



Temperature modulates the response of *Tetradismus obliquus* to high-frequency pulsed light

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Abstract

High-frequency pulsed light is gaining attention as a promising strategy for microalgal cultivation, providing several advantages over continuous illumination, including energy saving, cost reduction, and enhanced photosynthetic efficiency. Another important factor influencing photosynthetic performance and growth rate is temperature, as it directly affects the functioning of the electron transport chain. Accordingly, this study aimed to explore the interaction between pulsed light and temperature in *Tetradismus obliquus*, focusing on how varying light and dark periods affect photosynthetic performance and biomass accumulation. Respirometric experiments performed at three different temperatures revealed a clear effect of temperature on the photosynthetic rate under saturating light conditions, indicating a direct influence on the electron transport chain. As a result, at higher temperatures, shorter dark periods were sufficient to achieve efficient photon capture. These findings were further validated in continuously-operated photobioreactors, confirming the trends observed by photorespirometry.

Keywords *Tetradismus obliquus* · Pulsed light · Temperature · LEDs · Photorespirometry

Introduction

Microalgae are emerging as a sustainable source of food, feed, and bio-based products, due to their valuable biochemical composition. However, to enhance their market competitiveness, increasing production capacity through more efficient systems, and reducing operating costs through the optimisation of key process variables remain essential (Garrido-Cardenas et al. 2018; Kusmayadi et al. 2021).

Light plays a pivotal role under both photoautotrophic and mixotrophic conditions (Schulze et al. 2017). Although more expensive, artificial lighting enables precise control of photosynthetic photon flux density (PPFD), photoperiod, and light spectrum, thus allowing for stricter regulation of

microalgal growth, and resulting in increased productivity and biomass quality (Schulze et al. 2014). Still, in order to significantly reduce the energy costs associated with artificial lighting, improvements in energy conversion efficiency and optimisation of light delivery mechanisms need to be addressed (Borella et al. 2022). Light-emitting diodes (LEDs) offer several advantages, including long life-expectancy (up to 50,000 h), high stability, relatively low manufacturing costs, and the ability to withstand frequent on–off switching (Chen et al. 2011). Notably, their emission can be finely tuned through semiconductor selection, allowing for precise wavelength targeting, and thus increasing the overall energy efficiency and reducing energy loss as heat (Carvalho et al. 2011). In this context, LEDs can be optimised to emit red and blue light, which are essential to support growth and metabolic activity in photosynthetic organisms and are just as effective for photosynthesis as the full spectrum (Keeling 2013; Park and Dinh 2019).

Beyond spectral optimisation, another major challenge in microalgal cultivation is concerned with the light delivery within the photobioreactor (Borella et al. 2022). In this regard, due to its ability to better adapt to the time scales of photosynthetic reactions and in order to save energy by minimising wasted irradiation, high frequency pulsed light (or flashing light) has emerged as a promising strategy

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compared to conventional continuous illumination (Lunka and Bayless 2013; Abu-Ghosh et al. 2016).

By taking advantage of the fast switching properties of LEDs, high-frequency pulsed light can be implemented. This implies the use of short pulses with very high peak light intensities, which can result in deeper light penetration within photobioreactors, thereby decreasing light attenuation (Abu-Ghosh et al. 2016; Martín-Girela et al. 2017). To avoid photoinhibition due to the high intensity of these pulses, it is essential to carefully tune the flashing light settings to match the kinetics of photosynthetic processes and the energy dissipation mechanisms (Schulze et al. 2017). Even though the enhancement of photosynthesis in fluctuating light has been known for a long time (Kok 1953), over the last years there has been a growing interest for such a topic, also thanks to the availability of specialized pulsed LED lamps. From a biological perspective, many interacting factors play a role, as a result of the evolved ability of the photosynthetic microorganisms to survive in dynamic environment, where acclimation also play a pivotal role (Subba Rao 2006). The physiological basis of the response to fluctuating light kinetics is related to the regeneration of intermediate electron acceptors in the plastoquinone (PQ) pool at the level of the electron transport chain (Stitt and Zhu 2014). More specifically, it seems that the prevalence of plastoquinol prevents the reopening of the reaction centre of photosystem II, thus making the system unable to process incoming photons efficiently, leading to poor energy conversion and potential photoinhibition (Vejrazka et al. 2011; Hüner et al. 2012).

