Mexican Journal of Biotechnology

Journal homepage: www.mexjbiotechnol.com
ISSN:2448-6590

ORIGINAL RESEARCH

*Mexican Journal of Biotechnology 2025, 10(3):48-65*

# Optimizing nitrogen source and concentration for enhanced biomass and bioproducts in *Scenedesmus dimorphus*

# Optimización de la fuente y concentración de nitrógeno para mejorar la biomasa y bioproductos en *Scenedesmus dimorphus*

Lilia Tapia-López[1,2], Jorge Isaac Chairez-Oria[1], Luis Carlos Fernández-Linares[1*]

[1] Unidad Profesional Interdisciplinaria de Biotecnología, Instituto Politécnico Nacional, Mexico.
[2] Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Mexico.

*Corresponding author
E-mail address:lfernand36@gmail.com (L. C. Fernández-Linares)

Article history:
Received: 17 March 2025 / Received in revised form: 20 June 2025 / Accepted: 7 July 2025 / Published online: 18 July 2025.
https://doi.org/10.29267/mxjb.2025.10.3.48-65

## ABSTRACT

Microalgae are photosynthetic unicellular microorganisms with high biomass productivity and potential for producing value-added compounds such as pigments, proteins, lipids, and carbohydrates. *Scenedesmus dimorphus* has been extensively studied for biodiesel production and biotechnological applications; its biochemical composition is influenced by nitrogen source and concentration. This study investigates the effects of ammonium and nitrate (1, 2, 4, 8, 16 mM) on biomass productivity and macromolecule accumulation in *S. dimorphus*, cultivated in 450 mL photobioreactors using modified BG11 medium. Results indicate that ammonium had an inhibitory effect on *S. dimorphus* at all tested concentrations, whereas all nitrate concentrations supported growth, with the highest biomass productivity at 4 mM. Protein and carbohydrate productivity peaked at 8 mM, while lipid accumulation was highest at 1 mM. Additionally, nitrate concentrations between 1 and 4 mM increased polyunsaturated fatty acid content and reduced chain length. Despite these variations, all cultures yielded lipids suitable for biodiesel production. Selecting nitrate concentration based on target products could reduce nitrogen input costs by 55-94%, improving the economic feasibility of large-scale *S. dimorphus* cultivation.

**Keywords:** biodiesel, microalgae, nitrate, *Scenedesmus dimorphus.*

**RESUMEN**

Las microalgas son microorganismos fotosintéticos con alta productividad de biomasa y capacidad para sintetizar compuestos de valor, como proteínas, lípidos y carbohidratos. *Scenedesmus dimorphus* es estudiada para la producción de biodiésel y aplicaciones biotecnológicas; su composición bioquímica varía según la fuente y concentración de nitrógeno. Este estudio evaluó el efecto del amonio y el nitrato (1, 2, 4, 8 y 16 mM) en la biomasa y acumulación de macromoléculas en *S. dimorphus*, cultivada en fotobiorreactores de 450 mL con medio BG11 modificado. Los resultados muestran que el amonio inhibió el crecimiento en todas las concentraciones, mientras que el nitrato favoreció la producción de biomasa, con la mayor productividad a 4 mM. La producción de proteínas y carbohidratos fue máxima con 8 mM, mientras que la acumulación de lípidos alcanzó su punto más alto con 1 mM. Además, concentraciones de nitrato entre 1 y 4 mM aumentaron el contenido de ácidos grasos poliinsaturados y redujeron la longitud de la cadena. A pesar de estas variaciones, todos los cultivos produjeron lípidos adecuados para biodiésel. La selección de la concentración de nitrato basada en los productos objetivo podría reducir, entre 55-94%, el costo de los insumos nitrogenados, mejorando la viabilidad económica del cultivo de *S. dimorphus* a gran escala.

**Palabras clave:** biodiésel, microalga, nitrato, *Scenedesmus dimorphus.*

## 1. Introduction

Microalgae are unicellular, photosynthetic microorganisms that thrive in aquatic environments (Thoré *et al*., 2023). They have garnered global interest due to their applications in biopharmaceuticals, food, and renewable energy (Umashree *et al*., 2023). The genus *Scenedesmus* is extensively studied for biodiesel production (Arumugam *et al*., 2013; Mandotra *et al*., 2016; El-Sheekh *et al*., 2018), a fuel derived from the transesterification of lipids from oleaginous organisms. Biodiesel is biodegradable, non-toxic, and compatible with direct injection engines (Folayan *et al*., 2019). Its properties depend on fatty acid methyl esters (FAME) composition and must comply with both the American Standard for Testing Materials (ASTM) and the European Standard (EN) standards.

Despite its potential, the large-scale production of microalgae is still economically challenging. Strategies to reduce costs include enhancing biomass productivity, increasing target bioproduct yields, and improving downstream processing (Thoré *et al*., 2023). Biomass productivity and composition are influenced by various factors such as temperature, pH, light intensity, and nitrogen source and concentration (Ram *et al*., 2019; Santhakumaran *et al*., 2020). Nitrogen is a critical nutrient for both biomass and lipid production in microalgae (Arumugam *et al*., 2013). While ammonium is rapidly assimilated by cells for amino acid synthesis, it can be toxic to plants and photosynthetic microorganisms (Collos and Harrison, 2014; Wang *et al*., 2016). Microalgae exhibit species-specific

responses to nitrogen sources. For instance, *Chlorella vulgaris* grows similarly in ammonium sulfate and potassium nitrate media (Tam and Wong, 1996), while *Scenedesmus vacuolatus* exhibits maximum biomass production with nitrate (Gupta *et al*., 2019). Similarly, *Scenedesmus bijugatus* shows enhanced growth with potassium or sodium nitrate compared to other nitrogen sources (Arumugam *et al*., 2013). Some microalgae like *Chlorella* sp. and *Quadrigula* sp. can grow in a culture media with a mixture of nitrate ($NO_3^-$), nitrite ($NO_2^-$), ammonium ($NH_4^+$) and urea (Velázquez-Sánchez *et al*., 2023). This study evaluates the impact of nitrogen sources and concentrations on the growth and biochemical composition of *S. dimorphus* to optimize its cultivation in BG11 medium under photoautotrophic conditions.

## 2. Materials and methods

### 2.1. Microalgae strain and growth conditions

*S. dimorphus* strain was obtained from the Continental Algae Laboratory, Universidad Nacional Autónoma de Mexico. Cultures were maintained in BG11 medium (Rippka *et al*., 1979), in a 1 L photobioreactor (PBR) with a 900 mL working volume and a constant airflow rate of 1 vvm. Illumination was provided by white, fluorescent lamps (ECOSMART, 23 W) at an intensity of 180 µmol photons/m²s. The photoperiod was 12:12 h, and the temperature was controlled at 24 ± 1°C (Ramírez-López *et al*., 2016).

