SUPPLEMENT

REVISTA DE Biología Tropical

https://doi.org/10.15517/rev.biol.trop..v73iS1.63637

Interplay between light and temperature on growth of *Chlorella sorokiniana* (Chlorellaceae) cultures under laboratory conditions

Ana Margarita Silva Benavides[1,2]; https://orcid.org/0000-0001-9006-9391
Giuseppe Torzillo[2,3]; https://orcid.org/0000-0003-3621-8303

1. Escuela de Biología, Universidad de Costa Rica, San Pedro de Montes de Oca, San José, Costa Rica; ana.silva@ucr.ac.cr (*Correspondence)
2. Centro de Investigación en Ciencias del Mar y Limnología, Universidad de Costa Rica, San Pedro de Montes de Oca, San Jose, Costa Rica; giuseppe.torzillo@cnr.it
3. Consiglio Nazionale delle Ricerche-Istituto per la Bioeconomia, Via Madonna del Piano 10, Sesto Fiorentino, I-50019 Firenze, Italy.

Received 06-V-2024. Corrected 11-XI-2024. Accepted 24-I-2025.

ABSTRACT

Introduction: The relationship between light and temperature on the growth of *Chlorella sorokiniana* (Chlorophyceae) cultures was investigated under laboratory conditions.

Objective: The aim of this study was to evaluate the influence of different temperature and light intensities on growth, productivity, chlorophyll of *Chlorella sorokiniana* UTEX 1230 in laboratory conditions.

Methods: The cultures were exposed to a combination of two light irradiances (100 and 200 µmol photons m^{-2} s^{-1}) and 5 different temperatures (20 °C, 25 °C, 30 °C, 40 °C, 45 °C).

Results: At 100 µmol photons m^{-2} $s^{-1,}$ the culture growth did not differ significantly within 20 °C and 35 °C range. At this irradiance, the maximum attained biomass dry weight was 3.9 g/l after 9 days of cultivation under continuous light. Cultures grown under 200 µmol photons m^{-2} $s^{-1,}$showed much larger differences in their growth. Their final dry weight changed according to the following temperatures, 30 °C (6.19 g/l), 25 °C (5.24 g/l), 35 °C (4.33 g/l), 40 °C (2.50 g/l) 45 °C. Therefore, the optimal temperature for productivity of cultures of *Chlorella sorokiniana*, strongly changed according to the light intensities at which cultures were exposed. At 100 µmol photons m^{-2} $s^{-1,}$ a large plateau for optimal growth was observed between 20 °C and 35 °C, while at 200 µmol photons m^{-2} s^{-1} a clear optimal temperature for productivity was observed at 30 °C.

Conclusions: It was interesting to note that culture grown at 20 °C, performed well under 100 µmol photons m^{-2} s^{-1}, while when exposed to 200 µmol photons m^{-2} s^{-1} they were unable to grow. No growth was achieved at 45 °C. Therefore, 40 °C represented the upper limit to appreciate the growth in *Chlorella sorokiniana* strain UTEX 1230, while the temperature lower limit changed with light irradiance.

Key words: *Chlorella sorokiniana*; growth productivity; light intensity; temperature.

RESUMEN

Interacción entre la luz y la temperatura en el crecimiento de cultivos de *Chlorella sorokiniana* (Chlorellaceae) en condiciones de laboratorio

Introducción: Se investigó la relación entre la luz y la temperatura sobre la productividad de cultivos de la microalga *Chlorella sorokiniana* (Chlorophyceae).

Objetivo: evaluar la influencia de diferentes temperaturas e intensidades de luz sobre el crecimiento, la productividad y la clorofila *a+b* de *Chlorella sorokiniana* UTEX 1230 en condiciones de laboratorio.
Métodos: Los cultivos se expusieron a una combinación de dos irradiaciones de luz (100 y 200 µmol fotones m^{-2} s^{-1}) y cinco temperaturas (20 °C, 25 °C, 30 °C, 40 °C, 45 °C).
Resultados: A 100 µmol fotones m^{-2} s^{-1}, el crecimiento del cultivo no presentó diferencias significativas entre las temperaturas de 20 °C y 35 °C. Con esta irradiación, el peso seco máximo de la biomasa alcanzó 3.9 g/l después de 9 días de cultivo con luz continua. Los cultivos expuestos a una irradiación de 200 µmol fotones m^{-2} s^{-1} mostraron mayores diferencias en su crecimiento. El peso seco final cambió según las siguientes temperaturas, 30 °C (6.19 g/l), 25 °C (5.24 g/l), 35 °C (4.33 g/l), 40 °C (2.50 g/l), 45°C (0.00 g/l). Por consiguiente, la temperatura óptima para la productividad de los cultivos de *Chlorella sorokiniana* cambió de acuerdo con las intensidades de luz que fueron expuestos los cultivos. A 100 µmol fotones m^{-2} s^{-1} se presentó un crecimiento óptimo entre 20 °C y 35 °C, mientras que a 200 µmol fotones m-2 s-1 se observó una temperatura óptima para la productividad a 30 °C.
Conclusiones: Los resultados demuestran que los cultivos a 20 °C y 100 µmol fotones m^{-2} s^{-1} mostraron un óptimo crecimiento; sin embargo, este fue menor cuando se expuso a 200 µmol fotones m^{-2} s^{-1}. No se observó crecimiento de los cultivos a 45 °C. Por consiguiente, la temperatura a 40°C representó el límite superior para obtener una buena productividad en *Chlorella sorokiniana* cepa UTEX 1230, mientras que el límite inferior de temperatura cambió con la irradiación de la luz.

