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The effects of light and temperature on microalgal growth and nutrient removal: an experimental and mathematical approach†

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Cultivation of microalgae and cyanobacteria has been intensified in the last decades, due to the numerous applications described for these microorganisms. However, the high process costs associated with biomass production systems reduce the economic feasibility of microalgal/cyanobacterial cultivation. A better understanding of the effects of light and temperature on growth kinetics will contribute to the improvement of biomass productivities and reduce the costs associated with the optimization of culture parameters. In this study, the effects of average daily light irradiance and temperature on growth and nutrient removal were assessed using *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*. Additionally, a mathematical model relating specific growth rates with these variables was developed. Both kinetic growth parameters and nutrient removal had similar responses to light and temperature: increasing light supply, higher specific growth rates, biomass productivities and nutrient removal efficiencies were achieved. Among the studied temperatures, all microorganisms presented higher biomass productivities and nutrient removal efficiencies at 25 °C. Regarding the results from the mathematical model, the optimal temperature for the selected microorganisms was 25.3 ± 1.1 °C. On the other hand, the optimal average daily light irradiances varied with the species, being 208, 258, 178 and 140 μE m^{-2} s^{-1} for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*, respectively.

1. Introduction

Microalgae are a broad category of photosynthetic microorganisms, comprising single-cell eukaryotic microalgae and prokaryotic cyanobacteria. Cultivation of these photosynthetic microorganisms has gained much attention in the last decades, due to the huge potential of these microorganisms in such a variety of applications. When growing autotrophically, microalgae and cyanobacteria uptake CO_2 from the atmosphere and/or flue gas emissions, reducing the concentration of this greenhouse gas in the atmosphere.[1] Additionally, these microorganisms assimilate nitrogen and phosphorus, the main contributors to the eutrophication phenomenon, playing an important role in the remediation of water resources.[2,3] Due to the rich composition of microalgal/cyanobacterial cells, their biomass can then be used in different applications, such as human food and animal feed, production of drugs, cosmetics, functional food, biofuels and fertilizers.[4–7] Despite the numerous applications described for microalgae and cyanobacteria, cultivation of these microorganisms still presents some challenges regarding the achievement of high biomass productivities at reduced costs. Accordingly, optimization of cultivation parameters in order to obtain an economically viable process with increased biomass productivities becomes necessary. Microalgal/cyanobacterial growth can be affected by several factors, both biotic and abiotic. Biotic factors include the presence of pathogens, such as bacteria, fungi and viruses, and the competition by other microalgae, whereas abiotic factors include light, temperature, pH, salinity, nutrient qualitative and quantitative profiles, dissolved oxygen concentration and the presence of toxic compounds. Additionally, microalgal and cyanobacterial growth can be influenced by operational conditions, such as hydraulic residence time, harvesting rates, gas transfer and mixing.[8–11] Among these parameters, light supply and temperature appear as the most important factors influencing microalgal and cyanobacterial growth. In fact, photoautotrophic growth is driven by light supply, the energy source that is used to convert inorganic carbon into organic matter, and changes in temperature can easily affect microalgal/cyanobacterial growth since the metabolic activity of these photosynthetic microorganisms can be ceased by extreme temperatures. Furthermore, interaction between these variables in outdoor cultures determines the biochemical profile of the resulting biomass and growth state.[12]

In this study, the effects of light supply (average daily light irradiance) and temperature on biomass production and nutrient uptake were assessed for the microalgae *Chlorella*

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vulgaris and *Pseudokirchneriella subcapitata* and the cyanobacteria *Synechocystis salina* and *Microcystis aeruginosa*. Selection of these microorganisms was based on the following factors:[13–16] (i) these microalgae and cyanobacteria can be easily grown in laboratory cultures; and (ii) several authors have reported the use of these microorganisms in a wide variety of biotechnological applications, such as CO_2 capture, wastewater treatment, biofuels production and synthesis of bioactive compounds. Additionally, due to the wide diversity of microalgal and cyanobacterial species, the study and optimization of culture parameters for all these microorganisms under different light and temperature conditions is very difficult. In this sense, mathematical modelling of these variables constitutes an important tool for growth prediction and characterization. Mathematical models describing the effect of light supply and temperature on microalgal/cyanobacterial growth have already been reported in the literature.[17–20] However, only a few studies have considered both variables simultaneously.[21–23] Accordingly, a kinetic growth model was developed to determine optimal light and temperature conditions for the selected microorganisms.

2. Materials and methods

2.1. Microorganisms and culture medium

The microalgae *C. vulgaris* CCAP 211/11B and *P. subcapitata* CCAP 278/4 were obtained from Culture Collection of Algae and Protozoa (United Kingdom), while the cyanobacteria *S. salina* LEGE 06079 and *M. aeruginosa* LEGE 91344 were obtained from the Laboratory of Ecotoxicology, Genomic and Evolution – CIIMAR (Centre of Marine and Environmental Research of the University of Porto, Portugal). Stock solutions of these microorganisms were prepared in OECD (Organisation for Economic Co-operation and Development) test medium,[24] with the following composition (per litre): 15 mg $NaNO_3$, 12 mg $MgCl_2 \cdot 6H_2O$, 18 mg $CaCl_2 \cdot 2H_2O$, 15 mg $MgSO_4 \cdot 7H_2O$, 1.6 mg KH_2PO_4, 0.08 mg $FeCl_3 \cdot 6H_2O$, 0.1 mg $Na_2EDTA \cdot 2H_2O$, 0.185 mg H_3BO_3, 0.415 mg $MnCl_2 \cdot 4H_2O$, 3 μg $ZnCl_2$, 1.5 μg $CoCl_2 \cdot 6H_2O$, 0.01 μg $CuCl_2 \cdot 2H_2O$, 7 μg $Na_2MoO_4 \cdot 2H_2O$ and 50 mg $NaHCO_3$. The cells were incubated in 500 mL flasks at room temperature, under continuous fluorescent light with an irradiance of 120 μE m^{-2} s^{-1} (corresponding average daily light irradiance is 120 μE m^{-2} s^{-1}) at the surface of the flasks. Agitation was obtained by bubbling atmospheric air (filtered through 0.22 μm cellulose acetate membranes, Orange Scientific, Belgium) at the bottom of the flasks.

