

Effect of Different Culture Media on Growth of Microalgae
***Haematococcus* sp. TISTR 9450RE**

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Abstract

This study aimed to select the appropriate medium for supporting the best growth of *Haematococcus* sp. TISTR 9450RE. They were BG-11, Bold's Basal, CA and N8. The algae were cultured in 500 ml Erlenmeyer flask containing 200 ml of each medium. The initial cell concentration was 0.1 OD at 680 nm (equivalent to 0.07 g/L of cell dry weight). All flasks were shaken continuously at 100 rpm. At 7 and 14 days of cultivation, the biomass content of 0.1 and 0.13 g/L were obtained from BG-11 and Bold's Basal medium, respectively. Whereas slightly lower cell concentration at day 7 were 0.09 and 0.1 g/L from CA and N8 medium, and at day 14 were 0.11 and 0.12 g/L, respectively. The highest biomass content was found in BG-11 0.32 g/L after 28 days cultivation, with 4.57 times increasing from day 0, followed by Bold's Basal, N8 and CA at 0.26, 0.24 and 0.22 g/L, with 3.43, 3.25 and 3.14 times, respectively. Due to cell division, the highest absorbance of 0.44 at day 28 was found in N8, but biomass was less. The pH value of BG-11, Bold's Basal, CA and N8 at the end of cultivation were 11.06, 10.92, 10.74 and 7.49, respectively. The results revealed that BG-11 was the suitable medium for culturing *Haematococcus* sp. TISTR 9450RE and used for further study of carotenoid production from *Haematococcus* sp. TISTR 9450RE.

Keywords: *Haematococcus* sp., biomass, BG-11, Bold's Basal, CA, N8

Introduction

Algae are aquatic photosynthetic organisms that contain chlorophyll as important compound for photosynthesis. Most of them resemble plants and occur in variety of forms (single cell or multiple cells) and sizes. Algae are primary producers in the ecosystem's food chain, responsible for capturing light energy from the sun, using simple substrates, carbon dioxide and water and releasing oxygen into the environment.

In this study, freshwater microalgae *Haematococcus* sp. TISTR 9450RE was obtained from Thailand Institute of Scientific and Technological Research. This microalga has thick cell walls and mostly round or oval cells. It is able to accumulate large amount of natural astaxanthin under stress environmental condition such as lack of nutrients, high salt, high temperature, high light intensity and UV radiation from the sun. Lorenz and Cysewski [1] reported that astaxanthin content presented in *Haematococcus* sp. was 0.2% of dry weight, whereas Lee and Soh [2] observed the higher content at 4% of dry weight.

In recent years microalgae *Haematococcus* sp. has been widely studied. It has been considered as a possible natural source of food and human supplements, antioxidant for beauty products, animal feed and future energy. Mangels *et al.* [3] report that carotenoids from algae have many important properties such as anti-free radicals property which help protect plants from inappropriate conditions or preventive property from infection and sun damage. Carotenoids can be used as food supplements to increase immunity, prevent tumor, reduce the risk of eye aging, cancers and cardiovascular diseases. Astaxanthin in the subgroup of xanthophyll is more antioxidants effect in water and oil than other substances. It can also be used in the cosmetic industry, as well as in animal and poultry feeds. It can increase color intensity of egg yolks and color of meat scales for higher price selling [4]. . As mentioned above, *Haematococcus* sp. can accumulate high percentages of astaxanthin making it more interesting for researchers. The aims of this study is therefore, to determine appropriate culture medium for *Haematococcus* cultivation.

Materials and Methods

Preparation of inoculum

Haematococcus sp. TISTR 9450RE was obtained from the culture collection of Thailand Institute of Scientific and Technological Research (TISTR), Thailand. The algal volume of 6 ml was grown in 200 ml of BG-11 medium [5] in a 500 ml Erlenmeyer flask. The inoculum was pre-cultivated in a shaking incubator (100 rpm) at 28 ± 2 °C and followed by illumination at 1000 lux cold daylight for 14 days. Then, the inoculum cells were transferred into a 12 L carboy tank containing of 10 L of fresh BG-11 medium. Air was supplied through 0.2 µm filter and cultivation was prepared as described above.

Suitable medium selection for growth enhancement of *Haematococcus* sp.

Growth experiments were done using four culture media; BG-11 [5], Bold's Basal [6], CA [7] and N8 [8]. The compositions of culture media were shown in Table 1. The algae was cultivated in a 500 ml Erlenmeyer flask containing 200 ml of each medium. The initial algal concentration was 0.1 (OD_{680nm}). The cultures were placed a shaking incubator with 100 rpm, incubated at 28 ± 2 °C and illuminated at 1000 lux cold daylight for 28 days. The biomass dry weight (grams per liter) and absorbance of biomass at 680 nm were measured in day 0, 7, 14, 21 and 28 of cultivation.