Palabras claves: *Chlorella sorokiniana*; crecimiento; productividad; intensidad lumínica; temperatura.

INTRODUCTION

Microalgae are important as primary producers in both fresh and marine waters. They use light energy and carbon dioxide to produce biomass. Among microalgae *Chlorella sorokiniana* is a unicellular green microalga not flagellated, spherical, 2–10 µm in diameter, belonging to the phylum Chlorophyta (Schubert, 2003). *C. sorokiniana* represents a sub-species first isolated in 1953 by Sorokin, and originally considered as a thermotolerant mutant of *Chlorella pyrenoidosa* (Kunz, 1972; Sorokin & Meyer, 1953). This taxonomic classification was thereafter revised during the late 1980s and early 1990s by chloroplast 16S rDNA and 18S rRNA profilin which identified *C. sorokiniana* as a separate species (Dorr & Huss, 1990; Kessler & Huss, 1992). This microalga has been proposed as source lipids for biodiesel production, food supplements and metabolites with pharmacological activities, extraction of high-value compounds such as fatty acids, pigments (carotenoids, chlorophyll) (Becker, 2013; Borowitzka, 2013; Liu & Hu, 2013). It has also been suggested as a source of proteins in aquafeed (Chun-Yen et al., 2023) and as a dietary supplement meal, can have a positive influence on the growth, antioxidant status, and immune response of rainbow trout main food of rotifers as live food for larvae of marine fish (Chen et al., 2021; Lee et al., 2001) In recent years, *C. sorokiniana* has been extensively investigated for the wastewater treatment coupled to the production of biodiesel (Eladel et al., 2019).

Light and temperature are two important fundamental environmental factors for the autotrophic growth of microalgae since they can affect both growth productivity and cellular biochemical composition (de la Peña, 2007; Huesemann et al., 2018; Ugwu et al., 2007; Yang et al., 2024. It is well known that the growth rate of microalgae increases with the increase of temperature and light intensity up to an optimum value, and thereafter growth decreases. Usually, the decrease in growth is much sharper when the temperature surpasses the optimal value than when it decreases from the optimum (Converti et al., 2009; Ras et al., 2013).

In the literature there is enough information on the effect of a single environmental factor in particular temperature for *C. sorokiniana*, however the information on the interaction between light and temperature is scanter (Cuaresma et al., 2012; Moronta et al., 2006; Ugwu et al., 2007). These two environmental factors are strategic for the outdoor cultivation

of microalgae, particularly light which can fluctuate of one order of magnitude during the day. The combination of low temperature and high light conditions in the morning hours outdoors represents a frequent situation occurring particularly in desert areas where light intensity rises at a rate much higher than temperature making photosynthetic apparatus prone to photoinhibition with consequent reduction of daily productivity (Torzillo et al., 1998; Vonshak et al., 2001). *C. sorokiniana*, thanks to its biochemical plasticity, can play an important role in biorefinery (Sharma et al., 2022).

It is well known that the average productivity of the most common industrial strains including *C. sorokiniana* is far lower than maximal theoretical estimations, suggesting that the identification of factors limiting biomass yield is crucial to make algal-derived bioproducts economically viable on the industrial scale (Benedetti et al, 2018; Masojidek et al., 2013). To acquire further information on the growth of *C. sorokiniana* we exposed cells to a combination of different temperatures ranging from 20 °C to 45 °C, both at 100 and 200 μmol $m^{-2}s^{-1}$. Culture performance was followed with both measurement of growth, and by chlorophyll fluorescence changes to monitor the physiological status of the cultures under different conditions.

MATERIALS AND METHODS

Organism and culture conditions: *C. sorokiniana* (Utex 1230) was obtained from the Utex Culture Collections. The inoculum was grown in BG-11 medium (Rippka et al., 1979) in glass columns (5 cm internal diameter), 400 mL working volume, at 28 °C, bubbled with a mixture of air/CO_2 (97:3, v/v). The pH of culture medium was maintained at 7.5 ± 0.1. Cultures were illuminated with 70 μmol photons m^{-2} s^{-1} with cool white, fluorescent lights (Dulux L, 55W/840, Osram, Italy).

Experimental conditions: C. *sorokiniana* was grown in a batch mode. All experiments were conducted using triplicate 400 ml glass columns. Experiments were carried out under continuous illumination of 100 and 200 μmol photons m^{-2} s^{-1}, and at 6 different temperatures (20, 25, 30, 35, 40 and 45 °C). The photon flux density (PFD) was measured on the surface of tubes using the LI-250A equipped with a flat quantum sensor (LI-COR Biosciences, NE, USA). Cultures were grown under continuous illumination (24/00). Each tube was bubbled with a sterilized mixture of air/CO_2 (97: 3, v/v) at the rate of 51 min^{-1} to maintain turbulence and to keep the pH between 7.0 and 7.5. The flask cultures were inoculated with an initial dry weight of about 100 mgL^{-1}. Both culture medium and glass columns were autoclaved for 45 min at 121 °C to prevent any contamination during the growth experiments.

