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Effects of light intensity and temperature on photoautotrophic growth of a green microalga, *Chlorococcum littorale*

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ABSTRACT

A highly CO_2-tolerant green alga, *Chlorococcum littorale*, was investigated at temperatures ranging from 8 to 28 °C, light intensities from 30 to 170 $\mu mol\,m^{-2}\,s^{-1}$, a constant CO_2 concentration of 5% (v/v) and atmospheric pressure. The experimental results showed that a specific growth rate μ, defined in terms of cell growth rate under a logarithmic growth phase, increased with temperature up to the maximum value (*ca.* 22 °C), while the μ decreased at higher temperatures. These promotion and inhibition of the cell growth rate were expressed by both a multiple linear regression and a mathematical model taking account of the Arrhenius activation/deactivation energies. Light intensity affected on the cell growth was independently treated in the mathematical model. The proposed growth model agreed well with the experimental data to within 6.6 %, which provides good correlation for both temperature and light intensity effects on the microalgal cell growth.

1. Introduction

Algae have many potentials to be studied with a view toward food science and technology [1], energy [4,7], environment and global warming issues [12], since they can produce valuable metabolites like carbohydrates, proteins, lipids and pigments using highly efficient photosynthesis processes [16]. In these days, researches on microalgae for multifunctional applications have been increased, which can be derived from their advantages of fast growth and good environmental adaptability over normal land biomass resources. High-density growth can also be one of their advantages that could allow efficient production of the above-mentioned biochemicals in opened and/or closed culture systems [14,19].

Among many microalgal species existing in the nature, *Chlorococcum littorale* is one of candidates leading to high density cultures, because the cells can thrive at concentration growing up to 84 g/dm^3 by using a flat-panel photobioreactor from literature [2]. They also possess a high CO_2-tolerance up to 65 % of CO_2 concentration [5,9,10], although most microalgae thrive at suitable CO_2 concentrations, likely from 1 to 5% (v/v). The reason for the high CO_2-tolerance can be evaluated by the growth rate in the logarithmic growth phase under photoautotrophic culture systems. Kurano and Miyachi collected experimental data for evaluating CO_2-tolerance and applied to a growth inhibition model describing the growth rate of *C. littorale* [8]. The model was able to represent the experimental growth rate data at CO_2 concentrations lower than 20%, however, results at higher CO_2 concentrations do not allow sufficient evaluation. Our previous report found that the cell growth model agreed well with the experimental data under rich HCO_3-/CO_2 conditions [10]. Moreover, at these conditions, production of fatty acids were also promoted during nitrate deficient conditions in culture media based on artificial sea water and low dissolved oxygen concentrations to prevent photorespiration [9,11].

It is fundamentally another issue of debate to evaluate effects of temperature and light intensity on the *C. littorale* growth. The growth rate of this strain in the logarithmic growth phase was highly maintained at even extra-high light intensities (<2000 $\mu mol\,m^{-2}\,s^{-1}$), which was reported in literature [8]. Although temperature effect was also investigated [8], the interaction between the effects of light intensity and temperature was not reported on the *C. littorale* growth. For *Nannochloropsis oculata*, however, the interaction between temperature and light intensity was not observed from both experimental data analyzed by a statistical regression [17], although the findings are different from the report on *Microcystis aeruginosa* growth [20].

Evaluation of complex parameters related to the microalgal growth rate was needed to develop culture systems. For this aim, a green microalga *C. littorale* was chosen for study because the strain possesses a high CO_2 uptake rate [2] in addition to the above-mentioned advantages [6]. Experiments were planned to prevent

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photorespiration with having ignored influence of dissolved oxygen gas and to develop a new growth model applicable to a wide range of temperature and light intensity conditions. To evaluate both temperature and light intensity effects and their interaction on *C. littorale* growth, a multiple linear regression analysis was firstly examined using the experimental data. For this regression process, a simple experimental design method was used for setting the experimental conditions of the variables to examine the correlation between the two variables with respect to cultural growth rate. Since a lot of mathematical models have been proposed to elucidate temperature and light intensity effects, models that describe the obtained experimental data with high accuracy was secondly examined for evaluating *C. littorale* growth.

