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RESEARCH ARTICLE

OPTIMISING NUTRIENTS ENHANCING BIOMASS AND LIPID CONTENT OF *Nitzschia palea* IN CSI MEDIA

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ABSTRACT

Effect of different carbon source, nitrogen source and different concentration of silica, iron and salt in the growth medium on growth, biomass and lipid content of *N. palea* (Diatom) were investigated. The addition of high concentration of iron in Csi media significantly increase the growth, biomass and lipid content of *N. palea.* 0.05% (glucose), 0.05% (urea and nitrogen starvation), silica (100 mg/L and 20 mg/L), Ferric ethylene diamine tetra acetate (0.026Mm and 0.023mM) and salt (20ppt and 40ppt) showed a marginal rise in biomass and lipid content respectively. Among these, silica concentration and salt concentration played a major role in yielding highest biomass (0.838 ± 0.019 g L^{-1} dw, 0.88 ± 0.018 g L^{-1} dw) as well as highest lipid content (68.5 ± 0.84 %, 66.7 ± 1.12%) respectively. The present work highlights the importance of optimising culture media (Csi) in enhancing both biomass and lipid content to provide *N. palea* as efficient stain for biodiesel production.

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INTRODUCTION

Diatoms, unicellular microalgae possessing a silicon-based cell wall and belonging to the class Bacillariophyceae, are an ecologically successful taxonomic group of phytoplankton. These microalgae represent a large fraction of global primary productivity (Falkowski *et al.,* 1998; Brzezinski *et al.,* 2002; Granum *et al.,* 2005) and play fundamental roles in global nutrient cycling of carbon, nitrogen, phosphorus, and silicon (Werner, 1977; Tréguer *et al.,* 1995; Søndergaard and Jeppesen, 2005; Wilhelm *et al.,* 2006). In addition to their fundamental role in global nutrient cycles, diatoms represent a potential bioprocess platform for synthesizing biofuels and other value-added products (Hildebrand *et al.,* 2012). Biofuels are arguably more sustainable, technically feasible and environment friendly than the fossil fuels. They are relatively low net emitters of greenhouse gases (Slade and Bauen, 2013). Diatoms produce oil drops that are stored intracellularly as a reserve material during the vegetative period of growth, with percentages that vary from less than 23% to greater than 45% of dry cell weight (Hu *et al.,* 2008). Nutrient is the most important regulator of microalgal growth and lipid accumulation. Many microalgal species can synthesize large quantities of lipid and carbohydrates along with other bioactive molecules under varied nutrient supplement conditions. These newly synthesized lipids and carbohydrates can be directly converted to biodiesel and bioethanol, respectively (Scott *et al.,* 2010; Hu *et al.,* 2008; Griffiths and Harrison, 2009). Diatoms stress or unfavourable condition leads to the production of more lipids. Environmental parameters like pH, temperature, light, nitrogen, carbon, silicon, phosphorus, iron, salt concentration, etc affect the lipid composition of the diatoms. Different approaches have been used to enhance the biomass yield and lipid accumulation in microalgae for the last few years. All of them have concluded that improved culture strategies are needed to obtain high quality of oil in microalgal cells (Hu *et al.,* 2008; Abou-Shanab *et al.,* 2014; Guschina and Harwood, 2006). For economical production of biodiesel from microalgae, biomass and lipid content play an important role. Hence this study deals with the enhancement of biomass and lipid content of *N. palea* (Diatom) by altering the nutrient compositions of culture media.

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MATERIALS AND METHODS

Experimental setup

The experiment was carried out in 2 L flask containing 1 L of Csi medium pH 7.0 (Mc Laughlin and Zahl, 1959), with different nutrient compositions (carbon source, nitrogen source, silica concentration, iron concentration and salt concentration). The culture was cultivated under controlled conditions of 12 hours light and 12 hours dark cycle for 14

days at a constant temperature of 25 ± 1°C with a light intensity of 42µmoles/m^2/s. All the flasks were inoculated with uniform volume (10% v/v) of the *N. palea* and triplicates were maintained. The growth curve of *N. palea* (OD at 665nm) at different nutrient compositions were recorded continuously at an interval of 2 days. At 15th day the biomass was harvested, where the total biomass and total lipid content was determined.

