Vol 5 | Issue 1 | 2014 | 30-35.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

EFFECT OF NUTRIENTS ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF THE MARINE DIATOM, CHAETOCEROS SIMPLEX (OSTENFELD, 1901)

Annadurai Hemalatha, Panneerselvam Karthikeyan, Kalyanasundram Girija, Chinnadurai Saranya, Perumal Anantharaman*, Pitchai Sampathkumar

CAS in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai – 608 502, Tamilnadu, India.

ABSTRACT

The growth and biochemical composition of microalgae are considered to its potential utilization for various purposes. Culture conditions could amend the growth and biochemical composition of microalgae. The marine diatom *Chaetoceros simplex* has been examined for its growth and biochemical composition in different nitrate, phosphate and silicate concentrations. Growth, protein and carbohydrate content of *C. simplex* were significantly changed among the concentrations of macronutrients investigated. The maximum cell density, 18.23×10^5 cells ml^{-1} was reached in 1764 µM nitrate, 18.21×10^5 cells ml^{-1} was reached in 72.4 µM phosphate and 19.56×10^5 cells ml^{-1} was reached in 212 µM silicate concentration. The growth rate showed increasing trends with increase up to the concentration of silicate 265 µM, nitrate 2646 µM and phosphate 90.5 µM. The carbohydrate and chlorophyll 'a' content showed increasing trends with increase in concentration up to 1764 µM nitrate, 72.4 µM phosphate and 212 µM silicate. The protein showed increasing trends with increase in concentration of nitrate phosphate and silicate up to 2205 µM nitrate, 90.5 µM phosphate and 265 µM silicate. Based on the results of the present study, *Chaetoceros simplex* culture could be used as larval feed or other purposes cultured using the above nutrient concentrations.

Keywords: Growth rate, Biochemical composition, *Chaetoceros simplex*, Phosphate, Nitrate, Silicate.

INTRODUCTION

Microalgae are simple photosynthetic organisms which are widespread in nature, playing key roles as primary producers in marine, freshwater and sub-aerial terrestrial systems [1]. Beside their remarkable environmental importance, some economic uses of microalgae have been recognized for decades [2]. Microalgae contribute a wide range of commercial products from consumables such as feedstocks, essential oils and drugs, to energy resources and means for carbon capture through biofuel production [3-5]. Microalgae growth and composition may be influenced by nutrients like nitrate, phosphate and silicate [6,7]. The influence of nitrate and phosphate concentrations on growth, extracellular polysaccharide production, fatty acid profile of marine diatoms has reported [8-10]. Modifications in culture medium such as nitrogen, phosphorus and silicate concentrations affect the growth rate of microalgae, cellular composition, fatty acid profile of the lipid fraction, as well as the final yield of the *Isochrysis galbana* [11]. There are various culture media to provide the nutrients for the growth of diatom. In the production of diatom with certain desired characteristics, the composition of the culture medium is a fundamental factor. The relationship between the nutrients used and the composition of the microalgal cells was examined by Becker, 1994 [12]. The aquaculture industry is rapidly growing and the larval rearing technology has a significant role. The early larval stages of crustacean and fishes require nutritionally balanced diet. In this view, the present study was carried out to develop optimum culture conditions of major nutrient concentrations in the media for *C. simplex*.

MATERIALS AND METHODS

Microalgal culture *Cheatoceros simplex* strain was obtained from Rajiv Gandhi Centre for Aquaculture (RGCA), Sirkazhi, Tamilnadu, India. The unialgal culture was maintained under the laboratory conditions with F/2 media [13].

