Effects of Light Intensity, Photoperiod and Nitrate Levels on Biomass Production in Green Algae *Scenedesmus dimorphus*

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Abstract

The effects of three different photoperiods 12:12, 16:8 and 8:16 hours light: dark (LD), light intensity of 3000, 5000 and 7000 lux and nitrate levels of 1.47, 2.94 and 4.41 mmol/l on growth rate (cell number) and biomass of *Scenedesmus dimorphus* was studied. The algal cells were cultured at 28±2°C in BBM culture medium during one month and cells were counted every three days. By the end of experiments the lowest cell concentration (9.3×10^6 cells/ml) was observed at 12:12 LD, 1.47 mmol/L nitrate and 3000 lux light intensity and the highest cell concentration at 8:16 LD, 2.97 mmol/L nitrate and 3000 lux light intensity. The lowest algal biomass (1.38 g/l) was observed at 12:12 LD, 3000 lux light intensity and 1.47 mmol/l nitrate levels while the highest biomass (5.2 g/l) at 8:16 LD, 7000 lux light intensity of and 2.94 mmol/l nitrate level.

Keywords: *Scenedesmus dimorphus*, Light intensity, Photoperiod, Biomass production, Light quality, Growth rate

Introduction

Algae are photosynthetic organism divided into major groups of micro and macro algae (Rosenberg et al., 2008). Microalgae are considered as the most basic energy sources of an aquatic ecosystem (Walker et al., 2005). Environmental factors such as pH and light availability strongly influence the growth rate and biomass production in microalgae. Growth rate is the most important indicator of ecological success or adaptation of a species to environmental changes (Rivkin, 1989; Isik et al., 2006). Currently several groups of algae are used in industries, and green algae are more important in this aspect (Mata, 2010). Nowadays, the science of biotechnology have focused on developing effective stimuli to improve growth rate, properties of biochemical components and pigments in algae. As a photosynthetic organism green algae require an aqueous medium in the form of water, light, CO_2 and range of certain minerals (Balat, 2010). Algae contain valuable chemicals like vitamins, carotenoids, proteins, poly-

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saccharides, fatty acids with anti-inflammatory, anti-cancer, antifungal, antioxidative and antibacterial activities (Pereira, 2018; Shalaby, 2011). They also sustain immune system stimulatory features (Hanna et al., 2008). *Scenedesmus* has significant potentials in biotechnology (Soltani et al., 2002) with astonishing tolerance to acid condition (Soltani et al., 1992). Due to high protein contents the algae is mass cultured in aquaculture as a protein source (Kianmehr, 2005). This species is used as standard organism in water technology and management (Zochleder et al., 1986). Several authors have studied the effects of light quality and quantity on growth and biochemical properties of algae. Velichkova et al. (2013) cultured *Scenedesmus dimorphus* in two different culture media (BBM and 3N-BBN) and studied its potentials for biofuel production. Liang et al. (2009) used glucose as carbon source in *Chlorella vulgaris* biomass production under light and dark condition and achieved maximum productivity under light condition. Dittamart et al. (2014) applied different organic carbon supplements in mixotrophic culture of *Scenedesmus* sp. to enhance biomass and lipid production in AARL G022 and reported a photoperiod of 16:8 LD condition as optimum. The aim of the present study was finding out optimum light intensity and photoperiod to induce higher growth rate in *Scenedesmus dimorphus* to be used in larger and industrial scales.

Materials and Methods

Scenedesmus dimorphus was provided by the Clean Nature Explorers Company (CNE Company, Rasht, Iran). All equipment was sterilized prior to commencement of the experiments to eliminate potential contaminant risk.

