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Kinetics of photoautotrophic cell growth of the marine microalgae Chlorella minutissima. Influence of white, blue and red LEDs in cultivation

Amaral, M. S.[a*], Zorn, S. M. F. E.[a], Pedro, G. A.[a], Bredda, E. H.[b], Prata, A. M. R.[a], Silva, M. B.[a,b]

[a] Lorena Engineering School – USP, Lorena, Brasil

[b] Engineering College Guaratinguetá Paulista StateUniversity, UNESP, Guaratinguetá, Brasil
*mateusamaral@dequi.eel.usp.br

***Abstract*— Microalgae are photosynthetic microorganisms considered as a promising feedstock for biofuel production due to numerous advantages, such as: high growth rate, low land occupation, great atmospheric CO_2 capturing efficiency, and high lipid accumulation. Many researchers all around the world studies the light wavelength influence over cell growth and lipid production for different microalgae species. Therefore, the present work performed three photoautotrophic cultivation of marine microalgae *Chlorella minutissima* under LED illumination of three different colors (white, blue and red) in 2 L flasks. For each light color employed it was determined: cell density, volumetric biomass productivity, growth rate and lipid content. The highest cell density and volumetric biomass productivity was 265,34 mg.L^{-1} and 52,9 mg.L.$^{-1}$.d^{-1} under White LED. Meanwhile, the highest growth rate was 0,67 d^{-1} under Blue LED. On the other hand, the highest lipid percentage was achieved when the cultures were grown using the Red LED (21,1%).**

Keywords—Microalgae; Chlorella minutissima; White light; Blue light; Red light; Cell growth kinetics

I. INTRODUCTION

Microalgae are photosynthetic microorganisms that can transform the solar energy into the carbon storage products (lipid accumulation) reducing CO_2 emissions. Lipids extracted from microalgal biomass can be converted in biodiesel. Moreover, the use of biomass from microalgae as an energy source has important advantages compared to other natural sources, like high growth rate and no need for arable lands [1],[2].

The Light Emitting Diodes (LEDs) are efficient light source for rapid production of microalgae biomass in photobioreactors in order to produce biofuel [3]. The light wavelengths used to illuminate microalgae cultivations affect differently cell growth and lipid accumulation. Among all possible light colors, blue and red are more suitable for microalgae growth [4].

According to [5], *Chlorella* species are considered as a promising candidate for commercial lipid production because it is considered as one of the most robust species for cultivation in open ponds due to its capability of resisting to contamination. Thus, this study aimed to performing the cultivation of the marine microalgae *Chlorella minutissima* under different color lights (white, blue and red) using LEDs lamps in order to obtain kinetics parameters of cell growth and lipid content.

II. MATERIALS AND METHODS

A. *Microalgae strain*

All experiments were performed with the Chlor-CF strain of marine microalgae *Chlorella minutissima*, isolated in Cabo Frio (Rio de Janeiro-Brazil). Strain was kindly provided by the Seaweed Culture Collection of Oceanographic Institute at Universidade de São Paulo (SP, São Paulo, Brazil). All the other reagents used were of analytical grade.

B. *Microalgae cultivation*

Photoautotrophic cultivation experiments were performed in three flat bottom flasks (with 2 L working volumes each), stirred with sterile air (filtered with 0.22-μm filter and with aeration rate of 1 vvm), kept at 27±1°C. During cultivation, the flasks were continually illuminated under different color lights (white, blue and red) using LED lamps allocated beside the flasks, see **Figure 1**.Flasks were inoculated by adjusting the initial cell densityto 22 mg/L and the culturewas maintained in a modified Guillard f/2 medium without silica [6], during the cultivations, until stationary phase. Microalgae growth was monitored by measuring the optical density of the algal medium with spectrophotometer (Quimis 08980PT) at a wavelength 540 nm [7]. Measurements were taken daily and each reactor were measured in replicate.

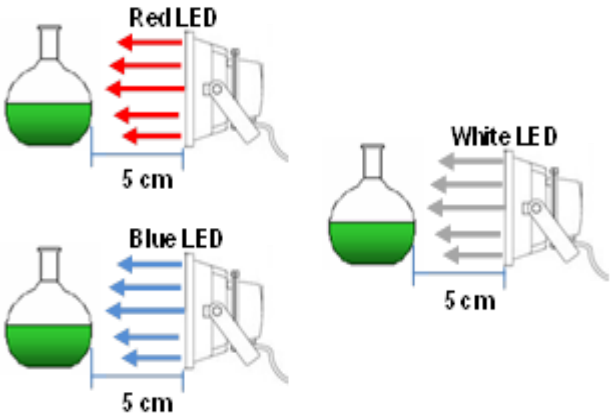

Figure 1: *Experimental set-up.*

C. *Growth evaluation*

Microalgae growth was evaluated daily by optical density measurements at 540 nm in replicates, which was converted into dry cell weight per liter of culture by a regression equation derived previously. Specific growth rate (GR) and volumetric biomass productivity (VBP) were calculated using the cell density (mg/L).