Results and Discussion

Growth performance of *Haematococcus* sp. TISTR 9450RE in four different media; BG-11, Bold's Basal, CA and N8, were shown in Table 2 and Figure 1. At day 7 and 14 of cultivation, biomass yield of algae cultivated in BG-11 and Bold's Basal presented the same value at 0.10 and 0.13 g/L. In CA and N8 medium similar biomass yields were obtained at 0.09 and 0.10 on day 7 and 0.11 and 0.12 g/L on day 14 respectively. The biomass concentrations in all culture media were higher on day 21 of cultivation ($p < 0.05$). On day 28 of cultivation, the highest biomass yield was found in BG-11 (0.32 g/L) with 4.57 times increasing from day 0. However, the biomass yields in Bold's Basal, N8 and CA were not significantly different on day 28 of cultivation (0.26, 0.24 and 0.22 g/L, respectively).

Table 1. Nutrient composition of different culture media: BG-11, Bold's Basal, CA and N8

Constituents	BG-11 (mg/L)	Bold's Basal (mg/L)	CA (mg/L)	N8 (mg/L)
NaNO ₃	1500	250	-	-
MgSO ₄ .7H ₂ O	75	75	20	50
CaCl ₂ .2H ₂ O	36	25	-	10
Citric acid	6	-	-	-
Na ₂ CO ₃	20	-	-	-
K ₂ HPO ₄ .3H ₂ O	40	98	-	-
Fe-EDTA	7	-	-	10
KH ₂ PO ₄	-	175	-	740
NaCl	-	25	-	-
EDTA	-	50	-	-
KOH	-	31	-	-
FeSO ₄ .7H ₂ O	-	4.98	-	-
H ₃ BO ₃	-	11.42	-	-
ZnSO ₄ .7H ₂ O	0.22	8.82	22	3.2
MoO ₃	-	0.71	-	-
Co(NO ₃) ₂ .6H ₂ O	0.05	0.49	-	-
MnCl ₂ .4H ₂ O	1.81	1.44	36	12.98
CuSO ₄ .5H ₂ O	0.08	1.57	-	1.83
Ca(NO ₃).4H ₂ O	-	-	20	-
KNO ₃	-	-	100	1000
NH ₄ NO ₃	-	-	50	-
β-glycerophosphate.5H ₂ O	-	-	30	-
HEPES	-	-	400	-
Vitamin B12	-	-	0.0001	-
Biotin	-	-	0.0001	-
Thiamine HCL	-	-	0.01	-
FeCl ₃ .6H ₂ O	-	-	196	-
CoCl ₂ .6H ₂ O	-	-	4	-
Na ₂ MoO ₄	0.39	-	3	-
Na ₂ EDTA.2H ₂ O	-	-	1000	-
Fe(NH ₄) ₂ (SO ₄) ₂ .6H ₂ O	-	-	702	-
Na ₂ HPO ₄ .2H ₂ O	-	-	-	260
Na ₂ SiO ₃	-	-	-	58
Al ₂ (SO ₄) ₃ .18H ₂ O	-	-	-	3.58
H ₃ BO ₃	2.86	-	-	-

The absorbance values for the first 14 days of culture were not significantly different, but were higher on 21 day of culture. The BG-11 medium had the highest absorption (0.37), followed by N8, CA and Bold's Basal (0.27, 0.25 and 0.21). On day 28 of culture, the highest absorbance was 0.44 in N8, followed by BG-11, CA and Bold's Basal; 0.33, 0.31 and 0.27, respectively. In N8 medium, the algae were in the cell division phase resulting in higher light absorption but less biomass production than in the other media. The culture pH on day 0 of BG-11, Bold's Basal, CA and N8 respectively were 7.86, 7.32, 8.11 and 6.79. The pH of each culture medium during cultivation period was not significantly different. The culture pH at the end of the experiment in BG-11, Bold's Basal, CA and N8 respectively were 11.06, 10.92, 10.74 and 7.49. The culture pH in different media and cultivation period as shown in Table 6.

Table 2: Biomass yield (g/L cell dry weight) and absorbance of *Haematococcus* sp. TISTR 9450RE cultured in four different media

Remark : ¹ is the number that increases from day 0 of cultivation.