Accordingly, pulsed light timing must be carefully designed to involve a very short period of light, with adequate flash intensity to excite reaction centre of photosystem II without triggering energy dissipation mechanisms, followed by a longer dark period, on the order of milliseconds, to allow the regeneration of the PQ pool (Schulze et al. 2017). When properly tuned, particularly in terms of frequency and duty cycle (Φ), pulsed light may lead the photosynthetic apparatus to operate near its maximum capacity, giving rise to the so-called “flashing light effect” (FLE), a phenomenon characterised by comparable or enhanced growth performance compared to continuous illumination at equivalent average light intensities (Abu-Ghosh et al. 2016).

Originally, pulsed light was successfully used to simulate the light–dark cycles caused by turbulent mixing, using low frequencies and high duty cycles (Sforza et al. 2012; Sionato et al. 2013; Vejrazka et al. 2013). Conflicting data were obtained, however, when higher frequencies were investigated. Schulze et al. (2020) reported no improvement in biomass growth compared to continuous light, despite testing a wide range of frequencies, duty cycles and intensities.

In contrast, other works have reported increased levels of proteins, PUFA, violaxanthin and β -carotene (Lima et al. 2021). Borella et al. (2022) first investigated the effect of

pulsed light on continuous cultures with experiments on *Arthrospira maxima*, demonstrating that the FLE can be achieved within a specific range of light periods (between 100–200 μ s), highlighting the advantages of continuous over batch cultivation (Barbera et al. 2019). Further experiments on *A. maxima* identified an optimal dark period of approximately 5 ms to prevent photosystem overload, and demonstrated that pulsed light enhanced biomass concentration and photosynthetic efficiency in reactors of increasing thickness, providing the basis for a model capable of predicting microalgal growth under high-frequency and high intensity pulsed light (Saccardo et al. 2024; Porcelli et al. 2025).

Besides light, temperature is another crucial variable regulating microalgal physiology, influencing photosynthetic activity, nutrient assimilation, CO₂ fixation, cellular composition, and growth rates (Daneshvar et al. 2021). Photosynthetic performance and growth generally increase with temperature up to a species-specific optimum, with a temperature coefficient Q_{10} of approximately 2, indicating that photosynthesis and growth rates approximately double for every 10 °C increase within the favourable range (Li 1980; Davison 1991). At temperatures above the optimum, photosynthesis and growth decrease due to heat-induced inactivation or denaturation of enzymes and other proteins involved in the photosynthetic machinery (Ras et al. 2013). Thermal tolerance, however, can vary widely among species, phyla and even strains, with optimal temperatures influenced by cultivation conditions such as indoor versus outdoor cultivation, open or closed systems, and light intensity (Daneshvar et al. 2021; Rossi et al. 2023).

In view of the above, it should be noticed that while the effects of pulsed light and temperature on microalgae growth have been investigated individually, their combined impact on photosynthesis and growth received little attention from the research community. Furthermore, how temperature interacts and modulates the photosynthetic response particularly at high intensity and high frequency pulsed light is still quite unexplored. Studying this interaction could therefore provide new insights for optimising microalgal productivity under high-frequency pulsed light regimes, thus reducing cost and enhancing the overall process sustainability.

In this work, *Tetrademus obliquus* was cultivated in photobioreactors operating in continuous mode under specially designed red and blue high frequency pulsed LEDs. Steady-state continuous systems intrinsically allow the establishment of acclimated cultures, giving a more reliable framework to study the effect of high frequency, high peak intensity pulsed. To evaluate the effects of temperature on light and dark periods, a photorespirometric protocol previously developed by Porcelli et al. (2025) was adopted and subsequently validated in a continuous system, to better ascertain the effect of acclimation. The aim was to identify the role of temperature on pulsed light duty cycle, showing

a possible different response to light and dark timings and offering perspectives for sustainable, energy-efficient microalgal production systems.

To our knowledge, this is the first study investigating how temperature influences the response of *T. obliquus* to high-frequency pulsed light, providing new insights into how temperature modulates the photosynthetic response under high-frequency pulsed light conditions, and eventually contributing to a more mechanistic understanding of microalgal productivity.

Materials and methods

Microorganism and cultivation conditions

Tetrademus obliquus (Turpin) M.J. Wynne (Wynne and Hallan 2015) (syn. *Acutodesmus obliquus*; strain SAG 276–7, Culture Collection of Algae at Göttingen University, Germany) was maintained and propagated at $24 \pm 1^\circ\text{C}$ in 250-mL Drechsel bottles containing sterilised BG11 medium (Rippka et al. 1979), sparged with a CO_2 -air mixture (5% v/v).

