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Optimizing Indigenous *Spirulina platensis* Culture: Unveiling the Role of Potassium Nitrate in Growth, Pigment, and Protein Synthesis

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ABSTRACT

Spirulina platensis is a filamentous cyanobacterium widely utilized as a human dietary supplement and as a protein-rich feed ingredient in aquaculture. This study evaluated the effect of potassium nitrate (KNO_3) on the growth performance, biomass yield, pigment composition, and protein content of a native strain of *S. platensis*. The experiment was conducted in nitrogen-free Zarrouk medium supplemented with four KNO_3 concentrations (1.0, 1.5, 2.0, and 2.5g L^{-1}). Cultures were maintained under photoautotrophic batch conditions for four days, with continuous illumination (4,500 lux) and salinity of 15 ppt. Results showed that KNO_3 concentration significantly ($P< 0.05$) influenced growth rate, dry weight, protein, chlorophyll-a, and carotenoid levels. The highest specific growth rate (0.839 day^{-1}) and biomass concentration (1.392g L^{-1}) were achieved at 2.5g L^{-1} KNO_3, representing a 1.3-fold increase compared to the lowest concentration (1.0 g L^{-1}). Similarly, chlorophyll-a, carotenoid, and protein contents peaked at 2.5 g L^{-1} KNO_3, though values were not significantly different ($P> 0.05$) from those observed at 2.0 g L^{-1}. These findings suggest that KNO_3 concentrations between 2.0 and 2.5g L^{-1} provide optimal conditions for maximizing biomass production, protein accumulation, and pigment synthesis in *S. platensis*. This range may serve as a practical benchmark for scaling up cost-effective *Spirulina* cultivation for nutritional and industrial applications.

INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes that have gained considerable attention in recent years due to their potential role in alleviating global malnutrition. These microalgae are reported to possess high protein content and a well-balanced nutritional profile, providing nearly all essential amino acids recommended by the Food and Agriculture Organization (FAO) **(Andrade *et al.*, 2018)**. Among cyanobacteria,

Spirulina platensis has been highlighted for its abundance of single-cell proteins, phycocyanin, carotenoids, chlorophylls, and polyunsaturated fatty acids **(Mousavi *et al.*, 2022; Abozied *et al.*, 2024)**. *Spirulina* contains up to 60–70% protein on a dry weight basis, depending on culture conditions, underscoring its nutritional superiority **(Ladjal-ettoumi *et al.*, 2024)**. Owing to these properties, *Spirulina* is widely marketed as a dietary supplement, aquaculture feed, and raw material for the pharmaceutical industry **(Vo *et al.*, 2015; El-Sheekh *et al.*, 2023)**.

The growth and biochemical composition of cyanobacteria, particularly *S. platensis,* are influenced by several physicochemical factors such as pH, salinity, light intensity, and nutrient availability **(Vo *et al.*, 2015)**. Among these, nutrient supply is the most critical factor, as it strongly affects growth rate and biomass composition. Carbon, nitrogen, and phosphorus are considered primary nutrients essential for microalgal growth and metabolism **(Su, 2021)**. In particular, nitrogen plays a key role in the synthesis of structural and functional proteins, including enzymes, peptides, chlorophyll, and nucleic acids **(Yaakob *et al.*, 2021)**. Hence, maintaining optimal nitrogen levels in growth media is vital for maximizing productivity.

Nitrate is one of the most commonly utilized nitrogen sources in synthetic media for microalgal cultivation, as it enhances both biomass accumulation and pigment production **(Yuniarti *et al.*, 2023)**. Different nitrate salts—such as ammonium nitrate, sodium nitrate, calcium nitrate, and potassium nitrate—have been reported to significantly influence microalgal biomass yield and biochemical composition **(Nahidian *et al.*, 2018)**. Among these, potassium nitrate (KNO_3) and sodium nitrate ($NaNO_3$) are regarded as particularly effective for promoting cell growth and pigment synthesis **(Yuniarti *et al.*, 2023)**. Besides increasing dry biomass, nitrate supplementation can enhance photosynthetic pigment levels, which are essential for efficient metabolic activity.

