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# Modeling of phycocyanin production from *Spirulina platensis* using different light-emitting diodes

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#### ABSTRACT

Phycocyanin is a high-value chromo-protein used in various industries. In this research, a simulation study of kinetics is carried out to identify different light spectra effects on microbial growth and phycocyanin production from the cyanobacteria *Spirulina platensis*. The results are compared with experimental data obtained from previous studies, and an acceptable accuracy is achieved in all the evaluated light spectra. Particular emphasis was placed on determining axial kinetic velocities simulated at different spectra regarding the latency, exponential and stationary microbial growth phases. According to the results obtained, cells grown in exponential phase lighted with red spectrum tend to resist the photo-limitation to a greater degree than cell exposure to white, green and yellow light. The latter is because phycocyanin allows a more excellent wavelength absorption from the red light. Contrarily, the light intensity for all spectra is reduced by around 80% at the inner bioreactor area regarding the intensity reached on the equipment surface during the stationary phase. Also, cell growth and phycocyanin production kinetic rates tend to be close to zero for all spectra, considering more than 50% of the inner bioreactor zone. This finding found in this research may be a key factor for the design of new photo-bioreactors so that these dark areas could be overcome by installing rotating internal lighting systems to guarantee the photosynthesis process of cyanobacteria in all regions of the bioreactor and thus avoid the phenomenon of photo-limitation due to low light intensities.

#### 1. Introduction

Sustainable production is one of the current challenges facing various economic sectors, especially in times of crisis due to the current COVID-19 pandemic. The United Nations Organization has proposed the 2030 plan. The sustainable development goals (SDGs) seek to create a common framework for various countries, international organizations, and industries. These goals are focused on health, industry, infrastructure and innovation and environment conservation. It is here where biotechnology plays a vital role in the development of the plans, as mentioned earlier. Based on the above, cyanobacteria is one of the most promising applications that allows sustainable processes aligned with the SDGs for industries such as food, agriculture, pharmaceuticals and health (Chisti, 2020). The photosynthetic and energy potential of cyanobacteria is high due to its more efficient sunlight than higher plants. That is why cyanobacteria can use the photon energy provided by light, carbon dioxide and water for compounds production such as biomass or

high-value secondary metabolites, in addition to efficiently mitigating the CO<sub>2</sub> from industrial and atmospheric sources (Yousuf, 2020; Priyadarshani and Rath, 2012; Prates et al., 2018).

Phycocyanin is one of the main phycobiliproteins characterized by being a light-collecting pigment. This metabolite generates beneficial biological effects for humans, with a high antioxidant and anti-inflammatory (Fratelli et al., 2021) and anti-cancer (Jiang et al., 2018) power. An antiviral potential has even been proven, and its possible usefulness in treating disorders related to Coronavirus infections caused by the recent COVID-19 outbreak caused by SARS-CoV2. The latter is possible thanks to its nutraceutical, anti-inflammatory, immune-stimulatory and immuno-modulating properties, being a preventive health method that requires more research (Ratha et al., 2021). Phycocyanin derived from *S. platensis* is used in industries as a colorant in food, sweets, beverages, dairy products and jellies. Its use extends to the cosmetic industry in color in lipsticks and eyeliners (Caicedo et al., 2020).

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However, optimization studies are required to increase the phycocyanin production from *S. platensis* to achieve its industrialization. The preceding argued that closed systems volumetric productivity has been higher than in open reactors. A typical example of the above is closed photobioreactors, which can have an efficiency up to 20 times greater than open ponds. However, the use of closed photobioreactors on a large scale still needs to be studied in greater detail to seek improvements that facilitate the process's profitability due to high production costs (Xie et al., 2015).

One of the most determining factors is supplying a suitable light intensity and spectrum to the equipment since light exerts a strong influence on the metabolites and biomass, allowing to maximize the growth and concentration of products (Kasiri et al., 2015; Kandilian et al., 2016; Huang et al., 2012; Luo et al., 2003; Park and Dinh, 2019; Naichia and Jen, 2009). For metabolites such as C-phycocyanin in closed systems, artificial light is used more than sunlight because this source is controllable, allowing better efficiency and a more standardized process, which translates into higher productivity (Prates et al., 2018). However, few studies have focused on modeling the effect of light intensity on S. platensis kinetics. The influence of different types of LED light on phycocyanin production has been little addressed from the computational simulation. The previous supported that previous studies have reported that more than 50% of conventional light is not used during cyanobacteria growth. In contrast, a LED light system provides a specific spectrum in which cyanobacteria can maximize their photosynthetic metabolism (Naichia and Jen, 2009). In addition to the above, the use of LEDs in the cyanobacteria culturing consumes less than 50% of energy than that used by a conventional fluorescent light system (Narukawa et al., 2006). Properly supplying LED light is a challenge in industrial bioprocessing. The above, considering the light transmission limitations to all bioreactor zones, which can be affected by the biomass concentration growing in different cell growth kinetics stages. In such a way, the phycocyanin production regulated by LED light should be a helpful process on a large scale. Achieving its industrialization implies identifying optimal operating conditions for the product synthesis. That is why mathematical simulations and models should predict biomass growth and phycocyanin production by considering the effect of different light sources and limiting substrate concentrations (Del Rio-Chanona and Zhang, 2018). Motivated by the above, this research focuses on determining by simulation the axial distribution of cell growth rates and phycocyanin production considering the effects of different spectra regarding the latency, exponential and stationary microbial growth phases. Simulation study of kinetics is carried out in this research to identify different light spectra effect on microbial growth and phycocyanin production from the cyanobacteria Spirulina platensis.

