International Journal of Ecology and Environmental Sciences
www.ecologyjournal.in
Online ISSN: 2664-7133; Print ISSN: 2664-7125; Impact Factor: RJIF 5.28
Received: 21-01-2021; Accepted: 06-02-2021; Published: 02-03-2021
Volume 3; Issue 1; 2021; Page No. 361-364

Biochemical variations of microalga *Nannochloropsis oculata* in different culture media

KA Anisha, Gijo Ittoop, Aneykutty Joseph*
Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Fine Arts Avenue, Cochin, Kerala, India

Abstract
Nannochloropsis oculata, was grown in four different culture media with different composition and was analyzed to study the effect of media on the biochemical composition of the microalgae. The species was cultured in Walne's, Miquel's, Chu#10 and f/2 media simultaneously, keeping the physicochemical factors viz. temperature (22ºC), light intensity (2000 lux), pH (8.0) and salinity (35ppt.) constant. The microalgae were harvested in the late exponential phase of growth to carry out the biochemical analysis to find out chlorophyll *a*, protein, lipid and carbohydrate content. The results revealed that the culture media has significant effect on the biochemical composition of the micro algae thus influencing its nutritional status. The highest protein content was observed in the alga grown in Miquel's medium (32.05%). The carbohydrate was highest when grown in Chu#10 medium (19.12%) and lipid content was highest when grown in f/2 (18.93%) medium. The protein and lipid content showed statistically significant variation in different media. *N. oculata* grown in Walne's medium gave the maximum cell count, cell density and chlorophyll *a* content. But the protein, lipid and carbohydrate content of the species were significantly low compared to other culture media. The result showed that the nutritional status of the microalgae depends on the composition of the culture media used.

Keywords: *Nannochloropsis oculata*, culture medium, biochemical composition, growth kinetics, chlorophyll

Introduction
In all the aquatic system micro algae forms the basis of the food pyramid as they are used as feed by all the other organisms either directly or indirectly. Therefore aquaculture industry rely on microalgal culture, since they are required to feed the larval and young stages of majority of cultivable finfish and shell fish for better survival and growth. Aquaculture of filtering molluscs, echinoderms, crustaceans (especially penaeid shrimps), and small larvae of fish, generally require microalgae either as direct feed or as feed for the zooplankton such as which is in turn used as feed by these species [1, 2]. Since the per capita demand for fish and seafood show a double fold increase in the last 50 years reaching to 20 kg in 2015, aquaculture is the only scope in front of the world to meet this demand. In fact most of the fish consumed comes from the aquaculture systems in recent years [3]. Microalgae are considered as a sustainable feedstock [4] and are sought after mainly for the lipid contents and carotenoids. It is preferred in most of the aquaculture industry because of the better economic management, water management and health management [5]. In these circumstances, research on nutritional status of the microalgae which can be mass produced and used in the aquaculture systems for the better production from the aquaculture systems, is of great significance. The most widely used algae are *Nannochloropsis oculata, Isochrysis galbana, Pavlova lutheri, Pavlova salina, Tetraselmis striata, Dunaliella salina, Chlorella virginica, Chaetoceros* sp., *Skeletonema* sp., *Phaeodactylum* sp. and *Thalassiosira* sp., etc. [6, 7]. But poor larval survival still remains to be bottle neck in the development of aquaculture industry, mainly because of inadequacy in the larval nutrition. Several studies have been conducted on the modulation of nutritional status of the microalgae to meet the nutritional requirement of the particular species of larvae. This is made possible by modulations in the culture strategies as well as the composition of the media [7, 8].

Nannochloropsis species are widely used as food in aquaculture for growing small zooplankton such as rotifers copepods, cladocerans and *Artemia*. It is also used extensively in reef tanks for feeding SPS corals and other filter-feeders that require extremely small phytoplankton to thrive. Because of its high content of eicosapentaenoic acid (EPA), *Nannochloropsis* sp. has also been proposed as a source of dietary polyunsaturated fatty acid [9].