### 2.2. Experimental design

The study assessed the effect of nitrogen source on *S. dimorphus* growth by substituting the standard BG11 nitrate ($NO_3^-$) source with either sodium nitrate ($NaNO_3$) (Meyer, Mexico) or ammonium chloride ($NH_4Cl$) (Meyer, México) at concentrations of 1, 2, 4, 8, and 16 mM. All experiments were performed in triplicate in 450 mL PBRs containing 300 mL of medium. Inocula were prepared from exponentially growing cultures in BG11 medium (1.5 g/L $NaNO_3$). The cells were harvested via centrifugation, washed three times with nitrogen-free BG11 medium, and resuspended to achieve an optical density (O.D.) of 1.0 at 600 nm. Cultures were inoculated at 10% v/v (0.036 g/L) and maintained under the conditions described in Section 2.1. Growth was monitored daily via O.D. at 600 nm (HACH DR6000 UV-VIS). Also, pH was measured (pH Meter, Jenway 3520), ammonium and nitrate concentration were also determined as described Ramírez-López *et al*. (2016). Biomass, lipids, carbohydrates, proteins, and pigment productivities were analyzed after 10 days. The theoretical biomass (Eze *et al*., 2018) and specific growth rate (Shanthi *et al*., 2018) were also calculated.

### 2.3. Analytical methods

Biomass was quantified gravimetrically using glass microfiber membranes (0.7 µm pore size) and a moisture analyzer (AandD Weighing MS-70) (Fernández-Linares *et al*., 2017).

Biomass productivity was calculated according to equation 1 (Costa *et al*., 2018):

$$P_X = \frac{X_1 - X_0}{t_1 - t_0} \qquad \text{(Eq. 1)}$$

Where: $P_x$ = biomass productivity; $X_1$= the dry biomass (g/L) at time $t_1$ (d), and $X_0$= the initial dry biomass (g/L) at initial time $t_0$ (d).
Total pigments were extracted from the cell pellet of 1.0 mL of culture by adding 1 mL of methanol and incubating the mixture at 60°C for 10 min in the dark. The methanolic extract was then stored at 4°C for 24 h. Subsequently, the absorbance was measured at 470, 653, and 666 nm using a UV-VIS spectrophotometer. The concentrations of chlorophyll a, chlorophyll b, carotenoids and total pigments, were estimated using equations 2, 3, 4 and 5 (Wellburn, 1994), as detailed below:

$$\text{Chlorophyll} - \text{a}\ \left(\frac{\mu g}{mL}\right) = \ 15.65\ (\text{ABS666}) \ - \ 7.34\ (\text{ABS653}) \qquad \text{(Eq. 2)}$$

$$\text{Chlorophyll} - \text{b}\ \left(\frac{\mu g}{mL}\right) = 27.05\ (\text{ABS653}) \ - \ 11.21\ (\text{ABS666}) \qquad \text{(Eq. 3)}$$

$$\text{Carotenoids}\ \left(\frac{\mu g}{mL}\right) = \ [1000(\text{ABS470}) \ - \ 2.86\ (\text{Chla}) \ - \ 129.2(\text{Chlb})]\ /\ 221 \qquad \text{(Eq. 4)}$$

$$\text{Total pigments}\ \left(\frac{\mu g}{mL}\right) = \text{Chlorophyll} - \text{a} + \text{Chlorophyll} - \text{b} + \ \text{Carotenoids} \qquad \text{(Eq. 5)}$$

Proteins were analyzed using the Lowry method (Jain *et al*., 2021). The cell pellet from 1.0 mL of culture was hydrolyzed with 1 mL of 1 N NaOH (Meyer, México) at 90°C for 2 h. Then, 1 mL of reagent D (a mixture of 2% $Na_2CO_3$ (Meyer, México) in 0.1N NaOH, 2% $CuSO_4 \cdot 5H_2O$ (Meyer, México), and 2% $NKC_4H_4O_6$ (Meyer, México), in a 100:1:1 ratio) was added to 200 µL of the hydrolysate. The mixture was incubated at room temperature for 10 min. Next, 100 µL of 1N Folin-Ciocalteau (Sigma, EE. UU.) reagent was added, and the solution was incubated for an additional 30 min. Absorbance was measured at 750 nm using a Hatch DR 6000 spectrophotometer.

Total carbohydrates were determined using the Dubois method. The cell pellet from 1 mL of culture was hydrolyzed with 1 mL of 2N HCl (Meyer, México) at 100°C for 2 h. Then, 200 µL of the hydrolysate were reacted with 200 µL of 5% (m/v) phenol (Meyer, México) and 1 mL of concentrated $H_2SO_4$ (Meyer, México). The mixture was incubated at room temperature for 10 min, vortexed, and further incubated for 30 min. Absorbance was measured at 490 nm using a Hatch DR 6000 spectrophotometer (Fernández-Linares *et al*., 2017).

Lipid determination was performed using a cell pellet obtained from 30 mL of microalgae culture. The pellet was hydrolyzed with 5 mL of 0.4 N HCl (Meyer, México) at 90°C for 2 h in a thermoblock. Lipids were extracted by adding 10 mL of isopropanol (Meyer, México) and stirring the mixture at 250 rpm for 1 h at room temperature. Afterward, 15 mL of hexane were added, and the mixture was stirred under the same conditions. Then, 5 mL of distilled water were added, and the mixture was transferred to a separatory funnel. The oil phase was eluted through a column containing anhydrous sodium sulfate (Meyer, México) and a glass microfiber membrane (Ahlstrom, 0.7 µm pore size). The oil was recovered in a vial,

and the solvent evaporated at 45°C for 48 h. Total lipids were determined gravimetrically (Ramírez-López *et al*., 2016).

### 2.4. Fatty acid methyl esters profile determination and biodiesel quality estimations

The lipids extracted from *S. dimorphus* (Section 2.3) were transesterified with 1 mL of methanolic HCl solution (0.5 N), as described by Ramírez *et al*. (2016). The FAMEs profile was determined by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890B GC System and 5977B MSD, equipped with a flame-ionization detector and an ultra-inert column (Agilent JandW HP-5ms, 30 m x 0.25 mm, 0.25 µm). The injector and detector temperatures were maintained at 250°C and 280°C, respectively. The oven temperature was initially set to 50°C for 1 min., then increased at a rate of 25°C/min until reaching 200°C. Afterward, the temperature increased at a rate of 3°C/min until reaching 230°C, where it was held for 8 min. Hydrogen and air were used as carrier gases, with flow rates of 40 and 450 mL/min, respectively. For each measurement, 1 µL of the sample was injected. Calibration curves for each FAME were constructed using the analytical standard Supelco 37 Component FAME Mix.

The obtained FAMEs profiles were used to estimate the biodiesel quality using BiodieselAnalyzer© software version 2.2(Iran), and the results were compared to biodiesel standards recommended by the European Standards (EN14214) and the American Society for Testing and Materials (ASTM D6751).

### 2.5. Statistical analysis

A one-way analysis of variance (ANOVA) was conducted to assess the differences among the treatments using Minitab® software (EE. UU.). Comparisons between means were performed using Tukey's test at a 95% confidence level.

