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In vitro study on the stress induced biomass and lipid production by marine diatom, *Chaetoceros muellerii* OQ390046

Ambily V.^a, Praveen Kumar C.S.^{a,b}. and Sylas V.P.^{a,b*}

^a*School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala – 686 560, India.*

^b*Advanced Centre of Environmental Studies and Sustainable Development,
Mahatma Gandhi University, Kottayam, Kerala – 686 560, India*

**Email: sylas@mgu.ac.in*

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Abstract

Microalgae and diatoms, as microscopic unicellular algae, present a promising alternative for biodiesel production due to their high lipid content and rapid growth rates. This study aimed to evaluate the effects of varying salinity levels and different concentrations of silicate and nitrate on the biomass and lipid production of the marine diatom *Chaetoceros muellerii* OQ390046. Cultures were grown in f/2 media enriched with NaCl at concentrations of 15 ppt, 30 ppt, 45 ppt, and 60 ppt, and with Na₂SiO₃ at 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, and 0.5 mM, along with NaNO₃ at concentrations of 5 mM, 10 mM, 15 mM, 20 mM, 25 mM, and 30 mM, and KH₂PO₄ at 5 mM, 10 mM, 15 mM, 20 mM, 25 mM, and 30 mM. The cultures were maintained under controlled conditions in a plant growth chamber. The results indicated that, under varying salinity conditions, the highest biomass production (6.7 g/L) was achieved at 45 ppt salinity, while the maximum lipid content (3.2 mg/L) was observed at 60 ppt. Among the tested silicate concentrations, the highest biomass (5.7 g/L) was recorded with 0.05 mM Na₂SiO₃, and the highest lipid production (10.4 mg/L) was noted with 0.02 mM Na₂SiO₃. Furthermore, the study revealed that light intensity also played a crucial role in maximizing production; 1000 lux was optimal for biomass accumulation, whereas 100 lux was most effective for enhancing lipid production. These findings highlight the potential for optimizing culture conditions to improve both biomass and lipid yields in *Chaetoceros muellerii*, thus advancing its application in biodiesel production.

Key words: Marine diatom, nutrients, culture media, biomass, total lipid, bioenergy.

1. Introduction

In recent years, there has been a significant surge in interest regarding microalgal cultivation for the production of biofuels, functional foods, and nutraceuticals. This growing interest is driven by the potential of microalgae to serve as a sustainable and high-yielding feedstock. A key aspect of this development is the adoption of various biorefinery techniques, particularly biomass-based biorefineries. These systems are designed to fractionate biomass into diverse chemical components, which are then converted into valuable end products through biochemical or

thermochemical processes. Such processes produce metabolites, fuels, chemicals, and other marketable products, enhancing the economic viability of microalgal cultivation (Udayan *et al.*, 2021, Hingsamer *et al.*, 2019). Marine microalgae, known for their superior photosynthetic efficiency, rapid growth rates, and high productivity of biomass and lipids, have emerged as one of the most promising feedstocks for biofuel production. Their ability to produce substantial amounts of lipids in a short timeframe makes them highly attractive for biofuel applications (Costa *et al.*, 2019, Liu 2021). While green algae have traditionally been favored due to their ease of cultivation and close relation to terrestrial plants, diatoms have recently garnered significant attention. Diatoms are recognized for their high content of neutral lipids, which are critical for biofuel production (Hildebrand *et al.*, 2012, Pires *et al.*, 2020).

Despite their promising potential, the commercialization of microalgae-based biofuels is hampered by several technical and economic challenges, including the difficulties associated with scaling up production, efficient biomass harvesting, and downstream processing. These challenges are particularly pronounced in large-scale operations, where maintaining optimal conditions for microalgae growth and lipid production can be complex and costly (Park *et al.*, 2023, Zhang *et al.*, 2024). Marine diatoms are particularly well-suited for cultivation in large raceway ponds due to their remarkable tolerance to a wide range of salinity variations. This tolerance enables them to thrive in conditions where many other microalgae might struggle, making them ideal candidates for large-scale, open-pond systems. However, despite this adaptability, the practical application of diatoms is frequently constrained by the need to optimize various growth conditions to maximize lipid synthesis (Liu 2021, Wang *et al.*, 2023).

Critical factors influencing diatom growth and productivity include light intensity, pH, turbulence, salinity, nutrient availability, and temperature. Each of these factors must be carefully controlled to ensure optimal growth and lipid accumulation. For instance, light intensity has a significant impact on photosynthetic efficiency and lipid production, with both high and low intensities potentially limiting growth or altering lipid composition (Xia *et al.*, 2022). Similarly, pH levels must be maintained within an optimal range to prevent stress that could inhibit growth or lipid biosynthesis (Tremblay *et al.*, 2022, Huang *et al.*, 2023).

Salinity is another crucial factor, as it affects not only the growth rate but also the lipid profile of diatoms. Marine diatoms' ability to withstand a wide range of salinity levels allows them to be cultivated in diverse environments, but specific salinity conditions are often required to optimize lipid production (Chen *et al.*, 2024). Nutrient availability is also essential; deficiencies in nutrients such as nitrogen and phosphorus can limit growth and reduce lipid content. To address these deficiencies, controlled cultures often require the addition of nutrient supplements to simulate optimal growth conditions (Liu 2021, Kumar *et al.*, 2023). Moreover, temperature plays a pivotal role in metabolic processes and overall productivity. Diatoms typically have a specific temperature range where their growth and lipid production are maximized. Deviations from this range can lead to suboptimal growth and reduced lipid yields (Lee, *et al.*, 2023, Zheng *et al.*, 2024). Therefore, successful commercialization of diatom-based biofuels necessitates a comprehensive understanding and precise control of these factors to balance growth and lipid production effectively. Continuous advancements in technology and a better understanding of these parameters are crucial for overcoming the current barriers to large-scale microalgae biofuel production (Song *et al.*, 2023, Wang *et al.*, 2023).

C. muellerii has emerged as a highly promising candidate for biofuel production, owing to its impressive biomass and lipid productivity, advantageous fatty acid methyl ester profile, and adaptability to a range of environmental conditions. This species thrives under varying salinity, temperature, and nutrient levels (Amoatey *et al.*, 2023), which positions it as a versatile option for biofuel applications. Despite its potential, achieving optimal lipid content and growth rates in microalgae like *C. muellerii* requires precise cultivation strategies, particularly in optimizing light, nutrient availability, and temperature (Huang *et al.*, 2023, Zhu *et al.*, 2024). Key to this optimization is balancing nutrient composition in the growth medium to enhance both biomass and lipid yield. Understanding how different environmental factors and nutrient levels impact neutral lipid regulation across various algal species is crucial for advancing biodiesel productivity (Mairet *et al.*, 2011, Song *et al.*, 2023). This study aims to address this knowledge gap by investigating the optimal environmental conditions and nutrient concentrations that maximize both biomass and lipid production in *C. muellerii*. By delving into these factors, the research seeks to pave the way for more efficient and sustainable microalgae-based biofuel systems.

2. Materials and methods

The marine diatom *C. muellerii* was obtained from the Central Marine Fisheries Research Institute, (CMFRI), Kochi, Kerala, India. The stock cultures were maintained in F/2 medium (pH 6.00) (Guillard 1975) composed (per litre) of 75 mg NaNO₃, 5 mg NaH₂PO₄H₂O, 30 mg Na₂SiO₃9H₂O, 3.15 mg FeCl₃6H₂O, 4.36 mg Na₂EDTAH₂O, 0.18 mg MnCl₂4H₂O, 0.022 mg ZnSO₄ 7H₂O, 0.01 mg CoCl₂6H₂O, 0.0098 mg CuSO₄5H₂O, 0.0063 mg Na₂MoO₄2H₂O, 0.1 mg thiamine, 0.05 mg biotin and 0.05 mg cyanocobalamin and incubated at a temperature of 25°C in a thermostatically controlled plant growth chamber, illuminated with cool white fluorescence lamps at an intensity of 30μEm⁻²s⁻¹ under a photoperiod of 16:8h. The culture vessels were agitated with a shaker within the plant growth chamber to avoid the settling and sticking of the diatom cultures onto the walls of the conical flasks. The shaking assures the uniform distribution of nutrients incorporated within the culture medium.