Intermittent illumination was provided by a LED lamp with an optimised red and blue (R/B) spectrum (Tormena et al. 2026). To prevent nutrient limitation, the growth medium was modified to contain twice the standard concentrations of all nutrients, except for K_2HPO_4 , which was supplied at four times the standard concentration. The medium was additionally buffered with 10 mM HEPES at pH 8 to maintain the pH of the culture between 7 and 8.

For continuous experiments, the microorganism was cultivated photoautotrophically in vertical flat-plate polycarbonate photobioreactors (PBRs) with a working volume of 200 mL, an optical light path length of 3.3 cm and an illuminated area of 60 cm^2 . Fresh medium was constantly introduced into the culture at the same flow rate, Q (mL day^{-1}), corresponding to the removal of excess biomass suspension, using a multichannel peristaltic pump (205S/CA, Watson Marlow Fluid Technology Group), thus keeping the volume of the reactor (V_{PBR}) constant.

The residence time (τ), defined as the ratio between the reactor volume and the inlet flow rate, was set to 1.3 days. Culture mixing was achieved through a magnetic stirrer at the bottom of the reactor and bubbling of a 1 L h^{-1} CO_2 air (5% v/v) mixture. No evident cell adhesion was observed. To ensure pH stability, the culture pH was measured daily with a calibrated pH probe (HI991001, Hanna Instruments), thereby verifying its maintenance within the defined range. Intermittent front illumination was provided by a high frequency pulsed R/B LED lamp while the stainless-steel lateral walls of the photobioreactor minimised lateral light diffusion. The use of continuous

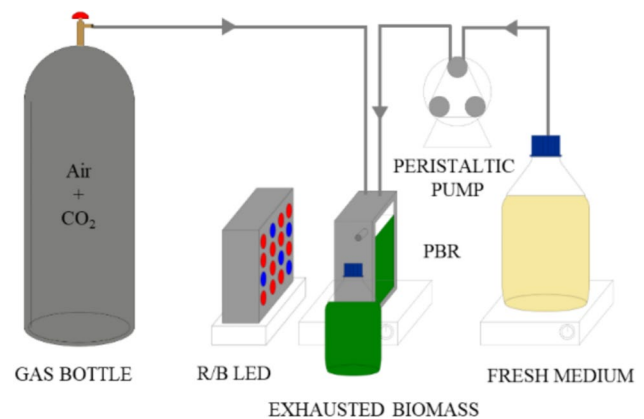


Fig. 1 Schematic representation of the experimental setup used to perform the experiments in continuous mode

cultivation offers advantages over batch mode, providing stable conditions over time and allowing microalgae to acclimate to high-frequency pulsed light (Borella et al. 2022). The experimental set up used for the experiments in continuous mode is depicted in Fig. 1.

Biomass concentration was monitored daily to assess the achievement of steady-state (SS) conditions. Specifically, optical density (OD) at 750 nm and dry weight (DW) measures were performed. The DW of the biomass was determined gravimetrically using pre-dried (110°C for at least 10 min) and pre-weighed cellulose nitrate membrane filters (ReliaDisc membrane filter, pore size $0.45\text{ }\mu\text{m}$, Ahlstrom-Munksjö, Germany). After filtering a specific volume of algal culture, the filter was dried in the oven at 110°C for 4 h and subsequently weighed. The weight difference was then measured and the DW for each sample calculated based on the volume passed through the membrane filter. At steady state, a minimum of three independent experimental replicates were collected.

To investigate the effect of temperature on light (t_l) and dark periods (t_d), two sets of photorespirometric experiments were carried out under pulsed light. Specifically, three different temperatures were tested: 20°C , 30°C , and 36°C , representing the optimal condition and two extremes, all within the range of conditions tolerated by the species (Martínez et al. 1999; Guedes et al. 2011; Venancio et al. 2020). In each experimental set, the intensity of the light pulse (I_{pulse}) was kept constant. Depending on the variable under investigation, either the light or dark period was fixed while the other was systematically varied. The average light intensity (I_{av} , $\mu\text{mol photons m}^{-2}\text{ s}^{-1}$), defined as the integral light intensity over the flashing cycle (t_c), varied accordingly.

