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SYNERGISTIC EFFECTS OF NITROGEN DEPRIVATION AND HIGH IRRADIANCE TO ENHANCE BIOMASS AND LIPID PRODUCTION IN *NANNOCHLOROPSIS*

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ABSTRACT

Nannochloropsis, due to its high lipid content, small size and growth rate, can be exploited for the production of biofuel and other value-added products. This study analyzed synergistic responses of high irradiance and nitrogen deprivation on *Nannochloropsis* cells to enhance biomass and lipid production. The growth of *Nannochloropsis* was first optimized under different irradiance 50, 100, 150, 200 and 250 µmol photons m^{-2} s^{-1}. The maximum specific growth rate (0.25 day^{-1}) and dry weight (0.67 g L^{-1}) were obtained ($p<0.05$) at photosynthetically active radiation (PAR) of 200 µmol photons m^{-2} s^{-1}, further increase in light intensity decreased the growth rate and dry weight up to 0.2 day^{-1} and 0.41g L^{-1}, respectively. The stress of high light for a short period increased the lipid content by 51.1% and decreased the protein content by 35.2% over the control values. In the second phase of the study, the combined stress of high light intensity of 200 µmol photons m^{-2} s^{-1} PAR and nitrate depletion (50%, 75%, 100% N^-) were studied and observed significant ($p<0.05$) increase in lipid content from 21.3±1.4% to 43.2±1.56% DCW and decrease in protein from 30.7±1.3% to 16.3±1.2% DCW, respectively. The highest lipid productivity (140 mg L^{-1} d^{-1}) had been observed in a culture grown at 75% N^- starved cells ($p<0.05$). However, the further increase of nitrate deprivation to 100% decreased the lipid productivity to 107 mg L^{-1} d^{-1}. Maximum accumulations of TG (19.6% DCW) were observed on 6 days (early stationary phase) in a 100% nitrogen deprived medium, although significant accumulation was observed on 4 days and 6 days of nitrate reduction.

Keywords: Nitrogen deprivation, light intensity, *Nannochloropsis*, lipid productivity, biomass

INTRODUCTION

Depleting energy reserves, soaring fuel prices and adverse environmental impact have raised a global debate on the search for a sustainable source of energy. Recently biofuel has been recognized as a promising renewable feedstock for the long-term replacement of fossil fuels and increasing the security of energy supply. Among numerous biomass feedstock's, microalgae are considered a realistic approach to supplant fossil fuels **(Schenk *et al.*, 2008)**. Since the use of terrestrial plants for biofuel generation may lead to biodiversity loss, raising the prices of food crops and consequently affected the food security and global food market. Unlike terrestrial crops, they can grow in wastewater, saltwater and non-arable land **(Rashid *et al.*, 2013; Razzak *et al.*, 2013; Malcata, 2011)**. In addition, they have a higher growth rate (10-50 times to conventional crops), high photosynthetic efficiency to fix CO_2, higher yield per hectare, higher lipid content (50%–70% dry cell weight) and wide seasonal tolerance compared to other energy crops.
Furthermore, microalgae use sunlight and carbon dioxide to produce valuable commodities that could be used to produce biofuel and additional feedstock for foods, feeds, and chemicals **(Spolaore 2006; Chisti 2007)**. Several marine microalgae such as *Botryococcus, Phaeactulum, Isochrysis, Nannochloropsis, Chlorella sp., Arthrospira sp.* have been exploited for biodiesel feedstock due to its high lipid/carbohydrate content, desirable composition and have high lipid/carbohydrate accumulation capability in adverse conditions **(Bondioli *et al.*, 2012; San Pedro *et al.*, 2013, Mishra and Prasad 2021)**. Currently, oleaginous marine microalgae species have been considered a promising biodiesel feedstock due to their high ability to produce storage materials in adverse conditions.
Nannochloropsis sp. is a photoautotrophic oleaginous marine microalga belonging to Eustigmatophyceae. Since the century, it has been used in aquaculture as feeds for bivalves and larva of fish, crustaceans, and molluscs due to its high nutritional value, small size and rapid growth rate **(Camacho-Rodríguez *et al.*, 2017)**. It possesses excellent metabolic and photosynthetic pathways that can be exploited to produce biofuel and other value-added products in pharmaceuticals, nutraceuticals, and aquaculture industries. In addition, due to their small size and higher growth rate, they are used as an emerging model in genetic engineering **(Radakovits *et al.*, 2010; Kilian *et al.*, 2011)**. It is known for its high lipid content and tolerance to extreme environmental conditions. It has a remarkable ability to accumulate lipids up to 65–70% of its dry weight under adverse environmental conditions **(Slocombe, 2015)**. Generally, lipids were accumulated in triacylglycerols (TAG) forms consisting of saturated and monosaturated fatty acids and thus may consider as a suitable precursor for biodiesel production. In addition, the growth characteristic and biochemical composition of biomass are greatly affected by the various nutritional and environmental factors (nitrogen, light supply, temperature, pH and salinity which may consequently result in oxidative stress **(Kim *et al.*, 2014; Bartley *et al.*, 2016)**. Nitrogen is the vital constituent of macromolecules such as enzymes, proteins, chlorophyll and genetic materials. Several researchers documented that nitrogen limitation significantly affects photosynthesis efficiency, growth rate and cell division **(Cai et al., 2013; Hu, 2013)**. Nitrogen starvation is considered an essential inducer for lipid accumulation in microalgae cells. The oxidative stress induced by nitrogen starvation leads to switching the cellular metabolism towards synthesizing triacylglycerols (TAGs) stored in systolic lipid bodies. Production of these storage materials is species-specific; generally, oleaginous species preferred lipid accumulation (up to 60%) **(Slocombe, 2015)**. The lipid productivity could be increased by genetic engineering of strain and innovation of culture technologies. It had hypothesized that high irradiance also stimulates the growth and synthesis of lipid, especially triglycerides **(Roessler 1990; Hu *et al.*, 2008)**. The stress of high light intensity (before photoinhibition) causes energy imbalance, stimulating a higher TAG production **(Parmar *et al.*, 2011)** and antioxidant.
The study's objective was to determine the synergistic effects of nitrogen deprivation and high irradiance to enhance biomass and lipid production in *Nannochloropsis* under controlled laboratory conditions. Therefore, the first optimum photosynthetic active radiation (PAR) required to produce higher biomass and lipid was investigated. Then the cells were inoculated to nitrogen deprived (50% N^-, 75% N^- and 100%N^-) culture medium to accumulate lipid and carbohydrate. This work would enable devising strategies to facilitate the search for an approach to optimize *Nannochloropsis* cultivation with enhanced lipid productivity. Thereby increasing its potential for its utilization in biofuel industries.