2.1. Optimization of growth and lipid production in *C. muellerii*

Effects of nutrient concentrations, salinity, light intensity, pH, and temperature

An aliquot of 1 mL containing 1×10^6 to $10^6 \times 10^6$ cells of *C. muellerii* was inoculated into 50 mL of sterilized medium, which was supplemented with varying concentrations of sodium silicate (0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM, 0.05 mM, 0.06 mM) and a control (without Na₂SiO₃). Sodium nitrate was tested at concentrations of 5 mM, 10 mM, 15 mM, 20 mM, 25 mM, and 30 mM, alongside a control (without NaNO₃). The phosphate concentrations investigated were 5 mM, 10 mM, 15 mM, 20 mM, 25 mM, and 30 mM, with a control lacking KH₂PO₄. Salinity levels were set at 10 ppt, 20 ppt, 30 ppt, 40 ppt, and 50 ppt, with 30 ppt serving as the control to reflect the typical salinity range of seawater (30-35 ppt). Light intensity was varied at 100 lux, 500 lux, 1000 lux, 2000 lux, 3000 lux, and 4000 lux. The pH levels tested were 5, 6, 7, 8, 9, and 10, with adjustments made using 0.1 N HCl or 0.1 N NaOH. Temperature conditions were set at 20°C, 25°C, 30°C, 35°C, and 40°C. All experimental conditions were replicated in triplicate.

A 50 mL aliquot of the marine diatom *C. muellerii* was inoculated into 200 mL of culture medium within 500 mL conical flasks. These flasks were maintained in a plant growth chamber (LABLINE Special LSI-134-PGC) under various experimental conditions for a period of 15 days.

Cultures were incubated in a shaker (Orbitek-LEIL; Scigenics Biotech Pvt. India) with a light intensity of $68 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, provided by fluorescent tubes, and subjected to an alternating light/dark cycle of 16:8 hours. Experimental conditions were replicated in triplicate. Biomass and total lipid content were measured every three days using standard methods by Folch *et al.* 1957, and biochemical analyses were performed at five-day intervals. Data were reported as mean values.

2.2. Biomass estimation

Centrifugation was performed to pellet the culture by spinning at 4000 rpm for 10 minutes. The resulting pellet was subjected to five washes with distilled water to remove residual salts. Following washing, the sample was transferred onto pre-dried GF/C glass fiber filter paper and dried in a hot air oven at 60°C overnight. After drying, the sample was cooled in a desiccator, and the dry biomass was subsequently weighed.

2.3. Estimation of total lipid

Total lipid content was determined using the Bligh and Dyer 1959 method. An aliquot of 10 mL of diatom culture was centrifuged at 4000 rpm for 10 minutes, and the pellet was then oven-dried at 40°C until completely dry. To the dried pellet, 4 mL of methanol was added, and the mixture was shaken at 160 rpm for 1 hour. Following this, 8 mL of chloroform was added, and the mixture was shaken for an additional 2 hours at 160 rpm. The sample was then centrifuged, and the supernatant was collected. The residue was subjected to a second extraction with a chloroform mixture (1:2) overnight. After centrifugation, the supernatant was combined with the previously collected extract and washed with 0.88% potassium chloride to remove any debris. The lower chloroform layer was centrifuged again to remove residual solids and water. The lipid fraction was then dried and re-dissolved in 5 mL of chloroform, which was transferred to a pre-weighed tube. The final weight was recorded after drying at 50°C. Total lipid content was measured gravimetrically using an electronic balance, and lipid content was calculated based on the weight of the dried lipid.

2.4. Estimation of carbohydrate

Glucose solutions with concentrations ranging from 0.02 to 0.14 mg/mL were prepared from a 1 mg/mL stock solution. Each sample was aliquot into two test tubes, with volumes of 0.1

mL and 0.2 mL, respectively, making a total volume of 1 mL per tube. To each tube, 1 mL of 5% phenol solution and 5 mL of 96% sulfuric acid were added. The mixtures were thoroughly shaken and incubated for 10 minutes. After incubation, the absorbance was measured at 490 nm after an additional 20 minutes of incubation in a water bath maintained at 25 to 30°C. A distilled water blank was prepared and used as a control, with its absorbance also recorded. The concentration of total carbohydrate in the samples was determined using the standard curve generated from various glucose concentrations (Dubois *et al.*, 1956).

2.5. Estimation of total protein

The protocol established by Lowry *et al.* (1951) reported various dilutions of bovine serum albumin (BSA) were prepared from a stock solution of 1 mg/mL to achieve final BSA concentrations ranging from 0.1 to 0.8 mg/mL in a total volume of 5 mL. For each dilution, 0.2 mL was pipetted into separate test tubes, and 2 mL of alkaline copper sulfate reagent was added. The alkaline copper sulfate reagent was prepared by combining 2 mL of reagent B (a mixture of 1.56% copper sulfate solution and 2.37% sodium potassium tartrate solution) with 100 mL of reagent A (2% sodium carbonate in 0.1 N NaOH). The solutions were mixed thoroughly and allowed to react for 10 minutes at room temperature. Subsequently, 0.2 mL of 1 N Folin-Ciocalteu reagent was added to each tube and the mixtures were incubated for an additional 30 minutes. Absorbance was then measured at 660 nm, using distilled water blank to set zero absorbance. Total protein concentrations in the marine diatom biomass samples were calculated using the standard curve generated from the known BSA concentrations.

2.6. Estimation of Chlorophyll

Chlorophyll content was quantified following the method outlined by Basu *et al.*, (2013). A 2 mL aliquot of the diatom culture was centrifuged at 4000 rpm for 10 minutes. The resulting pellet was then treated with 5 mL of methanol and subjected to ultrasonic extraction using a Soltec Sonica ultrasonic cleaner for 7 minutes. After extraction, the sample was centrifuged again, and the supernatant was analyzed using a UV/Vis spectrophotometer at wavelengths of 652 nm and

665 nm. Chlorophyll concentrations in the biomass were calculated based on the absorbance readings obtained.

$$\text{Chlorophyll a} = 34.9 \times A_{652} - 15.28 \times A_{665}$$

2.7. Statistical analysis

Analysis of variance (ANOVA) was used to evaluate whether or not there was a significant difference in the antioxidant assays, carotenoid content and potential antimicrobial analyses. The statistical analysis was conducted by using Statistical Package for Social Science (SPSS) 21 version.

3. Results and discussion

3.1. Effect of salinity on biomass and lipid production

Among the tested salinity concentrations, the highest biomass of 9.233 g/L was recorded at 40 ppt on the 12th day of the experiment, surpassing the control by 2.5 g/L (Fig. 1). The minimum biomass of 0.2 g/L was observed on the first day of the experiment at 15 ppt. Lipid accumulation peaked at 60 ppt, with concentrations of 7.9 mg/L, 6.6 mg/L, and 5.7 mg/L recorded on the 5th, 6th, and 4th days, respectively. In contrast, the lowest lipid accumulation of 0.2 mg/L was noted on the first day at 15 ppt. Control conditions consistently showed lower lipid levels compared to the experimental salinities. These findings suggest that increased salinity levels enhance lipid accumulation, likely due to osmotic stress induced by elevated salt concentrations. This observation is supported by recent studies, including work by Park *et al.* (2023) demonstrated that high salinity conditions significantly increase lipid production in various microalgae due to stress-induced metabolic changes. Similarly, Li *et al.* (2022) reported that increased salinity upregulated lipid biosynthesis pathways in *Chlorella vulgaris*. Statistical analysis confirmed significant differences in lipid production across different salinity levels ($p \leq 0.05$) [28].

