

# Effect of photosynthetically active radiation on *Rhodomonas salina* (Wisłouch) D.R.A.Hill & R.Wetherbee 1989, growth using a mathematical model under laboratory conditions

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**Abstract.** *Rhodomonas salina* (Wisłouch) D.R.A.Hill & R.Wetherbee 1989 is a marine microalga of great importance in aquaculture, used as high-quality live feed. However, light is one of the main factors limiting microalgae growth, as light availability decreases with increasing biomass concentration. Therefore, in microalgae production, factors related to optimal light supply (e.g., light intensity and optical path) must be considered to maximize biomass productivity and cultivation efficiency. For this reason, this study evaluated the effect of light on the growth rate of *R. salina* under culture conditions and correlated the experimental data with a mathematical model developed to generate different operational scenarios and support the design of a photobioreactor prototype suitable for microalgae production in aquaculture. To this end, *R. salina* was cultivated in the laboratory in triplicate under four initial irradiances ( $I_0 = 70, 80, 90,$  and  $100 \mu\text{E m}^{-2} \text{s}^{-1}$ ) at a temperature of  $20^\circ\text{C}$ . The model parameters were determined as  $I_k 28.7 \mu\text{E m}^{-2} \text{s}^{-1}$  and  $K_s 0.17 \text{ m}^2 \text{g}^{-1}$ . Based on these results, Molina's mathematical model was used to predict growth rate as a function of effective irradiance ( $R^2 = 0.966$ ). These results provide useful information for the proper design of photobioreactors, enabling the optimization of biomass productivity under different light and optical path conditions.

**Key Words:** *R. salina*, growth rate, biomass productivity, average irradiance.

**Introduction.** Aquaculture is one of the fastest-growing food production sectors (Boyd et al 2020), and in order to maintain this progress, it is necessary to optimize processes to ensure its sustainability. This is the case with microalgae, photosynthetic microorganisms used in aquaculture to feed zooplankton, mollusks, fish larvae, and crustaceans (Kaparapu 2018).

Microalgae are used for a variety of purposes, from live feed to the extraction of bioactive compounds such as pigments, DHA, polysaccharides, and food colourings (Cai et al 2020). An example of this is the marine microalga *Rhodomonas salina* (Wisłouch) D.R.A.Hill & R.Wetherbee 1989, which has several attributes of interest for aquaculture, such as: lack of cell wall and high content of proteins, lipids and polyunsaturated fatty acids (Guevara et al 2020), used as high-quality live feed for copepods (Drillet et al 2006), rotifers (Guevara et al 2011), and mollusks (Yamamoto et al 2020) as well as being a source of phycoerythrin with potential in food, pharmaceutical and cosmetic applications (Xie et al 2021). *R. salina* has a spherical equivalent diameter of  $6 \mu\text{m}$  to  $9 \mu\text{m}$  (Barreiro et al 2005) and can adapt to a salinity range of 5 to 50 PSU at irradiance levels of  $100\text{-}115 \mu\text{E m}^{-2} \text{s}^{-1}$  (Jepsen et al 2019). Vu et al (2016) suggest cultivating *R. salina* in a range of irradiance between  $60\text{-}100 \mu\text{E m}^{-2} \text{s}^{-1}$  and excess nutrients, as this represents a solution for large-scale production, as is the case with photobioreactors.

Large-scale microalgae production has had major drawbacks related to contamination, pathogens, nutrient deficiency,  $\text{CO}_2$ , oxygen control, temperature, and light (Molina et al 2019). Light is one of the most influential factors in the growth rate of microalgae (Lehmuskero et al 2018; Ahangar et al 2023). In order to optimize the cultivation of *R. salina*, studies have been conducted on the effect of light on growth, such

as that by Vu et al (2016). However, despite the importance of this variable in cultivation, no studies have been reported on modelling the effect of light on growth parameters in *R. Salina*.