2. Materials and methods

A green unicellular alga *C. littorale* (NBRC 102761) originally isolated by Marine Biotechnology Institute Co., Ltd. was used as received from National Institute of Technology and Evaluation (NBRC, Japan). Culture medium was based on the Daigo IMK medium (Nihon-Seiyaku Co., Ltd.) in artificial seawater made by mixing the Daigo SP (Nihon-Seiyaku Co., Ltd.) with the distilled water. The detailed experimental procedure was shown elsewhere [10]. A 10 cm^3 of preculture sample maintained at 22 °C and a photosynthetic photon flux density (PPFD) of *ca.* 14 $\mu mol\,m^{-2}\,s^{-1}$ using a LED light source (TAITEC corporation, LC-LED450W) was added aseptically to a 200 cm^3 of fresh culture medium within a flask. The flask was placed on the LED light source, illuminated at a given intensity, *I*, within an uncertainty of ±5 $\mu mol\,m^{-2}\,s^{-1}$ in an air bath (TAITEC, BR-43) where temperature, *T*, was controlled at a given temperature to within an uncertainty of ±0.5 °C. A flow rate of bubbling gas was regulated at 100 $cm^3\,min^{-1}$ at 100 kPa and CO_2 concentration was fixed at 5% by mixing CO_2 (99.5%) and N_2 (99.9%). Growth rate was monitored by means of changes in turbidity of the culture medium (optical density at 750 nm: OD_{750}) using a spectrophotometer (JASCO, V-570). Biomass concentration *X* [g-dry weight/dm^3] at a given incubation time was determined from a calibration curve between the available OD_{750} and the biomass concentration (g-dry weight/dm^3). Initial biomass concentration, X_0, was *ca.* 1.5 ± 1.0 mg/dm^3 for each experiment.

Fig. 1 and Table 1 show the experimental temperature and light intensity conditions chosen in this work. This work focused on temperature and light intensity as key variables to optimize the culture conditions of *C. littorale*. Since there is a possibility of having interactive relation between these two variables, a simple experimental design method was used for setting the experimental conditions of the variables to examine the correlation between the two variables with respect to cultural growth rate. Firstly, we set a central condition as 22 °C and 100 $\mu mol\,m^{-2}\,s^{-1}$ with considering our experimental set up capability, then set a unit scale as 3 °C for temperature and 35 $\mu mol\,m^{-2}\,s^{-1}$ for light intensity by considering the weight of effect of each variable on algal cultivation. Consequently, we varied the temperature form −2 to +2 scales and the light intensity from −2 to +2 scales with 1.0 interval, leading to 9 conditions as shown by filled square symbols in Fig. 1.

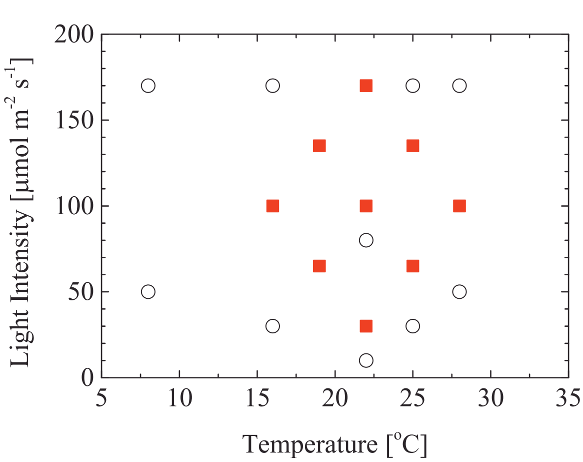

Fig. 1. Experimental temperature and light intensity conditions chosen in this study. Symbol of square and circle show the conditions selected with the experimental design method and the extra experiments, respectively.

Table 1
Correlation of the relative growth rate μ under different temperature and light intensity conditions.

Run	Number of data	Temperature [°C]	Light intensity [$\mu mol\,m^{-2}\,s^{-1}$]	μ [h^{-1}]	R^2 [–]
1	6	16	100	0.066	0.995
2	6	19	65	0.073	0.998
3	5	19	135	0.087	0.996
4	6	22	30	0.051	0.997
5	4	22	100	0.091	0.983
6	6	22	170	0.110	0.999
7	6	22	65	0.077	0.995
8	6	25	135	0.103	0.992
9	4	28	100	0.037	0.993

