Environmental Health Engineering and Management Journal 2015, 2(3), 141–147

http ehemj.com

Environmental Health Engineering and Management Journal

Original Article

Environmental Health Engineering and Management

Open Access Publish Free

Optimization of culture media for lipid production by *Nannochloropsis oculata* for Biodiesel production

Mohammad Malakootian[1*], Behnam Hatami[2], Shidwash Dowlatshahi[3], Ahmad Rajabizadeh[3]

[1]Professor, Environmental Health Engineering Research Center, Department of Environmental Health, Kerman University of Medical Sciences, Kerman, Iran

[2]MSc, Department of Environmental Health, Kerman University of Medical Sciences, Kerman, Iran

[3]Instructure, Environmental Health Engineering Research Center, Department of Environmental Health, Kerman University of Medical Sciences, Kerman, Iran

Abstract

Background: This study quantified the effect of four popular culture media in a biodiesel production study on the qualitative and quantitative lipid content, dry biomass, and lipid productivity of *Nannochloropsis oculata*.

Methods: Culture of microalgae was done separately in Walne, F/2, Sato, and TMRL media. In the logarithmic and stationary growth phases, biomass production and lipid accumulation of microalgae were measured and the constituents were identified by gas chromatography.

Results: *N. oculata* exhibited the highest rate of cell growth and biomass productivity of 0.2616 day^{-1} and 2.652 gl^{-1} in the Walne medium. The highest level of biomass conversion into lipids in TMRL medium revealed a cell dry weight of 37.22%. Walne medium proved to have the most efficient lipid productivity which was 0.1057 gl^{-1} day^{-1}. The highest amount of triacylglycerol (TAG) was obtained in Sato medium in the stationary growth phase and was 75.25% of the fatty acids.

Conclusion: The present study provides a practical benchmark, which allows the introduction of Walne as a suitable culture medium for *N. oculata* in biodiesel studies.

Keywords: *Nannochloropsis oculata*, Culture, Medium, Biofuel, Biodiesel, Lipid productivity

Citation: Malakootian M, Hatami B, Dowlatshahi S, Rajabizadeh A. Optimization of culture media for lipid production by Nannochloropsis oculata for Biodiesel production. Environmental Health Engineering and Management Journal 2015; 2(3): 141–147.

Article History:
Received: 14 June 2015
Accepted: 7 September 2015
ePublished: 12 September 2015

***Correspondence to:**
Mohammad Malakootian
Email: m.malakotian@gmail.com

Introduction

Energy crisis is one of the most society' s daunting challenge, as a result of the quick development of human activities and rapid depletion of fossil fuels (1,2). The use of fossil fuels as an energy source is intimately linked with the ever-increasing emission of carbon dioxide (3), climatic changes and global warming effects (4). In the past decade, attempts have been made to control these effects by reducing the level of CO_2 in the atmosphere, implementing the microalgae and producing renewable energy (5-8). Biodiesel as a sustainable and environmentally friendly alternative is a renewable, non-toxic, biodegradable and CO_2 neutral energy source (1,9-11). Microalgae are photosynthetic organisms and are a promising source for biodiesel production (12,13). Reacting microalgal oil in the form of triacylglycerol (TAG) with simple alcohol (known as "transesterification"), results in the formation of a chemical composition known as alkyl ester or biodiesel (14-16). In addition to fuel production, microalgae are commercially important for aquaculture and the food industry because they possess valuable products such as fatty acids, steroids, carotenoids and polysaccharides (17). *Nannochloropsis oculata* is a marine unicellular microalgae belonging to the Eustigmatophyceae class (18-21), alongside their ability to synthesize polyunsaturated fatty acids (PUFAs) and carotenoids for human and marine aquaculture consumption, they can also accumulate large amounts of neutral lipids in the form of TAG (13,22-25). In order to exploit these microalgae for biodiesel production and reduce the total cost of it, it is necessary to optimize biomass and lipid accumulation by obtaining a better understanding of the essential parameters contributing to the microalgae culture media (1,26,27). The composition of the culture media affects the specific growth rate, the maximum level of biomass production and change the biochemical composition of the biomass and lipids (5). For example, nutrient stress conditions is one of the most efficient ways of increasing lipid accumulation in cells and storage in the form of TAG with change in fatty acid (FA) composition in single cell microalgae (1,11). Studies have