Several studies have examined the effect of nitrate sources on *S. platensis*. For instance, **Costa *et al.* (2001)** demonstrated that supplementation with $NaNO_3$ and NH_4NO_3 improved growth rate and pigment content. **Fakhri *et al.* (2020)** reported that calcium nitrate positively influenced biomass and protein levels, while **Mousavi *et al.* (2022)** found that KNO_3 promoted biomass formation and phycocyanin production. Despite these findings, little is known about the effects of KNO_3 on other key pigments, particularly chlorophyll-a and carotenoids.

Since nitrogen availability is directly linked to chlorophyll synthesis, variations in nitrate concentration may impact photosynthetic efficiency and biomass quality **(Pozzobon *et al.*, 2021)**. Chlorophyll-a and carotenoids are the dominant pigments in *Spirulina*, and their abundance reflects culture health and productivity. Moreover, protein content—a key determinant of nutritional quality—is strongly correlated with nitrogen supply **(Markou *et al.*, 2014)**.

Although KNO_3 has been identified as a promising nitrogen source, comprehensive information on its effects across a wider range of growth parameters—including biomass yield, photosynthetic pigments, and protein content—remains limited. This knowledge gap could restrict its optimization for industrial-scale production, despite *Spirulina's* growing nutritional and commercial importance.

In this context, the present study systematically evaluated the influence of different KNO_3 concentrations on growth performance, biomass yield, photosynthetic pigment composition (chlorophyll-a and carotenoids), and protein content of indigenous *S. platensis*. Identifying the optimal nitrate concentration will contribute to enhancing biomass productivity and cost-effectiveness in large-scale *Spirulina* cultivation. Furthermore, the findings will provide valuable insights for nitrogen management strategies aimed at improving biomass and protein yield in microalgal culture systems.

MATERIALS AND METHODS

Strain and culture medium

The indigenous strain of *Spirulina platensis* applied in this experiment was acquired from the Institute for Mariculture Development and Research, Gondol, Bali, Indonesia (Fig. 1). The starter culture was grown in Zarrouk medium without the addition of trace metal solution. The microalgae were cultured at 29±2°C under constant light of 4,500 lux. Cultivation was carried out at a salinity of 15 ppt, and the culture was maintained under continuous aeration with sterilized air for favorable growth.

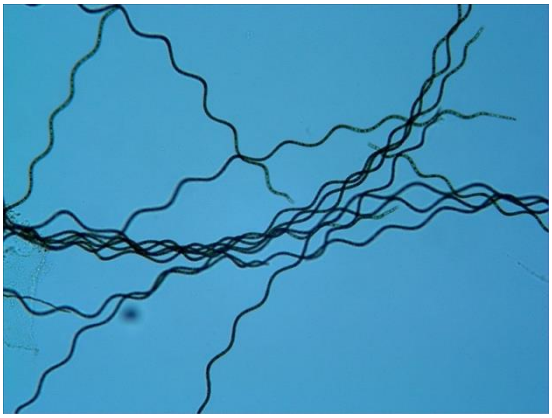

Fig. 1. Morphology of indigenous *Spirulina platensis*. The cells were monitored under a light microscope with 40x magnification.

Experimental cultivation conditions

S. platensis were grown in 0.5L Erlenmeyer flasks, each containing 0.4L of medium as the working volume. Three-day-old of the algal culture was used as an inoculant. A batch system with nitrogen-free Zarrouk medium was used with four potassium nitrate concentrations (KNO_3) (1, 1.5, 2, and 2.5g L^{-1}) with three replicates. KNO_3 was used as a replacement for sodium nitrate in the Zarrouk medium. Nitrate and nitrogen concentrations in all treatments are shown in Table (1). Strains were cultured at

29±2°C with continuous illumination under 4,500 lux light intensity, 15 ppt salinity, and continuously aerated. The initial biomass concentration used in all treatments was 0.11g L^{-1}. In this study, specific growth rate, biomass, chlorophyll*a*, carotenoid, and protein were measured.

Table 1. Nitrogen and nitrate content in various potassium nitrate concentrations

KNO_3 concentrations (g L^{-1})	Nitrate (mg L^{-1})	Nitrogen (mg L^{-1})
1.0	613.24	138.47
1.5	919.85	207.71
2.0	1,226.47	276.94
2.5	1,533.09	346.18

Measurement of biomass

A sample volume of 25mL microalgae suspension was filtered through the pre-dried filter paper (GF/C, 9cm in diameter) [A] and rinsed with 50mL of fresh water to eliminate residual salts. The filter paper was subsequently oven-dried at 105°C for another two hours to reach a consistent dry weight [B]. The dry weight (g L^{-1}) was calculated using the method outlined by **Fakhri *et al*. (2021)**.