2.2. Experimental setup and cultivation conditions

Batch experiments were performed in 500 mL flasks (VWR, Portugal) with a working volume of 400 mL. As the growth medium described above presents a very low concentration of nitrogen and phosphorus, concentrations of these elements were increased to simulate the concentrations commonly present in a secondary treated effluent. Therefore, cells were cultivated for 12 days in the culture medium described above, but with the following concentrations of $NaNO_3$ and KH_2PO_4: 250 and 45 mg L^{-1}, respectively.[25] In this study, nitrate was used as nitrogen source because this is the most thermodynamically stable form of inorganic nitrogen[8] and also because it is the most abundant nitrogen form in the tertiary treatment step of wastewater treatment plants, where microalgae can play an important remediation role.[25] The experimental conditions were the following: (i) initial cell concentration of approximately 1.0×10^6 cells per mL, which corresponds to a biomass (cell dry weight – dw) concentration of about 0.05–0.08 g_{dw} L^{-1}; (ii) initial pH was set at 7; (iii) continuous aeration with the injection of atmospheric air (filtered through 0.22 μm cellulose acetate membranes, Orange Scientific, Belgium) at the bottom of the flasks. The assays were carried out under different temperatures (15, 25 and 35 °C) and incident light irradiances (36 and 180 μE m^{-2} s^{-1}). The temperatures of 15, 25 and 35 °C were selected to simulate average temperatures observed in cold, warm and tropical regions, respectively. Light irradiance values were selected to observe the effect of low and high irradiance levels. Selection of this specific range of light irradiance values has taken into account the possible values that can be achieved using artificial light. For each temperature and irradiance value, different light cycles were evaluated: 10 : 14, 14 : 10, and 24 : 0 (light : dark ratio). The light : dark ratio of 24 : 0 was used because it promotes continuous photoautotrophic growth. To reduce production costs in terms of light requirements, the light : dark ratios of 10 : 14 and 14 : 10 were applied to simulate the number of light hours during winter and summer time, respectively. For each studied condition, two independent experiments were performed. Taking into account the light irradiances and light : dark ratios evaluated in this study, the corresponding average daily light irradiances are presented in Table 1.

2.3. Growth monitoring and kinetic growth parameters

Duplicate samples were collected at 24 h intervals and biomass concentration was determined by measuring optical density at 750 nm, OD_{750},[26] using a V-1200 spectrophotometer (VWR, Portugal). The relationship between OD_{750} and biomass concentration (X, mg_{dw} L^{-1}) for all microorganisms was established by linear regression, using the previously determined expressions.[27] Biomass concentration values were used to determine specific growth rates (μ, d^{-1}) and biomass productivities (P, mg_{dw} L^{-1} d^{-1}). Specific growth rates were determined according to eqn (1):[28]

Table 1 Average daily light irradiances evaluated in this study considering light irradiance and light : dark ratio values applied to the selected cultures

Light irradiance (μE m^{-2} s^{-1})	Light : dark ratio (h : h)	Average daily light irradiance (μE m^{-2} s^{-1})
36	10 : 14	15
	14 : 10	21
	24 : 0	36
180	10 : 14	75
	14 : 10	105
	24 : 0	180

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (1)$$

where X_2 and X_1 correspond to biomass concentration (in mg_{dw} L^{-1}) at times t_2 and t_1 (in days), the end and beginning of the exponential growth phase, respectively. Biomass productivities achieved in the exponential growth phase were calculated from the variation in biomass concentration within the exponential growth phase, as shown in eqn (2):[28,29]

$$P = \frac{X_2 - X_1}{t_2 - t_1} \quad (2)$$

2.4. Nutrients removal

Nutrients removal was determined by quantification of nitrogen and phosphorus in the culture medium. For each analytical assay, one-millilitre samples from each culture were collected in the first and last day of culturing. Samples were centrifuged at 16 500*g* for 10 min and supernatants were stored at −20 °C until being analysed. Nitrate concentration was determined through UV spectroscopy at 220 nm using a T80 UV/VIS Spectrophotometer (PG Instruments, UK), according to the method proposed by Collos *et al.*[30] On the other hand, inorganic phosphate quantification was performed by measuring absorbance at 820 nm of a phosphomolybdate complex formed by reaction of inorganic phosphate with ammonium molybdate in a Synergy™ HT 96-well microplate reader (Biotek Instruments, Inc., USA), as proposed by Lee *et al.*[31] Nutrients concentration in the first and last day of culturing were used to determine average removal rates (RR, in mg_S L^{-1} d^{-1}) and nutrients removal efficiencies (R, in %). Average removal rates were calculated as follows:[32]

$$RR = \frac{S_f - S_i}{t_f - t_i} \quad (3)$$

where S_f and S_i correspond to nutrients concentration (in mg_S L^{-1}) at times t_f and t_i (in days), the end and beginning of cultivation time, respectively. Nutrients removal efficiencies were determined according to eqn (4):

$$\%\ R = \frac{S_i - S_f}{S_i} \times 100 \quad (4)$$

Additionally, for each nutrient a mass balance was written and the mass fractions (α, in g_S $g_{dw}{}^{-1}$) of nitrogen and phosphorus incorporated in microalgal/cyanobacterial biomass were determined. This mass balance was determined according to eqn (5):[33]

$$\frac{dS}{dt} = -\alpha \frac{dX}{dt} \quad (5)$$

where S corresponds to nutrients concentration (in g_S L^{-1}). By integrating eqn (5) over the cultivation time, eqn (6) was obtained:

$$(S_i - S_f) = \alpha(X_f - X_i) \quad (6)$$

2.5. Modelling of microalgal growth

To determine the optimal growth conditions (average daily light irradiance and temperature) for the selected microalgae and cyanobacteria, a kinetic growth model was developed. Development of this model was based on specific growth rates determined for each of the studied microorganisms when grown under different light and temperature conditions. These data were obtained in this study and in other studies reported in the literature, as it is possible to see in Table S1 from the ESI.†

The behaviour of specific growth rates for increasing average daily light irradiance values was described according to the model proposed by Steele:[20]

$$\mu = \frac{\mu_{max} I}{I_{opt}} e^{\left(1 - \frac{I}{I_{opt}}\right)} \quad (7)$$

where μ_{max} corresponds to the maximum specific growth rate (in d^{-1}) achieved by the studied microorganisms, I denotes average daily light irradiance (in μE m^{-2} s^{-1}) and I_{opt} corresponds to the optimal value of average daily light irradiance (in μE m^{-2} s^{-1}) for microalgal/cyanobacterial growth.

On the other hand, the behaviour of specific growth rates for different temperatures was assumed to follow a skewed normal distribution, as reported by Dauta *et al.*:[34]

$$\mu = \mu_{max} e^{-\frac{(T - T_{opt})^2}{2\sigma^2}} \quad (8)$$

where T is the temperature (in °C), T_{opt} is the optimal temperature (in °C) for microalgal/cyanobacterial growth and σ is the standard deviation associated to the optimal temperature (in °C).

Eqn (7) and (8) were used to establish a two-dimensional model, resulting in the following expression:

$$\mu = \frac{\mu_{max} I}{I_{opt}} e^{\left(1 - \frac{I}{I_{opt}}\right)} e^{-\frac{(T - T_{opt})^2}{2\sigma^2}} \quad (9)$$

This expression was linearized (eqn (10)) and the parameters μ_{max}, I_{opt}, T_{opt} and σ were determined by minimizing the sum of squared residuals using the Solver supplement of Microsoft Excel 2013.

$$\ln \mu = \ln \mu_{max} + \ln \frac{I}{I_{opt}} + 1 - \frac{I}{I_{opt}} - \frac{(T - T_{opt})^2}{2\sigma^2} \quad (10)$$

The quality of the model fits was evaluated by calculating the root mean squared error (RMSE), a performance index that measures the agreement between data obtained experimentally and predicted values:

$$RMSE = \sqrt{\frac{\sum (z - \hat{z})^2}{n}} \quad (11)$$

where z denotes the experimental values, $\hat{z}$ the predicted values by the model and n the data size.

