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ORIGINAL ARTICLE

# Modeling the effects of light wavelength on the growth of *Nostoc ellipsosporum*

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## Abstract

Mathematical models provide information about population dynamics under different conditions. In the study, four models were evaluated and employed to describe the growth kinetics of *Nostoc ellipsosporum* with different light wavelengths: Baranyi-Roberts, Modified Gompertz, Modified Logistic, and Richards. *N. ellipsosporum* was grown in BG-11 liquid medium for 9 days, using 12 hours of photoperiod and the following treatments: white light (400-800 nm), red light (650-800 nm), yellow light (550-580 nm) and blue light (460-480 nm). Each experiment was performed in triplicate. The optical density (OD) was measured on days 1, 3, 5, 7 and 9, using a spectrophotometer at 650 nm. The maximum cell growth was obtained under white light ($OD_{650}$ : 0.090 ± 0.008), followed by the yellow light ($OD_{650}$ : 0.057 ± 0.004). Conversely, blue light showed a marked inhibitory effect on the growth of *N. ellipsosporum* ($OD_{650}$ : 0.009 ± 0.001). The results revealed that the Baranyi-Roberts model had a better fit with the experimental data from *N. ellipsosporum* growth in all four treatments. The findings from this modeling study could be used in several biotechnological applications that require the production of *N. ellipsosporum* and its bioproducts.

**Keywords:** cyanobacteria; light; mathematical model; microbial growth.

## Introduction

Cyanobacteria are gram-negative prokaryotes that represent the oldest group of autophototrophic organisms able to perform oxygenic photosynthesis (Makhalanyane *et al*., 2015).They have an evolutionary history of 3.5 billion years and are the ancestors of all plastids. They also serve as the only source of biogenic oxygen and a major source of fixed carbon and nitrogen (Pang *et al*., 2018). These microalgae are widely distributed in aquatic and terrestrial niches, this dominance is due to their metabolism, plasticity and structural conservation (Abed *et al*., 2009). Cyanobacteria are known for

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their complex photosynthetic systems, which can channel the absorbed solar energy into diverse forms of energy along with producing several metabolic products with biotechnological applications, such as proteins, exopolysaccharides (EPS), pigments, lipids for third generation biofuels, biofilters, antioxidants, biofertilizers and others (Delattre *et al*., 2016; Guo *et al*., 2016; Margarites *et al*., 2016; Pereyra & Ferrari, 2016; Ryu *et al*., 2017; Garlapati *et al*., 2019).

Among the cyanobacteria, the species from the genus *Nostoc* stand out as ideal candidates for biopharmaceutical applications (Cibichakravarthy *et al*., 2019). There are over 40 different *Nostoc* species that produce more than 120 bioproducts with antiviral, antioxidant, anticancer, antifungal and antimicrobial activities (Shalaby *et al*., 2019). These species are found in gelatinous colonies, composed of filaments called "trichomes"surrounded by a thin mucilaginous sheath that excrete EPS, which released is mainly concerned with protecting cells from physical or chemical stress and for anchorage purposes (Garlapati *et al*., 2019). They are known for their ability to lie dormant for long periods of time and recover metabolic activity when rehydrated with water (*Nostoc ellipsosporum*, 2018). The present study focuses in *Nostoc ellipsosporum*, a species known for its unusual chemicophysical stability with a prospective role in metallic nanoparticles production (Khanna *et al*., 2019), and its potent virucidal activity against a broad spectrum of enveloped viruses, including human immunodeficiency virus (HIV), influenza virus and herpes simplex virus, thanks to the cyanovirin-N protein produced by this microorganism (Xiong *et al*., 2010; Singh *et al*., 2017). Due to the type of applications, *N. ellipsosporum* requires innocuous culture conditions in order to avoid contamination, and axenic conditions to ensure the dominance of the species. Therefore, large-scale microalgae cultivation systems, such as open raceway ponds, are not suitable for this species, because they often suffer losses in productivity or alterations in the biochemical production as a result of contamination by parasites, predators or competitor microalgae that could inhibit the growth of *N. ellipsosporum* (Fuentes *et al*., 2016; McBride *et al*., 2016; Novoveska *et al*., 2016; Supriyanto *et al*., 2019).

A crucial point in the biotechnological process of *Nostoc* is to identify the optimal growth conditions that result in higher biomass accumulation, and improvement of polysaccharide production and structural features (Chiu *et al*., 2008; Da Silva Ferreira & Sant'anna, 2017). Hence, an increasing number of researchers are focusing on how to increase cyanobacterial bioproducts yields by optimizing specific culture conditions, such as light, pH, carbon source and nitrogen source (Andersen, 2005; Xu *et al*., 2019). This is closely linked to the species physiology and the requirements of each growth

phase, lag and exponential (Crnkovic *et al*., 2017; Cui *et al*., 2017). Cellular division during these growth phases depends on light and nutrient availability, as those are vital for the photosynthetic system (Barsanti & Gualtieri, 2006; Celekli *et al*., 2009; De Oliveira *et al*., 2014; Crnkovic *et al*., 2017). During this photosynthetic process, the sunlight is trapped by the pigments stored in the multilaminar complex named thylakoids and then elated to the core of photosystems I and II (Nozzi *et al*., 2013).

Cyanobacterial photosystems are mainly composed by chlorophyll a, which maximum absorption wavelength is 420 nm, and phycobiliproteins, such as phycocyanin and phycoerythrin, that absorb light at 610 nm and 570 nm, respectively (Barsanti & Gualtieri, 2006). Visible light or white light contains all wavelengths from about 400 nm to 800 nm. Thus, white light is able to stimulate all the photosynthetic pigments from photoautotrophic organisms (Hai *et al*., 2000). However, in terms of light quality, it has been shown that different wavelengths can enhance the sustainability of cyanobacteria cultures according to the target of production (Pagels *et al*., 2019). For instance, multiple studies reported that red light (650 nm) and blue light could promote the production of EPS or accelerate metabolic paths in cyanobacteria from genera *Nostoc*, *Synechocystis* and *Synechococcus* (Bland & Angenent, 2016; Han *et al*., 2017a; Han *et al*., 2017b; Ooms *et al*., 2017), and the microalgae *Chlorella* (Atta *et al*., 2013).