## 3. Results

### 3.1. Effect of nitrogen source and concentration on biomass productivity

*S. dimorphus* growth was negligible or null when $NH_4^+$ (1 to 16 mM) was supplemented in BG11 medium, where the pH dropped from 7 to 4. Conversely, media with $NO_3^-$ became alkaline (pH 7 to 10), and the specific growth rates were 0.19-0.26/d (Table 1). After 10 days of culture, biomass productivity of *S. dimorphus* increased proportionally with nitrate concentration from 1 to 4 mM ($R^2 = 0.9847$) and significantly decreased when the concentration was doubled (8 mM). Biomass productivities in media with 8 mM (53.6 ± 11.8 mg/Ld) and 16 mM (63.9 ± 8.9 mg/Ld) were comparable to that obtained in 2 mM (56.2 ± 4.4 mg/Ld). Therefore, the highest biomass production was achieved with 4 mM nitrate (83.6 ± 2.9 mg/L), whose relationship with theoretical biomass production (887.5 mg/L) was 94.2%.

**Table 1**. Biomass production and specific growth rate of *Scenedesmus dimorphus* during 10 days under different nitrate concentrations.

| Supply nitrate (mM) | Assimilated nitrate (mM) | Theoretical biomass (mg/L) | Biomass productivity (mg/L d) | Specific growth rate (1/d) |
|---|---|---|---|---|
| 1 | 1 | 221.9 | $34.4 \pm 2.2^{c}$ | $0.19 \pm 0^{c}$ |
| 2 | 2 | 443.8 | $56.2 \pm 4.4^{b}$ | $0.23 \pm 0^{b}$ |
| 4 | 4 | 887.5 | $83.6 \pm 2.9^{a}$ | $0.26 \pm 0^{a}$ |
| 8 | 5.8 | 1288.3 | $53.6 \pm 11.8^{b}$ | $0.23 \pm 0^{b}$ |
| 16 | 8.1 | 1803.6 | $63.9 \pm 8.9^{b}$ | $0.24 \pm 0^{ab}$ |

The different superscript letters indicate a significant difference between nitrate concentration according to Tukey's HSD test ($p \leq 0.05$).

### 3.2. Effect of nitrate concentration on bioproducts

Protein productivity appeared to be positively correlated with the initial nitrate concentration, ranging from 1 to 4 mM ($R^2 = 0.9812$). It showed a four-fold increase when the $NO_3^-$ concentration was raised from 2 to 4 mM, but when the concentration was doubled from 4 to 8 mM, the increase was only 21%. Therefore, the highest protein productivity was observed in the 8 and 16 mM media (39.94 and 39.99 mg/Ld, respectively), which were significantly higher than that obtained in the 4 mM medium (32.9 mg/Ld) (Fig. 1). Similarly, total pigment productivity increased significantly with nitrate concentrations of 1, 2, and 4 mM ($R^2 = 0.9865$), reaching 280.2 ± 13.2, 398.4 ± 4.2, and 802.1 ± 0.3 µg/L·d, respectively. However, no further significant increase was observed at higher concentrations (8 and 16 mM), which resulted in 786.1 ± 1.4 and 870 ± 3 µg/L·d, respectively. Just like protein and total pigment productivity, carbohydrate productivity in *S. dimorphus* increased with $NO_3^-$ concentration ($R^2 = 0.9535$) between 1 and 4 mM. At higher nitrate concentrations, no significant differences were observed (Fig. 1). On the other hand, the highest lipid productivity was obtained in the 1 mM $NO_3^-$ culture medium (the lowest nitrate concentration), although it was not significantly higher than in other culture media.

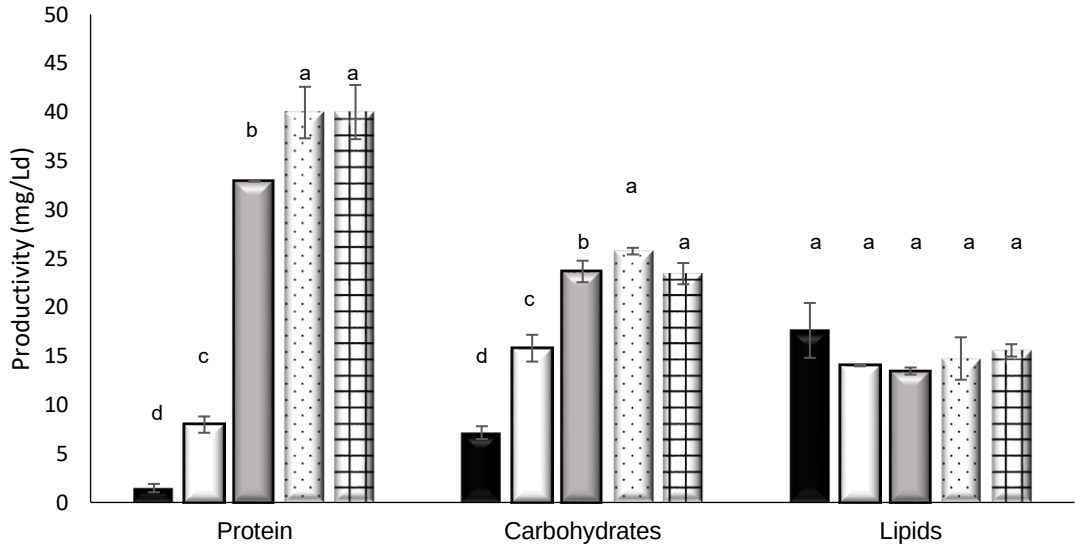


**Fig. 1**. Effect of nitrogen concentration (mM) on *S. dimorphus* productivity. $1NO_3^-$ (black bars), $2NO_3^-$ (empty bars), $4NO_3^-$ (gray bars), $8NO_3^-$ (dotted bars), 16 $NO_3^-$ (grid bars). The different superscript letters indicate a significant difference between nitrate concentration according to Tukey's HSD test ($p \leq 0.05$).

### 3.3. Effect of nitrate concentration on fatty acid profile

Nitrate concentration between 1 and 4 mM appeared to influence the fatty acid profile of *S. dimorphus*, specifically the percentage of polyunsaturated fatty acids ($R^2 = 0.9781$) and chain length ($R^2 = 0.8603$). Fatty acid profiles consisted primarily of saturated fatty acids (70.3 – 83.3%), with carbon chains ranging from C13:0 to C20:0. These were primarily composed of arachidonic acid (C20:0) and palmitic acid (C16:0). Monounsaturated fatty acids (10.4 – 24.4%), such as oleic acid (C18:1) and palmitoleic acid (C16:1), were also present. Linoleic acid (C18:2), the only polyunsaturated fatty acid, showed an increasing concentration with higher nitrate concentrations (Table 2).

### 3.4. Estimation of biodiesel properties

The potential use of *S. dimorphus* lipids for biodiesel production was assessed by determining biodiesel properties (Table 3) using the Biodiesel Analyzer software. The cetane number (CN) is a key property of diesel fuels, indicating the ease of ignition. According to ASTM and EN standards, the minimum required CN is 47 and 51, respectively. The CN of biodiesel derived from *S. dimorphus* ranged from 67 to 70.5. Another important property of biodiesel is the higher heating value (HHV), which indicates the energy content of the fuel. Higher HHV corresponds to lower fuel consumption. The HHVs of biodiesel from *S. dimorphus* ranged from 39.8 to 40.0 MJ/kg. The iodine value (IV) indicates the level of unsaturation in oil and suggests the oxidative stability of the fuel. The estimated IV for *S. dimorphus* in all cultures ranged from 17.3 to 30 g $I_2$/100 g, aligning with international standards. Therefore, the oxidation stability of the biodiesel also met the required standards. The lipids from *S. dimorphus* grown in BG11 medium with 1 and 2 mM $NO_3^-$ would produce

biodiesel with the highest oxidative stability. Kinematic viscosity (kV) and density are key properties for biodiesel utilization in engines. Both parameters fell within the required ranges specified by EN14214 and ASTM D6751. Similarly, the cloud and pour points, important properties for cold weather performance, met the required standards.