Experimental Design

The experiments were conducted in 250 ml conical flasks with 100 ml algal cultures. Totally three triplicate experiments at five different concentrations for

Corresponding Author: **Perumal Anantharaman Email:-** drparaman64@gmail.com

each nutrient were conducted separately for 16 days under temperature 25 ± 1ºC, 68 µmol photon m^{-2} s^{-1} light intensity, pH 8.0-8.4 and Salinity 30 psu and at five different concentrations of nitrate, (441, 882 (F/2 media), 1323, 1764, 2205 and 2646 µM), phosphate viz. (18.1, 36.2 (F/2 media), 54.3, 72.4, 90.5 and 108.6 µM) and silicate (53, 106 (F/2 media), 159, 212, 265 and 318 µM). Each experiment was conducted in triplicate. Cell were counted at every 24 h interval with haemocytometer (0.1 mm depth) under a light microscope and expressed as 10^5 cells/ml. The growth rate (µ) was calculated by the formula of OECD, 2002 [14].

$$\mu = \text{In}\ (N_1 - N_0) / t_1 - t_0$$

Where N_0 and N_1 is cell density values at times t_0 and t_1.

Chlorophyll 'a' concentration was determined by the modified method of Strickland & Parsons [15]. Briefly, 5 ml of 90% acetone was added to 2 ml of algal culture and vortexed for 1 min and kept at 4ºC under dark for 24 h. Then the samples were centrifuged at 5000 rpm for 10 min. Supernatant was read at 630, 645 and 660 nm in UV-Vis. Spectrophotometer (Perkin-Elmer Lamda 25). Acetone used as blank. Ten-milliliter aliquots of algal cultures were collected by centrifugation at 5000 × g for 10 min. Protein and carbohydrate contents were estimated at the stationary phase of culture (those treatments reached stationary phase by cell density). Protein was determined by the Lowry's method [16] using Bovine Serum Albumin (BSA) as a standard. Carbohydrate was estimated in pellets by the phenol-sulphuric acid method [17], using glucose as standard.

Statistical Analysis

Data obtained in the present study was statistically analyzed by one way analysis of variance using SPSS 16.0 software.

RESULTS

Cell Density

Nitrate on cell density: The maximum cell density, 18.23×10^5 cells ml^{-1} was reached in 1764 µM nitrate concentration followed by 17.89×10^5 cells ml^{-1} in 1323 µM at 11th day of culture. The higher concentrations (2205 µM and 2646 µM) showed fast growth during the early period of culture and later there observed a sudden decline in the growth from the 10th day of the culture period. In general, the cell density showed an increasing trend with an increase in concentration up to 1764 µM and there after it showed decreasing trend. The cell density, 17.56×10^5 cells ml^{-1} was observed in 882 µM concentration at 11 days aged culture (Fig. 1). The statistical analysis with one way ANOVA revealed that effect of nitrate concentration on cell density of C. simplex was significantly differed at P<0.05 (Table 1).

Phosphate on cell density: The maximum cell density, (18.21×10^5 cells ml^{-1}) was reached in 72.4 µM phosphate concentration followed by 17.89×10^5 cells ml^{-1} in 54.3 µM on the 11th day of culture. Higher concentration of phosphate showed less growth compared to other concentrations (Fig. 2). One way ANOVA inferred that effect of phosphate concentration on cell density of *C. simplex* was significantly differed at P<0.05 (Table 1).

Silicate on cell density: The maximum cell density, 19.56×10^5 cells ml^{-1} was reached in 212 µM silicate concentration followed by 18.95×10^5 cells ml^{-1} in 159 µM in 10 days aged culture. Lower concentration (50% of F/2 concentration) shown minimum cell count and actual F/2 media concentration (106 µM) shown medium cell density than other high concentrations of silicate (Fig. 3). One way ANOVA revealed that effect of silicate concentration on cell density of *C. simplex* was significantly differed at P<0.05 (Table 1).

Specific Growth Rate: The growth rate showed increasing trends with increase in concentration of nitrate and silicate whereas phosphate showed an increasing trend up to 90.5 µM and there after it decreased. The 265 µM concentration silicate recorded maximum (2.02 ± 0.07 µ. Day^{-1}) followed by 2646 µM nitrate (1.93 ± 0.07 µ. Day^{1}) and 90.5 µM phosphate (1.90±0.08 µ. Day^{-1}). Minimum growth rate (1.26 ± 0.07 µ. Day^{-1}) was observed at the lowest concentration of silicate (53 µM). The growth rate was higher in the higher concentrations when compared to the actual F/2 media composition (Fig. 4).

