World Journal of Fish and Marine Sciences 4 (6): 679-683, 2012
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2012.04.06.64238

Growth, Protein and Carotenoids Content of the Microalgae, *Chaetoceros calcitrans* under Different Concentrations of Salinity

[1]*Najme Meigoli Nezhad,* [2]*Mehran Javaheri Baboli and* [3]*Khosrow Aein Jamshid*

[1]Young Researchers Club, Science and Research Branch, Islamic Azad University, Khuzestan, Iran
[2]Department of Fisheries Science, Ahvaz Branch, Islamic Azad University, Khuzestan, Iran
[3]National Shrimp Research Institute, Bushehr, Iran

Abstract: The influence of salinity on the growth, protein and carotenoid content of marine microalgae *Chaetoceros calcitrans* were investigated. The obtained results showed no significant change in growth rate under the selected salinities (10, 27 and 40 ppt) ($p>0.05$) but the protein content had a significant change under the three studied concentrations of salinities ($p<0.05$). Maximum growth rate of *C. calcitrans* was observed in Conway medium in 10ppt salinity and the highest cell density was achieved at 27 ppt salinity. The highest level of total carotenoid content was 5.8 µg/g fresh weight and the maximum of protein content was recorded at the 10ppt in 60% of dry weight.

Key words: Carotenoid · Chaetoceros calcitrans · Conway · Protein · Salinity

INTRODUCTION

Microalgae are the major food source for many aquatic organisms and the main live feed component in marine hatchery operations. The marine diatom *Chaetoceros calcitrans* is considered as the most popular strain used in hatcheries, especially for shrimp larvae. This species gives vital energy and organic nutrients for the growth and development of larvae and juveniles [1]. Microalgae chemical composition is frequently determined with the objective to provide the necessary nutritional balance for the captive animals [2].

The literature suggests that, within fairly wide tolerance and different levels of salinity are associated with small changes in the gross chemical composition of marine microalgae [3]. Generally, the carotenoids are observed with the photosynthetic membranes of all photosynthetic organisms. They mainly function in light harvesting, especially in very dim spectrally unusual light available [4]. The carotene level depends on the algae species and environmental conditions; there was an increase in the carotene level in stress conditions [5]. For microbial proteins to be measured accurately, the cells must be pretreated to fully release the intracellular proteins. Pretreatments typically involve disrupting the cells by physical or chemical means [6]. However, comparison of the protein content among algae is difficult because of methodology [7]. In addition; the most common methods used for protein determination in algae are the methods of Lowry *et al.* [8] and Bradford [9]. The Lowry method is one of the most accurate methods for quantifying proteins [10]. Results determined that carotene pigments were the most important photosynthetic pigments and they prevented chlorophyll and thylakoid membrane from the damage of absorbed energy by photo oxidation [11]. These decrease pigment at high salinity (58 ppt) [3] Raghavan *et al.* [1]\ have indicated, a salinity of 25, temperature between 20 and 25°C and addition of carbon dioxide seems more adequate for enhanced growth of *C. calcitrans* and high biochemical composition. It shows that the lowest salinity and light intensity have the highest effect in protein contents [12]. The results of Raghavan [1] reported a decrease in the protein content with an increase in salinity. The result shows that a salinity of 25 is optimum for *C. calcitrans* in terms of growth and chemical composition. The aims of this study were to investigate the effect of varying the salinity concentrations in the

Corresponding Author: Najme Meigoli nezhad, Young Researchers Club, Science and Research Branch, Islamic Azad University, Khuzestan, Iran. Tel: +98-917 9888160, Fax: +98-771-353 4219.

Conway medium on the chemical composition of specie *C. calcitrans* and to determine the optimum salinity for maximum production of protein.

MATERIALS AND METHODS

Microalgae culture was obtained from the algal stock collection of Iranian National Shrimp Research Institute, at Bandargah Research Station in Bushehr, Iran and maintained under laboratory conditions. Protein analysis was performed at Bushehr University of Medical Sciences in Department of Biochemistry.

Algal Culture: The growth of *Chaetoceros calcitrans* was investigated in three levels of salinity (10, 27, 40 ppt) in Conway medium [13] in 9 flasks of 3.5 L within 15 days. The condition was kept at 25±2°C, light intensity of 2000 lux and light period of 24 hrs. All the treatments had three replicates. Cell numbers were determined daily by placing an aliquot of well-mixed culture suspension on a Neubauer haemocytometer. The cells were counted in five small squares in the centre block [14].During this period the physical parameters were recorded every two days. Biochemical compounds were analyzed on the sixth day, in the range of the stationary phase, in Conway medium. The changes in the cell density and specific growth rate (μ: d^{-1}) were calculated according to [15,16].