Dry weight: Dry weight was performed on triplicate 10 mL samples. Samples were taken at 24 hours intervals to determine the cell dry weight increase. Each sample was filtered through 0.7 μm pore size pre-weighted membranes (Whatman grade GF/F filters, Maidstone, England) and then dried at 105 °C for 3 h. They were then transferred in a desiccator to equilibrate them to room temperature, and thereafter the filters were weighed. Biomass productivity (mg l^{-1} d^{-1}) was calculated according to the equation x_1-x_0 /Δt where x_1 and x_0 (g/l) were the dry weight at intervals of 24 hours (Δ*t*).

Pigments: To measure cell chlorophyll *a/b,* and total carotenoids contents, a 10 ml aliquot of each culture was centrifuged for 5 min at 4000 x g. Five ml of 90 % acetone (v/v) were then added to the pellet, mixed in the vortex for 5 min, centrifuged and determined spectrophotometrically according to Lichtenthaler (1987) using a Beckman DU-640 spectrophotometer.

Chlorophyll fluorescence: Daily measurements of the fluorescence parameter F_v/F_m, i.e., the maximum photochemical quantum yield of PSII, were performed with portable pulse-amplitude-modulation fluorometer (PAM 2500, H. Walz, Effeltrich, Germany). For

this purpose, a volume of 1.5 ml of algal culture taken directly from the culture tubes, was incubated in the dark for 10–15 min in a 2 ml culture volume cuvette. PSII minimum fluorescence (F_0) was measured with a low-intensity modulated measuring beam (0.3 µmol m^{-2} s^{-1}) from light-emitting diodes (peak wavelength at 650 nm, frequency 600 Hz). A saturating light pulse was then administered to reach F_m level (Masojidek et al., 2011; Maxwell & Johnson, 2000; Torzillo et al., 1998).

Statistical analysis: Data gathered from laboratory batch cultures were treated statistically by two-way analysis of variance (ANOVA). Bonferroni's post-test was performed to determine the statistical significance ($P < 0.05$) of the effects of light and temperature and interaction of both factors on growth, productivity, chlorophyll *a+b*. The statistical analysis was carried out using GraphPad Prism 5 (GraphPad Software, Inc., California, USA).

RESULTS

In Fig. 1, the growth curves of *C. sorokiniana* cells grown at different temperatures ranging from 20 °C to 45 °C and at two light irradiances, 100 µmol photons m^{-2} s^{-1} (Fig. 1A) and 200 µmol photons m^{-2} s^{-1} are shown. (Fig. 1B).

Maximum dry weight reached by the cultures was about double in the cultures grown at double irradiance (100 vs 200 µmol photons $m^{-2}s^{-1}$). However, the optimal temperature for growth showed a broad range in cultures grown under 100 µmol photons $m^{-2}s^{-1}$), spanning from 20 °C to 35 °C, and peaking at 25 °C, while in cultures grown under 200 µmol photons $m^{-2}s^{-1}$, the optimal growth was found at 30 °C (Fig. 1B) ($P < 0.001$). An interesting feature was the different lower temperature thresholds for growth. Cultures grew well at 20 °C, under 100 µmol photons $m^{-2}s^{-1}$, while no appreciable growth was obtained at the same temperature but with double irradiance ($P < 0.001$). When the culture temperature was raised to 45 °C no appreciable growth was achieved under both the irradiances (Fig. 2A, Fig. 2B) ($P > 0.05$).

Productivities achieved at different growth temperatures increased with the irradiance from 100 µmol photons $m^{-2}s^{-1}$ (Fig. 2A) to 200 µmol photons $m^{-2}s^{-1}$ (Fig. 2B) ($P < 0.001$). Interestingly, the optimal temperature for growth was recorded at 25 °C at the lower light irradiance (Fig. 2A), and at 30 °C at higher irradiance (Fig. 2B), that is, there was a shift of 5 °C in the optimal temperature for growth between the two tested irradiances. Moreover, under 100 µmol photons $m^{-2}s^{-1}$ (Fig. 2A) the differences in productivities within the range of temperature of 20 °C and 35 °C, although significant, ($P < 0.05$) were not as high as that found under 200 µmol photons $m^{-2}s^{-1}$ In addition, under the higher irradiance (Fig. 2B) the decline in productivity was more marked than that found with a lower irradiance (Fig. 2A) as the temperature moved away from the optimum. Surprisingly, at 20 °C and 200 µmol photons $m^{-2}s^{-1}$ the productivity was negligible (Fig. 2B), most likely as a

