

Optimization of *Parachlorella kessleri* growth under different environmental conditions for lipid production

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Abstract

The sustainable development of polymers and biofuels through photosynthetic biomass cultivation has become a growing global priority driven by environmentally green procedures. The unicellular green algae *Parachlorella kessleri* has shown potential for lipid and biopolymer production. In this study, a *P. kessleri* strain (PDP-01) was isolated from the Yamuna River in Delhi. The organism was cultured and characterized using morphological, biochemical and molecular techniques for identification. This study was performed to examine the influence of media pH, incubation temperature, photoperiod, and CO₂ concentration on the growth characteristics of *P. kessleri*. A one-factor-at-one-time approach was used to assess the effect of parameters on cell reproduction. Lipid and polyhydroxyalkanoate (PHA) content was additionally quantified from biomass. The findings indicated that the maximum biomass production occurred in a 16-day-old culture under the following conditions: pH 8, a temperature of 25°C, a photoperiod of 16:8 (light:dark), and 2% CO₂ concentration. Under these conditions, the strain accumulated 17% lipid and 11% PHA of dry cell weight demonstrating its dual potential for lipid and biopolymer co-production. To our knowledge, this is the first report of an indigenous *P. kessleri* isolate from the Yamuna river, Delhi characterized and optimized for biomass, lipid and PHA production, highlighting its biorefinery potential for sustainable bioplastic application.

1. Introduction

Microalgae have emerged as a promising and sustainable biological platform for the production of high value bioproducts such as biodiesel, bioplastics, and bioenergy [1, 2, 3]. This growing interest is driven by the global demand for sustainable alternatives to fossil fuels and conventional plastics, both of which pose significant environmental challenges [4]. The growth dynamics of microalgae are highly strain-specific and strongly influenced by environmental factors such as pH, light intensity, photoperiod, temperature, and CO₂ availability [5, 6]. These parameters are critical in determining microalgal growth rates, adaptability, and biomass productivity, which are closely linked to photosynthetic efficiency [7, 8]. Optimizing these parameters is therefore essential to maximize yield, lower production costs, and enhance overall efficiency [9, 10].

Among various microalgal strains, *P. kessleri* has gained significant attention for its industrial potential due to its ability to produce both starch, lipids and polyhydroxyalkanoates (PHA), making it a valuable resource for a wide range of bioproducts [11, 12, 13]. Notable for its high lipid content, rapid growth rate, and substantial biomass productivity, *P. kessleri* ensures a reliable and abundant raw material supply [14, 15]. Additionally, its adaptability to diverse cultivation media, including brewery waste [16], swine effluent [17], agro-industrial byproducts, and wastewater, underscores its potential for resource recovery and environmental sustainability [18]. Recent advances have further highlighted the genetic traceability and biopolymer-producing capacity of *P. kessleri* with CRISPR/Cas9-mediated gene editing successfully employed to develop mutant strains with significant improved lipid productivity [19].

Despite its promising applications, previous research has primarily focused on optimizing individual growth parameters such as pH [20], photoperiod [21], and temperature [22], often overlooking the intricate interactions among multiple factors. This narrow approach limits the scalability of *P. kessleri* for industrial applications. To address this gap, the present study systematically evaluated the combined effects of pH, temperature, photoperiod, and CO₂ concentration on the growth dynamics and biomass productivity of *P. kessleri*. To address this gap, the present study systematically evaluated the combined effects of pH, temperature, photoperiod, CO₂ concentration on the growth of, biomass productivity, lipid content, and PHA accumulation of freshwater-isolated *P. kessleri* strain from Yamuna river, Delhi. To the best of our knowledge this is the first study to report concurrent lipid and PHA co-production from Indigenous Indian *P. kessleri* isolate optimized multi-parameter cultivation condition, establishing a strong foundation for its bio-refinery based industrial exploitation.

2. Material and Method

2.1 Chemicals

Analytical-grade chemicals such as Bold's Basal Medium, ampicillin, Lugol's solution, Nile red, CTAB buffer, and PCR reagents were sourced from Sigma-Aldrich. The equipment used included a Magnus MLXi Plus microscope, REMI C852 centrifuge, and Lasany Instruments UV-VIS spectrophotometer (Model No. LI 295).

2.2 Sample collection, Isolation and Growth Conditions

A microalgal sample was isolated from the stream of river Yamuna at Okhla, Delhi (latitude 28.52° N, longitude 77.28° E). Water samples were collected using the grab sampling method in a sterile white bottle. To reduce the microbial load and facilitate isolation, the sample was diluted with sterile distilled water and transported to the laboratory, where it was processed on the same day. The streak plate method was used to culture the algae in Bold Basal Medium (BBM) in petri plates. Successive rounds of streaking were performed on BBM plates supplemented with ampicillin to eliminate bacterial contaminants and obtain a purified (axenic) culture. Ampicillin, a β -lactam antibiotic, selectively inhibits bacterial cell wall synthesis without affecting algal cells, thereby aiding in the purification of the algal culture. The resulting axenic culture was maintained in BBM and incubated at 25°C under continuous illumination (100 lux) with constant shaking at 150 rpm on an orbital shaker [15].

2.3 Characterization of *P. kessleri*

2.3.1 Light microscopy

The algal sample was transferred onto a clean glass slide using a pipette, followed by addition of Lugol's solution to enhance visibility. A coverslip was gently placed over each sample to prepare a wet mount. The algal cells were examined under a bright-field microscope at 100x magnification, and the features and characteristics of the microalgal isolate PDP-01 were documented. Species identification was

achieved by comparing the observed characteristics with the original species descriptions available in the Algae Base [23, 24].

2.3.2 Fluorescent Microscopy

Intracellular lipid droplets were identified using Nile Red (9-(diethylamino)-5H-benzo[a]phenoxazine-5-one) fluorescence staining. Microalgae biomass was first collected by centrifugation at 250 g for 10 minutes and resuspended in 200 µL of distilled water by vortex mixing. The cell suspension was then treated with a 1% (w/v) Nile Red solution and incubated for 10 min under dark conditions. Excess dye was removed by washing, and lipid accumulation was visualized using fluorescence microscopy within an excitation range of 535–650 nm [24, 25].

