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## OPTIMIZATION OF MEDIA FOR VEGETATIVE STAGE OF *HAEMATOCOCCUS PLUVIALIS* FLOTOW, COLLECTED FROM PITHORAGARH DISTRICT, UTTARAKHAND, INDIA

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### Abstract:

The photosynthetic microalgae *Haematococcus pluvialis* Flotow is one of the best source of the carotenoid astaxanthin. Astaxanthin provides health benefits to humans and that is also used in mariculture feed to enhance the color of salmon flesh. The vegetative stage optimization is one of the difficult and most important step in astaxanthin production process. The potent strain of *Haematococcus pluvialis* Flotow collected from Pithoragarh district, Uttarakhand, India. Pithoragarh is located at 29.58°N 80.22°E. It has an average elevation of 1,514 metres (4,967 feet). In the present study, we optimised the effect of pH, light, NaNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>, NaHCO<sub>3</sub> for enhanced the vegetative growth of *Haematococcus pluvialis*. The maximum level of growth rate, chlorophyll a, chlorophyll b and carotenoid were observed in pH – 7.0, light - 12 hrs Light : 12 hrs Dark, NaNO<sub>3</sub> – 0.3 M, K<sub>2</sub>HPO<sub>4</sub> – 0.08M, NaHCO<sub>3</sub> – 0.08 M. on 40<sup>th</sup> day of incubation period.

### KEY WORDS:

*Haematococcus pluvialis*, Vegetative stage, Astaxanthin, pH, light, NaNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>, NaHCO<sub>3</sub>

### INTRODUCTION:

In recent years the microalga *Haematococcus pluvialis* Flotow has been considered as a possible natural source for the production of astaxanthin and it has been widely studied (Bubrick, 1991). Astaxanthin is a high – value carotenoid pigment with applications in nutraceuticals, cosmetics, food and feed industries (Guerin *et al.*, 2003).

The unicellular fresh water microalga, *H. pluvialis* (Volvocales, Chlorophyceae) is green-colored, biflagellate, and motile in its vegetative stage (Fan *et al.*, 1994). This microalga shows low growth rates and low final cell densities under optimal growth conditions (Fabregas *et al.*, 2000). In its growth stages, it has both motile and non-motile forms (Boussiba, 2000).

Both of the main physical and chemical parameters, especially nutrient medium and light, directly control the growth rate of *H. pluvialis*. Different studies have been performed on the growth conditions of *H. pluvialis* (Fan L *et al.*, 1994; Fabregas *et al.*, 2000; Hata *et al.*, 2001). This comprehensive study on the determination of the culture medium and the light intensity was carried out to maximize the growth of *H. pluvialis* for batch cultivations.

There are several reports on the optimization of culture medium in *Haematococcus*, but most of them mainly focused on the optimum concentration of KNO<sub>3</sub> and NaNO<sub>3</sub> (Borowitzka *et al.*, 1991;

## OPTIMIZATION OF MEDIA FOR VEGETATIVE STAGE OF *HAEMATOCOCCUS PLUVIALIS* FLOTOW.....

Fabregas *et al.*, 2000; Gong and Chen, 1997; Orosa *et al.*, 2005).

In the present study, the effects of various nitrogen compounds, pH, light, NaNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>, NaHCO<sub>3</sub> were investigated to improve the vegetative growth of batch *H. pluvialis* cultures.

### 2. MATERIALS AND METHODS

#### Sample collection

The green algal culture of *Haematococcus pluvialis* Flotow was collected from Pithoragarh district, Uttarakhand, India. Pithoragarh is located at 29.58°N 80.22°E. It has an average elevation of 1,514 metres (4,967 feet). Auxenic culture was obtained after antibiotic treatment (Droop, 1967)