#### 2. Materials and methods

Light intensity plays an essential role in cyanobacteria growth and mainly affects the photosynthesis process (Wang et al., 2016). However, it has been shown that spectrum and light intensity significantly influence biomass and phycocyanin production (Chen et al., 2010; Wang et al., 2016; Kilimtzidi et al., 2019; Wicaksono et al., 2019). Based on the above, this research proposes a mathematical model to simulate microbial growth and phycocyanin formation from *Spirulina platensis* cyanobacteria, taking into account light intensity at different spectra. Main equations resulted by considering a mass balance in a dynamic batch type bioreactor. Based on the latter, it is not regarded as mass input or output to the bioreactor, so that the process is governed mainly by the generation and consumption rates of species in the system. Results were compared with experimental data carried out by previous research, which showed a detailed influence of the spectra of red, white, green and yellow light (Chen et al., 2010).

If no mass is added and removed, the *Spirulina platensis* cyanobacteria growth in a bioreactor operated in batch mode is defined by the biomass formation rate  $Q_x$  as seen in Eq. (1):

$$\frac{dX}{dt} = \mu_T X = Q_x \tag{1}$$

The microbial growth rate  $\mu_T$  is limited by the substrate in a bioreactor. So that the specific growth rate is usually expressed according to the Monod model (Contois, 1959). However, the population density X also exerts a significant effect on cell growth rates and phycocyanin production. That is why a modified model was used in this research to simulate the specific cell formation rate under the effects of *Spirulina platensis* microbial density (Monod, 1949). In addition to the above and to include the light intensity effects on the growth and phycocyanin production, the following model is proposed in this research, including the aforementioned simultaneous effects, according to the light spectrum:

$$\mu_T = \mu_I \mu_C \tag{2}$$

Where  $\mu_I$  is the cell growth rate that takes into account the light intensity impacts for each spectrum and  $\mu_c$  is the cyanobacteria formation rate under the population density effect on growth kinetics. The latter is calculated in this research using the following model:

$$\mu_c = \mu_{\max} \frac{S}{k_s X + S} \tag{3}$$

Where  $\mu_{max}$  is the maximum microbial growth rate,  $k_S$  is a saturation constant. An essential characteristic of the Contois model is that cyanobacteria growth rate depends on both the substrate concentration and cell number. The inhibition effects are captured at high biomass concentrations. This phenomenon may be present in the phycocyanin formation since the high concentration of *Spirulina platensis* can compete with the surface area available to capture the light spectrum by the cells. The light intensity effect on the growth and phycocyanin production from the cyanobacteria *Spirulina platensis* are calculated from the Aiba model (Aiba, 1982):

$$\mu_I = \frac{I(z)}{k_{SI} + I(z) + \frac{I(z)^2}{k_I}} \tag{4}$$

Where I(z) is the light intensity emitted by a light beam towards the surface of the bioreactor and is calculated using the Beer-Lambert law.  $k_{SI}$  is a light intensity saturation constant and  $k_i$  is an inhibition constant. However, the light intensity emitted depends on the distance and the population density. That is why the law above is modified to calculate the light intensity in each z-position of the bioreactor based on its length L and taking into account the cell density (Zhang et al., 2015):

$$I(z) = I_0[\exp(-\tau Xz) + \exp(-\tau X(L-z))]$$
 (5)

In this mathematical model,  $\tau$  refers to the cell absorption coefficient and  $I_0$  is the incident light intensity constant. Previous studies suggest that phycocyanin content decreases with nitrogen depletion in the culture medium (Del Rio-Chanona and Zhang, 2018). The latter is since *Spirulina platensis* cells use phycocyanin as a nitrogen source once the latter has been depleted in the cyanobacteria growth medium (Eriksen, 2008). As mentioned before, mass is not fed and extracted from the system while the bioreactor is operated in batch mode. Therefore, substrate modeling results only from cell volumetric uptake rate ( $Q_s$ ). For the reasons presented here, the modeling of the limiting substrate based on nitrogen content is proposed in such a way that:

$$\frac{dS}{dt} = -q_s X = Q_s \tag{6}$$

Where  $q_s$  refers to nitrogen uptake rate and is calculated according to (Bekirogullari et al., 2017):