Analytical Methods: Determination of quantitative variation of algal carotenoids has been carried out by using spectrophotometric methods [11]. Intracellular content of carotenoids were extracted from the cells in the filter by 12 ml of acetone (90%) by reading the absorbance at 470, 645 and 662 nm as described by [11,12,17].

Protein contents were analyzed according to the methods of Lowry *et al.* [8]. First 500 cc of the algal samples centrifuged at 3000 rpm during 10 minutes and washed with 1% (w/v, g/100 ml) aqueous NaCl solution. The samples were centrifuged again and freeze-dried. The dry biomass was analyzed immediately or stored at -80°C up to 10-days prior to analysis. The Lowry method [8] was used to measure the protein content of the pretreated biomass. For protein analysis, 20 mg aliquots of the freeze-dried biomass were suspended for 20-min in 10 ml of lysis buffer in a Falcon tube to facilitate the extraction of proteins. The absorbance of the sample was measured at 750 nm by using a spectrophotometer. The protein content (% of dry weight) was calculated using the following equation:

$$\text{Protein (\%w/w)} = \frac{\text{CVD}}{\text{M}} \times 100$$

Where C is protein concentration (mg L^{-1}) obtained from the calibration curve, V is the volume (L) of the lysis buffer used to resuspend the biomass, D is the dilution factor and m is the amount of biomass (mg) [6].

Statistical Analyses: The data were analyzed using one-way analysis of variance (ANOVA) Tukey test. All statistical analysis was done using the statistical analysis SPSS15.

RESULTS AND DISCUSSION

Cell Density: *Chaetoceros* cell density and growth rates in salinity with the experimental range of 10-40 ppt. There was no significant change in the growth rates of *C. calcitrans* over the entire range of salinity. The highest algae cell density was calculated under salinity 27 ppt, 81.33×10^3 cells per ml was counted (Fig, 1 and Table 1). Tukey test results indicated that there are significant differences ($P>0.05$) between cell densities in salinities of 27 and 40 ppt and also between 27 and 10 ppt, but no significant differences was observed between 10 and 40ppt ($P>0.05$).

Maximum specific growth rate in Conway medium was in salinity 10 ppt, with specific rates 0.27 per day. The growth rate has been set to the 0.22 and 0.18 per day in salinities of 27 and 40 ppt, respectively. According to the specific growth rate, the doubling time has not shown any significant difference ($P>0.05$) in all treatments in the Conway medium. The highest density of cell doubling time in salinity 40 ppt in Conway was 4.3 per day and the minimum of it was belonged to 10 ppt salinity level.

Raghavan *et al.* [1] have reported that the optimum growth of *C. calcitrans* was observed in salinity of 25, temperature between 20 and 25 °C in addition of carbon dioxide, which is adequate for enhancing the growth of *C. calcitrans* and high biochemical composition of protein, lipids and carbohydrates. The Conway medium could be useful for the high algal production and successful operation of a hatchery system.

Depends on the absolute salinity, salinity shift and duration of salinity stress, the algal response to salinity is changes species-specific. The species-specificity response and ambiguity are most and least pronounced at salinity decrease from the normal seawater levels to 10-18 and 5-8 ppt respectively [18].

Table 1: specific growth rate and doubling time in different treatments

Salinity	Max density (day)	Density maximum (10^3 cells/ml)	Growth rate, μ (dG^{-1})	Doubling time
10	7	61.3±20.3[a]	0.27±0.01[a]	2.5±0.1[a]
27	7	81.33±20.2[b]	0.22±0.05[b]	3.3±0.7[b]
40	7	57.33±11.7[c]	0.18±0.04[c]	4.3±1.3[c]

The Values (mean ± standard error) with different superscript letters in the same column are significantly different (P < 0.05). SGR: specific growth rate.

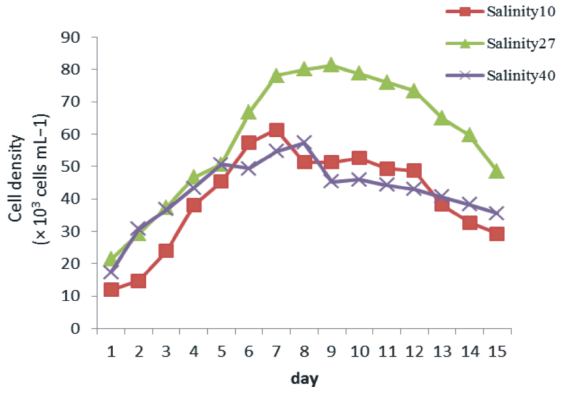

Fig. 1: The variation of Chaetoceros calcitrans cell density under salinities of 10, 27 and 40ppt in Conway medium

