dissolved oxygen levels may also contribute to stabilizing water quality and nutrient transformation processes during phytoremediation.

Dissolved oxygen (DO) was measured using a calibrated DO meter prior to and during the experimental period. The DO meter was calibrated according to the manufacturer's instructions before measurement to minimize instrumental error. Although continuous aeration was supplied at 1.0 L/min in each 4 L reactor, the measured DO values remained within the range of 5.67–5.77 mg/L. This condition was likely influenced by oxygen consumption from heterotrophic microbial activity associated with organic matter degradation in tilapia aquaculture wastewater. Residual feed, fish feces, and dissolved organic compounds in the wastewater may increase biological oxygen demand, thereby reducing the accumulation of dissolved oxygen despite continuous aeration. In addition, oxygen was also utilized in microbial nitrification and aerobic decomposition processes during the phytoremediation period. Therefore, the observed DO values reflected the balance between oxygen input from aeration and oxygen consumption by biological processes in the reactor system.

Salinity values were relatively uniform across all treatments, with an average value of 0 – 2 ppt. This result indicates that salinity conditions were consistently maintained throughout the experimental period and remained within the tolerance range for *Spirulina* sp. cultivation. The stability of salinity among treatments suggests that variations in nutrient removal efficiency and growth performance were primarily influenced by wastewater concentration rather than changes in environmental salinity conditions.

The Relative Growth of *Spirulina* sp.

The growth of *Spirulina* sp. increased from the second to the fourth day of observation. Subsequently, from the fifth to the seventh day, growth entered the stationary phase. The highest relative growth percentage was observed in the positive control on day 9, reaching 716.9%, indicating a substantial increase in biomass or cell density compared with the initial culture condition. This value represents the percentage increase relative to day 0 and should not be interpreted as the specific relative growth (μ) (Figure 2). Statistical analysis revealed that the relative growth in the positive control was greater than in the other treatments ($p < 0.05$).

Based on the data obtained, biomass generally increased during the early cultivation period on day 2 and remained at a steady level until the stationary phase between days 5 and 7. The exponential phase reflects a balanced condition between nutrient availability and increasing cell numbers (Adamia et al., 2018). Meanwhile, the stationary phase indicates relatively constant growth without significant increase, generally due to nutrient limitation in the culture medium (Kurashvili et al., 2018).

Nitrogen is an essential nutrient that plays a key role in supporting the growth of *Spirulina* sp. High nitrogen concentrations are known to strongly influence *Spirulina* cell abundance (Nawaz et al., 2024). Adequate nutrient availability enables *Spirulina* sp. to grow optimally and reach maximum biomass production. However, on day 8, a decline in growth or cell death was observed, presumably due to nutrient depletion. In addition to nutrient

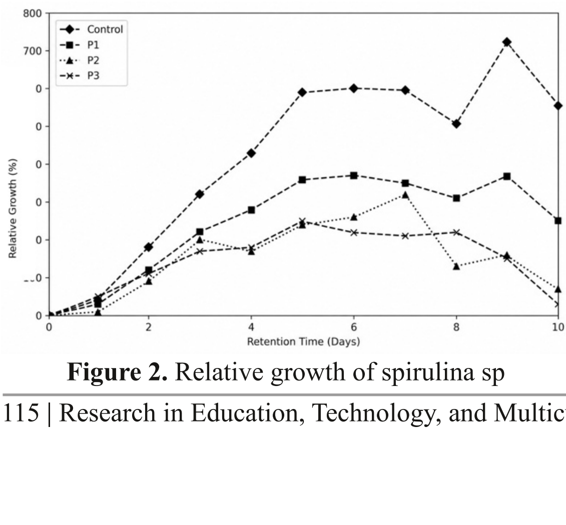


**Figure 2.** Relative growth of spirulina sp

deficiency, excessive nutrient concentrations may inhibit the growth of *Spirulina* sp. by forming toxic compounds that impair cellular growth.

The growth curve of *Spirulina* sp. showed a fluctuating pattern, characterized by a sharp decline in biomass accumulation on day 6, followed by an increase on day 8, particularly in several treatment groups. This phenomenon may be associated with physiological adaptation responses of *Spirulina* sp. to changing environmental conditions and nutrient availability during the cultivation period. In microalgal cultures, temporary decreases in cell density or relative growth are commonly observed during the transition from the exponential phase to the stationary phase due to nutrient limitation, metabolic stress, or environmental fluctuations (El-Sheekh et al., 2023).