Biomass (dry weight, g L^{-1}) = (B-A) x 1000/Sample volume

Growth analysis

The calculation of specific growth rate (μ) and doubling time (dt) followed the logarithmic growth model outlined by **Fakhri *et al*. (2021)**.

$\mu\ (day^{-1}) = [\ln(X2) - \ln(X1)]/(t2 - t1)$

Where, X_1 and X_2 represent the dry weight at initial time t_1 and final time t_2, respectively. Based on the computed μ value, the doubling time was subsequently derived using the appropriate mathematical expression.

$dt\ (day) = \ln(2)/\mu$

Determination of pigment

The quantification of photosynthetic pigments, specifically chlorophyll-*a* and carotenoids, was carried out using methanol-based extraction, as modified from the method reported by **Arifin *et al*. (2024)**. A 10mL aliquot of the algal culture was centrifuged at 4,000*g* for 10 minutes. The pellet was then mixed with 10mL of absolute methanol, vortexed for 30 seconds, and incubated at 70°C for 10 minutes in the absence of light. Following incubation, the mixture was vortexed again and subjected to a second centrifugation. The absorbance of the solution was quantified at wavelengths of 470 and 665nm utilizing a spectrophotometer (Spectroquant Pharo 300, Germany). Chlorophyll-*a*

was determined based on the equation established by **Ritchie (2006)**. On the other hand, carotenoid levels were calculated according to the method described by **Wellburn (1994)**.

Chl *a* (µg mL^{-1}) = $12.9447 \times A_{665}$

Carotenoid (µg mL^{-1}) = $[1000 \times A_{470} - 2.86 \times \text{Chl } a]/221$

Measurement of protein

The Lowry method was utilized to evaluate the protein content **(Lowry *et al.* 1951)**. Several reagents were prepared for this assay, including: Reagent A (5% sodium carbonate), Reagent B (1% copper sulfate pentahydrate), and Reagent C (2% potassium sodium tartrate tetrahydrate). Reagent D was freshly made by combining 50mL of Reagent A with 1mL of both Reagents B and C. Additionally, a standard solution of bovine serum albumin (BSA), 1 N NaOH, and Folin-Ciocalteau reagent were prepared. The concentration of BSA was 2mg mL^{-1}. To initiate the assay, 0.5mL of 1 N NaOH was combined with an equivalent volume of microalgal suspension and subsequently heated at 100°C for 10 minutes.. Once cooled, Reagent D (2.5mL) was added to each sample, mixed thoroughly using a vortex mixer for 10 seconds, and allowed to stand for 10 minutes. Afterward, Folin-Ciocalteu reagent (0.5mL) was introduced, followed by another 10-second vortexing. The reaction mixture was then left to incubate for 30 minutes at room temperature. Finally, the mixture was measured spectrophotometrically at the absorbance of 750nm (Spectroquant Pharo 300, Germany).

Data analysis

To analyze the data obtained, statistical evaluations were carried out using IBM SPSS Statistics version 27. The impact of different KNO_3 concentrations was assessed through one-way analysis of variance, and significant differences across treatment groups subsequently analyzed using Tukey's post hoc test. A *P*-value of less than 0.05 was deemed to signify statistical significance.

RESULTS

Influence of potassium nitrate on growth and biomass

The growth of *S. platensis* cultured in Zarrouk medium with varying potassium nitrate concentrations (1.0, 1.5, 2.0, and 2.5g L^{-1}) is presented in Fig. (2). The results showed that the cultures entered the logarithmic phase rapidly, with no clear lag phase observed across treatments. Growth patterns were similar at all KNO_3 concentrations, with the highest increase occurring between days 2 and 3. Maximum biomass was reached on day 3, followed by a decline on day 4, indicating that the cells were able to effectively utilize potassium nitrate as a nitrogen source to support rapid growth.

