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Comparative growth dynamics and morphological plasticity of *Chlorella vulgaris* under autotrophic, mixotrophic, and heterotrophic regimes supplemented with indole-3-acetic acid and glycerol

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ABSTRACT

The effects of cultivation mode, photoperiod regime, indole-3-acetic acid (IAA), and glycerol supplementation on the growth performance and morphology of *Chlorella vulgaris* were evaluated under autotrophic, mixotrophic, and heterotrophic culture conditions. The experiment was arranged in a completely randomized design consisting of six treatments with three replicates. Cultures were maintained for seven days under continuous light, continuous darkness, and a 12 h light:12 h dark photoperiod, with selected treatments supplemented with 1 mg L^{-1} IAA and 10 mL L^{-1} glycerol. Cell density, cell size, growth rate, doubling time and biomass productivity/dry weight were determined in this study. Temperature, pH, nitrate, and phosphate concentrations were also monitored throughout the cultivation period. Significant differences in growth performance and cell morphology were observed among treatments ($p < 0.05$). The autotrophic control (KA) produced the highest cell density, reaching $389.73 \pm 1.92 \times 10^4$ cells mL^{-1} on day 5, whereas the mixotrophic treatment supplemented with IAA and glycerol (M) exhibited the lowest cell density ($144.00 \pm 4.05 \times 10^4$ cells mL^{-1}). In contrast, the largest mean cell diameter was recorded in treatment M (6.20 ± 0.10 µm), followed by the heterotrophic treatment supplemented with IAA and glycerol (H) (6.00 ± 0.06 µm). Water quality parameters remained within suitable ranges for microalgal growth throughout the experiment. Residual nitrate and phosphate concentrations were generally lower in treatments with higher cell densities, indicating greater nutrient utilization. The results demonstrate that cultivation mode and photoperiod exerted a stronger influence on biomass production than IAA and glycerol supplementation under the conditions tested. Continuous illumination favored cell proliferation, whereas the combination of glycerol and IAA promoted cellular enlargement. These findings provide insight into the trade-off between population growth and cell morphology in *C. vulgaris* cultivation systems.

Keywords: *Chlorella vulgaris*, autotrophic cultivation, heterotrophic cultivation, mixotrophic cultivation, indole-3-acetic acid, glycerol, cell density, cell morphology.

INTRODUCTION

The rapid expansion of global aquaculture has increased the demand for sustainable feed ingredients that can support animal growth while reducing dependence on conventional fishmeal and fish oil resources (Ai et al., 2025). Microalgae have received considerable attention as alternative feed ingredients because of their high nutritional value and their capacity to produce a wide range of bioactive compounds. Among the available species, *Chlorella vulgaris* is one of the most extensively cultivated microalgae due to its high protein content, favorable amino acid profile, essential fatty acids, pigments, vitamins, and antioxidant compounds (Heo et al., 2025; Wang et al., 2024). In addition to its nutritional value, *C. vulgaris* has been incorporated into aquaculture production systems as a functional ingredient capable of improving growth performance, immune responses, and stress resistance in aquatic organisms (Ahmad et al., 2018; Mueller et al., 2024).

Despite these advantages, large-scale production of *C. vulgaris* remains constrained by the relatively high operational costs associated with conventional autotrophic cultivation. Biomass production in autotrophic systems depends primarily on light availability and photosynthetic carbon fixation, which can limit productivity under suboptimal environmental conditions (Taikhao and Phunpruch, 2025). As a result, alternative cultivation strategies have been explored to enhance biomass accumulation and improve production efficiency. Among these approaches, mixotrophic and heterotrophic cultivation have attracted considerable interest because they allow microalgae to utilize both inorganic and organic carbon sources, thereby reducing their dependence on photosynthesis alone (Castillo et al., 2021).

Glycerol is one of the most widely investigated organic carbon sources for mixotrophic and heterotrophic microalgal cultivation. As an abundant by-product of biodiesel production, glycerol is inexpensive, readily available, and can be assimilated by several microalgal species (Rincon et al., 2024). Previous studies have reported that glycerol supplementation may enhance biomass production, alter cellular composition, and influence physiological responses depending on species, concentration, and cultivation conditions (Rana and Prajapati, 2021). However, the effectiveness of glycerol supplementation varies considerably among studies, suggesting that its impact is strongly influenced by environmental factors, including light availability and cultivation mode.