Culture condition

Light intensity was adjusted to 3000, 5000 and 7000 lux by Lux Meter TES 1336A. Mean water temperature was 28±2°C. The BBM culture medium with slight modification was prepared for culture. The Design-Expert software suggested 17 experimental treatments and 3 factors. The first factor (light) was adjusted between 3000-70000 lux with binary level of 5000 lux. The second factor (nitrate) was used with normal concentration 2.94 mmol/l, half normal (1.47 mmol/l) and 1.5 normal (4.41 mmol/l). The third factor was different photoperiod (light/dark) with 3 levels of 12:12 (LD), 8:16 (LD) and 16:8 (LD) hours light: dark. The samples were cultivated in 4 liter containers using BBM medium. Culture container aerated regularly to maintain algal cells suspended. Culture period lasted for 30 days and algal cells were counted twice in a week in this period using a Thoma counting chamber. The cultured algae harvested by keeping the containers in dark room. Cells started to settle down at the bottom of the containers overnight. The settled algae cells were released into a falcon, centrifuged at 2500 rpm and dried by freeze drier and then weighted.

Results

The biomass of algae cells in light intensities of 3000, 5000 and 7000 lux as variable factor were plotted against nitrate levels (2.94, 1.47 and 4.41 mmol/l) and photoperiod (12:12, 8:16 and 16:8 hours LD). Considering the rotations in figures there is no direct relationship between nitrate concentration and darkness at fixed light intensity. The red areas reflect the highest yield obtained and cell number at 7000 lux light intensity. The results are presented in Figure 1. The BC variables (light intensity and photoperiod) were kept constant and the variable A or nitrate was changed. As shown in Fig-