Growth parameters are summarized in Table (2); data present specific growth rate, doubling time, and dry weight across treatments. Statistical analysis revealed that KNO_3

concentration significantly affected growth rate, biomass accumulation, and doubling time ($P< 0.05$). The best performance was recorded at 2.5g L^{-1}, yielding a maximum biomass concentration of 1.392g L^{-1}, a minimum doubling time of 0.824 days, and a specific growth rate of 0.839 day^{-1}. However, no significant differences ($P> 0.05$) were observed between 2.0 and 2.5g L^{-1} treatments. Increasing the KNO_3 concentration from 1.0 to 2.5g L^{-1} improved the growth rate by 10.2% and biomass yield by 26.6%. These findings highlight the high nitrate uptake capacity of *S. platensis*, which enhances its metabolic activity and biomass productivity. Consistent with this, increased potassium nitrate levels also elevated the nitrogen content of the medium (Table 1), further supporting enhanced cell growth.

Table 2. Specific growth rate, doubling time, and biomass of *Spirulina platensis* at various potassium nitrate concentrations

KNO_3 concentration (g L^{-1})	Specific growth rate (day^{-1})	Doubling time (day)	Dry weight (g L^{-1})
1.0	0.762 ± 0.031^{a}	0.911 ± 0.037^{a}	1.099 ± 0.032^{a}
1.5	0.808 ± 0.015^{ab}	0.857 ± 0.016^{a}	1.266 ± 0.044^{b}
2.0	0.823 ± 0.022^{b}	0.838 ± 0.022^{ab}	1.326 ± 0.014^{bc}
2.5	0.839 ± 0.022^{b}	0.824 ± 0.021^{b}	1.392 ± 0.020^{c}

Note: Means denoted by identical superscript characters are not statistically different ($P>0.05$).

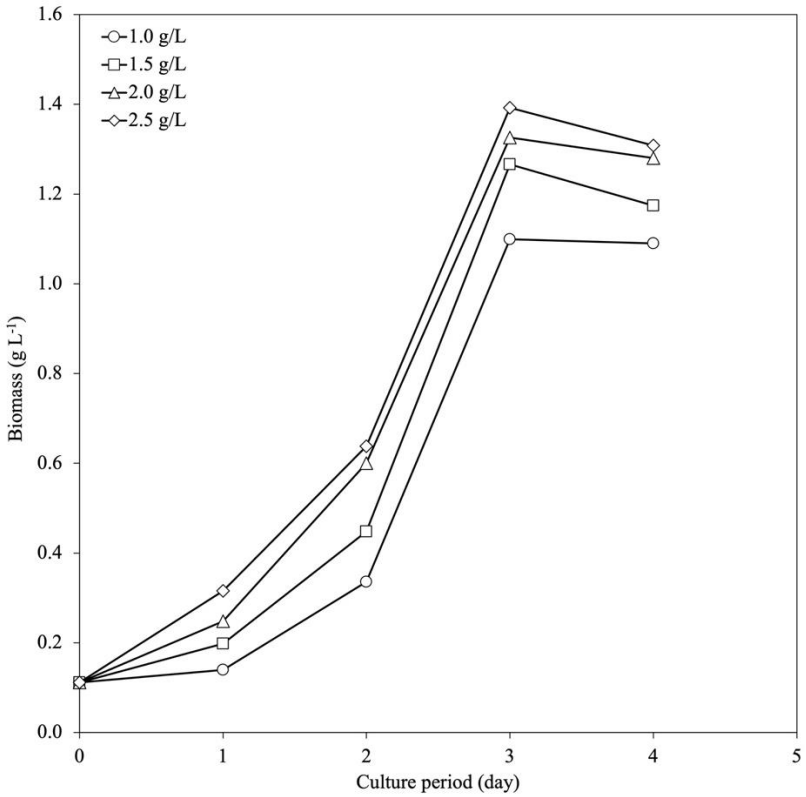

Fig. 2. Growth of *Spirulina platensis* at different concentrations of potassium nitrate over a 4-day culture period. The standard deviation (n = 3) is reflected by the error bars.

Influence of potassium nitrate on photosynthetic pigment

Chlorophyll-a and carotenoid contents at different potassium nitrate concentrations are presented in Fig. (3). The results showed that varying KNO_3 concentrations had a significant effect on both pigments in *S. platensis* ($P< 0.05$). Increasing KNO_3 from 1.0 to 2.5g L^{-1} led to a marked rise in chlorophyll-a and carotenoid levels. However, no significant difference ($P> 0.05$) was observed between the 2.0 and 2.5g L^{-1} treatments, where chlorophyll-a reached 4.98µg mL^{-1} and carotenoids 2.98µg mL^{-1} at 2.0g L^{-1}. The highest values—6.0µg mL^{-1} chlorophyll-a and 3.28µg mL^{-1} carotenoids—were obtained at 2.5g L^{-1} KNO_3.

