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The Effects of Wavelength and Salinity on Biomass Production from *Haematococcus pluvialis*

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Abstract

Currently, microalgae have emerged as promising source of high-value products due to its biomass productivity, high photosynthetic efficiency and non-competitive

environment for growth. In this work, biomass production from *Haematococcus pluvialis* was studied at three salt concentrations (0, 0.45 and 0.9% v/v) and blue, white and red wavelengths in order to evaluate the effects of these factors on microalgae growth. A 3^2 experimental design was performed and its results were analyzed by Manova test with 95% of reliability. The results obtained suggest that the best combination for biomass production consists of red wavelength and salt concentration of 0% v/v with a maximum productivity of 8172.2 mg/mL.

Keywords: Wavelengths, salinity, biomass, microalgae

1 Introduction

The growing of global demand for energy, depletion of resources and environmental pollution problems have led to search for suitable alternatives such as biomass production from microalgae or agricultural wastes [1]. Biomass utilization is an ever-trending process that is globally used for heating, cooking and energy production purposes, due to its sustainable nature, physicochemical characteristics, abundance and effective role in carbon dioxide atmospheric sequestration [2, 3]. Microalgae are single-celled or colonial photosynthetic organisms that are naturally present in different aquatic/humid environments [4]. They only need a liquid medium, some nutrients and sunlight to stimulate the growth of biomass, which makes feasible the use of land inappropriate for cultivating food products [5]. The interest for these organisms lies in their potential to produce biomass for food, feed and fine chemicals but mainly for the possibility of synthetizing biofuels from microalgae biomass [6]. The microalgae growth during photosynthesis is influenced by environmental parameters such as light and salinity. Salinity is an intricate stress that affects various physiological and biochemical mechanisms associated with the development of microalgae [7]. On the other hand, the effects of various LED light wavelengths and intensities have been previously investigated [8]. The aim of this work is to evaluate the effect of both salinity and light wavelength on growth of *Haematococcus pluvialis* in order to determinate the most suitable conditions for biomass production.

2 Materials and Methods

Culture methods

Haematococcus pluvialis strain was obtained from strains bank of industrial biotechnology research group, previously acquired from NUTRE Company. For preparing axenic culture of microalgae, metronidazole was used as an antibacterial and anti-parasitic agent. Three concentrations of metronidazole (3, 6, and 9 μg/mL) were evaluated in 1 L bioreactor for 8 days with photoperiod of 12 h light /12 h darkness, at a temperature of 24 ° C ± 1 ° C. After changes in culture appearance, adaptation to Bold medium was carried out according to Perales-Vela et al. [9]. For evaluating salinity concentrations and wavelengths, horizontal photobioreactors of

7 L were prepared, using 10 L capacity containers with plastic lid with two air intakes supplied by a high-power aquarium pump.

Biomass quantification

Dry weight methodology was used for quantifying biomass production from microalgae. Initially, calibration of 0.20µ nitrocellulose membranes with 45 mm diameter was performed during 24 hours at temperature of 70 °C. Then, they were sent to the oven and weighted after 30 minutes (W_1). The membrane filtration technique was implemented by passing 10 mL of sample (V_m) through this. Membranes were put back into oven for 12 hours at a temperature of 70 ° C and weighed to obtain the W_2. Finally, biomass production was calculated using Equation 1.

$$\text{Biomass (dry weight)} = \frac{W_2 - W_1}{V_m} \cdot 1000 \quad (1)$$

Experimental design

In this research, a 3^2 experimental design was stablished with two factors corresponding to salinity and wavelength, under levels represented by three concentrations of NaCl and three light spectra as shown Figure 1. The evaluations were carried out in 7 L reactors implementing white light as a reference parameter because it is a normal condition for growth. The culture was developed during 12 days in two phases: the first 5 days cell growth was stimulated and then, it was subjected to stress by prolonged exposure to light. The lamps implemented were fluorescent light located approximately 15 cm from photobioreactor. Different concentrations of NaCl were added after the fifth day of cultivation: 0, 0.45, and 9 % v/v implementing as reference 0% v/v.