In addition to organic carbon supplementation, phytohormones have been investigated as growth regulators in microalgal cultivation systems. Indole-3-acetic acid (IAA), a naturally occurring auxin, has been reported to influence cell growth, biomass accumulation, and cellular morphology in several microalgal species (Hakim et al., 2023). Responses to exogenous IAA are often species-specific and concentration-dependent. While some studies have reported enhanced growth following auxin supplementation, others have observed neutral or inhibitory effects, indicating that the interaction between phytohormones and cultivation conditions remains insufficiently understood.

Although the effects of glycerol supplementation and phytohormone application have been examined separately in many studies, limited information is available regarding their combined influence under different photoperiod regimes. Furthermore, relatively few studies have simultaneously evaluated growth performance, cell morphology, and nutrient utilization in *C. vulgaris* cultivated under autotrophic, mixotrophic, and heterotrophic conditions. Understanding these responses is important for developing cultivation strategies that improve biomass productivity while maintaining desirable cellular characteristics for downstream applications.

Although glycerol supplementation and phytohormone application have individually been reported to influence microalgal growth, information regarding their combined effects on cell proliferation and morphology under contrasting photoperiod regimes remains limited. Therefore, the present study evaluated the growth performance, cell diameter, and nutrient utilization of *Chlorella vulgaris* cultivated under autotrophic, mixotrophic, and heterotrophic conditions supplemented with glycerol and IAA. The study aimed to determine how cultivation mode and additive supplementation influence cell proliferation and morphology, and to identify cultivation conditions that support efficient biomass production.

MATERIALS AND METHODS

Microalgae strain and culture preparation

Chlorella vulgaris was obtained from the culture collection of the Brackishwater Aquaculture Development Center (BBPBAP), Jepara, Central Java, Indonesia. Stock cultures were maintained in Conwy medium prepared with seawater at a salinity of 30 ppt. Cultures were incubated at 25 ± 1 °C under continuous illumination provided by cool-white fluorescent lamps at an intensity of approximately 3,000 lux and supplied with continuous aeration. Prior to the experiment, cultures were acclimatized under laboratory conditions and standardized to an initial density of 10.0 × 10^6 cells mL^{-1}. This density was used as the starting concentration for all experimental treatments.

Culture conditions and experimental design

The experiment was conducted using a completely randomized design (CRD) consisting of six treatments with three replicates per treatment (n = 3). Cultivation was performed in 1.000 mL borosilicate Erlenmeyer flasks containing 600 mL working volume. Each culture vessel contained 200 mL of sterilized enriched seawater medium and 400 mL of *C. vulgaris* inoculum. Continuous aeration was provided throughout the cultivation period using an oil-free air compressor. All treatments received the same basal nutrient enrichment consisting of walne (1 mL L^{-1}), trace metal (1 mL L^{-1}), and vitamin B12 (0.5 mL L^{-1}). Indole-3-acetic acid (IAA) was applied at a concentration of 1 mg L^{-1} or 1 mL L^{-1} and glycerol at a concentration of 10 mL L^{-1}.

The experiment consisted of six cultivation treatments representing autotrophic, mixotrophic, and heterotrophic conditions with or without supplementation of IAA and glycerol. The autotrophic control (KA) was maintained under continuous illumination (24 h light) without the addition of IAA or glycerol, whereas the autotrophic treatment (A) received 1 mg L^{-1} IAA under the same photoperiod. The mixotrophic control (KM) was cultivated under a 12 h light:12 h dark photoperiod and supplemented with 10 mL L^{-1} glycerol. The corresponding mixotrophic treatment (M) received both 1 mg L^{-1} IAA and 10 mL L^{-1} glycerol under the same photoperiod regime. For heterotrophic cultivation, the heterotrophic control (KH) was maintained under continuous darkness (24 h dark) and supplemented with 10 mL L^{-1} glycerol, while the heterotrophic treatment (H) received both 1 mg L^{-1} IAA and 10 mL L^{-1} glycerol under continuous darkness. All cultures were maintained for seven days under batch-culture conditions. For heterotrophic treatments, culture racks were completely wrapped with black plastic curtains to prevent light penetration.

Growth performance

Cell density

Cell density was determined daily using cell counter. Samples were collected from each culture vessel at the same time each day and fixed with Lugol's iodine solution prior to counting and calculated according to the following equation:

$$Cell\ density\ (cells\ mL^{-1}) = \frac{N}{n} \times 10^4 \times DF \quad (1)$$

where: N is the total number of cells counted, n is the number of counting squares observed, and DF is the dilution factor.