Chlorophyll 'a'

The Chlorophyll 'a' content showed increasing trends with increase in concentration up to 1764 µM nitrate, 72.4 µM phosphate and 212 µM silicate and there after it decreased. The 212 µM concentration silicate recorded maximum (2.38 ± 0.16 pg. $cell^{-1}$) followed by 1764 µM nitrate (2.36 ± 0.13 pg. cell $^{-1}$) and 72.4 µM phosphate (1.35 ± 0.03 pg. $cell^{-1}$). The highest concentration of silicate (265 and 318 µM) recorded minimum chlorophyll 'a' which was much lower than that of the chlorophyll 'a' content recorded at the lowest (53 µM) and actual (106 µM) concentration of silicate in the media (Fig. 5).

Protein

The protein showed increasing trends with increase in concentration of nitrate, phosphate and silicate up to 2205 µM nitrate, 90.5 µM phosphate and 265 µM silicate and there after it decreased. The 2205 µM concentration nitrate recorded maximum (4.72 ± 0.53 pg. $cell^{-1}$) followed by 90.5 µM phosphate (3.83 ± 0.35 pg. $cell^{-1}$) and 265 µM silicate (3.66 ± 0.22 pg. $cell^{-1}$). Minimum protein was observed at the lowest concentration of all the nutrients. The protein content was significantly higher (P≥0. 05) in the higher concentrations when compared to the actual F/2 media (Fig. 6).

Carbohydrate

The carbohydrate showed increasing trends with increase in concentration of nitrate, phosphate and silicate up to 1764 µM nitrate, 72.4 µM phosphate and 212 µM silicate and there after it decreased. The 1764 µM concentration nitrate recorded maximum (0.85 ± 0.10 pg. $cell^{-1}$) followed by 72.4 µM phosphate

(0.75 ± 0.04 pg. cell^{-1}) and 212 µM silicate (0.55 ± 0.05 pg. cell^{-1}). Minimum protein was observed at the lowest concentration of nitrate (441 µM) and silicate (53 µM) nutrients and whereas in phosphate the highest concentration (108.6 µM) showed lowest carbohydrate (Fig. 7).

Table 1. ANOVA results (between the concentrations) for cell densities with different nutrients

Nutrient	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Nitrate	Between Groups	51.31	5	10.26	0.22	0.95	2.33
	Within Groups	3617.80	78	46.38			
Phosphate	Between Groups	32.04	5	6.4	0.13	0.98	2.32
	Within Groups	4083.19	85	48.03			
Silicate	Between Groups	77.44	5	15.48	0.3	0.91	2.32
	Within Groups	4386.03	85	51.6			

Fig 1. Effect of Nitrate concentration on cell density (growth) (Mean ± SD) of *C. simplex*

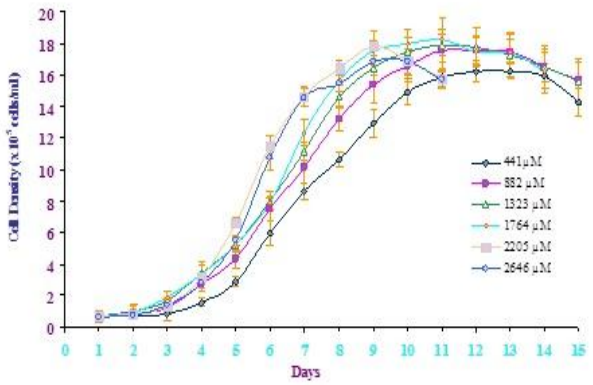


Fig 2. Effect of Phosphate concentration on cell density (growth) (Mean ± SD) of *C. simplex*

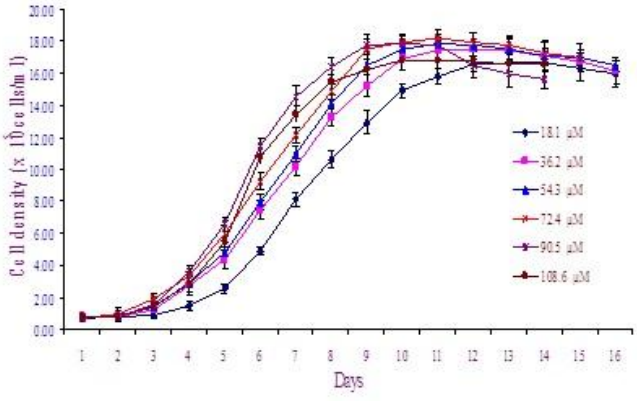


Fig 3. Effect of silicate concentration on cell density (Mean ± SD) of *C. simplex*