Time t_c was described as the sum of t_l and t_d , while the duty cycle (φ) was defined as the ratio between t_l over t_c . The experimental conditions employed are summarised in Tables 1 and 2. The range of light intensities and the time

Table 1 Flashing light conditions used to investigate the effect of dark period on *T. obliquus*

I_{pulse} [$\mu\text{mol photons m}^{-2} \text{ s}^{-1}$]	I_{av} [$\mu\text{mol photons m}^{-2} \text{ s}^{-1}$]	Duty cycle (ϕ)	Light period t_l [μs]	Dark period t_d [ms]
8000	35	0.0043	35	8
	55	0.0069		5
	92	0.0115		3
	335	0.0419		0.8

scale of the pulses were chosen based on the previous investigation (Porcelli et al. 2025).

To validate the results of the photorespirometric tests, additional experiments were conducted in continuous mode at the same three temperatures employed previously, specifically modifying the duration of the dark period (Table 3).

Lighting apparatus

The LED lighting apparatus was a custom-made prototype based on a high-power light source and a pulsed current driver system. The source had an emitting area of 70×100 mm and consisted of 72 LEDs with two different wavelengths: 450 nm (blue) and 660 nm (red). The LEDs were subdivided into two independent channels: the first was composed of 24 blue LEDs, and the second of 48 red LEDs. This configuration allowed different currents to be applied to the blue and red LEDs. The pulsed current driver was custom-made and consisted of two separate outputs, one for each channel.

Each output was equipped with a current control circuit that could be configured both in terms of current amplitude and pulse timing. The current amplitude was set through a voltage reference, while a pulsed signal enabled and disabled the output of each channel to generate the final pulsed current.

This configuration allowed independent adjustment of both current level and pulse duration for each channel. Both the voltage references and the pulsed signals were provided by a microcontroller. The output current was set between 10 mA and 1 A with 0.24 mA steps, with a minimum switch-on time of 10 μs .

Pulsed light photorespirometric protocol

Photorespirometric tests were carried out following a protocol originally described by Sforza et al. (2019) and subsequently adapted for pulsed light (Porcelli et al. 2025).

Dissolved oxygen concentration was monitored using an oximeter (HI6421, Hanna Instruments) at 10-s intervals on

Table 2 Flashing light conditions used to assess the effect of light period on *T. obliquus*

I_{pulse} [$\mu\text{mol photons m}^{-2} \text{ s}^{-1}$]	I_{av} [$\mu\text{mol photons m}^{-2} \text{ s}^{-1}$]	Duty cycle (ϕ)	Light period t_l [μs]	Dark period t_d [ms]
8000	40	0.0049	25	5
	55	0.0060	35	
	79	0.0099	50	
	110	0.0138	70	
	126	0.0157	80	
	157	0.0196	100	
	307	0.0384	200	
	352	0.0439	230	

properly diluted biomass samples collected at steady state and acclimated to the tested pulsed light intensity. During the photorespirometry tests, the culture was exposed to light–dark cycles of about 10:10 min each. According to the experimental design, either the light or the dark period was systematically varied while keeping the other constant, with at least three replicates performed for each experimental condition. Oxygen production rate (OPR, $\text{mg L}^{-1} \text{ day}^{-1}$) and oxygen consumption rate (OCR, $\text{mg L}^{-1} \text{ day}^{-1}$) were calculated considering the contribution of oxygen mass transfer and expressed as specific rates by dividing by the initial biomass concentration (measured as DW). For the investigation of dark periods, the gross OPR was assessed by adding the absolute value of OCR, representing the basal respiration occurring in the light, to the net OPR.

Finally, for each experimental point, both gross and net OPR values were first corrected for variations in light intensity by dividing by the average light intensity (I_{avint} , i.e., the integral light intensity over the flashing cycle along the light path of the chamber). To minimise variability between replicate tests, the corrected gross and net OPR values were then normalised to the maximum OPR observed within each dataset, ensuring consistent comparison across experimental points.

Experimental data collected during the investigation of light periods were fitted using Haldane kinetic model, while Monod kinetics was applied to data obtained from the variation of dark periods (Muloiwa et al. 2020).

