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EMPHASIZING THE IMPACT OF LIGHT SOURCE FOR THE PRODUCTION OF ASTAXANTHIN FROM *HAEMATOCOCCUSS PLUVIALIS* AUGMENTATION

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ABSTRACT

Haematococcus pluvialis is freshwater green algae having the neutraceutical values and very case sensitive to high temperature and pH. Many factors affect the growth and astaxanthin production of *H. pluvialis* in open pond system. This study dealt with the experiment carried out in closed conical flask with adequate essential nutrients and exposed to direct sunlight for the duration of 10 light/ 14 dark days. The outcome of this experiment revealed that all the environmental factors standardized and provided in correct ratio for the production of astaxanthin. This is the one of the most economical method of optimizing astaxanthin production through augmentation process because light plays a crucial role when compared to other internal and external factors in the synthesis of chlorophyll in turn converts into astaxanthin during maturation of the cell. Production of astaxanthin from *H. pluvialis* depends on duration of direct light/dark exposure were been standardized and optimized.

KEYWORDS: ***Haematococcus pluvialis,*** **light source, microalgae, astaxanthin, augmentation, pH, temperature**

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INTRODUCTION

Extensive studies on the carotenoid astaxanthin (which are having pharmaceutical values) production by the alga *Haematococcus pluvialis* has indicated that the formation of this carotenoid was greatly stimulated under growth limiting conditions[1], where morphological and biochemical transformations occur from green motile cells into inert red cysts[2]. Rapid production of *H. pluvialis* is delayed by the slow growing nature of this alga. As the astaxanthin content determines the product quality, the algal growth rate of the alga is closely related to its productivity. Microalgae have been extensively studied for various purposes, such as the production of biomass as a source of valuable chemicals or health foods[3] and in wastewater treatment[4]. Recently, the green microalga *H. pluvialis* has been extensively studied. However, one of the main problems[5], in the production of astaxanthin from *Haematococcus* is the contamination of the algal culture with fast-growing unicellular green and or blue-green algae due to the relative slowing nature[6]. *Haematococcus* cells are sensitive to high hydrodynamic stress and changes in cell morphology are evident under various environmental conditions[7]. Astaxanthin is known for it's a superior antioxidant activity, than the carotenoids[8]. Inspite of the fact that various organisms are able to producing astaxanthin, only a small number of these organisms are cultivated commercially. One of the most trustful organisms for astaxanthin production is the freshwater microalgae *H. pluvialis* which accumulates high concentrations of this carotenoid in its cellular content than the others[9].

Haematococcus pluvialis grows in BM (Bold's basal medium) on the combination of KH_2PO_4, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, $NaNO_3$, K_2HPO_4, NaCl, $Na_2EDTA.\ 2H_2O$, KOH, $FeSO_4.7H_2O$, H_2SO_4 (concentrated) Trace Metal Solution are H_3BO_3, $MnCl_2.4H_2O$ $ZnSO_4.7H_2O$, $Na_2MoO_4.2H_2O$, $CuSO_4.5H_2O$, Co $(NO_3)_2.6H_2O$[10]. The macro and micro nutrients are utilized by *H. pluvialis* during its active the growth phase as well as at maturity provide the valuable carotenoid product. The commercial use of *H. pluvialis* extracts can support an amazing feedstock for Astaxanthin production[5]. By the side of present, the algal species are being used for high-yield production of astaxanthin, and the fact that lipid production is associated with astaxanthin accumulation in *H. pluvialis* represents a vision for the additional use of astaxanthin production byproducts[11]. Temperature control allows lowering of the temperature during the night to decrease respiratory activity[12]. In addition, in the early morning temperature can be quickly increased to optimal condition.

Role of pH on Micro algae

pH plays important role in culturing *Haematococcus pluvialis.* Because the culture is very case sensitive and they yield more with optimum temperature and Lighting conditions. The micro algae utilize the pH as best source and start to grow.

Role of Light intensity on Micro algae

The second step was to cultivate the different algal strains used for screening under different light conditions. The aim was to determine the influence of low and high light irradiance on the total algal accumulation. Optimum light conditions resulted in higher biomass in almost all cultures, whereas the uncontrolled lighting shows poor result of growing *Haematococcus pluvialis*.

Role of Nutrient on Micro algae

Previous studies have shown that nutrient conditions is must for growing Microalgae, such as nitrogen malnourishment which can persuade higher biomass production in algal species. Depletion of nutrient will lead Algae to stop their growth and prone to death phase.