Media	Cultivation time (day)									
	0		7		14		21		28	
	Biomass	absorbance	Biomass	absorbance	Biomass	absorbance	Biomass	absorbance	Biomass	absorbance
BG-11	0.07±0.01 ^A	0.10±0.00 ^A	0.10±0.00 ^A (1.43) ¹	0.10±0.01 ^B (1.0)	0.13±0.00 ^A (1.86)	0.14±0.02 ^A (1.4)	0.25±0.00 ^A (3.57)	0.37±0.02 ^A (3.7)	0.32±0.11 ^A (4.57)	0.33±0.01 ^B (3.3)
Bold's Basal	0.07±0.01 ^A	0.10±0.00 ^A	0.10±0.01 ^A (1.43)	0.13±0.01 ^A (1.3)	0.13±0.01 ^A (1.86)	0.16±0.01 ^A (1.6)	0.16±0.01 ^C (2.29)	0.21±0.03 ^C (2.1)	0.24±0.00 ^B (3.43)	0.27±0.02 ^C (2.7)
CA	0.07±0.01 ^A	0.10±0.00 ^A	0.09±0.01 ^A (1.29)	0.13±0.01 ^A (1.3)	0.11±0.01 ^A (1.57)	0.13±0.00 ^A (1.3)	0.18±0.00 ^{BC} (2.57)	0.25±0.04 ^{BC} (2.5)	0.22±0.00 ^B (3.14)	0.31±0.02 ^B (3.1)
N8	0.08±0.01 ^A	0.10±0.00 ^A	0.10±0.01 ^A (1.25)	0.13±0.02 ^A (1.3)	0.12±0.01 ^A (1.5)	0.13±0.01 ^A (1.3)	0.21±0.00 ^B (2.63)	0.27±0.03 ^B (2.7)	0.26±0.02 ^B (3.25)	0.44±0.04 ^A (4.4)

Means in the same column with different letters were significantly different (P < 0.05).

Means in the same column with same letters were not significantly different (P > 0.05).

Table 3: pH of culture media during cultivation of *Haematococcus* sp. TISTR 9450RE for 28 days

Media	Cultivation time (day)				
	0	7	14	21	28
BG-11	7.86±0.00	8.13±0.01	8.17±0.02	9.34±0.02	11.06±0.01
Bold's Basal	7.32±0.00	7.94±0.01	7.90±0.01	8.31±0.03	10.92±0.02
CA	8.11±0.00	8.10±0.01	7.35±0.00	10.28±0.04	10.74±0.02
N8	6.79±0.00	6.92±0.02	6.52±0.01	7.05±0.03	7.49±0.04

The highest biomass of *Haematococcus* sp. TISTR 9450RE was obtained in BG-11, comparing with Bold's Basal, N8 and CA. It might be due to the richer nutrient contained in this media such as sodium nitrate, dipotassium hydrogen phosphate and sodium carbonate. Wang [9] also observed the similar result when using BG-11 medium for studying microalgae growth. However, other works were focused in Bold's Basal medium [10-12].