Mass and energy balances

The photobioreactor used in this study was approximated as a continuous stirred-tank reactor (CSTR), as complete mixing was achieved through magnetic stirring at the bottom and sparging with an air- CO_2 mixture (5% v/v). Accordingly, volumetric biomass productivity ($P_{x,v}$, $\text{g L}^{-1} \text{ day}^{-1}$), based on a mass balance, is defined as:

Table 3 Flashing light conditions employed to validate the photorespirometric profiles

I_{pulse} [$\mu\text{mol photons m}^{-2} \text{s}^{-1}$]	I_{av} [$\mu\text{mol photons m}^{-2} \text{s}^{-1}$]	Duty cycle (φ)	Light period t_l [μs]	Dark period t_d [ms]
6300	300	0.0476	100	2
15500		0.0196		5

$$P_{x,v} = \frac{C_{x,\text{out}}}{\tau} \quad (1)$$

where $C_{x,\text{out}}$ is the biomass concentration in the outlet stream.

Photosynthetic efficiency (η_{par}) was calculated from energy balances considering the Photosynthetically Active Radiation (PAR, 400–700 nm) as:

$$\eta_{\text{par}} = \frac{C_{x,\text{out}} \cdot Q \cdot LHV}{PPFD \cdot E_p \cdot A_{PBR}} \quad (2)$$

In Eq. 2, Q is the inlet flow rate (L s^{-1}), LHV is the lower heating value of microalgal biomass (kJ g^{-1}), assumed equal to 22 kJ g^{-1} based on literature data (Barbera et al. 2015), $PPFD$ ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) represents the incident photon flux density, namely the average light intensity (I_{av}) during one flashing cycle (back irradiance was not considered for pulsed light), A_{PBR} is the illuminated surface area of the photobioreactor (m^2), and E_p is the average energy per mole of photons ($\text{kJ } \mu\text{mol}^{-1}$). E_p was determined by integrating the emission spectrum of the red and blue pulsed LEDs and subsequently calculating the average energy of photons using Planck's law.

Statistical analysis

Statistical analyses were performed on data acquired at steady state. Depending on the dataset, one-way or two-way ANOVA was applied to evaluate main and interaction effects. Homogeneity of variances was assessed using Levene's test. When significant differences were detected ($p < 0.05$), pairwise comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test. All analyses were performed using OriginPro 2025b (OriginLab, USA). Data are reported as mean $\pm$ standard deviation (SD).

Results

Combined effect of pulsed light and temperature on light periods via respirometry

The experimental design of the photorespirometric tests was based on a protocol previously adapted for pulsed light by

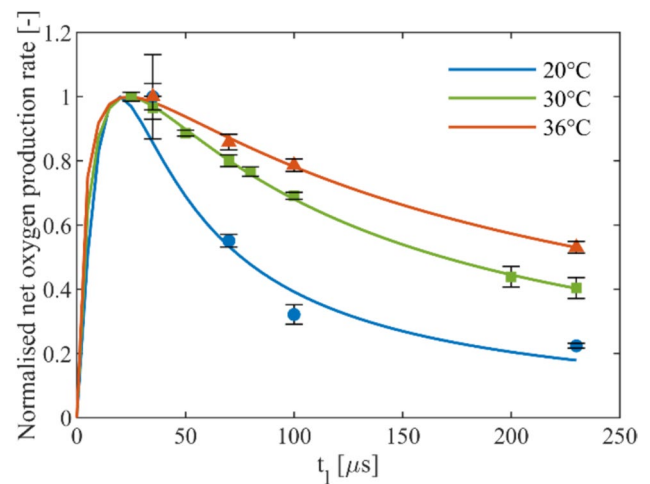


Fig. 2 Normalised net oxygen production rate as a function of light period, ranging from 25 μs to 230 μs , under a fixed dark period of 5 ms and a fixed intensity of the light pulse of $8000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Data represent the mean of 4 replicates per condition; error bars indicate standard deviation

Porcelli et al. (2025), proven to be effective for the rapid and reliable acquisition of a large amount of data, as well as crucial in identifying the optimal light and dark time values for *Arthrospira maxima*.

A preliminary series of photorespirometric tests was carried out to investigate the effect of three different temperatures, namely 20 °C, 30 °C and 36 °C, on light periods. A dark period of 5 ms and an intensity of the light pulse equal to $8000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ were selected and kept constant, based on the work by Porcelli et al. (2025). The light periods, instead, were systematically varied with values and timescale defined according to previous studies (Borella et al. 2022; Porcelli et al. 2025). The pulsed light configuration was optimised to align with the reaction kinetics of the linear electron transport chain, with a dark period sufficiently long to allow the oxidation of plastoquinol and thus prevent the over-reduction of the plastoquinone pool, and microsecond-scale light periods to efficiently reduce photosystem II without triggering energy dissipation pathways (Schulze et al. 2017; Zarmi et al. 2020).