Overall, the growth, survival, and production of cyanobacteria bioproducts have been shown to be affected by some abiotic factors such as different light wavelength bands (Sinha *et al*., 2003). Thus, there is a need for evaluating the different light wavelengths that may affect the growth and synthesis of bioactive compounds in order to commercially exploit *Nostoc ellipsosporum* (Kokabi *et al*., 2019). This type of research signifies an inexpensive alternative to enhance biomass production without increasing the nutrient supply (Johnson *et al*., 2014; Han *et al*., 2015; Khajepour *et al*., 2015; Gaytán-Luna *et al*., 2016; Crnkovic *et al*., 2017; Da Silva Ferreira & Sant'anna, 2017).

Microalgae growth can be monitored employing the linear relationship between optical density (OD) and cell density during the exponential phase (Griffiths *et al*., 2011; Liu *et al*., 2011), provided by the direct correlation between OD and the concentration of photosynthetic pigments like chlorophyll and phycobiliproteins (Richmond, 2004; Algal culturing techniques, 2005; Delattre *et al*., 2016). Therefore, microbial biomass and microbial pigments can be estimated by measuring absorption at certain wavelength (Da Silva Ferreira & Sant'anna, 2017). The results can be used to analyze the effect of environmental variables on microalgae growth at

different scales (Chiu *et al*., 2008; Ryu *et al*., 2017). Once the variables affecting the microalgae physiology are understood, growth can be mathematically modeled (Halmi *et al*., 2014; Park & Lee, 2016; Lotfi *et al*., 2017). This approach has been used in monitoring the growth of *Chlorella vulgaris* (Infante *et al*., 2012), *Spirulina platensis* (Kim & Lee, 2018), and *Synechocystis* sp (Schuurmans *et al*., 2017).

A typical microbial growth curve shows sigmoidal behavior and exhibits four growth phases (lag, exponential, stationary, and cell death). During the lag phase, microorganism adapt to culture conditions. Through the exponential phase, the number of cells and the rate of population increase due to rapid cell division. The stationary phase is characterized by growth-limiting conditions, the growth rate and death rate are equal, thus, the number of individuals does not increase. Finally, there is a cell death phase that results from environmental stress.

Mathematical modeling is an essential and powerful tool to study and analyze microalgae growth under different physiological conditions. Mathematical models complement *in vivo* and *in vitro* experimental approaches and can also be used to design experiments. This tool has been utilized to provide insight into industries such as food and medicine (Li *et al*., 2007). The use of mathematical models to describe and predict the behavior of microorganisms (growth, survival, and inactivation), occurs in two stages. First, an approximation using primary models to explain microorganism growth over time is used, these models can be deterministic or stochastic depending on the type of parameters and variables. Then, secondary models are brought up to describe the relationships between the parameters from the primary model and the environmental factors that affect the development of the microorganisms (Swinnen *et al*., 2004). Furthermore, growth kinetics combined with deterministic primary models have been used in empiric models with known functions such as Logistic, Gompertz, Richards, Schnute, Stannard, Ratkowsky, Weibull and Von Bertalanffy. Some of these models have modified versions using parameters with biological meaning (Halmi *et al*., 2014; Zwietering *et al*., 1990). Moreover, some primary models have a mechanistic conception and include parameters and functions with biological meaning like Baranyi-Roberts (1994, 1995) and Huang (2013) models.

In this sense, the aim of the present study was to monitor the growth of *N. ellipsosporum* under different light wavelengths and compare four deterministic primary models used to describe the species growth, three modified empirical: Logistic, Gompertz and Richards (Zwietering *et al*., 1990), and one mechanistic: Baranyi-Roberts (Baranyi &

Roberts, 1994, 1995) (**Table 1**), as a way to contribute to cyanobacteria biotechnological development. The modified growth models: Logistic, Gompertz, and Baranyi-Roberts are based on the maximum specific growth rate ($\mu_{max}$), i.e. the increase in the number of cells over time, the latency phase and the time. On the other hand, the modified Richards growth model introduces an additional parameter for adjustment ($v$), which must always have a positive value considering that cellular growth is related to the binary fission of the parental cells in the culture medium and its minimum value has to be zero (Zwietering *et al*., 1990; Baranyi & Roberts, 1994; Cayré *et al*., 2007; Merli & Perazzi, 2017), since there cannot exist a negative number of cells (Cayré *et al*., 2007; Trejos *et al*., 2009; Infante *et al*., 2012; Castillo *et al*., 2017). At the same time, the mathematical model, as an abstraction of reality, must show an adjustment in its curves in order to closely represent the behavior of the experimental data, as it is the product of a complex interaction between environmental factors and the biology of the analyzed microorganism (Zwietering *et al*., 1990; Castillo *et al*., 2017; Merli & Perazzi, 2017).

## Materials and methods

### Strain information

For growth experiments, *Nostoc ellipsosporum* was used (Göttingen Univ. B1453-79), provided by the Institute of Vegetal Biochemestry and Photosynthesis (IBVF) from the Center of Scientific Research Isla de Cartuja, Seville (Spain). *N. ellipsosporum* Rabenhorst ex Bornet & Flahault belongs to the Prokaryotic domain, Eubacteria kingdom, Negibacteria subkingdom, Cyanobacteria phylum, Cyanophyceae class, Nostocophycidae subclass, Nostocales order and Nostocaceae family. They have a highly metabolic plasticity which allow them to endure high temperatures and acid environmental conditions, and inhabit different areas such as Great Britain, Czech Republic, Spain, Turkey, North-America, Argentina, Bangladesh, India, Iraq, Nepal and New Zealand (*Nostoc ellipsosporum*, 2018).