**Table 2.** Effect of nitrate concentration on fatty acid profile of *S. dimorphus*.

| FAME Supelco 37 | | 1mM $NO_3^-$ | 2mM $NO_3^-$ | 4mM $NO_3^-$ | 8mM $NO_3^-$ | 16mM $NO_3^-$ |
|---|---|---|---|---|---|---|
| Tridecanoic | C13:0 | 0.0 ± 0.0 $^{c}$ | 0.0 ± 0.0$^{c}$ | 0.5 ±0.03$^{b}$ | 0.5 ±0.04$^{b}$ | 0.6 ± 0.04$^{a}$ |
| Myristic | C14:0 | 0.0 ± 0.0$^{c}$ | 0.8 ± 0.08$^{a}$ | 0.3 ±0.02$^{b}$ | 0.4 ±0.02$^{b}$ | 0.7 ±.007$^{a}$ |
| Myristoleic | C14:1 | 0.0 ± 0.0$^{c}$ | 0.0 ±0.0$^{c}$ | 0.5 ±0.02$^{b}$ | 0.0 ± 0.0$^{c}$ | 1.6 ±0.08$^{a}$ |
| Palmitic | C16:0 | 15.3 ±0.9$^{b}$ | 20.8 ± 1.5$^{a}$ | 17.4 ±1.3$^{ab}$ | 15.9 ±1.7$^{b}$ | 16.8 ±1.4$^{b}$ |
| Palmitoleic | C16:1 | 1.6 ±0.13$^{c}$ | 1.4 ±0.15$^{c}$ | 2.3 ±0.11$^{b}$ | 0.9 ±0.11$^{d}$ | 3.0 ±0.08$^{a}$ |
| cis-10-heptadecenoic | C17:1 | 0.0±0.0$^{c}$ | 0.72 ±0.12$^{b}$ | 3.7 ±0.41$^{a}$ | 3.9 ±0.05$^{a}$ | 3.7 ±0.09$^{a}$ |
| Stearic | C18:0 | 4.8 ± 0.8$^{c}$ | 9.1 ± 0.7$^{a}$ | 4.1 ±0.3$^{c}$ | 6.6 ±0.7$^{b}$ | 4.4 ±0.4$^{c}$ |
| Oleic | C18:1 | 22.8 ±1.2$^{a}$ | 13.8 ±0.9$^{b}$ | 13.9 ±3.0$^{b}$ | 5.6 ±0.4$^{c}$ | 12.7 ±0.7$^{b}$ |
| Linoleic | C18:2 | 2.7 ± 0.2$^{c}$ | 3.9 ±0.2$^{c}$ | 9.0 ±0.9$^{a}$ | 6.3 ±0.4$^{b}$ | 8.7 ±0.9$^{a}$ |
| Arachidic | C20:0 | 52.8 ± 4.2 $^{ab}$ | 49.6 ± 3.4$^{ab}$ | 48.3 ± 3.7$^{ab}$ | 59.9 ± 5.5$^{a}$ | 47.8 ± 3.8$^{b}$ |
| Saturated | | 72.9 ± 3.0 $^{ab}$ | 80.2 ± 1.0 $^{ab}$ | 70.5 ± 1.7 $^{b}$ | 83.3 ± 7.6 $^{a}$ | 70.3 ± 5.3 $^{b}$ |
| Monounsaturated | | 24.4 ± 1.4 $^{a}$ | 15.9 ± 0.9 $^{c}$ | 20.4 ± 2.5 $^{bc}$ | 10.4 ±0.3 $^{d}$ | 21.0 ± 0.8 $^{ab}$ |
| Polyunsaturated | | 2.7±0.2 $^{c}$ | 3.9 ±0.3 $^{c}$ | 9 .0 ±1.0 $^{a}$ | 6.3 ±0.4 $^{b}$ | 8.7 ±1.0 $^{a}$ |

Data are expressed as mean ± standard deviation. Significant differences were determined by Tukey´s test. The different superscript letters indicate a significant difference at $p \leq 0.05$.

**Table 3.** Prediction of the effect of nitrate concentration on the properties of biodiesel obtained with lipids from *S. dimorphus* grown in BG11.

| Biodiesel property | Nitrate (mM) | | | | | EN 14214 | ASTM D 6751 |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 8 | 16 | | |
| Cetane number | 68.3 | 69.4 | 67.0 | 70.5 | 67.2 | >51 | >47 |
| High heating value | 39.9 | 39.9 | 39.8 | 40.0 | 39.8 | - | - |
| Iodine value (g $I_2$/100 g) | 27.0 | 20.8 | 31.3 | 17.3 | 30.0 | ≤120 | - |
| Oxidation stability (h) | 46.3 | 32.5 | 15.6 | 21.4 | 16.2 | 3 | 6 |
| Density (g/cm$^3$) | 0.8 | 0.8 | 0.9 | 0.9 | 0.9 | | 0.86-0.90 |
| Kinematic viscosity (mm$^2$/s) | 4.9 | 4.8 | 4.7 | 5.0 | 4.7 | 3.5-5 | 1.9-6 |
| Cloud point (°C) | 3.0 | 5.9 | 4.1 | 3.4 | 3.8 | - | -3 to 12 |
| Pour point (°C) | -3.6 | -0.5 | -2.3 | -3.2 | -2.6 | -15 to 6 | ≥-10 |

European Standards (EN14214). American Society for Testing and Materials (ASTM D6751).

## 4. Discussion

### 4.1. Effect of the source and concentration of nitrogen on the biomass productivity

In aquatic ecosystems, nitrogen is mainly present in inorganic forms such as ammonium, nitrite, and nitrate. Ammonium is typically consumed first, as its assimilation is easier and faster than other nitrogen sources (Hellebust and Ahmad, 1989; Ritchie, 2013; Sanz-Luque *et al*., 2015; Raven and Giordano, 2016). However, in this study, ammonium had an inhibitory effect on the growth of *S. dimorphus*. Similarly, Ho *et al*. (2015) reported the lowest biomass production of *S. obliquus* in the presence of $NH_4^+$, attributing it to a decrease in pH from 7 to 5. During the assimilation of one mole of $NH_4^+$ into organic matter, 1.3 $H^+$ are excreted by the cells (Shahar *et al*., 2020). If the environment is a closed system with a poorly buffered medium, cellular damage may occur due to acidification (Raven and Giordano, 2016), as observed in this study. To prevent acidity-related damage to *S. dimorphus* during $NH_4^+$ metabolism, it is essential to neutralize $H^+$ or increase the buffer capacity of the BG11 medium. It is known that the assimilation of one mole of nitrate generates 0.7 $OH^-$ (Raven and Giordano, 2016), these could neutralize the acidity of the medium. In this sense, as complementary evidence, *S. dimorphus* was grown in two culture media with a mixture of $NH_4^+$ (1 and 2 mM) with 4 mM $NO_3^-$. In both cultures the initial pH was 7.2, after three days, it decreased to 4.0. On the fifth day, pH increased to 9, only in the culture with 1 mM $NH_4^+$ and 4 mM $NO_3^-$. This mixture successfully prevented acidification damage and allowed biomass production (1170 mg/L).