2.6. Statistical analysis

For each parameter, the average and standard deviation were calculated. The statistical significance of the results was evaluated using the Student's paired *t*-test to investigate whether the differences between the studied cultures could be considered significant. This analysis was performed using the statistical software SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Statistical tests were carried out at a significance level of 0.05.

3. Results and discussion

3.1. Influence of light supply and temperature on microalgal/cyanobacterial growth

When growing autotrophically, microalgae and cyanobacteria strongly depend on light supply and temperature.[8,9] These environmental factors influence growth dynamics (Fig. S1, ESI†), including the specific growth rates and biomass productivities, and also nutrients uptake from the culture medium. Fig. 1 shows the effect of average daily light irradiance and temperature on specific growth rates of the microalgae *C. vulgaris* and *P. subcapitata* (A and B) and the cyanobacteria *S. salina* and *M. aeruginosa* (C and D). Maximum biomass concentrations and biomass productivities achieved in the exponential growth phase under these conditions are shown in Table 2. Specific growth rates determined for the studied microorganisms ranged from 0.0188 ± 0.0033 d^{-1} (for *P. subcapitata* grown at 35 °C with an average daily light irradiance of 15 μE m^{-2} s^{-1}) to 1.19 ± 0.04 d^{-1} (for *C. vulgaris* grown at 25 °C with an average daily light irradiance of 180 μE m^{-2} s^{-1}). Regarding light supply, an increase in average daily light irradiance resulted in statistically higher ($p < 0.05$) specific growth rates. Several studies have already reported the increase of specific growth rates with increasing light supplies.[12,35,36] A positive relationship between specific growth rates and average daily light irradiance is not surprising, since microalgal/cyanobacterial growth is mainly autotrophic, requiring light as the major energy source. These results indicate that higher light supplies favoured the photosynthetic activity of the studied microorganisms, which was confirmed by the increase observed in average pH of the studied cultures: from 8.12 ± 0.29 (at 15 μE m^{-2} s^{-1}) to 8.76 ± 1.03 (at 180 μE m^{-2} s^{-1}). The increase in pH of the culture medium is related to an increase in carbon uptake by microalgae or cyanobacteria and, hence, in photosynthetic activity.[37] Culturing temperature also contributed to considerable changes in the specific growth rates of the studied microorganisms. Specific growth rates determined at 25 °C were statistically higher than those determined at 15 ($p < 0.001$) and 35 °C ($p = 0.001$). However, no statistical differences ($p = 0.087$) were observed between specific growth rates determined at 15 and 35 °C. These results indicate that the growth of the studied microorganisms in response to different temperatures may follow a normal distribution function, being the optimal culturing temperature approximately 25 °C. Evidence that the optimal temperature for autotrophic microalgal/cyanobacterial growth is near 25 °C was also given by the increase observed in pH and dissolved oxygen concentration at this temperature: for cultures performed at 15, 25 and 35 °C average pH of the culture medium was 8.32 ± 0.43, 8.91 ± 0.91 and 8.09 ± 0.82, respectively, whereas average dissolved oxygen concentration was 3.8 ± 1.1, 6.5 ± 0.4 and 4.8 ± 1.0 mg_{O_2} L^{-1}, respectively. A similar behaviour was observed by James *et al.*[38] when evaluating the effect of temperature on the growth and fatty acid and amino acid composition of two microalgae belonging to the genera *Chlorella* and *Nannochloropsis*. For temperatures ranging from 15 to 35 °C, an increase in specific growth rates was observed until 25 °C, while for higher temperatures specific growth rates started decreasing. Similarly, when evaluating the optimum temperature and salinity conditions for the growth of *Chlorella ellipsoidea* and *Nannochloris oculata*, Cho *et al.*[39] demonstrated that keeping a constant salinity of 10, an increase in

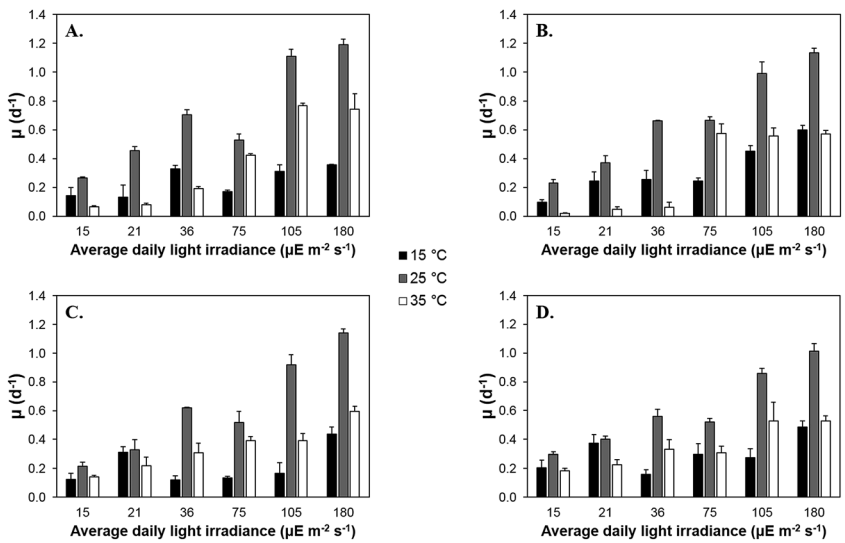

Fig. 1 Specific growth rates, in d^{-1}, determined for *C. vulgaris* (A), *P. subcapitata* (B), *S. salina* (C) and *M. aeruginosa* (D) under different light and temperature conditions. Error bars correspond to the standard deviation of two independent experiments.

Table 2 Maximum biomass concentrations (X_{max}, in $mg_{dw}\ L^{-1}$) and biomass productivities achieved in the exponential growth phase (P, in $mg_{dw}\ L^{-1}\ d^{-1}$) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light and temperature conditions[a]