MATERIALS AND METHODS

Experimental organism and growth medium

The starter culture of marine microalgae *Nannochloropsis sp.* was obtained from the Center of Marine and Fishery Research Institute (CMFRI) Kochi, India. The *Nannochloropsis* cells were cultured in autoclaved natural seawater, supplemented with f/2 medium (Guillard, 1975). The f/2 culture medium was composed of 75 mg L^{-1} $NaNO_3$, 5 mg L^{-1} $NaH_2PO_4H_2O$, 1 ml L^{-1} of trace metal solution (3.15 mg L^{-1} $FeCl_36H_2O$, 4.36 mg L^{-1} Na_2EDTA, 0.0098 mg L^{-1} $CuSO_45H_2O$, 0.0063 mg L^{-1} $Na_2MoO_42H_2O$, 0.022 mg L^{-1} $ZnSO_47H_2O$, 0.01 mg L^{-1} $CoCl_26H_2O$, 0.18 mg L^{-1} $MnCl_2$ $4H_2O$) and 0.5 ml L^{-1} of vitamin solution (0.001 mg L^{-1} vitamin B_{12}, 0.2 mg L^{-1} vitamin B_1, 0.001 mg biotin^{-1}).

Experimental Design

The batch culture of *Nannochloropsis* was maintained at a controlled temperature of 22±1^{0}C, pH 8-8.2 under the photosynthetically active radiation (PAR) of 75µmol photons m^{-2} s^{-1} with a 16/8 h of light/dark period and referred to as control. Exponentially grown *Nannochloropsis* culture was exposed to different light intensities 50, 100, 150, 200 and 250 µmol photons m^{-2} s^{-1} and then sequentially subjected to nitrogen-deprived medium (50% N^-, 75% N^- and 100% N^-**)**. For particular stress conditions, cells were harvested by centrifugation (3,000 rpm for 5 min) and resuspended in the corresponding experimental medium. The stress of nitrogen was given for 5 days, and then cells were harvested by centrifugation, freeze-dried and analyzed for their biochemical compositions. Manual shaking of cultures was done 3-4 times regularly. The early exponential phase cultures were used for each experiment.

Determination of growth and biomass productivity

The growth of *Nannochloropsis* cells was estimated in terms of specific growth rate and dry cell weight (DCW), according to **Guillard (1975)**. The growth of cells was estimated by recording the absorbance of culture (A_{680nm}) with the help of a UV-VIS spectrophotometer (model 1700, Shimadzu, Japan). The specific growth rate of each culture was calculated from the slope of the linear regression of time and natural log optical density (OD) in the exponential growth phase.

$$K= (In\ N_1\text{-}In\ N_0)/ (t_1\text{-}\ t_0) \qquad (1)$$

Where N_0 was cell concentration at OD 680 nm at the beginning of the exponential phase (t_0) and N_1 represents cell concentration at OD 680 nm in time (t_1) of the exponential phase **(Arredondo et al., 2017)**

For dry weight measurement, culture samples (10 ml) were centrifuged at 5000 rpm for 5 min, and after rinsing twice with ammonium formate buffer, the pellets were dried 6 h in an oven at 100 ^{0}C.