Biochemical composition of *C. muellerii* was assessed during the log, stationary, and decline phases of growth. The salinity of the medium significantly influenced protein production. Maximum protein levels were observed during the log phase at 30 ppt, followed by 50 ppt and 20 ppt. Statistical analysis confirmed significant differences in protein production across the various salinities ($p < 0.05$). Similarly, carbohydrate production peaked at 40 ppt on the 10th day, reaching 33.600 mg/L, with subsequent values of 30.133 mg/L at 30 ppt and 26.333 mg/L at 20 ppt on the same day (Fig. 3). Carbohydrate levels declined after the 10th day across all salinity treatments. Additionally, salinity impacted chlorophyll production in *C. muellerii*, with the highest chlorophyll concentration of 8.807 mg/L observed at 20 ppt on the 10th day (Fig. 1). These results align with recent findings by Liu (2021) reported that salinity variations significantly affect biochemical compositions in microalgae, including protein and carbohydrate yields. Furthermore, Zhang *et al.* (2022) demonstrated that optimal chlorophyll production in *Chaetoceros muellerii* is influenced by specific salinity levels, corroborating the observed patterns in this study.

Salinity plays a crucial role in the growth and metabolic processes of plants, notably affecting central metabolic pathways such as photosynthesis (Mario *et al.*, (2014). The growth dynamics of microalgae in natural marine environments typically show a significant increase in cell proliferation around days 5 to 7 of the cultivation period, with the culture duration extending from two to three weeks. In the present study, the growth of *Chaetoceros muellerii* showed a gradual increase from the initiation of the culture, reaching peak biomass on the 9th day across all tested salinity conditions. Similar findings by Huang (2013) indicated that high salinity concentrations can markedly impact the physiological processes of microorganisms, although the growth responses to salinity are species-specific and exhibit considerable variability. Further research into the salinity tolerance of different microalgal species is essential for optimizing their cultivation in saline environments and understanding their metabolic adaptations (Smith *et al.*, 2018, Johnson and Wen (2010).

The growth rate and biochemical composition of algae can be significantly influenced by deviations in salinity from their natural or acclimated conditions. Variations in salt concentration in the culture medium affect both the biochemical and physiological properties of microalgae and diatoms. For instance, exposure to saline environments can induce salinity stress in diatom cells, leading to an increase in total lipid content Kirrolia *et al.*, (2011). Similarly, Pal and lipton (2022)

investigated the impact of sodium chloride on *Nannochloropsis* sp., revealing that the maximum total fatty acid (TFA) content reached 47.0% dry weight (DW), with an average lipid yield of 360 mg TFA L⁻¹ day⁻¹, achieved at a NaCl concentration of 13 g L⁻¹. Furthermore, the freshwater alga *Scenedesmus* exhibited a progressive increase in lipid content under salt stress conditions, reflecting the adaptation of this species to salinity variations. However, it is important to note that excessively high salinity can impair diatom cell growth and alter cellular morphology and structure due to osmotic pressure imbalances between the culture medium and the diatom cells (Nguyen and Rittmann, 2024), Zhang *et al.*, (2024). This highlights the need for careful optimization of salinity levels to balance growth and lipid accumulation in microalgal cultures.

The maximum protein content observed during the experiment was 87.19 mg/L and 83.19 mg/L at salinities of 50 ppt and 30 ppt, respectively, on the 10th day (Fig. 3). In contrast, carbohydrate content was significantly higher at salinities of 40 ppt and 30 ppt, with values of 33.60 mg/L and 30.13 mg/L, respectively. Chlorophyll content peaked at 10 ppt, reaching 8.80 mg/L. Statistical analysis indicated significant differences in protein, carbohydrate, and chlorophyll production across the various salinity levels ($p < 0.05$). These findings underscore the influence of salinity on the biochemical composition of microalgae, suggesting that different salinity levels can distinctly affect the synthesis of key cellular components. Recent studies corroborate these results, showing that salinity gradients can modulate the biochemical profiles of microalgae, influencing their protein, carbohydrate, and pigment contents (Li *et al.*, 2023, Zhang *et al.*, 2024).

The marine diatom *C. muellerii* exhibited remarkable salinity tolerance, thriving across a range of salinities (10, 20, 30, 40, and 50 ppt) as depicted in Figure 1. The data indicate that biomass increased proportionally at salinities of 20 ppt and 40 ppt, while the lowest biomass concentration was recorded on the third day of the experiment at 30 ppt. The exponential growth phase commenced shortly after the sixth day. Notably, at a salinity of 30 g/kg, *C. muellerii* demonstrated significant growth Li *et al.*, (2023). In a similar context, *Nannochloropsis salina*, a promising bioenergy feedstock, showed substantial increases in growth rate, biomass, and lipid content when cultured at salinities between 22 and 34 g/kg, with higher salinities effectively reducing populations of non-target algae and grazers Bartley *et al.*, (2013). The biomass

production of *Thalassiosira weissflogii* was slower at salinities of 25 and 30 psu compared to 35 to 50 psu. The study found significant differences in biomass production during various culture phases, with the highest dry biomass observed at 25-30 psu during the stationary phase (Garcia *et al.*, (2012). In the present study, lipid production was notably high on the 16th day of the experiment at 40 ppt, whereas lipid concentrations were comparatively lower at 10 and 20 ppt (Fig. 1). Lipid content was minimal during the logarithmic phase, increased progressively, and peaked during the decline phase across all tested salinities. These findings align with recent research emphasizing the role of salinity in modulating lipid production and stress responses in microalgae.

Salinity has a profound impact on protein synthesis and the overall biochemical composition of microalgae Kirrolia *et al.*, (2011). In the present study, protein production was significantly higher at salinities of 50 ppt and 30 ppt compared to other tested salinities (Fig. 3). Notably, protein concentrations were elevated during the logarithmic growth phase and decreased during the stationary phase, regardless of the salinity levels. The elevated salinities typically influence protein production in microalgal cells. Rai *et al.*, (2015) supporting the observed trend in the present study. Carbohydrate production was notably affected by salinity, with the highest carbohydrate levels recorded during the logarithmic phase at 40 ppt salinity. The high salinity ranges enhance the synthesis of soluble sugars, which play a crucial role in osmotic regulation within cells (Gill and Tuteja, 2010). This adaptation suggests that increased carbohydrate production at 40 ppt may reflect a microalgal response to high salinity conditions. This finding is consistent with another study which reported a similar increase in carbohydrate content in *Scenedesmus quadricauda* under high salinity stress (Kirrolia, *et al.*, (2011). These observations highlight the ability of microalgae to adapt their biochemical composition in response to varying salinity levels, demonstrating their potential for cultivation in saline environments (Smith *et al.*, (2018) Johnson and Wen (2010).

3.2. Effect of silicate on biomass and lipids

The impact of varying sodium silicate concentrations on the biomass of the marine diatom *C. muellerii* was evaluated using F/2 medium. The highest biomass production was achieved with a sodium silicate concentration of 0.05 mM, which significantly outperformed other concentrations. Specifically, biomass production increased progressively with sodium silicate

concentrations up to 0.05 mM, but began to decline at 0.06 mM. The maximum biomass of 5.667 g/L was recorded on the 9th day of the experiment at 0.05 mM, 0.04 mM, and 0.03 mM sodium silicate, representing a substantial increase of 5 g/L compared to the control, which contained no sodium silicate (Fig. 1).

In terms of lipid content, *C. muellerii* exhibited peak levels on the 9th and 12th days of the experiment. Specifically, lipid contents were 10.500 mg/L and 9.267 mg/L, respectively, at sodium silicate concentrations of 0.02 mM and 0.03 mM. Notably, all experimental concentrations of sodium silicate resulted in higher lipid contents compared to the control cultures (Fig. 1). These results underscore the positive influence of sodium silicate on both biomass and lipid accumulation in *C. muellerii*, aligning with recent studies that have demonstrated the role of silicate in enhancing microalgal productivity and lipid content (Zhang *et al.*, 2024, Lee *et al.*, 2024). Further research is warranted to explore the underlying mechanisms by which sodium silicate concentrations influence these metabolic pathways.