Optimization of parameters and growth conditions for vegetative growth

*H. pluvialis* incubated and grown at different concentrations of pH - 6.0, 6.5, 7.0, 7.5, 8.0; light - 24 hrs light (L), 24 hrs dark (D), 12 (L):12(D), 8 (L):16(D), 16(L):8(D); NaNO<sub>3</sub> - 0.1M, 0.2M, 0.3M, 0.4M, 0.5M; K<sub>2</sub>HPO<sub>4</sub> - 0.02M, 0.04M, 0.06M, 0.08M, 0.1M; NaHCO<sub>3</sub> - 0.02M, 0.04M, 0.06M, 0.08M, 0.1M. Five ml of auxenic culture was inoculated on 250 ml (1:50) bold basal medium, further algae grown on above mentioned parameters at 24°C. It was maintained at 30µEm<sup>-2</sup> s<sup>-1</sup> light irradiance. The cultures were mixed manually thrice a day.

#### Photomicrographs

Microscopic descriptions of the algae are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo II microscopic unit. For normal observations bright field was used.

#### Methods and measurement of growth rate, Chlorophyll a, Chlorophyll b and Carotenoids

UV-visible spectrophotometer (Hitachi U-2900) was used to estimate the different pigments such as Chlorophyll a, Chlorophyll b and Carotenoids using different methods such as Jeffrey and Humphrey, 1975 method for both Chlorophyll a and Chlorophyll b. MacKinney, 1941 method for Carotenoid. Growth rate was measured at 690 nm.

### 3. RESULTS AND DISCUSSION

#### Morphology

*Haematococcus pluvialis* derived from an inoculum with typical characteristics of motile stage, with biflagellate cells. (Fig. 1).

