Plant Archives Vol. 19, Supplement 2, 2019 pp. 173–175 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

VARIABILITY IN BIOCHEMICAL COMPOSITION OF MICROALGAE *ISOCHRYSIS GALBANA* UNDER NITRATE DEPRIVATION

Neha mishra[1], Rashmi Srivastava[2,] Shraddha Tripathi[2] and Neetu Mishra[*2]

[1]Department of Home Science, University of Allahabad
[2]Centre of Food Technology, University of Allahabad and
[3]Sam Higginbottom University of Agriculture, Technology and Sciences
Corresponding author: Neetu Mishra
neetum1976@gmail.com

Abstract

The present work studied the effects of nitrogen limitation on the growth and biochemical composition (protein carbohydrate, lipid and pigments) of marine microalgae *Isochrysis galbana*. Stress of nitrogen limitations was given for 5 days and harvested freeze-dried algal biomass was analyzed for their growth and biochemical compositions. The growth of *Isochrysis galbana* cells were estimated by measuring optical density at 710nm and dry weight of filtered cells was incinerated at 550ºC in a muffle oven after every two days. The biochemical components protein, lipid, carbohydrate and pigment were measured by Lowry, Bligh and Dryer, Dubois and Strickland and Parsons Method respectively. The result of present study found that stress of nitrogen deprivation shifts the metabolic pathways towards accumulation carbohydrate content (15.8%DW-22.2%DW) and lipid content (23.3%DW-37.9%DW). These valuable compounds exhibit potential applications in human health and biofuel industry.

Keywords **:** *Isochrysis galbana,* light intensity, biochemical composition, pigments.

Introduction

Over the last decades microalgae have attracted the interest of many researches due to its structurally diverse bioactive compounds, more efficient photosynthetic machinery, ability to grow in non arable land, non-potable water and adverse environment condition with higher biomass productivity (Searchinger *et al.,* 2008; Rawat *et al.,* 2011). In marine world, microalgae are the primary producers of biomass and accumulates to higher organisms via food chain**.** They produce number of primary and secondary metabolites which exhibit wide variety of biological activities including antitumor, antibacterial, antioxidant properties, anti-inflammatory and hypochloesteromic (Ibañez and Cifuentes 2013).

Isochrysis galbana is a brown microalgae widely used as a feed in aquaculture since long time due to its high content of polyunsaturated fatty acid (PUFA) especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These are the essential fatty acids required for proper development of fetal, development of infant brain and eye, healthy heart, regulation of cellular inflammation and other metabolic activities. *Isochrysis galbana* is a golden brown marine microalgae belonging to the class haptophyta. They are unicellular organism with spherical/ellipsoidal motile cell of size 5-6µm long and 2-4µm wide **(**Hori and Green, 1991) with absence of a tough cell wall (Jeffrey *et al*., 1994; Liu and Lin, 2001). They are photoautotrophic organism, usually have two flagella, more or less equal and one smooth haptonema (hence the name of class) (Raposo *et al.,* 2014).

Isochrysis galbana **belonging to this systematic:**

Phylum	:	Eukaryota
Class	:	Haptophyceae
Order	:	Isochrysidales
Family	:	Isochrysidaceae
Genus	:	Isochrysis
Species	:	*Isochrysis galbana*

Recently, it have been found that *Isochrysis* species could be a valuable source of biologically active material includes indispensable amino acids, essential fatty acids, soluble and insoluble polysaccharides, photosynthetic pigments, carotenoids and phenolic compounds (Plaza *et al*., 2009; Guedes *et al.,* 2011) which have wide range of application in field of food, pharmaceutical and biofuel industry. The chemical composition of microalgae depends on growth phase, environmental and culture conditions.

Since, nitrogen is the key constituent of, photosynthetic pigments, enzymes and genetic materials and plays important role in photosynthesis, respiration and growth. The stressful condition such as limitation in nutrient concentration affects the growth and biochemical composition of microalgae. Therefore, aim of present study is to evaluate the nutrient stress of nitrogen limitation on growth and biochemical composition of *Isochrysis galbana.*

Materials and Methods

Algal culture

The starter culture of marine microalgae *Isochrysis galbana* was obtained from Center of Marine and Fishery Research Institute (CMFRI) Kochi, India.