Nitrate was identified as the most suitable nitrogen source for *S. dimorphus* growth in BG11 medium. The highest biomass productivity was achieved with 4 mM $NO_3^-$, whereas higher nitrate concentrations led to a decline in productivity. This trend has also been observed in other *Scenedesmus* species. For instance, *S. bijugatus* was cultivated in a modified basal soil extract medium with nitrate concentrations of 5, 10, 15, and 20 mM. The highest biomass productivity (15.5 mg/Ld) was obtained at 5 and 10 mM $NO_3^-$, while higher concentrations (15 and 20 mM) resulted in decreased productivity (Arumugam *et al*., 2013). Similarly, Gupta *et al*. (2019) investigated the effect of nitrogen concentration on *Scenedesmus vacuolatus* biomass production in BG11 medium with $NO_3^-$ ranging from 0 to 25 mM. The highest biomass yields were recorded at 10 mM (0.47 g/L) and 5 mM (0.40 g/L), whereas lower yields were observed at 15, 20, and 25 mM $NO_3^-$. In both species, nitrate concentrations above 15 mM negatively impacted biomass production. However, in *S. dimorphus*, *S. bijugatus*, and *S. vacuolatus*, nitrate concentrations of 4–5 mM were optimal for biomass production. This could be attributed to the ability of microalgae to uptake and accumulate nitrate from the culture medium (Ma *et al*., 2018). Subsequently, they can utilize the stored nitrate under nitrogen starvation conditions, as seen in cultures with 1 and 2 mM $NO_3^-$, where biomass production exceeded theoretical values by 55% and 27%, respectively (Table 1).

On the other hand, in cultures with 8 and 16 mM $NO_3^-$, biomass productivity was lower despite the availability of nitrate. Rani and Maróti (2021) reported that nitrate removal efficiency in *Chlamydomonas* sp. cultures is influenced by light intensity. They cultivated *Chlamydomonas* in TAP medium with 5 and 10 mM $NO_3^-$ under different light conditions, including white light at three intensities (50, 100, and 250 µmol/m²s). In cultures exposed to 50

and 100 µmol/m²s, nitrate removal efficiency decreased by approximately 50% when nitrate concentration was doubled from 5 to 10 mM. However, at 250 µmol/m²s, the decrease was only 20% (Rani and Maróti, 2023). This effect may be due to self-shading, where microalgae absorb available light for biomass production, leading to light attenuation in the inner regions of photobioreactors, ultimately limiting growth (Saccardo *et al*., 2022).

To evaluate light penetration at different phases of *S. dimorphus* growth, measurements were taken in a 20-cm deep open pond. During the first three days, microalgal cells were fully exposed to light. However, as biomass concentration increased, light penetration gradually declined, reaching 54% by day 10. These findings highlight the importance of optimizing nitrate concentration to maximize *S. dimorphus* biomass production while minimizing input costs. Specifically, reducing nitrate concentration in BG11 medium from 17.6 to 4 mM could result in an estimated 61.5% reduction in total culture medium costs.

### 4.2. Effect of nitrogen source and concentration on the value-added products

Nitrogen is essential for the synthesis of proteins, pigments, nucleic acids, and energy molecules like ATP. In addition, it regulates its own assimilation (Ranadheer *et al*., 2019). Nitrate limitation induces stress in microalgae, leading to slower growth rates and reduced photosynthetic capacity due to decreased enzyme activity and amino acid production (Ma *et al*., 2018). Conversely, high $NO_3^-$ concentrations enhance protein synthesis, with excess nitrogen stored as protein reserves. Liang *et al*. (2023) conducted a proteomic resource allocation analysis in *Graesiella emersonii* grown in BG11 medium under nitrate starvation and abundance (0.2, 0.7, 1.4, 2.4, 4.7, and 9.4 mM). They demonstrated that excess nitrate was stored as proteins to maintain metabolic reserves, as these proteins were involved in amino acid metabolism, terpenoid biosynthesis, energy metabolism, and protein translation (Liang *et al*., 2023). This phenomenon may explain why *S. dimorphus* continued growing for several days in BG11 medium even after nitrogen depletion (1, 2, and 4 mM $NO_3^-$). Similarly, it could account for the higher protein productivity observed in cultures with 8 and 16 mM $NO_3^-$, despite their lower biomass productivity compared to cultures with 4 mM $NO_3^-$.

Microalgal biomass with high protein content has been used as a nutritional supplement in aquaculture, enriching feed for fish species such as Nile tilapia (*Oreochromis niloticus*) (Abdel-Tawwab *et al*., 2022) and rainbow trout (*Oncorhynchus mykiss*) (Tomás-Almenar *et al*., 2018). Additionally, *Scenedesmus* biomass has been incorporated into bread formulations, imparting a green-yellow coloration without significantly affecting texture parameters such as hardness, chewiness, and resilience (García-Segovia *et al*., 2017). Protein concentrates derived from microalgae biomass also hold potential applications in beverages, food products, and pharmaceutical formulations (Kumar *et al*., 2022). However, large-scale production of microalgal proteins remains costly. Therefore, optimizing nitrogen source and concentration could serve as a cost-effective strategy for enhancing the production of proteins and other macromolecules.

The relationship between *S. dimorphus* total pigment productivity and initial $NO_3^-$ concentration is likely due to the nitrogen requirement for chlorophyll synthesis (Ram *et al*., 2019). The chemical structure of chlorophyll consists of four pyrrole rings, each containing four

carbon atoms and one nitrogen atom, arranged as a porphyrin macrocycle around a central magnesium ion (Scheer, 2004). A similar trend has been observed in *Chlorella vulgaris*, where chlorophyll content increased with nitrate availability. In cultures with 1.12 mM $NO_3^-$, chlorophyll concentration was 10 mg/g, rising to 35 mg/g at 4.03 mM $NO_3^-$ (Cho *et al*., 2019). This effect has been attributed to nitrogen availability, which enhances photosynthetic efficiency through the activation of photosystem II (PSII) (Cho *et al*., 2019). Ranadheer *et al*. (2019) also demonstrated that increasing nitrate concentration from 2.94 to 8.82 mM enhanced PSII efficiency, leading to improved biomass and value-added product yields in *Scenedesmus* sp.