Cell size

Cell size was measured by its diameter. Cell diameter was also measured each day, corresponding to know the differential of cell size each day of growth phase. Measurements were performed using a calibrated ocular micrometer at 400× magnification. For each replicate, 30 cells were selected randomly and measured to obtain the mean cell diameter, expressed in micrometers (μm).

Growth rate

The growth performance of Chlorella vulgaris was evaluated by calculating the specific growth rate () during the cultivation period. Cell density data obtained from daily observations were used for the calculation. Specific growth rate was determined according to the following equation:

$$\mu = (ln\ Nt - ln\ N0) / t \quad (2)$$

where: μ is the specific growth rate (day^{-1}), $N0$ is the initial cell density (cells mL^{-1}), Nt is the cell density at time t (cells mL^{-1}), and t is the cultivation period (days).

Doubling time

Doubling time (DT) was calculated to estimate the time required for the microalgal population to

double during the exponential growth phase. The doubling time was calculated using the following equation:

$$DT\ (day)\ =\ 0.693\ /\ \mu \qquad (3)$$

where: *DT* is the doubling time (days), 0.693 is the natural logarithm of 2.

Lower DT values indicate faster cell division and greater growth performance.

Dry weight

Biomass production was determined using the dry weight method at the end of the cultivation period. A 50 mL culture sample was collected from each experimental unit and centrifuged at 4000 rpm for 15 min. The resulting biomass pellet was washed twice with distilled water to remove residual salts and culture medium components. The biomass was then transferred into a pre-weighed aluminum dish and dried in a hot-air oven at 105 °C until a constant weight was achieved. After drying, the samples were cooled in a desiccator and weighed using an analytical balance. Dry weight was calculated according to the following equation:

$$DW\ (gL^{-1})\ =\ Wf\ -\ Wi \qquad (4)$$

where: *DW* is the dry biomass weight (gL^{-1}), *Wf* is the final weight of the dish containing dried biomass (mg), and *Wi* is the initial weight of the empty dish (mg).

Water quality analysis

Temperature and pH were monitored daily using a digital pH/temperature meter (HI-98107, Hanna Instruments). Nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations were measured at the beginning as lag phase (day 0), exponential phase (day 5) and stationary phase (day 7) of the cultivation period.

Samples were centrifuged at 4.000 rpm for 15 min to separate the biomass from the culture medium. The resulting supernatant was analyzed spectrophotometrically according to standard methods described by the American Public Health Association (Baird et al., 2017). Nitrate was determined using the cadmium reduction method, while phosphate was measured using the ascorbic acid method.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene tests, respectively. Differences among treatments were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected ($p < 0.05$), treatment means were compared using Tukey's honestly significant difference (HSD) test. All statistical analyses were performed using SPSS version 26.0.

RESULTS

Growth performance and cell density of *Chlorella vulgaris*

The growth performance of *Chlorella vulgaris* under different cultivation modes and supplementation treatments is presented in Table 1. Cell density increased progressively in all treatments during the cultivation period and reached its peak on Day 5, followed by a decline toward the end of the experiment. Significant differences in growth performance were observed among treatments ($p < 0.05$).

The highest cell density was recorded in the autotrophic control treatment (KA), reaching $389.73 \pm 1.92 \times 10^4$ cells mL^{-1}, followed by the autotrophic treatment supplemented with indole-3-acetic acid (IAA) (A) with $277.17 \pm 3.38 \times 10^4$ cells mL^{-1}. Heterotrophic treatments produced intermediate cell densities, with KH and H attaining $247.43 \pm 5.56 \times 10^4$ and $197.00 \pm 1.73 \times 10^4$ cells mL^{-1}, respectively. The lowest cell densities were observed under mixotrophic conditions, particularly in treatment M ($144.00 \pm 4.05 \times 10^4$ cells mL^{-1}), which differed significantly from all other treatments (Table 1).

A similar trend was observed for growth rate. Treatments A, KH, and KA exhibited the highest growth rates, reaching 0.565 ± 0.010, 0.554 ± 0.017, and 0.535 ± 0.007 day^{-1}, respectively, with no significant differences among these treatments ($p > 0.05$). In contrast, treatment M showed the lowest growth rate (0.290 ± 0.009 day^{-1}), while treatments H and KM displayed intermediate values of 0.424 ± 0.012 and 0.398 ± 0.008 day^{-1}, respectively.