### Culture conditions

*N. ellipsosporum* was cultivated in BD flasks with 30 ml of BG-11 liquid medium for 9 days. The inoculum concentration was $1.1 \times 10^4$ cells/ml, and was isolated from a pre-culture at exponential phase and counted using a Neubauer chamber. The glass paper wrapping method was used to deduce the effect of light wavelengths on *N. ellipsosporum* biomass production, this technique is based on the principle that the color glass paper allows only a selective wavelength of light to pass through it; i.e., if red colored glass

**Table 1.** Mathematical models used in the present study: Baranyi-Roberts, modified Gompertz, modified logistic and modified Richards.

| Model | Expression |
|---|---|
| Modified logistic | $y(t) = \frac{A}{1 + e^{\frac{4\mu_{max}}{A}(\lambda - t) + 2}}$ |
| Modified Gompertz | $y(t) = Ae^{-e^{\frac{\mu_{max}e}{A}(\lambda - t) + 1}}$ |
| Modified Richards | $y(t) = A\left[1 + ve^{(1+v)}\, e^{\frac{\mu_{max}}{A}(1+v)(1+1)(\lambda - t)}\right]^{-1/v}$ |
| Modified-Roberts | $y(t) = A + \mu_{max}\, t + \ln\left(e^{-\mu_{max}t} + e^{-h_0} - e^{-\mu_{max}t - h_0}\right) - \ln\left(1 + e^{\mu_{max}t + 1\left(e^{-\mu_{max}t} + e^{-h_0} - e^{-\mu_{max}t - h}\right)} - 1\right) / e^{y_{max} - A}$ |

$y(t) = \ln(x(t))$, where $x(t)$: cell density at moment $t$.
$\mu_{max}$: maximum specific growth rate, $\lambda$: lag time,
$A$: growth inferior asymptote
$v$: adjustment parameter, $h_0 = \mu_{max}\lambda$.

paper is used, then only red color light wavelength will pass through the paper (Rehman & Dixit, 2019). Each glass paper was wrapped around the flask and 10 cm kept away from the light source (Kang *et al*., 2015; Ojit *et al*., 2015; Sanmartín *et al*., 2017; Rather & Singh, 2018; Znad *et al*., 2018). Fluorescent lamps adjusted to 1200 lux using a lux meter were employed. A total of 12 experimental units were analyzed with the following treatments: white light (400-800 nm) as control treatment with no colored glass paper; red light (650-800 nm); yellow light (550-580 nm); and blue light (460-480 nm). Each experiment was performed in triplicate. The experimental units were kept under in controlled conditions such as 23 °C and 12 h of photoperiod, according to the Biotechnology Laboratory at the Federal University of São Carlos (UFSCar) in Brazil (Camargo & Lombardi, 2018). On days 1, 3, 5, 7, and 9, optical densities (OD) were measured using 1 ml of *N. ellipsosporum* liquid culture and a spectrophotometer (Mettler Toledo) at 650 nm.

## Data processing

All the results were analyzed by means of (one-way) ANOVA followed by Tukey HSD multiple comparisons. All statistical analyses were performed using SPSS (V. 21) software. $P < 0.05$ was considered statistically significant.

All four chosen models, Baranyi-Roberts (Baranyi & Roberts, 1994, 1995; Baranyi *et al*., 1995), modified Gompertz (Zwietering *et al*., 1990), modified Logistics (Gompertz, 1825; Zwietering *et al*., 1990) and Richards (Richards, 1959; Zwietering *et al*., 1990), consider biological meaningful parameters such as $\mu_{max}$ (maximum specific growth rate) and $\lambda$ (lag time). Growth data was adjusted using a non-linear regression and the CurveExpert Basic 2.1.0 software, which employs the Marquardt algorithm. This algorithm minimizes the Residual Sums of Squares between predicted and experimental values, while providing correlation coefficient and standard error. The obtained data was adjusted to the four models, and the models were compared employing a F-test (Motulsky & Ransnas, 1987).

## Results and Discussion

Cyanobacteria began an adaptive diversification 3.5 billion years ago by optimizing oxygenic photosynthesis, managing to expand their ecological niches (Dhar *et al*., 2015). Such changes led to the evolution of a wide spectrum of photosynthetic pigments with specialized functions in order to compete with other autotrophic organisms (Morales *et al*., 2017; Benítez *et al*., 2018). Oxygenic photosynthetic organisms like cyanobacteria contain two types of reaction centers, P700 from photosystem I and P680 from photosystem II (Singh *et al*., 2015). Photosystems have chlorophyll as their main pigment and phycobilisomes (PBS) as light-harvesting antennae to optimize the absorption of light energy (Bland & Angenent, 2016; Hagemann *et al*., 2016). PBS are supramolecular assemblies of water-soluble proteins named phycobiliproteins (PBP) and polypeptides (Ho *et al*., 2017). PBP are heterodimers of two structurally related but distinctly specialized polypeptides, $\alpha$ and $\beta$, made up of seven alpha-helices, each, which allow the complementary chromatic adaptation (Singh *et al*., 2015).

Cyanobacteria often feature an extensive range of colored pigments due to these highly fluorescent and covalently bound phycobiliproteins (Arteni *et al*., 2009). Some of these proteins are phycocyanin (CPC) with an absorption spectrum around 610-620 nm (blue), allophycocyanin (APC), having an absorption spectrum around 650-655 nm (blue grey), and phycoerythrin (CPE) around 540-570 nm (red) (Tiwari *et al*., 2015; Shukla *et al*., 2016; Garlapati *et al*., 2019). The absorption spectrum of phycobiliproteins is determined by an open-chain tetrapyrrole chromophore and the environment (Singh & Singh, 2015).

As mentioned before, phycobiliproteins have a very important role in regulating the distribution of the absorbed light energy between photosynthetic systems I and II, reason for which phycobiliproteins go under rapid changes when light is modified. This physiological mechanism allows cyanobacteria to adapt fast to environmental conditions. Therefore, light quality determines the intensity of all vital processes affecting yield and quality of the biomass obtained (Bland & Angenent, 2016; Chen *et al*., 2016; Cepoi, 2019). In this scenario, the effect of different light wavelengths (i.e., red, yellow, blue and white) on the growth of *Nostoc ellipsosporum* was evaluated and the obtained results are presented next. Growth was monitored based on the optical density ($OD_{650}$) over a 9-day incubation period.

## Laboratory results

As shown in **Fig. 1**, *N. ellipsosporum* had significantly distinct levels of cell growth when cultivated under different light wavelengths.