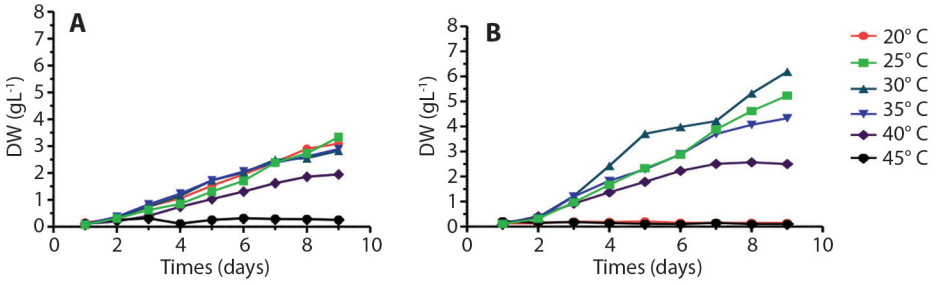

Fig. 1. Dry weight increase attained at different temperatures by *C. sorokiniana* UTEX 1230. **A.** PFD = 100 µmol photons $m^{-2}s^{-1}$, **B.** PFD= 200 µmol photons $m^{-2}s^{-1}$

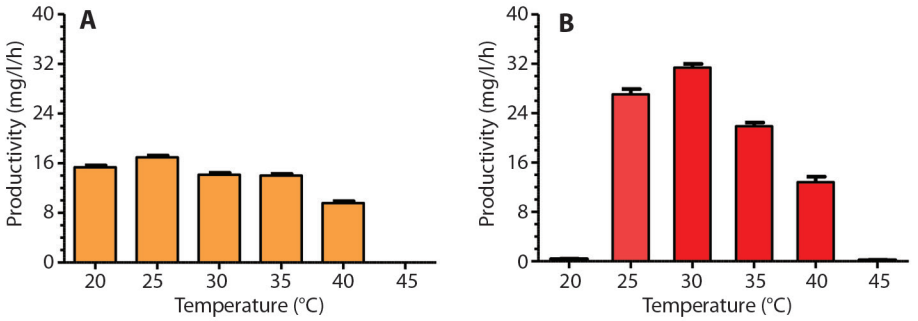

Fig. 2. Productivity ($mgL^{-1}day^{-1}$) of *C. sorokiniana* UTEX 1 230 cultures grown at different temperatures and light intensities, **A.** PFD = 100 and **B.** PFD = 200 µmol photons $m^{-2}s^{-1}$. No appreciable productivity was achieved at 45 °C both at 100, and 200 µmol photons $m^{-2}s^{-1}$, and at 20 °C under 200 µmol photons $m^{-2}s^{-1}$.

result of high irradiance combined to low temperature ($P < 0.001$). Both at 100 and 200 µmol photons $m^{-2}s^{-1,}$ no productivity was detected at 45 °C indicating that 40 °C is the upper limit for growth productivity ($P > 0.05$).

Chlorophyll fluorescence: In Fig. 3, the changes in the Fv/Fm ratio of the cultures grown at different temperatures and under the two irradiances are shown. The F_v/F_m ratio of culture grown at 100 µmol photons m^{-2} s^{-1} remained close to the optimal value (0.7 ± 0.05) within the temperatures range 20–40 °C, with a tendency to diminish as the temperature increased. The highest value 0.744 ($P < 0.05$) was attained by the cultures grown at 25 °C and 100 µmol photons m^{-2} s^{-1}.

However, when cultures were exposed to 45 °C, the decline of F/Fm value become evident indicating the inhibition of the photosynthetic activity, in line to what observed by growth measurements (Fig. 3A). The F_v/F_m ratio measured during the 8 consecutive days of culture grown under 200 µmol photons m^{-2} s^{-1} resulted generally inferior to that of culture grown at 100 µmol photons m^{-2} s^{-1}($P < 0.001$). Under 200 µmol photons $m^{-2}s^{-1}$ the lowest values were observed at 45 °C, 0.11 (average of 8 days). F_v/F_m values significantly lower than optimal were recorded at 20 °C, particularly under 200 µmol photons m^{-2} s^{-1}: at this temperature, F_v/F_m was 0.382 (average) with

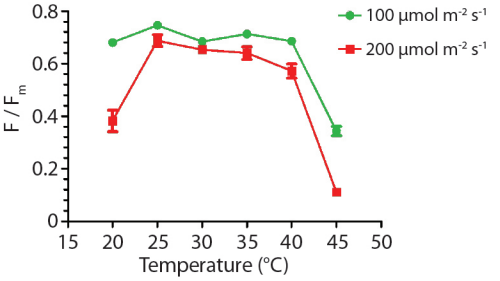

Fig. 3. Maximum photochemical efficiency of PSII (F_v/F_m), of *C. sorokiniana* Utex 1230 cells grown at both 100 and 200 µmol photons m^{-2} s^{-1} and different temperatures.

tendency to reduction during the cultivation period. Therefore, the combination of suboptimal temperature and higher light caused a negative synergistic effect on the maximum photochemical quantum yield of PSII, at both 20 °C (Fig. 3).