The doubling time results showed an inverse relationship with growth rate. The

Table 1. Growth performance of *Chlorella vulgaris* at the peak exponential growth phase (Day 5) under different cultivation modes and supplementation treatments

Treatment	Cultivation mode	Supplementation	Cell density ($\times 10^4$ cells mL^{-1})	Growth rate (day^{-1})	Doubling time (days)	Dry weight ($g\ L^{-1}$)
KA	Autotrophic (24 h light)	None (Control)	389.73 ± 1.92^{f}	0.535 ± 0.007^{a}	1.294 ± 0.017^{c}	1.055 ± 0.073^{a}
A	Autotrophic (24 h light)	IAA	277.17 ± 3.38^{e}	0.565 ± 0.010^{a}	1.227 ± 0.021^{c}	0.987 ± 0.050^{a}
KH	Heterotrophic (24 h dark)	Glycerol (Control)	247.43 ± 5.56^{d}	0.554 ± 0.017^{a}	1.252 ± 0.039^{c}	0.841 ± 0.039^{b}
H	Heterotrophic (24 h dark)	Glycerol + IAA	197.00 ± 1.73^{c}	0.424 ± 0.012^{b}	1.634 ± 0.047^{b}	0.723 ± 0.014^{c}
KM	Mixotrophic (12 h light: 12 h dark)	Glycerol (Control)	170.50 ± 1.25^{b}	0.398 ± 0.008^{b}	1.744 ± 0.035^{b}	0.689 ± 0.011^{c}
M	Mixotrophic (12 h light: 12 h dark)	Glycerol + IAA	144.00 ± 4.05^{a}	0.290 ± 0.009^{c}	2.391 ± 0.074^{a}	0.563 ± 0.034^{d}

Note: Values are presented as mean ± standard deviation (n = 3). Different superscript letters within the same column indicate significant differences among treatments according to Tukey's HSD test following one-way ANOVA at $p < 0.05$.

shortest doubling times were observed in treatments A (1.227 ± 0.021 days), KH (1.252 ± 0.039 days), and KA (1.294 ± 0.017 days), indicating rapid cell proliferation. Conversely, treatment M required the longest time for population doubling (2.391 ± 0.074 days), followed by KM (1.744 ± 0.035 days) and H (1.634 ± 0.047 days). These results indicate that autotrophic and heterotrophic cultures supported faster cell division than mixotrophic cultures under the conditions tested.

Dry weight measurements further supported the observed growth responses. The highest dry biomass concentration was obtained in treatment KA (1.055 ± 0.073 g L^{-1}), followed by A (0.987 ± 0.050 g L^{-1}) and KH (0.841 ± 0.039 g L^{-1}). Lower dry biomass concentrations were recorded in H (0.723 ± 0.014 g L^{-1}) and KM (0.689 ± 0.011 g L^{-1}), whereas the lowest biomass accumulation was observed in treatment M (0.563 ± 0.034 g L^{-1}). Dry weight values generally followed the pattern observed for cell density, indicating that treatments with greater cell proliferation also produced higher biomass concentrations. The autotrophic treatments (KA and A) generated significantly greater dry biomass than the heterotrophic and mixotrophic treatments, while treatment M exhibited the lowest biomass production among all cultivation regimes.

The autotrophic control treatment consistently exhibited the best growth performance, characterized by the highest cell density and dry biomass, together with a relatively high growth rate and short doubling time. In contrast, the mixotrophic treatment supplemented with glycerol and IAA produced the lowest growth performance among all treatments, suggesting that the combined supplementation did not effectively support cell proliferation and biomass accumulation under the cultivation conditions applied in this study.

Cell size of *Chlorella vulgaris*

Significant differences in cell size were observed among cultivation treatments on day 5 (Figure 1). The largest mean cell diameter was recorded in the mixotrophic treatment supplemented with glycerol and IAA (M), reaching 6.20 ± 0.10 μm. The heterotrophic treatment supplemented with glycerol and IAA (H) also exhibited relatively large cells, with a mean diameter of 6.00 ± 0.06 μm. In these treatments shown that glycerol and IAA addition help to increased cell diameter, and the photoperiodic of mixotrophic worked the best in this category.