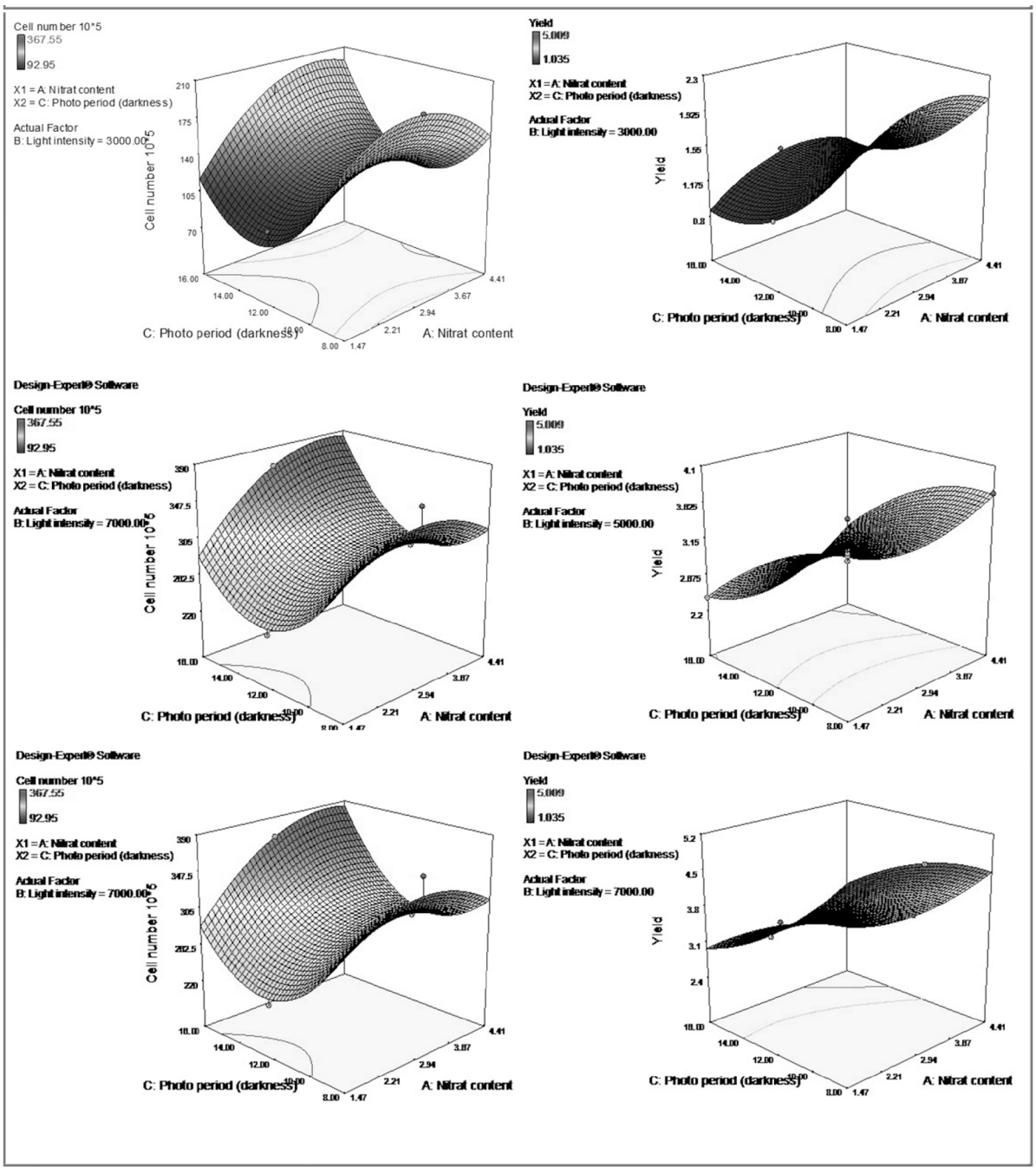

Fig. 1. Surface plot of the algal biomass (g/l) and algal concentration (cell/ml) light intensity as variable factor (3000, 5000, 7000 lux) and photoperiod and nitrate as fixed factors.