Chlorophyll: Total cellular chlorophyll was strongly modified by both light intensity and temperature (Fig. 4A, Fig. 4B) ($P < 0.001$). In general, the amount of chlorophyll accumulated by the cells resulted higher in cultures grown under 100 µmol photons $m^{-2}s^{-1}$ (Fig. 4A). Under this light irradiance, the highest accumulation was observed within 20 °C and 35 °C, with a significant difference ($P < 0.05$) in favor of the culture grown at 30 °C (Fig. 4A). A 40 °C chlorophyll accumulation showed a

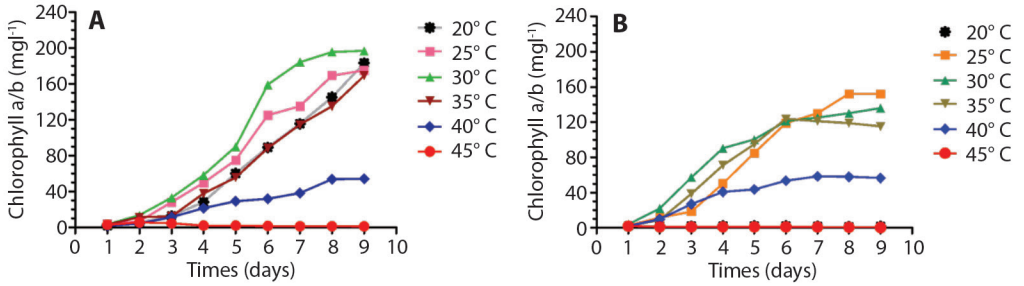

Fig. 4. Total chlorophyll (a+b) accumulations attained in culture of *C. sorokiniana* exposed at two light irradiances,100 µmol photons $m^{-2}s^{-1}$ **A.**, and of 200 µmol photons $m^{-2}s^{-1}$, **B.** at different temperatures.

strong drop, and no new synthesis was attained at 45 °C. (Fig. 4A). In cultures grown under 200 µmol photons $m^{-2}s^{-1}$, no significant differences were observed within 25 °C and 35 °C ($P > 0.05$), while at 40 °C chlorophyll showed a strong reduction, and a further rise of temperature to 45 °C caused a total inhibition of chlorophyll synthesis (Fig. 4B). The exposure of cultures to double level of irradiance at 20 °C caused an inhibition of synthesis of chlorophyll (Fig. 4B). Data gathered with chlorophyll measurements align with those of growth. Indeed, no growth was attained at 45 °C under both the light irradiances and similarly no growth, and thus no productivity could be detected at 20 °C under double the amount of light (Fig. 4B).

Total carotenoids: Total carotenoids of the cells varied under both light regimes and at various temperatures. The data reported in Fig. 5 show that a higher content of total carotenoid was recorded at a lower irradiance ($P < 0.001$).

However, a different pattern was observed with temperature. Under 100 µmol photons $m^{-2}s^{-1}$, the amount of carotenoids decreased linearly to zero when the growth temperature was increased to 45 °C. Under 200 µmol $m^{-2}s^{-1}$, the higher carotenoid content was attained by cultures grown within the 25 °C and 40 °C range, while no significant amounts of carotenoids were recorded at both 20 °C and 45 °C. (Fig. 5).

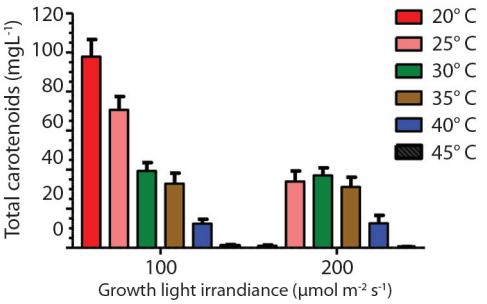

Fig. 5. Total carotenoid content detected in culture of *C. sorokiniana* exposed to two light irradiances (100 and 200 µmol photons $m^{-2}s^{-1}$) and temperatures from 20 to 45 °C.

DISCUSSION

Microalgal grown outdoors are subjected to suboptimal culture conditions, particularly light and temperature, two environmental factors that are difficult to govern even through the adoption of an appropriate culture system. Cultures grown in open ponds usually suffer from low temperatures, particularly in the morning hours, while in closed systems they are usually subjected to excessive temperature. Microalgal cultures, grown both in open and closed systems, suffer from excessive light irradiance during the central hour of the day (Torzillo & Vonshak, 2013). Therefore, an important aspect of algal biotechnology is to develop strains that can grow well both under suboptimal and supra-optimal temperatures, that is, within 20 °C and 40 °C. Culture temperatures close to 40 °C can be frequently recorded even in open ponds, particularly in greenhouses in

summer (Torzillo et al., 2021), while 20 °C and lower values are usually recorded in the early morning hours and over the day at the end of culture season. However, it is important to consider that cultures outdoors are subjected to the interaction of two or more environmental factors, usually light and temperature. Therefore, the development of strains that are capable of growing under variable light and temperature conditions, and that preserve a standard biochemical composition, as usually required by the market, are important prerequisites to consider to properly address the selection of suitable strains (Seyfabadi et al., 2011). In this respect, *C. sorokiniana* has evidenced a broad range of temperatures for growth, particularly under lower irradiance ranging from 20 °C to 40 °C. Interestingly, the productivity reached at 20 °C under low irradiance was comparable to that reached at 25 °C (optimal). This is an important feature of the strain for growing it in temperate regions. Actually, this strain is successfully grown in thin-layer cascades in Central Europe (Grivalsky et al., 2019). Moreover, the ability to resist until 40 °C adds further advantages in the choice of this strain outdoors.

