International Journal of Innovation in Agriculture Sciences and Rural Development
Vol.1, NO.1 , P : 38 - 42
Received: 23 Nov 2018
Accepted: 20 Feb 2019

Mass Cultivation Phytoplankton Nannochloropsis oculata in the indoor and outdoor systems

Salavatian,S.M [*1] , Rufchaei ,R

Email*: salavatian_2002@yahoo.com

1- Inland waters Aquaculture Research Center, Iranian Fisheries Science Research Institute (IFSRI), Agriculture Research Education and Extension Organization (AREEO), Bandar-e Anzali, .Iran

Abstract

This study was carried out to culture *Nannochloropsis oculata* marine algae at Gomishan workshop located in Golestan province in the autumn and winter. The algae were transferred from Kuwait to research center of Khozestan province to culture and feed the marine fish culture. The culture was established in an experimental –scale at indoor 250ml at 20 liter tanks in liquid cultivated medium f/2 (45×10^6 cells ml^{-1}) and outdoor at an average density of 16×10^6 cells ml^{-1} in liquid cultivation medium TMRL. Water was directly pumped from a nearby shore and filtered by filtration systems and was used to produce liquid cultivated medium. No significant difference in temperature, pH, salinity, light or dissolved oxygen (DO) were observed between treatments. The temperature in all the process was 25±2˚C and algal light intensity was above 3500±350 lux and salinity was 30-32 ppt. Aeration was provided from air blower pumps and transferred with PVC pipe. Rotifers (B*rachionus plicatilis)* mono culture was maintained in 500 liters out door tanks which were to be fed by Striped Mullet larvae. The size (15-20µ) and cells wall structure of *Nannochloropsis oculata* in compartion with *chlorellas* algae cell wall are very suitable to feed and digestion for Striped Mullet larvae.In other words , the high amount of polyunsaturated fatty acids (PUFA)in this algae is most essential for all the marine fish larvae. Acording to Sethi et al. (2004) stability of larvae during the culture in compartion with other scientific reports and results of this project in the past years was very good, which shows a positive role of these algae to feed the Striped Mullet larvae.

Key word: *Nannochloropsis oculata*, Mass Cultivation, indoor, Outdoor, Iran

Introduction

Microalgae are an indispensable food source for all growth stages of bivalves and for the larvae of some crustacean and fish species in aquaculture. They are also eaten by zooplankton reared as food for the larvae and juveniles of some crustacean and fish species. HUFA are essential for marine fish larvae (Watanabe *et al*., 1983; Witt *et al.*, 1984; Sureshkumar *et al*., 2014; Watanabe and Kiron, 1994; Melina *et al*.,2016). Docosahexaenoic acid (DHA; 22:6 (n_3)) has a significant influence on larval stress resistance (Kraul *et al*., 1991: Kraul *et al*., 1993). High cell density cultures of *Nannochloropsis* (e.g.

6×10^8 cells ml^{-1}). have clear advantages; a) eliminating a concentration stage required when low cell densities of algae are to be fed to high density cultures of rotifers (Kobayashi et al., 2008) low density cultures in open raceways become easily contaminated; b) maintenance of monoalgal cultures is usually more readily accomplished in high cell densities; c) The productivity of open systems is much less stable than that of enclosed reactors which are less susceptible to daily, as well as seasonal, variations in temperature; d) low density cultures burden production costs in requiring large reactor and culture volumes. One of the most important needs for any marine fish or crustacean hatchery is to obtain a constant high quality supply of live food microorganisms at a reasonable cost (Herrero et al., 1991).Marine fish larvae's survival and growth rates are mainly related to the quality of live microorganisms supplied (Hirayama & Funamoto, 1983; James & Abu-Rezq, 1988; Ferreiro et al., 1991; Korstad et al., 1995). At present, the only commercial source of eicosapentaenoic acid (EPA) is marine fish oil. However, there are several limitations for using fish oil as a source of EPA such as peculiar taste, outdoor, stability problems and high purification cost. Because fish themselves obtain ω-3 fatty acids from zooplankton that has fed on algae, cultivation of microalgae is an attractive alternative for the production of EPA.

