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Astaxanthin Production from *Haematococcus pluvialis:* Effects of Light Wavelength and Salinity

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Abstract

Haematococcus pluvialis has attracted much global attention due to its ability to produce huge amounts of astaxanthin, a carotenoid pigment widely applied in pharmaceutical, cosmetic and food industries. In this work, production of astaxanthin from *Haematococcus pluvialis* was performed under three different salt concentrations (0, 0.45 and 0.9% v/v) and wavelengths (blue, white and red) in order to evaluate the effects of salinity and light wavelengths on microalgae growth. A multifactorial 3^2 experimental design was used and its results were studied by Manova analyzer with 95% of reliability. According to the results, the highest amount of astaxanthin (9.72 μg/mL) was obtained at 0.45 %v/v of salt concentration and blue wavelength.

Keywords: Wavelengths, salinity, astaxanthin, microalgae, *Haematococcus*

1 Introduction

Microalgae have been considered as a promising eco-friendly alternative source of

renewable energy because of its photosynthetic efficiency, biomass productivity and oil content [1]. For growth of microalgae, they require liquid medium, some nutrients and sunlight [2]. These organism are source of biomass, lipids, carbohydrate, pigments, antioxidants and proteins [3]. They have been extensively studied for various purposes including the production of biomass as a source of valuable chemicals, in health food and feed, and in wastewater treatment [4]. Among these products based on microalgae is the production of astaxanthin, a natural ketocarotenoid that is a secondary metabolite with powerful properties as antioxidant [5]. Therefore, this carotenoid has great commercial potential in aquaculture, pharmaceutical, cosmetics, and health supplement industries [6]. The freshwater green microalga *Haematococcus pluvialis* is widely accepted as the best natural resource of astaxanthin [7]. Biological process based on the microalgae *Haematococcus pluvialis* is challenged with low cell densities, even though *H. pluvialis* produces the highest level of astaxanthin (1.5-3.0% dry weight) compared to other astaxanthin producers [8]. For enhancing astaxanthin production, many stresses have been considered such as nutrient deficiency, high light intensity, high salinity, hormone treatment and supplementary oxidative stress [7]. 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 astaxanthin production.

2 Materials and Methods

Culture methods

Haematococcus pluvialis was purchased from the Culture Collection of Algae, University of Texas at Austin, USA. The axenic culture of microalgae was prepared using metronidazole as antibacterial and anti-parasitic agent at three different concentrations (3, 6, and 9 μg/mL). A bioreactor with capacity of 1 L was used for carrying out this procedure during 8 days and photoperiod of 12 h light/ 12 darkness. The changes in culture appearance were observed by optical microscopy. Methodology based on Perales-Vela et al. [9] was considered to perform adaptation to Bold medium. The effect of salinity and wavelengths were evaluated in horizontal photobioreactors of 7 L, which were made with 10 L capacity containers with plastic lid and two air intakes supplied by a pump.

Pigments and carotenoids quantification

Quantification of pigments was developed by Wellburn method, modified by Lichtenthaler. A volume of 2 mL of sample was centrifuged at 3500 r.p.m. during 10 minutes. The supernatant was discarded by adding a volume of 2 mL of methanol. Then, samples were mixed, sent to bain-marie for 10 minutes at 70 °C and centrifuged at 3500 r.p.m. for 10 minutes. The liquid obtained was carefully decanted and measurements were made in a JENWAY spectrophotometer at 666, 653 and 470 nm. For determining chlorophylls a and b, as well as total carotenoids, methanol-based extracts and equations proposed by Lichtenthaler and Wellburn (1983) were used:

$$C_a = (15.65\, A_{666} - 7.34\, A_{653}) \cdot (U/V) \quad (1)$$

$$C_b = (27.05\, A_{653} - 11.21\, A_{666}) \cdot (U/V) \quad (2)$$

$$\text{Astaxanthin} = ((1000\, A_{470} - 2.86\, C_a - 129.2\, C_b)/245) \cdot (U/V) \quad (3)$$

Where C_a and C_b represent the concentrations of chlorophyll a and chlorophyll b, respectively, expressed in μg per ml of extract; U is the volume of methanol in mL and V is the volume of sample in mL.