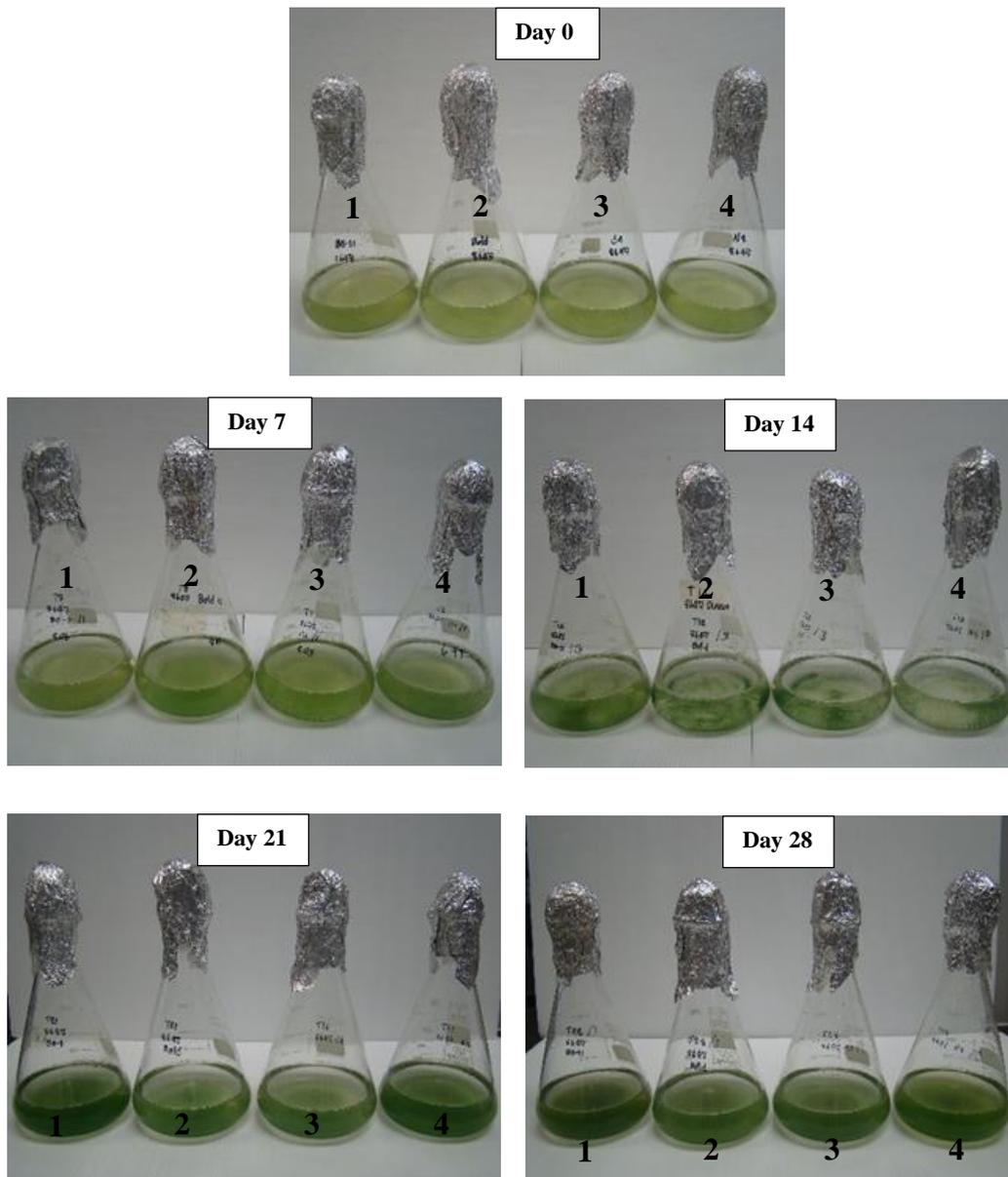


Fig. 1 Characteristics of algae in BG-11 (1), Bold's Basal (2), CA (3) and N8 (4) during cultivation for 28 days

Conclusions

The BG-11 medium supported the highest biomass production of 0.32 g/L. Biomass productions from Bold's Basal, N8 and CA were not different at 0.26, 0.24 and 0.22 g/L respectively. Therefore, BG-11 was used as the culture medium for further experiments.

Acknowledgements

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References

- [1] Loren R.T. and Cysewski G.R. (2000) Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends Biotechnol. 18,160-167.
- [2] Lee Y.K. and Soh C.W. (1991) Accumulation of astaxanthin in *Haematococcus lacustris* (Chlorophyta). J Phycol 27:575-577.
- [3] Mangels A.R, Holden J.M., Beecher G.R., Foman M.R. and Lanza E. (1993) Carotenoid content of fruits and vegetables: an evaluation of analytic data. Journal of American Dietetic Association 93(5):527-527.
- [4] Johnson E.A. and Schroeder W.A. (1996) Microbial carotenoids. J. Adv. Biochem. Eng. Biotechnol. 53:119-178.
- [5] Stainer R.Y, Kunisawa R, Mandel M. and Cohen-Bazize G. (1971) Purification and properties of unicellular blue-green algae (order Chlorococcales). J. Bact. Rev. 35: 171-205.
- [6] Stein, J.R. (1973) Handbook of phycological methods. Culture methods and growth measurements. J. Cambridge University Press. 448 pp.
- [7] Ichimura T. and Watanabe M. (1974) The *Closterium calosporum* complex from the Ryukyu Islands-Variation and taxonomical problems. J. Mem. Nat. Sci. Mus. Tokyo, 7, 89-102, pls. 13-14.
- [8] Vonshak A. (1986) Laboratory techniques for the cultivation of microalgae. In Richmond, A. (ed.), Handbook of microalgae mass culture. CRC Press, Boca Raton, Florida, pp. 117-145.

- [9] Wang J., Han D., Milton R., Sommerfeld, Lu C. and Hu Q. (2013) Effect of initial biomass density on growth and astaxanthin production of *Haematococcus pluvialis* in an outdoor photobioreactor. *J. Appl Phycol* 25:253-260, DOI 10.1007/s10811-012-9859-4.
- [10] Fabregas J., Dominguez A., Regueiro M., Maseda A. and Otero A. (2000) Optimization of culture medium for the continuous cultivation of the microalga *Haematococcus pluvialis*. *J. springer-Verlag* 53: 530-535.
- [11] Dragoş N., Bercea V., Bica A., Druga B., Nicoara A. and Coman C. (2011) Astaxanthin production from a new strain of *Haematococcus pluvialis* grown in batch culture. *J. Annals of RSCB*.
- [12] Hanan N.A., Al-Shorgani N.K., Shukor H., Rahman A.N. and Kalil M.S. (2013) Pre-optimization conditions for *Haematococcus pluvialis* growth. *Int. J. Adv. Sci. Eng. Info. Technol.*, 3 (2).