$$q_s = \frac{\mu_T}{y_{xx}} \tag{7}$$

In Eq. (7), the term  $y_{xs}$  is the cyanobacteria cells produced according to the limiting substrate utilized (nitrogen). If phycocyanin is not added and extracted while cell growth occurs, the overall metabolite production results only from kinetics rate and light intensity impacts. Based on the results refereed, the volumetric velocity of phycocyanin formation is appropriately determined by relating it to biomass growth kinetics (Chen et al., 2010). However, the phycocyanin production in a bioreactor depends on the phycocyanin formation rate due to cyanobacteria growth  $q_p$  and phycocyanin uptake velocity due to limiting substrate depletion  $q_d$  (Zhang et al., 2015). Therefore, if both phenomena are considered, the phycocyanin produced by the cyanobacteria *Spirulina platensis* is calculated using the following differential equation:

$$\frac{dP}{dt} = q_p X - q_d P = Q_p \tag{8}$$

As mentioned before, the phycocyanin formation kinetics  $q_p$  also depends on light intensity impacts. That is why the latter is calculated according to a proposed production coefficient  $\nu_{pl}$  considering the light intensity:

$$q_p = v_{pl}\mu_T \tag{9}$$

Where  $v_{pI}$  is calculated considering the Aiba model (Aiba, 1982) and taking into account the axial light intensity effect I(z) and its saturation  $k_{Sp}$ :

$$v_{pl} = v_p \frac{I(z)}{k_{Sp} + I(z) + \frac{I(z)^2}{k_{to}}}$$
(10)

Previous studies suggest that phycocyanin production increases with light intensity. However, at higher levels, the output of the mentioned metabolite can be inhibited (Aiba, 1982). For the reasons above, this research includes the term  $k_{ip}$  that considers the photo-inhibition of phycocyanin production. The constant  $\nu_p$  refers to the phycocyanin production yield. The phycocyanin uptake rate  $q_d$  is considered to depend on nitrogen in the culturing medium and is calculated as (Zhang et al., 2015):

$$q_d = \frac{kd}{k_{Mn} + S} \tag{11}$$

Where kd is a specific phycocyanin uptake rate and  $k_{Mp}$  is a limiting nitrogen constant. By replacing Eqs (2) - (4) and (9) - (10) in Eqs (1) and (8), respectively, and considering the light intensity I as a function that depends on the z position as shown in Eq. (5), model results with concentrations dependent on the position z and time t. That is why, in this research, a methodology is used to solve Eqs (4) and (10) in a generalized way, using the trapezoid rule Del Rio-Chanona and Zhang, 2018). The above, to facilitate the numerical calculation process to solve the proposed differential equations and couple the cyanobacteria growth and phycocyanin production rates to the physical phenomenon of light intensity, mediated by the modified Beer-Lambert law. Using the trapezoid rule, Eqs (4) and ((10) simplify to:

$$\mu_{I} = \frac{1}{20} \left[ \frac{I_{(z_{0})}}{I_{(z_{0})} + K_{sI} + \frac{I_{(z_{0})}^{2}}{K_{i}}} + 2 \sum_{n=1}^{9} \left( \frac{I_{(z_{n})}}{I_{(z_{n})} + K_{sI} + \frac{I_{(z_{n})}^{2}}{K_{i}}} \right) + \frac{I_{(L)}}{I_{(L)} + K_{sI} + \frac{I_{(L)}^{2}}{K_{i}}} \right]$$

$$v_{pl} = \frac{v_p}{20} \left[ \frac{I_{(z_0)}}{I_{(z_0)} + k_{Sp} + \frac{I_{(z_0)}^2}{K_{lp}}} + 2\sum_{n=1}^{9} \left( \frac{I_{(z_n)}}{I_{(z_n)} + k_{Sp} + \frac{I_{(z_n)}^2}{K_{lp}}} \right) + \frac{I_{(L)}}{I_{(L)} + k_{Sp} + \frac{I_{(L)}^2}{K_{lp}}} \right]$$
(13)

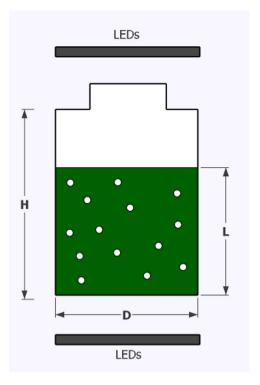


Fig. 1. Setup conditions for simulations of biomass and phycocyanin kinetics.

Once placed in its discretized form, Eqs. (12) and (13) are coupled to the differential equations proposed in this investigation. The latter is solved by the Runge-Kutta numerical method using the Matlab R2017b software.