shown that the quality and quantity of microalgae lipid content can be changed as a result of changes in growth conditions (temperature, light intensity) or medium composition (nitrogen, phosphate and iron concentration) (15,28,29). Furthermore, in order to achieve mass cultivation of microalgal biomass in an industrial scale, optimization of the appropriate culture medium is one of the most important factors (7).

This study quantified the effect of 4 popular culture media in a biodiesel production study on the qualitative and quantitative lipid content, dry biomass, and lipid productivity of *N. oculata*.

Methods

Microalgae

N. oculata was obtained from the research institute for Aquaculture in the south of the country in the form of stock culture with high density (25×10^6 cell/ml). This microalga is a eukaryotic photosynthetic microorganism and given its simple structure, it has a fast growth rate (28). According to the study of Chen et al (13), which determined the effects of cell density on microalgae growth and lipid composition, this study used microalgae with high density.

Culture conditions of *N. oculata*

In order to determine the most appropriate medium, microalgae was cultured separately in four media namely Walne, F/2, Sato, and TMRL. Table 1 shows the composition of each medium including every elemental nutrient and concentration. In all the media, the Gillard vitamins were used. Growth experiments were repeated three times using a 2L-Erlenmeyer flask and in refrigerated incubators equipped with temperature and light intensity. The optimum value of temperature, 20°C, was chosen on the basis of data reported in the literature (28) and according to the study of Banerjee et al (30) and Sen et al (31), the light intensity would be 70 μE m^{-2} s^{-1}. In order to achieve higher efficiency, on the basis of Chiu et al study (14), air flow containing 2% of carbon dioxide was used for aeration, after being saturated in water and passed through a 0.45 μm filter. To avoid any kind of pollution during the different steps, the media and containers were sterilized in an autoclave at a temperature of 121°C.

Microalgae cell counting and dry weight

Cell density (cells mL^{-1}) was measured using a ultraviolet-visible spectrophotometer(UV-Vis) spectrophotometer (Shimadzu Corporation) at an absorbance wavelength of 680 nm. Each sample was diluted to give an absorbance in the range of 0.1–1.0, if optical density was greater than 1.0 (14).

Microalgae dry weight per liter (g L^{-1}) was measured according to the method previously reported (15). Microalgae cells were collected by centrifugation of wet biomass for 30 minutes in 15°C with 3000 rpm. The dry weight of marine microalgae samples was affected by salt absorbed on the cell surface and its presence in the intercellular water ensured error in estimating the amount of biomass. This explains the differentiations in the amount of dry cell weight in various papers. Hence, before gravimetric analysis, to remove salts, the centrifuged cells were again solved in 200 ml Ammonium format (0.5M, pH 8.0, adjusted with 1M NaOH) and centrifuged under the mentioned circumstances (30,32). The microalgae pellet was dried at 100°C for 4 hours for dry weight measurement (13,30,32).

Measurement of growth rate

Specific growth rate of microalgae in logarithmic phase was calculated as follows:

$$\mu = \frac{\ln N_f - \ln N_i}{t_2 - t_1} \quad \text{(Eq. 1)}$$

Where; μ (day^{-1}) is the specific growth rate, $\ln N_i$ and $\ln N_f$ are the cell densities (cell/ml) at the beginning and end of the logarithmic growth phase, respectively and t is the time (day) (1,14,5,30).