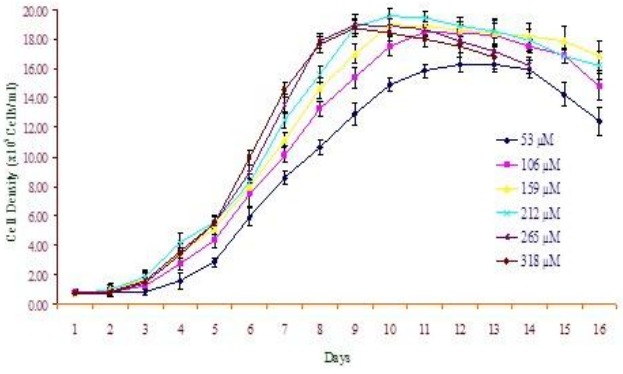


Fig 4. Effect of different concentrations of nutrients on growth rate of *C. simplex*

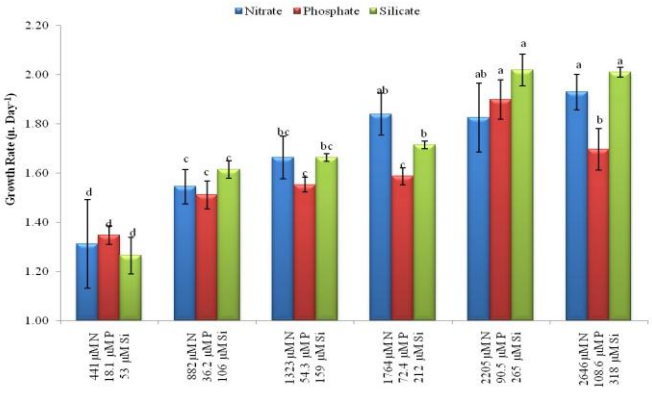


Fig 5. Effect of different concentrations of nutrients on Chlorophyll 'a' of *C. simplex*

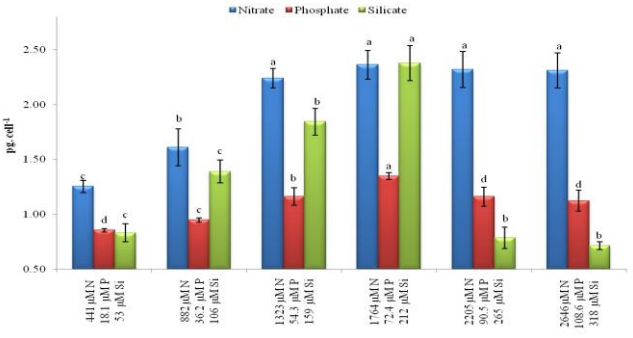


Fig 6. Effect of different concentrations of nutrients on protein content of *C. simplex*