2.4 Molecular Identification

2.4.1 DNA Extraction

Genomic DNA was isolated from algal samples using a modified CTAB-based method adapted from Doyle [26]. The samples were suspended in CTAB extraction buffer and incubated at 60°C for 1 h with intermittent mixing. After cooling to ambient temperature, chloroform:isoamyl alcohol (24:1, v/v) was added, and the mixture was gently mixed for 15 min, followed by centrifugation at room temperature at approximately 16,000 g for 10 min. The aqueous phase was carefully transferred to a new tube and combined with an equal volume of chilled isopropanol to precipitate the DNA, then incubated at – 20°C for 20–30 min. The DNA was pelleted by centrifugation at 4°C for 10 min, washed with 70% ethanol, and resuspended in TE buffer. DNA integrity and quality were assessed using 0.8% agarose gel electrophoresis prior to PCR amplification [20].

2.4.2 PCR Amplification

Amplification of partial 18S rRNA gene sequences was carried out using a thermal cycler. PCR was performed with the forward primer A (5'-AACCTGGTTGATCCTGCCAG-3') and the reverse primer SSU-inR1 (5'-CACCAGACTTGCCCTCCA-3'), which target conserved regions of the 18S rDNA. Each 20 µL PCR reaction contained 1 µL of genomic DNA template along with the SSU-inR1 primers. The amplification program consisted of an initial denaturation step at 94°C for 30 s, followed by annealing at 55°C for 30 s and extension at 72°C for 1 min, with a final extension at 72°C for 7 min [23, 27].

2.4.3 Sequence alignment and phylogenetic analysis

The generated Internal Transcribed Spacer (ITS) sequence was initially examined and manually refined using BioEdit software (version 7.2.5) to eliminate ambiguous and non-aligned regions at both the 5' and 3' termini. Molecular identification of the microalgal isolate was then performed by comparing the edited sequence against reference sequences available in the National Center for Biotechnology Information (NCBI) database using the BLAST (Basic Local Alignment Search Tool) algorithm [23, 27].

2.5 Effect of cultivation factor on the growth behavior of *P. kessleri*

This study aimed to identify the key factors that influence microalgae growth under controlled conditions. A one factor at one time experiment was conducted in triplicates, focusing on pH, temperature, photoperiod, and CO₂ concentration. The pH levels were varied at 6, 7, 8, 9, and 10, while temperature conditions were set at 25°C, 30°C, 35°C, 40°C, and 45°C. The photo period was adjusted to different light/dark cycles, including 8:16, 10:14, 12:12, 14:10, and 16:8 hours, while CO₂ concentration was tested at 1%, 2%, 3%, 4%, and 5%. All experiments were maintained at a consistent light intensity of 100 Lux (Orbit) throughout the study. The cultivation process was carried out in 250 mL Erlenmeyer flasks, each containing a working volume of 100 mL of bold basal medium as the growth medium. The experiment spanned a total duration of 16 days, during which growth was periodically monitored to evaluate the impact of different parameters on microalgae proliferation.

2.6 Growth Kinetics

The growth of *P. kessleri* was tracked by recording the optical density (OD) at 670 nm every three days using a UV spectrophotometer. For biomass quantification, 100 mL culture samples were collected on the sixteenth day and centrifuged at 27.95 x g for 10 minutes to separate the algal biomass. After weighing, the wet biomass was dried at 40°C in a hot air oven until it reached a stable weight, allowing calculation of dry biomass yield (g L⁻¹). The data obtained was used to analyze specific growth rates (μ) and biomass productivity (P) as per Eqs. 1 and 2 respectively. One factor at one time (OFAT) approach was utilized to determine the optimum conditions for maximum biomass amount.

Specific growth rate (μ) = $\frac{\ln x_2 - \ln x_1}{t_2 - t_1}$ (1)

Where x₁ and x₂ denote biomass concentrations (g L⁻¹) measured at time t₁ and t₂.

Biomass productivity: $P = \frac{x_f - x_i}{t}$ (2)

Where “x_f” and “x_i” represents final and initial biomass concentrations (g L⁻¹) respectively and “t” represented cultivation period in days

2.7 Validation of Growth at Optimized Condition

After determining the optimum levels for all four input variables, a comparative growth experiment was conducted under optimized and non-optimized conditions. All experiments were performed in triplicate using 250 mL Erlenmeyer flasks with a 100 mL working volume. For the preparation of experimental culture, 1 mL of actively growing *P. kessleri* sp. inoculum (exponential phase) was aseptically transferred into 99 mL of sterile distilled water, yielding a total working volume of 100 mL. The optimized conditions were set at pH 8, 25°C temperature, a 16:8 h photoperiod (light–dark), and 2% CO₂ aeration. Optical density (OD) was recorded at three-day intervals, and the dry cell weight (DCW) of the biomass was

measured on day 16 to evaluate growth differences between the optimized and non-optimized treatments.

2.8 Extraction and Estimation of Lipid

The extraction and estimation of total lipids were performed from sixteen days old dried algal biomass using the Bligh and Dyer method. The algal culture was centrifuged at 2795g for 10 minutes, after which the resulting pellet was freeze-dried. Following lyophilization, 0.1 g of the dried algal powder was precisely weighed and placed into a 5-mL glass vial. The empty glass bottle was weighed in advance of after which 2 mL methanol and 1 mL of chloroform were added, and the mixture was refrigerated for 12 hours to facilitate lipid extraction. After incubation, the mixture was centrifuged at 2795g, for 10 min. After centrifugation, the lower (bottom) organic phase containing chloroform and extracted lipids was carefully collected. The lipid content was quantified by calculating the ratio of extracted lipid weight to the total biomass weight, expressed as a percentage of dry cell weight using Eq. 3:

$$Lipid \% = \frac{W_l}{W_B} \times 100$$

3

where W_l represents the weight of the extracted lipid, and W_B is the total weight of the dry biomass [28].