Extraction and measurement of lipid content and triacylglycerol

The methanol-chloroform (1/1, V/V) extraction method was used to extract total lipids from the dried cells (13,33,34). To remove residual microalgae, the extraction

Table 1. The media composition used in this study (m_{mol}/L)

Element's nutrients	Medium			
	Walne	F/2	Sato	TMRL
$MnCl_2.4H_2o$	7.6717×10^{-4}	0.3452	5.1784×10^{-4}	-
$CoCl_2.6H_2o$	0.084	0.042	3.3623×10^{-4}	-
$CuSo_4.5H_2o$	0.0801	0.04	1.602×10^{-5}	-
$FeCl_3$	4.932×10^{-3}	0.0194	1.8934×10^{-5}	10^{-5}
$NaHCo_3$	-	-	1.9998	-
$(NH_4)_6Mo_7O_{24}.4H_2O$	7.2814×10^{-4}	4.8543×10^{-6}	-	-
$ZnCl_2$	0.154	0.0765	2.2012×10^{-4}	-
$NaNO_3$	1.1765	1.1765	0.8824	0.8824
H_3BO_3	0.5434	-	0.0556	-
$Na_2EDTA.2H_2O$	0.1208	0.0117	8.0593×10^{-3}	-
$NaH_2PO_4.2H_2O$	0.0755	0.0387	0.0193	0.0193

lipids were filtered on membranes with 0.45 µm mean pore diameter. After washing twice with methanol and its complete evaporation, gravimetric analysis was done and part of the lipid fraction expressed as the percent of dry cell weight (28). Lipid productivity was calculated by (9,35):

$$P_{Lipid}\left(gl^{-1}day^{-1}\right) = \frac{(C_f \times DCW_f)-(C_i \times DCW_i)}{T} \qquad \text{(Eq. 2)}$$

Where; P_{Lipid} is the lipid productivity, C_f and D_{CWf} are the lipid content (g/g) and biomass (g/l) of the microalgae in the final stationary growth phase, respectively; C_i and D_{CWi} are the lipid content (g/g) and biomass (g/l) of the microalgae in the initial stationary growth phase, respectively; and T is the cultivation time (day). After the measurement of total lipid, the dried lipid was solved in 0.4 ml of Isopropyl alcohol and the TAG was estimated by an enzymatic colorimetric method using a commercial kit (36).

Determination of fatty acid profiles

The direct esterification method was used to measure the fatty acid property. A mixture of 100 mg of lyophilized microalgae and 8 ml of KOH was sonicated for 3 minutes. For saponification, the mixture was heated to 100°C for 15 minutes and cooled to room temperature. For esterification, 8 ml of 0.7 N HCl in methanol and BF_3/CH_3OH was added to the mixture (14% V/V) and again was heated to 100°C for 15 minutes. After cooling to room temperature, to avoid emulsification, 2 ml of a saturated solution of sodium chloride was added. The FAMEs were extracted by adding aliquots of n-hexane. The FAMEs in the hexane layer were analyzed using standard gas chromatography (Agilent technologies 7890A-5975c) with a capillary column and a flame ionization detector. Nitrogen was used as the carrier gas and delivered at a rate of 1.5 mL min^{-1}. The temperature was programmed to increase from 130°C to 180°C at a rate of 10°C min^{-1} and thereafter ramped to 210°C at a rate of 2°C min^{-1}. The injector and detector were kept at 220°C and 250°C, respectively (1,35).

Statistics

All values were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) and in order to determine the statistical difference between media, the Tukey test was used. A value of $P<0.05$ was considered statistically significant (21,24).

Results

Effects of medium on cell growth rate of N. oculata

Figure 1 shows the cell growth rate of *N. oculata* in four different media. *N. oculata* exhibited the highest growth rate in the Walne medium. Except for the TMRL medium, in which there was no logarithmic phase, in other media, the microalgae after a lag phase of 24 hours, entered the logarithmic growth phase. The results of the one-way ANOVA showed that the observed differences between media types were significant ($P<0.05$). Tukey test also confirmed that they were not put in homogenous groups and the Walne medium had considerable difference with F/2 ($P=0.008$), Sato ($P=0.002$) and TMRL ($P=0.000$) media. There was no significant statistical difference between F/2, Sato and TMRL media ($P>0.05$).

