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STUDY OF AGITATION, COLOR AND STRESS LIGHT VARIABLES ON *Spirulina platensis* CULTURE IN A VERTICAL STIRRED REACTOR IN STANDARD MEDIUM

ESTUDIO DE LAS VARIABLES AGITACIÓN, COLOR DE LUZ Y ESTRÉS DE LUZ SOBRE EL CULTIVO DE *Spirulina platensis* EN UN REACTOR VERTICAL AGITADO EN MEDIO ESTÁNDAR

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Abstract

Spirulina platensis is a helical-shaped photosynthetic cyanobacterium. Its cultivation depends on light intensity, nutrients, pH, cell density, temperature, among others. Microalgae are capable of growing in open and closed reactors. In the present work, it was studied microalgae *Spirulina platensis* growth in a stirred tank reactor (STR), evaluating stirring rate, color and light stress. *Spirulina platensis* was kept in flasks with Schlösser standard solution at room temperature, orbital shaker and fluorescent lamps. The operating conditions of stirred vertical reactors (vertical axis) were: LED light externally adhered to the reactor walls, four Rushton agitators, and 2.5 L of Schlösser standard solution inoculated with *Spirulina platensis* resulting in an initial concentration of 0.15 OD. Within the study of rate variable, the highest rate of 114 rpm, showed a productivity (P) of 2.08 $mgL^{-1}h^{-1}$, concerning light color variable a high growth was observed in white light with a productivity of 2.85 $mgL^{-1}h^{-1}$ and, regarding stress variable, Flash function showed a productivity 41% lower than standard white light. It was shown that stirring rate has a positive influence and light stress affects negatively the microalgae growth.

Keywords: *Spirulina platensis*, Stirring rate, light color, light stress, vertical reactor.

Resumen

La *Spirulina platensis* es una cianobacteria fotosintética con forma de hélices. Para su cultivo, depende de la intensidad de luz, nutrientes, pH, densidad celular, temperatura, entre otros. Las microalgas pueden crecer en reactores abiertos y cerrados. El presente trabajo estudió el crecimiento de la microalga *Spirulina platensis* en un reactor tipo tanque agitado (STR), evaluando velocidad de agitación, color y estrés de luz. La *Spirulina platensis* se mantuvo en matraces con solución estándar Schlösser a temperatura ambiente, agitación orbital (shaker) y lámparas fluorescentes. Las condiciones de operación de los reactores verticales agitados (eje vertical) fueron: luz LED adherida externamente a las paredes del reactor, cuatro agitadores tipo Rushton, y 2.5 L de solución estándar Schlösser inoculados con *Spirulina platensis* resultando una concentración inicial de 0.15 de DO. En el estudio de la variable velocidad, la velocidad de 114 rpm mostró productividad (P) de 2.08 $mgL^{-1}h^{-1}$, en la variable color de luz se observó un crecimiento elevado en luz blanca con productividad de 2.85 $mgL^{-1}h^{-1}$ y en la variable estrés, la función Flash mostró productividad de 41% inferior al padrón (luz blanca). Se demostró que la velocidad de agitación influye positivamente y el stress de luz afecta negativamente el crecimiento de la microalga.

Palabras clave: *Spirulina platensis*, velocidad de agitación, color de luz, estrés de luz, reactor vertical.

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1 Introduction

Microalgae combine typical plant properties (photosynthesis and simplicity of nutritional requirements) with biotechnological attributes of microbial cells (rapid growth in liquid medium and ability to accumulate or secrete metabolites). This combination favors the use of these microorganisms for application in biotechnological processes (Del Campo *et al*., 2007; Johansen 2012), as well as a great economic and industrial potential. They are considered as a great source of proteins, pigments, vitamins and lipids, the latter being studied for biofuel production purposes (Avila-Leon *et al*., 2012).

Spirulina species are considered cyanobacteria (*Oscillatoriaceae* family) with helical-shaped structure and multicellular filaments. These species can be found in different environments, such as: saline lakes, soils, swamps, brackish water, seawater, hot springs and fresh water (Sonani *et al*., 2017). The cyanobacterium *Arthrosphira platensis*, commonly known as *Spirulina platensis*, is a photosynthetic microorganism that depends largely on light intensity, nutrient availability, pH, cellular density, temperature (Ferreira *et al*. 2012a), salinity and bicarbonate ions presence (Costa *et al*., 2002; Moheimani *et al*., 2015) for its cultivation. Cell growth of *Spirulina platensis* can be limited by high or low light intensity, photoinhibition or photolimitation (Bezerra *et al*., 2008; Liu *et al*., 2018).