Intermediate cell diameters were recorded in KM (5.90 ± 0.10 μm) and KH (5.80 ± 0.00 μm). The smallest cell diameters were observed under autotrophic conditions, with KA and A exhibiting mean diameters of 5.53 ± 0.00 μm and 5.37 ± 0.06 μm, respectively. Treatment A showed the smallest mean cell diameter among all treatments.

One-way analysis of variance (ANOVA) revealed significant differences in cell diameter among treatments on day 5 ($p < 0.05$). Tukey's HSD test indicated that treatment M produced significantly larger cells than the autotrophic treatments, while treatments KH and KM showed intermediate responses.

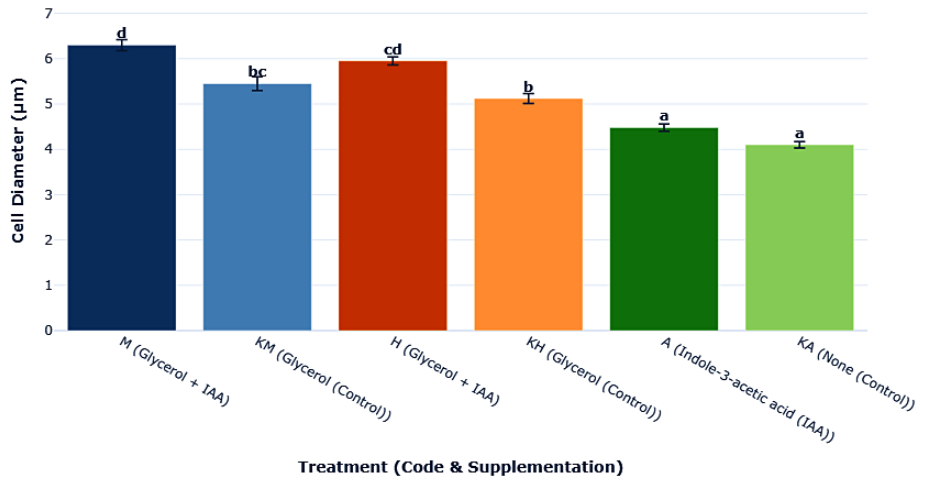

Figure 1. Cell diameter of *Chlorella vulgaris* under different cultivation modes and supplementation treatments

Water quality parameters

Water quality parameters remained within relatively stable ranges throughout the cultivation period across all treatments (Table 2). Culture temperature ranged from 28.0 to 29.7 °C, while pH values varied between 7.4 and 8.6.

At the end of the 7-day cultivation period, residual nitrate (NO_3^-) concentrations differed among treatments, ranging from 12.45 ± 0.85 to 28.90 ± 1.65 mg L^{-1}. The lowest nitrate concentration was recorded in the autotrophic KA, whereas the highest concentration was observed in the mixotrophic treatment supplemented with glycerol and IAA (M). Intermediate nitrate concentrations were measured in treatments A (15.10 ± 1.02 mg L^{-1}), KH (19.60 ± 1.15 mg L^{-1}), KM (22.34 ± 1.40 mg L^{-1}), and H (24.15 ± 1.38 mg L^{-1}).

Residual phosphate (PO_4^{3-}) concentrations ranged from 1.15 ± 0.12 to 2.85 ± 0.22 mg L^{-1}. Similar to nitrate, the lowest phosphate concentration was recorded in treatment KA, while the highest concentration was observed in treatment M. Treatments A, KH, KM, and H exhibited intermediate phosphate concentrations of 1.42 ± 0.09, 1.98 ± 0.14, 2.11 ± 0.18, and 2.40 ± 0.19 mg L^{-1}, respectively. The measured temperature, pH, nitrate, and phosphate values remained within the ranges observed during the experimental period for all cultivation treatments.

DISCUSSION

Effects of cultivation mode and IAA supplementation on cell growth

Cultivation mode significantly affected the growth performance of *C. vulgaris*. Among all treatments, the KA produced the highest cell density, indicating that continuous illumination without external organic carbon supplementation provided favorable conditions for cell proliferation. Published research reported enhanced growth of *C. vulgaris* under photoheterotrophic cultivation, whereas the present study observed lower

Table 2. Water quality parameters of the culture medium during the cultivation of Chlorella vulgaris