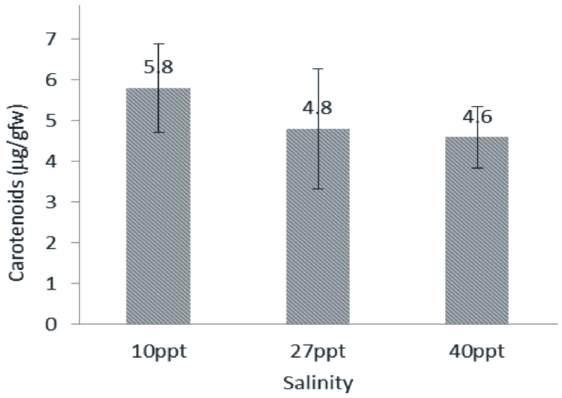

Fig. 2: The content of carotenoid under different concentrations of salinity of 10, 27 and 40 ppt in Conway medium

Lower dry biomass and SGR were reduced when the algae was stressed by high salinities [19]. Radchenko and Il'yash [18] observed that *Thalassiosira weissogii* proved capable to grow under conditions of strong hypotonic stress, which, together with the wide salinity range allowing its natural vegetation, points to the mechanisms adjusting the intracellular turgor pressure to the environmental turgor pressure. Gu *et al.* [20] have indicated algae *Nannochloropsis oculata* preferred the low salinity in media and had slow growth during the increase of salinity (Tr-1:35-15 g/l, Tr-2:35-25 g/l, Tr-3:35-35 g/l, Tr-4:35-45g/l and Tr-5:35-55 g/l). maybe algae would expend energy while attempting to maintain the turgor pressure and this resulted in a decrease in productivity or reduction in growth.

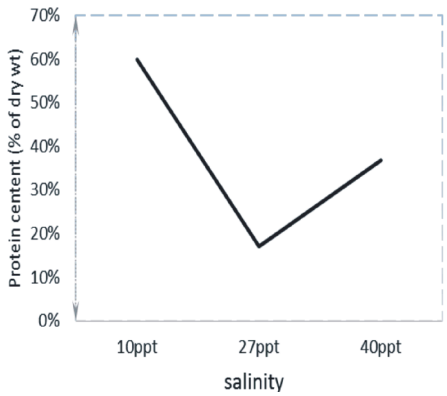

Fig. 3: The amount of protein content in Conway medium

Chemical Composition: One of the best parameters to monitor microalgae production is the estimation of growth, generally expressed in biomass and density increase, proteins, pigments and carbohydrates contents over a certain period of time [21].

The highest contents of carotenoid in salinity of 10ppt and were, 5.8 and 4.8 µg/g fresh weight respectively and the lowest of them, in salinity 40 ppt, was 4.68 µg/g fresh weight. There is no significant difference between the treatments (P>0.05). Salinity effect on carotenoid content did not show significant difference (P>0.05) (Fig. 2).

High pigment values were probably due to the high cell density [2]. Increasing salinity reacts by decreasing the amount of chlorophyll a per cell [3]. Gu et al [20] have indicated chlorophyll-a and carotenoid contents in *Nanochloropsis oculata* were the highest during the salinity change from 35 to 25 g/l and this was in accordance with the reports that low salinity could increase content chlorophyll-a and carotenoid production in algae *Dunaliella tertiolecta* and also *Tetraselmi chuii* [12].

Most proteins in Conway medium were 60% of dry weight under salinity 10 ppt. In salinity 40 ppt content protein is 38% dry weight. The lowest proteins were

measured in salinity 27 ppt which equals to 17% dry weight. During the studies performed, there is significant difference between treatments ($P<0.05$). Salinity effects on the protein show significant difference (Fig. 3) with increasing salinity and an increase or a decrease in total protein [22]. The results indicated that the amount of proteins have been increased by the salt stress. Its amount in 20ppt salinity and 2500 lux was the highest [12]. One of the main problems with protein analysis in algae is the protein extraction, done with different degrees of success by researchers. Differences in cell wall composition of algae and in procedures for protein extraction establish strong and negative effects on the final results [23]. *C. calcitrans* apparently directed the extra-assimilated carbon mainly to protein synthesis, indicating a positive effect on cell physiology. Probably, the cells were investing the excess of carbon assimilated much more in protein synthesis and growth rather than lipids and carbohydrates as reserve substances in microalgae [1].

ACKNOWLEDGMENTS

Thanks to Mr. Zendeh boudi from Shrimp Broodstoking Research Station of Bandarghah, Mr. Bargahi from Persian Gulf Marine Biotechnology Research Center, Bushehr University of Medical Sciences and Dara Bagheri PhD student of Tehran University. This article is based on master's thesis submitted to Islamic Azad University of Science and Research of Khuzestan branch.