Effects of medium on biomass and lipid production

Figure 2 shows the results of medium effects on biomass production in two logarithmic and stationary growth phases. Walne medium recorded the highest biomass production. The results of one-way ANOVA showed a large variation in biomass production between the culture media used. Tukey test also confirmed that they were not in homogenous groups and the Walne medium had a significant difference with the F/2 ($P=0.005$), Sato ($P=0.001$) and TMRL ($P=0.000$) media. Biomass production did not vary greatly between the F/2, Sato and TMRL media ($P>0.05$).

The results of medium effects on lipid production in 2 logarithmic and stationary growth phases are shown in Figure 3. The TMRL medium recorded the highest percentage of biomass conversion to lipid. The results of one-way ANOVA showed that the difference between lipid conversion percentages in the used media was significant. Also, the Tukey test showed that the differences between all media were significant ($P<0.001$) but between Walne and F/2 media, no significant differences were observed ($P=0.065$).

Effect of medium on fatty acids composition

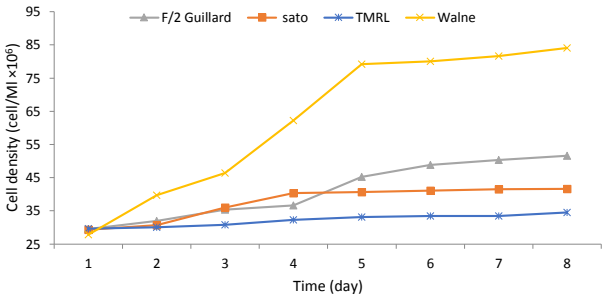

Figure 1. Cell growth rate of *Nannochloropsis oculata* in Walne, F/2, Sato and TMRL media.

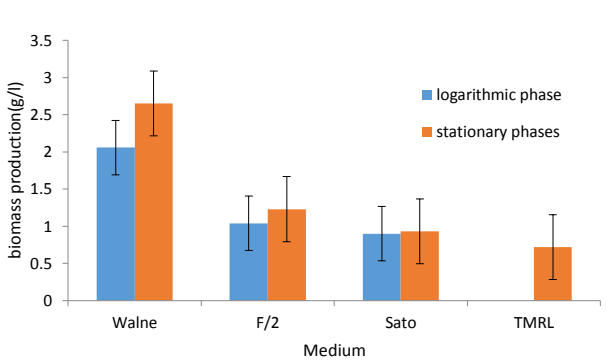

Figure 2. Biomass production by *Nannochloropsis oculata* in logarithmic and stationary growth phases in various media (in TMRL medium, there was no logarithmic growth phase).

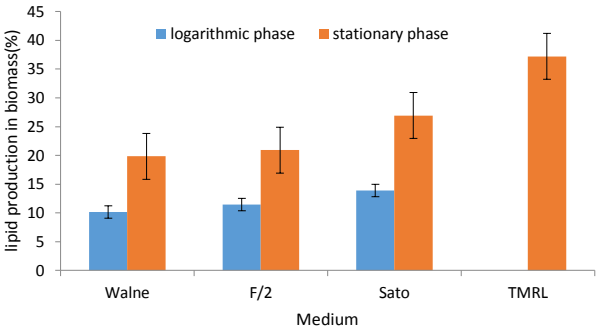

Figure 3. Lipid production by *Nannochloropsis oculata* in logarithmic and stationary growth phases in various mediums (in TMRL medium, there was no logarithmic growth phase).

The results of fatty acids composition of *N. oculata* grown in various media during logarithmic and stationary growth phases are shown in Table 2.

Discussion

Maximal cell densities, specific growth rates and biomass production

N, P, K, Mg, Ca, S, Fe, Cu, Mn, and Zn are essential elements for the growth of green algae added to culture media in the form of salts (37). Due to the existence of enough nutrients in the Walne and F/2 media, the logarithmic growth phase continued until the fifth day but it reached the end of the logarithmic phase on the fourth day, in the Sato medium. In the TMRL medium, the logarithmic phase was so short and could be ignored.