Role of temperature on Micro algae

Uncontrolled temperature like below 16°C and higher 35°C are dangerous for some species. If necessary, algal cultures can be cooled by air temperature with chilled air - conditioning units. The controlled temperature promotes more growth on *Haematococcus pluvialis.* The main aim behind this study was to identify pure form of algal culture, detection of astaxanthin, productions and maximum conversion of protoplast and chlorophyll into

astaxanthin components by optimizing the intrinsic and extrinsic factors especially light. Microalgae comprise the capacity to absorb light energy photons and to convert it into chemical energy through the formation of chemical bonds. The basic unit of the photosynthetic apparatus is the photo system. Light energy, i,e. photon is absorbed by carotenoid and chlorophyll pigments of the photo system antenna complex. So this investigation highlights the impact of light source for the growth and production of Astaxanthin from *H.pluvialis*.

METHODOLOGY

Seed culture conditions

Haematococcus pluvialis was obtained from the Culture Collection of Algae, Advance Center for Biotechnology, Anna University, and Chennai, India. The temperature is to be maintained at 24±1^{o} C. The seed culture was used as inoculums for the algal culture study with an initial concentration of 0.03±0.01 g L^{-1}.

Screening studies of Haematococcus pluvialis

The first step of the study was to screen and select productive algal culture suited for further investigations. For this purpose, different freshwater alga like *Chlorella, Volvox, Clamydomonas and H. pluvialis* representing the major algal classes were cultivated at low and moderate temperature for more than three month. The experiments were conducted under fully controlled environmental conditions[18].

Analytical Procedures

These experiments were carried out in triplicates. The seed culture of *Haematococcus pluvialis* were inoculated in 9 conical flask of 1ml in 50 ml of BM (Bold's basal medium). Three conical flasks of *H. pluvialis* culture was taken with pH-6.8 and kept near window day by night without disturbing them. Day by night combination is due to the synthesis of chlorophyll and photosynthetic activity during day time and respiration process during night time. The other 3 conical flask of same culture were kept inside incubator of uncontrolled light with pH-6.8 without disturbing them for few months. Then other 3 set of conical flask of same pH-6.8 were kept under controlled light condition by (10light/14dark). The macro and micro nutrients of BM medium enhance the augmentation of Astaxanthin. This is due to the major reason of direct sun light rays[18].

RESULTS

Culture Maintained in incubator without direct light source

Under this condition, the initial inoculated seed culture showing maximum synthesis of chlorophyll inside each individual cells. When it is incubated in incubator by providing optimum pH-6.8±1 , temperature 24°C±1, is providing all the essential nutrient components and only transmitted light allowed to pass through the glass door. The culture under this condition showed high rate of cell mortality of immatured cells and no astaxanthin production was noticed. Under the observation gives a authentication evidence that light source is one of the major factor which influence the growth of *Hematococcus pluvialis* and production of astaxanthin at minimal level (Figure 1).

Culture maintained under controlled conditions by exposure to the artificial light source (Electric light)

Haematococcus pluvialis culture under artificial light conditions which reveals there is no limitations for the synthesis of chlorophyll process was observed. Whereas the rate of conversion (augmentation) chlorophyll into astaxanthin is not quite promising when compared to the culture maximum exposed to matured light source by optimizing all other intrinsic and extrinsic factors. By this controlled setup of experiment there is moderate level of astaxanthin production were noticed by increasing the culture duration (Figure 2).

Culture and maintained under Natural light source

The *H. pluvialis* culture has been cultured and maintained in natural sunlight condition under 10±2 hours/day optimizing the internal and external factors proven the higher rate of astaxanthin synthesis during winter and spring

seasons. Thereby this research optimized and proves that natural sunlight and seasonal variation has a vital role on augmenting and synthesis of astaxanthin from *H. pluvialis* (Figure 3).