Mathematical modelling is considered the most important in relation to microalgal growth, as it can help predict light-dependent growth kinetics (Darvehei et al 2018). As microalgal biomass increases, the light passing through the culture is attenuated due to the absorption and scattering of the photon beam. This means that the cells are not exposed to a single irradiance, but to different irradiances that change over time, defined as the average irradiance ( $I_{av}$ ) in the microalgal culture (Fernández et al 2020). Darvehei et al (2018) highlight models that relate to light, with some studies using average irradiance ( $I_{av}$ ) and others using initial irradiance ( $I_0$ ), without reaching a consensus in the literature on which is more appropriate for correlating microalgal growth as a variable of great importance for obtaining the best possible result.

This study evaluated a mathematical model to analyze the effect of incident light on the cultivation of the microalga *R. salina*, focusing on the irradiance parameters required to obtain  $\mu_{max}/2$  ( $I_k$ ) and the extinction coefficient ( $K_a$ ). The model incorporated both average irradiance ( $I_{av}$ ) and initial irradiance ( $I_0$ ) with the aim of optimizing the production of this microalga.

**Material and Method.** The *R. salina* microalgal strain was obtained from the "Culture Collection of Algae at The University of Texas at Austin (UTEX)", identified with code UTEX LB 2763. The study was conducted at the Psychology and Primary Productivity Laboratory of the University of Nariño, Pasto, Colombia. Seawater was obtained from the Acuimar project at the University of Nariño (Tumaco, Colombia), filtered through a 1  $\mu\text{m}$  felt filter bag, and sterilized using ultraviolet (UV) radiation. A batch culture system was used, starting from 10 mL test tubes and scaled up to a final volume of 3 L. Guillard's F/2 culture medium (Coutteau 1996) was modified for the cultivation of *R. salina* according to the Laboratory of Phycology and Plankton at the University of Nariño. This medium contained the following concentrations:  $\text{NaNO}_3$  300  $\text{mg L}^{-1}$ ;  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  30  $\text{mg L}^{-1}$ ;  $\text{Na}_2\text{C}_{10}\text{H}_{14}\text{O}_8\text{N}_2 \cdot \text{H}_2\text{O}$  ( $\text{Na}_2\text{EDTA}$ ) 4.36  $\text{mg L}^{-1}$ ;  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  0.01  $\text{mg L}^{-1}$ ;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  0.01  $\text{mg L}^{-1}$ ;  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  3.15  $\text{mg L}^{-1}$ ;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  0.18  $\text{mg L}^{-1}$ ;  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  0.006  $\text{mg L}^{-1}$ ;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.022  $\text{mg L}^{-1}$ ; Thiamine HCl 0.1  $\text{mg L}^{-1}$ ; Biotin 0.0005  $\text{mg L}^{-1}$ ; Vitamin B<sub>12</sub> 0.0005  $\text{mg L}^{-1}$ . The biomass of the microalga *R. salina* was maintained at a temperature of 20°C and initial Photosynthetically Active Radiation (PAR) irradiances of 70, 80, 90, and 100  $\mu\text{E m}^{-2} \text{s}^{-1}$  distributed in four treatments with three replicates each, under artificial white LED light, salinity 25 UPS, photoperiod 24h, and pH control 8.0. The experimental units consisted of cylindrical 1 L glass containers with a working volume of 0.8 L of microalgae culture medium and continuous aeration for 8 days.

Biomass concentration was determined by measuring the optical density (OD). According to Lee et al (2013), it is recommended to use a wavelength outside the pigment absorption range, such as 750 nm. This wavelength has been previously used in studies with *Rhodomonas* species (Oostlander et al 2020; Latsos et al 2021b).

The daily growth of the *R. salina* culture was determined by measuring the absorbance at 750 nm of each experimental unit using a GENESYS 30 Visible spectrophotometer and applying the formula developed for this study:

$$\text{g L}^{-1} = 0.7893 \cdot \text{Abs}_{750} + 0.0094$$

Microalgal samples with absorbances values outside the linear range (0.8  $\text{g L}^{-1}$ ) were diluted before measurement by mixing 8 mL of seawater and 2 mL of microalgae from the culture.