Specific growth rate μ (d^{-1}) was calculated according to the Eq (1);

$$\mu = \frac{\ln X_2/X_1}{t_2 - t_1} \quad (1)$$

Volumetric biomass productivity, r_x (g l^{-1} d^{-1}) was calculated according to the Eq (2);

$$r_X = \frac{X_2 - X_1}{t_2 - t_1} \quad (2)$$

where X_1 and $X_2$are the biomass concentration (g l^{-1}) on days $t_1$and t_2, respectively.

D. *Harvesting and biomass determination*

Resulting biomass at the end of the culture process (stationary phase) was recovered by coagulation using 1 M sodium hydroxide, the supernatant was removed and cells were washed with a 0.6 M ammonium formate solution in order to remove sea salt, being subsequently lyophilized. Biomass productivity was determined by the dry weight biomass per days of cultivation.

E. *Lipid extraction*

Lipids were extracted from the lyophilized biomass according to a modified Bligh's and Dyer's method [8], using a mixture of chloroform, methanol and water as extracting solvent, in the following respective ratio: 1: 2: 0.8 (v/v/v). Subsequently, biomass was filtered and the obtained extract was dried in an evaporator to remove the solvent, and residual lipids were transferred to an incubator (60 °C) until constant weight was achieved. Quantification of lipids total weight was determined by the percentage of lipid per gram in 100 g of biomass.

III. Results and Discussion

Cell density measurements for each microalgae cultivation under white, blue and red light exposure are shown in Figure 2. It can be noted that the white light favored the cell growth more than the other light colors.Cell density achieved under white LED light in photoautotrophic cultivation was approximately the double compared with other cultivations.

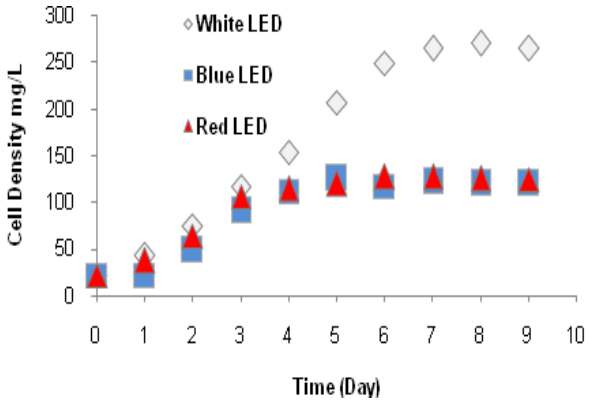

Figure 2: *Cell growth profile of chlorella minutissima cultivated under white, blue and red LED illumination.*

Cultivation illuminated by white LED reached the stationary phase after 7 days, resulting in a cell density of 265.34 mg / L, while both cultures illuminated by blue and red LED reached the stationary phase on the fifth day, reaching cellular densities of 120 mg / L and 125 Mg / L respectively.

Both blue and red LED cultures provided identical maximum biomass volumetric yields on the third day of cultivation (42.33 mg / Ld), while white LED cultivation took 5 days to reach maximum biomass volumetric productivity (52, 91 mg / Ld) as shown in Figure 3.

According to the test, cultures with bright white light tend to have longer lifetimes when compared to cultivations illuminated by blue and red colors.

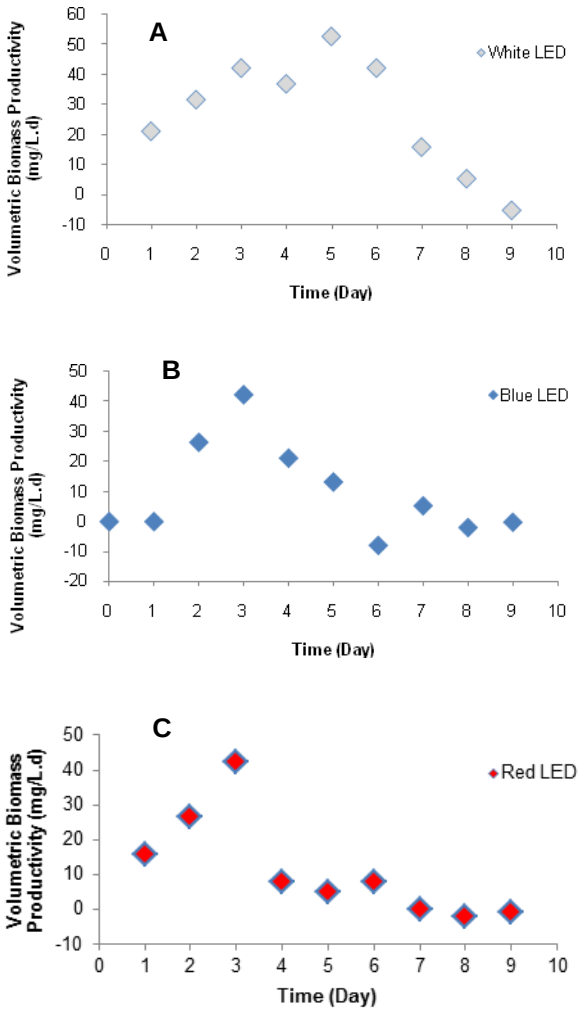

Figure 3: *Volumetric Biomass Productivity: A - White LED; B - Blue LED; C - Red LED.*

Although white LED-lighted culture reached the highest cell density, it was blue LED culture that achieved the highest growth rate, 0.79 d-1, followed by white LED (0.67 d-1) and Red LED (0.54 d-1):

Figure 4 shows the cell growth ratios for *Chlorella minutissima* microalgae for each illumination color (white, blue and red LEDs) throughout the days.