It is necessary to develop systems that facilitate microalgae cultivation (May-Cua *et al*., 2019), as well as it is necessary to know biological and engineering aspects.

The algal biomass production process can be divided into two stages:

1) Biological knowledge and operational parameters (cell growth)

2) Reactor design, obtaining and processing of the biomass produced (engineering aspects) (Vonshak 2002).

The availability and light intensity are factors that control photosynthetic cultures productivity (Rajesh *et al*., 2017).

During microalgae growth, the self-shading interferes with light availability; a diluted culture facilitates passage of light through the medium. Therefore, the increase in cell concentration decreases direct incidence of light, observing the decrease in growth rate (Vonshak 2002). Culture stirring increase movement of particles that are behind incidence of light, produces and maintains a uniform distribution, increases production rate and prevents sedimentation (Arruda *et al*., 2009). Some researchers perform stirring by recirculation system with peristaltic pumps, agitators with different geometries, rotary shaker or injection of air flow (Bautista-Monroy *et al*., 2019; Danesi *et al*., 2004; Ferreira *et al*., 2012b; Jácome *et al*., 2012; Sassano *et al*., 2010; Vonshak, 2002).

Researchers have carried out microalgae cultures in open lagoons and "raceway" reactors, but few species can be maintained in traditional open systems with contamination control (Rodríguez-Mata *et al*., 2019), using highly alkaline or saline selective environments. Fully enclosed photobioreactors have a greater opportunity to maintain a monoseptic culture and have possibility of using a greater algae variety than open reactors (Hongsthong *et al*., 2017; Robles-Heredia *et al*., 2016).

The main interest of this work was to study *Spirulina platensis* growth in Schlösser standard solution, evaluating the effect of three stirring rates (increasing light availability to the culture), four different light colors and three different types of light stress; aiming to prove being better than common light growth, using a stirred vertical reactor.

2 Materials and methods

Spirulina platensis was kept in 500 mL Erlenmayer flasks with Schlösser (1982) standard solution at room temperature, on an orbital shaker at 100 rpm and under 15 W fluorescent lamps (2240 Lx) illumination.

In order to determine optical density, a wavelength sweep was performed for cell concentration analysis. Biomass was centrifuged and re-suspended in 10 mL of distilled water, and measured in a HACH DR2000 spectrophotometer at a wavelength of 590 nm, using Schlösser medium as blank. The HACH DR2000 is a visible spectrophotometer with wavelength range between 400-900 nm and gas-filled tungsten source lamp.

A wavelength sweep was performed in a range of 480 to 760 nm in the HACH spectrophotometer to determine the greatest absorption point for *Spirulina platensis*, where biomass was centrifuged and re-suspended in 10 mL of distilled water, taking water as a blank.

2.1 Operation of stirred vertical reactors

Stirred vertical reactors (Fig. 1) were operated in batch mode with 2.5 L of operating volume, an average initial optical density (OD) (adimentional) of 0.1 of *Spirulina platensis*, using Schlösser standard solution with agitation (vertical axis) of 35 rpm, three agitators (Rushton type) of 4 cm in diameter and one of 6 cm in diameter. It was chosen as minimum agitation point 35 rpm, since at lower rates biomass sediments over time. In addition, rates of 78 and 114 rpm were used at room temperature of 25 ºC during light period under white light intensity of 3000 Lx, and without temperature control during dark period. To provide illumination, LED tapes were placed around glass reactors and set up in photoperiods of 12 h light, with different light colors intensities: R1-white 3000, R2-blue 3800, R3-green 3630 and R4-red 840 Lx. For the "light" variable analysis, an experimental run for each light color was carried out; all at the same time maintaining a stirring rate of 35 rpm, at room temperature of 30 ºC, this due to temperature variation during the year's season.

An environmental factor can cause stress in microorganism when it imposes physiological challenges that compromise microorganism survive and reproduction (Andrade-Linares *et al*., 2016). So, we expected to increase cell growth stressing microalgae with light color. The influence of flashing lights over the system was evaluated, where R1 reactor had white light with 3000 Lx, R2 with the "Smooth" function (red-blue-green lights in intervals of 1.8 seconds for each color) with a mean intensity of 2105 Lx, R3 with white light in "Strobe" function (decrease in intensity until dark in 5 second intervals) with a mean intensity of 1829 Lx, and R4 with "Flash" function (red-blue-green-purple-yellow-turquoise-white lights in intervals of 2.13 seconds for each color) with a mean intensity of 2213 Lx in this experiment.