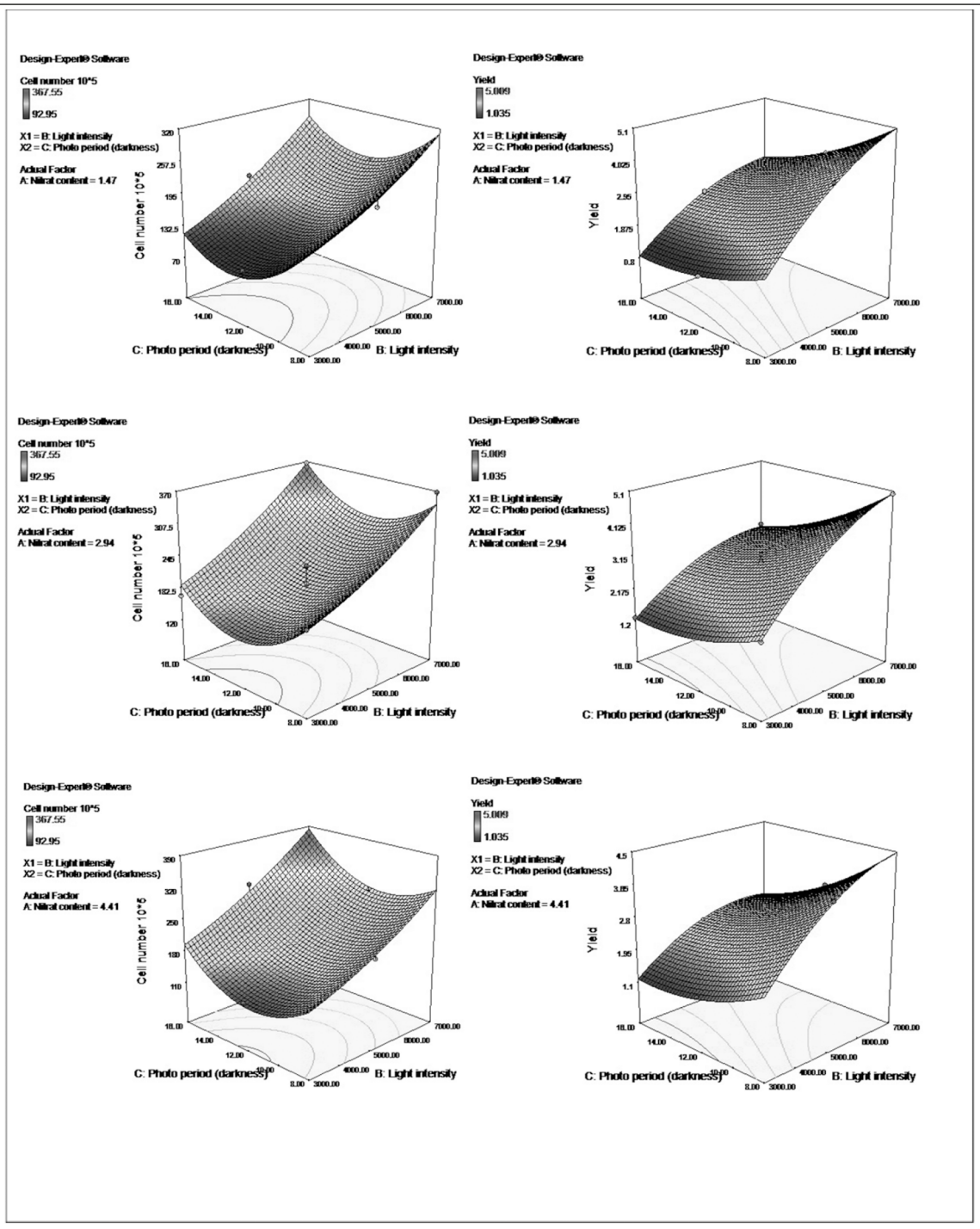

Fig. 2. Surface plot of the algal biomass (g/l) and algal concentration (cell/ml) with nitrate level as variable factor (1.47, 2.94, 4.41 mmol/l) and photoperiod and light intensity as fixed factors.

ure 2 increases in light intensity at different nitrate concentration resulted in higher biomass and yield. The highest cell number was observed at 4.41 mmol/l (red areas). The effects on cell number are plotted in Figure 2.

Biomass obtained at different photoperiod as variable factor were plotted against nitrate levels and light intensities. As shown in Figure 3 at 8 hours darkness increase in light intensity resulted in higher cell number and