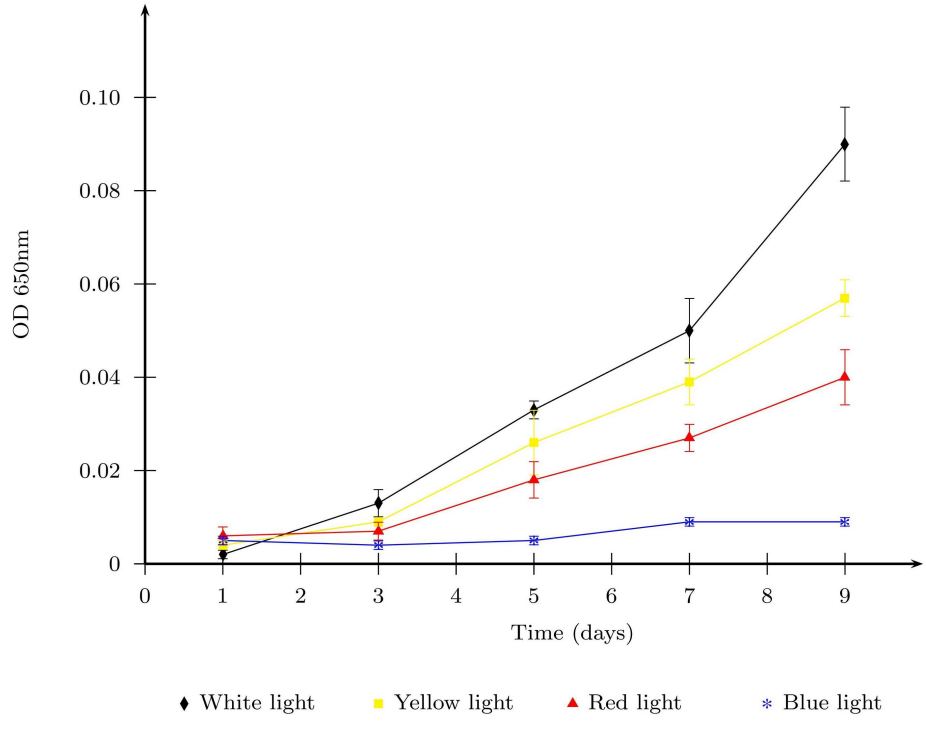


**Figure 1.** Growth curves from *N. ellipsosporum* under different light wavelength, treatments were as follow: white light (400-800 nm); red light (650-800 nm); yellow light (550-580 nm) and blue light (460-480 nm). OD: Optical density.

The ($OD_{650}$) of *N. ellipsosporum* was higher under white light conditions (0.090 ± 0.008), followed by yellow (0.057 ± 0.004), red (0.040 ± 0.006) and blue light (0.009 ± 0.001). These results may be due to the wider photo-spectrum distribution of white light (400-800 nm), which stimulates mostly all photosynthetic pigments from autotrophic microorganisms (Ho *et al*., 2014); as seen in microalgae *Navicula incerta* (Singh & Singh, 2015) and cyanobacterium *Nostoc flagelliforme* (Han *et al*., 2014). Similarly, Kim *et al* (2013) reported that the cell growth rate of the microalgae *Scenedesmus* sp. was the highest under white light conditions, followed by red, blue, and green light, respectively. Zhao *et al*., also stated that the highest maximum specific growth rate and biomass productivity of microalgae *Chlamydomonas* sp. was under white light, then green, blue, and red (Zhao *et al*., 2019). Nevertheless, Das *et al*. reported that the biomass productivity of *Spirulina platensis* was the highest under red light condition, yet the maximum specific growth rate of *Nannochloropsis* sp. was achieved under blue light (Das *et al*., 2011). Since this is not the present case, it can be said that the effect of light wavelength on biomass production in photoautotrophic microalgae is species-dependent (Ho *et al*., 2014).

The growth of *N. ellipsosporum* showed a progressive increase from day 3 until day 9, under white, red and yellow lights (Fig. 1). The maximum $OD_{650}$ values were observed on day 9 of the incubation period. On the other hand, under blue light, the growth was stationary, almost non-existing, and slow from day 5 to day 9.

*N. ellipsosporum* showed a similar trend in its growth curve under white light as previous growth curves reported by several authors in *Nostoc flageliforme*, *Synechocystis* sp. and *Microcystis aeruginosa*, exhibiting a lag phase during three days after inoculation and an exponential phase afterwards (Han *et al*., 2014; Bland & Angenent, 2016; Crettaz Minaglia *et al*., 2017). Overall, comparison of the $OD_{650}$ using (one-way) ANOVA and Tukey HSD multiple comparisons showed that the *N. ellipsosporum* cells grew significantly better ($P = 0.000$; 98 % correlation coefficient) under white light (control), and that blue light restricts cyanobacteria growth.

*N. ellipsosporum* photosynthetic system includes various pigments. The primary pigment is chlorophyll a, whose maximum absorption wavelength is 420 nm, and is stimulated by white and blue lights (Singh & Singh, 2015); since chlorophyll is the most abundant, the cyanobacterium grew better under the white light. Other pigments in the photosynthetic systems are phycocyanin with a maximum absorption at 620 nm, it is stimulated by white, red and yellow lights; and phycoerythrin whose maximum absorption wavelength is 570 nm and is stimulated by white and yellow lights (Barsanti & Gualtieri, 2006; Johnson *et al*., 2014). As described above, phycocyanin and

phycoerythrin are pigments that efficiently harvest light energy and transfer it to photosynthetic reaction centers. They are produced to increase the range of light absorption, once the cyanobacterium needs alternative ways to get light and energy in order to grow. They are directly related and influenced by the composition of the light provided to the culture (Pagels *et al*., 2019). In the present study there was a slight adaptation of *N. ellipsosporum* when exposed to red and yellow light under the established conditions according to the $OD_{650}$ values, which suggest some increase in these proteins' contents and a consequent effect on the cellular growth and function. Likewise, Tiwari *et al*. observed that higher quantity of phycocyanin is produced under red light and phycoerythrin under green light in *Nostoc muscorum*, in order to guarantee the photosynthetic efficiency (Tiwari *et al*., 2015). However, acclimation to the different light quality is not horizontal and it varies according to the species. For example, it has been described that, under blue light, some *Anabaena* species increase the total phycobiliprotein productivity (Pagels *et al*., 2019). The same outcome has been observed in *Nostoc sphaeroides* (Ma *et al*., 2015). Nonetheless, no adaptation under blue light was observed in this study.