Temperature (°C)	Average daily light irradiance ($\mu E\ m^{-2}\ s^{-1}$)	*C. vulgaris*		*P. subcapitata*		*S. salina*		*M. aeruginosa*	
		X_{max} ($mg_{dw}\ L^{-1}$)	P ($mg_{dw}\ L^{-1}\ d^{-1}$)	X_{max} ($mg_{dw}\ L^{-1}$)	P ($mg_{dw}\ L^{-1}\ d^{-1}$)	X_{max} ($mg_{dw}\ L^{-1}$)	P ($mg_{dw}\ L^{-1}\ d^{-1}$)	X_{max} ($mg_{dw}\ L^{-1}$)	P ($mg_{dw}\ L^{-1}\ d^{-1}$)
15	15	73.9 ± 4.5	6.91 ± 2.46	49.7 ± 13.1	3.60 ± 0.40	167 ± 1	4.66 ± 1.55	72.6 ± 1.0	7.85 ± 2.34
	21	107 ± 19	6.40 ± 4.24	70.8 ± 4	10.8 ± 3.4	173 ± 12	10.4 ± 1.2	109 ± 20	10.2 ± 1.5
	36	194 ± 52	17.5 ± 1.6	107 ± 25	10.1 ± 2.1	242 ± 13	5.12 ± 1.36	189 ± 29	5.37 ± 0.94
	75	331 ± 46	12.9 ± 1.0	113 ± 3	11.2 ± 0.9	349 ± 11	6.49 ± 0.58	211 ± 11	12.6 ± 3.3
	105	293 ± 20	15.4 ± 2.6	134 ± 5	23.4 ± 2.2	363 ± 20	6.03 ± 2.67	290 ± 7	10.2 ± 1.9
	180	588 ± 71	23.2 ± 0.4	459 ± 27	41.4 ± 1.9	501 ± 33	33.7 ± 0.6	458 ± 7	26.0 ± 1.5
25	15	414 ± 13	13.5 ± 0.3	234 ± 25	8.43 ± 0.94	426 ± 24	9.25 ± 1.39	406 ± 16	22.8 ± 1.3
	21	517 ± 11	29.4 ± 2.2	249 ± 13	16.5 ± 2.3	481 ± 19	17.4 ± 3.1	484 ± 7	30.4 ± 1.9
	36	828 ± 23	49.7 ± 3.9	426 ± 15	33.9 ± 0.7	738 ± 16	36.2 ± 1.4	742 ± 3	44.3 ± 2.8
	75	771 ± 11	31.7 ± 2.5	488 ± 13	32.6 ± 0.8	719 ± 39	27.9 ± 6.2	767 ± 17	40.8 ± 2.4
	105	$(1.08 \pm 0.14) \times 10^3$	95.5 ± 9.5	697 ± 7	82.4 ± 7.8	914 ± 30	78.0 ± 6.4	991 ± 7	97.4 ± 6.3
	180	$(1.35 \pm 0.13) \times 10^3$	125 ± 8	798 ± 36	110 ± 6	$(1.26 \pm 0.06) \times 10^3$	111 ± 6	$(1.17 \pm 0.06) \times 10^3$	120 ± 16
35	15	93.4 ± 6.5	4.57 ± 0.24	3.94 ± 0.49	0.206 ± 0.111	172 ± 1	6.49 ± 0.58	71.7 ± 2.5	9.08 ± 0.53
	21	108 ± 2	5.16 ± 0.70	12.7 ± 1.1	0.418 ± 0.232	228 ± 16	13.4 ± 3.3	131 ± 17	12.6 ± 3.3
	36	152 ± 10	13.4 ± 0.8	15.9 ± 2.5	2.32 ± 1.23	260 ± 25	17.0 ± 3.7	177 ± 8	16.8 ± 3.7
	75	396 ± 29	31.8 ± 1.0	190 ± 5	22.2 ± 2.0	309 ± 7	26.5 ± 2.0	220 ± 26	17.4 ± 2.7
	105	527 ± 28	50.1 ± 0.9	366 ± 24	31.6 ± 4.2	461 ± 12	30.4 ± 4.1	391 ± 7	40.4 ± 6.3
	180	518 ± 58	48.7 ± 7.9	290 ± 19	30.2 ± 0.7	436 ± 20	38.2 ± 3.8	371 ± 26	39.8 ± 11.4

[a] Values are presented as the mean ± standard deviation of two independent experiments.

temperatures from 15 to 25 °C results in increased specific growth rates and, when temperature is increased to 30 °C, specific growth rates tend to decrease. Average specific growth rates determined for *Chlorella pyrenoidosa* grown under a temperature range of 10 to 35 °C also increased until the temperature of 25 °C, starting decreasing when culturing temperature was set at 30 and 35 °C.[40]

The influence of light supply and temperature on maximum biomass concentrations and biomass productivities was similar to the one observed for specific growth rates (Table 2). In this study maximum biomass concentration values ranged from 3.94 ± 0.49 (determined for *P. subcapitata* grown at 35 °C with an average daily light irradiance of 15 μE m^{-2} s^{-1}) to $(1.35 \pm 0.13) \times 10^3$ mg_{dw} L^{-1} (determined for *C. vulgaris* grown at 25 °C with an average daily light irradiance of 180 μE m^{-2} s^{-1}). Minimum and maximum biomass productivities were determined for the same microorganisms in the same conditions: 0.206 ± 0.111 (for *P. subcapitata* grown at 35 °C with an average daily light irradiance of 15 μE m^{-2} s^{-1}) and 125 ± 8 mg_{dw} L^{-1} d^{-1} (for *C. vulgaris* grown at 25 °C with an average daily light irradiance of 180 μE m^{-2} s^{-1}), respectively. As for specific growth rates, an increase in average daily light irradiance from 15 to 180 μE m^{-2} s^{-1} resulted in statistically higher ($p < 0.05$) maximum biomass concentrations and biomass productivities. Ugwu *et al.*[41] demonstrated that an increase in light irradiance results in an increase in biomass productivities when growing *Chlorella sorokiniana* with average daily light irradiances ranging from 100 to 250 μE m^{-2} s^{-1}. Regarding the effects of temperature, statistically higher ($p < 0.05$) maximum biomass concentrations and biomass productivities were determined for cultures grown at 25 °C. In the case of cultures grown at 15 and 35 °C, no statistical difference ($p > 0.05$) was observed in both maximum biomass concentrations and biomass productivities. Han *et al.*[42] found that cultivation of *C. pyrenoidosa* at 22, 30 and 36 °C resulted in biomass productivities of 120 ± 2, 141 ± 1 and 125 ± 2 mg L^{-1} d^{-1}, respectively.

Comparing kinetic growth parameters determined for the studied microorganisms, it was possible to observe that *C. vulgaris* achieved the highest specific growth rate, maximum biomass concentration and biomass productivity when cultured at 25 °C under an average daily light irradiance of 180 μE m^{-2} s^{-1}. In the same culturing conditions specific growth rates determined for *P. subcapitata* and *S. salina* were not statistically different ($p > 0.05$) from the one determined for *C. vulgaris*. In the case of *M. aeruginosa*, specific growth rate determined in these conditions was statistically lower ($p < 0.05$). Regarding maximum biomass concentrations and biomass productivities, values determined for *S. salina* and *M. aeruginosa* were not statistically different ($p > 0.05$) from those determined for *C. vulgaris*. However, statistically lower ($p < 0.05$) values were determined for *P. subcapitata*.

3.2. Influence of light supply and temperature on nutrients removal

To evaluate the influence of light supply and temperature on nitrogen and phosphorus removal, concentrations of these nutrients in the first and last day of culturing were determined and average removal rates and removal efficiencies were obtained. These results are shown in Table 3, for nitrogen, and Table 4, for phosphorus.