It was interesting to note that optimal productivity was shifted by 5 °C, that is, from 25 °C to 30 °C when the light irradiance was increased to 200 μmol m^{-2}-s^{-1} evidencing the synergistic effect of light and temperature. This finding confirmed that the selection of strains must include a combination of more than one factor to better evaluate their performance.

Another interesting finding was that growth of the cultures at 20 °C under lower light reached productivity comparable to that attained at 25 °C, and this was probably achieved by shifting the cellular metabolism toward the production of carbohydrates that were used as a sink to store the excess of reducing power (Torzillo et al., 1991). Surprisingly, no growth was achieved at 20 °C when cultures were grown under 200 μmol m^{-2}-s^{-1}. The measurement of chlorophyll fluorescence clearly indicated a strong depression of the F_v/F_m ratio indicating that cultures were photo inhibited. Indeed, F_v/F_m which is an indicator of photoinhibition (Masojidek et al., 2011; Torzillo et al., 1998; Vonshak et al., 1994) was very low, about 0.4, under the combination of light and low temperature. Conditions of suboptimal temperature and high light in low light acclimated cultures, as those growing outdoors, is a situation that can frequently occur in the morning hours where the rise of light is much faster than that of temperature creating the conditions of photoinhibition at relatively low light (Vonshak et al., 2001). Indeed, low temperatures in low acclimated cultures can make the photosynthetic apparatus prone to photoinhibition even in relatively low light irradiance due to an over reduction of the photosynthetic carriers (Torzillo & Vonshak, 2013; Vonshak et al., 2001).

Generally, the average productivity of the most common industrial strains, including *C. sorokiniana*, is much lower than the theoretical maximum, therefore it is mandatory to identify and assess the incidence of factors limiting productivity. According to Huesemann et al. (2018) working with *C. sorokiniana,* a key challenge in the development of an economically viable biofuels production process was the identification of strains that exhibit an annual biomass productivity of at least 25 g m^{-2} day^{-1} which is about double that achieved with present know-how on algal biotechnology.

The information gathered with this study on the interplay of environmental factors on the growth of *C. sorokiniana* point out the necessity to select robust strains resilient to changes in temperature and light irradiance. This species was able to growth within a wide range of temperatures, ranging from 20 °C to 40 °C, when exposed to low irradiance. Therefore, it is advisable for mass culture of this species, the choice of photobioreactor designs in which it is possible to achieve a high dilution rate of light irradiance, that is, vertical plates placed close to each other, or compact vertical tubular reactors to increase the cross-section of the reactors and diminish the incident light irradiance impinging on the surface of the reactor (Cuaresma et al., 2009; Slegers et al., 2011; Torzillo et al., 1991; Torzillo et al., 2022; Tredici et al., 2015).

In conclusion, the present findings demonstrated that *C. sorokiniana* Utex 1230 rather than to be a thermotolerant strain, by converse it can be grown under the suboptimal temperature of 20 °C without a remarkable loss in productivity, making it attractive for cultivating under temperate European regions where mesophilic commercial strains, such as *Arthrospira platensis*, are difficult to cultivate for extended.

Ethical statement: the authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

ACKNOWLEDGMENTS

We thank to Consiglio Nazionale delle Ricerche-Istituto per la Bioeconomia, Via Madonna del Piano 10, Sesto Fiorentino, I-50019 Florence, Italy for the laboratory support.

REFERENCES

Becker, W. (2013). Microalgae for human and animal nutrition. In A. Richmond & Q. Hu, (Eds.), *Handbook of microalgal culture: applied phycology and biotechnology* (pp. 461–503). John Wiley y Sons.

Benedetti, M., Vecchi, V., Barera, S., & Dall'Osto, L. (2018). Biomass from microalgae: the potential of domestication towards sustainable biofactories. *Microbial Cell Factories*, *17*, 173. https://doi.org/10.1186/s12934-018-1019-3

Borowitzka, M. (2013). High-value products from microalgae-their development and commercialization. *Journal of Applied Phycology*, *25*, 743–756.