One of the most important species belonging to this genus is *N. oculata*. In the present study, the variation in growth and nutritional status of this micro alga in different media would be investigated so as to identify a media, which support good growth and at the same time could provide a larval feed rich in PUFA and protein.

Materials and Methods
Pure culture of *N. oculata* maintained in f/2 medium was obtained from Central Marine Fisheries Research Institute, Kochi, for the present study. It was sub cultured in fresh f/2 medium prepared using filtered and sterilized seawater of 35 ppt salinity. The culture was maintained at a temperature of 22°C, pH 8 and a light intensity of 2000 lux with a photoperiod of 12L:12D. Contamination was checked every 3 to 4 days. Standard algal culture procedures were used throughout the study [10].

In the present study, Walne's [11] (Table 1), Miquel's [12] (Table 2), Chu#10 [13] (Table 3), and f/2 [14] (Table 4) media were used to study the difference in nutritional content of the algae. Culture was done in 2000 ml Haffkine flasks with four replications for each media. About 9 to 10 cells were inoculated per ml of the media. On the 9th day during the late exponential phase the culture was harvested using flocculation method by increasing the pH using sodium hydroxide [10]. Haemocytometer was used to estimate cell density.

Table 1: Walne's medium

Reagents	g/100 ml
Solution A	
Ferric chloride	0.130
Manganese chloride	0.036
Boric acid	3.360
Sodium EDTA	4.500
Sodium orthophosphate	2.000
Sodium nitrate	10.00
Solution B	
Zinc chloride	2.100
Cobalt chloride	2.000
Ammonium molybdate	0.900
Copper sulphate	2.000
Solution C	
Thiamine HCl	0.010
Cyanocobalamin	0.010

1ml of A, 0.5 ml of B and 0.1 ml of C were added to 1 liter of filtered and sterilized water.

Table 2: Miquel's medium

R Reagents	g/ 100ml
Solution A	
Potassium nitrate	2 20.2
Solution B	
Sodium orthophosphate	4.0
Calcium chloride	2.0
Ferric chloride	2.0
Hydrochloric acid	2.0 ml

0.55 ml of A and 0.50 ml of B were added to 1 liter of filtered and sterilized water.

Table 3: Chu#10 medium

Reagents	g/100 ml
Calcium nitrate	4.0
Potassium orthophosphate	0.5
Magnesium sulphate	2.5
Sodium carbonate	2.0
Sodium silicate	2.5
Ferric chloride	0.8

Added 1 ml of the solution to 1L of filtered and sterilized water.

Table 4: f/2 medium

Reagents	g/100 ml
Solution A	
Sodium nitrate	7.500
Sodium orthophosphate	0.500
Sodium silicate	3.000
Solution B	
Ferric chloride	0.315
Sodium EDTA	0.436
Copper sulphate	0.980
Zinc sulphate	2.200
Cobalt chloride	1.000
Manganese chloride	18.00
Sodium molybdate	0.630
Solution C	
Biotin	0.020
Cyanocobalamin	0.100
Thiamine HCl	0.100

Added 3 ml solution A, 1ml Solution B and 0.5 ml Solution C to 1 L of filtered and sterilized water.

Table 5: Biochemical composition of *Nannochloropsis oculata* in different media

Medium	Protein (%)	Lipid (%)	Carbohydrate (%)
Walne's	26.19± 6.08	10.03± 3.12	17.44± 3.13
Miquel's	32.05± 1.35	12.14± 3.36	17.76± 2.09
Chu	14.40± 2.3	15.57± 2.46	19.12± 2.78
f/2	23.61± 2.41	18.93± 1.27	15.73± 3.10

Chlorophyll

Chlorophyll *a* content was determined by the acetone extraction method [15]. About 10 ml of culture was taken and centrifuged. The pigments were extracted with 10 ml 100% acetone. After extraction the cell debris was removed by centrifugation. The absorbance of the solvent extract was measured at 663 nm and 645 nm against solvent blank. Later the chlorophyll content was estimated using the following equations.