The extinction coefficient ( $K_a$ ) was calculated from the average of the  $K_a a$  for each wavelength ( $\alpha$ ) between 400 and 700 nm using a spectrophotometer and the following expression (Morillas et al 2020):

$$K_a \alpha = \frac{\text{Abs}(\alpha)}{C_b \cdot p}$$

Where:

$K_a$ : Extinction coefficient

$\alpha$ : Wavelength

Abs ( $\alpha$ ): Absorbance at a given wavelength

$C_b$ : Biomass concentration ( $\text{g L}^{-1}$ )

$p$ : Optical path or cuvette diameter (0.01 m)

The average daily irradiance was determined using the following expression (Morillas et al 2020):

$$I_{av} = \frac{I_0}{K_a \cdot p \cdot C_b} (1 - \exp(-K_a \cdot p \cdot C_b))$$

Where:

$I_{av}$ : is the average light intensity ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )

$I_0$ : is the initial irradiance at the center of the culture and without water ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )

$K_a$ : is the extinction coefficient of the visible light spectrum ( $\text{m}^2 \text{g}^{-1}$ )

$C_b$ : biomass concentration ( $\text{g L}^{-1}$ )

$p$ : light path within the reactor (m)

The determination of  $I_k$ , defined as the irradiance required to achieve half the maximum growth rate ( $\mu_{\max}/2$ ), was obtained through a regression analysis between average irradiance and growth rate.

The Molina Model (Grima et al 1994) was used to evaluate the relationship between light and growth:

$$\mu = \frac{\mu_{\max} \cdot I_{av}^n}{I_k^n + I_{av}^n}$$

Where:

$\mu$ : is the specific growth rate,  $\text{day}^{-1}$

$\mu_{\max}$ : is the maximum specific growth rate,  $\text{day}^{-1}$

$I_{av}$ : is the average light intensity ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )

$I_k$ : is the irradiance required to obtain  $\mu_{\max}/2$  ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )

$n$ : is a shape parameter.

The parameter  $n$  was set based on the coefficient of determination ( $R^2$ ) that provided the best fit for the linear regression between the experimental growth rate and the Molina model.

A completely randomized design was used, and data are presented as mean  $\pm$  standard error (SE). Biomass productivity ( $P_b$ ) was analyzed using ANOVA after verifying normality, independence, and homogeneity of variances. All  $I_k$  and  $K_a$  data were subjected to a non-parametric Kruskal-Wallis test. In all cases, a significance level of 5% ( $\alpha=0.05$ ) was applied. The statistical software used was RStudio.

## Results and Discussion

**Biomass productivity.** The maximum biomass productivity ( $P_{b_{\max}}$ ) was directly proportional to the initial irradiance ( $p < 0.05$ ), with an irradiance of  $100 \mu\text{E m}^{-2} \text{s}^{-1}$  generating a  $P_{b_{\max}}$  of  $0.23 \text{ g L}^{-1} \text{ d}^{-1}$ , decreasing to  $0.13 \text{ g L}^{-1} \text{ d}^{-1}$  in the  $70 \mu\text{E m}^{-2} \text{s}^{-1}$  treatment (Figure 1).

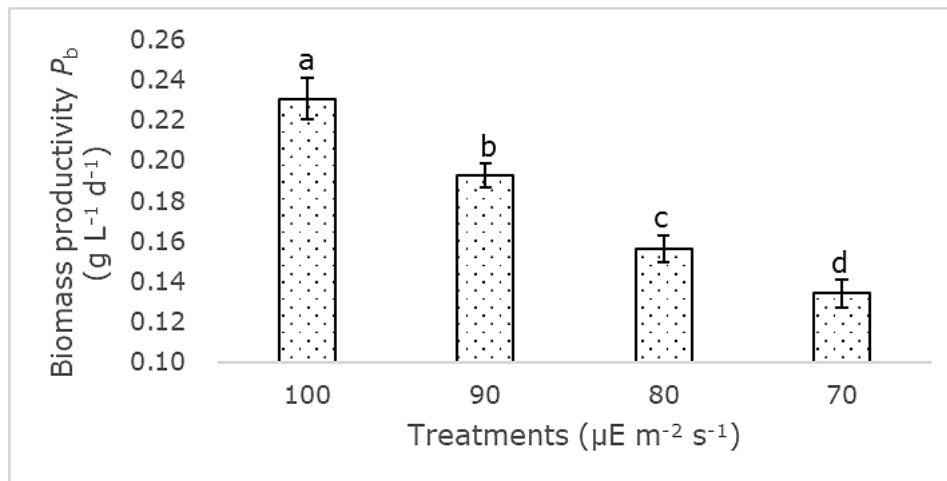


Figure 1. Maximum biomass productivity of *R. salina* cultivated under four initial irradiance levels.