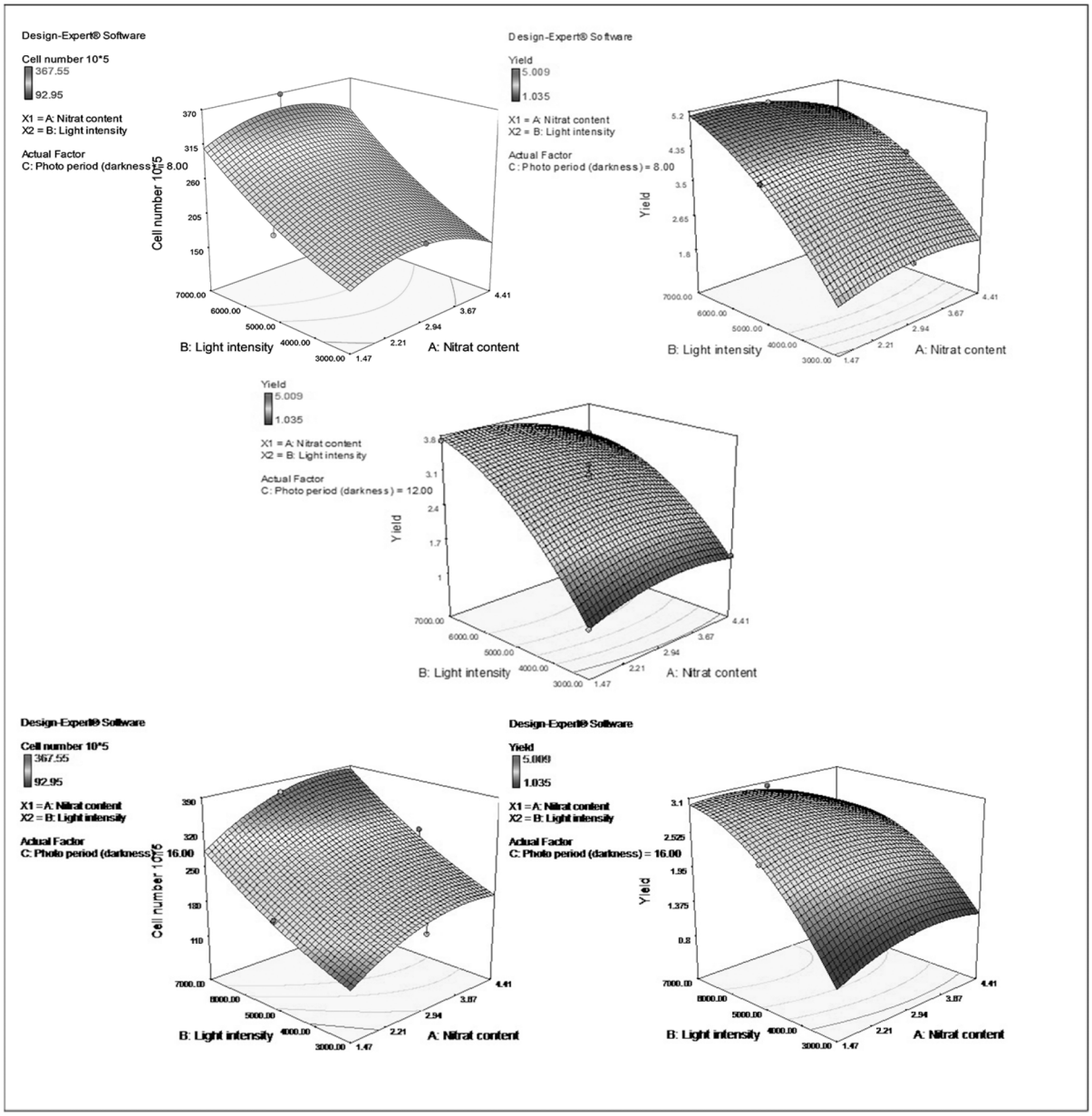

Fig. 3. Surface plot of the algal biomass (g/l) and algal concentration (cell/ml) at 8:16, 16:8, 12:12 photoperiod as variable factor and light intensities and nitrate levels as fixed factors.

the highest cell number was observed at 2.94 mmol/l nitrate concentration. Under this condition (upper right graph) biomass was also increased. Increaseing in light intensity resulted biomass increasing and yield. The results are presented in Figure 3.

Biomass of algae cells and growth rate related to photoperiod 12:00, 8:16 and 18:6 hours and nitrate levels, 1.47, 2.94 and 4.41 mmol/l were recorded for the period of 30 days and plotted which are presented. As light intensity increased the number of cells increased, correlations 0.787 and 0.782 yield. Also, a negative correlation coefficient occurs in longer dark period and high nitrate concentrations (Figure 4).