The increase in chlorophyll-a content reflects enhanced photosynthetic capacity, consistent with the observed improvements in biomass accumulation and growth rate. Carotenoid levels also rose in parallel with chlorophyll-a, serving both photoprotective and accessory light-harvesting roles. Together, these results indicate a strong regulatory link between nitrogen availability, chlorophyll synthesis, and carotenoid accumulation in *S. platensis*.

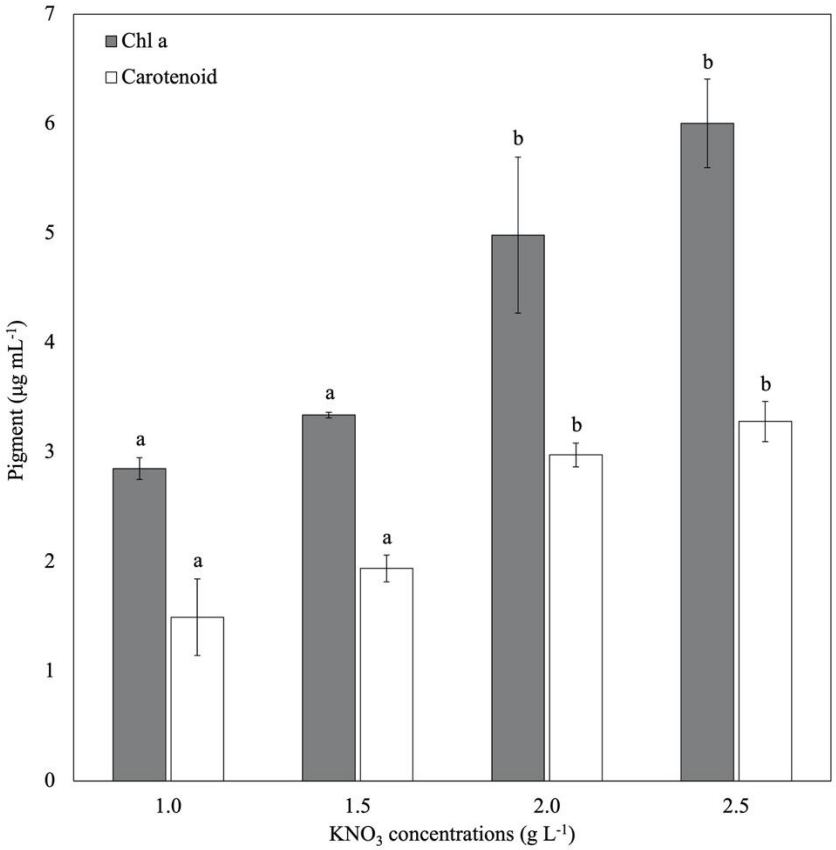

Fig. 3. Chl-*a* and carotenoid of *Spirulina platensis* at various concentrations of potassium nitrate over a 4-day culture period. The standard deviation (n = 3) is reflected by the error bars. Same letters above the bars are usually not significantly different from one another.

High potassium nitrate supply induced protein synthesis

Protein content at different potassium nitrate concentrations is shown in Fig. (4). The results demonstrated that protein accumulation in *S. platensis* was significantly affected by the initial KNO_3 concentration ($P< 0.05$). Increasing KNO_3 from 1.0 to 2.5g L^{-1} led to a progressive rise in protein production. The maximum protein content of 533.27µg mL^{-1} was recorded at 2.5g L^{-1} KNO_3, which was 1.8, 1.2, and 1.1 times higher than that obtained at 1.0, 1.5, and 2.0g L^{-1}, respectively. However, the difference between 2.0 and 2.5 g L^{-1} KNO_3 was not statistically significant ($P> 0.05$).

These findings confirm that nitrogen—particularly nitrate—is a key limiting factor for protein biosynthesis in *S. platensis*. Elevated KNO_3 concentrations enhanced protein production, underscoring the importance of nitrogen availability for efficient assimilation and metabolic activity.