Low nitrogen concentrations shift microalgal metabolism from protein synthesis toward carbohydrate and lipid accumulation (de Carvalho *et al*., 2022). This was evident in *S. dimorphus* lipid productivity, with the most significant increase occurring at 1 mM $NO_3^-$, although it was not significantly higher than in other culture media. Nitrogen starvation can enhance lipid and triacylglycerol (TAG) accumulation in microalgal cells by reducing thylakoid membrane content and stimulating phospholipid hydrolysis, leading to increased intracellular fatty acid acyl-CoA concentrations. Additionally, nitrogen starvation can activate diacylglycerol acyltransferase, which converts acyl-CoA into TAG (Xin *et al*., 2010). From a commercial perspective, these TAGs serve as potential feedstock for biodiesel production. Conversely, *S. dimorphus* carbohydrate productivity was closely linked to biomass productivity, as carbohydrates are structural components of the microalgal cell wall (cellulose, pectin, and sulfated polysaccharides) and function as storage molecules (starch) (González-Fernández and Ballesteros, 2012; de Farias Silva *et al*., 2019). Microalgal polysaccharides have applications in the food, feed, and nutraceutical industries (Selvaraj *et al*., 2023). Also, it serves as raw materials for biofuel production, including ethanol, butanol, methane, and hydrogen. However, the carbohydrate profile of *S. dimorphus* has not been extensively characterized (de Carvalho *et al*., 2022)

### 4.3. Effect of nitrate concentration on the fatty acid profile

The percentage of saturated fatty acids (SFA) in *S. dimorphus* was 6.5 times higher than in *Scenedesmus abundans* (11.6%), which was grown in Waris-H medium with $NaNO_3$ (González-Garcinuño *et al*., 2014). Conversely, the percentage of monounsaturated fatty acids (MUFA) was four times lower than in *S. abundans* (86.9%). El-Sheekh *et al*. (2018) reported that the lipid profile of *S. obliquus* grown in BBM medium contained 37.73% SFA, a lower percentage than that obtained in *S. dimorphus*, while the MUFA content of both species was similar.

The highest MUFA concentration in *S. dimorphus* was observed in cultures with 1 mM $NO_3^-$, which were subjected to nitrogen limitation for six days. In these conditions, oleic acid (C18:1) was the most abundant unsaturated fatty acid, accounting for 23% of the total fatty acids. A similar increase in oleic acid content (1–3%) has been reported in *Messastrum gracile* grown in F2 medium under nitrogen restriction compared to cultures without nitrogen limitation (Wan Afifudeen *et al*., 2021). This trend has also been observed in *Chlorella*, *Scenedesmus*, and *Spirulina* species (Ramesh Kumar *et al*., 2019). Microalgal oils with a high oleic acid content are highly valued in the food industry due to their potential benefits in reducing coronary heart disease, promoting high-density lipoprotein (HDL) levels, and

regulating both central and peripheral blood pressure (Kona *et al*., 2022). In the biodiesel industry, unsaturated fatty acids are desirable for improving fuel performance at low temperatures. However, increased unsaturation can negatively impact oxidation stability, making the biodiesel more prone to degradation over time (Hoekman *et al*., 2012).

### 4.4. Estimation of biodiesel properties

Through the transesterification process, triacylglycerols (TAGs) can be converted into fatty acid methyl esters (FAMEs), whose composition determines biodiesel quality (Trivedi *et al*., 2022). While production and post-production processes also influence biodiesel characteristics (Ramos *et al*., 2009), *S. dimorphus* grown in all nitrate-containing media produced FAMEs suitable for biodiesel that meets the quality standards set by EN 14214 and ASTM D6751. Notably, the biodiesel derived from *S. dimorphus* exhibited high oxidative stability, a critical property for fuel storage. Oxidative stability determines the resistance of biodiesel to degradation over time; unstable fuels can lead to increased viscosity, as well as the formation of gums, sediments, and other deposits that may compromise engine performance (Hoekman *et al*., 2012).

## 5. Conclusion

This study demonstrates that optimizing nitrogen concentration in BG11 medium significantly enhances *S. dimorphus* biomass production and the yield of value-added compounds. Maximum protein productivity was observed at 8 mM $NO_3^-$, whereas peak biomass productivity occurred at 4 mM $NO_3^-$, and the highest lipid productivity was achieved at 1 mM $NO_3^-$. These concentrations correspond to reductions in nitrogen input of approximately 55%, 77%, and 94%, respectively. These results underscore the potential for significantly enhancing the economic viability of large-scale *Scenedesmus dimorphus* cultivation through optimized nitrogen management. The lipid profile was notably influenced by nitrate concentration, with 1–4 mM $NO_3^-$ promoting polyunsaturated fatty acids (PUFAs) and reducing chain length, making lipids produced at 1 mM $NO_3^-$ particularly suitable for biodiesel. The biodiesel derived from *S. dimorphus* lipids met international standards (EN 14214 and ASTM D6751), exhibiting excellent oxidative stability, especially at 1 and 2 mM $NO_3^-$. These results have significant implications for the microalgae industry, offering a cost-effective strategy to optimize biomass and bioproduct yields for applications in bioenergy, nutrition, and biotechnology. However, this study was conducted under controlled photoautotrophic conditions in laboratory-scale photobioreactors, and results may vary in larger-scale systems or under different cultivation strategies, such as mixotrophic conditions. Future research should explore nitrogen optimization in industrial-scale systems, investigate the combined effects of nitrogen with other nutrients (e.g., phosphorus) or stress factors (e.g., light intensity, $CO_2$ levels), and evaluate the performance of *S. dimorphus* lipids in pilot-scale biodiesel production trials. By addressing these areas, the findings of this study can pave the way for more sustainable and economically viable microalgae-based industries.

## Acknowledgments

The authors are grateful for the funding received from the Secretariat of Education, Science, Technology and Innovation of Mexico City, grant SECTEI/032/2024; and the Secretaría de Investigación y Posgrado del Instituto Politécnico Nacional (SIP-IPN); SIP2024816 awarded to Luis Fernández.

## Author contribution

**Lilia Tapia-López**. Methodology, validation, formal analysis, investigation, visualization and writing – original draft. **Jorge Isaac Chairez-Oria** Methodology and formal analysis. **Luis Carlos Fernández-Linares** Conceptualization, methodology, resources, supervision, writing - review & editing, and project administration. All authors reviewed and approved the final version of the manuscript.

## Conflict of interest

The authors declare that there is no conflict of interest.

## References

Abdel-Tawwab, M., Gewida, A. G. A., Abdelghany, M. F., Mousa, M. A. A., Abdel-Razek, N., Abdel-Hamid, E. A. A., Shady, S. H. H., Mamoon, A., 2022. The green microalga *Scenedesmus quadricauda* modulates the performance, immune indices, and disease resistance of *Nile tilapia* fingerlings. Aquac. 560, 738550, https://doi.org/10.1016/j.aquaculture.2022.738550

Arumugam, M., Agarwal, A., Arya, M. C., Ahmed, Z., 2013. Influence of nitrogen sources on biomass productivity of microalgae *Scenedesmus bijugatus*. Bioresour. Technol. 131, 246–249. https://doi.org/10.1016/J.BIORTECH.2012.12.159

Cho, K., Cho, D.-H., Heo, J., Kim, U., Lee, Y. J., Choi, D.-Y., Kim, H.-S., 2019. Nitrogen modulation under chemostat cultivation mode induces biomass and lipid production by *Chlorella vulgaris* and reduces antenna pigment accumulation. Bioresour. Technol. 281, 118-125. https://doi.org/10.1016/j.biortech.2019.02.063

Collos, Y., Harrison, P. J., 2014. Acclimation and toxicity of high ammonium concentrations to unicellular algae. Mar. Pollut. Bull. 80(1–2), 8–23. https://doi.org/10.1016/J.MARPOLBUL.2014.01.006