Regarding nitrogen removal, maximum average removal rate, 2.89 ± 0.07 mg_N L^{-1} d^{-1}, was determined for *M. aeruginosa* grown at 25 °C, with an average daily light irradiance of 36 μE m^{-2} s^{-1}. On the other hand, maximum nitrogen removal efficiency achieved was 100% (for *C. vulgaris*, *P. subcapitata* and *M. aeruginosa* grown at 25 °C with an average daily light irradiance of 180 μE m^{-2} s^{-1}). The influence of light supply and temperature in these variables was very similar. In the case of average daily light irradiance, higher values resulted in statistically higher ($p < 0.05$) removal rates and removal efficiencies. In the study performed by Hu *et al.*,[43] nitrate uptake rates determined for *Synechococcus* sp. grown in nitrate-contaminated groundwater increased proportionally to increasing average daily light irradiance up to 100 μE m^{-2} s^{-1}. Regarding the effects of temperature, microalgal and cyanobacterial growth at 25 °C caused nitrogen removal rates and removal efficiencies statistically higher ($p < 0.05$) than those determined at 15 and 35 °C. The nitrogen removal rates and removal efficiencies were not statistically different ($p = 0.146$) between the extreme temperatures. Talbot and De la Noüe[44] demonstrated that cultivation of *Phormidium bohneri* in a secondary effluent from an activated sludge treatment plant at 30 °C for three days resulted in an effective removal of ammonia-nitrogen, whereas the same culture performed at 10 °C resulted in modest ammonia-nitrogen removal.

In the case of phosphorus removal, maximum average removal rate, 0.588 ± 0.029 mg_P L^{-1} d^{-1}, was determined for *C. vulgaris* grown at 25 °C with an average daily light irradiance of 180 μE m^{-2} s^{-1}. Phosphorus removal efficiencies ranged from 1.13 ± 0.03 (for *M. aeruginosa* grown at 15 °C, under the lowest average daily light irradiance) to 67.6 ± 7.1% (for *C. vulgaris* grown at 25 °C with an average daily light irradiance of 180 μE m^{-2} s^{-1}). These values were lower than those determined for nitrate, indicating that phosphorus assimilation is slower than nitrate-nitrogen assimilation. Different studies have already reported higher removal efficiencies for nitrogen than for phosphorus.[44,45] The influence of light supply and temperature on phosphorus removal rates and removal efficiencies was similar to the one observed for nitrogen removal. In general, an increase in the light supply resulted in increased phosphorus removal rates and removal efficiencies. Statistically higher ($p < 0.05$) removal rates and removal efficiencies were determined when light irradiance increased from 15 to 180 μE m^{-2} s^{-1}. In the study performed by Li *et al.*,[46] an increase in average daily light irradiance from 0 to 200 μE m^{-2} s^{-1} increased total phosphorus removal efficiencies from 65.8 to 87.0% (for *Chlorella kessleri*) and from 79.3 to 83.0% (for *Chlorella protothecoides*). The effects of temperature on phosphorus removal demonstrated that, in general, higher removal rates and removal efficiencies were obtained for cultures grown at 25 °C. However, these values were not statistically different ($p > 0.05$) from those determined for the other studied temperatures.

Table 3 Average nitrogen removal rates (RR, in $mg_N\ L^{-1}\ d^{-1}$) and nitrogen removal efficiencies (R, in %) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light and temperature conditions[a]

Temperature (°C)	Average daily light irradiance ($\mu E\ m^{-2}\ s^{-1}$)	*C. vulgaris*		*P. subcapitata*		*S. salina*		*M. aeruginosa*	
		RR ($mg_N\ L^{-1}\ d^{-1}$)	R (%)	RR ($mg_N\ L^{-1}\ d^{-1}$)	R (%)	RR ($mg_N\ L^{-1}\ d^{-1}$)	R (%)	RR ($mg_N\ L^{-1}\ d^{-1}$)	R (%)
15	15	0.658 ± 0.277	36.8 ± 9.6	0.115 ± 0.061	7.55 ± 3.62	0.278 ± 0.199	8.98 ± 6.55	0.497 ± 0.151	16.5 ± 4.7
	21	0.561 ± 0.035	37.9 ± 1.7	0.221 ± 0.098	16.5 ± 7.1	0.723 ± 0.161	25.3 ± 6.0	0.827 ± 0.250	27.1 ± 5.8
	36	1.67 ± 0.69	78.9 ± 6.0	0.472 ± 0.100	28.3 ± 5.8	0.816 ± 0.141	30.0 ± 5.8	1.21 ± 0.15	40.2 ± 4.9
	75	0.759 ± 0.225	24.8 ± 9.0	0.713 ± 0.474	25.3 ± 13.2	1.45 ± 0.33	45.7 ± 13.8	1.17 ± 0.12	41.1 ± 3.2
	105	2.11 ± 0.07	77.2 ± 5.6	1.69 ± 0.54	50.5 ± 10.0	2.32 ± 0.31	68.3 ± 5.0	1.87 ± 0.28	69.8 ± 3.3
	180	2.56 ± 0.49	93.4 ± 9.8	2.36 ± 0.25	79.1 ± 4.2	2.33 ± 0.27	75.0 ± 13.1	2.58 ± 0.34	85.3 ± 6.3
25	15	1.08 ± 0.03	42.3 ± 1.6	1.07 ± 0.21	43.5 ± 8.3	1.27 ± 0.02	48.5 ± 0.7	1.42 ± 0.04	53.6 ± 1.7
	21	1.69 ± 0.16	75.6 ± 5.8	1.24 ± 0.04	74.4 ± 2.9	1.86 ± 0.06	96.1 ± 0.9	1.82 ± 0.03	98.8 ± 1.4
	36	2.43 ± 0.38	97.1 ± 1.7	2.62 ± 0.08	88.0 ± 2.7	2.83 ± 0.16	92.5 ± 1.0	2.89 ± 0.07	97.3 ± 1.1
	75	2.40 ± 0.05	86.2 ± 1.7	1.97 ± 0.02	68.9 ± 0.8	2.45 ± 0.02	86.1 ± 0.6	2.59 ± 0.03	89.8 ± 0.4
	105	2.78 ± 0.06	98.0 ± 2.0	2.16 ± 0.54	97.7 ± 2.5	2.54 ± 0.20	98.6 ± 0.4	2.43 ± 0.33	98.0 ± 0.6
	180	2.43 ± 0.40	100 ± 0	2.37 ± 0.18	100 ± 0	1.97 ± 0.19	99.1 ± 0.7	2.53 ± 0.21	100 ± 0
35	15	0	0	0	0	0	0	0	0
	21	0.131 ± 0.039	6.68 ± 1.93	0	0	0.0836 ± 0.0091	0	0.0115 ± 0.0006	0.0510 ± 0.0141
	36	0.482 ± 0.292	16.3 ± 8.2	0.0442 ± 0.0071	1.37 ± 0.75	0.330 ± 0.081	15.1 ± 3.0	0.0874 ± 0.0360	4.00 ± 1.55
	75	0.959 ± 0.558	37.0 ± 21.3	0.804 ± 0.246	30.9 ± 9.2	2.22 ± 0.87	58.7 ± 9.5	1.47 ± 0.11	53.5 ± 2.4
	105	1.60 ± 0.12	63.4 ± 4.8	1.75 ± 0.07	70.6 ± 2.7	1.29 ± 0.01	61.4 ± 0.6	1.85 ± 0.06	73.5 ± 1.6
	180	2.41 ± 0.04	88.6 ± 1.5	1.95 ± 0.05	78.1 ± 1.6	1.25 ± 0.12	63.8 ± 1.9	2.14 ± 0.02	91.1 ± 0.6

[a] Values are presented as the mean ± standard deviation of two independent experiments.