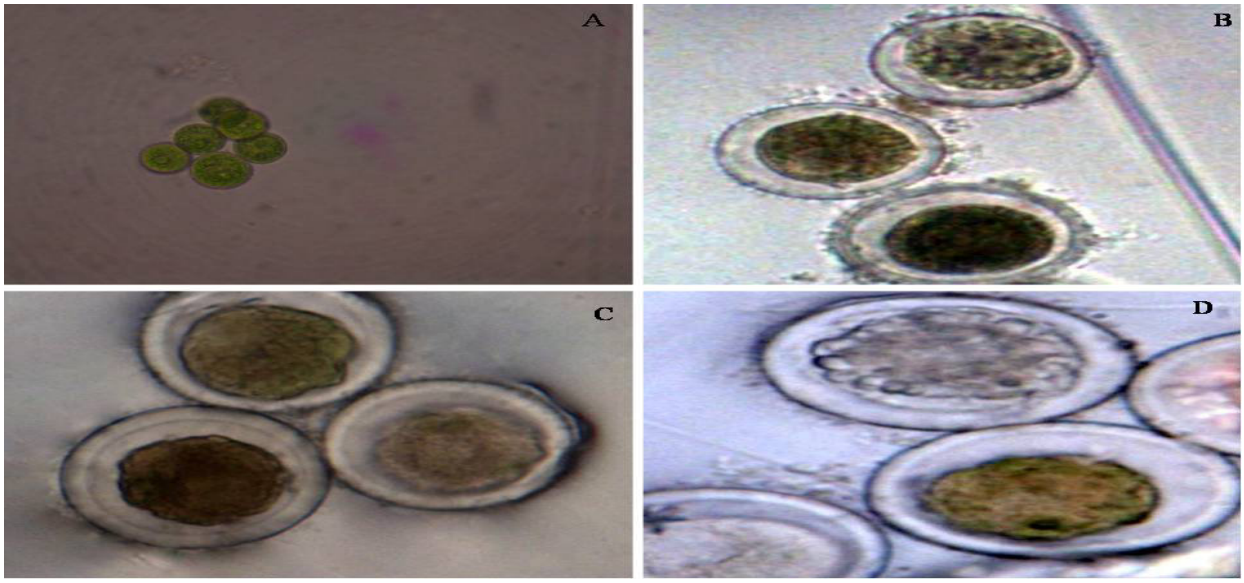

Figure 1
Seed culture of H. pluvialis, B; Culture after 30th day, C; Culture after 60th day, D; Culture after 90th day

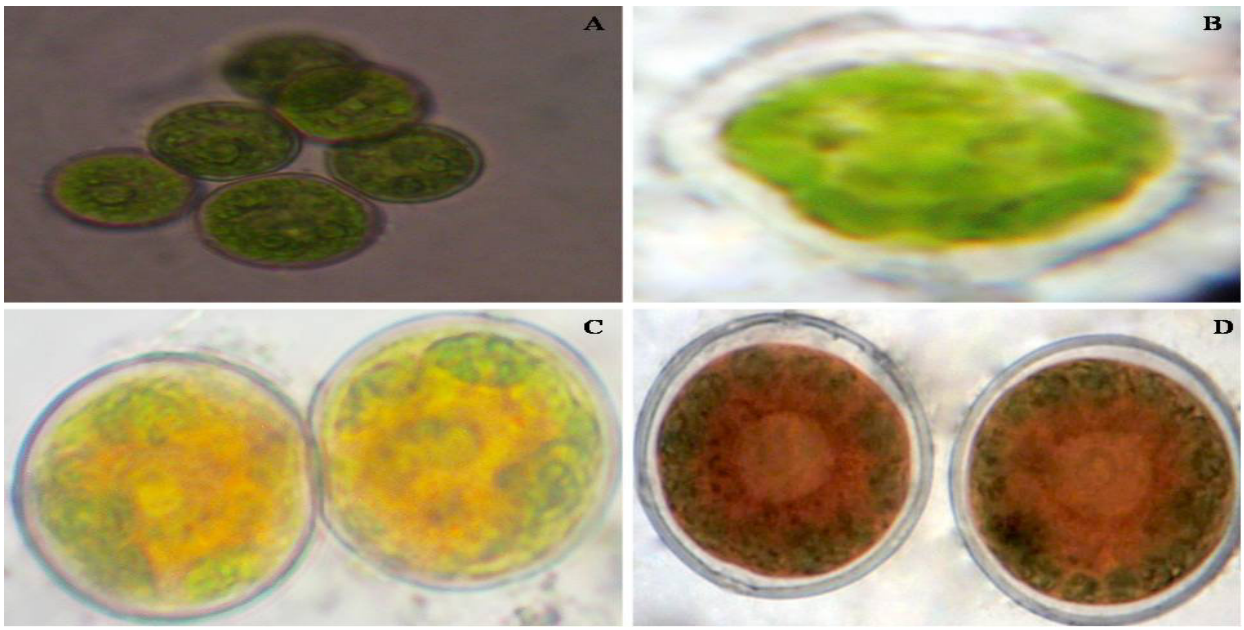

Figure 2
Seed culture of H. pluvialis, B; Culture after 30th day, C; Culture after 60th day, D; Culture after 90th day