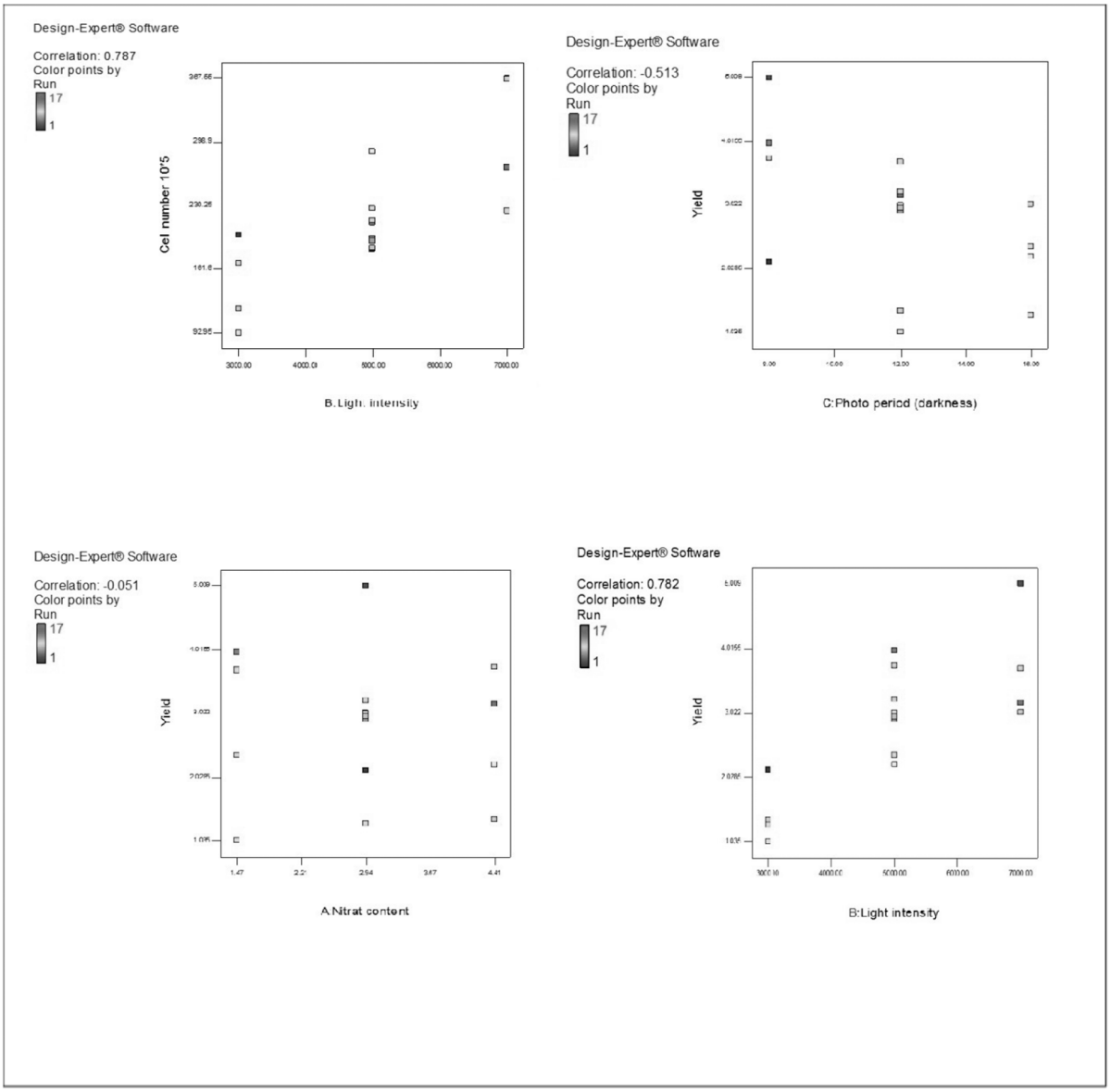

Fig. 4. Correlation matrix between cell number, light intensity, photoperiod and nitrate levels.

Discussion

Environmental factors such as light intensity and photoperiods influence the growth pattern of algae (Rai and Gupta, 2016), therefore vigilant regulation of these parameters is indispensable for optimal operation of algae culture system. The quality and quantity of light control grow rate, metabolism (Ruangsomboon, 2012) and biomass of algae (Sun et al., 2014). As a limiting factor, light should be adjusted properly (Xue et al., 2011). Nitrate levels are also critical in algae culture according to Pribyl et al. (2016). They changed nitrate level from 1.47 to 4.41 mmol/l and biomass production decreased. A similar result was achieved in present study where cell number increased but the total biomass decreased. Similar result has been reported by Guedes et al. (2010). Although increase in cell number was appar-