Experimental design

The experimental design selected to carry out this research is multifactorial 3^2 with two factors (salinity and wavelength), under three levels of concentrations of NaCl and light spectra. The experiments were performed in 7 L reactors with white light as reference parameter. Astaxanthin production required the development of culture during 12 days in two phases: stimulation of cell growth in first 5 days and then, it was subjected to stress by exposing to light (red, blue and white). Solution of NaCl was added after the fifth day of cultivation at different concentration: 0, 0.45, and 9 % v/v with 0% v/v as reference.

Statistical analysis

The Manova analysis was used to determine the incidence of salt concentration and wavelengths on variable of response, i.e., astaxanthin production with 95% of reliability using data analysis software Minitab and MS Excel.

3 Results and Discussion

Axenic culture

In order to obtain an axenic culture for *Haematococcus pluvialis* growth process and observe the effect of variables on growth kinetics, three concentrations (3, 6, and 9 μg / mL) of metronidazole were evaluated as is shown in Figure 1. During the first three days of culture, a shock reaction was observed, in which microorganisms that pretended to be controlled (protozoa) proliferated in the crop, however on the fifth day the presence of these organisms was greatly mitigated. Unfavorable effect on vegetative cells of *Haematococcus pluvialis* was not detected. After 8 days of culture, the presence of the protozoa was completely eliminated and an increase in microalgae population density was observed in all concentrations evaluated with respect to the control. Because of all concentrations of metronidazole showed good performance eliminating protozoa in the culture, it was determined to implement the lowest concentration for prevention treatments and the intermediate concentration for eliminating microorganisms in contaminated crops.

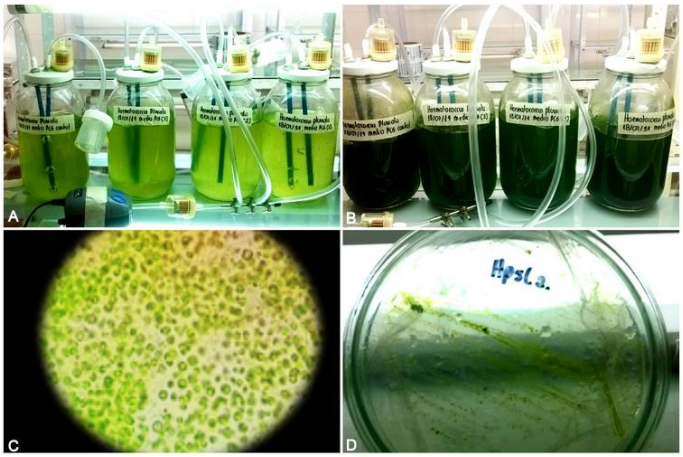

Figure 1. Evaluation of the effect of metronidazole on *Haematococcus pluvialis* culture: (A) appearance of reactors in the first days of culture, (B) change in coloration by growth increase, (C) microscopic observation of axenic culture and (D) conservation of microalga in solid medium.

Effect of light wavelengths

It was considered as a control parameter concentration of 0% NaCl. Figure 2 shows highest accumulation of astaxanthin with blue wavelength due to the function of this carotenoid is usually associated with photo-protection of cells by passive absorption of photons before they reach photosynthetic pigments, which reduce the amount of light transmitted to pigment-protein complexes associated with photosystem II, minimizing photo-inhibition and photo-destruction potentials caused by high-intensity blue lights [10]. Productivity obtained with this wavelength was 9.72 µg/mL, which is much higher than the maximum concentration (0.741 µg/mL) reported by Arias-Ramirez et al. [11] in previous work using stress due to nutrient limitation in *Haematococcus pluvialis*.

The effects of red and white light during exposure to light of 24-hour induced the generation of yellow colorations, which suggest production of primary carotenoids as β-carotene, lutein, violaxanthin, neoxanthin and zeaxanthin that are carotenoids precursors of yellow and orange coloration astaxanthin.

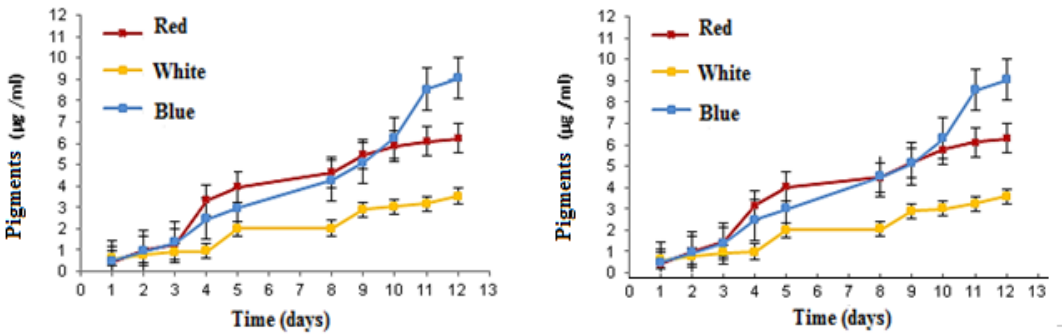

Figure 2. Astaxanthin production under blue, red and white wavelengths and concentration of 0% v/v of salinity in duplicate.

