Vol 3 Issue 4 Jan 2014

Impact Factor : 1.6772 (UIF)

ISSN No : 2249-894X

Monthly Multidisciplinary Research Journal

Review Of Research Journal

Chief Editors

Welcome to Review Of Research

RNI MAHMUL/2011/38595 **ISSN No.2249-894X**

Review Of Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

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Review Of Research
Vol. 3, Issue. 4, Jan. 2014
ISSN:-2249-894X
Available online at www.ror.isrj.net

ORIGINAL ARTICLE

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 tha color of salman 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, $NaNO_3$, K_2HPO_4, $NaHCO_3$ 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, $NaNO_3$ – 0.3 M, K_2HPO_4 – 0.08M, $NaHCO_3$ – 0.08 M. on 40th day of incubation period.

KEYWORDS:

Haematococcus pluvialis, Vegetative stage, Astaxanthin, pH, light, $NaNO_3$, K_2HPO_4, $NaHCO_3$

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 neutraceuticals, 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_3 and $NaNO_3$ (Borowitzka *et al*., 1991;

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

In the present study, the effects of various nitrogen compounds, pH, light, $NaNO_3$, K_2HPO_4, $NaHCO_3$ 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); $NaNO_3$ - 0.1M, 0.2M, 0.3M, 0.4M, 0.5M; K_2HPO_4 - 0.02M, 0.04M, 0.06M, 0.08M, 0.1M; $NaHCO_3$ - 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-2 s -1 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.

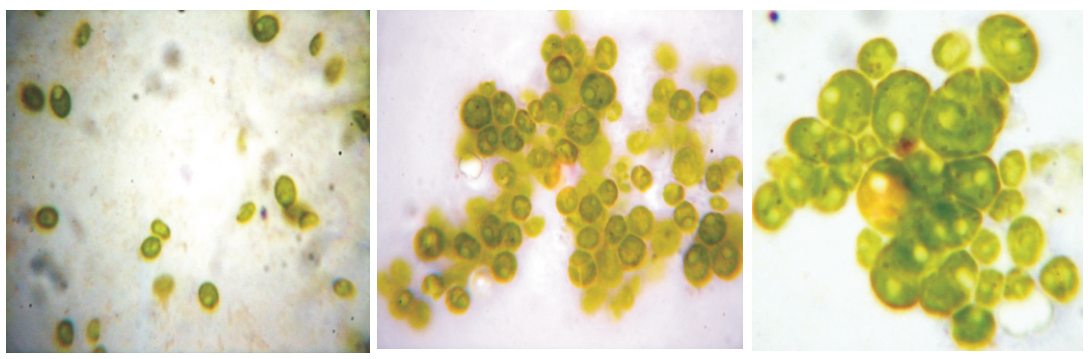
Methods and measurement of growth rate, Chlorophll a, Chlorophll b and Carotenoids

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).



10th Day **20th Day** **40th Day**

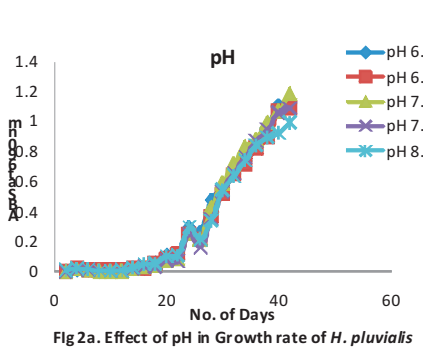
Fig. 1. Haematococcus pluvialis Flotow grown under favorable laboratory conditions.

Effect of growth rate in different parameters

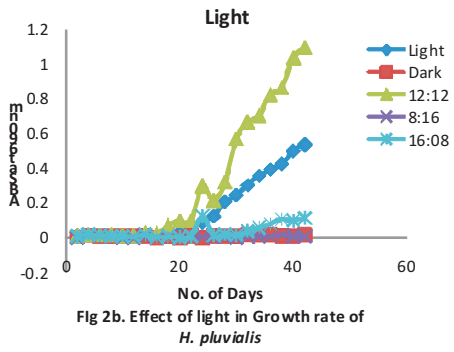
Haematococcus pluvialis survived in all the different parameters and concentrations of modified BBM media. Optical density was taken at 690 nm on 40th day. Maximum growth of 1.185 optical density (OD) was recorded at pH 7.0 (Fig. 2a.); 12 (L):12 (D) shows maximum growth of 1.095 (OD) (Fig. 2b.); 0.3M $NaNO_3$ is the best concentration compared to others 1.082 (OD) (Fig. 2c,). The highest optical density 0.459 was recorded at 0.08M K_2HPO_4 (Fig. 2d.). Maximum growth of 0.702 optical density (OD) was recorded at 0.08M $NaHCO_3$ (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).