A non-linear local optimization numerical technique was used in this research to determine the kinetic constants of the model. The above allows a minimized function, which relates the difference between the experimental data and those simulated with the proposed model. In such a way, a vector of kinetic constants is obtained that allows a well accurate regarding experimental data. In this way, the function to minimize J is expressed by the following expression (Moles et al., 2013):

$$J = \int_{0}^{t_f} (y_{msd}(t) - y(p, t)^T W(t) (y_{msd}(t) - y(p, t)) dt$$
 (14)

Restricted to:

$$f\left(\frac{dx}{dt}, x, y, p, v, t\right) = 0 \tag{15}$$

$$x(t_0) = x_0 \tag{16}$$

$$h(x, y, p, v) = 0 \tag{17}$$

$$g(x, y, p, v) \le 0 \tag{18}$$

$$p^L$$

Where p is the vector of kinetic constants for the mathematical model that simulates microbial growth, substrate consumption and phycocyanin production,  $y_{msd}$  are the experimental data of the process, and y(p,t) are the simulated results, W is a weighting diagonal matrix, x are the state variables (in this case, biomass, substrate and product),  $x_0$  are the state variables' initial conditionals, v is a vector of non-estimated parameters, f is the set of equality constraints of the algebraic and

**Table 1** Parameter estimation for the proposed model.

Parameter	Red	White	Green	Yellow
$\mu_{max}$ [d <sup>-1</sup> ]	2.500	1.800	1.117	1.100
$k_S [mgL^{-1}]$	115.49	115.49	115.49	115.49
$k_{SI}$ [ $\mu$ molm <sup>-2</sup> s <sup>-1</sup> ]	178.85	178.85	178.85	178.85
$k_i \ [\mu \text{molm}^{-2} \text{s}^{-1}]$	447.12	447.12	447.12	447.12
$\tau  [\mathrm{m}^2 \mathrm{g}^{-1}]$	0.289	0.373	0.40	0.45
$I_0 \ [\mu \text{molm}^{-2} \text{s}^{-1}]$	301.15	299.91	299.64	302.56
$y_{xs} [g/mg]x10^{-4}$	1.80	1.50	0.78	0.77
$v_p$ [g/g]	0.26	0.27	0.26	0.22
$k_{Sp}$ [ $\mu$ molm <sup>-2</sup> s <sup>-1</sup> ]	23.51	23.51	23.51	23.51
$k_{ip}$ [ $\mu$ molm <sup>-2</sup> s <sup>-1</sup> ]	800	800	800	800
kd [mgL <sup>-1</sup> d <sup>-1</sup> ]	1.5	0.28	0.28	0.28
$k_{Mp} [ mgL^{-1} ]$	16.89	16.89	16.89	16.89

differential equations that describe the system dynamics, h and g are the possible equality and inequality constraints that express the additional requirements for system performance. Finally  $p^L$  and  $p^U$  act as kinetic parameter limiters (Moles et al., 2013).

Fig. 1 shows the graphical schematization of the bioprocess proposed based on a cylindrical photobioreactor with a volume of  $500~\rm cm^3$  and a filling height of  $0.081~\rm m$  of Zarrouk culture medium stirred at  $120~\rm rpm$  with a temperature of  $30~\rm ^{\circ}C$  for five days of cell growth (Chen et al., 2010). Illumination is achieved through an LED lighting system that generates the different light spectra evaluated in this research: red, white, green and yellow.

#### 3. Results and discussion

#### 3.1. Parameter estimation and analysis of growth kinetics

The main objective of this research is to determine by simulation the axial kinetic velocities in a bioreactor operated at different LED light spectra. The previous, to evaluate its effects on microbial growth and phycocyanin production from the cyanobacterium *Spirulina platensis*. The above was carried out using a mathematical framework to predict the LED impact of light on cell growth and phycocyanin production. The results were compared with experimental data reported in previous investigations (Chen et al., 2010). It is shown in Table 1 the parameterization results allowing the best accurate between the simulated results by the equations proposed in this investigation and the experimental data.

The accuracy achieved related to cyanobacteria kinetics simulation is of utmost importance for the analysis of bioprocess. The information generated can be considered the starting point for the optimization of future processes. In addition to the above, it is possible to identify critical parameters for scale-up metabolite production, such as phycocyanin, using computer simulation techniques.

As mentioned before, a non-linear local optimization technique was used in this research to determine the kinetic constants shown in Table 1. The latter, for minimizing the difference between simulated and experimental results. The numerical treatment to solve the kinetic expressions for the growth and production of phycocyanin allowed the coupling of kinetics to the axial bioreactor positions and time. In such a way, the numerical solution allowed the simplification of ordinary differential equations to be solved simultaneously with the mentioned parameterization technique. Therefore, axial velocities are shown and explained in results Section 3.2 (Determination of axial growth velocities").

As observed in Figs. (2)-(5), the simulation results proved to represent the experimental data analyzed.