Growth condition

The experiments were carried out in 1litre conical flask containing 200-300ml of sterilized guillard media (Guillard, 1975), having nitrate concentration of 8mM, 2mM and 0.5mM. The biomass was harvested after 5 days by centrifugation at 10,000 rpm for 10 min.

Measurement of Algal growth

The growth of *Isochrysis galbana* is calculated by measuring optical density (O.D.) and dry weight. Optical density was measured daily at 710nm by UV/visible spectrophotometer. 50ml of culture samples were centrifuged at 5000 rpm for 5 min followed by twice rinsing with distilled water. The pellets were dried 6hr in an oven at

110 ºC and difference between the initial and final weight were taken as the dry weight of algal biomass (mg/l).

Biochemical Analysis

The crude protein was determined by modified Lowery method (Lowry et al 1991). The absorbance of the sample was taken at 650nm and the concentration was determined using standard curve: Total Protein Content = wt. of protein (from curve) X 100/ dry cell mass (mg). The content of carbohydrate is estimated by the modified by the phenol-sulfuric acid method of Dubois (DuBois *et al*., 1956). The optical density of the sample was determined against the blank at 490 nm in a UV-visible spectrophotometer. Carbohydrate Content (%) = wt. of carbohydrate (fromGlucose standard curve) X 100/ dry cell mass (g) and total lipid contents were analyzed gravimetrically after extraction with chloroform–methanol (2:1) modified by Bligh and Dyer (Bligh and Dyer, 1959). Pigments were extracted in acetone (90%) at 4 °C overnight and measured by spectrophotometric methods (Jeffrey and Humphrey, 1975).

Statistical Analysis

All experiments were done with three replicates and data represent the means ± SD. They were analyzed by one-way ANOVA and significant differences between treatments were tested using Duncan's multiple range test (DMRT). *P*-values <0.05 were considered significant. Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) 10.0.

Results and Discussion

Since nitrogen is a major component in many biological macromolecules like protein, chlorophyll, photosystem, enzymatic and genetic material (Hu *et al*., 2008). Nitrogen deprivation is a major limiting factor affecting the growth and biomass production.

Growth

The growth of *I. galbana* cells treated with different concentration of nitrate (80mg/L, 40mg/L, 20mg/L) were evaluated by measuring the absorbance at 710 nm. The growth curve under different nitrogen concentration elicited that initially up to 2 days there is no significant difference in growth which later on significantly (>0.05) lowered with nitrogen depletion in culture medium. In support, previous studies elicited that nitrogen is crucial nutrient for growth and its deprivation in medium limits the rate of cell division.

The dry weight of biomass after 5 days also depicts its growth as illustrated in figure 2. Biomass content significantly decreased with deprivation of nitrate concentration from 0.78 to 0.43 g L^{-1}. Result reveals that low levels of nitrogen in media reduced the productivity of microalgae as they may have an adverse effect on the, cell division, protein synthesis and metabolism.

Biochemical composition and Pigments

In figure 3, the variations of the determined biochemical compositions during the cultivation of *I. galbana* under different nitrate concentration were illustrated. With deprivation of nitrogen in medium, carbohydrate and lipid accumulates from 15.8+1.62% to 22.23+2.0% and 23.33+2.6% to 37.93+3.4% respectively. The result of present study was consistent of previous studies and reported nitrogen deprivation on culture medium leads to accumulation of lipid in many microalgae species such as *Neochloris oleoabundans, Nannochlorpsis sp., Chlorella muelleri and Scenedesmus sp*. (Blinová *et al*. 2015; Gao et al 2013; Ho 2014). This is reasoned that nitrate limitation affects the metabolic activity of cell and diverts the cell from protein synthesis to carbohydrate and lipid accumulation.