3. Results and discussion

Fig. 2 shows that a plot of the logarithmic relative biomass concentration, ln X/X_0, against incubation time, *t*, under different temperature and light intensity conditions. For each experiment, ln X/X_0 increased proportionally with incubation time, of which corresponds to a logarithmic growth phase. The growth rates increased with light intensity, but varied with temperature conditions. With regard this point, a multiple linear regression analysis was carried out for discussion. Because temperature and light intensity have dimension and the absolute values are different each other, a normalization technique was adopted to the experimental data as follows:

$$x_1 = \frac{(T - T_0)}{\Delta T} = \left(\frac{T - 22}{6}\right) \quad (1)$$

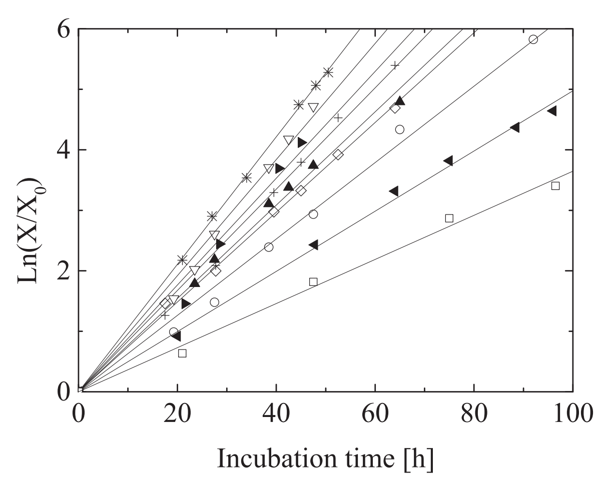

Fig. 2. Logarithmic relative concentration, ln (X/X_0), as a function of incubation time. Symbols show circles for Run 1, diamonds for Run 2, + marks for Run 3, left pointing triangles for Run4, right pointing triangles for Run 5, asterisks for Run 6, triangles for Run 7, upside-down triangles for Run 8 and squares for Run 9, respectively.

Table 2a
Correlation of the relative growth rate μ under different conditions.

Run	Number of experiments for the calculation	Number of variables in the model	Prob. > F
Model A	9	6	0.129
Model B	9	3	0.008

$$x_2 = \frac{(I - I_0)}{\Delta I} = \left(\frac{I - 100}{70}\right) \quad (2)$$

T_0, I_0, ΔT and ΔI were determined from the experimental set up to be 22 °C and 100 μmol m^{-2} s^{-1}, 6 °C and 70 μmol m^{-2} s^{-1}, respectively. Thus, an average of the normalized data for each x value was 0 and a maximum absolute deviation was 1.

To begin with discussion about interaction between temperature and light intensity on the *C. littorale* growth rate, the following equation was formulated:

$$y = a_0 + \sum_{i=1}^{2} a_i x_i + \sum_{i=1}^{2}\sum_{j=1}^{2} a_{ij} x_i x_j \quad (3)$$

where, x_1 and x_2 as defined in Eq. (3) mean variables of temperature and light intensity, respectively and a values show the coefficient of each variable. A term of $x_1 x_2$ shows the interaction between temperature and light intensity. y means a specific growth rate, μ, as defined in the following equation:

$$y = \mu = \frac{1}{X}\frac{dX}{dt} \quad (4)$$

where X and t are biomass concentration [g-dry weight/dm^3] and incubation time [h], respectively. Standardizing Eq. (4) with X_0 which is the initial biomass concentration leads to the following equation:

$$\mu = \frac{d \ln X/X_0}{dt} \quad (5)$$

The obtained specific growth rate, μ, under different temperature and light intensity conditions were listed in Table 1.

When a regression formula (Eq. (3)) includes unnecessary variable number, the expression was not explained statistically. Therefore, variable numbers were selected for the formulated regression. There are several ways for the selection of variable numbers. The method falls roughly into two categories. One is all possible regression and another is iteration technique which was adopted in this work because of convenient usefulness and the calculated load. Excel 2013 was used for this analysis.

If the F-test for the model after regression is significant at the 5% level (i.e., $P < 0.05$), there is evidence that the model can explain the variation in the response. The regression results as shown in Table 2a for the first calculation (Model A) using all terms in Eq. (3) show that the model cannot satisfactorily explain the effects of two factors on the *C. littorale* growth (Prob. < F = 0.129) although R^2 = (0.878) can be almost suitable. From the resulted coefficient

Table 2b
Calculation results for Model A and B.