In such a way, well accuracy is achieved regarding biomass

production, with values close to 0.43 gL $^{-1}$  of *Spirulina platensis* cells considering the red light spectrum at a time of 4 days, as shown in Fig. 2 (a). Also, the white light results are shown in Fig. 3(a), and a cell final level of 0.37 gL $^{-1}$  at the end of the five days of the process is reached. However, the simulations and experimental results suggest reducing cyanobacteria biomass for studies carried out with green and yellow LED light spectra, compared to those obtained with the red and white colors. The latter reached mean values of 0.196 and 0.185 gL $^{-1}$ , respectively, in 5 days.

Limiting substrate is depicted in Figs 2(b)–5(b). According to previous studies (Monod, 1949), nitrogen is directly related to phycocyanin productivity since once it is fully depleted, the mentioned metabolite is considerably decreased. The preceding, because once the stationary phase is reached, the cyanobacteria use the nitrogen reserves in the phycocyanin so that its production is reduced (Zhang et al., 2015). According to the experimental results referenced in this research, the phenomenon above can only be seen in cyanobacteria grown considering the red light (Fig. 2c). The latter is because, after 3.7 h, the nitrogen is completely exhausted, as shown in Fig. 2(b), significantly affecting the phycocyanin production. Interestingly, once cyanobacteria deplete all of the limiting substrate, the phycocyanin reduction phenomenon is also observed in the simulation results, as shown in Fig. 2(c). In such a way that the phycocyanin uptake term  $q_d$  included in Eq. (8) is of great importance to capture the behavior above.

In contrast, it should be mentioned that the blue spectrum was not considered in this study since blue light could slow down the growth of some cyanobacteria strains of *Spirulina sp.* according to the referenced experimental results. Even so, similar results (Park and Dinh, 2019; Chen et al., 2010) indicate that the red spectrum favors a high biomass and phycocyanin production, as can be observed in the simulations carried out in this investigation.

The red spectrum resulted in similar behavior to the experimental data regarding phycocyanin production, as observed in Fig. 2(c). Likewise, a maximum phycocyanin production of 0.08 gL $^{-1}$  is reached at four days of evolution. Similar results can be seen in Fig. 3(c). When phycocyanin is produced under the white spectrum, the simulations result in detail a slower production, reaching a concentration of approximately 0.06 gL $^{-1}$  on day 5. In the case of the green color, a maximum phycocyanin production is identified with an approximate value of 0.0379 gL $^{-1}$  in 5 days, as shown in Fig. 4(c). However, the phycocyanin production presents significant discrepancies concerning the mentioned spectra using the yellow spectrum, since a reduction of more than 65% is observed compared to the metabolite produced under the red light effect, obtaining a low efficiency for pigment production, with mean values of 0.0277 gL $^{-1}$  as shown in Fig. 5(c).

#### 3.2. Determination of axial growth velocities

Biomass growth and phycocyanin production rates ( $Q_X$  and  $Q_p$ , respectively) provide crucial information on cell metabolism and physiology, and their reliable calculation is desirable. Cell growth rate data indicate cell density and viability and can be used to design control strategies for bioreactor operation. In addition, axial  $Q_X$  and  $Q_p$  information is also necessary for bioreactor design and scale-up illuminating systems since it allows light transmission impact on metabolism.

The volumetric cyanobacteria growth rates  $Q_X$  and net phycocyanin formation  $Q_p$  exposed in Eqs. (1) and (8), respectively, were evaluated according to each light spectrum's spatial light intensity. The latter investigates each light spectrum effect on cell growth and phycocyanin kinetics from the *Spirulina platensis* cyanobacteria. The results are shown in Figs (6) - (8). In addition to the above and to identify the spectra effects on different cell growth phases, the results of biomass and substrate found in the latency phase (growth start), exponential phase

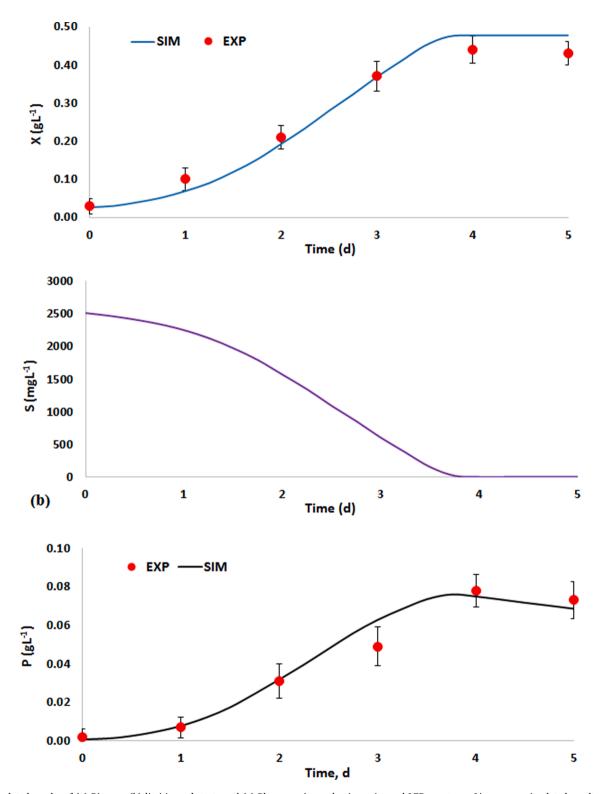


Fig. 2. Simulated results of (a) Biomass (b) limiting substrate and (c) Phycocyanin production using red LED spectrum. Lines mean simulated results and marks account from refereed experimental data.