These biomass productivity values are higher than those reported in other studies on *R. salina*, as established by Thoisen (2018) and Oostlander et al (2020), who report values ranging from 0.02 to 0.08  $\text{g L}^{-1} \text{d}^{-1}$  cultivated in a 250 L tubular photobioreactor with a tube diameter of 0.06 m, salinity of 30 PSU (artificial seawater), an ambient temperature of 19°C, and an initial irradiance ( $I_o$ ) between 100 and 250  $\mu\text{E m}^{-2} \text{s}^{-1}$ .

Other studies have reported  $P_{b_{\max}}$  values higher than those obtained in this study, such as Latsos et al (2021b), who achieved a  $P_{b_{\max}}$  of 1.17  $\text{g L}^{-1} \text{d}^{-1}$  in a flat panel airlift-loop photobioreactor with artificial light, an optical path of 1.4 cm, and a 24 h photoperiod. This may be attributed to the shorter optical path used compared to the present study (8 cm), which increases the  $I_{av}$  to a higher  $C_b$ . Additionally, the authors used an initial irradiance of 100  $\mu\text{E m}^{-2} \text{s}^{-1}$ , which was gradually increased to 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and a slightly higher temperature than used in this study (22°C). Another factor that could influence biomass growth and productivity is the color of the light used in the photobioreactor, as suggested by Latsos et al (2021a), who suggest that green light is better utilized by the photosynthetic pigments of *Rhodomonas sp.*, leading to higher  $P_b$ .

**Irradiance required to obtain  $\mu_{\max}/2$ ,  $I_k$ .** The variable  $I_k$  did not show significant differences between treatments ( $p > 0.05$ ), as indicated in Table 1.

Table 1

$I_k$  obtained different initial irradiances in the cultivation of the microalga *R. salina*

$I_k$ $\mu\text{E m}^{-2} \text{s}^{-1}$	Treatments $\mu\text{E m}^{-2} \text{s}^{-1}$			
	100	90	80	70
	28.40±0.35 <sup>a</sup>	28.95±0.25 <sup>a</sup>	28.96±0.28 <sup>a</sup>	29.04±0.25 <sup>a</sup>

Identical letters indicate no significant differences ( $p > 0.578$ ).

Since there was no significant difference between treatments,  $I_{av}$  was plotted against growth rate to estimate a single  $I_k$  with  $\mu_{\max}/2$  (1.2/2). Thus,  $I_k = 28.7 \mu\text{E m}^{-2} \text{s}^{-1}$  (Figure 2).

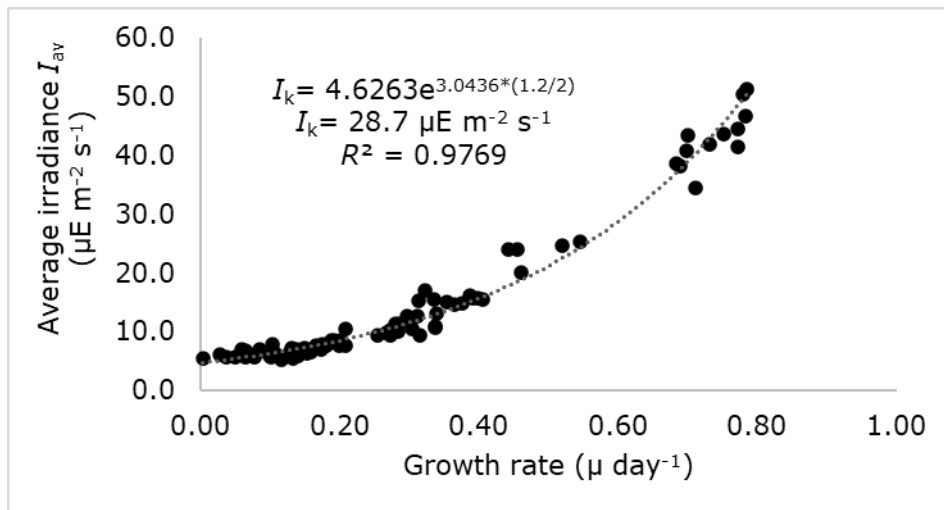


Figure 2. Relationship between growth rate ( $\mu \text{ day}^{-1}$ ) and average irradiance ( $\mu\text{E m}^{-2} \text{ s}^{-1}$ ).