Chlorophyll $a = (12.7 \times a_{663}) - (2.69 \times a_{645})$

Protein

Protein content of the microalgae was estimated using the technique dye binding method [16]. Bovine serum albumin was used as protein standard. Representative aliquots of culture were taken and cells were collected by centrifugation. The cells were homogenized in 50 ml tris buffer. Later protein was precipitated using 20% trichloroacetic acid. The protein was collected by centrifugation, and then washed in 90% ethanol in 20 ml tris. Absorbance at 595 nm was measured and graph was plotted.

Lipid

The lipid content of the algae was determined using Sulpho-phospho vaniline method [17]. 500 mg of dry algae was taken in a homogenizer and added 10 ml of chloroform- methanol mixture. The homogenate was filtered through Whatmann no.1 filter paper. It was then transferred to a separating funnel and kept overnight to form a biphasic layer. The lower lipid layer was taken and the volume was adjusted to 10 ml by adding chloroform. Measured 0.5 ml of extract into a clean tube and allowed to dry in vacuum desiccators over silica gel. Then added 0.5 ml of concentrated sulfuric acid and mixed well. Plugged with non-absorbent cotton. Heated in a boiling water bath for 10 minutes and the cooled at room temperature. 0.2 ml of lipid extract was taken in a test tube and added 5 ml of vanillin reagent, mixed well, and allowed to stand for 30 minutes. Later, measured the absorbance at 520 nm.

Carbohydrate

Carbohydrate was estimated using phenol-sulphuric acid method [18]. 100 mg of centrifuged sample pellet was taken and homogenized using mortar and pestle and it was diluted in 20 ml distilled water. 1ml of sample was taken in three test tubes each. Added 0.05 ml of 80% phenol to each test tube and shake well. Kept it for ten minutes. Added 5 ml conc. H_2SO_4 to each test tube. Shake well and kept for half an hour at room temperature. Later absorbance was read at 490 nm.

Statistical analysis

Statistical analysis of the data was carried out using analysis of variance technique using the software available in Microsoft Windows. Comparison of means using't' test was done [19].

Results and Discussion

In the present study the marine microalge *N. oculata* was cultured in four media such as Walne's, Miquel's, Chu#10 and f/2 media and cell numbers and cell density, chlorophyll *a*, protein, lipid and carbohydrate content were estimated to understand the modulations of these parameters in the different media.

The details in figure 1 reveals that the Walne's medium supported the maximum cell growth followed by Miquel's and f/2 medium. Walne's medium gave the best result for cholorophyll *a* also as shown in figure 2. The nutrient content as revealed in table 5 revealed that Miquel's media gave the best result for the protein content. Walne's medium gave moderate values for protein, but the lipid and carbohydrate content in the microalgae grown in this medium was less, compared to all the other media used. The nutrient content of *N. oculata* grown in Chu#10 medium showed poor values for all the nutrients studied. Algae grown in the f/2 medium gave moderate values for protein and highest value for lipid. The statistical analysis showed significant different between he values obtained in the different media at 5% level of significance.

The algal diets rich in carbohydrate was found to be best for larval scallops (*Patinopecten yessoensis)* [20] and juvenile oysters (*Ostrea edulis*) [21], if the polyunsaturated fatty acids are present in sufficient quantities. The microalgae with high protein content was found to give best growth for juvenile Pacific oyster (*Crassostrea gigas*) [22] and mussels (*Mytilus trossulus*) [23]. Thus it can be seen that the nutrient requirement of the target species of aquaculture are different and best results can be obtained by giving them microalgae having a biochemical composition meeting the requirement of these species. The nutrient content of the same species of the algae can be changed according to the requirement of the larvae to be fed by making small changes in the composition of media. For example, the carbohydrate level of the microalgae can be increased by limiting nitrate in the media [24]. In the present study the carbohydrate content of *N. oculata* grown in different media were not significantly different ($p \leq 0.5$). But Miquel's medium gave a significantly high content of protein showing its efficiency to produce microalgae with high protein content. Another phenomenon noted in the present study was the effect of Walne's medium. Although the microalgae grown in this medium showed maximum cell density and chlorophyll *a* content, the nutritional status of the micro algae produced was found to be inferior compared to other media. This reveals that the media which support a good growth of micro algae need not be the best media in terms of nutritional quality of the algae produced. The nutrient intake by the cell changes when cell density increases [25]. They also suggested that the biochemical composition of algae varies with media and some media may not be good for some species. Therefore, after selecting the micro algae for culture, it is necessary to find the best media for its culture. Thus it is proved that a manipulation in the biochemical composition of the microalgae *N. oculata* is possible through culture in different media. The results obtained in the present study are in accordance with the results of the study which revealed that different media could produce some difference in the biochemical composition of *N. oculata* [26]. The best media is always the one that gives a nutritional content in micro algae as per the requirement of the animal that is fed [27].