2.9 Extraction and Estimation of PHA

PHA was extracted from 100 mg of 16-day-old freeze dried *P. kessleri* biomass using sodium hypochlorite digestion followed by chloroform solvent extraction [36], Briefly the dried biomass was treated with sodium hypochlorite solution (10% v/v) for 1 h at room temperature with intermittent agitation to digest non-PHB cellular components. The resultant pellet was collected by centrifugation and washed sequentially with distilled water, acetone, and ethanol to remove residual impurities. PHA was subsequently dissolved in chloroform and recovered by precipitation with cold ethanol. The extracted PHA powder was dried at 40°C to constant weight. PHA content was quantified using the crotonic acid assay. Briefly, 10 ml of 36 N H₂SO₄ was added to the extracted PHA powder and heated at 100°C in a boiling water bath for 20 min, converting PHA to crotonic acid. The development of brown colouration confirmed the conversion of PHA to crotonic acid. Absorbance was measured at 235 nm using UV spectrophotometer against a crotonic acid standard curve [37] adapted from published calibration references. PHA content was expressed as a percentage of dry cell weight using the formula:

PHA content (%) = (weight of extracted PHA/ Dry biomass weight) * 100.....(4)

2.10 Statistical analysis

All experiments were performed in triplicate, and the results are presented as mean values. Differences between groups were assessed using one-way and two-way analysis of variance (ANOVA) in GraphPad

PRISM version 8.0, with a p-value of < 0.05 considered statistically significant.

3 Result and discussion

3.1 Analysis and Characterization of *P. kessleri*

The morphological features of the algal sample were like those of the genus *P. kessleri*. The individual cells of the colonies were in the range of 10 μm . Cells were dark green, spherical, and unicellular in shape, as shown in Fig. 1(A). Furthermore, Nile red staining of lipid granules shown in Fig. 1(B) confirms the presence of high lipid content in *P. kessleri*.

3.2 Identification of Microalgae Species

BLAST and phylogenetic analysis revealed that the 18S rDNA sequence of PDP_01 closely clustered with *P. kessleri*., showing 92% homology with related species *Dictyosphaerium* Sp. (GQ487252) and *Dictyosphaerium* Sp (MF664512). The small subunit ribosomal RNA sequence of *Parachlorella kessleri* PDP 01 was submitted to NCBI GenBank that can be accessed with accession number OR364517.

3.3 Effect of pH on growth of *P. kessleri*

The effect of pH on the cell number and mass of *P. kessleri* is depicted in Fig. 2(A) and 2(B). The data indicates that growth was highest at pH 8, with the lowest growth observed at pH 6. On the 16th day, the optical density of algal biomass reached 0.70 ± 0.021 , and the maximum dry weight was recorded at 0.52 ± 0.036 g. Similarly, it was observed that the growth of *P. kessleri*. and inorganic carbon (IC) concentration across various nitrogen levels and found that maintaining the pH at 8 enhanced algal growth and achieved carbon removal efficiency of up to 95% over 34 days, yielding 3.4 grams of dry biomass [29]. Additionally, another researcher also investigated algal biomass and extracellular polysaccharide production in both indoor and outdoor settings, observing that under indoor conditions, the highest biomass was produced at pH 8 in combination with 2% CO_2 , outperforming two other treatments [30]. These findings are consistent with existing literature, which demonstrates that pH 8 provides favorable conditions for enhanced growth rates and effective nitrogen removal from wastewater. Furthermore, this supports the strain's adaptability to slightly alkaline conditions, although it can also grow across a broader pH range.

3.4 Effect of Temperature on growth of *P. kessleri*.

Temperature is a critical determinant of microalgal physiology, and *P. kessleri* exhibited its highest biomass productivity at 25°C (Fig. 3(A) and 3(B)). At this temperature, the culture reached a maximum optical density of 0.69 ± 0.005 and a biomass yield of 0.023 ± 0.08 g, whereas growth declined markedly at elevated temperatures, with minimal performance recorded at 35°C (OD: 0.50 ± 0.038 ; biomass: 0.92 ± 0.09 g) and complete inhibition at 40–45°C. The superior growth at 25°C is likely attributable to two key factors: (1) enhanced photosynthetic and metabolic efficiency, as core enzymes such as RuBisCO and

components of the electron transport chain function optimally at moderate temperatures; and (2) minimal thermal stress, since temperatures above $\sim 28\text{--}30^\circ\text{C}$ are known to induce ROS accumulation, membrane destabilization, and photoinhibition, thereby suppressing biomass formation. These results are consistent with study [31], who also identified 25°C as the optimal growth temperature, and with the findings of [32], who reported that *P. kessleri* achieved the highest biomass concentrations ($2750\text{--}2765\text{ mg L}^{-1}$) among five algal species after seven days of cultivation at this temperature.

3.5 Effect of Photoperiod on growth of *P. Kessleri*.

The highest algal growth was observed under a 16:8-hour light-dark cycle, achieving the maximum growth rate of 1.22 ± 0.025 and biomass productivity of 0.067 ± 0.004 . The effect of photoperiod on number of cells and cell biomass are shown in Fig. 4(A) and 4(B) respectively. Consistent with our results, Schriber reported enhanced growth under a 16:8 day/night cycle. Similarly, another researcher examined the effects of different photoperiod treatments on algal biomass growth and found no significant difference between the 12:12 and 16:8 cycles [31]. Hidasi et al. studied triglyceride production under a 16:8 light-dark regime for *P. kessleri* in heterotrophic and autotrophic conditions, reporting that *P. kessleri* exhibited significantly greater triglyceride accumulation in heterotrophic conditions [15]. In contrast, Taleb et al. observed a 26% reduction in triacylglycerol (TAG) concentration with a day/night cycle compared to continuous illumination [33]. Magierek et al. and also investigated photoperiod versus continuous illumination, both concluding that continuous illumination supported higher algal growth [34].

3.6 Effect of CO₂ on growth of *P. kessleri*.

The change in cell numbers and biomass for the during the algal growth under different time period of CO₂ sparging is depicted in Fig. 5(A) and 5(B) respectively. In comparison to the other treatments, the 1-minute and 3-minute groups initially exhibited the highest growth from day 7. By day 10, there was no statistically significant difference in growth between the 1, 2, and 3-minute treatments compared to the 4 and 5-minute treatments. However, on day 13, the 4-minute treatment showed a statistically significant difference, followed by the 1, 3, 2, and 5-minute treatments. By day 16, the growth rates for the 2, 1, and 3-minute treatments were significantly higher than those of the 4 and 5-minute treatments, although no significant differences were observed between the 2, 1, and 3-minute groups. Similar to our study, another researcher investigated algal biomass and extracellular polysaccharide (EPS) production under three different CO₂ concentrations 0.04%, 2%, and 5% and found that algal biomass productivity and EPS production were highest at 2% CO₂ compared to the other two treatments [30].

