Advance Journal of Food Science and Technology 5(9): 1139-1142, 2013
DOI:10.19026/ajfst.5.3071
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: May 03, 2013 Accepted: June 01, 2013 Published: September 05, 2013

Research Article

Effect of Vitamin-B_{12} and Vitamin-H on the Growth and Astaxanthin Content of *Haematococcus pluvialis* CH-1

[1]Li-xin Li, [1]Zhi-wei Song, [1]You Zhan, [2]Shun-shan Duan, [1]Qian-shen Zhao and [3]Yan Liu
[1]School of Environment and Chemical Engineering, Heilongjiang University of Science and Technology, Harbin 150022, China
[2]Research Center of Hydrobiology, Jinan University, Guangzhou 510632, China
[3]College of Life Science and Technology, Harbin Normal University, Harbin 150025, China

Abstract: An economic microalgae *Haematococcus pluvialis* CH-1 was used as experimental material. An experiment of adding six grades of concentrations of Vitamin-B_{12} and Vitamin-H respectively was conducted. Cell density, biomass and astaxanthin content were measured. The results showed that the growth of *H. pluvialis* was accelerated significantly by adding of Vitamin-B_{12} and Vitamin-H, respectively. The optimal adding concentration of Vitamin-B_{12} and Vitamin-H respectively for *H. pluvialis* was 50 and 500 μg/L. Under the optimal concentration for *H. pluvialis*, cells density, biomass and astaxanthin content were enhanced with Vitamin-B_{12}: 29.5, 30.0 and 28.3%; Vitamin-H: 17.1, 29.2 and 22.6% higher than the blank, respectively. On the mass culture of motile cells of *H. pluvialis*, properly adding Vitamin-B_{12} and Vitamin-H, respectively was effective for increase cells density, biomass and astaxanthin content.

Keywords: *Haematococcus pluvialis*, vitamin-b_{12}, vitamin-H, astaxanthin

INTRODUCTION

The unicellular fresh water microalga, *Haematococcus pluvialis* Flotow (Volvocales, Chlorophyceae) is green-colored, biflagellate and motile in its vegetative stage. In its growth stages, it has both motile and non-motile forms (Esra *et al*., 2007). In the life cycle of *H. pluvialis*, green vegetative cells with two flagella can grow autotrophic ally in the light or heterotrophic ally in extreme environment, such as high temperature, nutrient deficiencies, the dark (Ranjbar *et al*., 2008). Most of the reports showed astaxanthin accumulation in encysted cells when grown in heterotrophic conditions under high light or salinity stress. The red secondary carotenoid astaxanthin, which is widely used as a color additive in aquaculture, food and feed industries and has recently, attracted attention as nutraceutical food, cosmetics, pharmaceuticals, due to its high antioxidant activity (Miki, 1991; Palozza and Krinsky, 1992; Fukuzawa *et al*., 1998; Martin and Mark, 2003; Bjerkeng and Johnsen, 1995). The Chlorophyte alga *Haematococcus pluvialis* is believed to accumulate the highest levels of astaxanthin in nature.

It is reported, adding VB_1, VB_{12} and VH into culture medium can effectively promote the growth of microalgae. Ford (1958) proved there is a close relationship between adding concentration of VB_{12} and cell division rate of *Isochrysis galbana*; The results showed proliferation effect order of adding VB_1, VB_{12} and VH for *Prorocentrum micans* was VB_1 >VH >VB_{12} (Wang *et al*., 1996). Liu *et al*. (2002) pointed out that growth promotion effect is the best for the transgenic *Anabaena* while adding VB_1, VB_{12} and VH together However; it is unknown how to influence on cell density, biomass and astaxanthin content of *H. pluvialis* when respectively adding two kinds of vitamins, VB_{12} and VH. In this study, the effect of different concentrations of VB_{12} and VH on cell density, biomass and astaxanthin content of *H.pluvialis* was studied and the results may provide the support of theory and technology for the high density cultivation of *H. pluvialis*.