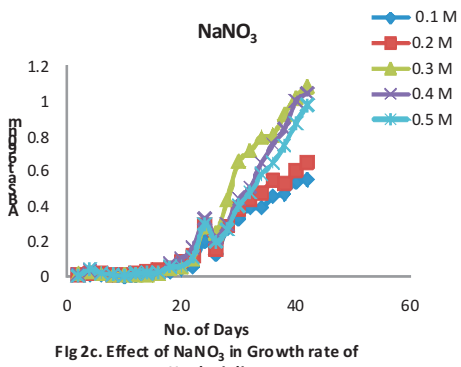
The maximum growth rate reported at pH 7.0; maximum cell number recorded 0.5M $NaNO_3$ and the maximum number of cells was reprted at 0.1M K_2HPO_4 (Nagaraj *et.al*., 2012).



Flg 2a. Effect of pH in Growth rate of *H. pluvialis*



Flg 2b. Effect of light in Growth rate of *H. pluvialis*



Flg 2c. Effect of $NaNO_3$ in Growth rate of *H. pluvialis*

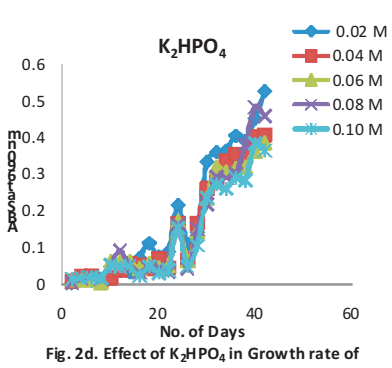


Fig. 2d. Effect of K_2HPO_4 in Growth rate of *H. pluvialis*

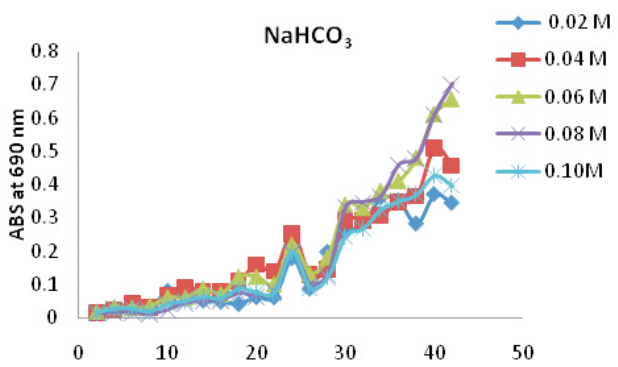


Fig. 2e. Effect of $NaHCO_3$ in Growth rate of *H. pluvialis*

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 µg ml^{-1} recorded at pH 7.0 (Fig. 3a.); 12 (L):12 (D) shows maximum Chl. a of 10.39 µg ml^{-1} (Fig. 3b.); 0.3M $NaNO_3$ is the best concentration compared to others which contain 10.54 µg ml^{-1} (Fig. 3c.). The highest Chl. a content 4.90 µg ml^{-1} was recorded at 0.08M K_2HPO_4 (Fig. 4d.). Maximum Chl. a content of 5.45 µg ml^{-1} was recorded at 0.08M $NaHCO_3$ (Fig. 3e.).

Nagaraj *et.al*., 2012 reported H. pluvialis grown well and showed maximum concentration Chl. a at 7.0 pH; 0.3 M $NaNO_3$ showed maximum amount of Chl. a; 0.01 and 0.08M K_2HPO_4 showed maximum concentration Chl a.