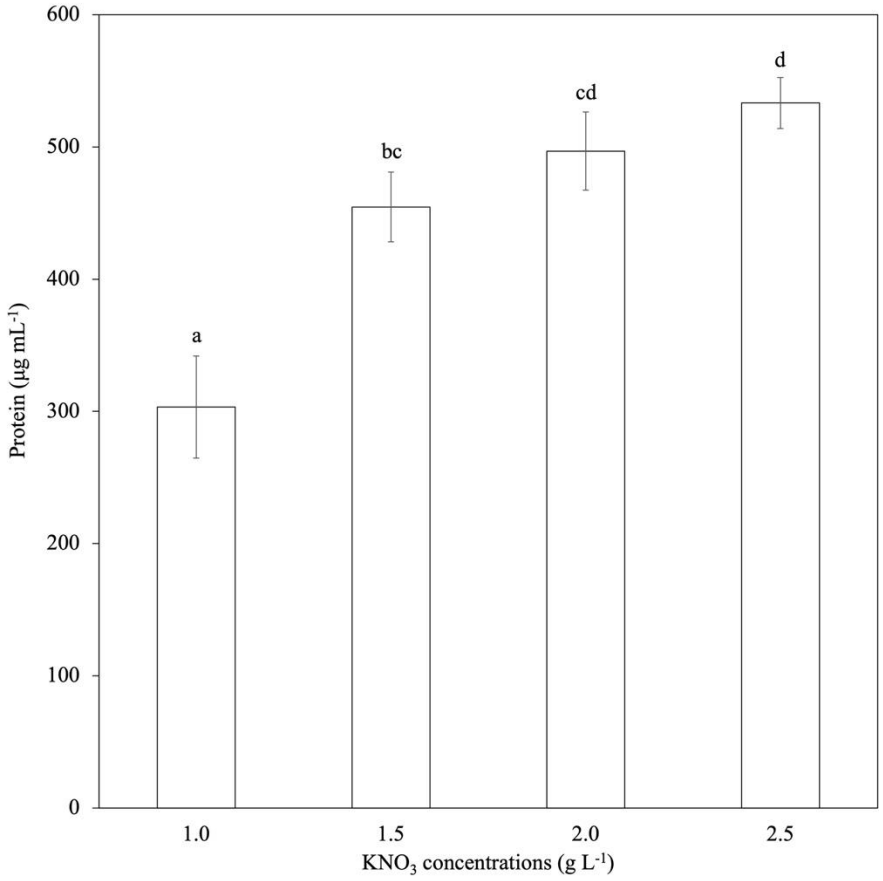

Fig. 4. Protein content of *Spirulina platensis* at various potassium nitrate concentrations over a 4-day culture period. The standard deviation (n = 3) is reflected by the error bars. Same letters above the bars are usually not significantly different from one another.

DISCUSSION

The results clearly demonstrated that potassium nitrate (KNO_3) is essential for enhancing the growth and nutritional composition of *S. platensis*. The strong linear correlation between KNO_3 concentration and growth performance up to 2.5 g L^{-1} highlights the nutrient-dependent nature of microalgal productivity. Similar findings were reported by **Costa *et al.* (2001)**, who observed that increasing sodium nitrate concentration from 0.01 M to 0.03 M led to a 21.7% increase in *S. platensis* biomass and a 23.8% improvement in specific growth rate. In contrast, **Mousavi *et al.* (2022)** found that high KNO_3 concentrations (25mM or 2.5g L^{-1}) inhibited *S. platensis* growth. This discrepancy suggests that strain origin plays a critical role in determining microalgal capacity to utilize potassium nitrate as a nitrogen source. Similarly, **Palanisamy *et al.* (2023)** reported that nitrate limitation reduced growth and biomass productivity in certain

microalgae due to decreased photosynthetic efficiency, concluding that optimal nitrogen availability improves light-harvesting efficiency and metabolic activity.

Nitrogen is a fundamental element for algal growth, serving as a key component of DNA, RNA, enzymes, and chlorophyll, and directly influencing photosynthetic efficiency **(Zarrinmehr *et al.*, 2020)**. Both nitrogen deficiency and excess can suppress algal growth, while optimal supplementation enhances photosynthesis, biomass accumulation, and dry weight **(Mousavi *et al.*, 2022)**. Nitrate is the primary nitrogen form absorbed by *Spirulina* **(Vo *et al.*, 2015)**. **Mousavi *et al.* (2022)** further reported that potassium nitrate supplementation improved biomass production compared to other nitrate sources, which aligns with the present study. KNO_3 provides a dual benefit: nitrate for protein and pigment synthesis, and potassium ions that help maintain cytosolic ionic balance, supporting metabolism, growth regulation, and photosynthesis **(Rodrigues *et al.*, 2010)**. Importantly, in this study, no additional trace element supplementation was required, suggesting that optimized KNO_3 inputs could reduce operational costs while improving economic feasibility for large-scale production.