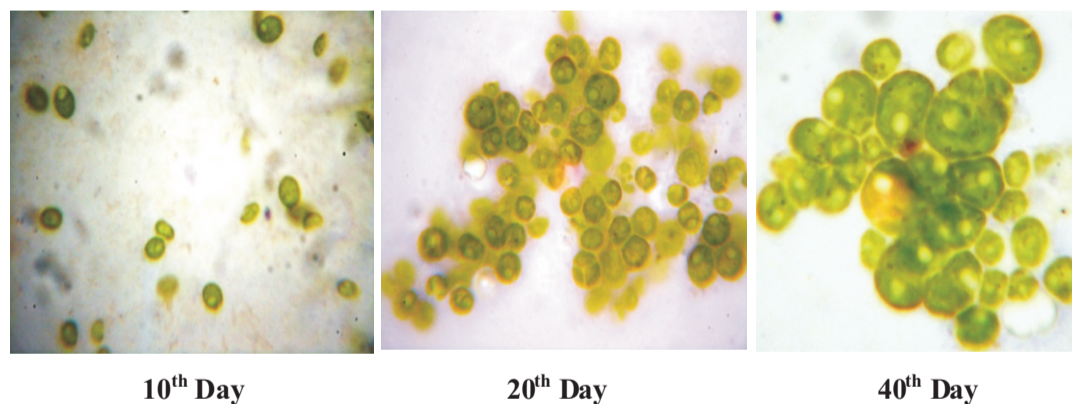


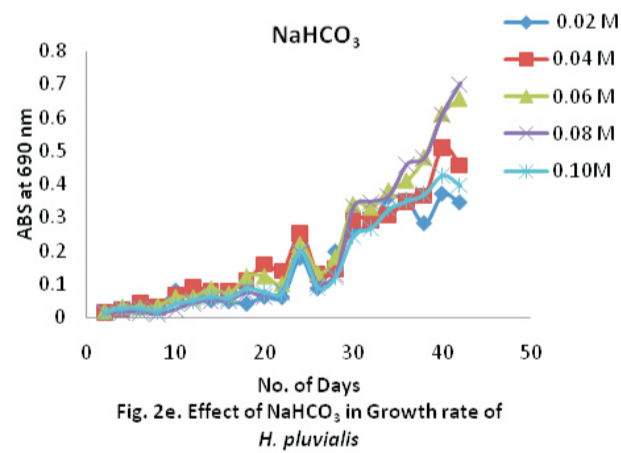
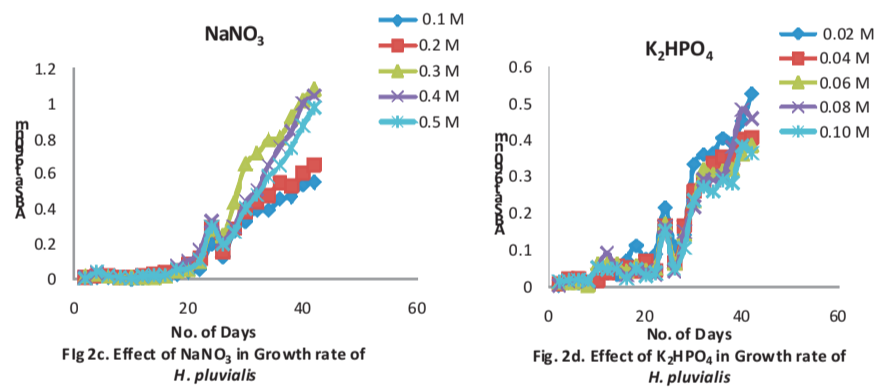
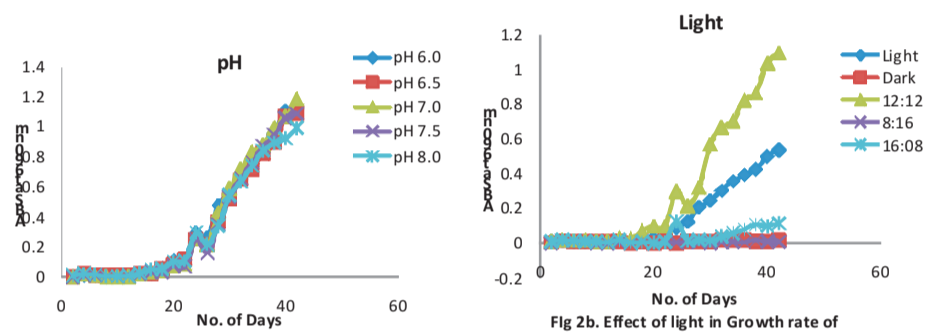
Fig. 1. *Haematococcus pluvialis* Flotow grown under favorable laboratory conditions.

**Effect of growth rate in different parameters**

*Haematococcus pluvialis* survived in all the different parameters and concentrations of modified BBM media. Optical density was taken at 690 nm on 40<sup>th</sup> day. Maximum growth of 1.185 optical density (OD) was recorded at pH 7.0 (Fig. 2a.); 12 (L):12 (D) shows maximum growth of 1.095 (OD) (Fig. 2b.); 0.3M NaNO<sub>3</sub> is the best concentration compared to others 1.082 (OD) (Fig. 2c.). The highest optical density 0.459 was recorded at 0.08M K<sub>2</sub>HPO<sub>4</sub> (Fig. 2d.). Maximum growth of 0.702 optical density (OD) was recorded at 0.08M NaHCO<sub>3</sub> (Fig. 2e.)

In commercial astaxanthin production from *H. pluvialis*, vegetative cultivation of the cells plays an important role. In some studies, different strengths of a growth medium were studied (Garcia-Malea *et al.*, 2005).

The maximum growth rate reported at pH 7.0; maximum cell number recorded 0.5M NaNO<sub>3</sub> and the maximum number of cells was reported at 0.1M K<sub>2</sub>HPO<sub>4</sub> (Nagaraj *et al.*, 2012).



**Effect of Chlorophyll a in different parameters**

*Haematococcus pluvialis* survived in all the different parameters and concentrations of modified BBM media. The Chlorophyll a was estimated by the above described method on every 40 days. Maximum Chlorophyll a content was  $11.46 \mu\text{g ml}^{-1}$  recorded at pH 7.0 (Fig. 3a.); 12 (L):12 (D) shows maximum Chl. a of  $10.39 \mu\text{g ml}^{-1}$  (Fig. 3b.); 0.3M  $\text{NaNO}_3$  is the best concentration compared to others which contain  $10.54 \mu\text{g ml}^{-1}$  (Fig. 3c.). The highest Chl. a content  $4.90 \mu\text{g ml}^{-1}$  was recorded at 0.08M  $\text{K}_2\text{HPO}_4$  (Fig. 4d.). Maximum Chl. a content of  $5.45 \mu\text{g ml}^{-1}$  was recorded at 0.08M  $\text{NaHCO}_3$ (Fig. 3e.).