The fact that a single  $I_k$  value was obtained does not agree with the findings reported by Sánchez et al (2008), who suggested that in the microalga *Scenedesmus almeriensis*,  $I_k$  depends on the initial irradiance ( $I_0$ ). In fact, the authors proposed an equation to estimate  $I_k$  dependent on  $I_0$ . The  $I_k$  parameter measures microalgal affinity to light (Grima et al 1994), which is related to photosynthetic efficiency and depends, to a certain extent, on the type and quantity of photosynthetic pigments. This could indicate that in *R. salina*, the photon flux required in  $I_k$  does not depend on the  $I_0$  used, and that changes in photosynthetic efficiency depend more on  $I_{av}$  than on  $I_0$ .

**Extinction coefficient,  $K_a$ .** The extinction coefficient at the beginning ( $K_{a0}$ ) and at the end ( $K_{a1}$ ) of the experiment did not vary between treatments. However, there were significant differences between  $K_{a0}$  and  $K_{a1}$  ( $p < 0.05$ ). Therefore, the prediction of  $K_a$  as a function of  $I_{av}$  could be obtained by plotting  $K_a$  against  $I_{av}$ , as shown in Figure 3. In addition, the highest biomass productivity was observed at  $I_{av} < 20 \mu\text{E m}^{-2} \text{ s}^{-1}$  in the four treatments where  $K_a$  remained constant. For practical purposes of the mathematical model, a  $K_a$  value of  $0.17 \text{ m}^2 \text{ g}^{-1}$  was used to estimate the variables of  $I_{av}$  and  $P_b$ .

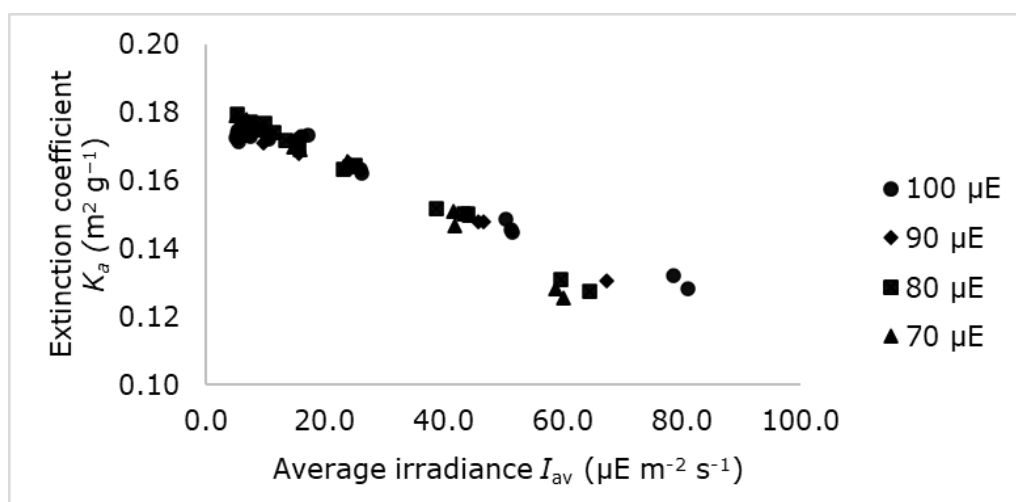


Figure 3. Extinction coefficient  $K_a$  as a function of  $I_{av}$  in *R. salina*.