Treatment regime	Temperature (°C)	pH	Final NO_3^- (mg L^{-1})	Final PO_4^{3-} (mg L^{-1})
KA (Autotrophic control)	29–29.7	7.8–8.6	12.45 ± 0.85	1.15 ± 0.12
A (Autotrophic + IAA)	28–29	8.1–8.5	15.10 ± 1.02	1.42 ± 0.09
KM (Mixotrophic control)	28–29.3	7.4–7.7	22.34 ± 1.40	2.11 ± 0.18
M (Mixotrophic + IAA + Glycerol)	28–29.3	7.6–7.9	28.90 ± 1.65	2.85 ± 0.22
KH (Heterotrophic control)	29–29.7	7.5–7.8	19.60 ± 1.15	1.98 ± 0.14
H (Heterotrophic + IAA + Glycerol)	29–29.5	7.6–7.8	24.15 ± 1.38	2.40 ± 0.19

Note: Values are presented as mean ± standard deviation (n = 3). Final nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations were measured at the end of the 7-day cultivation period.

cell densities in glycerol-supplemented cultures (Long et al., 2024). These contrasting results may be attributed to differences in carbon concentration, photoperiod regime, or nutrient composition.

The addition of IAA resulted in lower cell densities under autotrophic, heterotrophic, and mixotrophic conditions. Compared with their respective controls, treatments supplemented with IAA consistently exhibited reduced cell abundance at the end of the exponential growth phase. This response suggests that the concentration of IAA used in the present study did not promote cell proliferation under the tested conditions. Previous studies have reported variable responses of microalgae to exogenous auxins, with growth stimulation occurring only within specific concentration ranges and species-dependent conditions (Lin et al., 2021; Wang et al., 2021). Therefore, the inhibitory response observed in this study may reflect a concentration-dependent effect of IAA on *C. vulgaris*.

The inhibitory response observed in the present study may not be solely attributed to IAA supplementation. The lower cell densities recorded in heterotrophic and mixotrophic cultures may also indicate that the glycerol concentration applied in this study was not optimal for supporting cell proliferation. Although glycerol is widely used as an organic carbon source in microalgal cultivation, its effectiveness is highly dependent on species, cultivation mode, and dosage. Previous studies have demonstrated that glycerol can stimulate biomass production when supplied at appropriate concentrations; however, excessive or suboptimal concentrations may reduce growth performance by altering cellular metabolism, increasing osmotic stress, or causing metabolic imbalance (Rana & Prajapati, 2021). Under such conditions, cells may allocate more energy toward maintenance and carbon assimilation processes rather than cell division, resulting in lower population growth.

Furthermore, the combined application of glycerol and IAA may have intensified this response. Auxins such as IAA are known to exhibit concentration-dependent effects in microalgae, with growth stimulation occurring only within a narrow optimal range (Abd-El-Aziz et al., 2025). Concentrations above the physiological requirement may disrupt cellular regulation and lead to reduced growth rates (Pekkoh et al., 2024). Therefore, the lower cell densities observed in the heterotrophic and mixotrophic treatments could reflect a combined effect of non-optimal glycerol utilization and auxin-induced metabolic regulation rather than the action of IAA alone.

Lower cell densities were also observed in glycerol-supplemented cultures, particularly under mixotrophic conditions. The mixotrophic treatment supplemented with glycerol and IAA (M) produced the lowest cell density among all treatments. Although mixotrophic cultivation is often used to enhance biomass production, its effectiveness depends on the type and concentration of the organic carbon source as well as cultivation conditions (Higgins and VanderGheynst, 2014). The present results indicate that the combination of glycerol supplementation and the applied photoperiod did not improve cell proliferation relative to autotrophic cultivation.

The dry weight results closely followed the pattern observed for cell density. Cultures grown under autotrophic conditions produced the highest biomass accumulation, while mixotrophic cultures supplemented with glycerol and IAA exhibited the lowest dry biomass. This finding suggests that under the conditions tested, continuous illumination supported biomass production more effectively than glycerol supplementation. The consistency between cell density and dry weight data indicates that differences in biomass production were primarily associated with variations in cell proliferation rather than changes in cellular mass alone.

Changes in cell diameter under different cultivation conditions

In contrast to cell density, cell diameter increased in treatments receiving glycerol and IAA supplementation. The largest cells were observed in the mixotrophic treatment supplemented with glycerol and IAA (M), followed by the heterotrophic treatment receiving the same supplementation (H). Meanwhile, the smallest cell diameter was recorded in the KA.