3.3. Effect of silicate on biomass and lipid production

In diatoms, variations in nutrient sources significantly influence metabolic traits such as protein, carbohydrate, lipid, and amino acid levels, as well as growth rates (Mario *et al.*, 2014). In this study, the highest protein production for *C. muellerii* was recorded on the 10th day at a sodium silicate concentration of 0.06 mM, followed by 0.05 mM. Carbohydrate content was highest in the control group (246.01 mg/L). Among the experimental concentrations, 0.01 mM sodium silicate yielded the highest carbohydrate content at 218.86 mg/L, followed by 0.02 mM with 170.81 mg/L (Fig. 3). The maximum cellular chlorophyll content of 1.660 mg/L was observed at 0.06 mM sodium silicate. Protein content ranged from 2.18 to 16.597 mg/L across lower and higher concentrations of sodium silicate, respectively, likely due to enhanced nutrient availability in the culture media.

Carbohydrates in microalgae and diatoms are often viewed as crucial chemical energy reserves, with senescence and spore development frequently linked to increased energy reserves (Hemalatha *et al.*, 2014). In the present study, carbohydrate content decreased with increasing

sodium silicate concentrations. The highest carbohydrate levels were observed at lower silicate concentrations, which may reflect a greater accumulation of energy reserves under nutrient-limited conditions. These findings are consistent with recent literature that suggests nutrient availability and stress conditions play pivotal roles in the metabolic regulation of microalgae and diatoms (Smith *et al.*, (2018), Johnson and Wen (2010). Further research is needed to elucidate the mechanisms underlying these metabolic changes and their implications for optimizing diatom cultivation in various nutrient regimes. The results of the present study revealed that the biomass, lipid and biochemical composition of *C. muellerii* was changed significantly in altered nutrient concentrations and the enhanced production of biomass and lipid by the diatoms can be used for the production of biodiesel. Maximum protein production (16.59mg/L and 13.43 mg/L) was observed in 0.06mM and 0.05mM concentration of sodium silicate respectively. The higher concentration of carbohydrate (246.01 mg/L) was noted in culture medium without sodium silicate and very little amount of carbohydrate was noticed in culture medium having least concentration of sodium silicate (0.01mM). ANOVA results showed that there is significant difference in protein, carbohydrate and chlorophyll production in various silicate concentrations ($p < 0.05$).

The influence of varying concentrations of Na_2SiO_3 on biomass production and biochemical composition was assessed in the present study. Notably, biomass began to decline across all experimental concentrations after the 12th day. Among the tested concentrations, 0.06 mM Na_2SiO_3 resulted in the lowest biomass production, potentially due to increased stress responses associated with high nutrient levels in the culture medium, which could impair cell growth and reduce biomass accumulation (Courtois *et al.*, 2012). In addition, lipid concentrations were significantly affected by silicate and nitrate levels in the media. Under nitrogen deprivation conditions, lipid concentration increased, whereas silicate limitation led to a decrease in lipid content with lower silicate concentrations (Fig. 4). Similar trends have been observed in diatoms such as *Chaetoceros gracilis* and *Nitzschia sp.*, where lipid accumulation was enhanced under conditions of silicate limitation (Jiang *et al.*, 2015). The observed variations in lipid content are likely influenced by different culturing conditions and strains used in the experiments. The reduced lipid concentration in diatom cells under silicate-limited conditions can be attributed to metabolic acclimatization, which often leads to significant biochemical changes within the cells (Nguyen and

Rittmann, 2024). This metabolic adaptation highlights the complex interplay between nutrient availability and cellular metabolism in diatom cultures.

The variation in nutrient concentrations within the culture medium significantly affected protein concentrations. The highest protein production for *C. muellerii* was observed at a sodium silicate concentration of 0.06 mM. This result contrasts with findings on *Navicula germanopolonica* exhibited higher protein content (35.36%) in control conditions compared to experimental conditions (21%) (Leal et al., 2013). Such discrepancies can be attributed to factors such as species-specific responses, growth conditions, and the composition of the growth media. For *C. muellerii*, silicates are integral to cell wall structure, making silicate limitation a critical stress factor affecting this marine diatom. The reduced silicate levels in the medium led to changes in both protein content and the lipid-to-protein ratio in *Nitzschia perspicua*, highlighting the impact of silicate stress on protein metabolism. These changes are particularly pronounced under high-stress conditions (Jiang et al., 2015).

In contrast, higher carbohydrate concentrations were noted in control conditions, suggesting that nutrient deprivation promotes carbohydrate synthesis. This is consistent with the understanding that low nutrient levels can alter the cellular composition of biological compounds. Nutrient limitation, particularly in the context of the Calvin cycle, often results in decreased protein production and the accumulation of surplus energy as carbohydrates or lipids. These findings underscore the complex interplay between nutrient availability and metabolic processes in microalgae, emphasizing the need for optimized growth conditions to balance protein and carbohydrate production.

3.4. Effect of sodium nitrate on Biomass and lipid production

The diatom exhibited significant biomass enhancement when cultured with sodium nitrate concentrations of 25, 20, and 15 mM, yielding biomass values of 5.1, 4.2, and 3.9 g/L, respectively. These values represent increases of 3.4, 2.5, and 2.2 g/L compared to the control, which contained no sodium nitrate. The diatom also demonstrated maximum lipid accumulation of 3.2, 3.0, and 2.8 mg/L on the 9th, 12th, and 15th days of the experiment, respectively (Fig. 1). Notably, higher lipid content was observed at lower nitrate concentrations. As nitrate concentration increased in the

culture medium, biomass production also rose, while lipid accumulation showed a decreasing trend. Statistical analysis using ANOVA revealed significant differences in both biomass and lipid production across the various nitrate concentrations ($p < 0.05$). These findings indicate a trade-off between biomass and lipid accumulation in response to nitrate availability. Recent studies have similarly reported that nitrate concentrations can influence both biomass and lipid content in diatoms, with high nitrate levels often leading to increased biomass but reduced lipid content (Smith *et al.*, (2018), Johnson and Wen (2010). The observed patterns in lipid and biomass production under varying nitrate concentrations underscore the complex interplay between nutrient availability and metabolic pathways in diatom cultivation. Further research is needed to optimize nitrate levels for maximizing lipid production while maintaining robust biomass yields.

The concentration of nutrients in the culture medium significantly influenced protein content in the diatom cultures. Among the various nitrate concentrations tested, the highest protein content was observed at 30 mM (179.840 mg/L), followed by 25 mM (152.807 mg/L) and 15 mM (128.550 mg/L). Total carbohydrate content was also highest at 30 mM nitrate concentration (419.983 mg/L) and lowest at 5 mM (98.497 mg/L). The control, with no nitrate supplementation, exhibited a carbohydrate content of 76.717 mg/L on the 10th day of the experiment (Fig. 3). Chlorophyll content reached its peak at 30 mM nitrate concentration, measuring 4.867 mg/L on the 10th day. These results suggest that elevated nitrate levels enhance both protein and carbohydrate production, while also maximizing chlorophyll content. Recent studies corroborate these findings, indicating that nitrate availability can significantly impact the biochemical composition of diatoms, including protein, carbohydrate, and pigment levels (Zhang *et al.*, 2024, Lee *et al.*, 2024). These observations underscore the critical role of nutrient concentration in optimizing the growth and metabolic profiles of diatom cultures. Nitrogen limitation significantly influences lipid content in diatoms, as nitrogen is a crucial component of structural and functional proteins, constituting 7%–20% of cell dry weight. Nitrogen deficiency enhances lipid and triglyceride accumulation, as it halts protein synthesis and cell division, thereby stopping microalgal growth and promoting lipid storage (Converti *et al.*, 2009, Lombardi *et al.*, 1991). Since nitrogen is essential for proteins, DNA, and chlorophyll, its deficiency impacts biomolecule concentrations and induces stress (Song *et al.*, 2023).