Variable	Coefficient	Model A	Model B
–	a_0	0.0969	0.0888
x_1	a_1	−0.0064	–
x_2	a_2	0.0262	0.0262
x_{11}	a_{11}	−0.0440	−0.0349
x_{22}	a_{22}	−0.0440	–
x_{12}	a_{12}	−0.0151	–

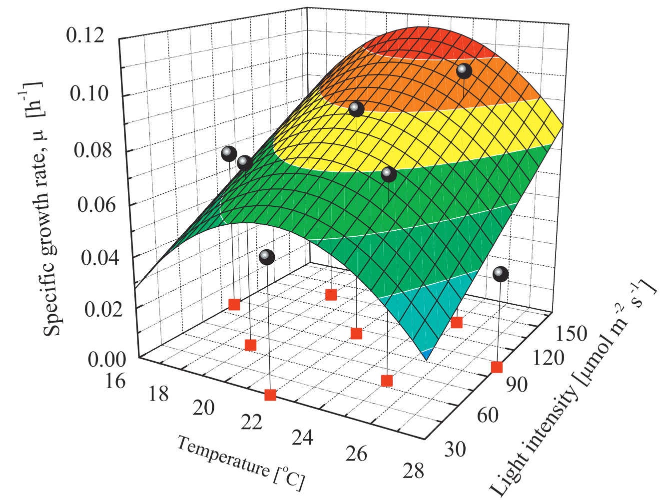

Fig. 3. Experimental data for *C. littorale* growth under different temperature and light intensity conditions accompanied with calculation results by model B.

values as shown in Table 2b, the effects of a_1 and a_{12} were decided to be too small to be removed for better regression. By iteration process, a_{22} can be removed, but if a_{11} was removed, the F value did not become feasible, then the iteration process quitted for the second calculation (Model B). The obtained results listed in Table 2a shows that the model B can satisfactorily explain the above effects (Prob. < F = 0.008). As mentioned above, the F-test for the model after regression is significant at the 5% level (i.e., $P < 0.05$) and there is evidence that the model B can explain the variation in the response. The correlated results using model B, of which fitting parameters were listed in Table 2b, revealed that the experimental data agreed well with the calculation results as shown in Fig. 3. From the correlation, an average relative deviation (ARD) as defined in Eq. (6) was within 14.6 %, meaning that the *C. littorale* growth was not strongly affected by the interaction between temperature and light intensity (a_{12}), which well supported for the discussion of *N. oculata* growth [17], although the findings are different from the report on *M. aeruginosa* growth [20].

$$\mathrm{ARD} = \frac{1}{N}\sum_i \left|\frac{x_{i,\mathrm{exp.}} - x_{i,\mathrm{calc.}}}{x_{i,\mathrm{exp.}}}\right| \times 100 \quad (6)$$

Because another evaluation technique was necessary to elucidate the effects between temperature and light intensity on *C. littorale* growth, the mathematical modeling was examined in

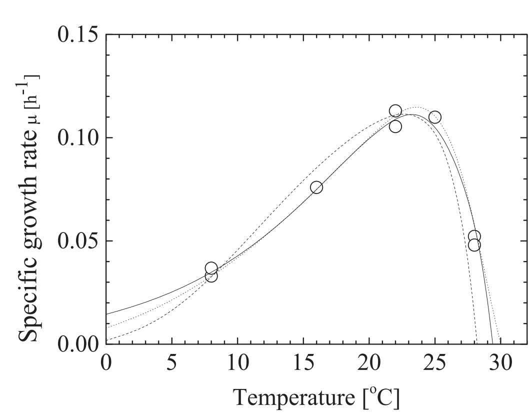

Fig. 4. Specific growth rate μ as a function of temperature. The results of fitting by Eqs. (7)–(9) to the experimental data show continuous line, dashed line and dotted line, respectively.

Table 3
Fitting parameters by each model and the resulted ARD.