Nagaraj *et.al.*, 2012 reported *H. pluvialis* grown well and showed maximum concentration Chl. a at 7.0 pH; 0.3 M  $\text{NaNO}_3$  showed maximum amount of Chl. a; 0.01 and 0.08 M  $\text{K}_2\text{HPO}_4$  showed maximum concentration Chl a.

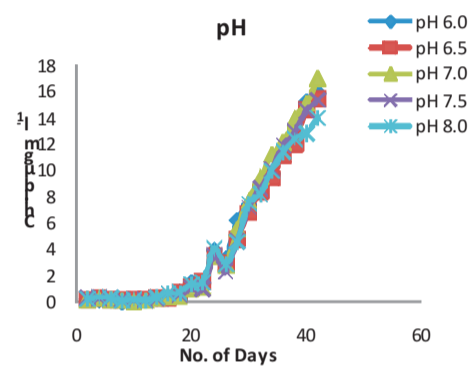


Fig. 3a. Effect of pH on Chl. a production of *H. pluvialis*

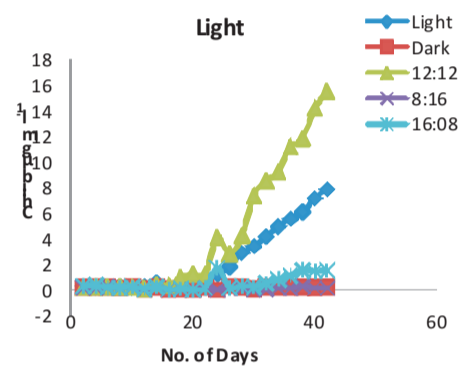


Fig. 3b. Effect of Light on Chl. a production of *H. pluvialis*

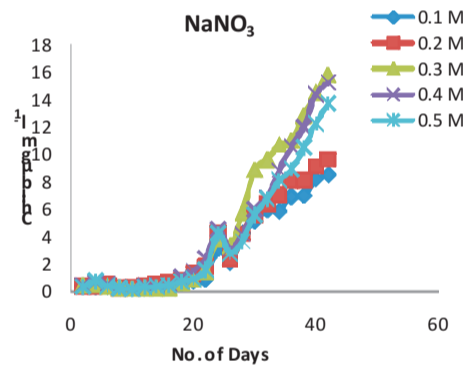


Fig. 3c. Effect of  $\text{NaNO}_3$  on Chl. a production of *H. pluvialis*

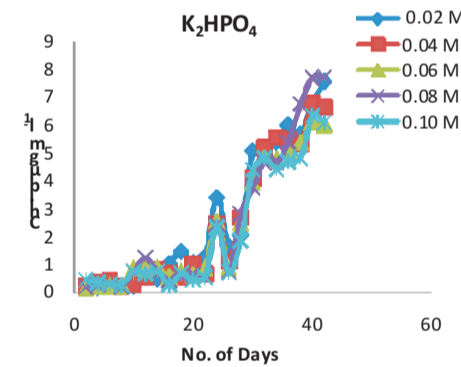


Fig. 3d. Effect of  $\text{K}_2\text{HPO}_4$  on Chl. a production of *H. pluvialis*