REFERENCES

1. Raghavan, G., C.K. Haridevi and C.P. Gopinathan, 2008. Growth and proximate composition of the *Chaetoceros calcitrans* under different temperature, salinity and carbon dioxide level. J. Aquaculture Research, pp: 1058.
2. Junior, A.M., E.B. Neto, M.L. Koening and E.E. Leça, 2007. Chemical compositon of three microalgae species for possible use in mariculture, Brazilian Archives of Biology and Technology. 50(3) : 461-467.
3. Renaud, S.M., D.L. Parry, T. Luong-Van, C. Kuo, A. Padovan and N. Sammy, 1991. Effect of light intensity on the proximate biochemical and fatty acid composition of *Isochrysis* sp. and *Nannochloropsis oculata* for use in tropical aquaculture. J. Appl. Phycol., 3: 43-53
4. Naguit, M.R.A. and W.L. Tisera, 2009. Pigment analysis on *Eucheuma Denticulatum* (Collins& Hervey) and *Kappaphycus alvarezii* (DOTY) Cultivars cultured at different depths. pp: 29-37.
5. Grung, M.F., M.L. D'souza and M. Borowitzka, 1992. Algal Carotenoids 1. Secondary Carotenoids. J. Appl. Phycol., 4: 165-171.
6. Gonzalez Lopez, C.V., C.C. Ceron Garcia and F.G. Fernandez, 2010. Protein measurements of microalgal and cyanobacterial biomass, journal homepage: www. elsevier. com/ locate/biortech. Bioresource Technology, pp: 7587-7591.
7. Berges, J.B., A.E. Fisher and P.J. Harrison, 1993. A comparison of Lowry, Bradford and Smith protein assays using different protein standards and protein isolated from the marine diatom *Thalassiosira pseudonana*. Marine Biology, 115: 187-193.
8. Lowry. O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.
9. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Analy. Biochem. 72: 248-254.
10. Peterson, G.L., 1979. Review of the Folin phenol protein quantitation method of Lowry, Rosebrough, Farr and Randall. Anal. Biochem. 100: 201-220.
11. Dere, S., T. Gunes and R. Sivaci, 1998. Spectrophotometric determination of chlorophyll - A, B and total carotenoid contents of some algae species using different solvents. Tr. J. of Botany, 22: 13-17.
12. Ghezelbash, F., T. Farboodnia and R. Heidari, 2008. Biochemical effects of different salinities and luminance on green microalgae *Tetraselmis chuii*. Research Journal of Biological Sciences, 3(2): 217-221.
13. Lavens, P. and P. Sorgeloos, 1996. Manual on the production and use of live food for aquaculture FAO Fisheries Technical Paper. No. 361. Rome, FAO. pp: 295.
14. Banerjee, S.W., E. Hew, H. Khatoon and M. Shariff, 2011. Growth and proximate composition of tropical marine *Chaetoceros calcitrans* and *Nannochloropsis oculata* cultured outdoors and under laboratory conditions. African Journal of Biotechnology. pp: 1376-1383.
15. Garcia, F., Y. Freile-Pelegrin and D. Robledo, 2007. Physiological characterization of *Dunaliella* sp. (Chlorophyta, Volvocales) from Yucatan, Mexico. Bioresource Technology, 98: 1359-65.

16. Amini Khoeyi, Z., J. Seyfabadi and Z. Ramezanpour, 2010. Effect of light intensity on growth and biomass of *Chlorella vulgaris* Microalgae. Iranian Journal of Fisheries, pp: 11-20.
17. Lichtenthaler, H.K. and A.R. Wellburn, 1985. Determination of total carotenoids and chlorophylls A and B of leaf in different solvents. Biol. Soc. Trans., 11: 591-592.
18. Radchenko, I. and L. Il'yash, 2006. Growth and photosynthetic activity of diatom *Thalassiosira weissflogii* at decreasing salinity. Biology Bulletin, 33: 242-247.
19. Ravelonandro, P.H., D.H. Ratianarivo, C. Joannis-Cassan, A. Isambert and M. Raherimandimby, 2011. Improvement of the growth of *Arthrospira* (Spirulina) platensis from *Toliara* (Madagascar): effect of agitation, salinity and CO_2 addition. Food and Bioproducts Processing, 89(3): 209-216.
20. Gu, N., Q. Lin and G. Qin, 2012. Effect of salinity change on biomass and biochemical composition of *Nannochloropsis oculata*. J. World Aquacult., 43: 97-105.
21. Becker, E.W., 1995. Microalgae: biotechnology and microbiology, Cambridge University Press, New York.
22. Fabregas, J., C. Herrero, J. Abalde and B. Cabezas, 1985. Growth, chlorophyll a and protein of the marine microalgae *Isochrysis galbana* in batch cultures with different salinities and high nutrient concentrations. Aquaculture, 50: 1-11.
23. Lourenço, S.O., E. Barbarino and J.C.D.E. Paula, 2002. Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical. Phycological Research, pp: 233-241.