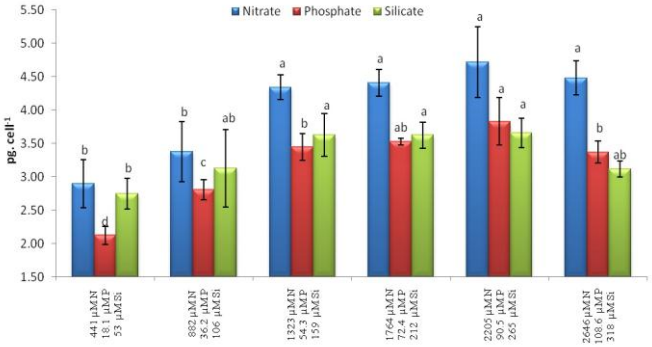
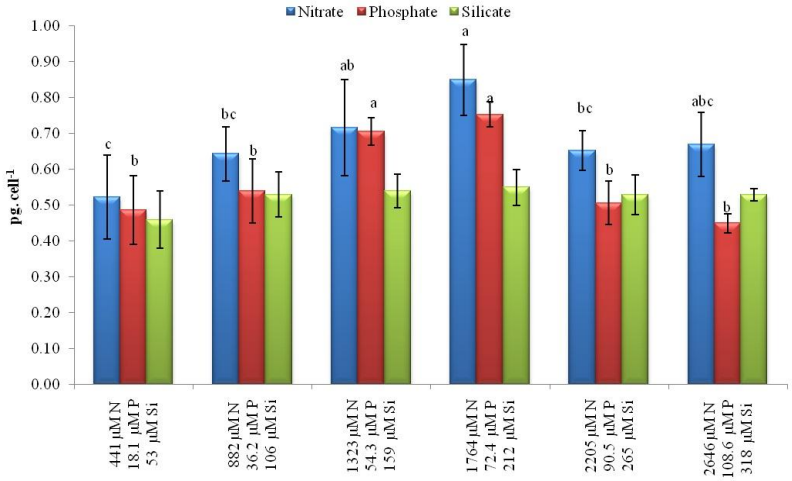


Fig 7. Effect of different concentrations of nutrients on carbohydrate content of *C.simplex*.



DISCUSSION

In the present study, increased cell densities 18.23×10^5 cells ml^{-1}, 18.21×10^5 cells ml^{-1} and 19.56×10^5 cells ml^{-1} were achieved at 1764 µM nitrate, 72.4 µM phosphate and 212 µM silicate concentration in the media respectively. Cucchiari et al. [18] reported that the cultures of *Fibrocapsa japonica* reached cell densities that were roughly 65% lower when grown in 200 mM nitrate and 7.3 mM phosphate, comparable to the densities reached in F/2 medium, which has a macronutrient concentration nearly five times higher.

Padhi et al. [19] obtained maximum growth of *Chaetoceros curvisetus* at 3 mM $NaNO_3$ and declined slightly at 6 mM $NaNO_3$. In the present study also the higher concentrations of nitrate and phosphate showed decreased growth when compared to the lower concentrations. Fabregas et al. [20] reported that biomass yield of *Tetraselmis suecica* did not increase under the high NaNO3 concentration but improved with the addition of CO_2. This shows the possibility of excess $NaNO_3$ may not be utilized due to limitation of other nutrients such as carbon and phosphate.

Chlorophyll '*a*' content showed a direct relationship with the nutrient concentration. For all experiments, the chlorophyll 'a' per cell varied as a function treatment time. Eriksen and Iversen [21] observed the response in nitrogen-starved cells of *Rhodomonas* sp. when fresh medium was added. They found that the content of chlorophyll '*a*' increased from 0.5 to 1.5 pg. $cell^{-1}$ during the first 23 h of incubation, but after the nitrate was exhausted, the chlorophyll decreased to 0.3 pg. $cell^{-1}$. In the present study, chlorophyll '*a*' content was recorded the maximum of 2.4 pg. $cell^{-1}$, 2.3 pg. $cell^{-1}$ and 1.34 pg. $cell^{-1}$ at 1764 µM nitrate, 72.4 µM phosphate and 212 µM silicate concentration in the media respectively.

Muggli & Harrison [22] noted much lower chlorophyll '*a'* in *S. costatum* (0.049 pg chlorophyll '*a'* $cell^{-1}$) at much lower nutrient concentrations (10 µM $NO3^-$, 1 µM PO_4^{-3} and 6 nM Fe^{+2}). De la Curz et al. [23] also reported that chlorophyll '*a*' content was directly related to cellular density and indirectly to irradiance level and concentration of nutrients. They recorded almost constant chlorophyll '*a*' content (1.35 ± 0.15 pg. $cell^{-1}$) in *Rhodomonas* sp. through the experiment. The present study has the similar results with Eker-Develi et al. [24] and Young & Beardall [25] where the chlorophyll '*a*' content per cell changed during the growth phase and it was the highest in early exponential growth under both high-nutrient and nutrient-limited conditions. Eker-Develi et al. [24] also recorded average Chlorophyll '*a*' of 0.8 pg. $cell^{-1}$ in *Prorocentrum micans* and 0.4 pg. $cell^{-1}$ in *Skeletonema Costatum* in their experiment with the effect of nutrients.