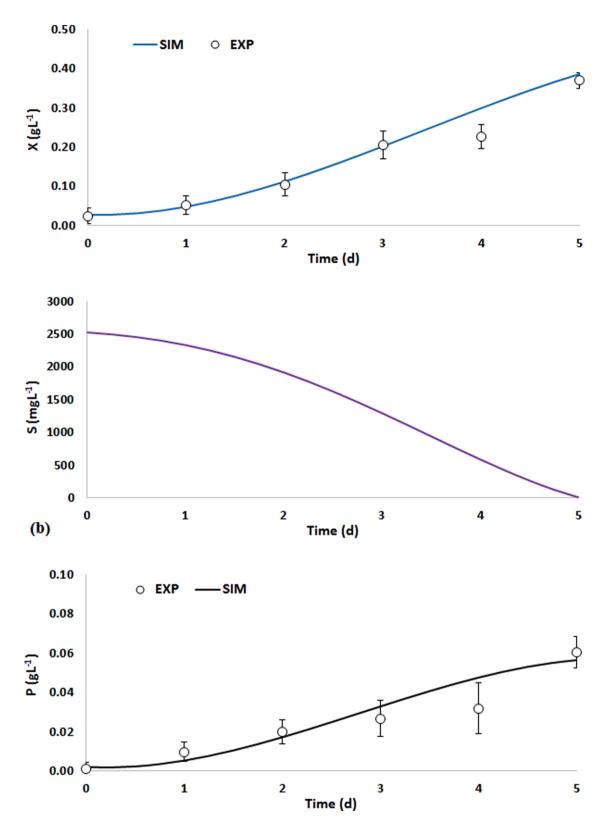


Fig. 3. Simulated results of (a) Biomass (b) limiting substrate and (c) Phycocyanin production using white LED spectrum. Lines mean simulated results and marks account from refereed experimental data.

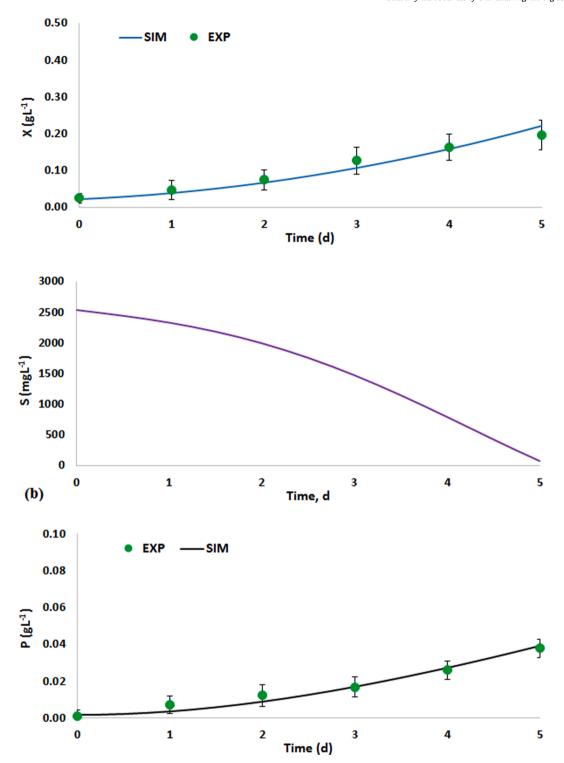


Fig. 4. Simulated results of (a) Biomass (b) limiting substrate and (c) Phycocyanin production using green LED spectrum. Lines mean simulated results and marks account from refereed experimental data.

(between 1 and 3 days of culture) and stationary phase (close to 4–5 days of culture) are used for the calculations mentioned before.

In the latency phase, it can be seen in Fig. 6 that there are no significant axial intensity effects on cell growth or phycocyanin production. In such a way, the light intensity in all the spectra remains constant from the bioreactor walls to the center of the equipment, presenting the red light the highest spatial intensity, as seen in Fig 6(a). However,

according to Fig. 7, the cell light absorption begins to be meaningful and concentrated once the exponential phase is reached, the more excellent absorption in red and white spectra, from biomass levels of 0.28 and 0.20  ${\rm gL}^{-1},$  respectively.