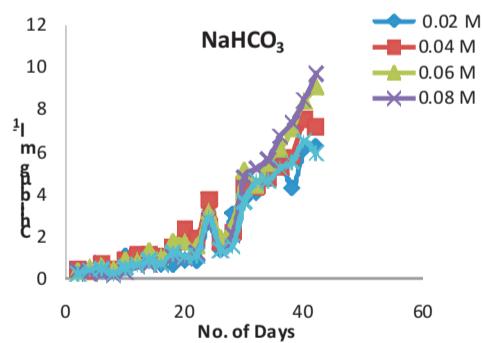
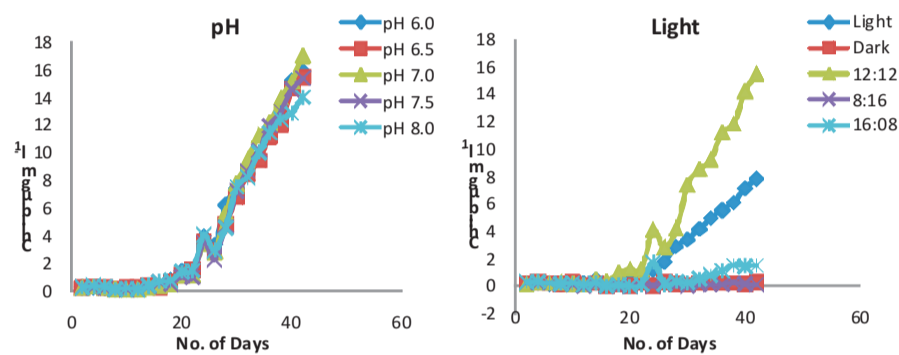
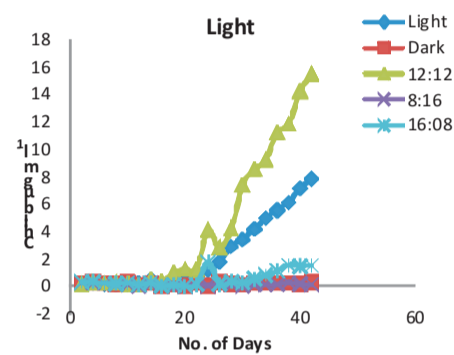
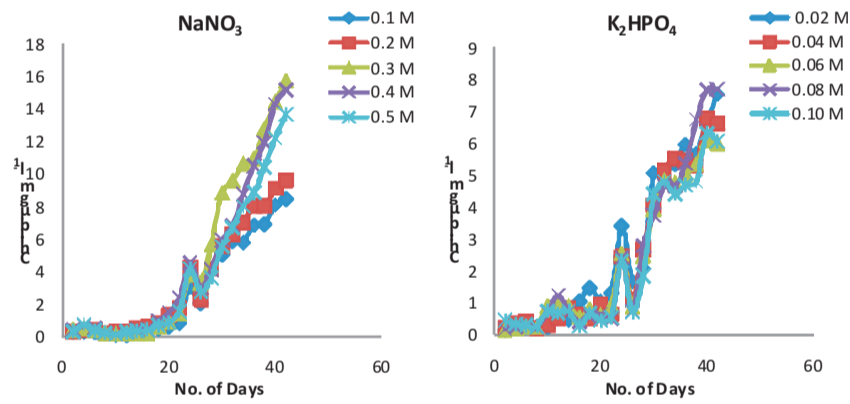
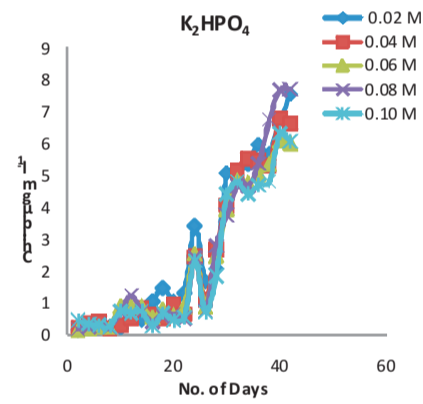
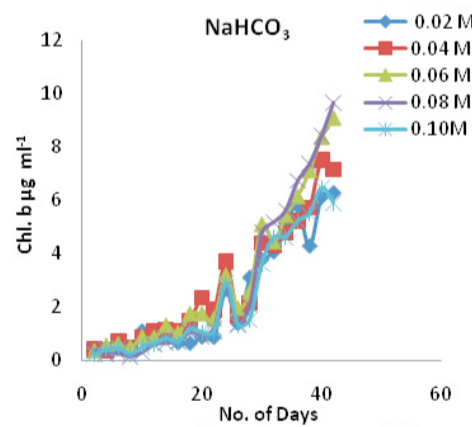


Fig. 3e. Effect of  $\text{NaHCO}_3$  on Chl. a...