Padhi et al. [19] reported that the biochemical composition of *Chaetoceros curvisetus* changed in response to varied nitrate and phosphate concentration. The protein contents of 3.8 pg. $cell^{1}$, 9.0 pg. $cell^{-1}$, 23 pg. $cell^{-1}$, 13.1 pg. $cell^{-1}$, 9.7 pg. $cell^{-1}$, 83.4 pg. $cell^{-1}$ of the marine diatoms, *Chaetoceros calcitrans, C. gracilis, Nitzchia closterium, Phaeodactylum tricornutum, Skeletonema costatum, Thalassiosira pseudonana* and *Tetraselmis chui* respectively were documented by Brown [26]. The protein content of *C. simplex* in the present investigation was ranged between 56.4 and 105.3pg.$cell^{-1}$ in the lower concentration of silicate and higher concentration of the nitrate respectively and it showed higher values than Brown [26] this may be due to the increased concentrations of nutrients in the culture media.

Carbohydrates are used as chemical energy reserves in diatoms [27, 28]. An increase in the energy reserves of microalgae is usually associated with senescence and spore formation [29, 30]. The carbohydrate content was decreasing with decreasing concentrations of nutrients in the present study and the maximum of 0.77 pg. $cell^{-1}$ was comparable to the reported value of the carbohydrate content of 26.84 ± 0.80 ng/10^4 cells in *Skeletonema costatum* by Yang et al. [31].

The results of the present study reveal that the growth and biochemical composition of *C. simplex* was changed significantly than f/2 and altered nutrient concentrations in f/2 media was produced nutritionally rich biomass for larval diet or other purposes.

ACKNOWLEDGEMENT

The authors are thankful to of CAS in Marine Biology and higher authorities of Annamalai University, Parangipettai for the facilities provided to carry out the research work. Authors are thankful to Centre of Marine Living Resources and Ecology, MoES, Govt. of India for providing financial support throughout the study period.