3.7 Growth of algae under optimized conditions

The results indicated that under optimal conditions, microalgae cell number reached approximately 825, whereas in the absence of these conditions, cell number was significantly lower at 212 (Fig. 6(A)). Furthermore, the dry biomass weight under optimized conditions was $0.29 \pm 0.07\text{ g L}^{-1}$, compared to $0.09 \pm 0.002\text{ g L}^{-1}$ in the unoptimized conditions (Fig. 6(B)).

3.8 Lipid content of microalgae

The biochemical composition of the 16-day-old stationary-phase culture of *P. kessleri* showed a lipid content of 17%. Beigbeder et al. reported that *P. kessleri* cultivated in Bold Basal Medium with NaHCO_3 at pH 8 exhibited a similar lipid content of 17% [21]. Gao et al. observed that lipid content in *P. kessleri* TY02 varied with culture duration and was more pronounced under nitrogen stress (N-) than in nitrogen-supplemented conditions (N+), ranging from 11.2% to 33.2% under N + and 12.5% to 45.5% under N- [23]. Pribyl et al. evaluated biomass productivity and lipid content in ten different strains over seven days, finding that *P. kessleri* achieved high biomass productivity (1.291 ± 0.032) with lipid contents of $51.06 \pm 1.27\%$ under nutrient-depleted conditions [28]. Nitrogen stress has been widely reported to enhance lipid accumulation in microalgae; for example, Li et al. found that nitrogen deficiency can induce lipid accumulation of up to 60% in five days [14]. Similarly, Fernandes et al. observed up to 28% lipid accumulation in seven days across three different media: fresh medium, 0.2 medium, and 0.1 medium [35].

3.9 PHA Production from *P. kessleri* Biomass

The production of PHA from 16-day-old freeze-dried *P. kessleri* biomass was confirmed by the development of a characteristic brown colouration upon acid treatment, indicative of PHA conversion to crotonic acid. The absorbance measured at 235 nm was 0.223, corresponding to a PHA content of 11% w/w of dry cell weight, equivalent to 11 mg PHA per 100 mg of algal biomass (0.11 mg/mg dry weight). PHA accumulation in microalgae has been reported across several species; Samantaray and Mallick (2012) recorded PHB yields of up to 10.2% in the cyanobacterium *Aulosira fertilissima* under nitrogen-limiting conditions [38], values comparable to those obtained in the present study. Similarly, Kavitha et al. (2011) reported PHA yields ranging from 7 to 14% from marine microalgal sources under nutrient stress [39]. Notably, the 11% PHA yield observed in the present study was achieved under standard optimized growth conditions – without additional nitrogen starvation or stress induction – suggesting that *P. kessleri* possesses an inherent capacity for intracellular carbon storage as PHA, making it particularly amenable for biorefinery applications. The concurrent accumulation of 17% lipid and 11% PHA from the same biomass under identical cultivation conditions represents a significant advantage from an industrial perspective. Co-extraction of lipids and PHA from a single algal batch can substantially reduce the overall production cost and improve process sustainability, aligning with the principles of circular bio-economy [40]. To the best of our knowledge, this is among the first reports documenting simultaneous lipid and PHA co-production from a freshwater-isolated Indian *P. kessleri* strain, underscoring the industrial and environmental significance of this isolate for sustainable bioplastic and biopolymer production.

To produce bioplastics from lipid-rich microalgae, it is essential to understand their growth behaviour under varying environmental conditions, as these directly influence biomass yield, lipid accumulation, and biopolymer content [40]. Microalgae-derived PHA has attracted growing interest as a sustainable alternative to petroleum-based plastics, owing to its biodegradability, biocompatibility, and thermoplastic

properties comparable to polypropylene [40]. Several studies have demonstrated PHA accumulation in green microalgae under optimized cultivation; Da Silva et al. [25] reported successful biopolymer production from *Parachlorella* species under wastewater treatment conditions [18], while Samantaray and Mallick (2012) documented PHB yields of up to 10.2% in cyanobacterial biomass [38]. In the present study, the highest cell density and biomass yield were achieved at pH 8, 25°C, a 16:8 h light-dark cycle, and 2% CO₂ concentration. Neutral lipids were confirmed through Nile Red staining, with lipid quantification showing 17% accumulation after 16 days of growth. In addition, PHA extraction from the same biomass yielded 11% w/w of dry cell weight, confirming the dual biopolymer-producing potential of this isolate. This isolate demonstrated tolerance to high CO₂ concentrations and a wide pH range, and exhibited growth under continuous illumination, collectively making it a strong candidate for bioplastic and other value-added bioproduct applications.

The findings suggest that *P. kessleri* is a promising candidate for bioplastic production due to its high biomass productivity, robust lipid accumulation, and adaptability to various environmental conditions. Its rapid growth, particularly under continuous illumination, ensures the large-scale biomass yields required for efficient bioplastic production. Additionally, *P. kessleri* is known for accumulating significant amounts of lipids, which serve as key precursors to produce polyhydroxyalkanoates (PHA). Its ability to thrive in diverse environments, including wastewater, makes it a sustainable and cost-effective option for industrial applications. From our perspective, bioplastics derived from *P. kessleri* have the potential to address global challenges by offering high biomass production at a reduced cost. However, it should be noted that no literature currently exists on bioplastic production from *P. kessleri*. To investigate this, the second phase of the study will be carried out in a photobioreactor to evaluate bioplastic production using *P. kessleri*.

4 Conclusion

The study identifies the key factors—pH, photoperiod, temperature and CO₂ concentration—that govern the growth rate of *P. kessleri*. The results suggest that maintaining a pH of 8, a temperature of 25°C, a photoperiod of 16:8, and the addition of 2% CO₂ can enhance the growth of *P. kessleri* for large-scale cultivation in photobioreactors. Biochemical analysis revealed that the strain contained 17% lipids in a 100 mg sample, underscoring its potential as a viable candidate for bioplastic production and other value-added applications. These findings not only improve the understanding of *P. kessleri*'s growth dynamics but also support its industrial scalability.