The highest protein concentration was observed at 30 mM and 25 mM NaNO_3 , measuring 179.84 mg/L and 152.80 mg/L, respectively (Fig. 3). Carbohydrate and chlorophyll concentrations were also highest at 30 mM on the 10th and 15th days. Nitrogen limitation is a common technique to boost reserve metabolite synthesis in microalgae, leading to increased carbohydrate and lipid accumulation while protein levels decrease (Xia *et al.*, 2022). In *C. muellerii*, protein and carbohydrate contents varied significantly with different NaNO_3 concentrations. High nitrate levels resulted in increased protein content, with a maximum of 179.84 mg/L at 30 mM, while carbohydrate content was highest at 30 mM (419.983 mg/L) and lowest at 5 mM (98.497 mg/L). Nitrogen availability influences microalgal composition, with nitrogen excess promoting both protein and lipid production and nitrogen scarcity leading to reduced nitrogen-containing molecules and increased lipid storage (Araujo *et al.*, 2022). While nitrogen deprivation is effective for enhancing lipid production, it can reduce protein and chlorophyll levels, impair photosynthesis, and slow growth rates (Ordog *et al.*, 2012).

3.5. Effect of Phosphate on Biomass and lipids

The diatom exhibited peak biomass on the 6th and 9th days, with maximum lipid accumulation also occurring on the 9th day. Phosphate is crucial as it is a key component of ATP, DNA, RNA, and phospholipids. Deficiency in phosphorus and nitrogen alters metabolic pathways, shifting lipid metabolism from membrane lipids to neutral lipids, thus increasing total lipid content in green algae (Hu *et al.*, 2008). For instance, reducing phosphate concentration from 2.0 mg/L to 0.1 mg/L significantly increased the total lipid content of *Scenedesmus sp.* from 23% to 53% (Converti *et al.*, 2009). Recent studies have further supported these findings, demonstrating that nutrient limitations continue to play a critical role in modulating lipid accumulation in microalgae Liu (2021), Zhang *et al.*, 2024).

Protein content peaked on the 10th day at a phosphate concentration of 30 mM, with the lowest protein levels recorded in phosphate-free media (Fig. 3). Carbohydrate content was highest at 25 mM and 30 mM phosphate concentrations, while chlorophyll content was maximum at 30 mM and 25 mM (7.90 and 5.79 mg/L, respectively). Phosphorus is essential for eukaryotic algae growth, biochemical synthesis, and development. In many environments, phosphorus is often the limiting nutrient rather than nitrogen (Dyhrman, 2016). Phosphorus limitation reduces Calvin-Benson cycle activity and light energy utilization for carbon fixation, leading to lower biomass

production and growth rates (Belotty *et al.*, 2013). Consistent with this, low biomass production was observed at 5 mM phosphate concentration (Fig. 1). The highest total lipid content was observed at the lowest phosphate concentration, with a decrease noted at 20 mM phosphate (Fig. 3). Phosphate deprivation typically induces an increase in neutral lipid accumulation as phospholipids are replaced by non-phosphorus lipids (Hakalin *et al.*, 2014). Recent studies further support these findings, showing that phosphorus limitation drives lipid accumulation in microalgae (Liu, 2021), Zhang *et al.*, 2024).

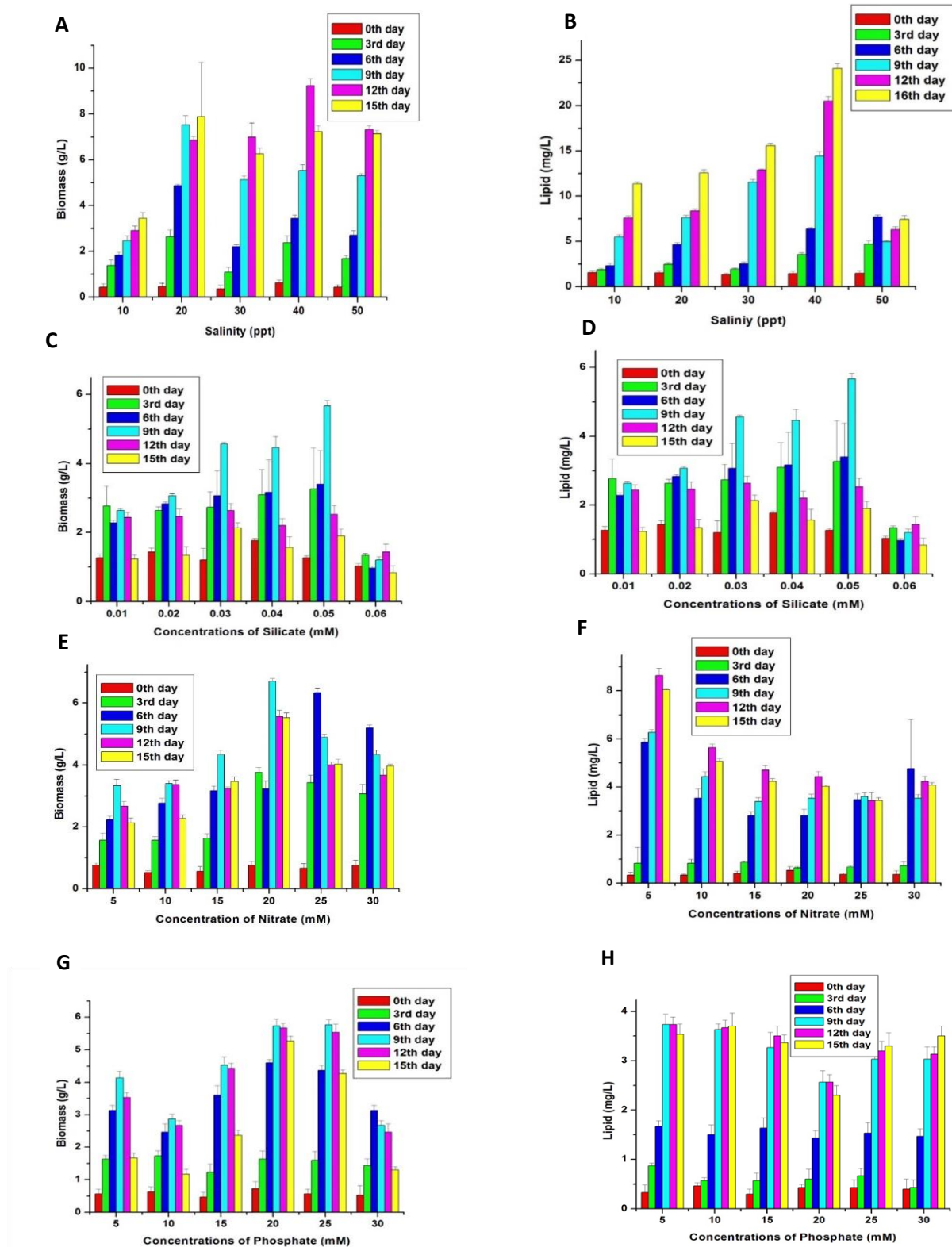


Fig. 1. Effect of salinity on biomass (A), lipids (B), effect of silicate on biomass (C), lipids (D), effect of sodium nitrate on biomass (E), lipids (F), effect of phosphate on biomass (G), lipids (H) in *C. muellerii* under various experimental conditions.

3.6. Effect of pH on biomass and lipid production of *C. muellerii*

pH is a crucial parameter in algal cultivation as it affects the solubility and availability of essential nutrients and CO₂, impacting algal metabolism (Chen *et al.*, 2024). In this study, maximum biomass was observed at pH 9 on the 9th day, while maximum lipid content was recorded at pH 7 (Fig. 2). Alkaline pH increases cell wall flexibility, which inhibits cell rupture and spore release, indirectly boosting triglyceride accumulation and reducing membrane-associated polar lipids. Acidic pH resulted in reduced biomass and lipid production compared to alkaline conditions. For *Chlorella vulgaris*, the optimal pH for biomass growth and lipid accumulation was found to be around 7.5 (Rai *et al.*, 2015). Recent research further supports that a neutral to slightly alkaline pH optimizes lipid production and growth in various microalgae species (Li *et al.*, 2023, Zhang *et al.*, 2024).