Biochemical composition

The crude protein was determined by spectrophotometer at 650 nm according to the modified Lowery method **(Lowry, 1951)** modified by Herbert **(Herbert, 1971)**. The carbohydrate content was estimated with phenol-sulfuric acid as described by the Dubois method with slight modification. The purple colour of the sample was determined against the blank at 490 nm in a UV-visible spectrophotometer. Lipid was extracted by solvents: chloroform-methanol-water with a volume ratio of 2:1:1 (v/v/v) according to Bligh and Dyer's method **(Bligh and Dyer, 1959; Fakhry and El Maghraby, 2015)**. Chlorophyll *a* and carotenoids were extracted at 4 ^{0}C with 80% acetone, and the amount was estimated according to the method of Ritchie **(Ritchie, 2006)**.

Lipid productivity

The lipid productivity was calculated according to Mata *et al.* (2016) by the following equation:

$$LP= (C_f \times DCW_f - C_i \times DCW_i)/T) \qquad (3)$$

Where LP is lipid productivity, and T is the cultivation time (d); Cf and Ci are the final and initial content (%) of algal lipid during cultivation time, and DCW_f and DCW_i are the final and initial biomass concentration (mg L^{-1}) of microalgae.

Statistical Analysis

All experiments were done with three replicates, and data represent the means ± SD. One-way ANOVA analyzed them, and significant differences between treatments were tested using Duncan's multiple range test (DMRT). Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) 10.0.

RESULTS

Optimization of high irradiance

Growth and biomass production in relation to irradiance

Light intensity is one of the essential vital factors influencing the growth rate and biomass composition. Effect of different irradiance 50, 100, 150, 200 and 250 µmol photons m^{-2} s^{-1} on growth parameters of *Nannochloropsis* were illustrated in figure 1. The significant effect ($p \leq 0.05$) of irradiance on specific growth rate (figure 1a) and biomass (figure 1b) were observed. In this work, maximum specific growth rate (0.25 day^{-1}) and dry weight (0.67 g L^{-1}) were obtained at photosynthetically active radiation (PAR) of 200 µmol photons m^{-2} s^{-1}, further increase in light intensity was found to decrease the growth rate and dry weight up to 0.2 day^{-1} and 0.41g L^{-1}, respectively. There had been a linear relationship between growth and irradiance up to 150 µmol photons m^{-2} s^{-1}. At 200 µmol photons, m^{-2} s^{-1} of PAR, no significant effect on growth enhancement were observed, as shown in figure 1. However, the further increase of irradiance has a negative impact on the growth of cells.

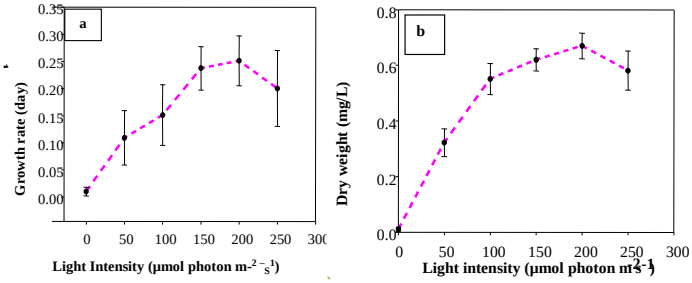


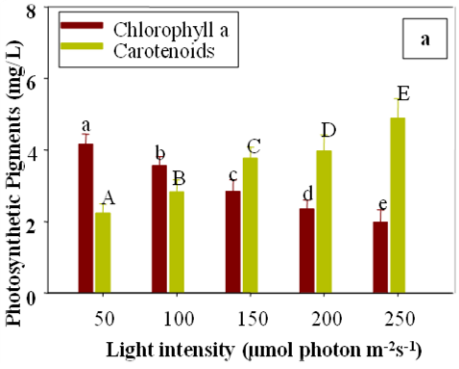
Figure 1 Growth of *Nannochloropsis* concerning the different light intensity

Photosynthetic pigments and biochemical composition

The effect of different light intensities on photosynthetic pigments of *Nannochloropsis* cells has *been* elicited in figure 2A. Results revealed that chlorophyll *a* content (primary photosynthetic pigment) continued to decrease from 4.65 mg L^{-1} to 1.98 mg L^{-1} and carotenoid content increased from 2.24 mg L^{-1} to 4.89 mg L^{-1} with an increase in irradiance (50 µmol photons m^{-2} s^{-1} to 250 µmol photons m^{-2} s^{-1}). Results of biochemical composition, as illustrated in figure 2B, which depicted that stress of high light for a short period increased the lipid content by 51.1% and decreased the protein content by 35.2% over the values of control. At 250 µmol photons, m^{-2} s^{-1} of PAR maximum accumulation of storage material was observed with lipid content of 32.5%. In contrast, the maximum protein content of 29.2% of DCW had been observed at a low light intensity of 50 µmol photons m^{-2} s^{-1}. The result of this work revealed that stress of high irradiance (200 µmol photons m^{-2} s^{-1}) favours the biomass production and accumulation of lipid at the expense of protein.