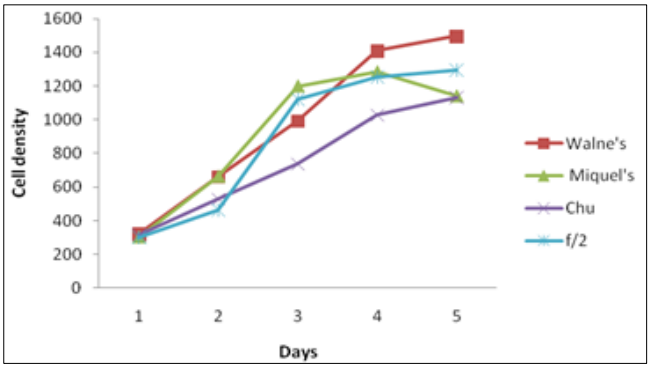

Fig 1: Growth kinetics of *Nannochloropsis oculata* in four culture media

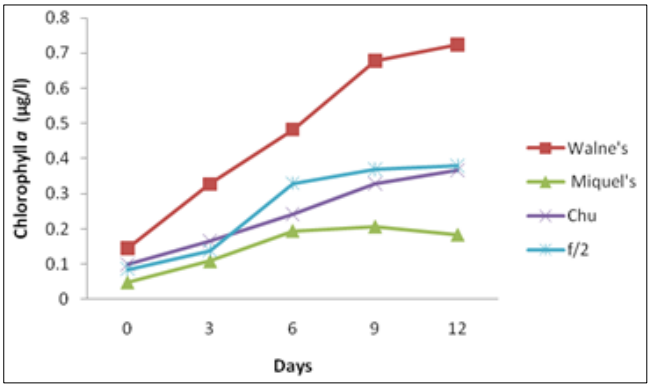

Fig 2: Chlorophyll *a* content of *Nannochloropsis oculata* in four culture media

Acknowledgements

The authors are deeply indebted to the Kerala Biotechnology Commission, Government of Kerala, Trivandrum and Department of Marine biology, Cochin University of Science and Technology, for the facilities provided.

References

1. Muller-Feuga A. The role of microalgae in aquaculture: situation and trends. J. Appl. Phycol. 2000; 12:527-534.
2. Welladsen H, Kent M, Mangott A. Shelf-life assessment of microalgae concentrates: Effect of cold preservation on microalgal nutrition profiles. Aquaculture. 2014; 430:241-247.
3. FAO. The state of world fisheries and aquaculture: Contributing to food security and nutrition for all. Food and Agriculture Organization of the United Nations, Rome, 2016.
4. Murray PM, Moane S, Collins C. Sustainable production of biologically active molecules of marine based origin. New Biotech. 2013; 30 (6):839-50.
5. New MB, Wagner CV. Freshwater prawn culture. Blackwell Science, Oxford, 2000, 1-11.
6. Muller-Feuga M. Handbook of Microalgal Culture. In: Richmond A. (Ed.). Biotechnology and Applied Phycology. Published by Blackwell Science, 2007, 352-363.
7. Divya S, Aanand S. Microalgae- A boon for larviculture of aquatic organisms. International J. Appl. Res. 2020; 6(5):138-143.