In photosynthetic organisms like *N. ellipsosporum*, carotenoids and xanthophylls serve as both light harvesting pigments and to protect cells from photooxidative damage. They absorb light from the green and blue regions (380-580 nm). Carotenoids production is related to the nutrient's availability in the genus *Nostoc* (Yang *et al*., 2019). When carotenoids are absent, the number of phycocyanin molecules connected to the phycobilisome nucleus is strongly reduced, thus, disturbing the organization of the thylakoid membrane and affecting the microalgae growth (Tóth *et al*., 2015). Under the experimental conditions of this study, *N. ellipsosporum* seems to be associated with low concentrations of carotenoids, similar to what has been described before (Loaiza *et al*., 2016; Rivera-González *et al*., 2017).

The inhibitory effect of blue light on the growth of *N. ellipsosporum* is comparable to the results from Ooms *et al*., where blue light also diminished *Synechococcus* sp. biomass production (Ooms *et al*., 2017); and to the results from Luimstra *et al.*, which showed that cyanobacterium *Synechocystis* sp. displayed lower $O_2$ and biomass production rates in blue light (Luimstra *et al.*, 2018, 2019). It was demonstrated that cyanobacteria have low photosynthetic efficiency in blue light because PBS does not absorb wavelengths under 450 nm, and hence blue light mainly excites the photosystem I, while fewer photons excite the photosystem II reaction center (Solhaugh *et al.*,2014; Kirilovsky, 2015).

Also, although blue light absorption is strong, a great part of this energy is diverted to other metabolic paths (Itoh *et al*., 2014; Teo *et al*., 2014),

resulting in cell division delays (Oldenhof *et al*., 2006). Furthermore, particular light wavelengths can enhance the production of specific metabolic products in closely related species. Han *et al*. showed in 2018 and 2019, that different light qualities influenced the gene expression of *N. flagelliforme*, and that cells treated under blue and red light showed more significant changes in carbohydrate metabolism, energy metabolism, amino acid metabolism, nucleotide metabolism, activities of key enzymes and signal transduction. Moreover, light quality was considered to be a key factor controlling the growth and exopolysaccharides biosynthesis (Han *et al*., 2018; Han *et al*., 2019).

## Mathematical modeling of data

Turbidimetry allows the detection of small particles that diffract light in a culture medium according to its concentration, within the detection limits determined by the spectrophotometer used. Since it is a fast and low-cost methodology, it has been widely used to study bacterial growth through the measurement of optical density (OD) and is used as a tool for mathematical modeling of cellular growth (Dalgaard *et al*., 1994; Dalgaard & Koutsoumanis, 2001; Santiesteban-López & López-Malo, 2008; Belda-Galbis *et al*., 2011; Gupta *et al*., 2012; Tevatia *et al*., 2012; Mytilinaios *et al*., 2014; Pla *et al*., 2015; Ahmad *et al*., 2015; Ibrahim *et al*., 2018; Silva *et al*., 2018; You *et al*., 2018; Charlebois & Balázsi, 2019).

Data from all four light wavelength treatments (white, yellow, red and blue), was adjusted to the selected models (Baranyi-Roberts, modified Gompertz, modified logistic and modified Richards). Estimation of the model with the better adjustment was based on correlation coefficient and the F-test (**Table 2**). An example of the adjustment is presented in **Fig. 2** using data from *N. ellipsosporum* growth under yellow light. According to the correlation coefficient and the F-test, Baranyi-Roberts model showed the best adjustment to experimental data from all four treatments. This is particularly interesting considering it is a mechanical-based model; however, biological parameters were used from the initial approach. In this model (Baranyi & Roberts, 1994, 1995), the starting point is that the variation of cell density $x(t)$ in time $t$ can be modeled from the initial value problem:

$$\frac{dx}{dt} = \mu_{max}\, \alpha(t)\, u(x)\, x, \qquad x(0) = x_0, \tag{1}$$

**Table 2.** Correlation coefficient for adjustments of Baranyi-Roberts, modified Gompertz, modified logistic and modified Richards models to *N. ellipsosporum* growth under different light wavelengths: white light (400-800 nm); red light (650-800 nm); yellow light (550-580 nm) and blue light (460-480 nm).

| Model | White light treatment | Yellow light treatment | Red light treatment | Blue light treatment |
|---|---|---|---|---|
| Baranyi-Roberts | 0.9974 | 0.9978 | 0.9903 | 0.9403 |
| Modified Gompertz | 0.9953 | 0.9964 | 0.9792 | 0.8579 |
| Modified Logistic | 0.9912 | 0.9977 | 0.9814 | 0.8662 |
| Modified Richards | 0.9953 | 0.9979 | 0.9842 | 0.8674 |

where $\mu_{max}$ is the maximum specific growth rate, $\alpha(t)$ is a function that describes the population adaptation to the new environment, $u(x)$ is a function that describes the inhibition growth at the end of the exponential phase and the beginning of the stationary phase.