The stress of high irradiance and nitrogen deprivation on *Nannochloropsis*

This is the second phase in which combined stress of high light intensity of 200 µmol photons m^{-2} s^{-1} PAR and nitrogen deprivation were studied. First, the cells were grown in nitrogen sufficient medium to acclimatize them in the nitrogen-rich medium. Then cells in the active phase were harvested and then resuspended in the corresponding nitrogen deprived medium. The stress was given for 5 days to ensure the physiological changes and lipid accumulation, as shown in figure 3. Then cells were harvested, freeze-dried and analyzed.



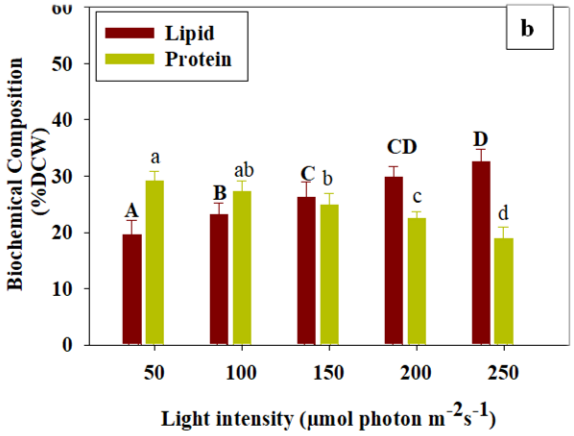
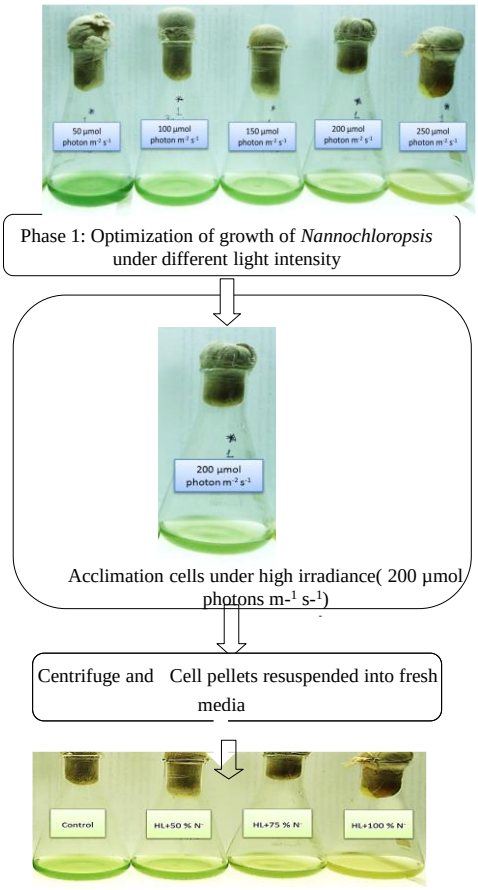


Figure 2 Biochemical composition of *Nannochrolopsis* at different light intensities



Phase 2: Lipid accumulation in different nitrate concentration

Figure 3 experimental design for the synergistic impact of light and nitrate deprivation on *Nannochloropsis* cells

Growth and biomass production

To evaluate the effect of nitrate deprivation on growth, absorbance at 680 nm was measured. Figure 4A shows the growth responses of *Nannochloropsis* cells cultivated in the culture medium with 80 mg L^{-1} (control), 40 mg L^{-1} (50% N^-), 20 mg L^{-1} (75% N^-) and 0 mg L^{-1} (100% N^-). The significant effect of nitrate deprivation on specific growth rate and biomass were observed. Culture growing with the highest level of sodium nitrate in the medium (80 mg L^{-1}) showed a significantly higher specific growth rate (0.217 day^{-1}) and dry cell weight (1.02 g L^{-1}) as shown in figure 4B and 4C, respectively. Nitrogen starvation for 2 days shows no significant effect on growth, and biomass yield varies from 0.33g L^{-1} to 0.22g L^{-1}. The biomass content significantly decreased with deprivation of nitrate in culture medium after 5 days from 1.02 to 0.43 g L^{-1} in a dose-dependent manner. Results revealed that deprivation of nitrate (40 mg L^{-1}, 20 mg L^{-1} and 0 mg L^{-1}) lowers growth in term dry weight by 19.6%, 38.2% and 57.8% over control values, respectively.