Effect of different carbon source on *N. palea*

1 L of Csi medium with 0.05 % concentration of different carbon source (C - source) was introduced into each of the flasks. The different carbon source added in Csi medium was glucose, sucrose, glycerine, maltose and starch. Here Csi medium without C source (exact composition of Csi medium) was taken as control.

Effect of different nitrogen source on *N. palea*

1 L of Csi medium with 0.05 % concentration of different nitrogen source (N - source) was introduced into each of the flasks. The different nitrogen source added in Csi medium was urea, peptone, sodium nitrate and yeast. Here Csi medium without N source (Ca (No3)2. 4H2O and KNO3) was taken as control.

Effect of different Silica concentration on *N. palea*

Various concentrations (10mg/L, 20mg/L, 40mg/L, 60mg/L, 80mg/L and 100mg/L) of silica were incorporated in the Csi medium used for the cultivation of *N. palea* . Silica concentration of 10mg/L served as the control.

Effect of different iron concentration on *N. palea*

1 L of Csi medium with varying concentration of Fe-EDTA (Fe) was introduced into each of the flasks. The different concentration of ferric ammonium citrate added in Csi medium were 0.009mM, 0.014mM, 0.017mM, 0.020mM, 0.023mM and 0.026mM. Here 0.014mM concentration of Fe-EDTA in Csi medium served as control.

Effect of different salt concentration on *N. palea*

1 L of Csi medium with varying concentration of sodium chloride (NaCl) was introduced into each of the flasks. The different concentration of sodium chloride added in Csi medium were 10ppt, 20ppt, 30ppt and 40ppt. Here addition of 10ppt concentration of NaCl in Csi medium served as control.

Statistical Analysis

All the experiments were performed in triplicates. The results were plotted in graph using Origin (Version 8.0), and their data were presented as mean values ± S.D using Microsoft excel. The growth of *N. palea* were statistically analysed by two way Anova using MATLAB (7.8 Version).

RESULTS

Effect of different carbon source on *N. palea*

The growth curve (Fig 1) of *N. palea* reaches its peak early on 10th day at all carbon source except under glucose on 12th day of incubation. This diatom supplemented with glucose attained maximum OD value of 0.288 ± 0.009 and showed reduced growth under other carbon source especially low at maltose (OD 0.154 ± 0.005). Among the carbon source, glucose enhanced significant (P<0.001) growth of *N. palea* in Csi medium. Fig 2: shows the production of total biomass and lipid content of *N. palea* in Csi medium under different carbon source. Compared to control, both biomass and lipid content was recorded greater under glucose of 0.784 ± 0.009 g L^{-1} dw, 47.1 ± 0.37 % followed by starch of 0.613 ± 0.01 g L^{-1} dw, 46.5 ± 0.21 % respectively and resulted low biomass as well as lipid content in all other carbon source.

Effect of different nitrogen source on *N. palea*

The growth curve (Fig 3) of *N. palea* reaches its peak on 10th day of incubation. It showed higher growth at urea (OD 0.271 ± 0.005) followed by $NaNO_3$ (OD 0.259 ± 0.007) and absence of nitrogen source (control) had no impact on biomass production. Significantly highest growth rate (P<0.001) was obtained for *N. palea* cultivated in Csi media with urea as nitrogen source. Compared to control, the total biomass was increased under all nitrogen source added for the growth of *N. palea* . Similar to growth rate, maximum biomass was obtained under urea (0.714 ± 0.015 g L^{-1} dw) and recorded minimum biomass at control (0.479 ± 0.008 g L^{-1} dw). The lipid content was recorded highest at control (49.6 ± 0.38 %) followed by $NaNO_3$ (48.3 ± 0.33 %) and recorded lowest under peptone (38.1 ± 0.29 %). Both biomass and lipid content (Fig 4) was found to be optimum in Csi medium with the addition of $NaNO_3$.