The highest protein production and carbohydrate content were observed at pH 9, while the lowest values were recorded at pH 5 (Fig. 3). Chlorophyll content peaked at pH 8 on the 15th day, with minimal values at pH 10. Algal cell membranes are permeable to H⁺ and OH⁻ ions, and extreme pH levels can disrupt cellular processes, with low pH being particularly detrimental (Johansen *et al.*, 1990). Most microalgae thrive at pH 7 to 8, with pH 7 being optimal for many species (Abalde *et al.*, 1995), Stottrup and McEvoy, 2008). *C. muellerii* exhibited maximum biomass and lipid production at pH 9, with lipid levels peaking on the 12th day. Lower biomass and lipid production were observed at pH 5 and 6, aligning with previous findings that *Nannochloropsis* species grow well at pH 8.4 (Spolaore *et al.*, 2006). High pH conditions generally lead to increased lipid production, though pH did not affect hydrocarbon content in *Botryococcus braunii* (Dayananda *et al.*, 2007). Protein production was highest in the log phase at pH 9, suggesting that high pH stress may drive protein synthesis. Carbohydrate synthesis was also enhanced under high pH conditions, likely due to stress-induced responses. Chlorophyll production was elevated at pH 8, likely due to high biomass (Balode *et al.*, 1998, Li *et al.*, 2022).

3.7. Effect of temperature on biomass and lipid production

The effect of temperature on biomass and lipid production in the marine diatom *C. muellerii* was investigated by culturing the diatom at various temperatures and analyzing the biomass and lipid content gravimetrically. Optimal biomass production occurred at 30°C, with substantial growth and lipid production also observed at 20°C and 25°C (Fig. 2). Although both biomass and lipid yields decreased with increasing temperature, *C. muellerii* tolerated up to 40°C, albeit with reduced productivity. Temperature significantly influences diatom growth rates, cell size, biochemical composition, and nutrient needs. Changes in temperature often alter the fatty acid unsaturation levels in lipids. For instance, *Botryococcus braunii* exhibited variations in lipid composition and increased polysaccharide accumulation at different growth temperatures (18°C, 25°C, and 32°C) (Hamid *et al.*, 2019). However, temperature changes did not affect extracellular lipid secretion (Kalacheva *et al.*, 2004). Recent studies have further elucidated how temperature fluctuations impact lipid metabolism and overall diatom productivity (Nguyen and Rittmann 2024). The highest protein production in *C. muellerii* was observed at 20°C and 30°C, while the lowest was recorded at 35°C. Maximum carbohydrate production occurred at 30°C, with minimum levels at 20°C and 40°C. The optimal biomass yield was also achieved at 30°C, consistent with findings by Rukminasari *et al.* 2019. Lower temperatures reduced biomass, aligning with the expectation that microalgal growth increases with temperature up to a threshold, beyond which it declines (Montagnes *et al.*, 2003).

In the current study, the highest biomass was noted on the 12th day at 30°C. Temperature significantly affects physiological and biochemical processes in algae, with high temperatures often leading to decreased protein content and increased lipid and carbohydrate levels. However, lipid content in this study was lower compared to some reports. Similar to findings by Wu *et al.* (2013) on *Monoraphidium sp.*, lipid content increased after the 9th day at all temperatures tested. Wah *et al.* (2015) reported highest lipid content at 23°C in *Amphora subacutiuscula*, with increased saturated fatty acids at lower temperatures. These variations may be due to temperature adaptation mechanisms. Recent studies further support these findings, demonstrating the complex

interplay between temperature, nutrient availability, and lipid accumulation in microalgae (Nagarajan *et al.*, 2023).

3.8. Effect of light intensity on biomass and lipid production

The energy source in the photoautotrophic growth phase is light, which organisms utilize to convert carbon dioxide into organic compounds. During photosynthesis, light is essential for the growth of marine diatoms, impacting their growth and composition significantly. Sufficient light intensity favors the overproduction of diatom lipids, likely due to the promotion of surplus photoassimilate storage that is subsequently transformed into chemical energy. The primary result of the experiment indicated that growth rate varied with different light intensities (lux). *C. muellerii* can be cultured in a laboratory at varying light intensities, with the optimal light intensity for maximum yield found to be 1000 lux. The maximum biomass was observed at this intensity, with considerable biomass concentration also noted at 1500 lux (Fig. 2). The highest lipid content in the tested light intensity conditions was recorded at 1000 lux. Light intensity influences the growth of algae and diatoms through its impact on photosynthesis (Stockenreiter *et al.*, 2013). Additionally, light intensity affects the cellular composition of algae. High light conditions have been shown to increase triacylglycerol synthesis while reducing galactolipid synthesis (Suklenik *et al.*, 1989). Light is necessary for the growth of microalgae; however, both excessive and insufficient light can impede growth rates. This study indicates that a light intensity range of 1000 to 1500 lux is optimal for producing maximum yield in *C. muellerii*.

Studies have demonstrated that the lipid content in microalgae, such as *Scenedesmus abundans*, increases with rising light intensity. Specifically, a study reported that when cultures were exposed to a light intensity of 6000 lux, the maximum lipid content of 32.77% was achieved. This was followed by lipid contents of 27.10% and 21.20% at light intensities of 5000 lux and 3000 lux, respectively (Xia *et al.*, 2022). This suggests that different microalgae and diatom species reach peak lipid content at varying light intensities, indicating diverse light utilization

efficiencies. Light intensity positively influences lipid accumulation in microalgae up to an optimal point. Beyond this, photoinhibition can occur, which damages the photosystems and reduces lipid synthesis. This phenomenon has been observed in various studies. For example, a study highlighted that excessive light intensity can lead to reactive oxygen species (ROS) formation, causing oxidative stress and impairing lipid accumulation (Solovchenko *et al.*, 2020). Similarly, another research demonstrated that extremely high light intensities could hinder photosynthetic efficiency and overall lipid productivity. Recent advances in understanding the relationship between light intensity and lipid accumulation have been made and noted that controlled light regimes could optimize lipid production by balancing light capture and photoprotection mechanisms (Zhang *et al.*, 2024). Furthermore, another study explored the genetic and metabolic responses of microalgae to varying light intensities, providing insights into how light regulation can be harnessed to enhance lipid biosynthesis (Li *et al.*, 2022). Light intensity is a critical factor for lipid accumulation in microalgae, its effects are optimal only within a specific range. Exceeding this range can lead to photoinhibition and diminished lipid production. Therefore, fine-tuning light conditions is essential for maximizing lipid yield in microalgal cultures.