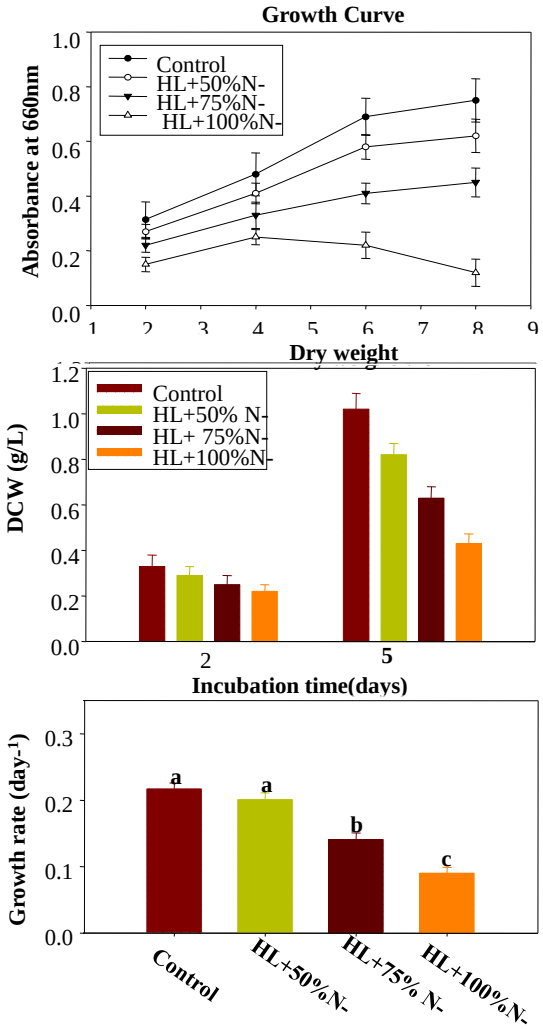


Figure 4 Growth response (A. absorbance at 660nm, B. Dry cell weight, C. Growth rate) of *Nannochloropsis* in different nitrate concentration

The growth curve was obtained by measuring optical density at 680 nm and reported a steadily decline in cell division with nitrogen deprivation. Figure 4A elicited that cell can grow even in complete nitrogen deprivation but with a reduced rate. It has shown that growth was comparable during the first two days, although, after the third day, significant differences in the growth phases were observed among treatments. The results indicated that nitrogen deprivation affects the growth negatively, but even with incomplete removal of nitrogen source in the medium, cells were able to grow.

This was confirmed by dry weight measurements, which observed no significant difference in the first two days. However, after 5 days, a significant difference ($p \leq 0.05$) was observed under different nitrogen deprived conditions. Inconsistent with this, several studies explained that cells grow and survive at the expense of the catabolism of intracellular metabolites **(Ho, 2014)**. A recent study hypothesized that under nitrogen starvation conditions, PUFAs are stored in TAG, allowing its rapid incorporation into plastid membranes upon more favourable growth conditions along with such translocation of TAG; EPA was also de novo synthesized **(Janssen *et al.*, 2019)**. Due to the enhanced formation of triacylglycerols (TAG) under nitrogen deprivation, light intensity and salinity increased cellular content of dry weight and lipids **(Pal *et al.*, 2011)**.

Photosynthetic pigment and biochemical composition

To test the possible consequences of N starvation on photosynthesis, measured photosynthetic pigments were after 5 days of N depletion. Since chlorophyll is a nitrogenous compound, its content and composition are greatly influenced by nitrate concentration in the growth medium. Figure 5 illustrated that with the limitation of nitrate content, photosynthetic pigments and carotenoids decreased significantly ($p \leq 0.05$). Reduction in nitrate in cultivation medium has reduced the total chlorophyll and carotenoids by about 3.55% to 0.95% DW and 3.35% to 2.1% DW, respectively. It has been hypothesized that a high irradiance supply under nitrogen-limited conditions increased the production of storage materials (lipid and carbohydrates). However, this accumulation of lipid and carbohydrate are species-specific. Figure 6A elicited that the combined effect of nitrogen deprivation and high PAR (200 µmol photons m^{-2} $s^{-1)}$ increase the lipid content from 21.3±1.4% to 43.2±1.56% DCW and decreases protein from 30.7±1.3% to 16.3±1.2% DCW,

respectively. It was also observed that there was a negative correlation between DCW and protein but a positive correlation between DCW and lipid accumulation, as shown in figure 6B and 6C.