Costa, S. S., Miranda, A. L., Andrade, B. B., Assis, D. de J., Souza, C. O., de Morais, M. G., Costa, J. A. V., Druzian, J. I., 2018. Influence of nitrogen on growth, biomass composition, production, and properties of polyhydroxyalkanoates (PHAs) by microalgae. Int. J. Biol. Macromol. 116, 552–562. https://doi.org/10.1016/J.IJBIOMAC.2018.05.064

de Carvalho S. M. A., Severo G. I., Held A. S. P., Silva C. S., García Pereira S. P., Oliveira S. L., Goldbeck R., 2022. Microalgae-based carbohydrates: A green innovative source of bioenergy. Bioresour. Technol. 344, 126304. https://doi.org/10.1016/J.BIORTECH.2021.126304

de Farias Silva, C. E., Sforza, E., Bertucco, A., 2019. Enhancing carbohydrate productivity in photosynthetic microorganism production: A comparison between cyanobacteria and microalgae and the effect of cultivation systems. In Advances in Feedstock Conversion Technologies for Alternative Fuels and Bioproducts. Elsevier. pp. 37–67). https://doi.org/10.1016/B978-0-12-817937-6.00003-5

El-Sheekh, M., Abomohra, A. E.-F., Eladel, H., Battah, M., Mohammed, S., 2018. Screening of different species of *Scenedesmus* isolated from Egyptian freshwater habitats for biodiesel production. Renew. Energy. 129, 114–120. https://doi.org/10.1016/J.RENENE.2018.05.099

Eze, V. C., Velasquez-Orta, S. B., Hernández-García, A., Monje-Ramírez, I., Orta-Ledesma, M. T., 2018. Kinetic modelling of microalgae cultivation for wastewater treatment and carbon dioxide sequestration. Algal Res. 32, 131–141. https://doi.org/10.1016/j.algal.2018.03.015

Fernández-Linares, L. C., Guerrero B. C., Durán P. E., Badillo C. J. A., 2017. Assessment of *Chlorella vulgaris* and indigenous microalgae biomass with treated wastewater as growth culture medium. Bioresour. Technol. 244, 400–406. https://doi.org/10.1016/J.BIORTECH.2017.07.141

Folayan, A. J., Anawe, P. A. L., Aladejare, A. E., Ayeni, A. O., 2019. Experimental investigation of the effect of fatty acids configuration, chain length, branching and degree of unsaturation on biodiesel fuel properties obtained from lauric oils, high-oleic and high-linoleic vegetable oil biomass. Energy Rep. 5, 793–806. https://doi.org/10.1016/j.egyr.2019.06.013

García-Segovia, P., Pagán-Moreno, M. J., Lara, I. F., Martínez-Monzó, J., 2017. Effect of microalgae incorporation on physicochemical and textural properties in wheat bread formulation. Food Sci. Technol. Int. 23(5), 437-447. https://doi.org/10.1177/1082013217700259

González-Fernández, C., Ballesteros, M., 2012. Linking microalgae and cyanobacteria culture conditions and key-enzymes for carbohydrate accumulation. In Biotechnol. Adv. Elsevier. 30 (6), pp. 1655-1661). https://doi.org/10.1016/j.biotechadv.2012.07.003

González-Garcinuño, Á., Tabernero, A., Sánchez-Álvarez, J. M., Martin del Valle, E. M., Galán, M. A., 2014. Effect of nitrogen source on growth and lipid accumulation in *Scenedesmus abundans* and *Chlorella ellipsoidea*. Bioresour. Technol. 173, 334–341. https://doi.org/10.1016/J.BIORTECH.2014.09.038

Gupta, N., Khare, P., Singh, D. P., 2019. Nitrogen-dependent metabolic regulation of lipid production in microalga *Scenedesmus vacuolatus*. Ecotox. Environ. Saf. 174, 706–713. https://doi.org/10.1016/J.ECOENV.2019.03.035

Hellebust, J. A., Ahmad, I., 1989. Regulation of nitrogen assimilation in green microalgae. Biol. Oceanogr. 6(3–4), 241–255. https://doi.org/10.1080/01965581.1988.10749529

Ho, S.-H., Xie, Y., Chan, M.-C., Liu, C.-C., Chen, C.-Y., Huang, C.-C., 2015. Effects of nitrogen source availability and bioreactor operating strategies on lutein production with *Scenedesmus obliquus* FSP-3. Bioresour. Technol. 184, 131-138. https://doi.org/10.1016/J.BIORTECH.2014.10.062

Hoekman, S. K., Broch, A., Robbins, C., Ceniceros, E., Natarajan, M., 2012. Review of biodiesel composition, properties, and specifications. Renew. Sustain. Energy Rev. 16(1), 143–169. https://doi.org/10.1016/J.RSER.2011.07.143

Jain, B. P., Goswami, S. K., Pandey, S., 2021. Chapter 4. Protein. In: Jain, B.P., Goswami, S.K. Pandey, S. Protocols in Biochemistry and Clinical Biochemistry. Academic Press, 31–48. https://doi.org/10.1016/B978-0-12-822007-8.00008-8

Kona, R., Katakojwala, R., Pallerla, P., Sripadi, P., Mohan, S. V., 2022. High oleic acid biosynthesis and its absolute quantification by GC/MS in oleaginous *Scenedesmus* sp. SVMIICT1 cultivated in dual stress phase. Algal Res. 67, 102865. https://doi.org/10.1016/J.ALGAL.2022.102865

Kumar, R., Hegde, A. S., Sharma, K., Parmar, P., Srivatsan, V., 2022. Microalgae as a sustainable source of edible proteins and bioactive peptides – current trends and future prospects. Food Res. Int. 157, 111338. https://doi.org/10.1016/J.FOODRES.2022.111338

Liang, L., Wang, Z., Ding, Y., Li, Y., Wen, X., 2023. Protein reserves elucidate the growth of microalgae under nitrogen deficiency. Algal Res. 75, 103269. https://doi.org/10.1016/J.ALGAL.2023.103269

Ma, N. L., Aziz, A., Teh, K. Y., Lam, S. S., Cha, T. S., 2018. Metabolites re-programming and physiological changes induced in *Scenedesmus regularis* under nitrate treatment. Sci. Rep. 8(1), 1–12. https://doi.org/10.1038/s41598-018-27894-0

Mandotra, S. K., Kumar, P., Suseela, M. R., Nayaka, S., Ramteke, P. W., 2016. Evaluation of fatty acid profile and biodiesel properties of microalga *Scenedesmus abundans* under the influence of phosphorus, pH and light intensities. Bioresour. Technol. 201, 222–229. https://doi.org/10.1016/J.BIORTECH.2015.11.042

Ram, S., Paliwal, C., Mishra, S., 2019. Growth medium and nitrogen stress sparked biochemical and carotenogenic alterations in *Scenedesmus* sp. CCNM 1028. Bioresour. Technol. Rep. 7, 100194. https://doi.org/10.1016/J.BITEB.2019.100194

Ramesh Kumar, B., Deviram, G., Mathimani, T., Duc, P. A., Pugazhendhi, A., 2019. Microalgae as rich source of polyunsaturated fatty acids. Biocatal. Agric. Biotechnol. 17, 583–588. https://doi.org/10.1016/J.BCAB.2019.01.017