N. oculata reached the highest cell density (84×10^6 cell/ml) (Figure 1) and highest biomass production (2.652 g/l) (Figure 2) in the Walne medium at the end of the stationary growth phase and conformed to the values reported by Olofsson et al (11), Solovchenko et al (25) and Chiu et al (14). According to Table 1, unlike other media, the Walne medium used two nitrogen sources (sodium nitrate and ammonium molybdate). Nitrogen as an important constituent of cellular protein and chlorophyll molecules are required for microalgae cell growth (21). Another reason for higher growth rate and biomass production in the Walne medium was the existence of ammonium which is a necessary element for the microalgae and its concentration in the Walne medium was 150 times more than the F/2 medium. This was not observed in other media. It takes more energy to assimilate NO3-N than to assimilate $NH4^+$-N, hence microalgae prefer $NH4^+$-N in the medium (21). On the other hand, in consideration of the role of phosphate in producing ATP required for photosynthesis and rapid microalgae growth (38), its concentration in Walne medium was double that of the F/2 medium and 4 times the other media. Copper is one of the required elements for microalgae and is an important part of the plastocyanin protein in the electron transport chain. The concentration of copper in the Walne medium is considerably higher than other media.

The fastest specific growth rate among the media was recorded in the Walne medium (0.2616 day^{-1}), while the F/2 and Sato media were 0.1066 day^{-1} and 0.08 day^{-1}, respectively. The specific growth rates found in the present study were not in the median range of other published studies. In the study of Chiu et al (14) and Banerjee et al (30) *on N. oculata* cultivation, the specific growth rate was 0.571 day^{-1} and 0.004 day^{-1}. Differences in growth rates compared to the other studies could be due to differences in culturing methods, reactor geometry, prior culture history, light intensity and temperature (15,20).

Lipid accumulation and lipid productivity

Algal biomass is composed of three main components namely carbohydrates, proteins and lipids (natural oils) (16). To increase the proportion of the biomass that contains a useful lipid, different strategies including nutrient starvation, bioprocess optimization and genetic modification (39,40) were used. Under stress conditions, photosynthetic activity in microalgae decreases and the excess energy might be stored in the form of valuable compounds such as lipids (17,24). Nutrient starvation is one

Table 2. Fatty acid composition in dry weight, percentage of *Nannochloropsis oculata* grown in Walne, F/2, Sato and TMRL medium during logarithmic and stationary phases

Fatty acid	Name	Medium						
		Walne		F/2		Sato		TMRL
		Logarithmic	Stationary	Logarithmic	Stationary	Logarithmic	Stationary	Stationary
C14:0	Myristic acid	3.5[a]	5.59	3.1	6.45	3.1	4.79	3.3
C15:0	-	0.2	0.34	0.1	0	0.2	0.12	0.2
C16:0	Palmitic acid	22.76	28.57	29.97	34.36	30.5	35.17	34.3
C16:1n-7	Palmitoleic acid	18.45	25.04	19.3	23.19	20.11	24.16	22.56
C18:0	Stearic acid	1.01	0.73	0.8	0.8	1	0.89	0.7
C18:1n-9	Oleic acid	6.3	5.24	7.4	7.85	9.6	11.01	7.5
C18:2n-6	Linoleic acid	3.15	1.12	1.7	0.23	2.4	1.15	1.2
C20:0	Eicosanoic acid	0.1	0	0.1	0.03	0.1	0	0.1
C20:4n-6	Arachidonic acid	3.73	2.46	3.25	1.1	2.98	0.76	3.21
C20:5n-3	Eicosapentaenoic acid (EPA)	36.11	22.19	27.6	19.98	24.2	16.17	17.3

[a] Values represent mean of two replicate samples.

of the stress conditions that can be applied to microalgae by culture medium modifications and therefore change the biochemical composition of the biomass. Nitrogen and phosphorus are the most common restrictive factors in the media that can lead to lipid accumulation. In nitrogen limiting media, the lipid content usually increases in the algae due to less susceptibility of lipid-synthesizing enzymes for disorganization than carbohydrate synthesizing enzymes due to nitrogen deprivation (2). Also, decreasing the nitrate concentration of the medium causes a decrease in the amount of chlorophyll II and limits biochemical protein synthesis (28,37). According to the results in Figure 3, the maximum percentage of converting biomass to lipid was attained in the TMRL medium (37.22% of dry cell weight). Also the results of lipid analysis in two logarithmic and stationary growth phases showed that lipid accumulation in microalgae cells had a direct relation to their growth phases and growth from the logarithmic to the stationary growth phase was accompanied by increase in lipid percentage. These results matched the results of Nigam et al (6), Hu and Gao (19) and Gouveia and Oliveira (4) studies.

