Investigation of the effect of solar irradiation and temperature on biomass production of H. pluvialis in photobioreactors under outdoor cultivation in Sri Lanka

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ABSTRACT-Complicated and changeable weather conditions and contamination from fungi, protozoa and bacteria increase difficulties in outdoor microalgae cultivation. In this paper, outdoor microalgae cultivation was investigated in Moratuwa city, Western province Sri Lanka. During December, when both the solar irradiation and temperature is high, the water spray system combined two agro shading nets, each with a shading rate of 40-50% can effectively reduce the temperature to 27±2 ^{0}C and control solar irradiation below 13500 lux in the 3 L vertical tubular photobioreactor. Under an initial biomass density of 0.2 g/l and an atmospheric air flowrate of 1 vvm, *H. pluvialis* had a maximum biomass accumulation of 0.45 g/l and the maximum specific growth rate of 0.020 g/l.day. In addition, reactor system and its design exhibited good performance, implying a potential scale- up opportunity.

Key words: *Haematococcus pluvialis;* Outdoor cultivation; Photobioreactor; Biomass accumulation

INTRODUCTION

Microalgae cultivation has earned a wide research interest because microalgae can produce valuable metabolites, such as pigments which can be used as natural ingredients in food products, vitamins and pharmaceuticals. Microalgae can also be used to remove heavy metals from wastewater, as soil conditioners, as biofuels and as biofertilizers (Vega-Estrada et al., 2005). *Haematococcus pluvialis* is a very important alga, because it was reported to be one of the best natural sources of astaxanthin. It may accumulate more than 3% astaxanthin by weight under environmentally and nutritionally specific conditions (Poonkum et al., 2015). Astaxanthin or 3,3-'dihydroxy-β-β-carotene-4,4'-dione is a red ketocarotenoid. This colourful, lipid-soluble compound is a useful pigmentation inducer in food, in the diets of fish and animals and also has beneficial clinical applications due to its high antioxidant activity (Vega-Estrada *et al.*, 2005).

Lack of robust microalgae strains and limitations on cultivation conditions are two important factors that cause the unreasonably high microalgae production cost. Utilizing the sunlight resource to magnify the cultivation of microalgae under outdoor conditions is an effective way to reduce the cost of microalgal biomass production. The current studies are mainly carried out at indoor labs as there are many difficulties in outdoor cultivation. The main difficulties for sustainable outdoor cultivation of microalgae are contamination from fungi, protozoa and bacteria and complex and changeable weather conditions (Huo et al., 2018). In addition, a variety of photobioreactors has been used and proposed for microalgae mass production. However, only a few bioengineering analyses of such reactors have been published.

In this work, a 3 L vertical tubular photobioreactor was designed and assembled to study the feasibility for outdoor cultivation of *H. pluvialis* and evaluate the effect of solar irradiation and temperature on biomass production in batch culture with vegetative cells of *H. pluvialis*.

METHODOLOGY

Algal strain and Inoculum culture preparation

The *H. pluvialis* for the cultivation was obtained from the sample from the culture collection of algae at the University of Texas (UTEX), Austin, USA. Prior to the outdoor cultivation of *H. pluvialis* the inoculum for the outdoor photobioreactor was prepared in the laboratory in 2 L glass reactor bottles with 1800 ml working volume of BBM – Bold's Basal medium. The culture media was prepared with the aeration supply of sterile air, filtered by 0.22 µm PTFE membranes at a rate of 1500 ml/min. For the preparation of the inoculum, the illumination conditions were provided with cool white LED source, with a photoperiod of 12/12 hr: light/dark cycle and the light intensity was maintained for each culture, as 1800 lux, in order to support the cell proliferation. After preparation of the inoculum under mild conditions without any stressing the inoculum was transferred to the outdoor reactor under sterile conditions.

Photobioreactor for the cultivation of *H. pluvialis* and cultivation conditions

Vertical tubular photobioreactor was made of clear acrylic plastic tubes with an outer diameter of 5 cm and a thickness of 0.04 cm. Height of the reactor is 2 m. The schematic diagram of the reactor is displayed in the Fig.1., The outdoor cultures were carried out under controlled conditions. The reactor setup was cooled using a water spray system to maintain the temperature between 24-29 ^{0}C and mixed by air bubbling. The cultivation media (70%-BBM & 30%-inoculum) in the reactors were prepared with an aeration supply of sterile air, filtered by 0.22 µm PTFE membranes at a rate of 1 vvm. Light intensity for the reactor cultures, was controlled below 13500 lux with the use of two agro shading nets each with a shading rate of 40-50%.

Determination of cell density

The dry biomass of the concentrated algal suspension was determined in duplicate by filtering 10 mL of the suspension onto a filter paper (0.4 µm pore size) with a predetermined dry weight of W_1 and dried the whole set in 24 h at 60 °C. The dried filter paper with algae was weighed, and the drying process repeated until an unchanged total weight of W_2 was achieved. The dry biomass in 10 mL concentrated algae liquid was calculated as $D = (W_2 – W_1)/10$.