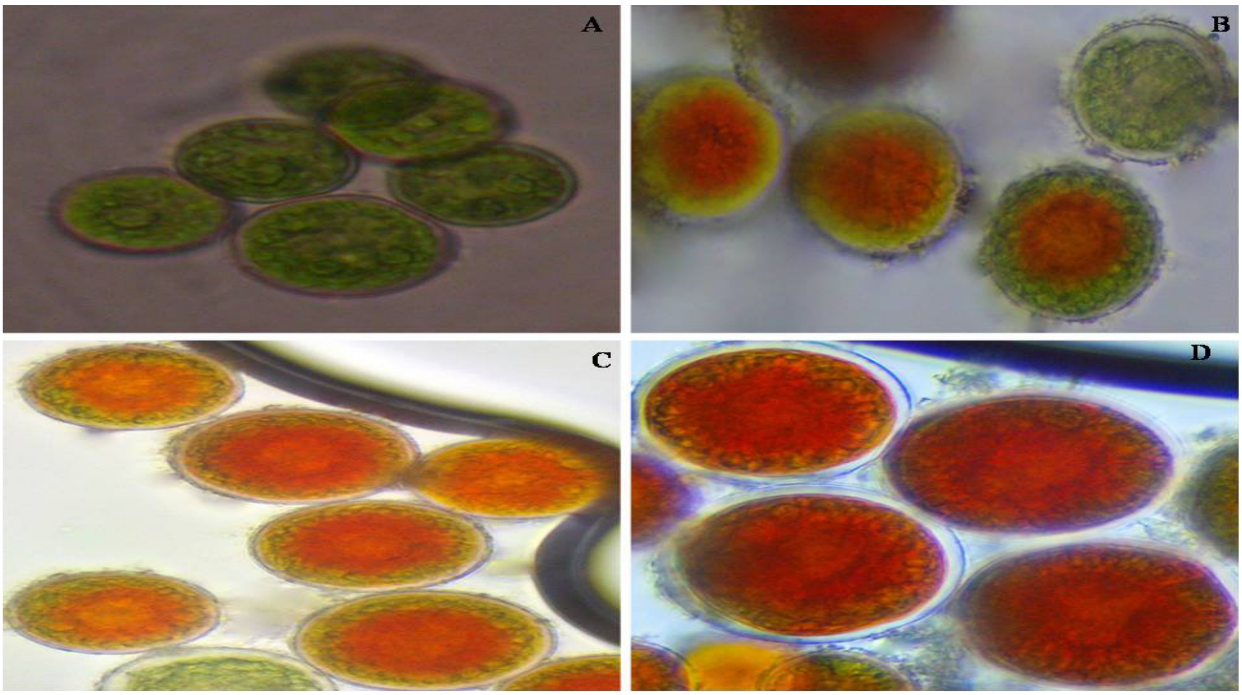

Figure 3
Seed culture of H. pluvialis, B; Culture after 30th day, C; Culture after 60th day, D; Culture after 90th day

DISCUSSION

Algae grow almost everywhere in the world. They are the dominant group of living systems in the aquatic ecosystem providing food and shelter to other organisms. In developing an optimal process for microalgal products, two major aspects are usually considered for improvement. One is the effect of environmental factors such as temperature, light intensity, pH, aeration and agitation, while another is the selection of a suitable nutrient medium[13]. Micro algae play a crucial role in stabilizing the aquatic ecosystem and it was suggested the specific light absorption coefficient of algae cultivated under light/dark cycles is higher than those cultivated under continuous light. Several experiments have been carried out by many researchers on the growth of *Haematococcus sp*. in order to obtain maximum growth rate and Astaxanthin production[7, 14]. Light is essential for the life cycle of *H. pluvialis*. Higher light intensities can lead to photoinhibition. Light penetration (which is inversely proportional to cell concentration) is another problem in the phototrophic cultivation of microalgae[15]. Under optimal growth conditions, light absorbed by antenna pigments is converted to chemical energy forming ATP and NADPH through a photosynthetic electron transport chain. This chemical energy is finally stored in starch by fixing CO_2 through the Calvin cycle[2]. Lowering the temperature level during the night leads to decrease the respiratory activity. Direct observation of cell morphology indicated that the cell tended to change from the vegetative to cyst stage more quickly under high level of sun light. The present study reports that a proper level of nutrients can also enhance the productivity of *H. pluvialis* cell biomass when sun rays in the form of light are supplied 10:14 ratio. However, the mass production of *H. pluvialis* is hindered by the slow growth of this organism. While the astaxanthin content determines the product quality, the algal growth rate is closely related to Astaxanthin productivity. The effect of light intensity is dependent on the nutritional state of the cultures. The interference between nutrient status and light intensity has been already reported in carotenogenesis studies[16], indicating the importance of using an

optimized culture medium for correct interpretation of experimental results[17].

CONCLUSION

Cultivation of microalgae seems quite easy. They are needed only simple nutrients such as ammonium, nitrates, phosphates, trace levels of certain metals and most importantly the carbon dioxide. Because the algae are autotrophic they grow on simple and cheap media. The only problem is that as the algae are phototrophic and light energy is the major limiting factor for their growth. This is nice on one hand, because this energy source (source) is free: But on the other hand, it is very difficult to expose the culture to a sufficient amount of light energy and to utilize this energy efficiently for biomass production. Thus optimizing the *H. pluvialis* culture using sunlight is the best option for cultivating the algae in an economically feasible way. This result of the present study clearly shows that natural light could promote high growth rate of *H. pluvialis* which acts as a neutraceutical and pharmaceutical factor for curing many disease.

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