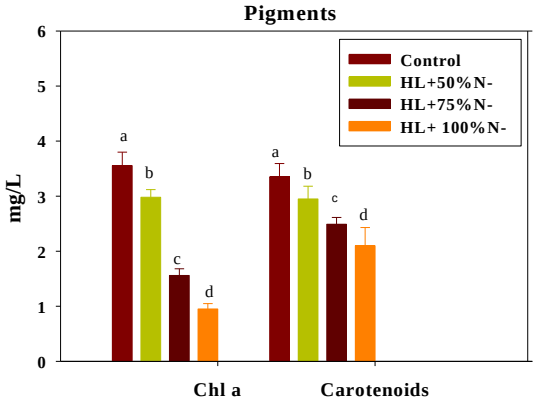


Figure 5 Variation of photosynthetic pigments with nitrate depletion

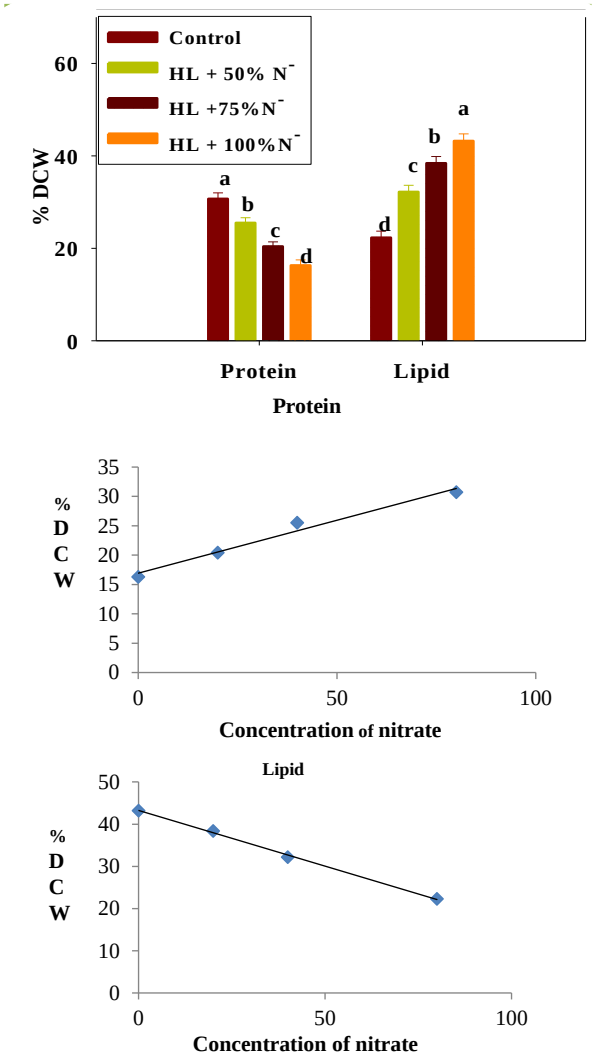


Figure 6 Effect of nitrate depletion on biochemical composition lipid and protein of *Nannochrolopsis* under high irradiance (200 umol photons m^{-2} s^{-1}) (A), % DCW trend shows increasing trend with protein in different nitrate concentration (B), % DCW trend shows increasing trend with lipid in different nitrate concentration (C)

It also examined the effect of nitrogen deprivation in TG accumulation at different growth phases. Figure 7 elicited that initially, on 2 days (early exponentially phase), there is no significant difference in TG accumulation. However, on 4 days and 6 days, reducing nitrogen in the medium produces a significant effect on TG content. Maximum accumulations of TG (19.6% DCW) were observed on 6 days (early stationary phase) in a 100% nitrogen deprived medium. In this study, lipid content and productivity as a function of nitrate deprivation was monitored under high irradiance of 200 µmol photons m^{-2} s^{-1} to increase lipid productivity. The highest lipid productivity (140 mg L^{-1} d^{-1}) had been observed in a culture grown at 75% N^- starved cells (figure 8).

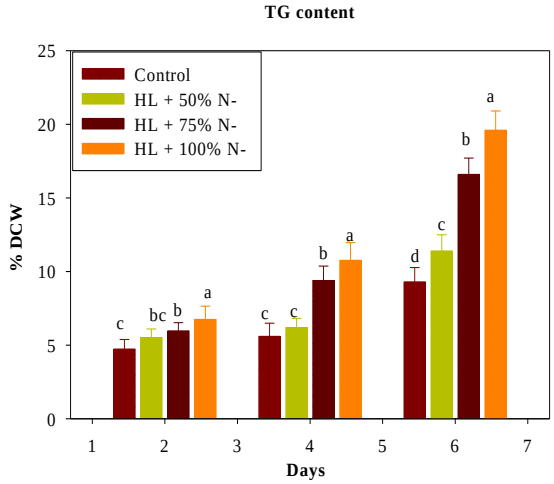


Figure 7 Accumulation of TG under different nitrate concentration

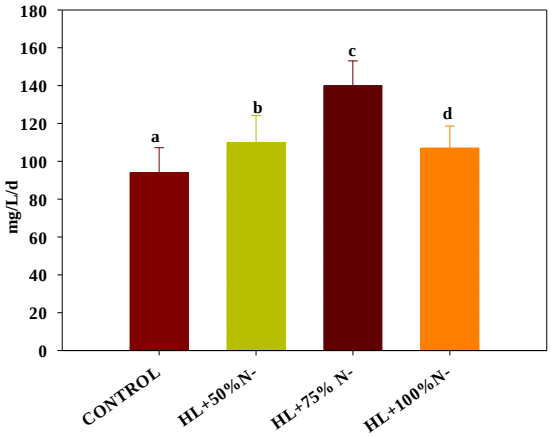


Figure 8 Combined effect of high irradiance and nitrate deprivation on lipid productivity