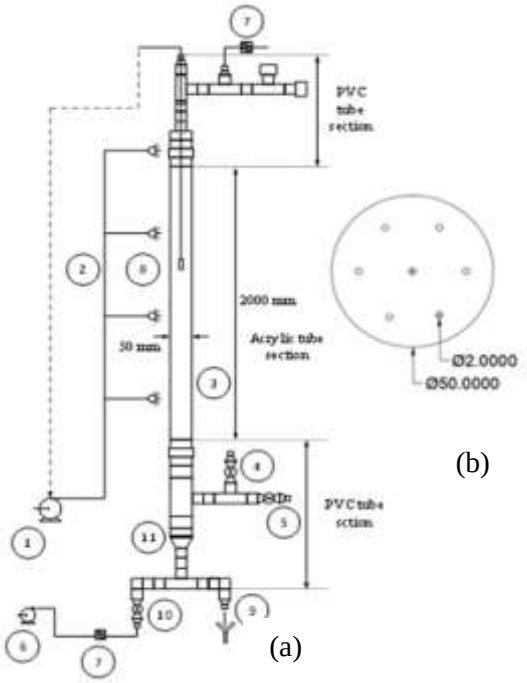

Figure 1. (a): Schematic diagram of the set-up of the vertical tubular photobioreactor with a working volume of 3000 ml. (1) water spray pump, (2) water spray system with water spray nozzles, (3) Acrylic tube, (4) Feed nozzle, (5) Sampling nozzle, (6) Air pump, (7) Air filter, (8) Temperature sensor with controller, (9) Main drain port, (10) Non return valve and, (11) Sparger and (b): Sparger design

RESULTS AND DISCUSSION

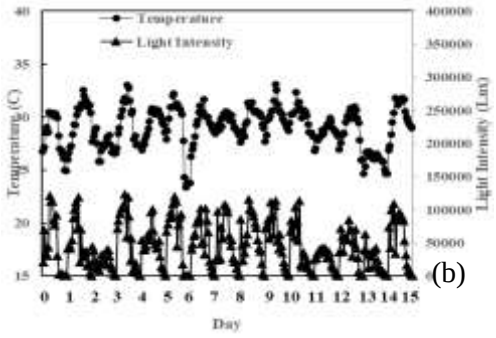

Figure 2. Variation of solar irradiation and temperature throughout the period of cultivation

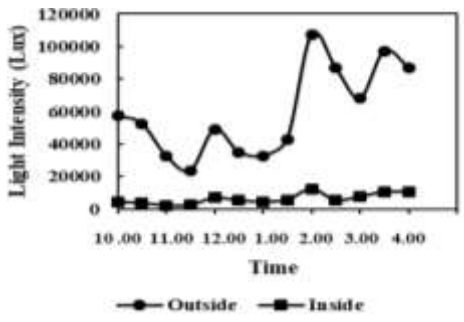

Figure 3. Variation of solar irradiation inside and outside the shading net throughout the day

Solar irradiation and temperature variation outside the shading net is shown in Fig.2. The two shading nets, each with a shading rate of 40-50% has effectively maintained light intensity inside the shading net well below the saturation limit, which is 13500 lux (Fig.3).

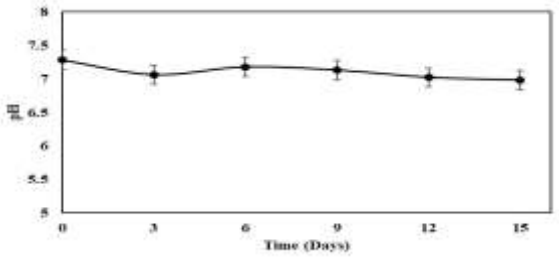

Figure 4. pH variation in the reactor

The pH of the culture medium lies in the acceptable range which is in the range of 6-8 as shown in Fig.4. (Values in literature fluctuate between 7-7.5)

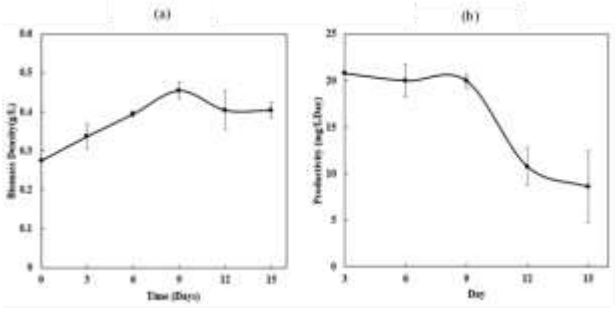

Figure 5. (a): Growth (b): Productivity of *H. pluvialis*

Maximum obtainable biomass density from the outdoor cultivation is 0.45 g/l under controlled temperature (27 ± 2^0C) and light intensity (two 40-50% shading nets). The inoculum used for the cultivation stage is 0.2 g/L. Variation of biomass productivity within the reactor is shown in Fig.5., Maximum reported biomass productivity was 0.0208 g/l.day. Several studies have described the conditions for the production of vegetative green *H. pluvialis*, in most cases productivity was up to 0.55 g/l.day under effective control of temperature in the range of 24-26 ^{0}C.

CONCLUSION

Adopting sunshade net and atomization spray combined system can effectively reduce the photobioreactor temperature and solar irradiance on the outdoor cultivation of *H. pluvialis* is discussed and the cultivation conditions were maintained according to Sri Lankan context. For the optimum growth solar irradiance was maintained under 13500 lux (4000-9000 lux) and temperature was maintained between 27 ± 2 ^{0}C and 0.0208 g/l.day of maximum biomass productivity was achieved.

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