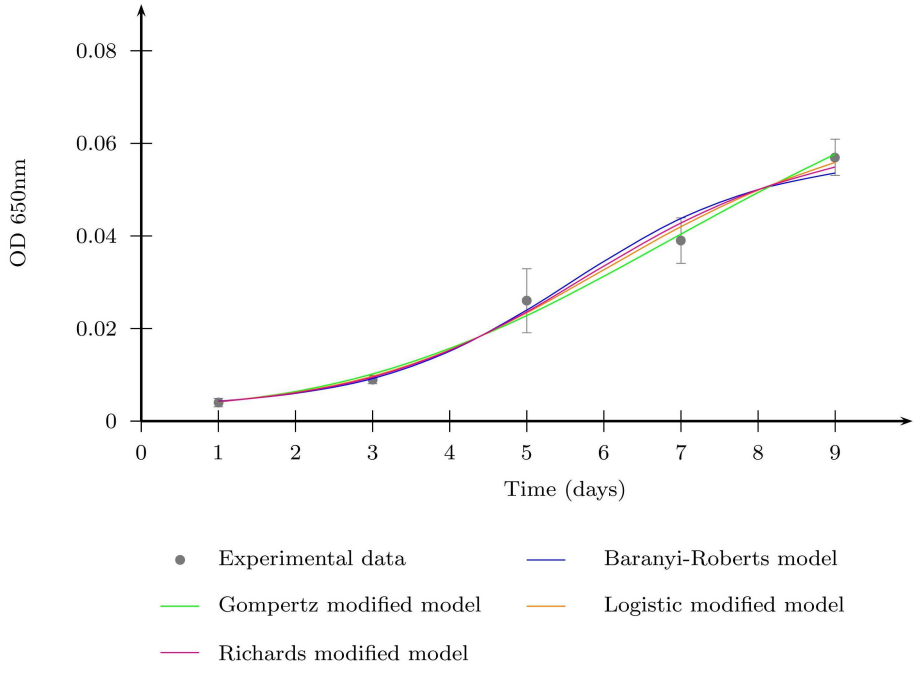


**Figure 2.** Growth curves from *N. ellipsosporum* using the mathematical models: Baranyi-Roberts, modified Gompertz, modified Logistic and modified Richards. All of them under yellow light treatment (550-580 nm). OD: optical density.

If the inhibition function has a Logistic form and $x_{max}$ represents the maximum density of the population, then:

$$u(x) = 1 - \frac{x}{x_{max}}. \tag{2}$$

Now, Baranyi & Roberts model (1994, 1995) supposes that the adaptation function $\alpha(t)$ indicates the required medium to ensure microorganism growth in the new environment, using Michaelis-Menten kinetics, then:

$$\alpha(t) = \frac{q(t)}{1+q(t)}, \tag{3}$$

where $q(t)$ represents the initial physiological state of the population. Under the assumption that $q(t)$ follows a first order kinetics, then:

$$\frac{dq}{dt} = v\ q, \qquad q(0) = q_0, \tag{4}$$

meaning that:

$$\alpha(t) = \frac{q_0}{q_0 + e^{-\mu_{max} t}}, \tag{5}$$

where $q_0$ indicates the initial physiological state of the inoculum and it was taken from $v = \mu_{max}$.

Changing the variable $y(t) = \ln(x(t))$, $y_0 = \ln(x_0)$, $y_{max} = \ln(x_{max})$, the solution to the corresponding initial value problem is given by:

$$y(t) = y_0 + \mu_{max} A(t) - \ln\left(1 + \frac{e^{\mu_{max} A(t)} - 1}{e^{y_{max} - y_0}}\right) \tag{6}$$

$$A(t) = t + \frac{1}{\mu_{max}} \ln\left(\frac{e^{-\mu_{max} t} + q_0}{1+q_0}\right). \tag{7}$$

If

$$h_0 = \ln\left(1 + \frac{1}{q_0}\right), \tag{8}$$

then

$$y(t) = y_0 + \mu_{max} t + \ln\left(e^{-\mu_{max} t} + e^{-h_0} - e^{-\mu_{max} t - h_0}\right) \tag{9}$$

$$- \ln\left(1 + \frac{e^{\mu_{max} t + \ln\left(e^{-\mu_{max} t} + e^{-h_0} - e^{-\mu_{max} t - h_0}\right)} - 1}{e^{y_{max} - y_0}}\right) \tag{10}$$

Using Baranyi-Roberts model (1994, 1995) as a base model, the effect of light wavelength on the growth of *Nostoc ellipsosporum* was adjusted (**Fig. 3**). The $\mu_{max}$ values (1/day) were between 0.5161 to 1.1883. Under the white light treatment (control) the lag phase was negative. Lag phase increased progressively in the yellow, red and blue light treatments (**Table 3**).

Over the last decades, the use of primary models to describe growth kinetics of microorganisms has increased, especially in microalgae research (Lacerda *et al*., 2011; Mohamed *et al*., 2014; Halmi *et al*., 2014). Among many mathematical models used, the Baranyi-Roberts model proved to be very useful in the present study, because its parameters have a direct biological interpretation, it can be applied to dynamic environmental conditions and can be adjusted to the experimental data.

A relationship between the natural logarithm of the OD and the natural logarithm of the cell density DC (cell/ml) was estimated in order to use the OD data in each treatment and calculate the respective cell concentration, considering that the optical density is directly proportional to the cell

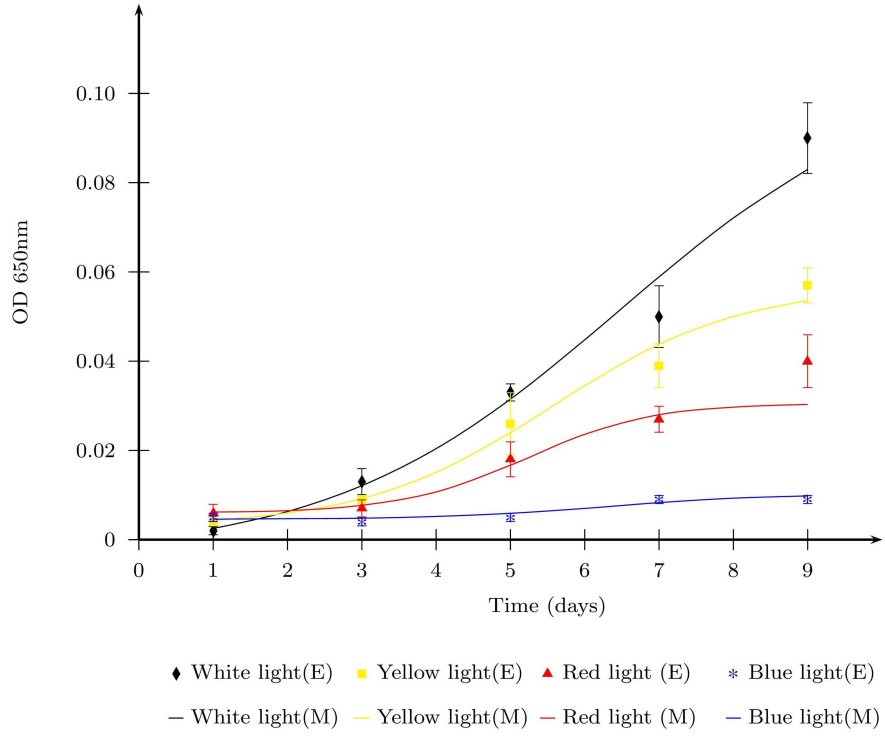


**Figure 3.** Growth curves from *N. ellipsosporum* under four light wavelength treatments. The curves were adjusted according to the Baranyi-Roberts mathematical model. OD: optical density, (E): Experimental, (M): Modelated.