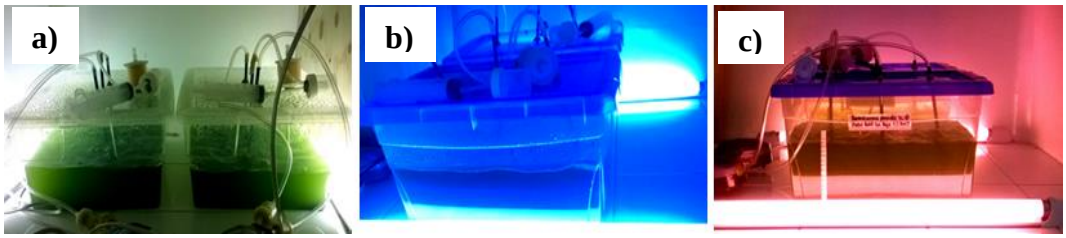

Figure 1. Microalgae culture under: a) white, b) blue and c) red light wavelengths

3 Results and Discussion

The study of *Haematococcus pluvialis* culture showed a phase of adaptation to new conditions during the first 3 days. The next day, an increase in cellular division of microalgae population is observed, especially in cells exposed to red light, which is suggested by an intense green coloration and formation of precipitates, characteristic of microalgae biomass. On the fifth day of cultivation, cells reproduction is mitigated mainly with white and blue light, while treatment with red

light affects growth but not limit it. During the following days, the crops have changes in the hue of their coloration becoming more yellow with the presence of slight orange and red precipitates.

Effect of light wavelengths

White light and salinity concentration of 0% v/v were established as control kinetics. As shown in Figure 2, microalgae biomass increased relatively low with days under this culture condition. Similar results were observed for blue light; due to both light wavelengths generate a photo-inhibition effect that mitigate the cell reproduction. On the other hand, red light decreased the doubling time and increased growth rate as observed in Table 1, generating a maximum biomass production of 8218.87 mg/mL, 51% and 65% higher than blue and white light, respectively. This is because red light improves accumulation of carbohydrates, the main component of microalgae cell wall and therefore affects their development [10].

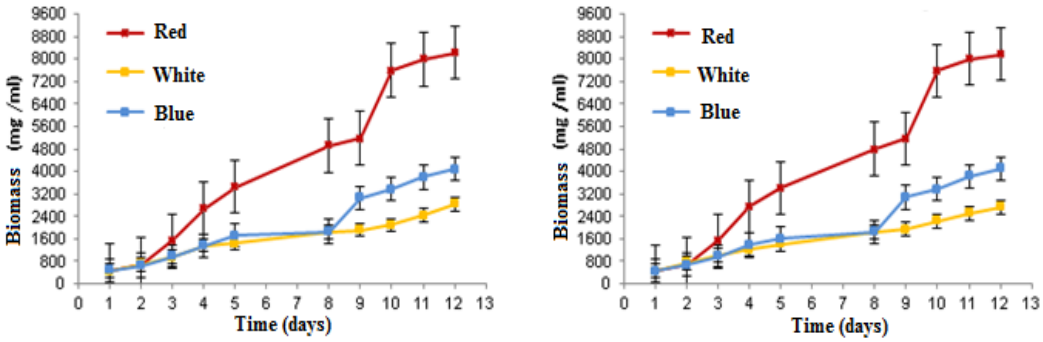

Figure 2. Biomass production under blue, red and white wavelengths and concentration of 0% v/v of salinity in duplicate.

Table 1. Kinetic parameters of *Haematococcus pluvialis* under blue, red and white wavelengths and concentration of 0% v/v of salinity in duplicate

Treatments	Reactor 1		Reactor 2	
	$\mu_{máx.}$ (day^{-1})	Doubling time (day)	$\mu_{máx.}$ (day^{-1})	Doubling time (day)
White light [0]% salinity	0.319	2.171	0.283	2.445
Blue light [0]% salinity	0.336	2.062	0.346	2.001
Red light [0]% salinity	0.816	0.849	0.805	0.861

Effect of salinity

The increase in salinity in culture medium was performed by adding NaCl on the fifth day of growth. Figure 3 shows the effect of concentration of 0.45%v/v on *Haematococcus pluvialis* cells, evidencing a decrease in cell replication, phenomenon previously reported by Serpa et al. [11].

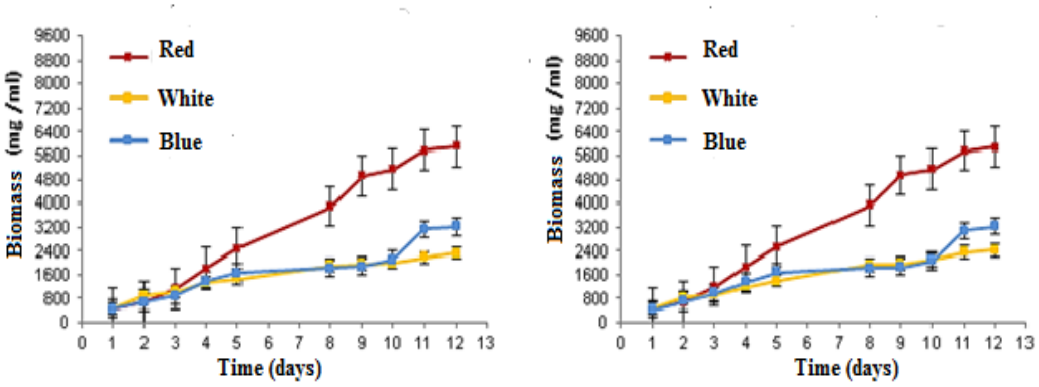

Figure 3. Biomass production under blue, red and white wavelengths and concentration of 0.45 % v/v of salinity in duplicate.