Chen, W., Luo, L., Han, D., Long, F., Chi, Q., & Hu, Q. (2021). Effect of dietary supplementation with *Chlorella sorokiniana* meal on the growth performance, antioxidant status, and immune response of rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Phycology*, *33*, 3113–3122. https://doi.org/10.1007/s10811-021-02541-w

Chun-Yen, C., Jhih-Ci, L., Yu-Han, C., Jih-Heng, C., Dillirani, N., Duu-Jong, L., & Jo-Shu, C. (2023). Optimizing heterotrophic production of *Chlorella sorokiniana* SU-9 proteins potentially used as a sustainable protein substitute in Aquafeed. *Bioresource Technology*, *370*, 128538. https://doi.org/10.1016/j.biortech.2022.128538

Converti A., Casazza, A. A., Ortiz, E. Y., Perego, P., & Borghi, M.. (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing*, *48*, 1146–1151. https://doi.org/10.1016/j.cep.2009.03.006

Cuaresma, M., Janssen, M., Vílchez, C., & Wijffels, R. (2009). Productivity of *Chlorella sorokiniana* in a short light-path (SLP) panel photobioreactor under high irradiance. *Biotechnology and Bioengineering*, *104*(2), 352–359. https://doi.org/10.1002/bit.22394

Cuaresma, M. F., Buffing, M. F., Janssen, M., Vílchez Lobato, C., & Wijffels, R. H. (2012). Performance of *Chlorella sorokiniana* under simulated extreme winter conditions. *Journal of Applied Phycology*, *24*(4), 693–699. https://doi.org/10.1007/s10811-011-9687-y

De La Peña, M. R. (2007). Cell growth and nutritive value of the tropical benthic diatom, *Amphora sp*., at varying levels of nutrients and light intensity, and different culture locations. *Journal of Applied Phycology, 19*, 647–655. https://doi.org/10.1007/s10811-007-9189-0

Dorr, R., & Huss, V. A. R. (1990). Characterization of nuclear-DNA in 12 species of *Chlorella* (Chlorococcales, Chlorophyta) by DNA reassociation. *Biosystems*, *24*(2), 145–155. https://doi.org/10.1016/0303-2647(90)90007-N

Eladel, H., Abomohra, A. E. F., Battah, M., Mohmmed, S., Radwan, A., & Abdelrahim, H. (2019). Evaluation of *Chlorella sorokiniana* isolated from local municipal wastewater for dual application in nutrient removal and biodiesel production. *Bioprocess and Biosystems Engineering*, *42*, 425–433. https://doi.org/10.1007/s00449-018-2046-5

Grivalsky, T., Ranglova, K., Câmara Manoel, J. A., Lakatos, G. E., Lhotsky, R., & Masojidek, J. (2019). Development of thin-lyer cascades for microalgae cultivation: milestones (review). *Folia Microbiologica*, *64*, 603–614. https://doi.org/10.1007/s12223-019-00739-7

Huesemann, M., Chavis, A., Edmundson S., Rye, D., Hobbs, S., Sun, N., & Wigmosta, M. (2018). Climate-simulated raceway pond culturing: quantifying the maximum achievable annual biomass productivity of *Chlorella sorokiniana* in the contiguous USA. *Journal Applied Phycology*, *30*, 287–298. https://doi.org/10.1007/s10811-017-1256-6

Kessler, E., & Huss, V. A. R. (1992). Comparative physiology and biochemistry and taxonomic assignment

of the *Chlorella* (Chlorophyceae) strains of the culture collection of the University of Texas at Austin. *Journal of Phycology*, *28*(4), 550–553. https://doi.org/10.1111/j.0022-3646.1992.00550.x

Kunz, W. F. (1972). Response of the alga *Chlorella sorokiniana* to 60 Co gamma radiation. *Nature*, *236*, 178–179. https://doi.org/10.1038/236178a0

Lee, J. S., Kim, D. K., Lee, J. P., Park, S. C., Koh, J. H., & Ohh, S. J. (2001). CO2 fixation by Chlorella KR-1 using flue gas and its utilization as a feedstuff for chicks. *Journal of Microbiology and Biotechnology*, *11*(5), 772–775.

Lichtenthaler, H. (1987). Chlorophyll and carotenoids: pigments of photosynthetic membranes. *Methods of Enzymology*, *148*, 350–382.

Liu, J., & Hu, Q. (2013). *Chlorella*: industrial production of cell mass and chemicals. In A. Richmond, & Q. Hu (Eds.), *Handbook of microalgal culture: applied phycology and biotechnology* (pp. 329–338). John Wiley y Sons.

Masojídek, J., Torzillo, G., & Koblízek, M. (2013). Photosynthesis in microalgae. In A. Richmond, & Q. Hu (Eds.), *Handbook of microalgal culture: applied phycology and biotechnology* (pp. 21–36). John Wiley y Sons.

Masojidek J., Vonshak, A., & Torzillo, G. (2011). Chlorophyll fluorescence applications in microalgal mass cultures. In D. J. Suggett, O. Prašil & M. A. Borowitzka (Eds.), *Chlorophyll a fluorescence in aquatic sciences: methods and applications developments in applied phycology* (pp. 277–292). Springer Science+Business Media B.V.

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence- a practical guide. *Journal of Experimental Botany*, *51*(345), 659–668. https://doi.org/10.1093/jexbot/51.345.659

Moronta, R., Mora, R., & Morales, E. (2006). Response of the microalga *Chlorella sorokiniana* to pH, salinity and temperature in axenic and non axenic conditions. *Revista de la Facultad de Agronomía*, *23*, 27–41.