Effect of salinity

Figure 3 shows an increase in astaxanthin production under all wavelengths with 0.45% v/v of salt concentration with respect to the control (0%v/v). Growth kinetics under white light represents more clearly the effect of this factor on response variable, because it is a normal condition for cultivation process. *Haematococcus pluvialis* is a microalgae of fresh water, therefore the increase in salinity generates an alteration of its environment, metabolizing a greater quantity of astaxanthin, as a protection mechanism against oxidative stress, which inhibit the propagation of reactive oxygen species and other free radicals, therefore preventing the harmful action of these at cellular level [12].

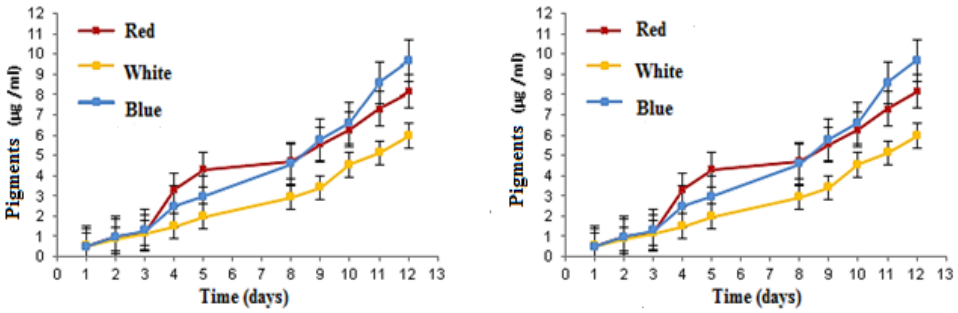

Figure 3. Astaxanthin production under blue, red and white wavelengths and concentration of 0.45 % v/v of salinity in duplicate.

Astaxanthin production increased in 45% with respect to control concentration under white with a maximum productivity of 5.960684 µg/mL, while blue and red wavelengths showed increments of 7 and 23% respectively. This is due to red and blue light have drastic effects on kinetics of *Haematococcus pluvialis* mitigating and generating stress, respectively, therefore their interaction with salinity generates a response more associated to these photoprotective effects.

Figure 4 shows astaxanthin production under salt concentration of 0.8% v/v and different light wavelengths. This concentration exhibited unfavorable effects on producing astaxanthin with a maximum reduction of 14% for red wavelength compared to control concentration. These results agree with reported by Sarada et al. [13], where concentrations of 1% or close to this value generated lysis and whitening of cells.

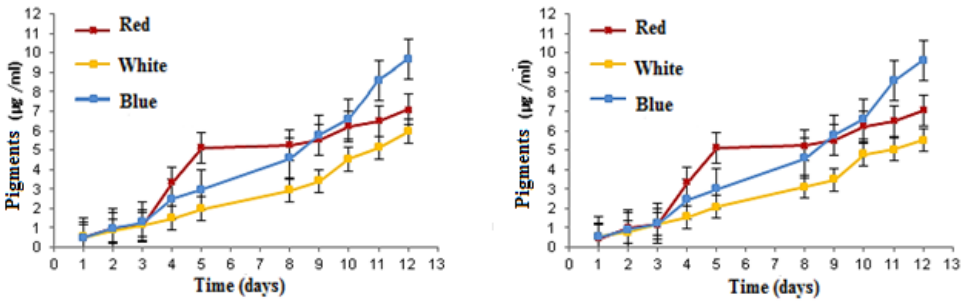

Figure 4. Astaxanthin production under blue, red and white wavelengths and concentration of 0.90 % v/v of salinity in duplicate.