However, the further increase of nitrate deprivation to 100% decreased the lipid productivity to 107 mg L^{-1} d^{-1}. The result that emerges from this study reveals that 75% nitrate deprivation in the medium under a high irradiance supply of 200 µmol photons m^{-2} s^{-1} favours the growth and lipid productivity in cells. The result that emerges from this study reveals a synergistic effect of 75% nitrate deprivation in the medium under a high irradiance supply of 200 µmol photons m^{-2} s^{-1}, favouring the growth and lipid productivity in *Nannochloropsis* cells.

DISCUSSION

Effect of different irradiance on *Nannochloropsis*

Growth in terms of specific growth rate and dry weight reveals that stress of high light intensity stimulates the growth of *Nannochloropsis* more efficiently than low light intensity; this could be because the light is the energy source for photoautotroph's **(Sforza *et al.*, 2012)**. Since *Nannochloropsis* is photoautotrophic, microalgae utilize light energy to fix carbon dioxide (CO_2) into valuable biomass. In the present study, there had been a linear relationship between growth and light intensity in an intensity-dependent manner up to a specific limit (200 µmol photons m^{-2} s^{-1}). Over this limit, further increase in light intensity did not result in growth enhancement, suggesting that the growth was saturated at 200 µmol photons m^{-2} s^{-1} and further increase of light intensity causes photoinhibition by disrupting the chloroplast and inactivating the enzymes required for CO_2 fixation resulting in slower growth rate **(Al-Qasmi *et al.*, 2012; Ho *et al.* 2012)**. Our results are inconsistent with previous studies, which reported that the stress of highlight intensity before photoinhibition increases the growth rate **(Xue *et al.*, 2011; Wahidin *et al.*, 2013)**.

Nannochloropsis have unusual photosynthetic apparatus that contains only chlorophyll *a*, which can become accustomed to a wide range of light intensities **(Sforza *et al.*, 2012)**. Since under high irradiance, cell synthesis less photosynthetic unit (chlorophyll *a*) and accumulates carotenoids to protect the photosynthetic apparatus from oxidative stress. The light intensity higher than this saturation point may cause damage to the photosynthetic receptor system. This photo-oxidation to photosynthesis apparatus would be avoided by decreasing the photosynthetic energy conversion efficiency in PSII, primary photosynthetic pigments and

increasing carotenoid content. Microalgae can tolerate high photosynthetic active irradiance with necessary cell adaptation, such as they increase their size and diverting the fixed carbon to lipid synthesis to survive in stressful environments **(Leal *et al*., 2013)**. The yield can be increased with multiple approaches, such as genetic engineering and process optimization, such as strain improvement. It has been reported that stress of high light intensity (before photoinhibition) causes energy imbalance, which would stimulate the growth and synthesis of lipid. Results showed the highest accumulation of lipid content at active photosynthetic radiance (PAR) of 250 µmol photons m^{-2} s^{-1}. This would be because more light energy supply induces carbon allocation for lipid synthesis in triglyceride (TAG), the primary storage product produced in stress conditions and can be easily reutilized to provide metabolic energy **(Masojidek *et al*., 2004; Sasaki and Nagano 2004)**. However, the highest yield has been observed at 200 µmol photons m^{-2} $s^{-1,}$ and further increase of irradiance lowers the yield. Therefore, the light intensity of 200 µmol photons m^{-2} s^{-1} would be optimized and ideal for performing all the experiments at this optimized irradiance (PAR).

Effect of nitrogen depletion on *Nannochloropsis*

Since nitrogen is a significant component in many biological macromolecules like protein, chlorophyll, DNA **(Hu *et al.*, 2008)**, nitrogen starvation has an adverse effect on the synthesis of macromolecules involves enzymes, proteins, nucleic acid, chlorophyll *a* and thereby cell division, metabolism and growth **(Nigam *et al.*, 2011)**. In this reference, *Nannochloropsis* were first grown at an intensity of 200 µmol of photons m^{-2} s^{-1} 3 days in an F/2 medium containing nitrogen (80 mg L^{-1} $NaNO_3$). Cells were then harvested and resuspended in the corresponding nitrogen deprived medium for 5 days. Then cells were harvested by centrifugation, freeze-dried and analyzed.