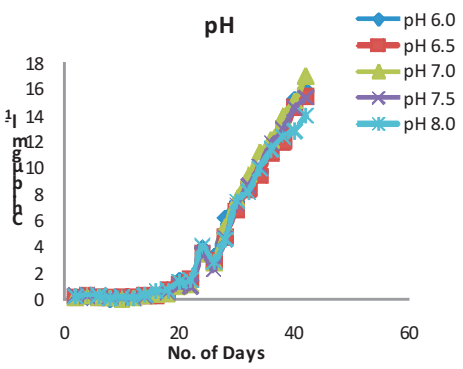


Fig. 3a. Effect of pH on Chl. a production of *H. pluvialis*

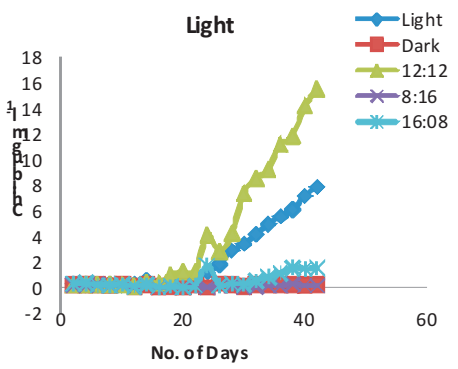


Fig. 3b. Effect of Light on Chl. a production of *H. pluvialis*

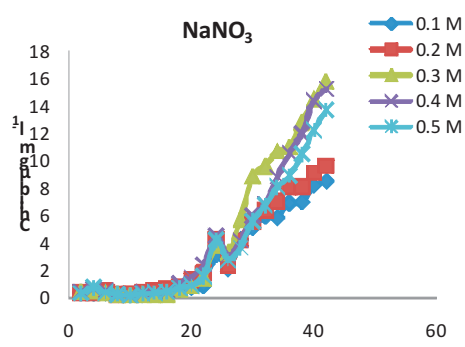


Fig. 3c. Effect of $NaNO_3$ on Chl. a production of *H. pluvialis*

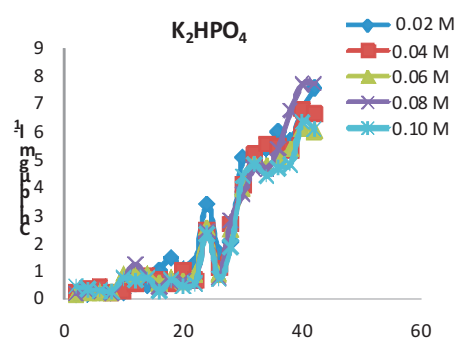


Fig. 3d. Effect of K_2HPO_4 on Chl. a production of *H. pluvialis*

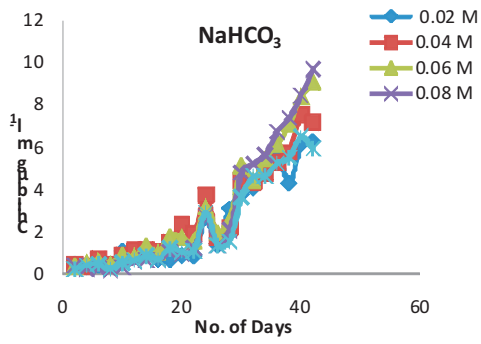


Fig. 3e. Effect of $NaHCO_3$ on Chl. a...

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 µg ml^{-1} recorded at pH 7.0 (Fig. 4a.); 12 (L):12 (D) shows maximum Chl. b of 15.49 µg ml^{-1} (Fig. 4b.); 0.3M $NaNO_3$ is the best concentration compared to others which contain 15.74 µg ml^{-1} (Fig. 4c,). The highest Chl. b content 7.70 µg ml^{-1} was recorded at 0.08M K_2HPO_4 (Fig. 4d.). Maximum Chl. b content of 9.67 µg ml^{-1} was recorded at 0.08M $NaHCO_3$ (Fig. 4e.).

H. pluvialis grown well and showed maximum concentration Chl. b at 7.0 pH; 0.3 M $NaNO_3$ showed maximum amount of Chl. a; 0.01 and 0.08M K_2HPO_4 showed maximum concentration Chl a. (Nagaraj *et.al.*, 2012)