It is possible to note that the maximum growth rate under white LED illumination is reached as early as the first day of cultivation, decreasing gradually in the following days.On the other hand, cultivation illuminated by the red LED reaches the maximum growth rate on the first cultivation day and maintains the same ratio for three days.Cultivation by blue LED takes two days to reach the maximum growth rate, suffering a dramatic decay on the two subsequent days.

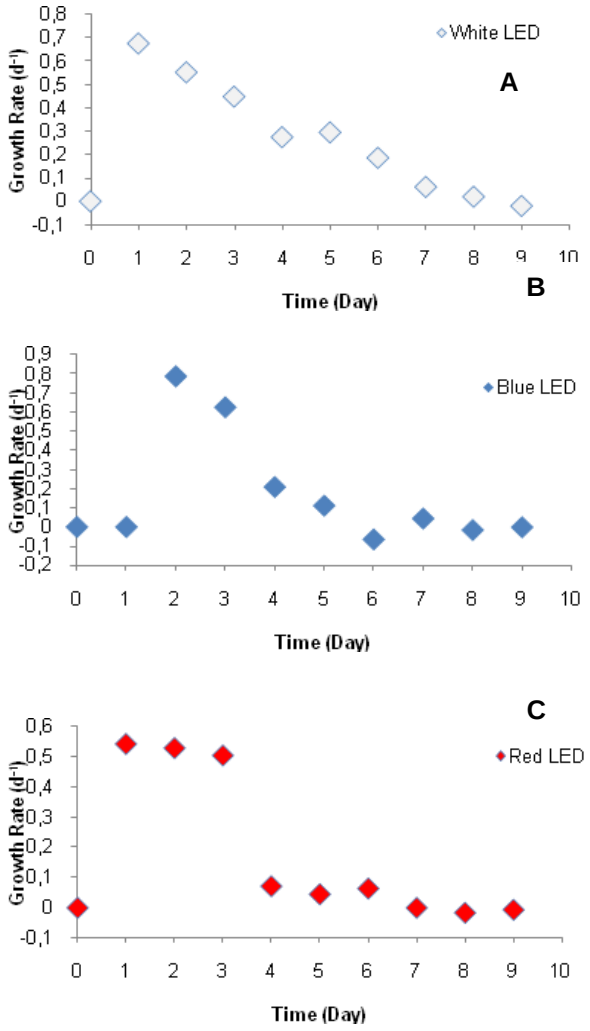

Figure 4: *Growth Ratio: A - White LED; B - Blue LED; C- Red LED.*

Color of the lighting influenced lipids accumulationof marine microalgae*Chlorella minutissima* studied in this work. Highest lipid content was observed in red LED cultivation (21.1%), followed by blue LED (17%) and white LED (13.2%). Figure 5 shows biomass lipid content formed for each crop (white, blue and red LEDs).

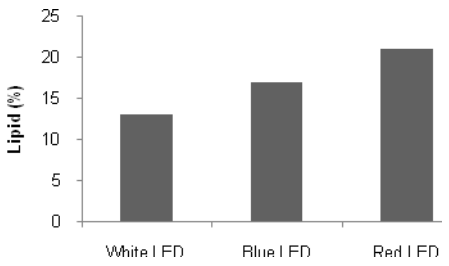

Figure 5: *Chlorella minutissima* lipids content

Higher cell density and, consequently, higher biomass volumetric productivity was achieved in the cultures illuminated by the white LED (265.34 mg / L and 52.91 mg / Ld respectively), while higher value for maximum growth rate was reached when using blue

LED (0.79 d-1).On the other hand, highest lipid content reached was 21.1% for the culture illuminated by red LED as summarized in Table 1.

Table 1: *Chlorella minutissimacellular density, volumetric productivity, growth rate and lipid content.*

	Cell Density ($mg.L^{-1}$)	Volumetric Biomass Productivity ($mg.L.^{-1}d^{-1}$)	Growth Rate (d^{-1})	Lipids (%)
White LED	265,34	52,91	0,67	13,2
Blue LED	120,3	42,33	0,79	17
Red LED	124,9	42,33	0,54	21,1

IV. Conclusion

Tests carried out showed that the kinetic parameters of biomass formation for marine microalga *Chlorella minutíssima* are influenced by the light color used in photoautotrophic cultures. It was possible to notice that the white light provided highest values of cell density and volumetric productivity, while blue light provided highest growth rate. However, accumulated lipid content in the biomass was maximized when red light was used.

Acknowledgment *(Heading 5)*

Capes and Oceanographic Institute - USP

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