Figure 4 illustrated that limitation of nitrogen significantly decreased all photosynthetic pigments viz. Chl *a* (3.55±0.15 to1.56±0.17), Chl *c* (2.23±0.18 to 0.48±0.15) and total carotenoids (3.35±0.18 to 2.19±0.19) respectively. The result of present study was supported by previous studies and reasoned that nitrogen is a key element for the synthesis of protein and its limitation lowers protein synthesis rate (Wang *et al*., 2013). Since proteins involves in photosystem reaction center and photosynthesis electron transport system therefore nitrogen limitation impairs the rate of photosynthesis too.

Conclusion

The result of our study indicated that nitrogen deprivation in culture medium significantly affect the growth and biochemical composition of *Isochrysis galbana*. The stress of nitrogen deficiency leads to the accumulation of lipid and carbohydrate on the expense of protein and growth. Therefore, nitrate deprivation in medium is reliable approach to achieve high lipid content in *Isochrysis galbana*, although further studies is recommended to analysis the effect of nitrogen depletion on fatty acid composition.

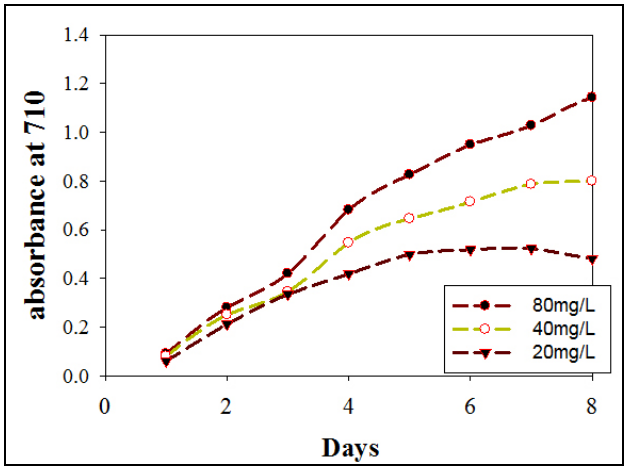

Fig. 1: Growth curve of *Isochrysis galbana* in different nitrogen concentration

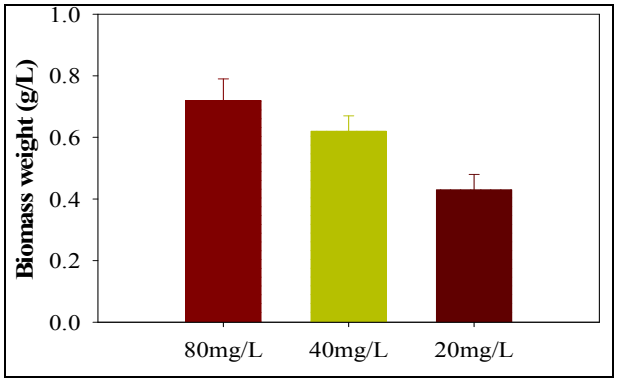

Fig. 2 : Dry weight of *Isochrysis galbana* in different nitrogen concentration

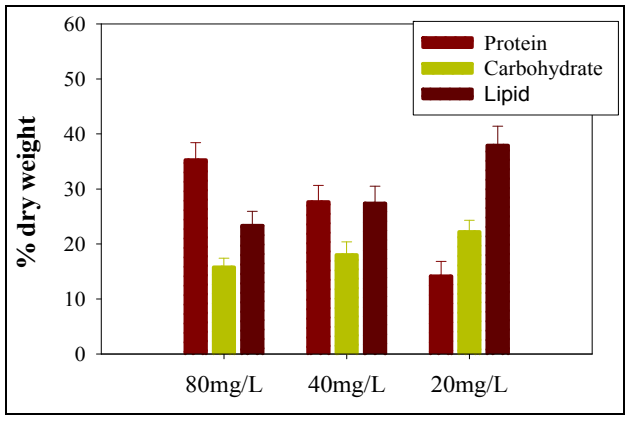

Fig. 3: Biochemical composition of *Isochrysis galbana* in different nitrogen concentration