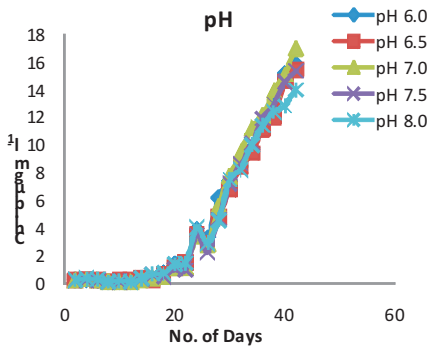


Fig. 4a. Effect of pH on Chl. b production of *H. pluvialis*

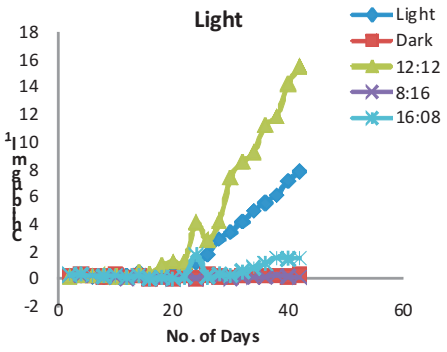


Fig. 4b. Effect of Light on Chl. b production of *H. pluvialis*

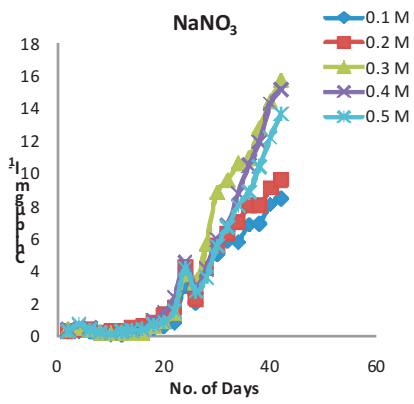


Fig. 4c. Effect of $NaNO_3$ on Chl.b production of *H. pluvialis*

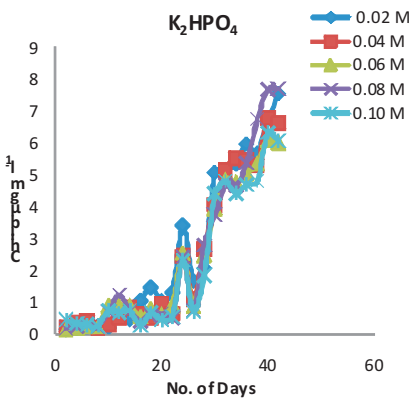


Fig. 4d. Effect of K_2HPO_4 on Chl. b Production of *H. pluvialis*

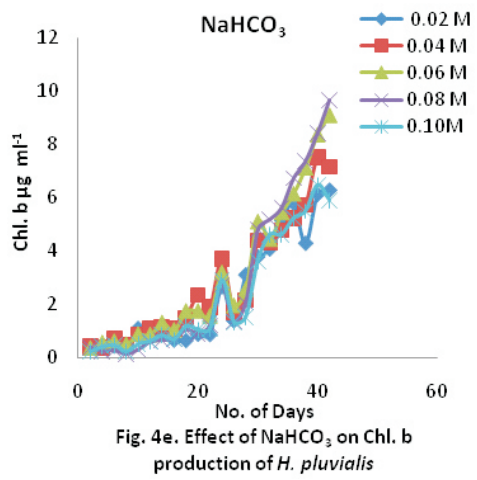


Fig. 4e. Effect of $NaHCO_3$ on Chl. b production of *H. pluvialis*

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^{-1} recorded at pH 7.0 (Fig. 5a.); 12 (L):12 (D) shows maximum Carotenoid of 125.7 mg L^{-1} (Fig. 5b.); 0.3M $NaNO_3$ is the best concentration compared to others which contain 123.7 mg L^{-1} (Fig. 5c,). The highest Carotenoid