8. Otero A, García D, Fábregas J. Factors controlling eicosapentaenoic acid production in semicontinuous cultures of marine microalgae. J. Appl. Phycol. 2006; 9(5):23-36.
9. Zittelli CG, Lavista F, Bastianini A, Rodolfi L, Vincenzini M, Tredici MR *et al*. Production of eicosapentaenoic acid by *Nannochloropsis* sp. Culture in outdoor tubular photobioreactors. J. Biotechnol. 1999; 70:299-312.
10. Andersen R.A. Algal culturing techniques. Elsevier Academic press, London, 2005, 578.
11. Walne PR. The culture of marine bivalve larvae. In: Wilbur K. M, Yonge C N. (Eds). Academic Press, New York, 1974, 197-210.
12. Miquel P. de la culture artificielle de diatomees. C.R. Academic Science. 1892; 94:1-780.
13. Chu FE, Dupuy JL, Webb KL. Polysaccharide composition of five algal species used as food for larvae of American oyster. Aquaculture. 1942; 29:241-252.
14. Guillard RL. Culture of phytoplankton for feeding marine invertebrates. In: Smith W L and Chanley M H. (Eds.). Culture of marine invertebrate animals, Plenum, Newyork, 1975, 29-60.
15. Lee Y, Huishen K. Basic culturing techniques. In: Richmond A. (Ed.). Handbook of microalgal culture. Biotechnology and Applied Phycology, 2005.
16. Bradford MM. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principles of Protein- Dye Binding. Anal. Biochem. 1976; 72:248-254.
17. Barner H, Black stock J. Estimation of lipids in marine mammals and tissues. Detailed investigation of Sulphophosphovanillin method for total lipids. J. Exp. Biol. Ecol. 1973; 12:103-118.
18. Dubios M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric determination for sugars and related substances. Anal. Chem. 1956; 28:350-6.
19. Snedecor GW, Cochran WG. Statistical Methods. Sixth edition. Lowa state University Press, Ames, 1968, 593.
20. Whyte JNC, Bourne N, Hodgson CA. Influence of algal diets on biochemical composition and energy reserves in *Patinopecten yessoensis* (Jay) larvae. Aquaculture. 1989; 78:333-347.
21. Enright CT, Newkirk GF, Craigie JS, Castell JD. Growth of juvenile *Ostrea edulis* L. fed *Chaetoceros calcitrans* Schütt of varied chemical composition. J. Exp. Mar. Biol. Ecol. 1986; 96:15-26.
22. Knuckey RM, Brown MR, Barrett SM, Hallegraeff GM. Isolation of new nanoplanktonic diatom strains and their evaluation as diets for the juvenile Pacific oyster. Aquaculture. 2002; 211:253-274.
23. Kreeger DA, Langdon CJ. Effect of Dietary Protein Content on Growth of Juvenile Mussels, *Mytilus trossulus* (Gould 1850). Biological Bulletin. 1993; 185(1):123-139.
24. Brown MR, Dunstan GA, Jeffrey SW, Volkman JK, Barrett SM, Leroi JM *et al*. The influence of irradiance on the biochemical composition of the prymnesiophyte *Isochrysis* sp. (clone T-ISO). J. Phycol. 1993; 29:601-612.
25. Pérez-morales A, Martínez-lópez JM, Camalich-carpizo. Dry weight, carbon, c/n ratio, hydrogen, and chlorophyll variation during exponential growth of selected microalgae species used in aquaculture. CICIMAR oceánides. 2015; 30(1):33- 43.
26. Renaud SM, Parry DL. Effect of salinity on growth, gross chemical composition and fatty acid composition of three species of marine microalgae. J. Appl. Phycol. 1994; 6(3):347-386.
27. Wikfors GH, Joseph W, Twarog JT, Ravenna U. Influence of chemical composition of algal food sources on growth of juvenile Oysters, *Crassostea virginica*. Biology Bulletin. 1984; 167:251-263.