The decline observed on day 6 was related to a temporary reduction in cellular activity caused by nutrient competition and physiological stress resulting from increased organic loading in the wastewater medium. Excessive nutrient concentrations, particularly nitrogen compounds above the optimum tolerance level, may induce osmotic stress and inhibit photosynthetic efficiency, thereby reducing biomass accumulation and chlorophyll synthesis (Choi et al., 2024). In addition, increasing biomass density during the cultivation period may reduce light penetration into the culture medium, limiting photosynthetic activity and temporarily suppressing microalgal growth (Srimongkol et al., 2022). Overall, the fluctuation pattern observed in this study indicates that wastewater concentration not only affects nutrient removal efficiency but also strongly influences the stability of *Spirulina* sp. growth dynamics during the phytoremediation process.

In addition to nutrient availability, light penetration may have contributed to the observed differences in *Spirulina* sp. growth among treatments. Although the external illumination was maintained at approximately 3000 lux under a 12:12 h light-dark photoperiod, the actual light intensity in the wastewater medium was likely lower due to suspended solids and dissolved organic matter. Increased turbidity at higher wastewater concentrations may have attenuated light penetration, thereby reducing the photon flux available for photosynthesis. Consequently, chlorophyll synthesis, carbon fixation, and biomass production may have been partially limited in treatments with higher wastewater proportions. Similar observations have been reported in previous studies, which demonstrated that reduced light penetration caused by elevated turbidity decreases photosynthetic efficiency and microalgal productivity in wastewater-based cultivation systems (El-Sheekh et al., 2023; Gonçalves et al., 2021).

Although an overall increase in *Spirulina* sp. biomass was observed during the initial cultivation period, the growth curves exhibited noticeable fluctuations rather than the typical sigmoid pattern commonly reported for microalgal cultures under controlled laboratory conditions. Several factors may have contributed to this response. First, the wastewater matrix contained suspended solids, residual feed particles, and dissolved organic matter that may have interfered with biomass measurements, particularly if optical-based methods were used. These suspended particles may contribute to fluctuations in optical density or apparent biomass values that do not exclusively represent *Spirulina* cell density (Li et al., 2021). Second, the heterogeneous composition of aquaculture wastewater may have produced temporal variations in nutrient availability and light penetration, resulting in alternating periods of physiological adaptation and biomass recovery (El-Sheekh et al., 2023). Therefore, the observed fluctuations in the present study likely reflected the combined effects of environmental adaptation, wastewater heterogeneity, and potential measurement interference rather than a continuous exponential growth phase. Accordingly, the term "growth trend" is considered more appropriate than "exponential growth phase" when describing the biomass dynamics of *Spirulina* sp. under wastewater cultivation conditions (Gonçalves et al., 2021).

**Total Chlorophyll Content**

At the end of the observation period, the highest chlorophyll content was found in the positive control treatment, while the lowest value was recorded in treatment P2 (Figure 3). Chlorophyll concentrations in the positive control and P2 treatments were 0,62 mg/L and 0,53 mg/L, respectively. Statistical analysis showed that the chlorophyll content in the positive control was not significantly different from that of treatment P1 ($p > 0.05$), but was significantly different from that of treatments P2 and P3 ($p < 0.05$).

The decrease in chlorophyll content may be influenced by the nutrient composition of the culture medium, particularly nitrogen. Excessively high nitrogen concentrations in the medium may inhibit chlorophyll synthesis and photosynthetic activity. (Gupta and Balomajumder, 2017) reported that nutrient