ent, it seems that algal cell did not grow optimally at lower photoperiod. Therefore reduction in final biomass could be justified which was the case in a study by Liang et al. (2013). The Response Surface Method (RSM) with Box- Behnken Design (BBD) was used to determine the optimum growth condition for *S. dimorphus*. In these types of graphs the interactions between parameters, responses and variables-responses are illustrated. Higher correlation coefficient shows effective interaction between the parameters. According to regression results, light intensity positively associated with cell number and growth rate of algae. Three dimensional plots illustrate the zones and extent of effects of each parameters on response. Considering the effects of photoperiod on cell concentration, a significant increase in cell concentration was observed at lower nitrate level (1.47mmol/l), highest light intensity (7000 lux) and 16: 8 LD. Singh and Singh (2015) and Pancha et al. (2015) reported similar results where increase in light intensity resulted in higher biomass production and yield. The lowest cell concentration was observed at 12:12 LD with 9.3×10^6 cells/ml and the highest at 16:8 LD and nitrate level of 2.94 mmol/l with 3.91×10^7 cells/ml. As shown in Figure 2 growth rate and biomass increased in various nitrate levels under 8:16 LD which resulted in significant increase in cell concentration. George et al. (2014) have reported similar trend in their study. Analysis of the effects of light intensity on growth rate showed the lowest growth rate at 3000 lux light intensity (2×10^6 cells/ml) and the highest growth at 7000 lux with 3.9×10^7 cells/ml a result which was observed in Scott et al. (2010). Nitrate also influenced the growth rate and cell concentration in combination with other environmental parameters. The lowest cell concentration was observed at the lowest nitrate level (1.47 mmol/l), 3.2×10^7 cells/ml and the highest at the 4.41mmol/l nitrate concentration about 3.9×10^7 cell/ml. Similar result has been reported by Sun et al. (2014) and confirmed that nitrate levels influence the production of algal biomass. In this study the lowest cell biomass was observed at 4.41 mmol/l nitrate (Mandotra et al., 2016) with 4.5 g/l and the highest at 2.94 mmol/l nitrate (5.2 g/l). As for light intensity and its effect on algal biomass the lowest biomass was recorded with 2.3 g/l at 3000 lux light intensity similar to Liang et al. (2013) and the highest with 5.2 g/l at 7000 lux which is in agreement with findings of Yeesang and Cheirsilp (2011). Photoperiod also influenced the biomass production. The lowest biomass (3.1 g/l) was observed at 16:8 light/dark condition and the highest (5.2 g/l) at 8:16 hours light/dark photoperiod.

Combination of environmental parameters such as light intensity, photoperiod and nitrate levels affect on growth rate and biomass production in different and rather unpredictable way. The lowest biomass for example was observed at higher nitrate level where the highest biomass was observed at lower nitrate level and lower photoperiod 8:16 LD. There are multiple interactions which directly or indirectly affect algal cell growth and

biomass production. Here we shed light into some of these parameters which impact algal growth individually and with other factors combination. Obviously there are several other factors or combinations of factors which impact growth rate and biomass production in algae. Therefore further study on this subject with different species of algae and different parameters suggested.

References

Balat H. (2010). Prospects of biofuels for a sustainable energy future: a critical assessment. Energy Education Sciences and Technology, Part A. 24: 85-111.

Dittamart D, Chayakorn P, Jeeraporn P, Yuwadee P. (2014). Effects of organic carbon source and light-dark period on growth and lipid accumulation of *Scenedesmus* sp. AARL G022. Maejo International Journal of Sciences and Technology. 8 (02): 198-206

George B, Pancha I, Desai C, Chokshi K, Paliwal C, Ghosh T. (2014). Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus* – a potential strain for biofuels production. Bioresource Technology. 171: 367-74.

Guedes AC, Meireles LA, Amaro HM, Malcata FX. (2010). Changes in lipid class and fatty acid composition of cultures of *Pavlova lutheri*, in response to light intensity. Journal of the American Oil Chemists Society. 87 (7): 791-801.

Hanaa HAEL-Baky, Hussein MM, Game SEl-Baroty. (2008). Algal extracts improve antioxidant defense abilities and salt tolerance of wheat plant immigrated with sea water. Electronic Journal of Environmental, Agricultural and Food Chemistry. 6: 2812-2832.

Isik O, Hizaric S, Sayin S, Gokpinar Durmaz Y. (2006). The effect of the environmental factors on the vitamin C (ascorbic acid), E (alpha-tocopherol), β-carotene contents and the fatty acid composition of *Spirulina platensis.* Journal of Fisheries and Aquatic Sciences. 3 (4): 257-261.

Kianmehr H. (2005). Biology of Algae, Ferdowsi University of Mashhad. 336 p.

Klyachko-Gurvich GL, Tsoglin LN, Doucha J, Kopetskii J, Shebalina IB, Semenenko VE. (1999). Desaturation of fatty acids as an adaptive response to shifts in light intensity. Physiologia Plantarum. 107: 240-249.

Liang K, Zhang Q, Gu M, Cong W. (2013). Effect of phosphorus on lipid accumulation in freshwater microalga *Chlorella* sp. Journal of Applied Phycology. 25: 311- 318.

Liang Y, Sarkany N, Cui Y. (2009). Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnology Letters. 31: 1043-1049.