The increase in cell size observed in glycerol-supplemented treatments may be associated with physiological changes related to carbon storage. Organic carbon sources can be utilized for the synthesis of storage compounds, including carbohydrates and lipids, which may contribute to increases in cellular volume (Ma et al., 2021; Narayanan et al., 2025). Similar increases in cell size under mixotrophic or heterotrophic cultivation have been reported in several microalgal species

supplied with external carbon sources (Rana and Prajapati, 2021).

An inverse trend was observed between cell density and cell diameter. Treatments that produced larger cells generally exhibited lower cell densities, whereas treatments with smaller cells achieved higher population densities. This pattern suggests that cultivation conditions favoring cellular enlargement did not necessarily promote rapid cell division. Similar relationships between growth rate and cell size have been described in microalgae, where environmental conditions can influence the balance between biomass accumulation and cell proliferation (Choe et al., 2025).

The response observed in IAA-supplemented cultures may also indicate that exogenous auxin influences cellular morphology in addition to growth performance. Previous studies have shown that phytohormones can modify microalgal cell characteristics, including cell size, biomass composition, and metabolite accumulation, although responses vary among species and cultivation conditions (Subramanian and Joseph, 2024).

Water quality and nutrient utilization

Water quality parameters remained within suitable ranges throughout the cultivation period. Temperature values ranged from 28.0 to 29.7 °C, while pH remained between 7.4 and 8.6. These conditions fall within the range commonly reported for successful cultivation of *Chlorella* species (Kalinina et al., 2023).

Differences in residual nitrate and phosphate concentrations were observed among treatments at the end of the experiment. The autotrophic control treatment exhibited the lowest residual nutrient concentrations, whereas the mixotrophic treatment supplemented with glycerol and IAA showed the highest remaining nitrate and phosphate levels. These results correspond with the observed growth patterns, where treatments with higher cell densities generally exhibited lower residual nutrient concentrations.

The reduction in nitrate and phosphate concentrations indicates active nutrient assimilation by *C. vulgaris* during cultivation. Nitrogen and phosphorus are essential elements for cellular processes, including protein synthesis, nucleic acid formation, and energy metabolism (Yaakob et al., 2021). Consequently, greater biomass production is typically associated with increased nutrient uptake from the culture medium. The nutrient profiles observed in this study therefore support the differences in growth performance recorded among cultivation treatments.

The present study was conducted using a single concentration of IAA and glycerol. Consequently, the observed responses cannot be used to determine the optimal dosage of either supplement for *Chlorella vulgaris* cultivation. In addition, growth performance was evaluated primarily through cell density, cell size, growth rate, cell doubling time, biomass productivity and nutrient utilization. Measurements of lipid, protein, carbohydrate content, photosynthetic performance, and metabolite accumulation would provide a more comprehensive understanding of the physiological responses to IAA and glycerol supplementation. Future studies should evaluate a broader range of IAA and glycerol concentrations under different photoperiod regimes and cultivation durations. Integrating growth analysis with biochemical and physiological assessments would help clarify whether the observed reduction in cell density is associated with changes in carbon allocation, metabolite production, or other cellular processes.

CONCLUSIONS

The present study demonstrated that cultivation mode, photoperiod regime, and supplementation with IAA and glycerol significantly affected the growth performance and cell morphology of *Chlorella vulgaris*. The highest cell density was obtained in the autotrophic control treatment cultivated under continuous illumination (KA), reaching $389.73 \pm 1.92 \times 10^4$ cells mL^{-1}. This finding indicates that continuous light exposure without exogenous carbon or phytohormone supplementation provided the most favorable conditions for cell proliferation under the experimental conditions evaluated. IAA supplementation reduced cell density across autotrophic, heterotrophic, and mixotrophic cultivation modes. Similarly, glycerol-containing treatments did not improve cell proliferation relative to the autotrophic control. However, cultures supplemented with glycerol and IAA exhibited larger cell diameters, particularly under mixotrophic and heterotrophic conditions, indicating that factors promoting cellular enlargement were not necessarily associated with increased population growth. Residual nitrate and phosphate concentrations reflected

differences in nutrient utilization among treatments, with lower residual nutrient levels observed in cultures achieving higher cell densities. Overall, the results suggest that cultivation mode and photoperiod exert a greater influence on biomass production than IAA and glycerol supplementation under the conditions tested. Further studies should evaluate a wider range of IAA and glycerol concentrations and examine their effects on biomass composition, including lipid, protein, and carbohydrate accumulation, to identify cultivation strategies that optimize both biomass yield and biomass quality in *C. vulgaris*.

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