Declarations

Conflict of Interest:

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Author Contribution

PR was involved in performing the experiments, collection of data, preparation of draft of the manuscript and editing. JS was involved in conceptualization of research and experiments, design of experiments. SS was involved in manuscript revision and data analysis. SG was responsible for data validation and analysis, preparation of manuscript, editing the draft and submission of manuscript.

Data Availability

All data supporting the findings of this study are available within the paper.

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Figures

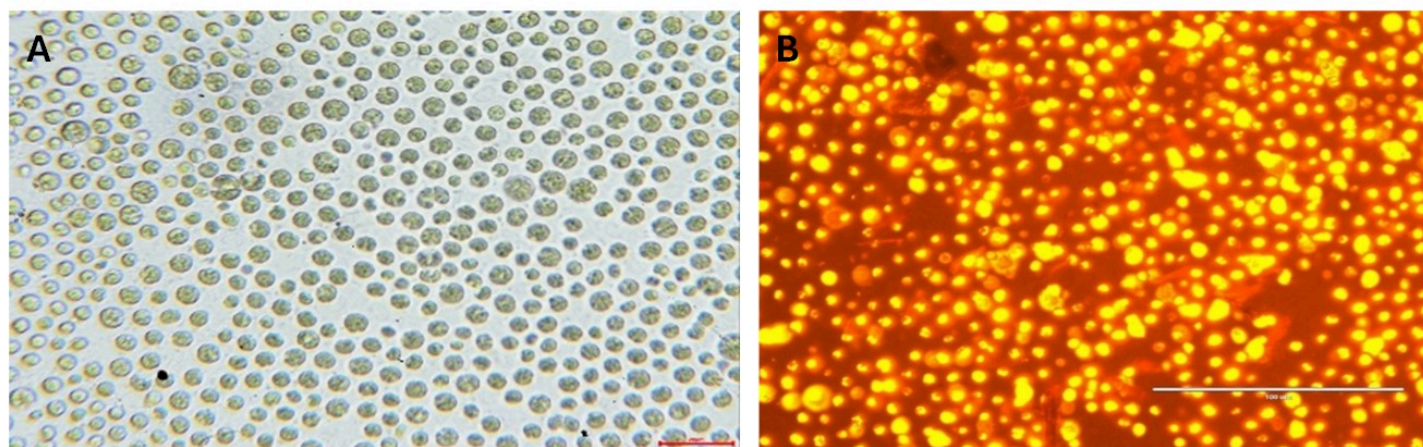


Figure 1

(A) Bright-field microscopic image of *P. kessleri*, illustrating its cellular morphology. (B) Fluorescent microscopic image of *P. kessleri* stained with Nile Red, highlighting intracellular lipid granules, which indicate lipid accumulation within the cells.

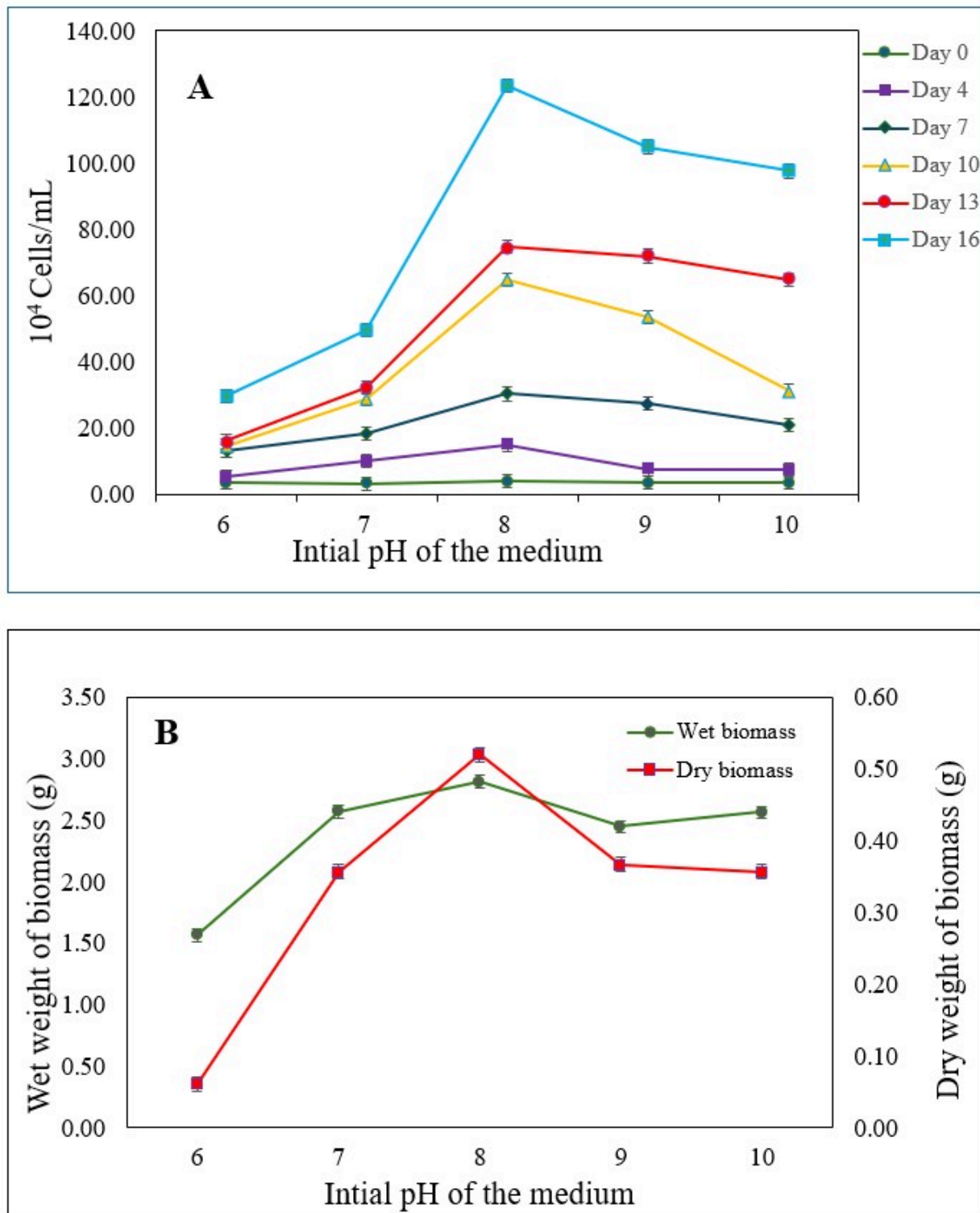


Figure 2

(A) Represents the growth curve of *P. kessleri* under different pH conditions, showing variations in growth over time. (B) Depicts the wet (x-axis) and dry biomass (y-axis) yield obtained at different pH levels, highlighting the impact of pH on biomass accumulation (n=3).