**Table 3.** Parameters adjusted according to the Baranyi-Roberts mathematical model for the growth of *N. ellipsosporum* under different light wavelengths.

| Parameter | White light | Yellow light | Red light | Blue light |
|---|---|---|---|---|
| $\mu_{max}$ (1/day) | 0.5161 | 0.7843 | 1.1883 | 0.9261 |
| λ (day) | | 2.0277 | 3.8561 | 5.4740 |

concentration and that it is a widely used parameter for monitoring cell growth (Infante *et al*., 2012; Vanegas & Ramírez, 2016). Only when a strain reaches the phase of cellular death, due to a high cell concentration, nutrient depletion and accumulation of toxic metabolites; the cell concentration reduces and the cellular biomass is precipitated, thus altering the OD of the culture medium.

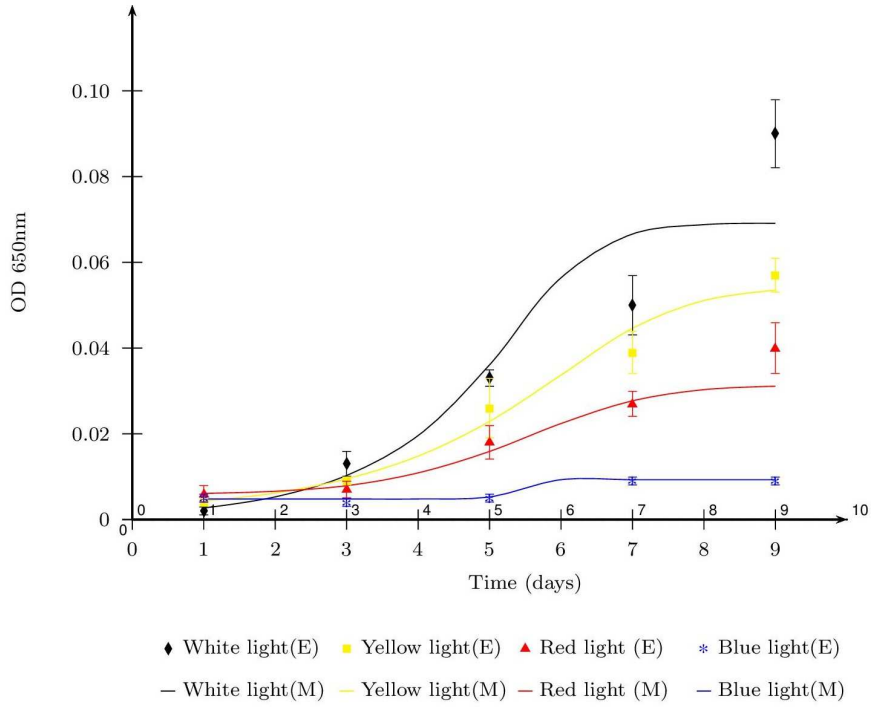


**Figure 4.** Growth curves from *N. ellipsosporum* under four light wavelength treatments. The curves were adjusted according to Baranyi-Roberts mathematical model (BR2): $y = 303978x + 2 \times 10^6$; where $x = \ln(OD)$, $y = \ln(CD)$. OD: optical density. CD:cell density.

**Table 4.** Parameters adjusted according to the Baranyi-Roberts mathematical model for the growth of *N. ellipsosporum* under different light wavelengths based on optical density data (BR1) and calibrated data (BR2).

| Parameter | White light | | Yellow light | | Red light | | Blue light | |
|---|---|---|---|---|---|---|---|---|
| **Model** | **BR1** | **BR2** | **BR1** | **BR2** | **BR1** | **BR2** | **BR1** | **BR2** |
| $\mu_{max}$ (1/day) | 0.5161 | 11.1966 | 0.7843 | 1.3748 | 1.1883 | 1.3646 | 0.9261 | 1.9799 |
| λ (day) | | 5.0705 | 2.0277 | 0.7227 | 3.8561 | 2.7663 | 5.4740 | |

This is why in batch cell culture systems, focused on cellular biomass production or its derivatives, cultivation is carried out until the phase of cellular death. It was found that when modeling the experimental data of OD using Baranyi-Roberts (BR1) a coefficient of determination value of $2.7 \times 10^{-4}$ is obtained, with a previous calibration curve (BR2) formed by the expression:

$$y = 303978x + 2 \times 10^{6}, \tag{11}$$

where: $x = \ln(OD)$, $y = \ln(CD)$. A coefficient of determination of $1.36 \times 10^{-3}$ was obtained, showing a better fit of BR2 with the growth curve of *N. ellipsosporum*. When applying this method to the data from each treatment, the following coefficients of determination were found: $1.32 \times 10^{-4}$ (white light BR1) and $7.32 \times 10^{-4}$ (white light BR2); $3.83 \times 10^{-5}$ (yellow light BR1) and $5.39 \times 10^{-5}$ (yellow light BR2); $9.65 \times 10^{-5}$ (BR1 red light) and $8.43 \times 10^{-5}$ (BR2 red light); $2.79 \times 10^{-6}$ (blue light BR1) and $1.02 \times 10^{-6}$ (blue light BR2). BR1 had a better fit with the red light and blue light treatments, while BR2 better represented the white light and yellow light treatments data (**Fig. 4**). **Table 4** shows the growth parameters of *N. ellipsosporum* under four treatments using different wavelengths of light and modeled with Baranyi-Roberts under conditions BR1 and BR2. This table shows that the white light treatment had the maximum growth rate $\mu_{max}$. The resulting data demonstrated that it is possible to extend the Baranyi-Roberts model to other types of growth indicators, such as absorbance.