The attenuation of light in microalgae cultures depends on the absorption coefficient, which is related to the content of photosynthetic pigments (Grima et al 1994). In *R. salina*, it was observed that as the average irradiance decreased, the pigment content increases ( $K_a$ ) as shown in figure 3, similar to the findings of Xie et al (2021), who point out that at light intensities of 20 and  $100 \mu\text{E m}^{-2} \text{ s}^{-1}$ , the phycoerythrin content in *R. salina* cultures

increased with culture time, which may be due to the decrease in photons per cell with increased cell growth (self-shading), at lower irradiance, cells increase their pigments to maximize light capture. However, in this study, average irradiance values below  $20 \mu\text{E m}^{-2} \text{s}^{-1}$  showed similar  $K_a$  values, which could explain the lack of significant differences in  $I_k$ , considering that  $K_a$  is independent of light when  $I_{av}$  is low and approaches  $I_k$ .

**Molina model.** Considering a  $K_a$  of  $0.17 \text{ m}^2 \text{ g}^{-1}$ , an equation for  $I_{av}$  was established as a function of biomass concentration ( $\text{g L}^{-1}$ ) as follows:

$$I_{av} = \frac{I_0}{(0.17).p.C_b.1000} (1 - \exp(-(0.17).p.C_b.1000))$$

A strong correlation was observed between the experimental data and Molina's model ( $R^2=0.966$ ), as shown in Figure 4.

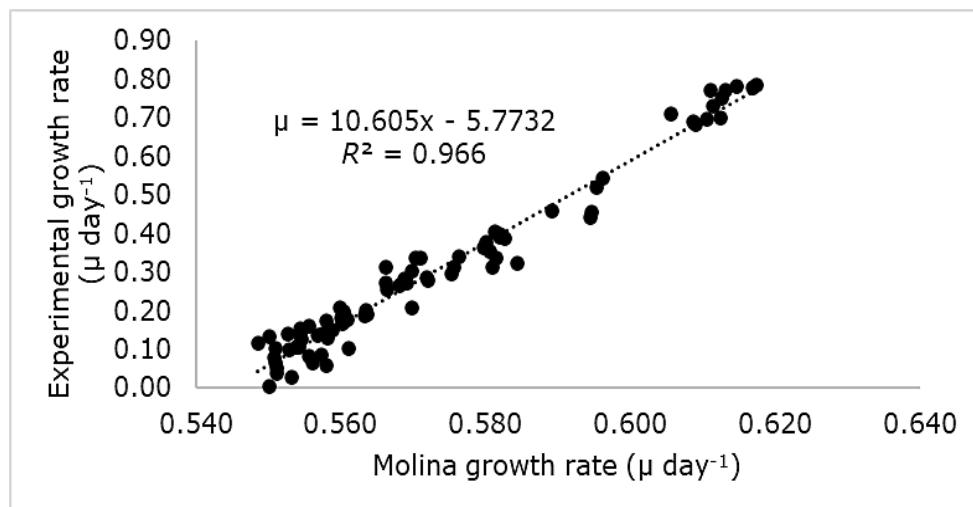


Figure 4. Linear regression between experimental and estimated growth rate ( $\mu \text{ day}^{-1}$ ) in *R. salina*.

In the final model, the average irradiance of the culture was used instead of the initial irradiance, considering  $I_{av}$  generates a good prediction with Molina's model and that both  $I_k$  and  $K_a$  depend on  $I_{av}$  rather than  $I_0$ . However, Darvehei et al (2018) suggested that there is no standardization regarding which parameter should be used to correlate microalgal growth with light energy.

Taking the above into account, the final mathematical model was formulated as follows:

$$\mu = 10.605 * \left[ \frac{1.2 \cdot \left( \frac{I_0}{(0.17).p.C_b.1000} * (1 - \exp(-(0.17).p.C_b.1000)) \right)^{0.1}}{28.7^{0.1} + \left( \frac{I_0}{(0.17).p.C_b.1000} * (1 - \exp(-(0.17).p.C_b.1000)) \right)^{0.1}} \right] - 5.7732$$

Where:

10.605: is the slope of the linear regression between the theoretical and experimental growth rate

-5.7732: is the intercept of the linear regression

$\mu$ : is the specific growth rate,  $\text{day}^{-1}$

1.2: is the maximum specific growth rate,  $\text{day}^{-1}$

$I_{av}$ : is the average irradiance ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )

28.7: is the irradiance required to obtain ( $\mu_{\max}/2$ ) ( $\mu\text{E m}^{-2} \text{s}^{-1}$ )

0.1: is a shape parameter.