Eq. (7)	A_0 [h^{-1}]	B_0 [h^{-1}]	E_a [kJ mol^{-1}]	E_b [kJ mol^{-1}]		ARD [%]
	0.15438	0.04529	71.48	198.5		3.86
Eq. (8)	b [K h$^{-1/2}$]	c [–]	T_{min} [K]	T_{max} [K]		ARD [%]
	0.20964	0.00061	274	303.7		10.2
Eq. (9)	A [K^{-1} h^{-1}]	α [–]	ΔG [kJ mol^{-1}]	T_{max} [K]	B [–]	ARD [%]
	0.00050	37	2.36	302.0	0.514	5.16

this study. Extra experiments were carried out for this purpose, which was shown in Fig. 1. Fig. 4 shows a specific growth rate μ as defined in Eq. (4) as a function of temperature at a light intensity of 170 μmol m^{-2} s^{-1} and a CO_2 concentration of 5% with diluted nitrogen gas. From the Fig. 4, the μ values increased with temperature up to *ca.* 22 °C, although those decreased at the higher temperature conditions.

For modeling the fundamental experimental data, the several mathematical models proposed in literatures were selected. Perez et al. examined Eq. (7) from *Phaeodactylum tricornutum* cultures [13] using the following equation:

$$\mu = A_0 \exp\left[\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right)\right] - B_0 \exp\left[\frac{-E_b}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right)\right] \quad (7)$$

The first term of the right-hand side shows a promotion effect on microalgal growth, while the second term shows an inhibition effect. The reference temperature (T_0) was arbitrary value in the literature and defined at the first treatment as 22 °C chosen in this work. Fitting parameters are frequency factors A_0 and B_0 and activation energies E_a and E_b, respectively.

Ratkowsky et al. proposed a subduplicate empirical model [15] as follows:

$$\mu = [b(T - T_{min})]^2\{-\exp[c(T - T_{min})]\} \quad (8)$$

T_{min} and T_{max} mean a minimum and a maximum temperature at which the cell can grow, respectively and b and c are constants.

Huang et al. proposed combination of Eyring model derived from the Arrhenius equation with the subduplicate empirical model [3] as defined in the following equation:

$$\mu = \mathrm{ATexp}^{-(\Delta G/RT)^a}\{1 - \exp[B(T - T_{max})]\} \quad (9)$$

ΔG means the Gibbs activation energy for cell growth and fitting parameters are A and B. The calculation results with these model were shown in Fig. 4. Available parameters are listed in Table 3. From the ARD analysis with Eq. (6), the Arrhenius-type equation Eq. (7) was the most suitable among the three models because the ARD value is the smallest (3.86%). However, the treatment of T_0 is an issue of debate, and thus, we examined how the fitting is affected by various T_0 as shown in Table 4. From the table, there are not large deviation with the various T_0 changed. Thus, the reference temperature of 22 °C was also adopted in the following section.

Next, we discussed about the effect of light intensity on the *C. littorale* growth with taking account of the above-mentioned Arrhenius-type equation that described the temperature effect

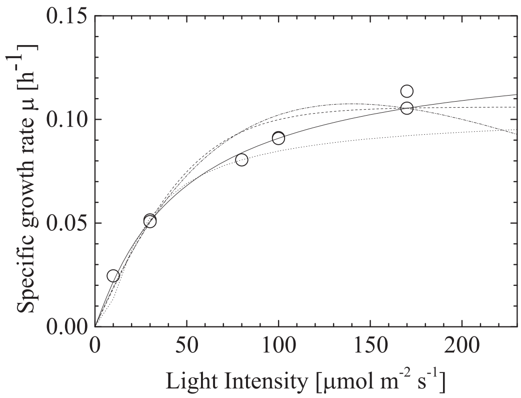

Fig. 5. Specific growth rate μ as a function of light intensity. The results of fitting by Eqs. (10)–(13) to the experimental data show dashed line, dotted line, continuous line and dashed-dotted line, respectively.

well. Fig. 5 shows the specific growth rate μ as a function of light intensity. The μ values increased with light intensity at low light intensities and then the slope was changed and gradually increased with light intensity at high light intensities. According to the experimental data, we selected models from the following 4 types of equations, which would satisfactorily explain the phenomenon.

$$\mu = \mu_m \tanh\left(\frac{I}{I_k}\right) \quad (10)$$

$$\mu = \mu_m \exp\left(\frac{-I}{I_k}\right) \quad (11)$$

$$\mu = \mu_m \frac{I}{K_I + I} \quad (12)$$

$$\mu = \mu_m\left(\frac{I}{I_{opt}}\right)\left\{1 - \exp\left(\frac{-I}{I_{opt}}\right)\right\} \quad (13)$$

Eqs. (10) and (11) include hyperbolic tangent and exponential functions, respectively. Both functions can be used for expression of activity of photosynthesis [8]. For Eq. (12), the functional form resembles the Monod type equation for modeling of enzymatic reactions [18]. Eq. (13) is extended for the expression of exponential function of Eq. (10). We used the four models for comparing with our obtained experimental data, which was shown in Fig. 5. All the models were fitted well with some errors. From the ARD analysis as shown in Table 5, the Monod-type equation in Eq. (12) showed the smallest (3.34%) among the four

Table 4
Fitting parameters of A_0,B_0, E_a and E_b in Eq. (7) estimated as a function of arbitrary T_0 and the resulted ARD.