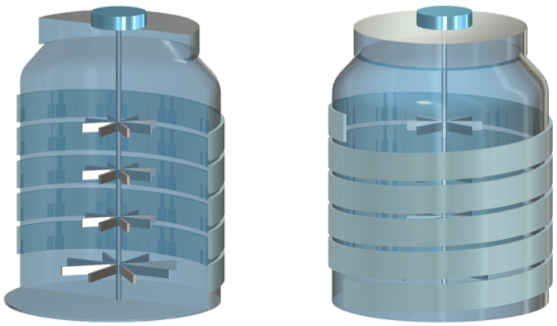

Fig. 1. Stirred vertical reactor with LED tapes.

Room temperature was 35ºC during summer season in São José do Rio Preto, São Paulo, Brazil.

pH correction was not necessary for Schlösser solution, since it remained at pH values close to 10.

Every 24 h aliquots of 10 mL were taken in all tests to determine OD and only at beginning and end of culture for dry biomass.

For cellular concentration analysis, biomass was centrifuged and re-suspended in 10 mL of distilled water. Afterwards, it was measured in a HACH DR2000 spectrophotometer at a wavelength of 590 nm, using water as a blank. Dry biomass was determined by gravimetry, where the sample used previously to determine OD was placed in a porcelain dish and dried at 110 ºC until constant weight.

A linear adjustment of optical density was made against biomass produced, in order to obtain a correlation model that explains microalgae growth, Eq. (1).

$$OD = 1.7437DB - 0.1727 \quad (1)$$

where:
OD = Optical density (absorbance 590 nm)
Db = Dry biomass (g L^{-1})

This correlation was used to determine biomass in terms of g L^{-1} in subsequent studies.

3 Results and discussion

Many authors use 560 nm (Liu *et al*., 2018; Morocho-Jácome *et al*., 2015) and 670 nm (Colla *et al*., 2007; Costa et al 2004; Walter *et al*., 2011) to measure OD, so it was decided to perform a wavelength sweep in order to know the higher absorption point. The wavelength sweep is shown in Fig. 2, where the highest results are observed at wavelength of 590 nm, hence it was decided to work with this value.

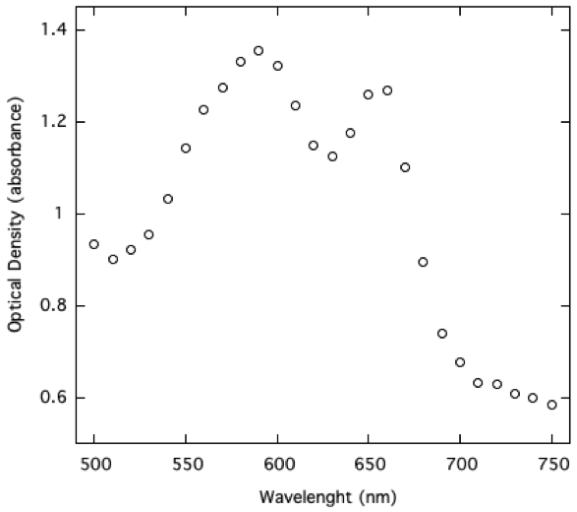

Fig. 2. Absorbance of microalgae *Spirulina platensis* in different wavelengths.

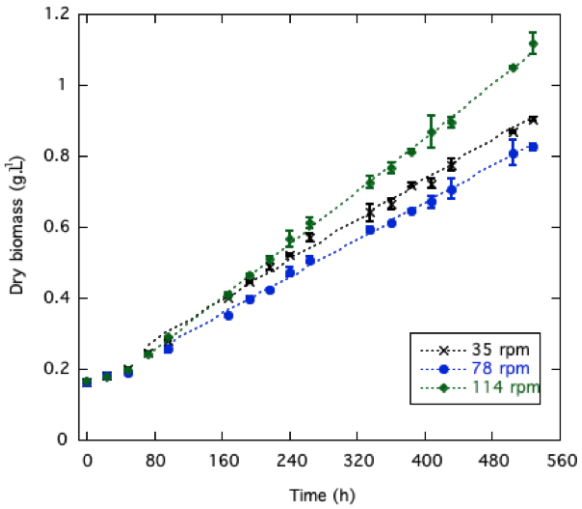

Fig. 3. Growth comparison at variable rate.