Figure 2 illustrates the results of the photorespirometric tests performed at three different temperatures. The normalised net OPR values, fitted using Haldane kinetics, are graphed as a function of the light period. For all the temperatures tested, the results indicate the presence of an optimal light period (approximately 20–40 μs), beyond which the normalised net OPR declines. However, the rate and extent of this decline differ with temperature, remaining less pronounced at higher temperatures.

Indeed, at 36 °C, the photosynthetic performance is markedly enhanced compared to 20 °C, where, particularly

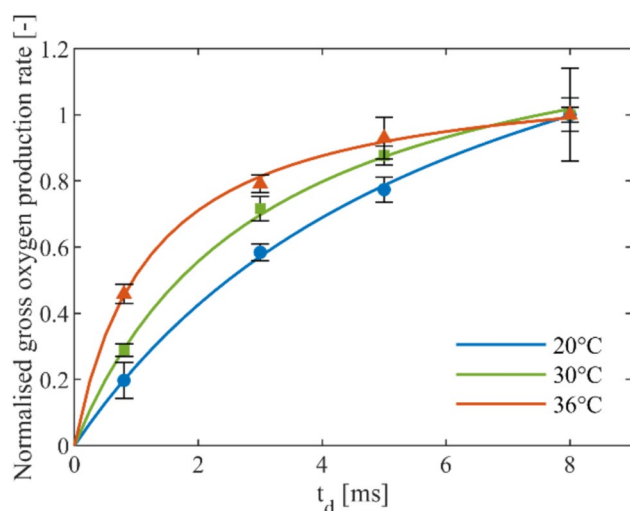


Fig. 3 Normalised gross oxygen production rate as a function of dark period, ranging from 0.8 ms to 8 ms, under a fixed light period of 35 μ s and a fixed intensity of light pulse of 8000 μ mol photons $m^{-2} s^{-1}$. Data represent the mean of 4 replicates per condition; error bars indicate standard deviation

under prolonged light pulse durations (e.g., 230 μ s), a clear decrease in the normalised net OPR is observed, ultimately leading to an almost complete cessation of the photosynthetic activity.

Combined effect of pulsed light and temperature on dark periods via respirometry

To verify the effect of temperature on the role of dark period, a second set of photorespirometric experiments was performed, employing the same three temperatures previously adopted. In this setup, the light pulse intensity was kept constant at 8000 μ mol photons $m^{-2} s^{-1}$, whereas, based on the results of the first series of experiments, a light pulse duration of 35 μ s was selected. The dark periods were systematically varied, thereby altering the average light intensity. The results, representing the normalised gross OPR as a function of dark time, were fitted using Monod kinetics and are shown in Fig. 3. The observed trends show a general increase in the normalised gross OPR when increasing the dark period, with the extent of this increase varying significantly with temperature. Notably, the increase is more pronounced at 36 °C compared to 30 °C and 20 °C. At longer dark times, however, the curves show a tendency toward a flattening, although a clear plateau is not fully reached.

The comparison between the curves at different temperatures clearly indicates that higher temperatures require shorter dark periods to reach the same normalised gross OPR level, suggesting that the photosynthetic performance improves more rapidly as temperature increases.

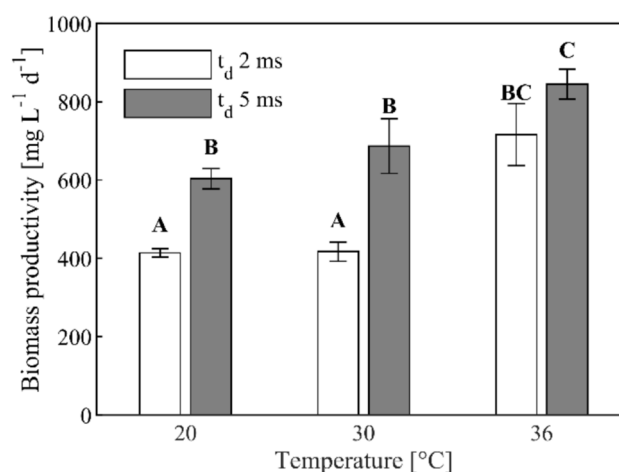


Fig. 4 Steady-state biomass productivity [$mg L^{-1} day^{-1}$] measured at different temperatures [°C]. For each temperature, two different dark periods were tested: 2 ms and 5 ms. Data represent the mean of 3 replicates per condition; error bars indicate standard deviation. Different letters above the bars indicate statistically significant differences among all temperature $\times$ dark period groups (two-way ANOVA followed by Tukey's HSD, $p < 0.05$)

For instance, at 36 °C, the OPR value reached with a dark period of 3 ms is higher than that observed at 20 °C with a dark period of 5 ms. Additionally, the graph reveals that the decrease in OPR associated with shorter dark periods (0.8–5 ms) is less pronounced at 36 °C and becomes progressively more evident at lower temperatures, first at 30 °C and then at 20 °C.