Table 4 Average phosphorus removal rates (RR, in $mg_P\ L^{-1}\ d^{-1}$) and phosphorus removal efficiencies (R, in %) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light and temperature conditions[a]

Temperature (°C)	Average daily light irradiance ($\mu E\ m^{-2}\ s^{-1}$)	*C. vulgaris*		*P. subcapitata*		*S. salina*		*M. aeruginosa*	
		RR ($mg_P\ L^{-1}\ d^{-1}$)	R (%)	RR ($mg_P\ L^{-1}\ d^{-1}$)	R (%)	RR ($mg_P\ L^{-1}\ d^{-1}$)	R (%)	RR ($mg_P\ L^{-1}\ d^{-1}$)	R (%)
15	15	0.110 ± 0.013	13.5 ± 1.6	0.0505 ± 0.0154	6.18 ± 1.74	0.0171 ± 0.0092	1.97 ± 1.09	0.00944 ± 0.00035	1.13 ± 0.03
	21	0.0934 ± 0.0607	11.8 ± 7.2	0.220 ± 0.044	26.2 ± 4.3	0.107 ± 0.026	10.9 ± 2.5	0.120 ± 0.060	12.4 ± 5.7
	36	0.265 ± 0.037	32.7 ± 4.5	0.158 ± 0.087	20.6 ± 12.2	0.126 ± 0.047	13.4 ± 5.1	0.182 ± 0.067	18.3 ± 6.2
	75	0.275 ± 0.025	29.5 ± 3.0	0.0751 ± 0.0061	9.47 ± 0.67	0.386 ± 0.089	44.6 ± 9.5	0.416 ± 0.031	26.3 ± 2.1
	105	0.255 ± 0.130	29.1 ± 12.3	0.157 ± 0.068	20.1 ± 9.7	0.215 ± 0.034	20.9 ± 4.4	0.389 ± 0.050	37.8 ± 0.9
	180	0.387 ± 0.010	44.2 ± 1.0	0.252 ± 0.073	27.5 ± 6.0	0.275 ± 0.008	29.1 ± 1.0	0.255 ± 0.027	21.4 ± 3.1
25	15	0.149 ± 0.035	16.9 ± 3.4	0.268 ± 0.115	17.5 ± 7.9	0.157 ± 0.007	17.3 ± 0.6	0.109 ± 0.081	13.4 ± 8.8
	21	0.258 ± 0.019	29.3 ± 1.6	0.223 ± 0.057	24.0 ± 9.6	0.222 ± 0.034	23.9 ± 3.0	0.279 ± 0.081	28.8 ± 6.6
	36	0.279 ± 0.092	29.3 ± 7.4	0.259 ± 0.056	34.2 ± 4.9	0.316 ± 0.034	35.4 ± 3.4	0.255 ± 0.068	29.7 ± 6.0
	75	0.240 ± 0.191	24.9 ± 18.4	0.235 ± 0.018	27.0 ± 2.0	0.231 ± 0.064	33.9 ± 0.6	0.218 ± 0.050	26.3 ± 5.7
	105	0.240 ± 0.074	31.5 ± 4.0	0.279 ± 0.020	32.7 ± 2.0	0.345 ± 0.035	32.0 ± 4.8	0.231 ± 0.039	25.8 ± 2.1
	180	0.588 ± 0.029	67.6 ± 7.1	0.393 ± 0.070	51.2 ± 4.8	0.348 ± 0.018	36.7 ± 4.3	0.357 ± 0.074	41.1 ± 9.2
35	15	0.0767 ± 0.0300	7.76 ± 2.60	0.0785 ± 0.0109	7.89 ± 0.67	0.0642 ± 0.0495	6.67 ± 4.98	0.063 ± 0.049	6.56 ± 4.90
	21	0.160 ± 0.017	16.4 ± 3.0	0.143 ± 0.026	14.6 ± 3.5	0.167 ± 0.029	16.8 ± 4.1	0.137 ± 0.027	13.1 ± 3.4
	36	0.171 ± 0.047	16.8 ± 3.9	0.184 ± 0.070	17.5 ± 5.6	0.188 ± 0.066	17.9 ± 5.4	0.157 ± 0.060	15.0 ± 5.1
	75	0.895 ± 0.015	21.0 ± 1.7	0.0968 ± 0.0213	9.84 ± 2.07	0.378 ± 0.006	42.9 ± 0.8	0.282 ± 0.030	26.1 ± 2.5
	105	0.316 ± 0.021	33.3 ± 2.0	0.241 ± 0.020	26.6 ± 2.2	0.194 ± 0.036	21.0 ± 4.6	0.352 ± 0.027	36.0 ± 2.5
	180	0.278 ± 0.063	38.3 ± 14.1	0.440 ± 0.067	38.7 ± 4.3	0.210 ± 0.046	22.7 ± 4.3	0.543 ± 0.072	54.2 ± 3.2

[a] Values are presented as the mean ± standard deviation of two independent experiments.

Table 5 Mass fractions of nitrogen (α_N, in $g_N\ g_{dw}^{-1}$) and phosphorus (α_P, in $g_P\ g_{dw}^{-1}$) incorporated in the biomass of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* obtained through the mass balance performed for each nutrient[a]

Temperature (°C)	Average daily light irradiance ($\mu E\ m^{-2}\ s^{-1}$)	*C. vulgaris*		*P. subcapitata*		*S. salina*		*M. aeruginosa*	
		α_N ($g_N\ g_{dw}^{-1}$)	α_P ($g_P\ g_{dw}^{-1}$)	α_N ($g_N\ g_{dw}^{-1}$)	α_P ($g_P\ g_{dw}^{-1}$)	α_N ($g_N\ g_{dw}^{-1}$)	α_P ($g_P\ g_{dw}^{-1}$)	α_N ($g_N\ g_{dw}^{-1}$)	α_P ($g_P\ g_{dw}^{-1}$)
15	15	0.142	0.0239	0.0278	0.0122	0.0505	0.00311	0.0950	0.00181
	21	0.0680	0.0113	0.0374	0.0372	0.116	0.0170	0.0941	0.0136
	36	0.102	0.0161	0.0498	0.0166	0.0689	0.0106	0.0772	0.0116
	75	0.0288	0.0105	0.0767	0.00807	0.0689	0.0184	0.0675	0.0240
	105	0.0892	0.0108	0.146	0.0136	0.100	0.00927	0.0748	0.0156
	180	0.0524	0.00793	0.0583	0.00623	0.0675	0.00797	0.0650	0.00643
25	15	0.0298	0.00412	0.0515	0.0129	0.0445	0.00548	0.0425	0.00326
	21	0.0373	0.00570	0.0558	0.0100	0.0560	0.00669	0.0452	0.00692
	36	0.0328	0.00377	0.0679	0.00672	0.0495	0.00552	0.0450	0.00397
	75	0.0349	0.00348	0.0444	0.0053	0.0441	0.00416	0.0390	0.00329
	105	0.0286	0.00248	0.0343	0.0044	0.0348	0.00473	0.0281	0.00266
	180	0.0200	0.00485	0.0329	0.00545	0.0189	0.00334	0.0245	0.00345
35	15	n.a.	0.0151	n.a.	0.219	n.a.	0.0130	n.a.	0.0158
	21	0.0192	0.0235	n.a.	0.124	0.00856	0.0171	0.000127	0.0139
	36	0.0452	0.0160	0.0660	0.275	0.0254	0.0145	0.00638	0.0115
	75	0.0286	0.00607	0.0494	0.00595	0.132	0.0224	0.0866	0.0167
	105	0.0343	0.00675	0.0534	0.00735	0.0420	0.00631	0.0214	0.00407
	180	0.0526	0.00608	0.0747	0.0169	0.0422	0.00711	0.0689	0.0175

[a] n.a. – not applicable.