REFERENCES

1. Graham LE, Graham JE, Wilcox LW, Algae, 2nd ed, Benjamin Cummings (Pearson), San Francisco, 2009, 720.
2. Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, 2006, 101(2), 87–96.
3. Nowak R. Microalgae hold the key to the biofuel conundrum, New Science, 197, 2008.
4. Deng X, Li Y, Fei X. Microalgae: a promising feedstock for biodiesel. *Afrrican Journal of Microbiology research*, 3, 2009, 1008–1014.
5. Venegas-Caleron M, Sayanova O, Napier JA. An alternative to fish oils: metabolic engineering of oil-seed crops to produce omega-3 long chain polyunsaturated fatty acids. *Progress in Lipid Research*, 49, 2010, 108–119.
6. Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likensn GE. Controlling eutrophication: nitrogen and phosphorus. *Science*, 323, 2009, 1014–1015
7. Paerl HW. Controlling eutrophication along the freshwater-marine continuum: dual nutrient (N and P) reductions are essential. *Estuaries and Coasts*, 32, 2009, 593-601.
8. Khoi CM, Guong VT, Merckx R. Growth of the diatom *Chaetoceros calcitrans* in sediment extracts from Artemia franciscana ponds at different concentrations of nitrogen and phosphorus. *Aquaculture*, 259, 2006, 354–364.
9. Liang Y, Beardall J, Heraud P. Effects of nitrogen source and UV radiation on the growth, chlorophyll fluorescence and fatty acid composition of *Phaeodactylum tricornutum* and *Chaetoceros muelleri* (Bacillariophyceae). *Journal of Photochemistry and Photobiology B: Biology*, 82, 2006, 161–172.
10. Staats N, Stal LJ, Mur LR. Exopolysaccharide production by the epipelic diatom *Cylindrotheca closterium*: effects of nutrient conditions, *Journal of Experimental Marine Biology and Ecology*, 249, 2000, 13 –27.
11. Sanchez S, Martınez ME, Espinola F. Biomass production and biochemical variability of the marine microalga *Isochrysis galbana* in relation to culture medium, *Journal of Biochemical Engineering*, 6, 2000, 13–18.
12. Becker EW. Chemical composition, Microalgae: Biotechnology and Microbiology. Cambridge University Press, New York, 1994, 177–195.
13. Guillard RRL. Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (Ed.), Culture of marine invertebrate animals, Plenum Press, New York, 1975, 26-60.
14. OECD, Algal growth inhibition tests, proposal for updating guideline, in: Guidelines for testing of chemicals, 2002, 201.
15. Strickland JDH, Parsons TR. A pratical handbook of seawater analysis, Bull Fish Res Board, Canada, 1972, 167-311.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RL. Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry*, 193, 1951, 265-275.
17. Dubois M, Gillies KA, Hamilton JK, Rebers PA, Smith F. Calorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28, 1956, 350–356.
18. Cucchiari E, Franca G, Antonella P, Cecilia T, Rossella P. Effect of salinity, temperature, organic and inorganic nutrients on growth of cultured *Fibrocapsa japonica* (Raphidophyceae) from the northern Adriatic Sea. *Harmful Algae*, 7, 2008, 405–414.
19. Padhi SB, Behera G, Swain PK, Behura SK, Dash PK. Effect of environmental factors on growth and biochemical composition of *Chetoceros curvisetus* for use in mariculture. *Indian Hydrobiology*,12(1), 2009, 58-64.
20. Fabregas J, Abaide J, Herres C, Cabezas BV, Veiga M. Growth of the marine alga Tetraselmis Suecica in batch cultures with different salinity and nutrients concentrations. *Aquaculture*, 42, 1984, 207–215.
21. Eriksen NT, Iversen LJJ. Photosynthetic pigments as nitrogen stores in the cryptophyte alga *Rhodomonas* sp, *Journal of Marine Biotechnology*, 3, 1995, 193–195.
22. Muggli DL, Harrison PJ., Effects of nitrogen source on the physiology and metal nutrition of *Emiliania huxleyi* grown under different iron and light conditions, *Marine Ecology Progress Series*, 130, 1996, 255–267.
23. De la Cruz FL, Valenzuela-Espinoza E, Millan-Nunez R, Trees CC, Santamaria-del-Angel E, Nunez-Cebrero F. Nutrient uptake, chlorophyll a and carbon fixation by *Rhodomonas* sp. (Cryptophyceae) cultured at different irradiance and nutrient concentrations. *Aquacultural Engineering*, 35, 2006, 51–60.
24. Elif Eker-Develi, Ahmet EK, Suleyman T, Effect of nutrients on culture dynamics of marine phytoplankton, *Aquatic Sciences*, 68, 2006, 28–39.
25. Young E, Beardall J. Rapid ammonium- and nitrate-induced perturbations to chl '*a'* fluorescence in nitrogen-stressed *Dunaliella tertiolecta* (Chlorophyta). *Journal of Phycology*, 39, 2003, 332–342.
26. Brown MR. The amino acid and sugar composition of 16 species of microalgae used in mariculture. *Aquaculture*, 145, 1991, 79-99.
27. Darley WM. Biochemical composition, in: D. Werner (Ed.) The Biology of Diatoms, Blackwell, Oxford, 1977, 198–223.
28. Werner D, Silicate metabolism, in: D. Wener (Ed.) The Biology of Diatoms, Blackwell, Oxford, 1977, 110-149.
29. MacLean RJ, Ultrastructure of *Spongiochloris typical* during senescence, *Journal of Phycology*, 4, 1968, 277–283.

30. French FW, Hargraves PE. Physiological characteristics of plankton diatom resting spores. *Marine Biology Letters*, 1, 1980, 185–195.
31. Yang S, Rudolf S, Wu S, Richard S, Kong YC. Physiological and cytological responses of the marine Diatom *skeletonema costatum* to 2,4-dichlorophenol. *Aquatic Toxicology*, 60, 2002, 33–41.