MATERIALS AND METHODS

Algal strain, medium and cultivation conditions: *Haematococcus pluvialis* CH-1 used in the present work was obtained from the Research Center of Hydrobiology of Jinan University (Guangzhou, China), Stock culture of *H. pluvialis* CH-1was grown photoautotrophic ally in BBM medium (Lorenz and Cysewski, 2000) at 24±1°C under 12 h: 12 h photoperiod (60 μmol/m^2/s) in 25 mL flask.. The cells of logarithmic growth phase were inoculated into 150 mL culture medium; inoculation density is 5-10×10^3 cells/mL.

Corresponding Author: Shun-shan Duan, Research Center of Hydrobiology, Jinan University, Guangzhou 510632, China, Tel.:+ 86-20-8523192; Fax: +86-20-85223192

Table 1: Treatments on three kinds of Vitamin with six concentration grades (μg/L)

	Concentration grades					
Treatment	0	1	2	3	4	5
VB_{12}	0	0.05	0.5	5	50	500
VH	0	0.5	5	50	500	5000

Experimental design: The experiment was conducted in the two groups, added-vitamin B_{12} groups and added-vitamin H groups; each group was divided into 6 concentrations grades treatment, including 3 replicates per treatment (Table 1). The concentration grade of 0 treatments was without any vitamin, which was the blank.

Measure indexes and methods:
Cell density: The sample was measured every 48 h, using hemocytometer counting.

Biomass: 10 mL algae liquid was filtrated by 0.45 μm cellulose acetate membrane every 6 d, then drying 36 h in 80°C, kept in a desiccators to room temperature, finally weighing by electronic balance (Sartorius BS210S).

Astaxanthin content: According to Boussiba's (Boussiba and Vonshak, 1991) method for the extraction and measure of astaxanthin: astaxanthin con/centration (mg/L) = OD_{490}×4.5

Data analysis: Analyses were done using the Excel program (Microsoft) and the SPSS software package version 11.5 of SPSS Inc. (Chicago, IL, USA.)

RESULTS AND DISCUSSION

Cell density: The results showed that, adding VB_{12} and VH respectively could obviously increase the cell density of *H. pluvialis* (Fig. 1). In VB_{12} group, cell density increased with the increase of the adding concentration of VB_{12}. When adding the concentration was 50 μg/L, the cell density was the maximum (Fig. 1a) and was significantly higher than other treatments. It reached 4.96×10^5cells/mL in the 18th day, enhanced 29.5% than the blank ($p<0.01$). But when the highest concentration (500 μg/L) was adding, the cell density was lower but still higher than the blank. The optimal concentration of adding VB_{12} was 50 μg/L.

In the adding VH test group, the cell density increased with the increase of the adding concentration of VH in 0.5-500 μg/L (Fig. 1b). When adding concentration was 500 μg/L, the cell density were higher than other treatments in the late culture period (12-22 d) and reached 4.36×10^5 cells/mL in the 22th day, enhanced 17.1% than the blank ($p<0.05$). When adding the concentration was 5000 μg/L, the cell density was lower than the blank.

The biomass: The effect of adding VB_{12} and VH for the biomass of *H. pluvialis* was showed in Fig. 2. In