These results have shown that the influence of light supply and temperature on nitrogen and phosphorus removal is similar to the one observed for specific growth rates, maximum biomass concentrations and biomass productivities, paralleling photosynthetic activity. Microalgae and cyanobacteria require high amounts of nitrogen and phosphorus for proteins, which account for 40–60% of cell dry weight, nucleic acids and phospholipids synthesis,[3] meaning that an increase in the photosynthetic activity may result in an increased assimilation of both nitrogen and phosphorus. Regarding the performance of the studied microorganisms in nitrogen and phosphorus removal, average removal rates and removal efficiencies were not statistically different ($p > 0.05$). Additionally, it was observed that the majority of cultures grown at 25 °C, under the highest light supplies have effectively removed nitrogen. These results constitute important findings for the application of microalgal/cyanobacterial cultures in the tertiary treatment step of wastewater treatment plants.

The mass balance written for nitrogen and phosphorus allowed the determination of the mass fractions of these nutrients in the biomass for each of the studied conditions (Table 5). Mass fractions of nitrogen and phosphorus were close to those reported in the typical composition of microalgal biomass ($CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$): 6.59 $g_N\ g_{dw}^{-1}$ and 1.33 $g_P\ g_{dw}^{-1}$ for nitrogen and phosphorus, respectively.[47] To have a better understanding about the effects of light and temperature on nitrogen and phosphorus contents on microalgal/cyanobacterial biomass, contour graphs relating these variables were obtained for the selected microorganisms (Fig. S2 and S3, ESI†). Additionally, these parameters were analysed through multiple linear regression to evaluate which parameters significantly influence nitrogen and phosphorus mass fractions (Table S2, ESI†). From these data, it is possible to conclude that the effect of light and temperature on the biochemical composition of microalgal/cyanobacterial biomass presented some differences between the studied microorganisms. These observations are in agreement with the study performed by Goldman,[48] who concluded that the relationship between nitrogen contents and temperature may be species specific. Regarding nitrogen mass fractions, temperature appears as the most important factor influencing this parameter: (i) in the case of *C. vulgaris* and *S. salina*, an increase in temperature results in lower nitrogen mass fractions; (ii) in *P. subcapitata*, both light and temperature have not significantly influenced ($p > 0.05$) nitrogen mass fractions; and (iii) in *M. aeruginosa*, an increase in light and temperature results in lower nitrogen mass fractions and, on the other hand, the simultaneous increase in both light and temperature results in higher nitrogen mass fractions. As for nitrogen mass fractions, phosphorus mass fractions were also mainly influenced by temperature: (i) in *C. vulgaris*, an increase in temperature results in a decrease of phosphorus mass fractions, with the minimum value reached at approximately 25 °C, and the simultaneous increase in both light and temperature results in lower phosphorus mass fractions; (ii) in *P. subcapitata*, phosphorus mass fractions had a similar behaviour to the one described for nitrogen mass fractions in *M. aeruginosa*; and (iii) in *S. salina* and *M. aeruginosa*, an increase in temperature results in a decrease of phosphorus mass fractions, with the minimum value reached at approximately 25 °C. These results indicate that environmental factors, such as light and

temperature, not only affect the photosynthetic activity and biomass productivities, but also cell metabolism and, consequently, biochemical composition, as previously reported by Hu.[9] The preponderance of temperature influence on nitrogen and phosphorus mass fractions behaviour suggests that these parameters were not strongly influenced by average daily light irradiance. Similar results were already reported by Mortensen *et al.*[49] In this study, nitrogen and phosphorus mass fractions determined for batch cultures of *Chaetoceros gracilis* grown with different light intensities at 28 °C were not statistically different. The decrease of nitrogen and phosphorus mass fractions with increasing temperatures, which was common for the majority of the selected microorganisms has already been reported in the literature. In the study performed by Fu *et al.*[50] an increase in temperature from 20 to 24 °C resulted in a decrease in nitrogen and phosphorus mass fractions in the cyanobacteria *Synechococcus* sp. The U-shape response observed for some microorganisms has also been described in the literature. According to Hu,[9] at temperatures below and above the optimal growth temperature, microalgae and cyanobacteria require higher amounts of nutrients, such as nitrogen and phosphorus, to achieve the same growth rates as those reported for optimal temperatures. Accordingly, nitrogen and phosphorus mass fractions tend to be lower at the optimal growth temperature, which was, in this study, around 25 °C.

3.3. Optimal light and temperature conditions determined through mathematical modelling

Optimal growth conditions (average daily light irradiance and temperature) for the selected microalgae and cyanobacteria were determined. For this, the model described by eqn (9) was applied and surface graphs (Fig. 2) relating specific growth rates with average daily light irradiance and temperature were obtained. Analysis of Fig. 2 shows that an increase in average daily light irradiance results in increased specific growth rates, with optimal average daily light irradiances varying according to the studied species. Regarding the effect of temperature on specific growth rates, Fig. 2 evidences a similar behaviour between the studied microorganisms. When temperature increases from 15 to 35 °C, specific growth rates tend to increase until

Table 6 Optimal growth conditions (average daily light irradiance and temperature) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* through mathematical modelling[a]

	C. vulgaris	*P. subcapitata*	*S. salina*	*M. aeruginosa*
μ_{max} (d^{-1})	1.30	1.21	1.14	1.02
I_{opt} ($\mu E\ m^{-2}\ s^{-1}$)	208	258	178	140
T_{opt} (°C)	25.4	23.7	26.4	25.6
σ (°C)	7.0	7.0	7.2	8.2
RMSE (d^{-1})	0.294	0.198	0.319	0.255
n	29	27	18	18
Model validation				
RMSE (d^{-1})	0.393	0.283	0.260	0.182
n	9	9	6	6

[a] These values were obtained through application of the developed model regarding the effect of light irradiance and temperature on specific growth rates. μ_{max} – maximum specific growth rate; I_{opt} – optimal average daily light irradiance value for microalgal/cyanobacterial growth; T_{opt} – optimal temperature for microalgal/cyanobacterial growth; σ – standard deviation associated to the optimal temperature; RMSE – root mean squared error; n – data size.