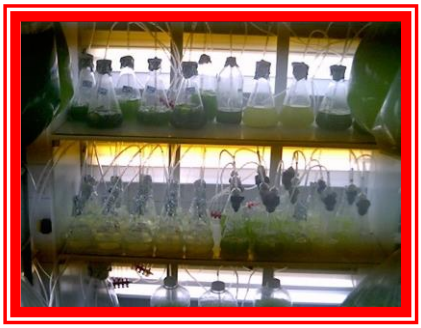
Material and methods

Nannochloropsis oculata was obtained from Kuwait to Kuzestan province research center. The culture was established in an experimental –scale at indoor 250 ml at 20 liter tanks in liquid cultivated medium f/2 (45×10^6 cells ml^{-1}) and outdoor at an average density of 16×10^6 cells ml^{-1} in liquid cultivation medium TMRL. Water was directly pumped from a nearby shore and filtered by filtration systems and was used to produce liquid cultivated medium. Cultures were kept in semi-continuous regime for 6 days with no significant difference in temperature, pH, salinity, light or dissolved oxygen (DO) were observed between treatments. Once the cultures were stabilized, samples were taken every 2 h during the light period and additionally were taken twice during the dark period, on the first and 12th h. The temperature in all the process was 25±2˚C and algal light intensity was above 3500±350 lux and salinity was 30-32 ppt (Rennolds et al.,1975; Rodolfi et al.,2003; Piri and Ordog ,1997). Aeration was provided from air blower pumps and transferred with PVC pipe. Rotifers (B*rachionus plicatilis)* mono culture was maintained in 500 liters outdoor tanks which were to be fed by Striped Mullet larvae. Cell density was calculated daily in the outflow of culture by microscope counting using an improved hematocytometer.

Results and discussion

Several species in the genus *Nannochloropsis* are commonly used as high-quality food organisms due to their high contents of EPA (Sukenik et al. 1993). All the *Nannochloropsis oculata* strains studied here exhibited a similar pattern of growth , achieving cell density of about 45×10^6 and 16×10^6 cells ml^{-1} consequently f/2 and TMRL mediums after 6 days of growth. The commonly used f/2 culture medium for *Nannochloropsis sp*. was shown to be limiting for both carbon and nitrogen sources. In fact, the medium has not carbon sources because autotrophic organisms are able to use the

atmospheric CO2. However, the addition of small concentrations of glucose and ammonium sulphate increased the cell growth rate. The enhanced growth of *Nannochloropsis* sp. by addition of CO2 was observed in the late growing phase probably due to carbon-limitation during this period. *Nannochloropsis sp*. grew best in culture aerated with the enriched CO2, while its biomass yield was the lowest with the ambient CO2 level. The effect of acetate on growth was so little that it can be ignored. A disadvantage of using an organic carbon source is the higher probability of contamination with non-phototrophic bacteria. A combined effect of both carbon and nitrogen sources was observed because an increase in nitrogen concentration was only beneficial with a corresponding increase in carbon concentration. The nutrient of TMRL medium was very low to compare with f/2 medium and it was depleted very soon. There are few reports of copepod culture with *N. oculata* as food. The microalgal densities were high in our experiments, although *N. oculata* is very small, so high number of cells per milliliter was expected. Use of baker's yeast promoted ciliate, bacteria and detritus proliferation, but in the present study, there were no yeasts because these products are also food sources for copepods (Zillioux,1969).The quantity of baker's yeast was the same as that used by other authors who combined it with *N. oculata* or other microalgae (Fulks and Main,1991). To improve culture conditions, the yeast should be divided into two or more partial rations during the day to lessen water quality deterioration. Microalgae have an important role as the primary source of vitamins in aquatic food chains where they are fed to zooplankton (e.g. rotifers, *Artemia*.) The sizes of *N. oculata* are satisfactory for larval rearing of marine fish because their nauplii are small, and are adequate as a first food for many smallmouth species, among which the ornamentals are abundant (Kuronuma and Fukusho,1984). As a general conclusion, we propose that small phytoplankton and zooplankton species with short life cycles and fast growth could be cultured with high-yield semi-intensive technologies. In tropical and subtropical countries with low costs of technically qualified labor and land, they could give good economic results in terms of highly stress-resistant larvae, good survival, growth and biomass productions when compared with enriched Artemia.



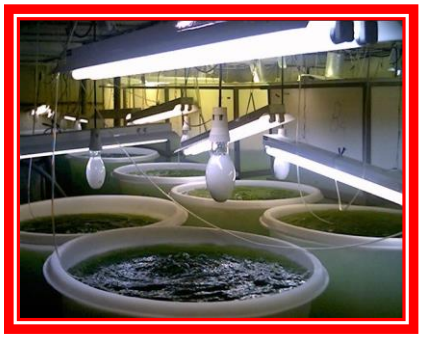


Fig1 . General view of the mass cultivation of *Nannochloropsis oculata* in the indoor and outdoor systems

References

1) Ferreiro, M. J., M. J. Fernandez-Reiriz, M. Planas, U. Labarta & J. L. Garrido, 1991. Biochemical composition of enriched and starved rotifers. Larvi '91 – Fish and Crustacean Larviculture Symposium. Europ. Aqua. Soc., Special Publication No. 15: 36– 38.

2) Fulks, W., Main, K.L. (Eds.), 1991. Rotifer and Microalgae Culture Systems. Proceedings of U.S.–Asia Workshop, Honolulu, Hawaii, January 28–31, 1991. Argent Laboratories, Redmond. 364 pp.