Ramírez-López, C., Chairez, I., Fernández-Linares, L., 2016. A novel culture medium designed for the simultaneous enhancement of biomass and lipid production by *Chlorella vulgaris* UTEX 26. Bioresour. Technol. 212, 207–216. https://doi.org/10.1016/J.BIORTECH.2016.04.051

Ramos, M. J., Fernández, C. M., Casas, A., Rodríguez, L., Pérez, Á., 2009. Influence of fatty acid composition of raw materials on biodiesel properties. Bioresour. Technol. 100(1), 261–268. https://doi.org/10.1016/J.BIORTECH.2008.06.039

Ranadheer, P., Kona, R., Sreeharsha, R. V., Venkata Mohan, S., 2019. Non-lethal nitrate supplementation enhances photosystem II efficiency in mixotrophic microalgae towards the synthesis of proteins and lipids. Bioresour Technol. 283, 373–377. https://doi.org/10.1016/J.BIORTECH.2019.03.089

Rani, V., Maróti, G., 2021. Assessment of nitrate removal capacity of two selected eukaryotic green microalgae. Cells. 10(9), 2490. https://doi.org/10.3390/CELLS10092490/S1

Raven, J.A., Giordano, M., 2016. Combined nitrogen. In: Borowitzka, M., Beardall, J., Raven, J. The Physiology of Microalgae. Dev. Appl. Phycol. Springer. pp 143-154. https://doi.org/10.1007/978-3-319-24945-2_7

Rippka, R., Deruelles, J., Waterbury J., Herdman M. Stanier R., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111, 1-61. http://dx.doi.org/10.1099/00221287-111-1-1

Ritchie, R. J., 2013. The ammonia transport, retention and futile cycling problem in cyanobacteria. Microb. Ecol. 65, 180–196. https://doi.org/10.1007/s00248-012-0111-1

Saccardo, A., Bezzo, F., Sforza, E., 2022. Microalgae growth in ultra-thin steady-state continuous photobioreactors: assessing self-shading effects. Front. Bioeng. Biotechnol. 10:977429. https://doi.org/10.3389/fbioe.2022.977429

Santhakumaran, P., Kookal, S. K., Mathew, L., Ray, J. G., 2020. Experimental evaluation of the culture parameters for optimum yield of lipids and other nutraceutically valuable compounds in *Chloroidium saccharophillum* (Kruger) comb. Nov. Renew. Energy. 147, 1082–1097. https://doi.org/10.1016/J.RENENE.2019.09.071

Sanz-Luque, E., Chamizo-Ampudia, A., Llamas, A., Galvan, A., Fernández, E., 2015. Understanding nitrate assimilation and its regulation in microalgae. Front. Plant Sci. 6. https://doi.org/10.3389/fpls.2015.00899

Scheer, H., 2004. Chlorophylls and carotenoids. Encyclopedia of Biological Chemistry, 430–437. https://doi.org/10.1016/B0-12-443710-9/00110-1

Selvaraj, S., Bains, A., Sharma, M., Chawla, P., Sridhar, K., 2023. Freshwater edible algae polysaccharides: A recent overview of novel extraction technologies, characterization, and future food applications. J. Polym. Environ. https://doi.org/10.1007/s10924-023-03049-9

Shahar, B., Shpigel, M., Barkan, R., Masasa, M., Neori, A., Chernov, H., Salomon, E., Kiflawi, M., Guttman, L., 2020. Changes in metabolism, growth and nutrient uptake of *Ulva fasciata* (Chlorophyta) in response to nitrogen source. Algal Res. 46, 101781. https://doi.org/10.1016/J.ALGAL.2019.101781

Shanthi, G., Premalatha, M., Anantharaman, N., 2018. Effects of l-amino acids as organic nitrogen source on the growth rate, biochemical composition and polyphenol content of *Spirulina platensis*. Algal Res. 35, 471–478. https://doi.org/10.1016/j.algal.2018.09.014

Tam, N. F. Y., Wong, Y. S., 1996. Effect of ammonia concentrations on growth of *Chlorella vulgaris* and nitrogen removal from media. Bioresour. Technol. 57(1), 45–50. https://doi.org/10.1016/0960-8524(96)00045-4

Thoré, E. S. J., Muylaert, K., Bertram, M. G., Brodin, T., 2023. Microalgae. Curr. Biol. 33(3), PR91–R95. https://doi.org/10.1016/J.CUB.2022.12.032

Tomás-Almenar, C., Larrán, A. M., De Mercado, E., Sanz-Calvo, M. A., Hernández, D., Riaño, B., García-González, M. C., 2018. *Scenedesmus almeriensis* from an integrated system waste-nutrient, as sustainable protein source for feed to rainbow trout (*Oncorhynchus mykiss*). Aquac. 497, 422-430. https://doi.org/10.1016/j.aquaculture.2018.08.011

Trivedi, J., Agrawal, D., Atray, N., Ray, A., 2022. Enhanced lipid production in *Scenedesmus obliquus* via nitrogen starvation in a two-stage cultivation process and evaluation for biodiesel production. Fuel. 316, 123418. https://doi.org/10.1016/j.fuel.2022.123418

Umashree, V. R., Anjana, K., Vidya, D., Vinod, B., Nayana, K., Sreelakshmi, M., Raja, R., Arunkumar, K., 2023. An introduction to algae materials. In: Arunkumar K., Arun A., Raja R, Palaniappan R. (Ed). Appl. Microbiol. Biotechnol. Algae Materials. Academic Press. pp 1–28. https://doi.org/10.1016/B978-0-443-18816-9.00021-6

Velázquez-Sánchez I. G., Tovar-Juárez, Estrada-Santos Y., Negrete-Moreno P.M, Herrera-Valencia V. A., Peraza-Echeverria S., Teco-Bravo J. I., 2023. Nitrogen and phosphorus removal coupled to $CO_2$ fixation by two green microalgae, *Chlorella* sp. and *Quadrigula* sp., native to the coast of Chiapas, Mexico. Mex. J. Biotechnol. 8(4):68-89. https://doi.org/10.29267/mxjb.2023.8.4.68

Wan Afifudeen, C. L., Loh, S. H., Aziz, A., Takahashi, K., Effendy, A. W. M., Cha, T. S., 2021. Double-high in palmitic and oleic acids accumulation in a non-model green microalga, *Messastrum gracile* SE-MC4 under nitrate-repletion and -starvation cultivations. Sci. Rep. 11(1), 1–14. https://doi.org/10.1038/s41598-020-79711-2

Wang, H., Hill, R. T., Zheng, T., Hu, X., Wang, B., 2016. Effects of bacterial communities on biofuel-producing microalgae: stimulation, inhibition and harvesting. Crit Rev Biotechnol. 36(2), 341–352. https://doi.org/10.3109/07388551.2014.961402

Wellburn, A. R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144(3), 307–313. https://doi.org/10.1016/S0176-1617(11)81192-2

Xin, L., Hong-ying, H., Ke, G., Ying-xue, S., 2010. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. Bioresour. Technol. 101 (14), 5494-5500. https://doi.org/10.1016/j.biortech.2010.02.016