Finally, a modified mathematical model was developed in a Microsoft Excel spreadsheet to predict  $I_{av}$ ,  $\mu$ , and  $Pb_{max}$  of *R. salina* between initial irradiances of 70 and 100  $\mu\text{E m}^{-2} \text{s}^{-1}$ , at a temperature of 20 °C, and with a defined optical path length.

Considering the maximum biomass productivity of 1.17  $\text{g L}^{-1} \text{d}^{-1}$  reported by Latsos et al (2021b) with *R. salina*, the model proposed in this study allowed us to generate an approximate prediction of  $Pb_{max}$  1.09  $\text{g L}^{-1}$  with an optical path length of 1.4 cm and initial irradiance of 100  $\mu\text{E m}^{-2} \text{s}^{-1}$  (Figure 5), a result similar to the  $Pb_{max}$  mentioned by the researchers.

This suggests that increasing biomass productivity in microalgae cultivation involves, on one hand, decreasing the optical path of light and, on the other hand, increasing the initial irradiance without affecting photoinhibition. However, authors such as Saccardo et al (2022) suggest that an excessive decrease in the optical path can lead to an increased photoinhibition, establishing a limit of 2 to 8 mm, and that to obtain an adequate light-dark cycle regime, a path greater than 35 mm should be maintained. This could indicate that larger optical path lengths would have an advantage over thinner reactors, such as in the case of handling and cleaning procedures, which could be difficult when the width of the photobioreactor is reduced. Therefore, the optical path length should be selected based on a combination of handling and productivity aspects.

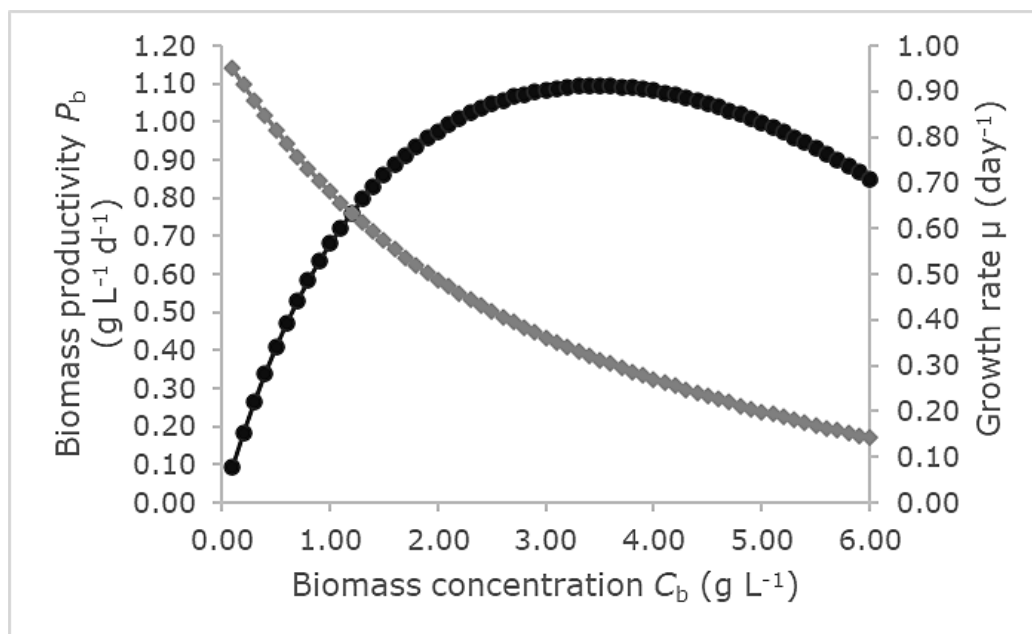


Figure 5. Biomass productivity prediction based on the modified model.

**Conclusions.** The modified model can be considered a useful tool for predicting  $Pb_{max}$  and the effect of  $I_{av}$  on  $\mu$ , as it provides design and optimization scenarios for the cultivation of the microalga *R. salina*. Its application ranges from the cultivation of this microalga, widely used in aquaculture, to the production of phycoerythrin.

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**Conflict of interest.** The authors declare that there is no conflict of interest.

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