Lastly, similar approaches have modeled the growth of various microorganisms under different conditions also using OD measurement and the modified Baranyi-Roberts model. For example, Mytilinaios *et al.* (2014),

examined the growth of *Escherichia coli* and *Salmonella typhimurium* under mild conditions of temperature, salt and pH; and concluded that a rearranged Baranyi-Roberts model could be used with time to detection data obtained from optical density measurements, producing highly accurate specific growth rates and lag phase durations (Mytilinaios *et al*., 2014). In 2005, Perni *et al*., found that the values of $\mu_{max}$ obtained by using the Gompertz, logistic and Baranyi-Roberts models, with absorbance and viable counts measurements of cell concentration, fitted the experimental data from the growth of *Listeria monocytogenes* and *Listeria innocua* well (Perni *et al*., 2005). Moreover, growth curves of *Bacillus cereus*, *L. monocytogenes* and *E. coli* with different initial concentrations were analyzed measuring the microbial growth by optical density, and using mathematical models that provided a good fitting for all growth curves, especially the Baranyi model (Pla *et al*., 2015). Furthermore, once again, the measurement of absorbance was used to monitor cell concentration in three species of lactic acid bacteria, to model their growth. Primary models Baranyi-Roberts and Gompertz were used, achieving a slightly better fit to the experimental data when using the first one (Silva *et al*., 2018).

## Conclusions

In this study, it was investigated the effects of three different light wavelengths (yellow, red and blue) and a control light wavelength (white) on *Nostoc ellipsosporum* growth. The experimental results indicated that white light 10 cm away and at 1200 lux was significantly more efficient for *N. ellipsosporum* growth compared to the other light wavelengths. The highest optical density ($OD_{650}$) value was read as 0.099 on day 9 of the incubation period. Growth under yellow and red light was registered as well. However, blue light inhibited *N. ellipsosporum* growth. Nevertheless, the present study only provides insight into the effect of light quality on the growth and biomass accumulation of *N. ellipsosporum*, if the production of specific bioproducts is required, further investigation must occur.

From the analyzed models, the one that proved to have a better fit with the experimental data from *N. ellipsosporum* in all four treatments was the Baranyi-Roberts model. Modeling provides a valuable quantitative approach to understanding different aspects of microalgae physiology and growth, this is a fundamental step for industrial exploitation.

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## Conflict of interest

The authors declare no conflict of interest.

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## Modelamiento de los efectos de la longitud de onda de la luz sobre el crecimiento de *Nostoc ellipsosporum*

**Resumen:** Los modelos matemáticos proveen información sobre las dinámicas poblacionales bajo diferentes condiciones. En el presente estudio, se evaluaron cuatro modelos (Baranyi-Roberts, Gompertz Modificado, Logístico Modificado y Richards) y se emplearon para describir la cinética de crecimiento de *Nostoc ellipsosporum* con diferentes longitudes de onda de luz. *N. ellipsosporum* creció en medio líquido BG-11 por 9 días, usando un fotoperiodo de 12 horas y los siguientes tratamientos: luz blanca (400-800 nm), luz roja (650-800 nm), luz amarilla (550-580 nm) y luz azul (460-480 nm). Cada experimento se llevó a cabo por triplicado. La densidad óptica (OD) se midió en los días 1, 3, 5, 7 y 9 usando un espectrofotómetro a 650 nm. El máximo crecimiento celular se obtuvo con la longitud de onda de la luz blanca ($OD_{650}$ : 0.090 ± 0.008), seguido de la luz amarilla ($OD_{650}$ : 0.057 ± 0.004). Por el contrario, la luz azul mostró un marcado efecto inhibitorio en el crecimiento de *N. ellipsosporum* ($OD_{650}$ : 0.009 ± 0.001). Los resultados revelaron que el modelo Baranyi-Roberts se ajustó mejor a los datos experimentales de crecimiento de *N. ellipsosporum* en los cuatro tratamientos. Los hallazgos de este estudio de modelación se pueden usar en diversas aplicaciones biotecnológicas que requieran la producción de *N. ellipsosporum* y sus bioproductos.

**Palabras clave:** cianobacterias; luz; modelo matemático; crecimiento microbiano.

## Modelamento dos efeitos da longitude de onda da luz sobre o crescimento de *Nostoc ellipsosporum*

**Resumo:** Os modelos matemáticos dao informações sobre as dinâmicas populacionais sob diferentes condições. No presente estudo, avaliaram-se quatro modelos (Baranyi-Roberts, Gompertz Modificado, Logístico Modificado y Richards) e foram aplicados para descrever a cinética de crescimento de *Nostoc ellipsosporum* com diferentes longitudes de onda de luz. *N. ellipsosporum* cresceu em meio líquido BG-11 por 9 dias, usando um fotoperíodo de 12 horas e os seguintes tratamentos: luz branca (400-800 nm), luz vermelha (650-800 nm), luz amarela (550-580 nm) e luz azul (460-480 nm). Cada experimento se realizou por triplicado. A densidade óptica (OD) foi medida nos dias 1, 3, 5, 7 e 9, usando um espectrofotômetro a 650 nm. O máximo crescimento celular foi obtido sob luz branca ($OD_{650}$ : 0.090 ± 0.008), seguido de luz amarela ($OD_{650}$ : 0.057 ± 0.004). Ao contrário, a luz azul mostrou um marcado efeito de inibição no crescimento de *N. ellipsosporum* ($OD_{650}$ : 0.009 ± 0.001). Os resultados revelaram que o modelo Baranyi-Roberts se ajustou melhor aos dados experimentais de crescimento de *N. ellipsosporum* nos quatro tratamentos. As descobertas de este estudo de modelação podem ser usadas em diversas aplicações biotecnológicas que requeram a produção de *N. ellipsosporum* e seus bioprodutos.

**Palavras-chave:** cianobactérias; luz; modelo matemático; crescimento microbiano.

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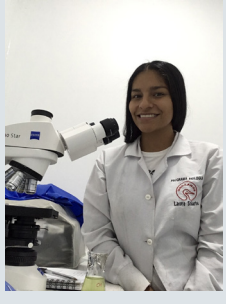

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