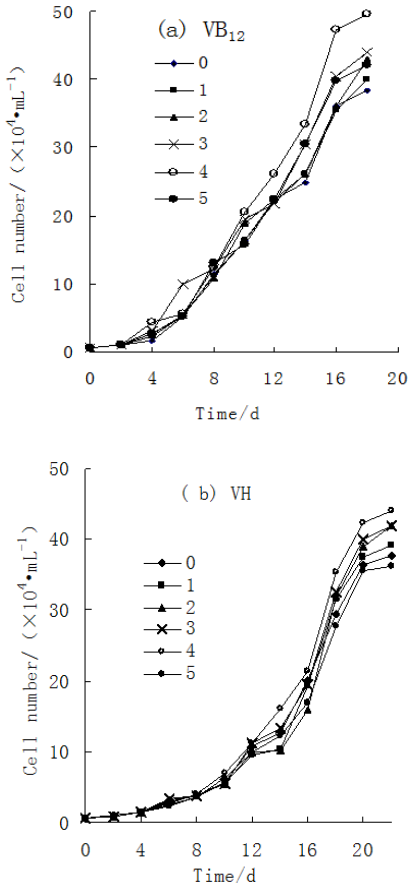

Fig. 1: Effects of Vitamin-B_{12} and Vitamin-H on cell density of the H. pluvialis

VB_{12} group, when the adding concentration is less than 50 μg/L, the biomass increased with the increase of the adding concentration and reached the maximum value 0.57 g/L at the concentration 50 μg/L, enhanced 30% than the blank. But when the adding concentration is 500 μg/L, the biomass significantly decreased (Fig. 2a). In the VH group, the biomass was significantly higher than other treatments ($p<0.01$) besides the blank, at the concentration level 500 μg/L, reached 0.54 g/L, enhanced 29.2% the blank (Fig. 2b). At the adding concentration 0.5 μg/L, the biomass was slightly lower than the blank.

It was concluded the trend of the effect (change) of adding VB_{12} and VH for the biomass of *H. pluvialis* are consistent with the trend of the effect (change) for the cell density, it indicated they were high correlation among the cell density and the biomass which can represent the growth rate of *H. pluvialis*.

Based on our research, higher vitamin concertration not always promting the growth of *H. pluvialis*, when the adding concentration exceeds a certain range, the effect of promotion declined obviously, even control the growth. It showed under the surfficent nutrient condition, growth rate of *H. pluvialis*