Continuous experiments

An additional series of experiments was performed under pulsed light conditions in photobioreactors operated in continuous mode, with the aim of validating the photorespirometric observations regarding the dark period duration. The objective was to assess whether the temperature-dependent pattern observed in photorespirometric tests, with higher temperatures requiring shorter dark periods to reach the same normalised gross OPR level, was reflected in long-term biomass accumulation. In addition, the analysis addressed whether the decrease in OPR associated with shorter dark periods was less pronounced at 36 °C and more evident at lower temperatures, particularly at 30 °C and 20 °C.

Since the temperature-related differences were particularly evident in a dark time range between 0.8 and 5 ms, continuous mode experiments were conducted using 2 ms and 5 ms as representative dark periods. Here, the average light intensity and the light period were kept constant and equal to 300 μ mol photons $m^{-2} s^{-1}$ and 100 μ s, respectively. The results, described as volumetric biomass productivity

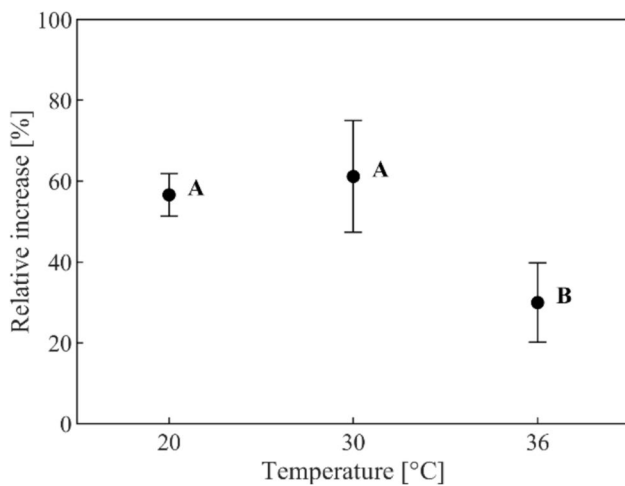


Fig. 5 Increase [%] in biomass concentration between 2 and 5 ms at different temperatures [°C]. Data represent the mean of 3 replicates per condition; error bars indicate standard deviation. Means that do not share a letter are significantly different (one-way ANOVA followed by Tukey's HSD, $p < 0.05$)

measured at different temperatures, are reported in Fig. 4. Consistent results based on photosynthetic efficiency are reported in the Supplementary Information.

To evaluate the effects of temperature and dark period duration on biomass productivity, a two-way ANOVA was performed. The analysis revealed a significant effect of temperature ($F(2,12) = 54.45$, $p < 0.0001$) and dark period duration ($F(1,12) = 73.48$, $p < 0.0001$), while their interaction was not statistically significant ($F(2,12) = 3.17$, $p = 0.078$). These statistical findings are reflected in the observed trends in biomass productivity. An overall increase is evident with increasing temperature under both dark periods. Across all temperatures tested, the longer dark period (5 ms) generally results in a higher biomass productivity compared to the shorter dark time (2 ms), except at 36 °C, where no significant difference between treatments is detected. At this temperature, the maximum productivity of $844.907 \pm 38.527 \text{ mg L}^{-1} \text{ day}^{-1}$ is achieved under the 5 ms condition. This outcome is consistent with the photorespirometric findings, which also indicated enhanced photosynthetic performance under longer dark periods at higher temperatures.

Subsequently, the relative increase in biomass concentration between the two dark periods (5 ms vs. 2 ms) was calculated for each temperature (Fig. 5). A one-way ANOVA revealed a significant effect of temperature on the relative increase in biomass ($F(2,6) = 14.42$, $p = 0.005$). The observed pattern reflects these statistical results, with the highest increases at 20 °C and 30 °C, and a significantly lower increase at 36 °C.