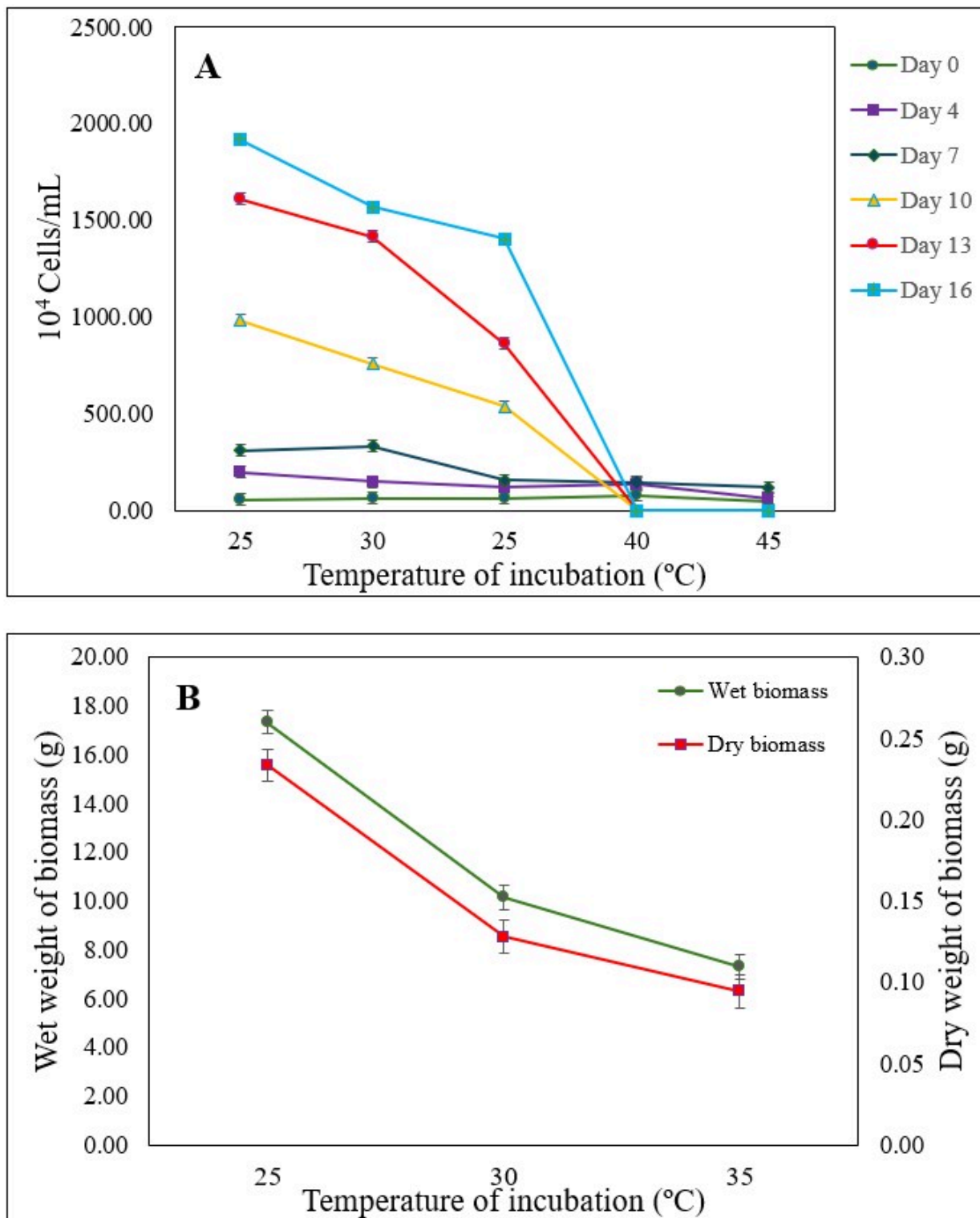


Figure 3

(A) Represents the growth curve of *P. kessleri* under different temperature conditions, illustrating how temperature influences its growth over time. (B) Shows wet (x-axis) and dry (y-axis) biomass yield obtained at different temperatures, indicating the impact of temperature variations on biomass accumulation (n=3).