3.1 *Vertical cylindrical reactors*

3.1.1 *Culture in Schlösser standard solution: rate variable*

Growth in the reactors was performed with white light and three different stirring rates (35, 78 and 114 rpm). It can be seen in Fig. 3 that the best results are found at 114 rpm of rate, with values of 1.1 g L^{-1}, subsequently, rate of 35 rpm is found with values of 0.9 g L^{-1} and finally, rate of 78 rpm with 0.82 g L^{-1}; all of them over a time of 528 h (22 days). Ravelonandro *et al.* (2011) obtained biomass production results of 1.8 g L^{-1} in 16 days, with an air bubbling system in modified Zarrouk medium and white fluorescent lights with an intensity of 600 Lx. However, it should be noted that productivity values obtained by aforementioned authors are greater compared with those obtained in this work. However, conditions used were different.

It is observed in Figure 3 that up to 72 hours the three tests remain in a lag or adaptation phase; afterwards, it was carried out an adjustment of biomass growth obtaining a linear behavior Eqs. 2-4.

$$Db = 0.16627 + 0.0014082 \cdot t \qquad R^2 = 0.99392, 35rpm \tag{2}$$

$$Db = 0.14363 + 0.0013089 \cdot t \qquad R^2 = 0.99675, 78rpm \tag{3}$$

$$Db = 0.10536 + 0.0018698 \cdot t \qquad R^2 = 0.99808, 114rpm \tag{4}$$

In Table 1 are shown the statistical analyzed values with ANOVA and Tukey test, where it is observed that on time 0 h there is no significant difference and on time 96 h there was found significant difference. Tukey test showed difference between reactors 35 rpm *vs* 78 rpm and 114 rpm *vs* 78 rpm, conversely from 240 to 504 h were found difference between all reactors.

3.1.2 *Culture in Schlösser standard solution: light variable*

Growth within reactors at different light colors (R1-white, R2-blue, R3-green, R4-red) is shown in Fig. 4. Under these conditions, it was observed that in white light there were obtained dry biomass results of 1.3 g L^{-1} in 456 h, followed by similar results of 0.9 g L^{-1} in 480 h in red light, and 0.93 g L^{-1} in 528 h in green light. Blue light reactor obtained a maximum dry biomass value of 0.55 g L^{-1} in 480 h.

Ravelonandro *et al.* (2008) worked with a closed system, where they obtained a production of 2.6 g L^{-1} in white light and 1.7 g L^{-1} in blue light, both results in 384 h (16 days) and air flow stirring. Wang *et al.* (2007) obtained 0.44 g L^{-1} of dry biomass in red light and 0.1 g L^{-1} in blue light, in 120 h (5 days) in a closed system.

Figure 4 shows a lag phase of 48 hours for white, green and red lights; however, blue light keeps that phase until 72 hours, followed by a slow growth. It was obtained the best biomass result in the white light. Data were adjusted to third and fourth degree equations that explain their behavior under stipulated conditions for these tests, Eqs. 5-8.

Table 1. ANOVA and Tukey statistical analysis for growth with different rate.

Time (h)	35 rpm	78 rpm	114 rpm	P Value ANOVA	Tukey Group comparison
0	0.1638± 0.0038	0.1633± 0.0028	0.1654± 0.0014	0.4959	-
80	0.2806± 0,0094	0.2560± 0.0033	0.2909± 0.0111	0.01247	35rpm-114rpm 35rpm-78rpm
240	0.5214± 0.0163	0.4733± 0.0028	0.5672± 0.0146	0.00238	All
400	0.7213± 0.0052	0.6714± 0.0113	0.8671± 0.0302	0.00077	All
504	0.8677± 0.8085	0.8085± 0.0137	1.0489± 0.0099	0.00019	All

- Does not apply, there is no significance difference.