**Effect of Chlorophyll b in different parameters**

*Haematococcus pluvialis* survived in all the different parameters and concentrations of modified BBM media. The Chlorophyll b was estimated by the above described method on every 40 days. Maximum Chlorophyll b content was  $17.01 \mu\text{g ml}^{-1}$  recorded at pH 7.0 (Fig. 4a.); 12 (L):12 (D) shows maximum Chl. b of  $15.49 \mu\text{g ml}^{-1}$  (Fig. 4b.); 0.3M  $\text{NaNO}_3$  is the best concentration compared to others which contain  $15.74 \mu\text{g ml}^{-1}$  (Fig. 4c.). The highest Chl. b content  $7.70 \mu\text{g ml}^{-1}$  was recorded at 0.08M  $\text{K}_2\text{HPO}_4$  (Fig. 4d.). Maximum Chl. b content of  $9.67 \mu\text{g ml}^{-1}$  was recorded at 0.08M  $\text{NaHCO}_3$  (Fig. 4e.).

*H. pluvialis* grown well and showed maximum concentration Chl. b at 7.0 pH; 0.3 M  $\text{NaNO}_3$  showed maximum amount of Chl. a; 0.01 and 0.08M  $\text{K}_2\text{HPO}_4$  showed maximum concentration Chl. a. (Nagaraj *et.al.*, 2012)

Fig. 4a. Effect of pH on Chl. b production of *H. pluvialis*Fig. 4b. Effect of Light on Chl. b production of *H. pluvialis*Fig. 4c. Effect of  $\text{NaNO}_3$  on Chl. b production of *H. pluvialis*Fig. 4d. Effect of  $\text{K}_2\text{HPO}_4$  on Chl. b Production of *H. pluvialis*Fig. 4e. Effect of  $\text{NaHCO}_3$  on Chl. b production of *H. pluvialis*

*Effect of Carotenoid in different parameters*

*Haematococcus pluvialis* survived in all the different parameters and concentrations of modified BBM media. The Carotenoid was estimated by the above described method on every 40 days. Maximum Carotenoid content was 139.2 mg L<sup>-1</sup> recorded at pH 7.0 (Fig. 5a.); 12 (L):12 (D) shows maximum Carotenoid of 125.7 mg L<sup>-1</sup> (Fig. 5b.); 0.3M NaNO<sub>3</sub> is the best concentration compared to others which contain 123.7 mg L<sup>-1</sup> (Fig. 5c.). The highest Carotenoid