3) Herrero, C., A. Cid, J. Fabregas & J. Abalde, 1991. Yields in biomass and chemical constituents of four commercially important marine microalgae with different culture media. Aqua. Eng. 10: 99–110.

4) Hirayama, K. & H. Funamoto, 1983. Supplementary effect of several nutrients on nutritive deficiency of baker's yeast for population growth of the rotifer Brachionus plicatilis. Bull. Jpn. Soc. Sci. Fish. 49: 505–510

5) James, C. M. & T. S. Abu-Rezq, 1988. Effect of different cell density of Chlorella capsulata and a marine Chlorella sp. for feeding the rotifer Brachionus plicatilis. Aquaculture 69: 43–56.

6) Kuronuma, K., Fukusho, K., 1984. Rearing of marine fish larvae in Japan. International Development Research Centre, Ottawa, IDRC-TS 47e. International Development Research Centre, Ottawa, Canada. 109 pp. – 79.

7) Kraul, S., Brittain, K., Cantrell, R., Nagao, T., Ako, H., Ogasawara, A., Kitagawa, H., 1993. Nutritional factors affecting stress resistance in the larval mahimahi Coryphaena hippurus. J. World Aquac. Soc. 24 (2), 186–193.

8) Kraul, S., Ako, H., Brittain, K., Ogasawara, A., Cantrell, R., Nagao, T., 1991. Comparison of copepods and enriched Artemia as feeds for larval mahimahi, Coryphaena hippurus. In: Lavens, P., Sorgeloos, P., Jaspers, E., Ollevier, F. (Eds.), Larvi'91 Fish and Crustacean Larviculture Symposium. Eur. Aquac. Soc. Spec. Publ., vol. 15. European Aquaculture Society, Ghent, Belgium, pp. 45– 47.

9) Korstad, J., A. Neyts, T. Danielsen, I. Overrein & Y. Olsen, 1995. Use of swimming speed and egg ratio as predictors of the status of rotifer cultures in aquaculture. Hydrobiologia 313/314: 395– 398.

10) Kobayashi T.; Toshiya N.; Akinori H. and Toshio T.,2008. Effect of combination feeding of Nannochloropsis and freshwater Chlorella on the fatty acid composition of Rotifer Brachionus plicatilis in a continues culture. Fisheries Science. 74:649-656.

11) Melina.I.Sahay.S.; Krishnika Arunachalam S.;Beena B. and Jayalakshmy N.,2016. Biochemical characterization of eight marine microalgae grown in batch cultures. Journal of Algal Biomass Utilization.7 (3):19-41.

12) Piri, Z.M.; and V. Ordog ,1997. Effect of some herbicides commonly used in Iranian agriculture on aquatic food chain. Ph.D. thesis to the hungarian academy of sciences.PP:19-30.

13) Rennolds,J.H; E.J,Middlebrooks; D.B,Procella and W.J,Grenney 1975 : Effects of temperature on growth constants of Selenastrum capricornutum.-J.water pollut.Control.Fed 47:242-0-2436.

14) Rodolfi,L.; G.C,Zittelli; L,Barsanti; G,Rosati; M.R,Tredici, 2003. Growth medium recycling in Nannochloropsis sP. Mass cultivatin. Biomolecular Engineering.20:243-248.

15)Sukenik A, Zmora O, Carmeli Y (1993) Biochemical quality of marine unicellular algae with special emphasis on lipid composition. II. Nannochloropsis sp. Aquaculture 117: 313–326.

16) Sureshkumar S.;Jasmin B.; Mujeeb Rahiman K.M. and Hatha Mohammed A.A.,2014. Growth enhancement of micro algae, Chaetoceros calcitransand and Nannochloropsis oculata using selected bacterial strains. International Journal of current microbiology and applied sciences.volum 3. Number 4 (2014).pp.352-359.

17)Watanabe, T., Kiron, V., 1994. Prospects in larval fish dietetics. Aquaculture 124, 223– 251.

Sorgeloos, P., Le´ger, P., Lavens, P., 1988. Improved larval rearing of European and Asian seabass, seabream, mahi- mahi, siganid and milkfish using enrichment diets for Brachionus and Artemia. World Aquac. 19 (4), 78

18) Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional value of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 34, 115– 143.

19)Witt, U., Quantz, G., Kuhlmann, D., Kattner, G., 1984. Survival and growth of turbot larvae Scophthalmus maximus L. reared on different food organisms with special regard to long-chain polyunsaturated fatty acids. Aquac. Eng. 3, 190– 1771.

20)Zillioux, E.J., 1969. A continuous recirculating culture system for planktonic copepods. Mar. Biol. 4, 215–218.