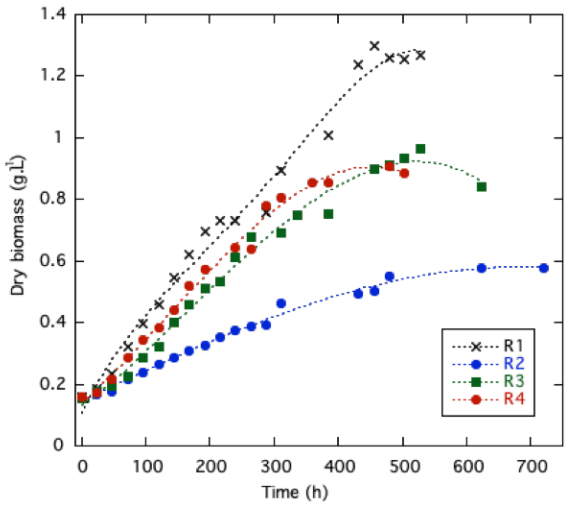

Fig. 4. Growth comparison with different light colors.

$$Db = 0.10656 + 0.0042547 \cdot t - 1.4357e^{-5)} \cdot t^2 + 34.123e^{-8} \cdot t^3 - 4.035e^{-11} \cdot t^4$$

$$R^2 = 0.99422 - R1 \quad (5)$$

$$Db = 0.14501 + 0.0010062 \cdot t - 1.2859e^{-7} \cdot t^2 - 6.0105e^{-10} \cdot t^3$$

$$R^2 = 0.99204 - R2 \quad (6)$$

$$Db = 0.13078 + 0.0015233 \cdot t + 2.855e^{-6} \cdot t^2 - 5.491e^{-9} \cdot t^3$$

$$R^2 = 0.99003 - R3 \quad (7)$$

$$Db = 0.14071 + 0.0018049 \cdot t + 3.2021e^{-6} \cdot t^2 - 7.6377e^{-9} \cdot t^3$$

$$R^2 = 0.9932 - R4 \quad (8)$$

In Table 2 are shown the statistical analyzed values with ANOVA and Tukey test, where it is observed that on time 0 h there is no significant difference. On time 72, 144 and 192 h there were found differences with the Tukey test on the 4 reactors. On time 312 and 480 h there were found differences between reactors R1 and R2, showing that R1 has the best growth and R2 the worst.

3.1.3 Culture in standard solution Schlösser: light stress variable

Fig. 5 shows a linear growth tendency from 48 h to 720 h; therefore, it is not possible to observe a microalga decreasing phase.

In Figure 5, a lag phase of 72 hours can be observed in white, smooth and strobe light (R1, R2 and R3 reactors respectively), and flash function light shows a lag phase of 48 h. It is better to have a short lag phase but, in this case, white light shows better results of biomass and growth. Data adjustment was done through second degree equations that allow to predict these results behavior under these tests conditions.

$$Db = 0.093309 + 0.0015031 \cdot t - 2.3227e^{-7} \cdot t^2$$

$$R^2 = 0.9935 - R1 \quad (9)$$

Table 2. ANOVA and Tukey statistical analysis for growth with different light colors.

Time (h)	White	Blue	Green	Red	P Value ANOVA	Tukey Group comparison
0	0.1575± 0.0049	0.1561± 0.0012	0.1544± 0.0012	0.1590± 0.0004	0.42472	-
72	0.3198± 0	0.2152± 0.0045	0.2264± 0.0008	0.2886± 0.0028	<0.0001	All
144	0.5467± 0.0069	0.2894± 0	0.4010± 0.0085	0.4397± 0.0057	<0.0001	All
192	0.6952± 0.0158	0.3273± 0.0024	0.5100± 0.0028	0.5705± 0.0122	<0.0001	All
312	0.8916± 0.0373	0.4626± 0.03	0.6923± 0.0053	0.8050± 0.0454	0.0008	White-Blue
480	0.6788± 0.0397	0.5504± 0.03	0.5054± 0.0134	0.5028± 0	0.00682	White-Blue

- Does not apply, there is no significance difference.

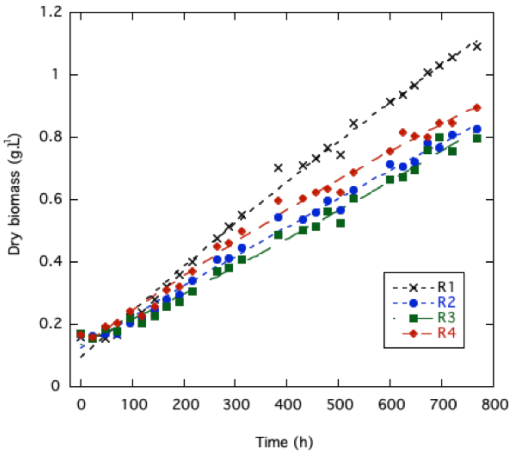

Fig. 5. Growth comparison with stress light.