Discussion

When analysing the effect of temperature on photosynthesis, it is generally observed that the photosynthetic rate increases progressively with rising temperature up to an optimum, beyond which it declines markedly (Davison 1991). However, this response is not uniform and strongly depends on the intensity of light supplied, with distinct behaviours under sub-saturating and saturating light conditions.

In the first series of photorespirometric experiments, where the dark period and the intensity of the light pulse were kept constant, increasing the light period led to a higher average light intensity and, consequently, to an increased total photon flux supplied to the system. Within this framework, the observed trends align with previous findings indicating that, under light-saturated conditions, elevated temperatures can significantly stimulate photosynthesis by accelerating temperature-dependent processes downstream of the primary photochemistry, such as electron transport, plastoquinone turnover, and carbon metabolism. Conversely, under light-limited (sub-saturating) conditions, the photosynthetic system is already constrained by the reduced photon availability, and further increases in temperature provide little or no additional benefit (Davison 1991; Hancke et al. 2008). In summary, although temperature is known to influence the overall photosynthetic rate, its effect is reduced under short light pulse conditions with sufficiently long dark periods (i.e., low average light intensity and low duty cycle). This suggests that temperature mainly affects the electron transport chain, which involves multiple enzymatic and protein-mediated steps, rather than the initial light-driven reactions.

With regard to the apparent optimal light period determined in this study, it appears to be associated with very short light pulses. However, due to technical limitations, it should be highlighted that further shortening could not be tested, as this would have resulted in too low an average light intensity, i.e., photolimiting conditions.

Beyond these technical constraints, the optimal light period, which ranges between 20–40 μs , contrasts with the 100–200 μs reported by Borella et al. (2022) on *A. maxima* under comparable pulsed light parameters. This discrepancy may be attributed to species-specific physiological differences, supported by the species-dependent capacity of the PQ pool to store plastoquinol, which may influence the threshold values for parameters such as frequency, duty cycle, and the average light intensity required to trigger the flashing light effect (Schulze et al. 2017).

Consistent with the aforementioned discussion on light periods, similar considerations regarding the influence of temperature can be extended to dark periods. In this

context, increasing the duration of the dark period leads to a decrease in the average light intensity, with shorter dark periods corresponding to higher average light intensities. As shown in Fig. 3, the temperature-dependent differences are most evident in the range of 0.8 to 5 ms, where higher temperatures result in a more rapid increase in normalised OPR. Under these light-saturated conditions, temperature has a pronounced effect on photosynthetic performance by accelerating enzymatic processes. In contrast, at longer dark periods, where the system becomes light-limited, the limited photon supply constrains any further benefit from increased temperature (Davison 1991; Hancke et al. 2008).

Further considerations should be made regarding the progressive flattening of the curves observed in the plot (Fig. 3), which tend towards a plateau around 8 ms, although OPR continues to increase at 20 °C and 30 °C, suggesting that the true plateau may lie beyond the measured values. This trend suggests that this dark period is long enough to enable the reoxidation of the PQ pool. As a result, all reaction centres can return to an open state, thereby optimising photon capture during the subsequent light flash. It should be noted, however, that the highest OPR measured in our experiments occurs at 8 ms under a relatively low average light intensity ($\sim 35 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), which is photolimiting. Consequently, shorter dark periods, while allowing only partial PQ reoxidation, provide a higher photon flux and are therefore preferable for maximising total photosynthetic activity.

The photorespirometric findings were subsequently confirmed in continuous-mode experiments, where the temperature-dependent response to dark interval duration was reflected in biomass accumulation.

Overall, these results demonstrate the importance of refining temperature and pulsed light parameters to maximise biomass productivity and photosynthetic efficiency, while reducing energy costs associated with artificial lighting. Furthermore, this approach provides for the first time insights into how temperature modulates the photosynthetic response of *T. obliquus* under high-frequency, high intensity pulsed light, highlighting the mechanistic understanding that may guide future studies on microalgal productivity. This approach suggests strong potential for implementation in large-scale photobioreactors, where the integration of high-frequency pulsed light with temperature control could significantly improve the overall performance, energy efficiency and sustainability of the system. However, as the effect of pulsed light is strongly geometry-dependent (Saccardo et al. 2024; Porcelli et al. 2025) a validation at larger scales should be carried out.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process During the preparation of this work, the authors used ChatGPT (OpenAI) to assist in grammar correction and to improve the clarity of expression. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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