The lipid content or biomass productions are not appropriate scales for microalgae lipid yields alone in biodiesel production and the most crucial comparative measure is lipid productivity that must be calculated using Equation 2 (15). The maximum lipid productivity was related to the Walne medium 0.1057 and for the F/2, Sato and TMRL was 0.0462, 0.0417, 0.023 gl^{-1} day^{-1} sequence, respectively. In Gouveia and Oliveira study (4), the lipid productivity was 0.09 gl^{-1} day^{-1} and in Griffiths and Harrison study (10), it was 0.082 gl^{-1} day^{-1} and matched the results obtained in this study.

Fatty acid compositions

Vegetable oils currently used for biodiesel productions are mainly C16 and C18. Olofsson et al (11) proposed myristic acid, palmitic acid, palmitoleic acid, stearic acid and oleic acid as important fatty acids for biodiesel production and consisted more than 45%-78% of all fatty acid compositions. According to the results of this study, these fatty acids consist of 65.7%, 72.65%, 76.02% and 68.38% of fatty acid composition in the dry weight of *N. oculata* grown in the Walne, F/2, Sato and TMRL media during the stationary phase.

Also, the microalgae oil in the form of TAG can be converted to biodiesel. The TAG of *N. oculata* consists of saturated and monounsaturated fatty acids and is mainly stored in vacuoles within the cell (11,24). In this study, TAG consisted of 64.78%, 71.88%, 75.25% and 67.96% of fatty acid composition in dry weight of *N. oculata* grown in the Walne, F/2, Sato and TMRL media during the stationary phase. Therefore, the Sato medium quantitatively provided the maximum amount of TAG for biodiesel production.

Conclusion

Lab scale experiments have an important role in developing biodiesel studies and finally scale-up production. In this study, a microalgae culture medium was surveyed as a major aspect of biodiesel production. The results showed that the best medium for *N. oculata* cultivation is Walne and the microalgae had maximum efficiency in the stationary growth phase. One advantage of this study is the use of *N. oculata* for aquaculture and human consumptions.

Acknowledgements

The work was financially supported by the Research and Technology Assistance Division of Kerman University of Medical Sciences, Kerman, Iran. This work was also partially supported by the Environmental Health Engineering Research Center of Kerman University of Medical Sciences, Kerman, Iran.

Ethical issues

We certify that all data collected during the study is presented in this manuscript and no data from the study has been or will be published separately.

Competing interests

Authors declare that they have no competing interests.

Authors' contributions

SD and BH conceived and designed the study. MM and AR performed the literature search and wrote the manuscript. All authors participated in the data acquisition, analysis and interpretation. All authors critically reviewed, refined and approved the manuscript.

References

1. Xin L, Hong-ying H, Yu-ping Z. Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. Bioresour Technol 2011; 102(3): 3098-102.
2. Rajasri Y, Ramgopal RS, Rao CS. Lipid accumulation studies in chlorella pyrenoidosa using customized photobioreactor - effect of nitrogen source, light intensity and mode of operation. International Journal of Engineering Research and Applications 2012; 2(3): 2446-53.
3. Xiaodong D, Yajun L, Xiaowen F. Effects of selective medium on lipid accumulation of chlorellas and screening of high lipid mutants through ultraviolet mutagenesis. African Journal of Agricultural Research 2011; 6(16): 3768-74.
4. Gouveia L, Oliveira A. Microalgae as a raw material for biofuels production. J Ind Microbiol Biotechnol 2009; 36(2): 269-74.
5. Sánchez S, Martínez M, Espinola F. Biomass production and biochemical variability of the marine microalga Isochrysis galbana in relation to culture medium. Biochem Eng J 2000; 6(1): 13-8.
6. Nigam S, Rai MP, Sharma R. Effect of nitrogen on growth and lipid content of chlorella pyrenoidosa.