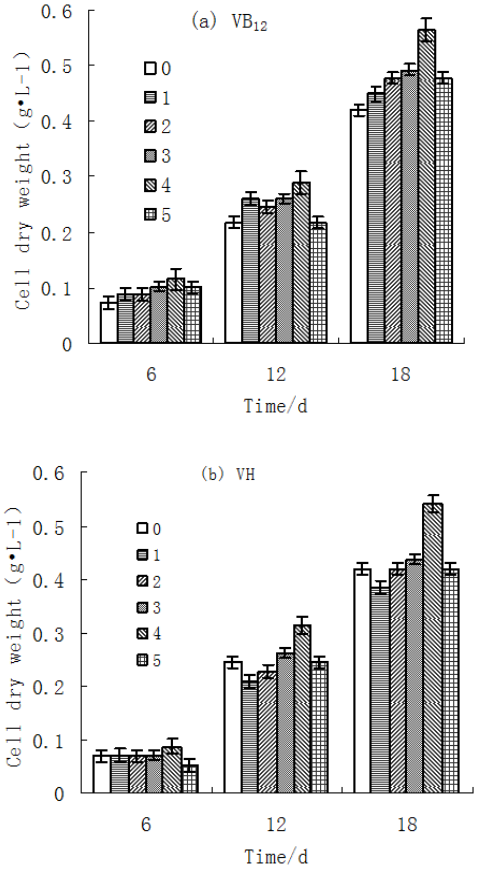

Fig. 2: Effects of Vitamin-B_{12} and Vitamin-H on the biomass of H. pluvialis

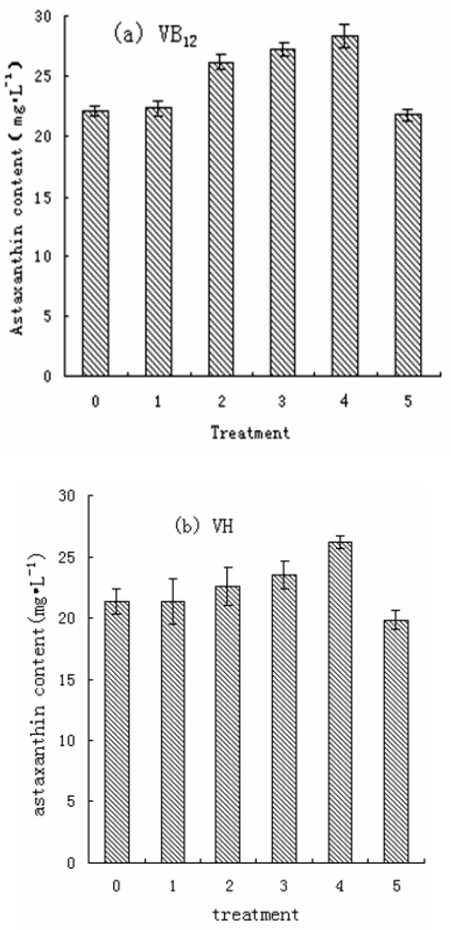

Fig. 3: Effects of Vitamin-B_{12} and Vitamin-H on Astaxanthin content of H. pluvialis

don't have obvious difference between low and high nutrient conditions. On the view of economy, adding VB_{12} to culture H. *pluvialis* is effective and economic.

The astaxanthin content: Respectively adding VB_{12} and VH made the changes of the astaxanthin content of *H. pluvialis* (Fig. 3). In VB_{12} group, the astaxanthin content increased with the increase of the adding concentration, reached maximum 28.35 mg/L at the adding concentration 50 μg/L, enhanced 28.3% than the blank (Fig. 3a). But when the highest concentration (500 μg/L) of VB_{12} was added, the astaxanthin content was lower than the blank. In VH group, the astaxanthin content increased with the increase of VH When adding concentration was 500 μg/L, the astaxanthin content was also higher than other treatments, enhanced 22.6% than the blank ($p<0.05$). When adding the concentration was 5000 μg/L, the astaxanthin content was lower than the blank (Fig. 3b).

H. pluvialis Flotow (Chlorophyceae) has a complex life-cycle involving several stages from motile flagellated zooids through to palmella and encysted stages. The conditions for the astaxanthin production are known to be considerably different from those for the growth of *H. pluvial is*. Therefore, to obtain high productivity of astaxanthin-hyperaccumulated *H. pluvialis* biomass, a two-stage culture system is likely to be more effective (Choi *et al*., 2002). The first stage is for high-rate growth of green motile cells under optimum conditions, astaxanthin is completely absent from the cells.

The second stage is for the hyperaccumulation of astaxanthin in red cells upon exposure of the cells to growth-limiting conditions, where a morphological and biochemical transformation occurs from green motile cells into inert red cysts. Various factors and/or methods promoting astaxanthin formation have been suggested: high irradiation, nitrogen deficiency, phosphate deficiency, magnesium deficiency, acetate addition, ferrous ion addition and salt addition or high temperature (Harker *et al*., 1996). Astaxanthin is synthesised and accumulated within oil droplets (Mendes-Pinto *et al*., 2001).

In the two test groups of adding vitamin, through comprehensive analysis, the rule of promoting astaxanthin content of *Haematococcus pluvialis* CH-1 and the rule of promoting the growth rate of *H. pluvialis* CH-1 is basically the consistent. It is

concluded that adding vitamin mainly promoting the growth rate of *H. pluvialis* CH-1 and indirectly increase the astaxanthin content., because adding the active micronutrient can promote to increase the content of cellular material in *H. pluvialis* CH-1,which can transfer into the astaxanthin under growth-limiting conditions.