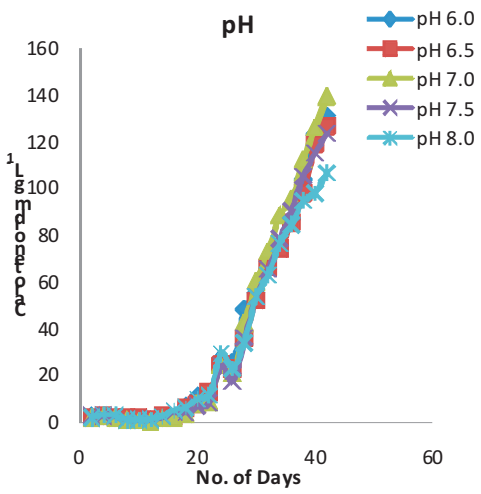


Fig. 5a. Effect of pH on Carotenoid production of *H. pluvialis*

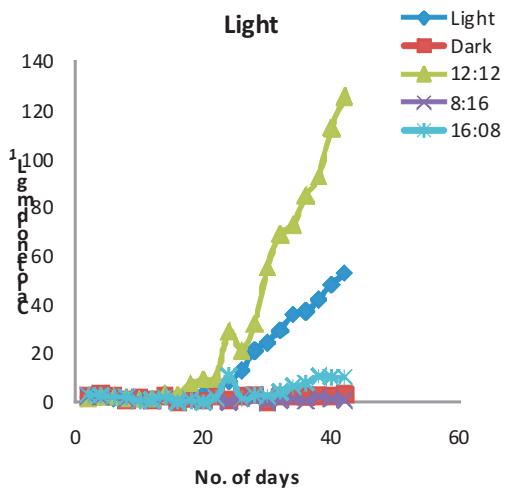


Fig. 5b. Effect of Light on Carotenoid production of *H. pluvialis*

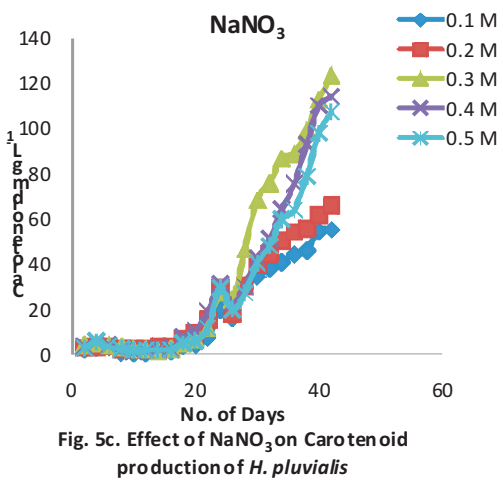


Fig. 5c. Effect of $NaNO_3$ on Carotenoid production of *H. pluvialis*

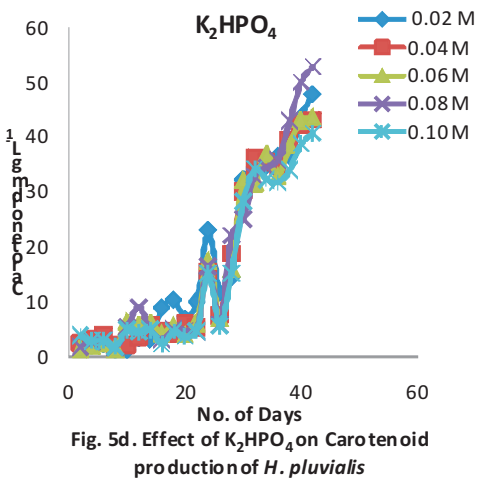


Fig. 5d. Effect of K_2HPO_4 on Carotenoid production of *H. pluvialis*

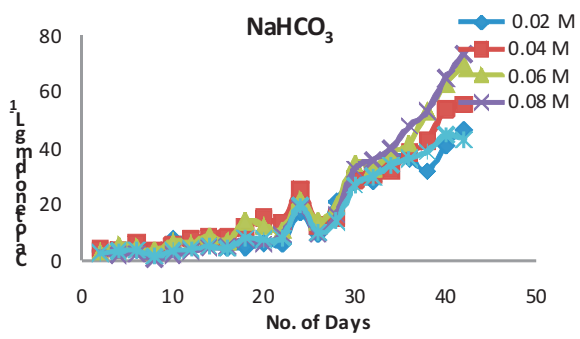


Fig. 5e. Effect of $NaHCO_3$ on Carotenoid...

content 52.9 mg L^{-1} was recorded at 0.08M K_2HPO_4 (Fig. 5d.). Maximum Carotenoid content of 73.6 mg L^{-1} was recorded at 0.08M $NaHCO_3$ (Fig. 5e.).

Nagaraj *et.al*., 2012 reported H. pluvialis pH 7.0 registered maximum carotenoid content; 0.2 M $NaNO_3$ favored the organisms for maximum accumulation of total carotenoids; A maximum level of carotenoids content recorded at 0.02M K_2HPO_4.

4. ACKNOWLEDGEMENTS

The authors expressed sincere thanks to DST - Science and Research Board (SERB), Ministry of Science and Technology, Government of India for their financial support

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