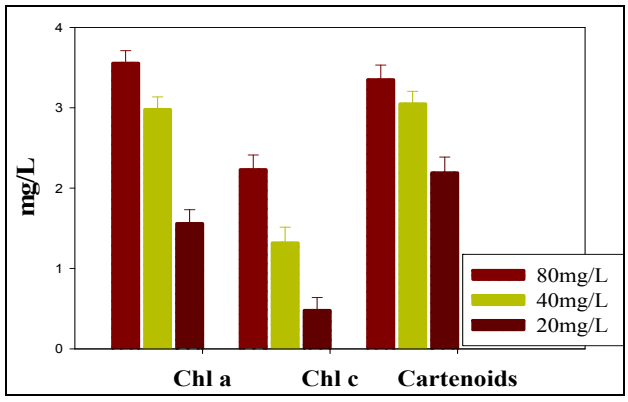

Fig. 4: Pigments of *Isochrysis galbana* in different nitrogen concentration

References

Searchinger, T.; Heimlich, R.; Houghton, R.A.; Dong, F.; Elobeid, A. and Fabiosa, J. (2008). Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. Sci., 319(5867): 1238–40.

Rawat, I.; Kumar, R.R.; Mutanda, T. and Bux, F. (2011). Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl energy. 88(10): 3411-24.

Ibañez, E. and Cifuentes, A. (2017). Benefits of using algae as natural sources of functional ingredients. J Sci Food Agric, 93(4): 03-9.

Hori, T. and Green, J.C. (1991). The ultrastructure of the flagellar root system of Isochrysis galbana (Prymnesiophyta). J. Mar. Biol. UK 71: 137-152.

Jeffrey, S.W.; Brown, M.R. and Volkman, J.K. (1994). Haptophyte as feedstocks in mariculture. *In* J.C. Green and B.S.C. Leadbeater (eds.), The Haptophyte Algae, Clarendon Press, Oxford, 287-302.

Liu, C.P. and Lin, L.P. (2001). Ultrastructural study and lipid formation of *Isochrysis sp.* Botanical Bulletin of Academia Sinica, 42: 207-214.

Raposo, M.F.; de Morais, R.M. and Bernardo de Morais, A.M. (2013). Bioactivity and applications of sulphated polysaccharides from marine microalgae. Mar Drugs. 11(1): 233-52.

Plaza, M.; Herrero, M.; Cifuentes, A. and Iba´n˜ez, E. (2009). Innovative natural functional ingredients from microalgae. J of Agr. Food. Chem., 57: 7159-7170.

Guedes A., Amaro, H.M. and Malcata, F.X. (2011). Microalgae as sources of high added-value compounds-a brief review of recent work. Biotech Progress. 27(3): 597-613.

Guillard, R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates, edited by Smith WL, Chanie M.H. (Plenum Press, New York), 29-60.

Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin Phenol reagent. J Biol. Chem, 193: 265-275.

DuBois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.T. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical chemistry. 28(3): 350-6.

Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. Canadian Biochem. Physiol. 37:911-917.

Jeffrey, S.T. and Humphrey, G.F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c 1 and c 2 in higher plants, algae and natural phytoplankton. Biochemie und Physiologie der Pflanzen. 167(2): 191-4.

Hu, Q.; Sommerfeld, M.; Jarvis, E.; Ghirardi, M.; Posewitz, M.; Seibert, M. and Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J., 54: 621–639.

Blinová, L.; Bartošová, A. and Gerulová, K. (2015). Cultivation of microalgae (Chlorella vulgaris) for biodiesel production. Research Papers Faculty of Materials Science and Technology Slovak University of Technology 23(36): 87-95.

Gao, Y.; Yang, M. and Wang, C. (2013). Nutrient deprivation enhances lipid content in marine microalgae. Bioresour Technol, 147: 484-491.

Ho, S.H.; Nakanishi, A.; Ye, X.; Chang, J.S.; Hara, K.; Hasunuma, T. and Kondo, A. (2014). Optimizing biodiesel production in marine *Chlamydomonas sp.* JSC4 through metabolic profiling and an innovative salinity-gradient strategy. Biotechnology for biofuels. 7(1): 97.

Wang, J.; Sommerfeld, M.R.; Lu, C. and Hu, Q. (2013). Combined effect of initial biomass density and nitrogen concentration on growth and astaxanthin production of *Haematococcus pluvialis* (Chlorophyta) in outdoor cultivation. Algae. 28: 193–202.