T_0 [K]	273	293	296 (T_{max})	313	333
A_0 [h^{-1}]	0.015	0.124	0.168	0.838	4.12
B_0 [h^{-1}]	0.000	0.025	0.056	4.60	436
E_a [kJ/mol]	71.2	71.4	71.5	72.4	71.0
E_b [kJ/mol]	198	199	199	199	199
ARD [%]	3.82	3.81	3.78	3.69	3.59

Table 5
Fitting parameters from each equation with ARD.

	μ_m [h^{-1}]	I_k [μmol m^{-2} s^{-1}]	ARD [%]
Eq. (10)	0.106	56.5	8.34
Eq. (11)	0.104	20.4	13.7
	μ_m [h^{-1}]	K_I [μmol m^{-2} s^{-1}]	ARD [%]
Eq. (12)	0.136	49.7	3.34
	μ_m [h^{-1}]	I_{opt} [μmol m^{-2} s^{-1}]	ARD [%]
Eq. (13)	0.108	140	7.79

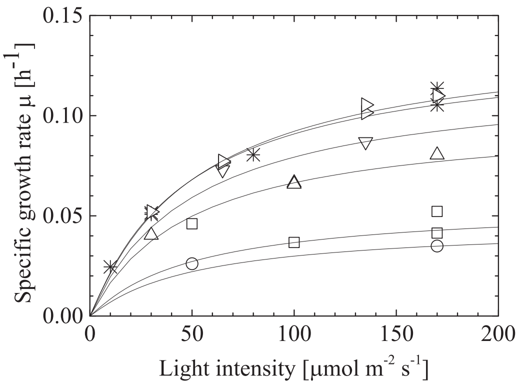

Fig. 6. Specific growth rate μ as a function of light intensity under different temperature conditions. In Figure, symbols show circles for 8 °C, triangles for 16 °C, upside-down triangles for 19 °C, asterisks for 22 °C, right pointing triangles for 25 °C and squares for 28 °C, respectively. Continuous line shows the correlation results by Eq. (12).

Table 6
Temperature dependence of fitting parameters with the resulted ARD.

Temperature [°C]	K_I [μmol m^{-2} s^{-1}]	μ_m [h^{-1}]	ARD [%]
8	53.2	0.046	7.48
16	50.0	0.100	3.09
19	51.5	0.120	4.07
22	49.7	0.136	3.34
25	54.0	0.142	1.64
28	52.9	0.056	15.5

models, which describes our experimental data the best. Thus, we chose the Eq. (12) for the next discussion.

Fig. 6 shows that the μ as a function of light intensity under different temperatures with correlation results using Eq. (12) The good agreement was observed in calculations with experimental data. The resulted fitting parameter using Eq. (12) depended on temperature was listed in Table 6. A half-saturation number of K_I was the almost same with the different temperature conditions, while maximum specific growth rate, μ_m, were varied with temperatures. The effect of temperature on the μ_m was plotted in Fig. 7. The μ_m value increased with temperature up to *ca.* 25 °C, while that decreased at the higher temperature conditions. Because the function was very similar to the Arrhenius-type model in Eq. (7), which was as shown in Fig. 4, here we used the following model for this expression:

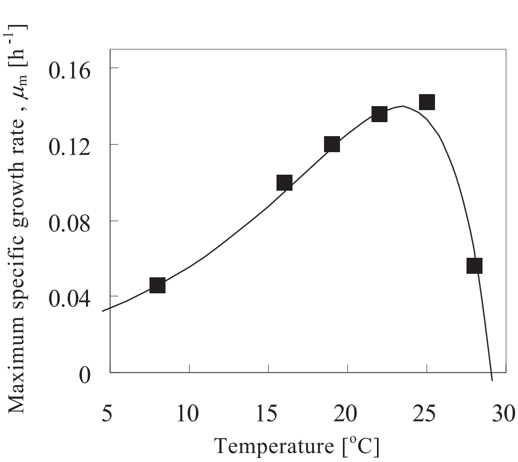

Fig. 7. Maximum specific growth rate, μ_m, as a function of temperature. Continuous line shows the correlation results by using Eq. (14).