Photosynthetic pigment accumulation also followed nitrate-dependent patterns. Chlorophyll-a content increased with higher nitrate concentrations, consistent with findings by **Lv *et al.* (2010)** in *Chlorella vulgaris* and **Fakhri *et al.* (2020)** in *S. platensis*. Since chlorophyll is a nitrogenous compound, its synthesis is highly dependent on nitrate availability **(Pancha *et al.*, 2014)**. As cell density increases, photosynthetic pigment levels also rise, demonstrating the link between nitrate concentration, growth rate, and chlorophyll synthesis. Similarly, carotenoid accumulation was positively influenced by nitrate supplementation. **Zarrinmehr *et al.* (2020)** reported increased carotenoid content in *Isochrysis galbana* with higher nitrate supply, while **Ponnuswamy *et al.* (2013)** emphasized that enhanced photosynthetic activity boosts carotenoid synthesis in *S. platensis*.

Nitrogen availability also directly influenced protein synthesis. **Pancha *et al.* (2014)** observed increased protein content in *I. galbana* with elevated nitrate concentrations, while **Fakhri *et al.* (2020)** reported similar effects using calcium nitrate in *S. platensis*. Nitrogen is a core component of proteins and enzymes, and its availability drives metabolic activity and protein biosynthesis **(Vo *et al.*, 2015; Zarrinmehr *et al.*, 2020)**. Moreover, **Alfadhly *et al*. (2022)** highlighted that protein synthesis in microalgae is influenced not only by nitrate but also by potassium, underscoring the dual role of KNO_3 in supporting both nitrogen assimilation and ionic balance.

Comparative evaluation and biotechnological implications

Compared with other nitrogen sources, KNO_3 exhibited a broader positive impact across key growth parameters, including cell density, pigment synthesis, and protein content. For example, **Fakhri *et al.* (2020)** reported improved protein synthesis with calcium nitrate, while **Costa *et al.* (2001)** observed enhanced pigment synthesis with sodium nitrate. In contrast, the present study demonstrates that KNO_3 can simultaneously

optimize biomass productivity, pigment accumulation, and protein content, offering a synergistic advantage for commercial cultivation.

Operationally, KNO_3 also improves the ionic balance and buffering capacity of culture media, ensuring stable growth conditions in large-scale photobioreactors. By serving as both a nitrogen and potassium source, it minimizes the need for additional mineral supplementation, thereby simplifying nutrient management and lowering costs.

This research reinforces the effectiveness of potassium nitrate as a superior nitrogen source for *S. platensis*. The observed improvements in growth rate, biomass yield, pigment content, and protein synthesis confirm the efficiency of KNO_3 as both a nitrate and potassium supplier. The dual nutritional role of KNO_3 provides a strategic advantage in microalgal biotechnology, offering a cost-effective and sustainable approach for industrial *Spirulina* cultivation. Future studies should focus on long-term culture trials and mechanistic investigations to refine nutrient optimization strategies and maximize production efficiency at commercial scales.

CONCLUSION

This study demonstrated that potassium nitrate supplementation significantly enhances the growth performance, pigment synthesis, and protein content of indigenous *S. platensis* under controlled culture conditions. Although the 2.5g L^{-1} KNO_3 treatment produced the highest numerical values for specific growth rate, biomass yield, chlorophyll-a, carotenoids, and protein content, these improvements were not statistically different from those obtained at 2.0g L^{-1}. This indicates that increasing the KNO_3 concentration beyond 2.0g L^{-1} does not provide additional biological benefits, and that 2.0 g L^{-1} represents a more efficient and cost-effective supplementation level for optimizing *S. platensis* cultivation.

The findings contribute to the broader understanding of nitrogen management in microalgal biotechnology and highlight the potential of KNO_3 as a dual nutrient source, supplying both nitrate and potassium. Future studies should aim to validate these results under long-term culture conditions and at larger production scales to develop robust, cost-effective nutrient optimization strategies for commercial *Spirulina* cultivation.

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