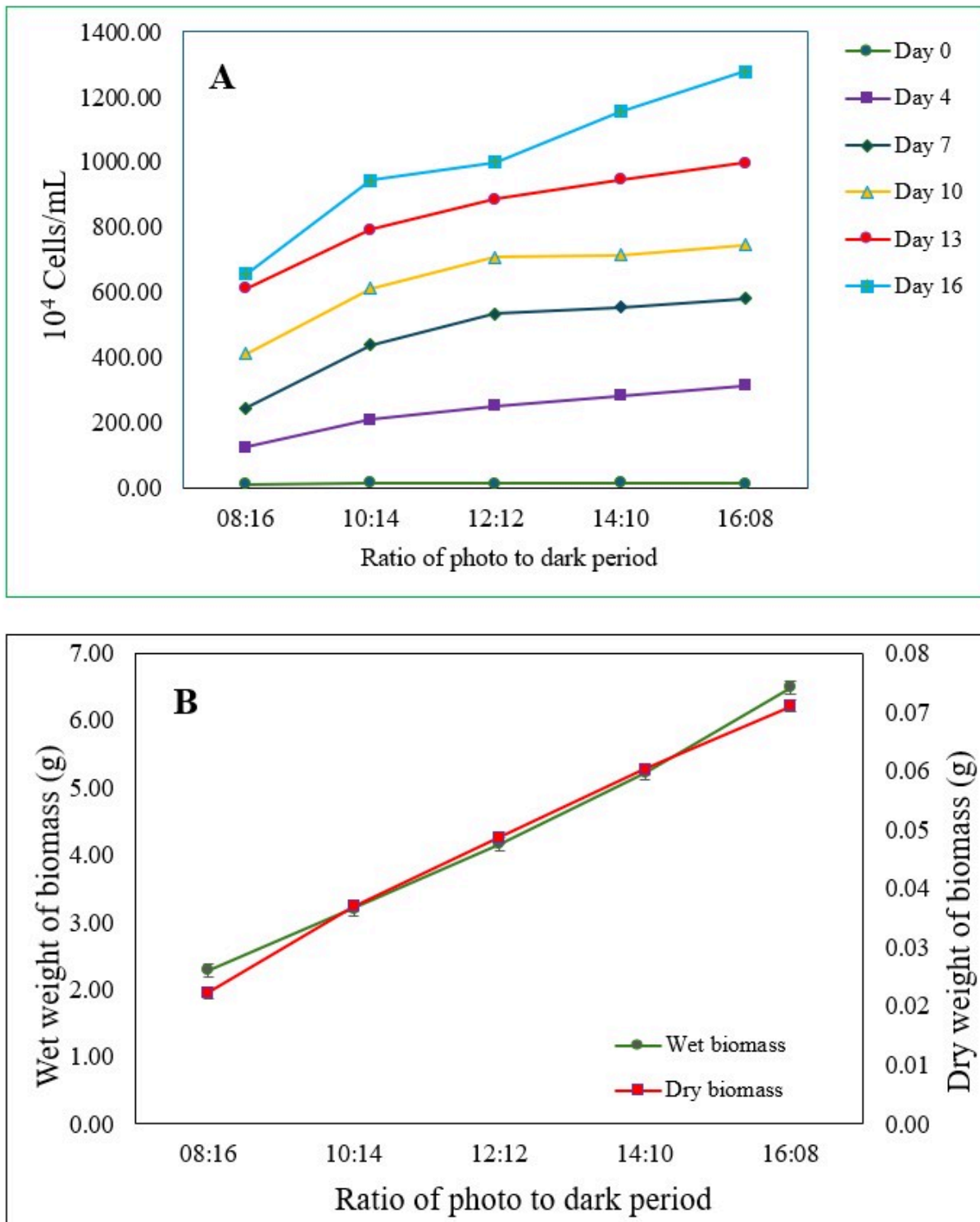


Figure 4

(A) Represents the growth profile of *P. kessleri* under varying light-dark cycles, demonstrating the impact of photoperiod on growth over time. (B) Depicts the wet (x-axis) and dry biomass (y-axis) yield obtained under different light-dark regimes, highlighting the effect of light exposure on biomass accumulation (n=3).

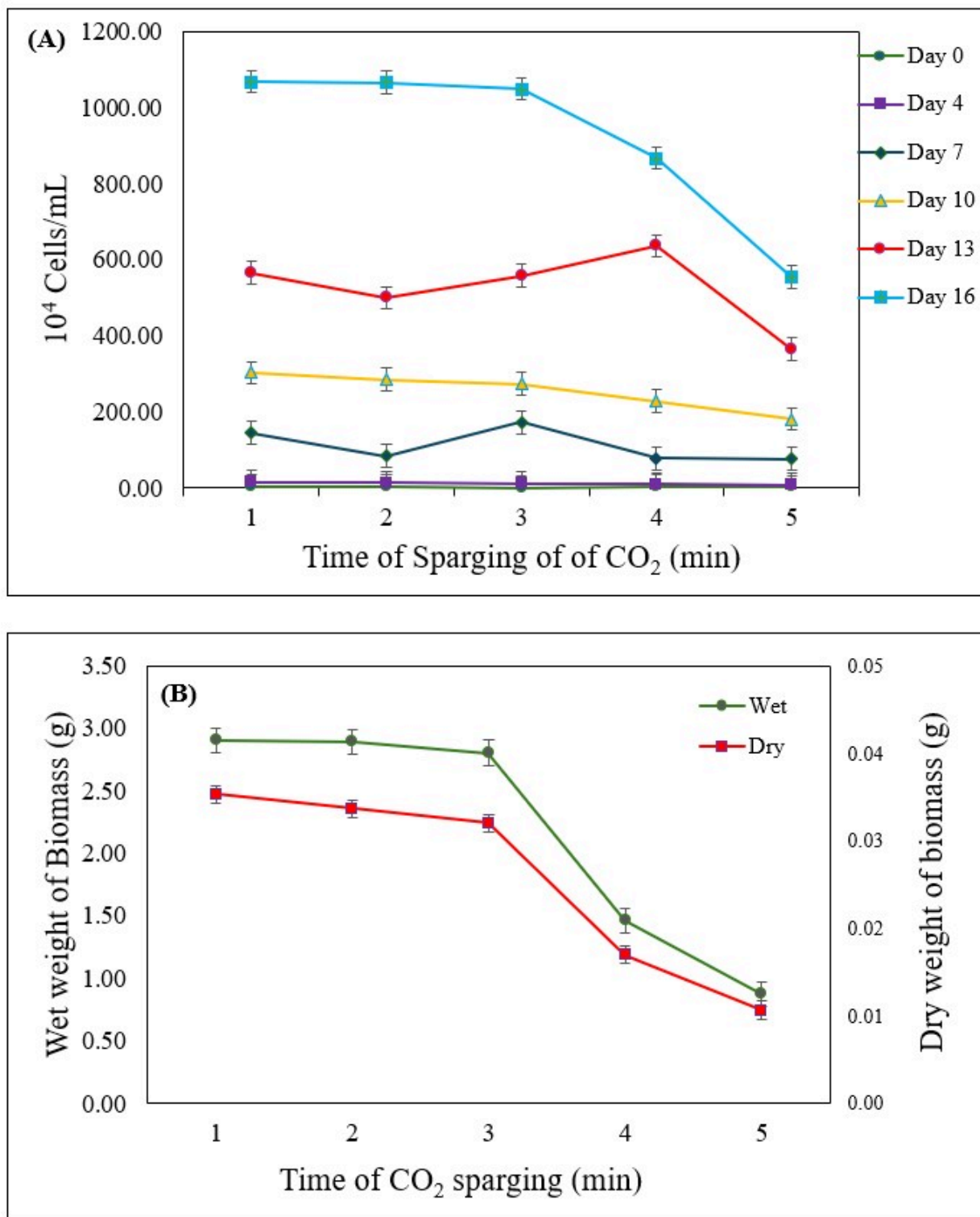


Figure 5

(A) Represents the growth curve of *P. kessleri* under different CO₂ concentrations, illustrating the impact of varying carbon dioxide levels on growth over time. (B) Shows the wet (x-axis) and dry biomass (y-axis) yield obtained at different CO₂ concentrations, highlighting the influence of CO₂ availability on biomass accumulation (n=3).