CONCLUSION

In BBM medium added VB_{12} and VH can obviously increase the growth of the *H. pluvialis* motile cell, respectively. Adding VB_{12} can significantly improve the cell density, biomass and astaxanthin content, while promotion effect of adding VH is lower. The suitable concentration of VB_{12} and VH is different, the optimal adding concentration of Vitamin-B_{12} and Vitamin-H respectively for *H. pluvialis* was 50 μg/L and 500 μg/L. Adding suitable concentration of VB_{12} can obviously improve astaxanthin content in the commercial production process of natural astaxanthin of *H. pluvialis.*

ACKNOWLEDGMENT

This study was supported by Key Project of NSFC-Guangdong Joint Fund (U1133003), Natural Science Foundation of China (41176104), Science and Technology Research Project of Heilongjiang Province Education Department (12513088), Promising Youngsters Training Program of Heilongjiang University of Science and Technology (Q20120201) and Foundation for Youth Scholar Key Teacher of General University by Heilongjiang Province of China (1155G4P).

REFERENCES

Bjerkeng, B. and G. Johnsen, 1995. Frozen storage quality of rainbow trout (Oncorhynchus mykiss) as affected by oxygen, illumination and fillet pigment. J. Food Sci., 60(2): 284-288.

Boussiba, S. and A. Vonshak, 1991. Astaxanthin accumulation in the green alga *Haematococcus pluvialis* . Plant Cell Physiol., 32(7): 1077-1082.

Choi, Y.E., Y.S. Yun and J.M. Park, 2002. Evaluation of factors promoting astaxanthin production by a unicellular green alga, haematococcus pluvialis, with fractional factorial design. Biotechnol. Prog., 18(6): 1170-1175.

Esra, I., V.S. Fazilet and C.D. Meltem, 2007. Effect of different culture media and light intensities on growth of *Haematococcus pluvialis*. Int. J. Nat. Eng. Sci., 1(3): 05-09.

Ford, J. E., 1958. B12-Vitamins and growth of the flagellate ochromonas mathamonsis. J. Gen. Microbiol., 19(1): 161-172.

Fukuzawa, K., Y. Inokami, A. Tokumura, J. Terao and A. Suzuki, 1998. Rate constants for quenching singlet oxygen and activities for inhibiting lipid peroxidation of carotenoids and α-tocopherol in liposomes. Lipids, 33(8): 751-756.

Harker M., A.J. Tsavalos and A.J. Young, 1996. Factor responsible for astaxanthin formation in the Chlorophyte *Haematococcus pluvialis*. Bioresource Technol., 55(3): 207-214.

Liu, Z. W., C. Zhang and Y. Guo, 2002. Effect of vitamin on culture of recombinant anabaena sp. PCC7120. Acta Hydrobiol. Sinica, 26(6): 722-724.

Lorenz, R.T. and G.R. Cysewski, 2000. Commercial potential for Haematococcus pluvialis as a natural source of astaxanthin. Trends Biotechnol., 18(4): 160-167.

Martin, G. and E. Mark, 2003. Haematococcus astaxanthin: Applications for human health and nutrition. Trends Biotechnol., 21(5): 210-216.

Mendes-Pinto, M.M., M.F.J. Raposo and J. Bowen, 2001. Evaluation of different cell disruption processes on encysted cells of *Haematococcus pluvialis*: Effects on astaxanthin recovery and implications for bio-availability. J. Appl. Phycol., 13(1): 19-24.

Miki, W., 1991. Biological functions and activities of animal carotenoids. Pure Appl. Chem., 63(1): 141-146.

Palozza, P. and N.L. Krinsky, 1992. Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. Arch. Biochem. Biophys., 297(2): 291-295.

Ranjbar, R., R. Inoue, H. Shiraishi, K. Tomohisa and K. Shigeo, 2008. High efficiency production of astaxanthin by autotrophic cultivation of *Haematococcus pluvialis* in a bubble column photobioreactor. Biochem. Eng. J., 39(3): 575-580.

Wang, Z.F., Q. Zhang and Y. Lu, 1996. The effects of nutrients, vitamins and trace metals on the growth of the red tide organism Prorocentrum micans. Donghai Mar. Sci., 14(3): 33-38.