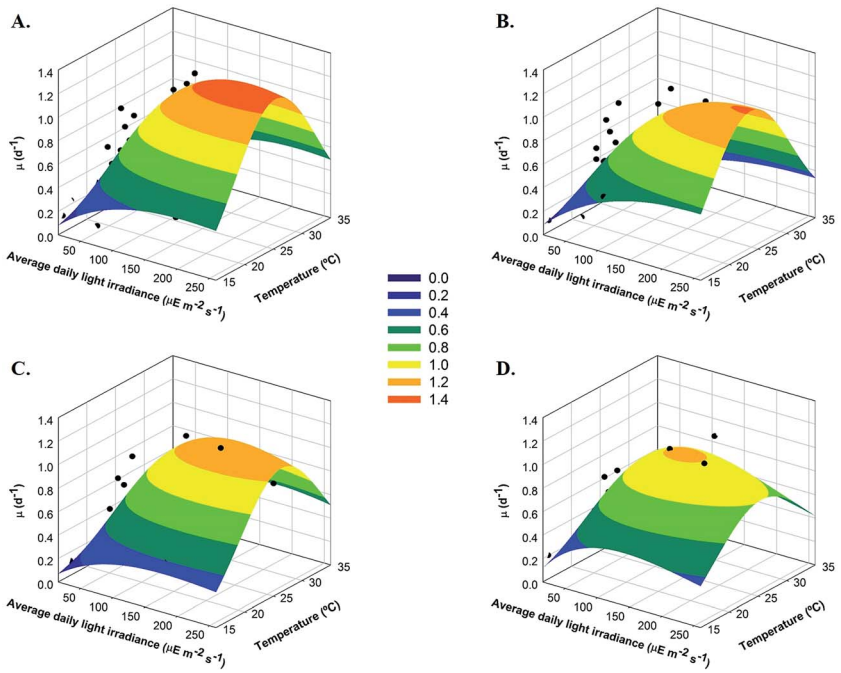

Fig. 2 Influence of average daily light irradiance and temperature on specific growth rates of *C. vulgaris* (A), *P. subcapitata* (B), *S. salina* (C) and *M. aeruginosa* (D). The dots correspond to the experimental data. The surface graphs were obtained through mathematical modelling.

approximately 25 °C, where specific growth rates start decreasing, reaching values close to those observed at 15 °C.

Optimal average daily light irradiance and temperature determined through mathematical modelling for each microorganism are shown in Table 6. For determination of these parameters, it was assumed that maximum specific growth rates achieved by each microorganism could not be lower than the maximum specific growth rate value determined for each microalgal/cyanobacterial strain: 1.30, 1.13, 1.14 and 1.02 d^{-1} for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*, respectively. Definition of this condition was based on the fact that each microalgal species usually presents a maximum specific growth rate, which is obtained under optimal growth conditions.[51] From Table 6, it is possible to observe that optimal temperatures determined for the studied microorganisms were very similar. T_{opt} values determined through mathematical modelling for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* were 25.4, 23.7, 26.4 and 25.6 °C, respectively. These values were slightly lower than optimal temperature determined for *C. vulgaris* growth in the study performed by Dauta *et al.*[34] In this study, for a maximum specific growth rate of 1.30 d^{-1}, optimal temperature determined for *C. vulgaris* was 30 °C. However, other studies reported optimal growth temperatures close to 25 °C. In the study performed by Claquin *et al.*,[52] average optimal temperature determined for eight species of marine microalgae (*Thalassiosira pseudonana*, *Skeletonema marinoi*, *Pseudo-nitzschia fraudulenta*, *Emiliania huxleyi*, *Isochrysis galbana*, *Isochrysis* aff. *galbana*, *Pavlova lutheri* and *Lepidodinium chlorophorum*) was 23.7 ± 3.1 °C, corresponding to a maximum specific growth rate of 1.27 ± 0.27 d^{-1}. Yang *et al.*[40] demonstrated that *C. vulgaris* can grow normally in the temperature range of 5 to 30 °C, being optimal growth temperature 25 °C. Through mathematical modelling, Aleya *et al.*[53] determined an optimal growth temperature for *Chlorella minutissima* of 28 °C, corresponding to a maximum specific growth rate of 0.7 d^{-1}. Regarding optimal average daily light irradiances determined using this model, Table 6 shows that I_{opt} values differ according to microalgal/cyanobacterial species, being 208, 258, 178 and 140 μE m^{-2} s^{-1} for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*, respectively. Similar orders of magnitude have already been reported in the literature for several microalgae and cyanobacteria. Optimal average daily light irradiance values determined by Dauta *et al.*[34] for *C. vulgaris*, *Fragilaria crotonensis*, *Staurastrum pingue* and *Synechocystis minima* ranged from 78 to 169 μE m^{-2} s^{-1}. On the other hand, optimal average daily light irradiances determined for *Selenastrum minutum*, *Coelastrum microporum f. astroidea* and *Cosmarium subprotumidum* ranged from 250 to 263 μE m^{-2} s^{-1}.[51] However, optimal average daily light irradiance determined for *C. vulgaris* and *P. subcapitata* surpassed the range of values assessed in this study, meaning that optimal growth of these microalgae is expected to occur for an average daily light irradiance of 208 and 258 μE m^{-2} s^{-1}, respectively. Although these results were not validated experimentally, it is possible to propose that the established models can be correctly applied to describe the response of specific growth rates of the studied microorganisms to light and temperature. In fact, optimal light and temperature conditions determined are in accordance with the ones already reported in the literature. Additionally, the low RMSE values determined (ranging from 0.198 to 0.319 d^{-1}) indicate that these models correctly fit to the experimental data. Nevertheless, the current models were validated by evaluating the RMSE values obtained between specific growth rates determined by these models and a validation data set composed by specific growth rates determined in different light and temperature conditions (Table S3, ESI†). With the current models, RMSE values determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* were 0.294, 0.198, 0.319 and 0.255 d^{-1}, respectively. On the other hand, RMSE determined through application of this model to data obtained from other studies (validation data set) was 0.393, 0.283, 0.260 and 0.182 d^{-1}, respectively. These results indicate that the developed model can be correctly applied to the studied microorganisms grown under light and temperature conditions within the range of those reported in this study. Additionally, in this study specific mathematical models were determined for different microalgal/cyanobacterial species. Determination of an adequate model that describes microalgal/cyanobacterial growth in relation to light supply and temperature may result in several savings, especially in the optimization of cultivation conditions.

4. Conclusions

In this study, the effects of average daily light irradiance and temperature on microalgal/cyanobacterial growth and nutrients (nitrogen and phosphorus) uptake was evaluated. The results have shown that increased light supplies favour both biomass productivities and nutrients removal. Regarding the temperature effect, it was observed that the studied microorganisms presented higher photosynthetic activity at 25 °C. Among the studied microorganisms, *C. vulgaris*, *S. salina* and *M. aeruginosa* have shown to be the most effective in biomass production. Development of a mathematical model able to describe the behaviour of specific growth rates in response to average daily light irradiance and temperature allowed the determination of optimal light and temperature conditions for the selected microalgae and cyanobacteria. This mathematical approach can be correctly applied to the selected microorganisms under light and temperature conditions within the range of those used in this study, providing the rapid determination of optimal growth conditions and reducing the time and costs associated to the optimization of culture parameters.

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