Table 7
The obtained fitting parameters with the resulted ARD.

A_0 [h^{-1}]	B_0 [h^{-1}]	E_a [kJ mol^{-1}]	E_b [kJ mol^{-1}]	ARD [%]
0.192	0.079	73.4	198.4	1.52

$$\mu_m = A_0 \exp\left[\frac{-E_a}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right)\right] - B_0 \exp\left[\frac{-E_b}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right)\right] \quad (14)$$

The parameters obtained from fitting to our experimental data were listed in Table 7. The model was a good agreement within ARD of 1.52%. From activation energy balance analysis, a deactivation energy (E_b = 198.4 kJ mol^{-1}) overcame an activation energy (E_a = 73.4 kJ mol^{-1}). From kinetic model for growth of *P. tricornutum*, E_a and E_b showed 117.0 kJ mol^{-1} and 163.0 kJ mol^{-1}, respectively [13] and the orders of activation energies were comparable between our results and literature [13]. Thus, the final expression of the growth model was shown as follows:

$$\mu = \mu_m \frac{I}{K_I + I} = \left[A_0 \exp\left\{\frac{-E_a}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right)\right\} - B_0 \exp\left\{\frac{-E_b}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right)\right\}\right]\frac{I}{K_I + I} \quad (15)$$

As mentioned in Table 6, K_I was independent of temperature and light intensity and assumed to be the averaged value (51.8 μmol m^{-2} s^{-1}). From the obtained parameters in Table 7, the results using the growth model was in Fig. 8. ARD for this calculation within 6.6% showed a good agreement between experimental and calculation to be achieved.

Consequently, the above-mentioned two analyses with regression and mathematical modeling showed that the cell growth of *C. littorale* can discriminate between temperature and light intensity effects. The temperature given for the maximum growth rate for this strain was *ca.* 25 °C and light intensity effect was expressed by the Monod-type equation. The results obtained by mathematical models were well supported by the multiple linear regression analysis.

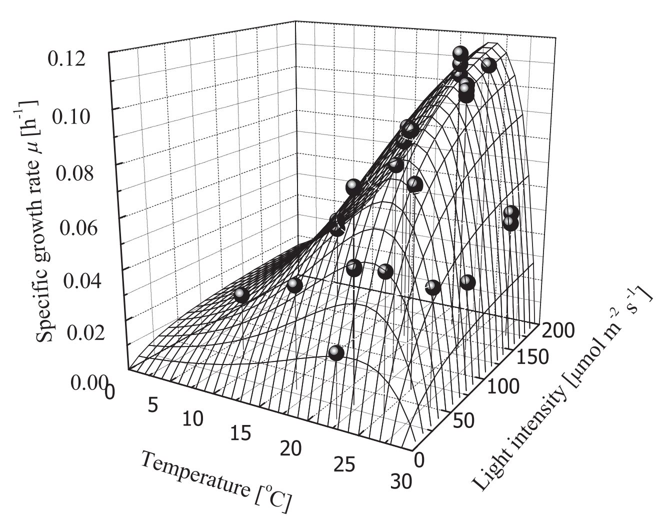

Fig. 8. Specific growth rate, μ, as a function of temperature and light intensity. Symbol of circle shows experimental data with continuous line showing correlation by Eq. (15).

4. Conclusions

A highly CO_2-tolerant green alga, *C. littorale*, was investigated for clarification of the effects of both temperatures and light intensities on the *C. littorale* growth. From analyses by a multiple linear regression, the *C. littorale* growth was affected independently by each temperature and light intensity and not strongly affected by the interaction between temperature and light intensity. A mathematical model taking account of the Arrhenius activation/deactivation energies expressed the temperature-dependent promotion and inhibition of the cell growth rate. Light intensity affected on the cell growth was independently treated in the mathematical model. The proposed growth model agreed well with the experimental data to within 6.6 %, which provides good correlation for both temperature and light intensity effects on the microalgal cell growth.

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