The growth curve was obtained by measuring optical density at 680 nm and reported a steadily decline in cell division with nitrogen deprivation. Figure 4 elicited that cells can grow even in complete deprivation of nitrogen but with a reduced rate. It has been shown that during the first two days, growth was comparable with other treatments, although, after the third day, significant differences in the growth were observed. The results indicated that nitrogen deprivation negatively affects growth, but cells could grow incomplete nitrogen removal in the medium. This was confirmed by measurements of the dry weight, which observed no significant difference in the first two days, although after 5 days, significant differences ($p \leq 0.05$) were observed under different nitrogen deprived conditions. Inconsistent with this, several studies explained that cells grow and survive at the expense of the catabolism of intracellular metabolites **(Ho 2014)**. Maximum growth rate (0.217 day^{-1}) were observed in control, and complete removal of nitrate in medium reduced the growth rate to 58.2% over control. To examine the possible consequences of N starvation on photosynthesis, photosynthetic pigments were measured after 5 days of N depletion. Since chlorophyll is a nitrogenous compound, its content and composition are greatly influenced by nitrate concentration in the growth medium. Several previous studies reported a decrease in the Chl*a* concentration under nitrogen scarcity in many microalgae species **(Wang *et al.*, 2013).**

Nitrogen starvation is considered an essential inducer for lipid accumulation in microalgae cells. It leads to switching the cellular metabolism towards the synthesis of triacylglycerides (TAGs) stored in systolic lipid bodies. Production of these storage materials is species-specific; generally, oleaginous species has excellent ability to accumulate lipids up to 65–70% of their dry weight under adverse environmental conditions. Similarly, the result of the present work showed that nitrogen starvation increased the accumulation of lipid and decreased protein content. When the nitrogen is limited in the culture medium, microalgae slow down the cell growth rate and shifts anabolic pathways from protein synthesis to produce reserve substances like carbohydrates or lipid **(Ho *et al.*, 2014)**. This behaviour is consistent with numerous studies that demonstrated that under N-limited conditions, lipid and carbohydrate contents were improved in many algal species like *Neochlorisoleoabundans* HK-129 **(Sun *et al.*, 2014)**. The general principle is that when there is insufficient nitrogen (N) in the medium for N assimilation and amino acid biosynthesis, excess carbon from photosynthesis is directed towards producing storage molecules such as especially triglyceride or starch **(Scott *et al.*, 2010)**. Previous studies indicated that lipid was the primary carbon sink in *Nannochloropsis* under nitrogen stress. Lipids were accumulated in triacylglycerols (TAG) forms consisting of saturated and monosaturated fatty acids and thus may consider as a suitable precursor for biodiesel production as readily converted into diesel through trans-esterification. Therefore, N limitation could increase lipid and triglyceride content in microalgal cells. However, it lowers the growth rate and lipid productivity, considered the main bottleneck in utilizing microalgae for biodiesel production. **Rodolfi *et al.* 2009** observed the positive effect of irradiance and nutrient deprivation on lipid accumulation and productivity. It reasoned that stress of high light intensity in nitrogen-limited conditions leads to an increase in the energy imbalance due to the accumulation of reluctant photosynthetic power, which would stimulate an even higher TAG production. This work reported that under the combined stress of nitrogen deprivation and high irradiance, lipid productivity increases from 140-240 mg L^{-1} d^{-1} in *Nannochloropsis sp.* **(Chiu *et al.*, 2009; Fakhry and El Maghraby 2015; Nigam *et al.*, 2011)**. However, 100% nitrogen deprivation decreased the lipid productivity because nitrogen starvation has an adverse effect on synthesizing macromolecules involving enzymes, proteins, nucleic acid, chlorophyll *a* and thereby cell division, metabolism and growth **(Nigam *et al.*, 2011)**.

CONCLUSION

Microorganisms can adjust under different environmental conditions via utilizing their defense system, which includes the generation of antioxidants, synthesis of proteins and secondary metabolites, and more accumulation of lipid and carbohydrates. The present study concludes that the high light intensity of up to 200 µmol photons m^{-2} s^{-1} has significantly raised the specific growth rate and dry weight. However, future increases in irradiance have decreasing effect on the growth rate. Therefore, the light intensity of 200 µmol photons m^{-2} s^{-1} would be optimized and ideal for performing all the experiments at this optimized irradiance (PAR). Nitrogen starvation is considered an essential inducer for lipid accumulation in microalgae cells. It observed that high irradiance supply under nitrogen-limited conditions increased the production of storage materials (lipid and carbohydrates). This work reported that lipid productivity increases from 140-240 mg L^{-1} d^{-1} in *Nannochloropsis sp* under the combined stress of nitrogen deprivation and high irradiance. So, for better accumulation of lipid in the studied organism, a combination of 75% nitrate deprivation in the medium under high irradiance supply of 200 µmol photons m^{-2} s^{-1} may have opted as a novel approach for more production of lipid and its further utilization in biofuel industries as an alternate for all available synthetic fuels.

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