Similar results have been found by (Rebolledo-Oyarce et al., 2019), who have investigated a radiation heat transfer model considering monochromatic light to evaluate its effects on the growth of *Dunaliella* 

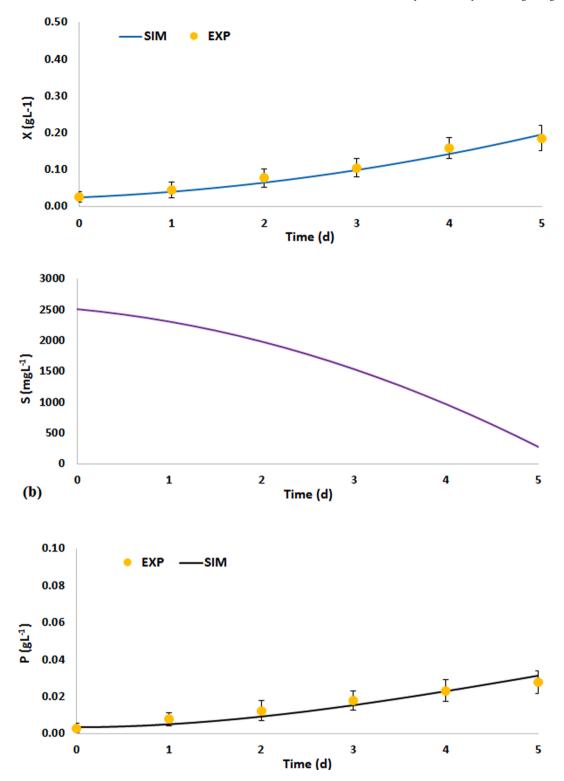


Fig. 5. Simulated results of (a) Biomass (b) limiting substrate and (c) Phycocyanin production using yellow LED spectrum. Lines mean simulated results and marks account from refereed experimental data.

*tertiolecta*. Results suggest the red color as one of the best spectra to maximize biomass yields. A lower absorption is observed in the green and red spectra using mean values of cell concentration in the order of 0.11 and  $0.09~{\rm gL}^{-1}$ , respectively, in this research. However, there may be critical light intensity levels in the center of the bioreactor that

significantly affect cell growth rates and phycocyanin synthesis (Naichia and Jen, 2009), (Fernández et al., 1998). This finding found in this research may be a critical factor in designing new photobioreactors, so the dark areas could be overcome by installing internal and rotating lighting systems to guarantee the cyanobacteria photosynthesis regions

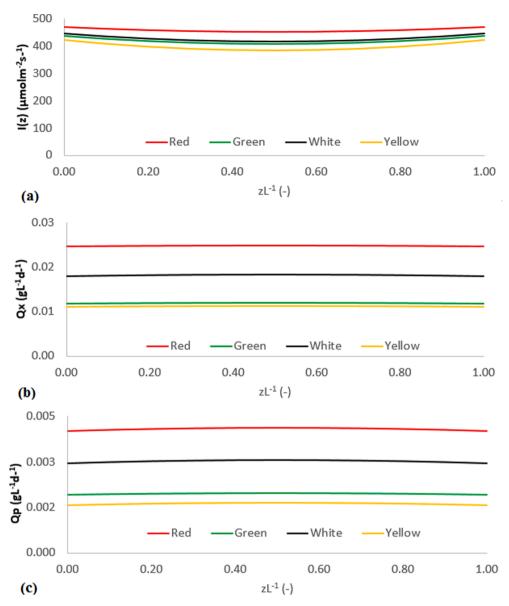


Fig. 6. Non-dimensional [zL<sup>-1</sup>] axial position effect on kinetic rates at different light spectrum for lag phase growth. (a) Light intensity, (b) Biomass Growth and (c) Phycocyanin production.

of the bioreactor and thus avoid the photo-limitation phenomenon due to low light intensities. The photo-limitation phenomenon found in this phase can be explained, according to the results obtained from examining the spatial light intensity effects on growth rates and phycocyanin production shown in Fig. 7(b)-(c), finding values below  $100~\mu molm^{-2}$  s<sup>-1</sup> in the center of the equipment (zL<sup>-1</sup> = 0.5) that considerably affect the photosynthesis process. In this zone of the fermenter, the kinetic speeds tend to be close to zero for all spectra. However, the results show a cellular tendency to resist limitation by light intensity. Microbial growth and metabolite synthesis are more significant than in the other spectra, regarding all axial positions of the photobioreactor, as seen in Fig. 7(b)-(c).

This finding could support the higher biomass and phycocyanin yields concerning the red light spectrum found in the referenced experimental data. The above is because phycocyanin allows a more excellent wavelength absorption from red light than the white, green and yellow spectra. The photo-limitation phenomenon is even more

pronounced in the stationary phase, in which cells initiate metabolic stress due to a shortage of nutrients. For all spectra, light attenuation is similar in this stationary phase due to the high density that blocks the spectra. The latter can be avoided with internal lighting, as mentioned (Hu and Sato, 2017). According to the results shown in Fig. 8, the growth and phycocyanin production is the only representative in areas closed to the bioreactor surface, where cells can still capture the light intensity. However, 60% of the bioreactor presents limitation by the light intensity in this stationary phase regarding all the mentioned spectra, leading to a cessation of production kinetic activities. The latter is because the light intensity is reduced by around 80% regarding the intensity reached on the surface of the equipment. Interestingly, the mentioned bioreactor zone is governed by the phycocyanin uptake (negative  $Q_p$  values), according to the results obtained for the red light spectrum, as observed in Fig. 8(c). The above is because the limiting substrate has been completely exhausted, and the cells use the nitrogen reserves in the phycocyanin.