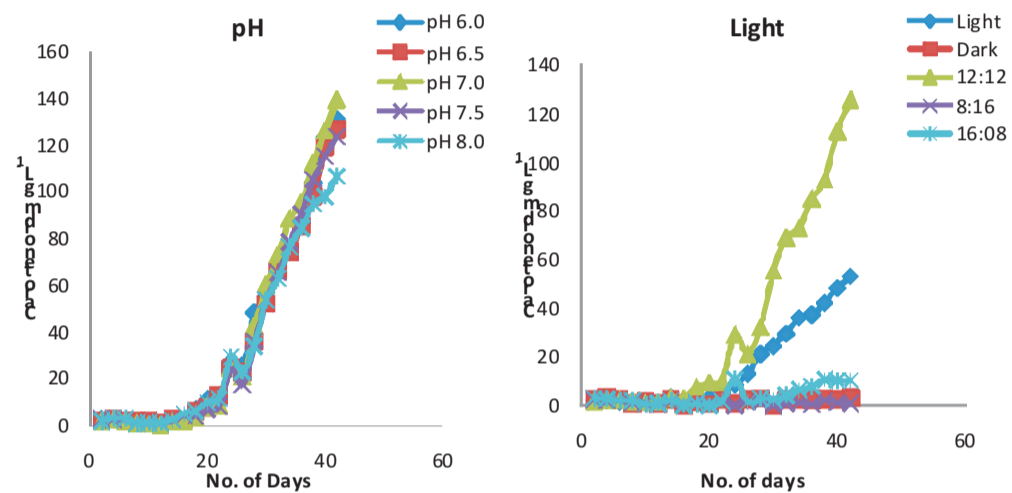


Fig. 5a. Effect of pH on Carotenoid production of *H. pluvialis*

Fig. 5b. Effect of Light on Carotenoid production of *H. pluvialis*

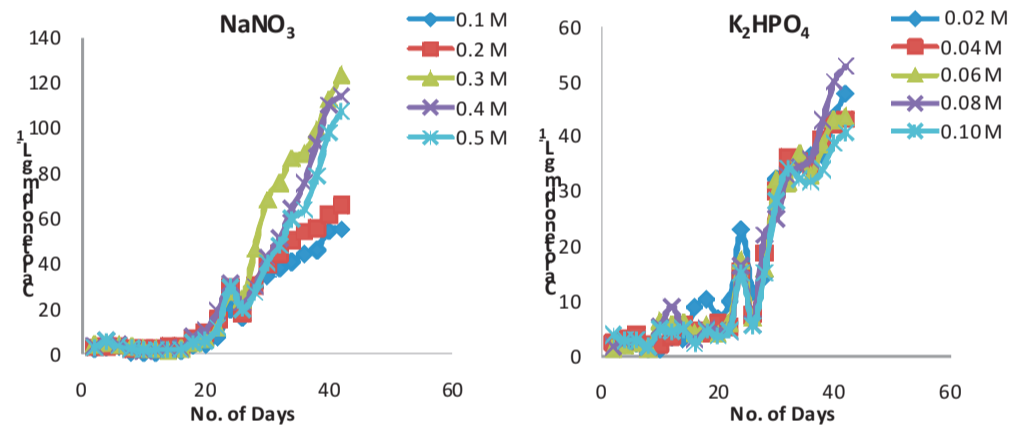


Fig. 5c. Effect of NaNO<sub>3</sub> on Carotenoid production of *H. pluvialis*

Fig. 5d. Effect of K<sub>2</sub>HPO<sub>4</sub> on Carotenoid production of *H. pluvialis*

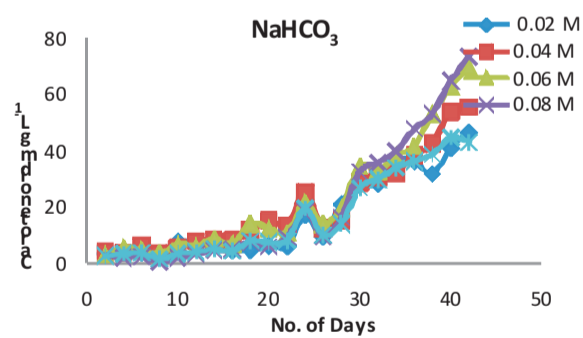


Fig. 5e. Effect of NaHCO<sub>3</sub> on Carotenoid...



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content  $52.9 \text{ mg L}^{-1}$  was recorded at  $0.08 \text{ M K}_2\text{HPO}_4$  (Fig. 5d.). Maximum Carotenoid content of  $73.6 \text{ mg L}^{-1}$  was recorded at  $0.08 \text{ M NaHCO}_3$  (Fig. 5e.).

Nagaraj *et al.*, 2012 reported *H. pluvialis* pH 7.0 registered maximum carotenoid content;  $0.2 \text{ M NaNO}_3$  favored the organisms for maximum accumulation of total carotenoids; A maximum level of carotenoids content recorded at  $0.02 \text{ M K}_2\text{HPO}_4$ .

#### 4. ACKNOWLEDGEMENTS

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