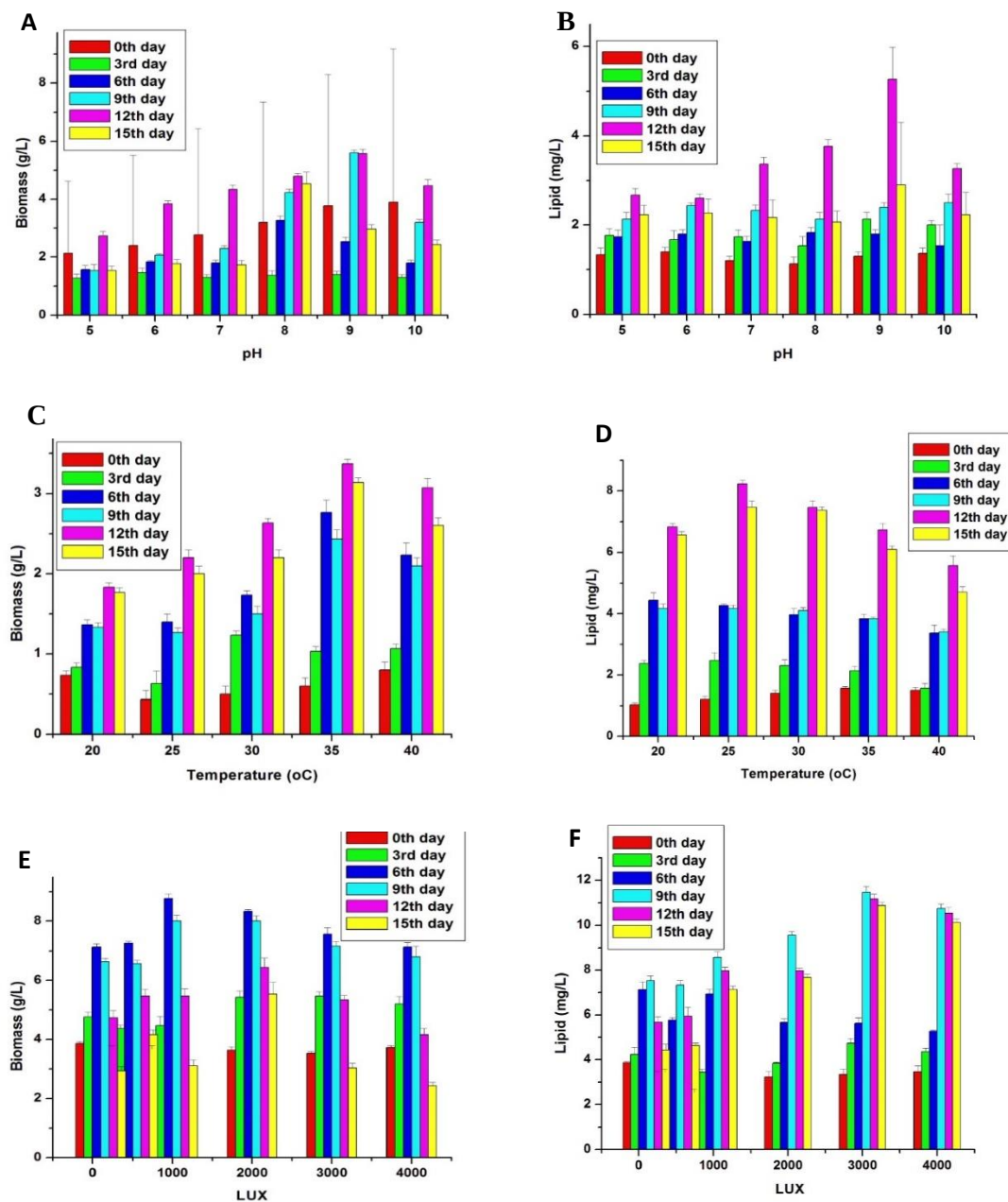


Fig. 2. Effect of pH on biomass (A), lipids (B), effect of temperature on biomass (C), lipids (D), effect of light intensity on biomass (E), lipids (F) in *C. muellerii* under various experimental conditions.

The highest protein content in *C. muellerii* cultures was observed on the 10th day of the experiment at a light intensity of 1000 lux. On the same day, the maximum carbohydrate content was recorded at a light intensity of 4000 lux. Additionally, a comparatively high chlorophyll concentration of 15.75 mg/L was noted at 100 lux (Fig. 3). Statistical analysis indicated significant differences in protein, carbohydrate, and chlorophyll production across different light intensities ($p < 0.05$). These findings align with previous research indicating that light intensity plays a crucial role in regulating the biochemical composition of microalgae. High light intensities can enhance the synthesis of specific biomolecules. The optimal light conditions could maximize protein and carbohydrate production in microalgae cultures. Similarly, a study reported that varying light intensities significantly affect chlorophyll accumulation and overall photosynthetic efficiency (Xu *et al.*, 2022). The relationship between light intensity and microalgal growth is complex, involving intricate regulatory mechanisms. According to recent studies, light intensity not only influences photosynthetic activity but also affects the allocation of resources towards different biochemical pathways. For instance, some studies highlighted that moderate light intensities could balance the production of proteins and carbohydrates, while extremely high or low intensities might disrupt this balance (Wang *et al.*, 2023). Understanding these dynamics is essential for optimizing culture conditions to achieve desired biochemical outputs. This study's results contribute to the growing body of knowledge on how light intensity affects the biochemical composition of microalgae, offering valuable insights for future research and biotechnological applications.

The study demonstrated that *C. muellerii* exhibited robust growth across the tested light intensities, with particularly favorable growth observed at light intensities of 1000 lux and 2000 lux. Light intensity significantly influences microalgae development and photosynthesis by modulating various physiological and biochemical mechanisms. Microalgae adapt to different light intensities through changes in pigment composition and quantity, growth rate, dark respiration rate, and the availability of essential fatty acids (Fabregas *et al.*, 2004). In comparison, *Chlorella ellipsoidea* achieves optimal growth at light intensities ranging from 2500 to 5000 lux (Hoff and Snell, 2008). In the present study, the optimum biomass production for *C. muellerii* was observed at light intensities of 1000 lux and 2000 lux. Chlorophyll production in microalgae is influenced by media composition and culture conditions.

Research on *Scenedesmus abundans* indicated that the highest lipid content of 32.77% dry weight (DW) was achieved under a light intensity of 6000 lux, while the lowest lipid content of 21.20% DW was recorded at 3000 lux (McLachlan *et al.*, 1995). Similarly, *Nannochloropsis oleoabundans* produced maximum lipid content when exposed to a light intensity of 14,800 lux (Xu *et al.*, 2022). These findings underscore the importance of optimizing light conditions to enhance the biochemical composition of different microalgae species. Recent studies have further elucidated the role of light intensity in microalgal metabolism. For instance, a study highlighted that controlled light conditions can significantly enhance lipid production in microalgae by optimizing photosynthetic efficiency and resource allocation (Wang *et al.*, 2023). Additionally, the genetic and metabolic adaptations of microalgae to varying light intensities, providing deeper insights into the mechanisms underlying light-driven biochemical changes (Li *et al.*, 2022). Optimal light intensity for maximum biomass and biochemical yield in *C. muellerii* was found to be between 1000 and 2000 lux. These results contribute to the broader understanding of light intensity's role in microalgal cultivation, offering valuable insights for enhancing microalgal productivity in biotechnological applications.