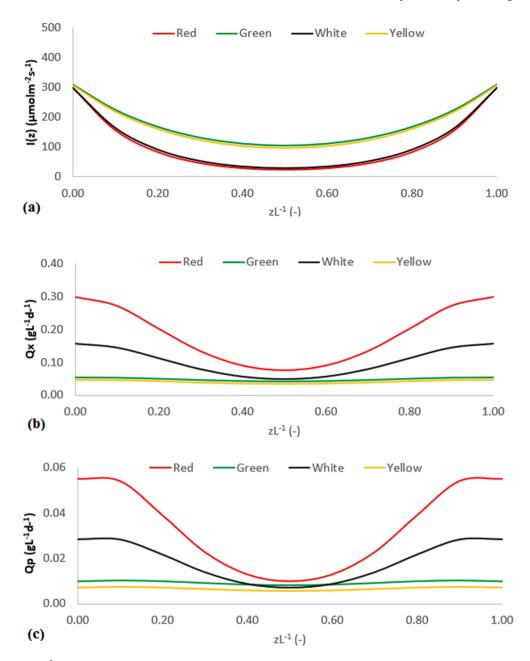


Fig. 7. Non-dimensional  $[zL^{-1}]$  axial position effect on kinetic rates at different light spectrum for the exponential growth phase. (a) Light intensity, (b) Biomass Growth and (c) Phycocyanin production.

One of the main challenges in cyanobacteria and cyanobacteria cultures for secondary metabolites is balancing the high compound and biomass production (Prates et al., 2018; Park and Dinh, 2019; Chen et al., 2010). Therefore, simulations to predict the phycocyanin productivity at a specific light condition are helpful for future optimization studies. Consequently, it can be a determining factor for industrial scale-up stages.

#### 4. Conclusions

Clear evidence related to LED light spectra effect on biomass and phycocyanin production from the cyanobacteria Spirulina platensis is shown in this research. Particular emphasis was placed on the cell growth and phycocyanin kinetics at different axial positions of the photobioreactor considering each light spectrum evaluated during the

latency, exponential and stationary phases. Results suggest the red color as one of the best spectra to maximize phycocyanin production rate. The latter due to the great affinity of phycocyanins to absorb the wavelength from the red spectrum, even under early photo-limiting conditions in the central zone of the photobioreactor. According to the information obtained, the axial distribution of cell growth rates and phycocyanin production are considerably affected by light intensity leading to a cessation of production kinetic activities at major internal bioreactor zones where light intensity transmission is weak. However, more detailed experimentation is required to confirm the findings elucidated in this research. Also, a more robust framework modeling should be of great interest to determine the flow pattern reached in a photobioreactor. The latter, for a better understanding of its influence on light intensity and kinetics. The model proposed in this research can be coupled to simulation models of photobioreactor hydrodynamics, using

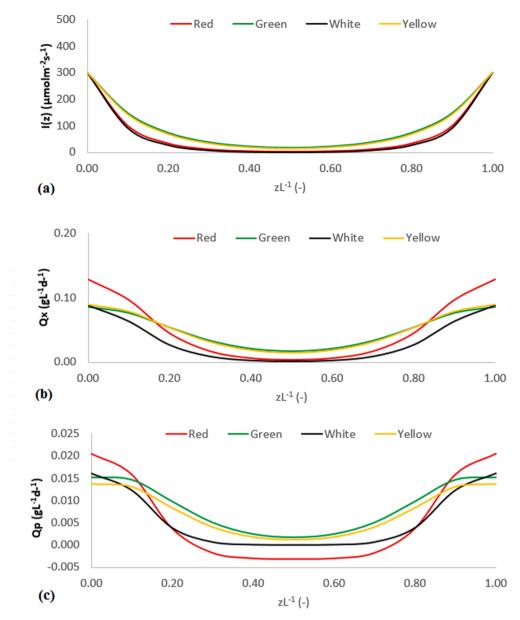


Fig. 8. Non-dimensional [zL<sup>-1</sup>] axial position effect on kinetic rates at different light spectrum for the stationary growth phase. (a) Light intensity, (b) Biomass Growth and (c) Phycocyanin production.

Computational Fluid Dynamics (CFD) to evaluate the spatial transmission of the light intensity of different devices commonly used on a large scale. The above for the optimization of industrial processes.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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