$$Db = 0.12283 + 0.0010161 \cdot t - 1.0804e^{-7} \cdot t^2$$

$$R^2 = 0.99122 - R2 \quad (10)$$

$$Db = 0.13466 + 0.00079055 \cdot t + 1.404e^{-7} \cdot t^2$$

$$R^2 = 0.99013 - R3 \quad (11)$$

$$Db = 0.12583 + 0.0011999 \cdot t - 2.5162e^{-7} \cdot t^2$$

$$R^2 = 0.99125 - R4 \quad (12)$$

Maximum growth in reactors with flashing lights was R1 1.1 g L^{-1}, R2 0.83 g L^{-1}, R3 0.78 g L^{-1} and R4 0.9 g L^{-1}, all in 768 h.

Biomass production rate for each reactor was R1 1.432 mg $L^{-1}h^{-1}$, R2 1.081 mg $L^{-1}h^{-1}$, R3 1.017 mg $L^{-1}h^{-1}$ and R4 1.028 mg $L^{-1}h^{-1}$, where reactor R1 has the highest rate.

Table 3 shows statistical test, where on time 0 h there is not significant difference, on time 48 h there is difference between Flash *vs* White and Smooth *vs* White. From 216 to 768 h all groups present differences between them.

The stress light did not show what was expected: to allow a better growth than that of common light color. The best result was obtained with white light.

Danesi *et al*. (2011) performed tests of influence of temperature on microalgae growth, showing that in values between 30 and 33 ºC highest productions of *Spirulina platensis* biomass were obtained, while in values close to 25 and 35 ºC there is a biomass decrease. This concurs with the results obtained in the present work, where tests carried out at 25 and 35 ºC show a lower productivity compared to tests carried out at 30 ºC. In tests carried out at 25 and 35 ºC the lag phase is about 72 h, while in tests carried out at 30 ºC it is only 48 h.

Table 4 displays dry biomass results and productivity for different usage conditions, where the best results were obtained in Schlösser medium with 2.85 mg $L^{-1}h^{-1}$ (white light).

All the obtained equations represent microalgae growth versus DO, with a significance level of 99%. These equations can be used to know the dry biomass amount along the microalgae growth process. The slopes were calculated after lag phase.

Table 3. ANOVA and Tukey statistical analysis for growth with different light stress.

Time (h)	White	Smooth	Strobe	Flash	P Value ANOVA	Tukey Group comparison
0	0.1594± 0.0133	0.1679± 0.0069	0.1703± 0.0004	0.1676± 0.0008	0.5622	-
48	0.1531± 0.0028	0.1694± 0.0127	0.1842± 0.0014	0.1927± 0.0028	0.01464	Flash-White Smooth-White
216	0.3985± 0.0113	0.3395± 0.0019	0.3076± 0.0014	0.3683± 0.0028	0.00044	All
528	0.8465± 0.014	0.6318± 0.0014	0.6049± 0.012	0.6873± 0.0029	<0.0001	All
768	1.0925± 0.0016	0.8280± 0.0016	0.7668± 0.0017	0.8962± 0.0025	<0.0001	All

- Does not apply, there is no significance difference.

Table 4. Operating conditions and obtained results in stirred vertical reactors at different usage conditions.

Stirring rate/ color & stress light	Dry biomass (g L-1)	Time (h)	Productivity (mg $L^{-1}h^{-1}$)
35 rpm	0.9	528	1.7
78 rpm	0.82	528	1.55
118 rpm	1.1	528	2.08
White	1.3	456	2.85
Blue	0.55	480	1.14
Green	0.93	528	1.76
Red	0.9	480	1.87
White	1.1	768	1.43
Smooth	0.83	768	1.08
Intensity decrease	0.78	768	1.02
Flash	0.9	768	1.17

Conclusions

Rate variable has influence on microalgae growth, it is not a value that represents a great variation on biomass production, however, the highest productivity results were found at 114 rpm of rate.

Light variable has influence on microalgae growth, where it was demonstrated that white light is the best option for microalgae growth; however, blue light has a negative influence.

Stress variable has a negative effect on microalgae growth.

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Nomenclature

P	productivity, $mgL^{-1}h^{-1}$
OD	optical density, adimensional
Db	dry biomass, gL^{-1}

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