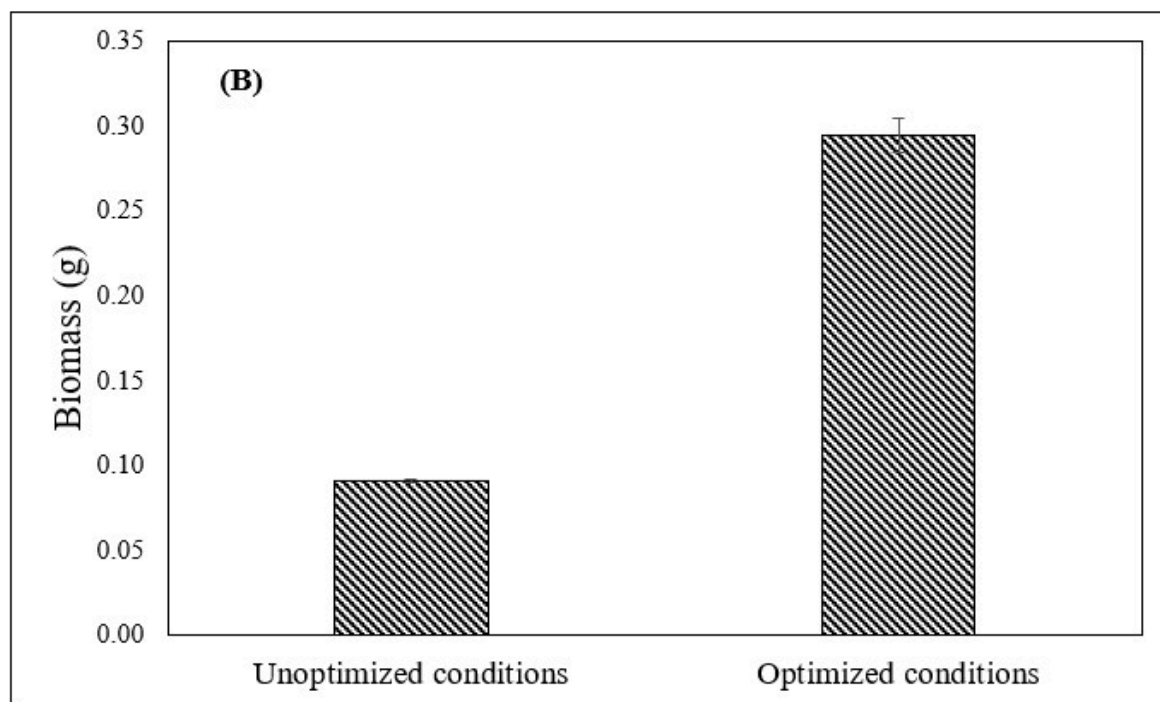
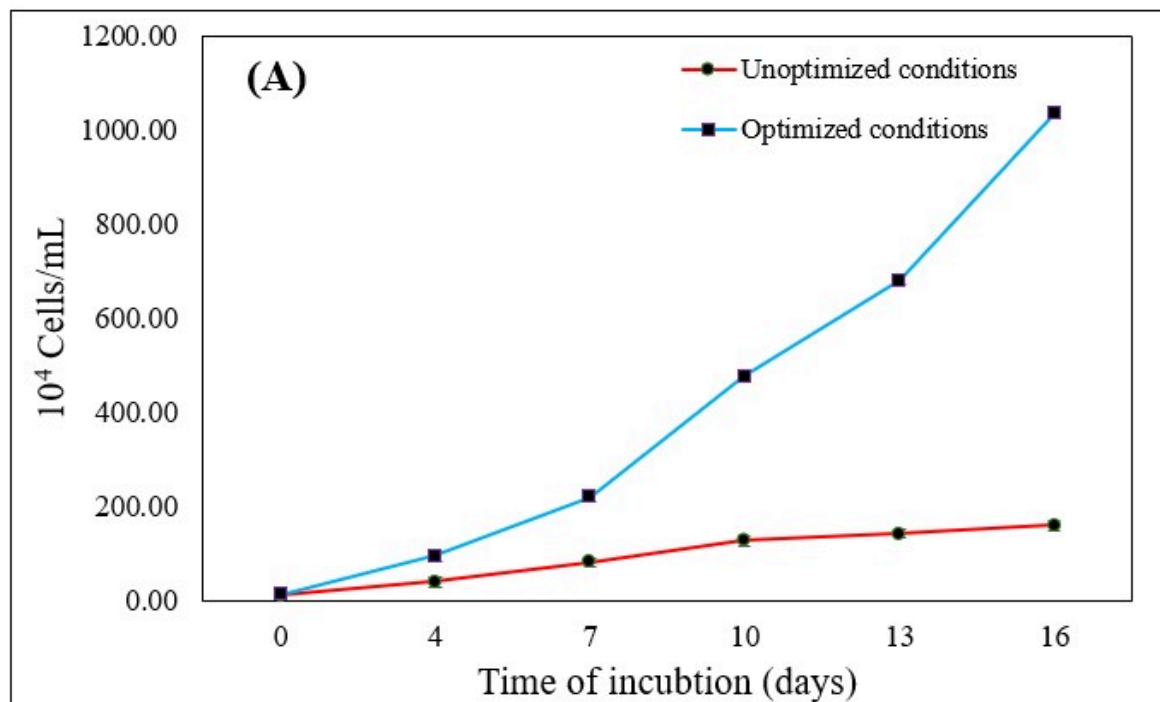


Figure 6

(A) shows the growth of *P. kessleri* under optimized and non-optimized conditions, while (B) illustrates the biomass growth after fifteen days under selected and non-selected parameters."

Supplementary Files

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- [floatimage1.png](#)