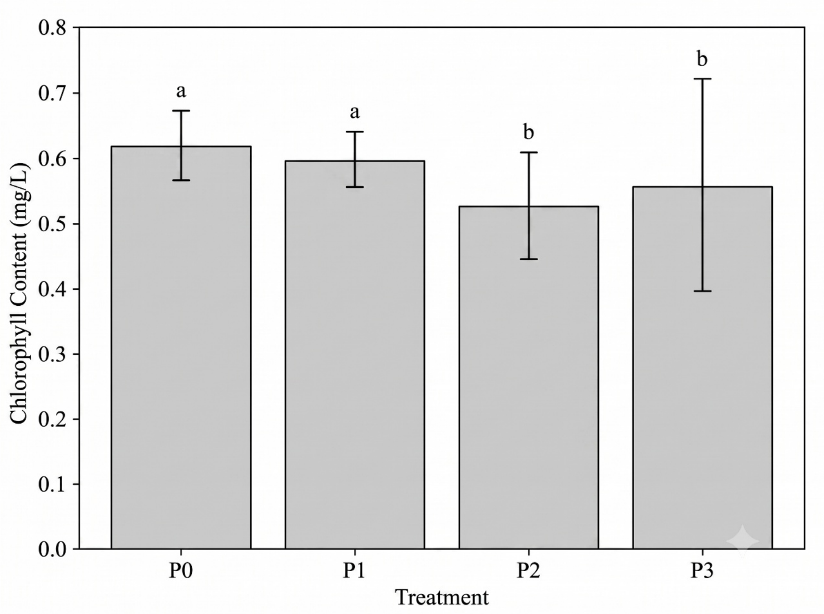


**Figure 3.** Chlorophyll content of spirulina sp.

concentrations of 50%, categorized as relatively high, could disrupt chlorophyll synthesis and the photosynthetic process. Furthermore, Nie et al. (2020) stated that microalgae are unable to grow optimally in organic wastewater media at concentrations exceeding 40%.

The present study provides evidence for the potential application of *Spirulina* sp. in the phytoremediation of tilapia aquaculture wastewater. Nevertheless, several limitations should be acknowledged. First, the external light intensity was maintained at approximately 3000 lux; however, the actual irradiance within the culture medium was not measured. Consequently, the influence of wastewater turbidity on photon availability and photosynthetic efficiency could not be quantitatively evaluated. Second, dissolved oxygen concentrations were monitored throughout the experiment, but the respective contributions of heterotrophic microbial respiration and nitrification processes to oxygen consumption were not directly quantified. Third, although treatment effects were statistically evaluated, the original replicate-level longitudinal dataset was not retained, preventing reanalysis using repeated measures statistical approaches to investigate the interaction between wastewater concentration and observation time. Future studies should preserve complete replicate-level datasets, quantify internal light intensity at different reactor depths, and evaluate microbial community dynamics to provide a more comprehensive understanding of the mechanisms governing nutrient removal and microalgal growth in aquaculture wastewater treatment systems.

## ■ CONCLUSION

The results of this research demonstrated that *Spirulina* sp.-based phytoremediation effectively reduced ammonia, nitrate, and phosphate concentrations in tilapia aquaculture wastewater. Statistical analysis showed that reductions in ammonia and phosphate concentrations differed significantly among treatments ($p < 0.05$), whereas nitrate removal did not differ significantly ($p > 0.05$). The highest nutrient removal efficiencies were observed at moderate wastewater concentrations, indicating the optimum tolerance range of *Spirulina* sp. for nutrient uptake and remediation activity. However, wastewater concentrations exceeding the optimum level negatively affected the physiological response of *Spirulina* sp., as indicated by decreased relative growth and chlorophyll content. These findings suggest that *Spirulina* sp. has considerable potential as an environmentally friendly phytoremediation agent for aquaculture wastewater treatment. However, optimization of wastewater concentration is necessary to maintain both remediation efficiency and microalgal growth performance. Future research should retain complete replicate-level measurements throughout the experimental period to facilitate the application of repeated measures or mixed-effects statistical analyses. Such approaches would enable a more comprehensive evaluation of the individual and interactive effects of wastewater concentration and observation time on nutrient removal efficiency, microalgal growth, and chlorophyll production.

## ■ DECLARATION OF GENERATIVE AI USAGE IN THE WRITING PROCESS

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing, grammar correction, text refinement, and improvement of manuscript readability. The AI tool was not used to generate, analyze, or interpret research data, nor to draw scientific conclusions. All outputs generated by the AI tool were critically reviewed, revised, and verified by the authors. The authors take full responsibility for the content and integrity of the published article.

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