Mandotra SK, Kumar P, Suseela MR, Nayaka S, Ramteke PW. (2015). Evaluation of fatty acid profile and biodiesel properties of microalga *Scenedesmus abundans* under the influence of phosphorus, pH and light intensities. Bioresource Technology. 201: 222-229.

Mata TM. (2010). Microalgae for biodiesel production and other applications: are view. Renew able and Sustainable Energy Reviews. 14: 217-232.

Pancha I, Chokshi K, Mishra S. (2015). En-

hanced biofuel production potential with nutritional stress amelioration through optimization of carbon source and light intensity in *Scenedesmus* sp. CCNM 1077. Bioresource Technololgy. 179:565-72.

Pereira L. (2018). Seaweeds as source of bioactive substances and skin care therapy cosmeceuticals, algotheraphy, and thalassotherapy. Cosmetics. 5: 68. 2-41.

Pribyl P, Pilny J, Cepak V, Kastanek P. (2016). The role of light and nitrogen in growth and carotenoid accumulation in *Scenedesmus* sp. Algal Research. 16: 69-75

Rai PM and Gupta Sh. (2016). Effect of media composition and light supply on biomass, lipid content and FAME profile for quality biofuel production from *Scenedesmus* abundans. Energy Conversion and Management. 141: 85-92.

Rivkin RB. (1989). Influence of irradiance and spectral quality on the carbon metabolism of phytoplankton. I. Photosynthesis, chemical composition and growth. Journal of Marine Ecology Progress Series. 5: 291-304.

Rosenberg JN, Oyler GA, Wilkinson L, Betenbaug MJ. (2008). A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Journal of Current Opinion in Biotechnology. 19: 430-436.

Ruangsomboon S. (2012). Effect of light, nutrient, cultivation time and salinity on lipid production of newly isolated strain of the green microalga, *Botryococcus braunii* KMITL 2. Bioresource Technology. 109: 261-265.

Scott SA, Davey MP, Dennis JS, Horst I, Howe CJ, Lea-Smith DJ, Smith AG. (2010). Biodiesel from algae: challenges and prospects. Journal of Current Opinion in Biotechnology. 21: 277-286.

Shalaby EA. (2011). Algae as promising organisms for environment and health. Plant Signal Behaviour. 6 (9): 1338-1350.

Singh SP and Singh P. (2015). Effect of temperature and light on the growth of algae species: A review. Renewable and Sustainable Energy Reviews. 50: 431-444

Soltani N, BaftehChi L, Shokravi Sh. (2002). Survey on industrial and pharmaceutical aspects of algae with stress on species from Iran, Research project report.

Soltani N, Khavarinezhad R, Riahi H. (1992). Effects of photoperiod on growth status and pigments of *Scenedesmus brevispin*. MSc. Thesis. Tarbiat Moalem University.

Sun X, Cao Y, Xu H, Liu Y, Sun J, Qiao D, Cao Y. (2014). Effect of nitrogen-starvation, light intensity and iron on triacylglyceride/carbohydrate production and fatty acid profile of *Neochloris oleoabundans* HK-129 by a two stage process. Bioresource Technology. 155: 204-212.

Velichkova k, SirakovI, Georgiev G. (2013). Cultivation of *Scenedesmus dimorphus* strain for biofuel production. Agricultural Science and Technology, 5 (2): 181-185

Walker TL, PurtonS, Becker DK, Collet C. (2005). Microalgae as bioreactors. Journal of Plant Cell Reports. 24: 629-641.

Xue S, Su Z, Cong W. (2011). Growth of *Spirulina platensis* enhanced under intermittent illumination. Journal of Biotechnology. 151 (3): 271-277.

Yeesang C and Cheirsilp B. (2011). Effect of nitrogen, salt, and iron content in the growth

medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. Bioresource Technology. 102: 3034- 3040.

Zochleder V, Wittenburg E, Abarzua S. (1986). Factors controlling the inhibitory effects of 3, 4- benzo (a) pyrence on the chlorococcal alga *Scenedesmus quadricauda*. Algological studies, Archive fur Hydrobiologie, Supplement. 43: 281-296.