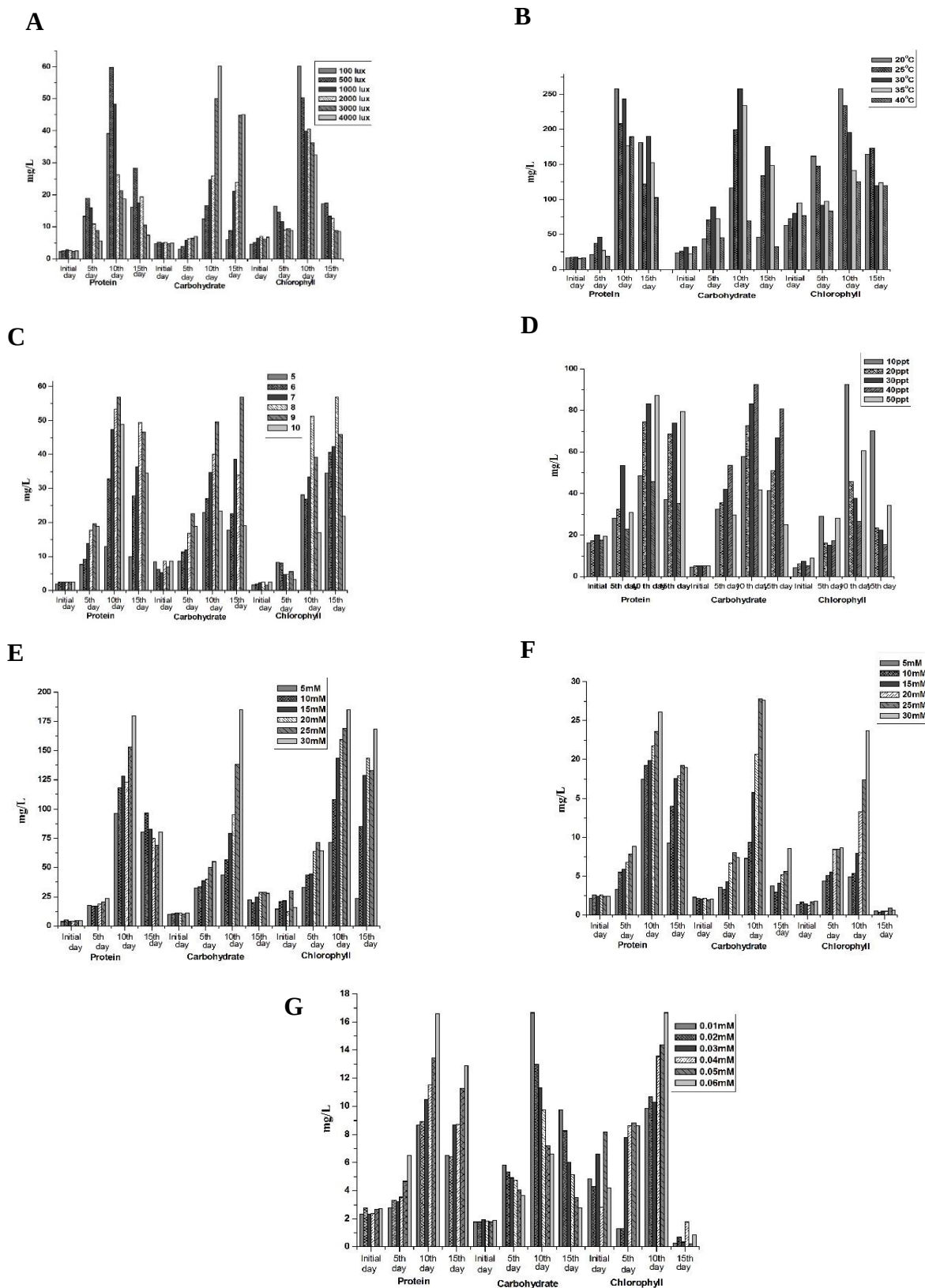


Fig. 3. Variation of protein, carbohydrate and chlorophyll in different parameters like (A) lux (B) temperature (C) pH (D) salinity (E) nitrate (F) phosphate and (G) Na₂SiO₃

Marine diatom growth is highly dependent on environmental conditions, with growth rates varying significantly across species. Key variables influencing diatom growth include salinity, pH, temperature, light, and nutrient availability (Banerjee *et al.*, 2010). Recent studies have shown that variations in temperature and pH significantly affect the lipid content of marine diatoms. Specifically, stress conditions such as extreme temperatures or pH levels lead to lipid accumulation while concurrently inhibiting diatom growth, thereby reducing overall lipid production. Nutrient composition in the culture medium is another critical factor regulating phytoplankton growth (Geider and La Roche, 2002). Nutrient limitations, particularly nitrogen (N) or phosphorus (P) deficiencies have been observed to induce an increase in carbohydrate or lipid content within diatoms. These findings underscore the complex interplay between environmental stressors and nutrient availability in shaping the metabolic responses of marine diatoms and demonstrated that diatoms subjected to nitrogen limitation exhibited a significant rise in lipid content, which has implications for biofuel production (Gao *et al.*, 2018). The adaptive responses highlight the potential of diatoms for biotechnological applications, especially in sustainable biofuel production and bioremediation efforts. Understanding the precise environmental and nutritional conditions that optimize diatom growth and lipid accumulation is crucial for harnessing their full potential in various industrial applications.

Table 1. Maximum Biomass and Lipid production of *C. muellerii* under different tested in vitro conditions.

parameters	Maximum biomass g/L	Maximum Lipid (mg/L)
Salinity	9.233(40 ppt)	21.100 (40ppt)
silicate	5 (0.05mM)	10.500 (0.02mM)
nitrate	6.7(25 mM)	8.6 (5mM)
Phosphate	5.76 (25)	3.5 (5mM)
pH	5.6 (9)	5.267 (9)
Temperature	3.3 (9)	8.20 (25)
Light Intensity	8.767 (1000 lux)	11.467 (2000 lux)

Increasing biomass concentration in diatom cultures is correlated with a decrease in total lipid content. Studies have shown that low sodium silicate concentrations result in higher lipid accumulation in diatoms, indicating a significant relationship between silicate availability, lipid metabolism, and cellular growth (Thajuddin *et al.*, 2015). Since silicates are a critical component of diatom cell walls, their concentration directly influences diatom physiology. For instance, providing a maximum of 2 mM Si to batch cultures can enhance lipid production, but excessive concentrations can negatively impact growth and approach the solubility limit. Environmental concentrations of silicic acid above 100 μ M have been observed to exert detrimental effects on certain diatom species, with particularly adverse outcomes noted at 400 μ M in laboratory settings (Hildebrand *et al.*, 2005).

Careful management of silicate levels is therefore crucial to maximize biomass accumulation without inducing negative side effects. The present study corroborates these findings, demonstrating that lower silicate concentrations can stimulate lipid production. Additionally, diatom biomass peaks have been associated with higher salinities, attributable to the mineral fraction within the culture medium (Garcia *et al.*, 2012). Recent researches further supports these observations, showing that optimized silicate and salinity conditions can significantly enhance lipid yields in diatom cultures and found that controlled silicate supplementation, tailored to avoid the solubility limit, promoted substantial lipid accumulation while maintaining healthy growth rates (Zhu *et al.*, 2024). These insights underscore the importance of precise environmental regulation in diatom cultivation, particularly for applications in biofuel production and bioproducts. Marine diatoms have been identified as a promising feedstock for biodiesel production due to their ability to accumulate lipids, which serve as energy storage molecules and can be converted into biodiesel. Nutrient stress, particularly nitrogen restriction, is one of the most extensively employed strategies to enhance the synthesis of reserve metabolites in specific microalgal species. Under nitrogen-limited conditions, cells shift their metabolism towards the synthesis of energy-storing substances such as lipids and carbohydrates, while reducing the synthesis of nitrogen-containing compounds like proteins. Achieving lipid overproduction during the scaling-up process is critical for the economic viability of biodiesel production from marine diatoms, and several effective approaches have been developed to assist in this effort.

Among these strategies, nutrient starvation is the most effective and widely used method to induce lipid accumulation. This approach exploits the natural response of diatoms to nutrient deficiency, triggering metabolic pathways that prioritize lipid synthesis. Other methods to promote lipid over accumulation include manipulating light intensity, temperature, carbon dioxide levels, salinity stress, and metal stress. For instance, enhancing light intensity has been shown to increase lipid production by providing more energy for photosynthesis, while specific temperature ranges can optimize enzymatic activities related to lipid biosynthesis (Wijffels, *et al.*, 2010). Elevated carbon dioxide levels can enhance carbon availability for lipid synthesis, and salinity stress can induce osmotic adjustments that favor lipid accumulation (Huang *et al.*, 2023). Combining these strategies can further optimize lipid production. For example, combining nitrogen starvation with elevated light intensity and controlled temperature can synergistically enhance lipid yields (Prajapati *et al.*, 2013). Recent advancements also explore the integration of genetic and metabolic engineering techniques to further boost lipid production in marine diatoms (Gong *et al.*, 2013). These multifaceted approaches underscore the potential of marine diatoms as a sustainable and scalable source of biodiesel.

4. Conclusions

Salt stress significantly influences cell growth and lipid formation in marine diatoms. Lipids act as secondary metabolites in algae, with stress or unfavorable conditions such as nutrient limitation, growth phase, temperature, and light intensity triggering increased lipid accumulation. In this study, evaluated the biomass and total lipid content of the marine diatom *C. muellerii* under various concentrations of sodium chloride, sodium silicate, and sodium nitrate in f/2 medium under laboratory conditions. The present study revealed that the maximum biomass of *C. muellerii* was achieved when the medium was amended with 45 ppt sodium chloride. In contrast, the highest lipid production, reaching 7.9 mg/L, was supported by 60 ppt sodium chloride. Among the different concentrations of sodium silicate and sodium nitrate, a sodium silicate concentration of 0.05 mM resulted in the highest biomass, while the maximum lipid production was recorded at a sodium silicate concentration of 0.02 mM. These findings indicate that fluctuations in salinity and silicate concentrations can significantly alter growth rates, biomass, and lipid accumulation in marine diatoms. *C. muellerii* emerges as a promising candidate for the production of biomass, lipids, proteins, and carbohydrates, with significant potential for biofuel applications. As a marine

organism, *C. muellerii* can be cultivated across a wide range of salinity concentrations, and minor adjustments in the culture medium can optimize lipid yields. This adaptability highlights the potential for scalable and efficient biofuel production using marine diatoms.

Conflict of interest statement

We declare that we have no conflict of interest.

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