Am J Biochem Biotechnol 2011; 7(3): 124-9.
7. Sankar M, Ramasubramanian V. Biomass production of commercial algae Chlorella vulgaris on different culture media. E-Journal of Life Science 2012; 1(1): 56-60.
8. Wang G, Wang T. Characterization of lipid components in two microalgae for biofuel application. J Am Oil Chem Soc 2012; 89(1): 135-43.
9. Li Y, Horsman M, Wang B, Wu N, Lan C. Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleoabundans. Appl Microbiol Biotechnol 2008; 81(4): 629-36.
10. Griffiths M, Harrison SL. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J Appl Phycol 2009; 21(5): 493-507.
11. Olofsson M, Lamela T, Nilsson E, Bergé JP, del Pino V, Uronen P, et al. Seasonal variation of lipids and fatty acids of the microalgae Nannochloropsis oculata grown in outdoor large-scale photobioreactors. Energies 2012; 5(5): 1577-92.
12. Banskota A, Stefanova R, Gallant P, McGinn P. Mono- and digalactosyldiacylglycerols: potent nitric oxide inhibitors from the marine microalga Nannochloropsis granulata. J Appl Phycol 2013; 25(2): 349-57.
13. Chen Y, Wang J, Liu T, Gao L. Effects of initial population density (IPD) on growth and lipid composition of Nannochloropsis sp. J Appl Phycol 2012; 24(6): 1623-7.
14. Chiu SY, Kao CY, Tsai MT, Ong SC, Chen CH, Lin CS. Lipid accumulation and CO2 utilization of Nannochloropsis oculata in response to CO2 aeration. Bioresour Technol 2009; 100(2): 833-8.
15. Huerlimann R, de Nys R, Heimann K. Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotechnol Bioeng 2010; 107(2): 245-56.
16. Karampudi S, Chowdhury K. Effect of media on algae growth for bio-fuel production. Notulae Scientia Biologicae 2011; 3(3): 33-41.
17. Recht L, Zarka A, Boussiba S. Patterns of carbohydrate and fatty acid changes under nitrogen starvation in the microalgae Haematococcus pluvialis and Nannochloropsis sp. Appl Microbiol Biotechnol 2012; 94(6): 1495-503.
18. Sandnes JM, Källqvist T, Wenner D, Gislerød HR. Combined influence of light and temperature on growth rates of Nannochloropsis oceanica: linking cellular responses to large-scale biomass production. J Appl Phycol 2005; 17(6): 515-25.
19. Hu H, Gao K. Response of growth and fatty acid compositions of Nannochloropsis sp. to environmental factors under elevated CO2 concentration. Biotechnol Lett 2006; 28(13): 987-92.
20. Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Optimization of Nannochloropsis oculata growth using the response surface method. J Chem Technol Biotechnol 2006; 81(6): 1049-56.
21. Durmaz Y. Vitamin E (α-tocopherol) production by the marine microalgae Nannochloropsis oculata (Eustigmatophyceae) in nitrogen limitation. Aquaculture 2007; 272(1–4): 717-22.
22. Zhang J, Liu S, Sun X, Yang G, Zhang X, Gao Z. Fatty acid composition analyses of the DCMU resistant mutants of Nannochloropsis oculata (Eustigmatophyceae). Journal of Ocean University of Qingdao 2003; 2(1): 65-8.
23. Solovchenko AE, Khozin-Goldberg I, Didi-Cohen S, Cohen Z, Merzlyak MN. Effects of light intensity and nitrogen starvation on growth, total fatty acids and arachidonic acid in the green microalga Parietochloris incisa. J Appl Phycol 2008; 20(3): 245-51.
24. Pal D, Khozin-Goldberg I, Cohen Z, Boussiba S. The effect of light, salinity, and nitrogen availability on lipid production by Nannochloropsis sp. Appl Microbiol Biotechnol 2011; 90(4): 1429-41.
25. Solovchenko A, Khozin-Goldberg I, Recht L, Boussiba S. Stress-induced changes in optical properties, pigment and fatty acid content of Nannochloropsis sp.: implications for non-destructive assay of total fatty acids. Mar Biotechnol 2011; 13(3): 527-35.
26. Simionato D, Sforza E, Corteggiani Carpinelli E, Bertucco A, Giacometti GM, Morosinotto T. Acclimation of Nannochloropsis gaditana to different illumination regimes: effects on lipids accumulation. Bioresour Technol 2011; 102(10): 6026-32.
27. Huang X, Huang Z, Wen W, Yan J. Effects of nitrogen supplementation of the culture medium on the growth, total lipid content and fatty acid profiles of three microalgae (Tetraselmis subcordiformis, Nannochloropsis oculata and Pavlova viridis). J Appl Phycol 2013; 25(1): 129-37.
28. Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M. Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. Chemical Engineering and Processing: Process Intensification 2009; 48(6): 1146-51.
29. Srinivas R, Ochs C. Effect of UV-A irradiance on lipid accumulation in Nannochloropsis oculata. Photochem Photobiol 2012; 88(3): 684-9.
30. Banerjee S, Hew WE, Khatoon H, Shariff M, Yusoff FM. Growth and proximate composition of tropical marine Chaetoceros calcitrans and Nannochloropsis oculata cultured outdoors and under laboratory conditions. Afr J Biotechnol 2011; 10(8): 1375-83.
31. Sen B, Koser M, Alp M, Erbas H. Studies on growth of marine microalgae in batch cultures:III. Nannochloropsis Oculata (Eustigmatophyceae). Asian Journal of Plant Science 2005; 4(6): 642-4.
32. Zhu CJ, Lee YK. Determination of biomass dry weight of marine microalgae. J Appl Phycol 1997; 9(2): 189-94.
33. Xin L, Hu HY, Ke G, Sun YX. Effects of different nitrogen and phosphorus concentrations on the

growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. Bioresour Technol 2010; 101(14): 5494-500.
34. Wan M, Liu P, Xia J, Rosenberg J, Oyler G, Betenbaugh M, et al. The effect of mixotrophy on microalgal growth, lipid content, and expression levels of three pathway genes in Chlorella sorokiniana. Appl Microbiol Biotechnol 2011; 91(3): 835-44.
35. Su CH, Chien LJ, Gomes J, Lin YS, Yu YK, Liou JS, et al. Factors affecting lipid accumulation by Nannochloropsis oculata in a two-stage cultivation process. J Appl Phycol 2011; 23(5): 903-8.
36. Xin L, Hong-Ying H, Jia Y, Yin-Hu W. Enhancement effect of ethyl-2-methyl acetoacetate on triacylglycerols production by a freshwater microalga, Scenedesmus sp. LX1. Bioresour Technol 2010; 101(24): 9819-21.
37. Mandalam RK, Palsson BO. Elemental balancing of biomass and medium composition enhances growth capacity in high-density Chlorella vulgaris cultures. Biotechnol Bioeng 1998; 59(5): 605-11.
38. Hu H, Zhou Q. Regulation of inorganic carbon acquisition by nitrogen and phosphorus levels in the Nannochloropsis sp. World J Microbiol Biotechnol 2010; 26(5): 957-61.
39. Van Wagenen J, Miller TW, Hobbs S, Hook P, Crowe B, Huesemann M. Effects of light and temperature on fatty acid production in Nannochloropsis salina. Energies 2012; 5(3): 731-40.
40. Malakootian M, Hatami B, Dowlatshahi S, Rajabizadeh A. Evaluation of factors affecting on lipid extraction for recovery of fatty acids from Nannochloropsis oculata micro-algae to biodiesel production. Environ Health Eng Manag J 2014; 1(1): 19-24.