Statistical analysis

Manova analysis for astaxanthin as response variable showed the incidence of both factors (salinity and wavelengths) on production of this pigment. The factor of wavelength exhibited greater effects than salinity on the bioproduction of astaxanthin with a 95% of reliability. The critical value of F for both factors was 4.2564953 and for interaction 3.633089, highly exceeded at the end of the crop with values of 27905.56 for wavelength, 3933.259 for salinity and 497.186 for interaction.

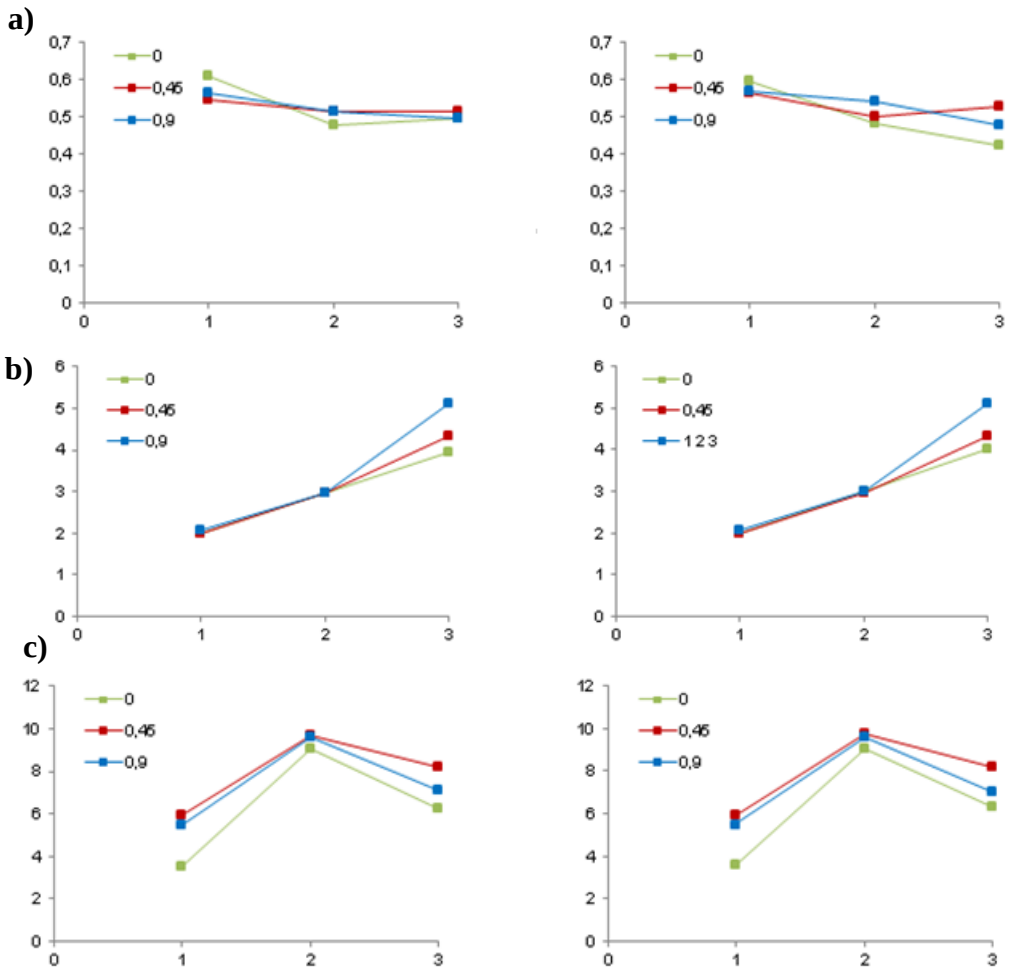

Figure 5. Interaction between factors on astaxanthin production response variable on days: a) 1, b) 5 and c) 12.

Figure 5 shows interaction of factors on response variable on days 1, 5 and 12, where blue, red and white wavelengths are represented by 1, 2 and 3, respectively. The treatment that indicates best effect on response variable is concentration of 0.45 % v/v and blue light, generating a maximum interaction level in the development of stress phase.

4 Conclusions

In this work, the effect of light wavelengths and salinity on astaxanthin production from *Haematococcus pluvialis* was evaluated. The most suitable conditions for producing highest amount of this pigment correspond to blue wavelength and salt concentration of 0.45% v/v with a maximum productivity of 9.72 μg/mL. In addition, it was observed that metronidazole does not cause adverse effects on culture development; hence, it can be used as antibacterial and antiparasitic agent to obtain an axenic culture.

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