Ras, M., Steyer, J. P., & Bernard, O. (2013). Temperature effect on microalgae: A crucial factor for outdoor production. *Reviews in Environmental Science and Biotechnology*, *12*, 153–164. https://doi.org/10.1007/s11157-013-9310-6

Rippka, R., Deruelles, J., Waterbury, B., Herdman, M., & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of Cyanobacteria. *Journal of General Microbiology, 111*(1), 1–61. https://doi.org/10.1099/00221287-111-1-1

Seyfabadi, J., Ramezanpour, Z., & Amini-Khoeyi, Z. (2011). Protein, fatty acid, and pigment content of *Chlorella vulgaris* under different light regimes. *Journal of Applied Phycology*, *23*, 721–726. https://doi.org/10.1007/s10811-010-9569-8

Sharma, P., Srinivas Gujjala, L. K., Varjani, S., & Kumar, S. (2022). Emerging microalgae-based technologies in biorefinery and risk assessment issues: bioeconomy for sustainable development. *Science of the Total Environoment*, *813*, 152417. https://doi.org/10.1016/j.scitotenv.2021.152417

Schubert, E. (2003). Nonmotile coccoid and colonial green algae. In J. D. Wehr, & R. Sheath (Eds.), *Freshwater algae of North America: ecology and classification* (2nd ed., pp. 253–307). Academic Press. https://doi.org/10.1016/B978-0-12-385876-4.00007-4

Sleger, P. M., Wijffels, R. H., van Straten, G., & van Boxtel, A. J. B. (2011). Design scenarios for flat panel photobioreactors. *Applied Energy*, *88*(10), 3342–3353. https://doi.org/10.1016/j.apenergy.2010.12.037

Sorokin, C., & Myers, J. (1953). A high-temperature strain of *Chlorella*. *Science*, *117*(3039), 330–331. https://doi.org/10.1126/science.117.3039.330

Torzillo, G., Bernardini, P., & Masojidek, J. (1998). Online monitoring of chlorophyll fluorescence to assess the extent of photoinhibition of photosynthesis induced by high oxygen concentration and low temperature and its effect on the productivity of outdoor cultures of *Spirulina platensis* (Cyanobacteria). *Journal of Phycology*, *34*(3), 504–510. https://doi.org/10.1046/j.1529-8817.1998.340504.x

Torzillo, G., Chini Zittelli, G., Silva Benavides, A. M., Ranglova, K., & Masojidek, J. (2021). Culturing of microalgae for food applications. In T. Lafarga & G. Acién (Eds.), *Cultured Microalgae for the Food Industry* (pp. 1–48). Academic Press. https://doi.org/10.1016/B978-0-12-821080-2.00002-2

Torzillo, G., Sacchi, A., & Materassi, R. (1991). Temperature as an important factor affecting productivity and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioreactors. *Bioresource Technology*, *38*(2–3), 95–100. https://doi.org/10.1016/0960-8524(91)90137-9

Torzillo, G., & Vonshak, A. (2013). Environmental stress physiology with references to mass cultures. In A. Richmond (Ed.), *Handbook of microalgal mass cultures* (pp. 90–113). Blackwell Science.

Torzillo G., Zittelli, G. C., Cicchi, B., Diano, M., Parente, M., Silva-Benavides, A. M., Esposito, E., & Touloupakis, E. (2022). Effect of plate distance on light conversion efficiency of a *Synechocystis* culture grown outdoors in a multiplate photobioreactor. *Science of the Total Environment*, *842*, 156840. https://doi.org/10.1016/j.scitotenv.2022.156840

Tredici, M. R., Bassi, N., Prussi, M., Biondi, N., Rodolfi, L., Chini Zittelli, G., & Sampietro, G. (2015). Energy balance of algal biomass production in a 1-ha "Green

Wall panel" plant: how to produce algal biomass in a closed reactor achieving a high net energy ratio. *Applied Energy*, *154*, 1103–1111. https://doi.org/10.1016/j.apenergy.2015.01.086

Ugwu C. U., Aoyagi H., & Uchiyama H. (2007). Influence of irradiance, dissolved oxygen concentration, and temperature on the growth of *Chlorella sorokiniana*. *Photosynthetica*, *45*(2), 309–311. https://doi.org/10.1007/s11099-007-0052-y

Vonshak, A., Torzillo, G., Masojidek, J., & Boussiba, S. (2001). Sub-optimal morning temperature induces photoinhibition in dense outdoor cultures of the alga *Monodus subterraneus* (Eustigmatophyta). *Plant Cell Environment*, *24*(10), 1113–1118. https://doi.org/10.1046/j.0016-8025.2001.00759.x

Vonshak, A., Torzillo, G., & Tomaselli, L. (1994). Use of chlorophyll fluorescence to estimate the effect of photoinhibition in outdoor cultures of *Spirulina platensis*. *Journal of Applied Phycology*, *6*, 31–34. https://doi.org/10.1007/BF02185901

Yang, J., Dou, S., Liu, X., Zhu, L., Liu, K., Zhang, Y., Li, L., Liu, G., & Yang, M. (2024). Enhanced starch accumulation in *Chlorella sorokiniana* as sugar platform and the expression profiling of key regulatory proteins. *Industrial Crops & Products*, *213*, 118433. https://doi.org/10.1016/j.indcrop.2024.118433