The growth rate in all treatments shows a decrease in its value as shown in Table 2. In addition, it is observed an increase in doubling time due to the effect of salinity on metabolism of *Haematococcus pluvialis*. The cellular responses to saline stress are regulatory and seem to depend on a variety of mechanisms linked to the modification in the balance of abscisic acid, which causes an inhibition of growth or entry to a latency state [12].

Table 2. Kinetic parameters of *Haematococcus pluvialis* under blue, red and white wavelengths and concentration of 0.45% v/v of salinity in duplicate

Treatments	Reactor 1		Reactor 2	
	$\mu_{máx.}$ (day^{-1})	Doubling time (day)	$\mu_{máx.}$ (day^{-1})	Doubling time (day)
White light [0.45]% salinity	0,255	2.714	0.260	2.665
Blue light [0.45]% salinity	0,185	3,748	0.216	3.216
Red light [0.45]% salinity	0,478	1,450	0.444	1.562

Figure 4 shows that concentration of 0.9% v/v presents a strong effect under all light wavelengths, decreasing the concentration of cells even under red light by 30% in relation to the concentration obtained without exposure to the increase in salinity.

The vegetative cells of *Haematococcus pluvialis* are sensitive even at low concentrations of salinity, therefore concentrations of NaCl above or close to 1% are harmful and cause cells bleaching and limit cell reproduction. Table 3 summarizes kinetic parameters obtained at this salinity concentration, in which highest value for maximum growth rate is reached when red light is used in microalgae cultivation.

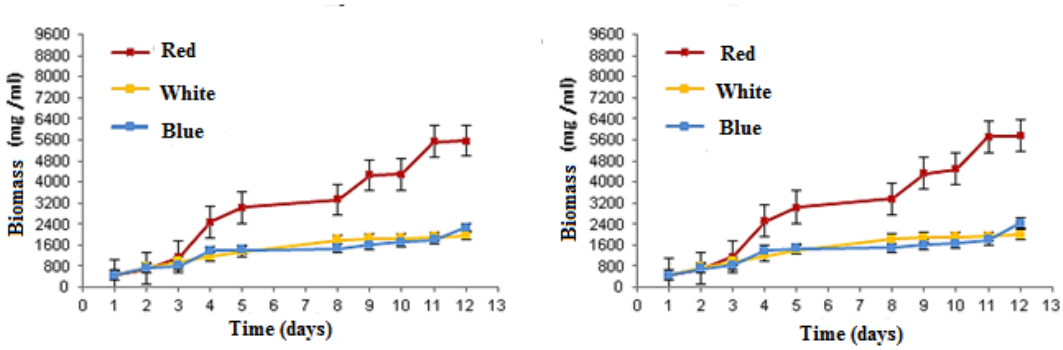

Figure 4. Biomass production under blue, red and white wavelengths and concentration of 0.90 % v/v of salinity in duplicate.

Table 3. Kinetic parameters of *Haematococcus pluvialis* under blue, red and white wavelengths and concentration of 0.90% v/v of salinity in duplicate

Treatments	Reactor 1		Reactor 2	
	$\mu_{máx.}$ (day^{-1})	Doubling time (day)	$\mu_{máx.}$ (day^{-1})	Doubling time (day)
White light [0.90]% salinity	0.185	3.741	0.187	3.713
Blue light [0.90]% salinity	0.136	5.099	0.173	4.015
Red light [0.90]% salinity	0.247	2.802	0.254	2.723

Statistical analysis

The Manova analysis allows to determine the presence of significant differences between the levels of each factor and the incidence of both factors on biomass production as variable of response. The critical value of F for factors was 4.256 and for interaction was 3.633. As the cultivation time elapses, the experimental value of F greatly exceeds the critical value, for which it shows statistically significant differences of treatments on the response variable.

4 Conclusions

This work attempted to study the effect of light wavelengths and salinity on biomass

production from *Haematococcus pluvialis.* The use of metronidazole as an antibacterial and anti-parasitic agent in microalgae cultures is feasible since it does not represent adverse effects on culture development, establishing 3 μg / mL as optimal concentration. The combination that showed the best effect on producing biomass from *Haematococcus pluvialis* incorporates red wavelength and salt concentration of 0% v/v with a maximum productivity 8172.2 mg/mL.

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