Effect of different Silica concentration on *N. palea*

The growth curve (Fig 5) of *N. palea* increases according to the increasing concentration of silica and reaches its peak early on 10th day at all silica concentration except at 100 mg/L of silica concentration which reaches on 12th day of incubation. It attained maximum OD value of 0.341 ±0.009 under addition of 100 mg/L of silica in Csi medium and significantly higher (P<0.5) than the other silica concentrations. It grew more slowly (OD 0.244 ± 0.009) when silicon was limited (10 mg/L silica concentration). Compared to control, both biomass and lipid content (Fig 6) *N. palea* in Csi medium under different silica concentration increases according to the increasing concentration of silica (20 mg/L to 100 mg/L) but showed variation in lipid content. Maximum biomass was recorded in 100 mg/L (0.838 ± 0.009 g L^{-1} dw) of silica concentration and minimum at control (0.54 ± 0.009 g L^{-1} dw). Higher percentage of lipid content was recorded in silicate non-limited cultures (20 mg/L and 40mg/L silica concentration) of 68.5 ± 0.84 % and 66.3 ± 0.58 % respectively. Here optimum biomass with high lipid content was observed at 20 mg/L concentration of silica.

Effect of different iron concentration on *N. palea*

The growth curve (Fig 7) of *N. palea* increases according to the increasing concentration of iron and reaches its peak early on 10th day at all concentration except at 0.026 mM of iron on 12th day of incubation. The growth rate of *N. palea* attained maximum OD value of 0.312 ± 0.005 under addition of 0.026 mM of iron in Csi medium showed significantly (P<0.1) highest than the other concentrations. Here cultures grown under iron limitation showed a decrease in growth rate.

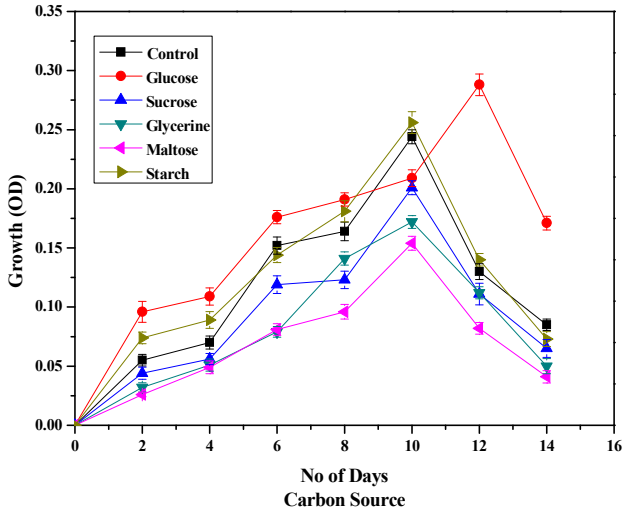

Fig.1. Effect of different carbon source on the growth of *N. palea*

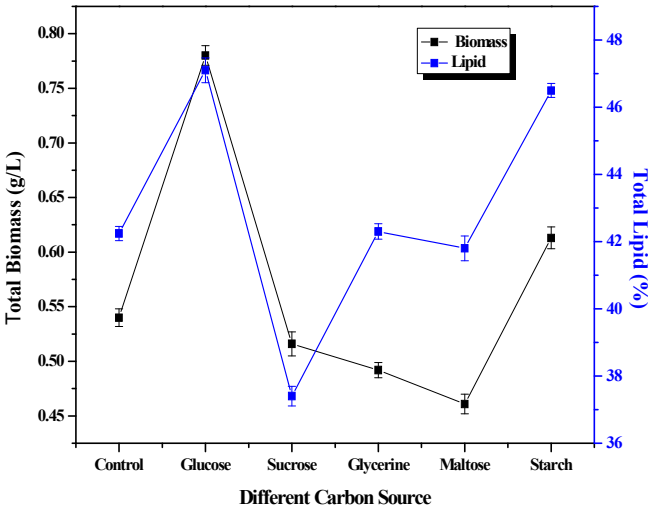

Fig.2. Effect of different carbon source on the biomass and lipid content of *N. palea*

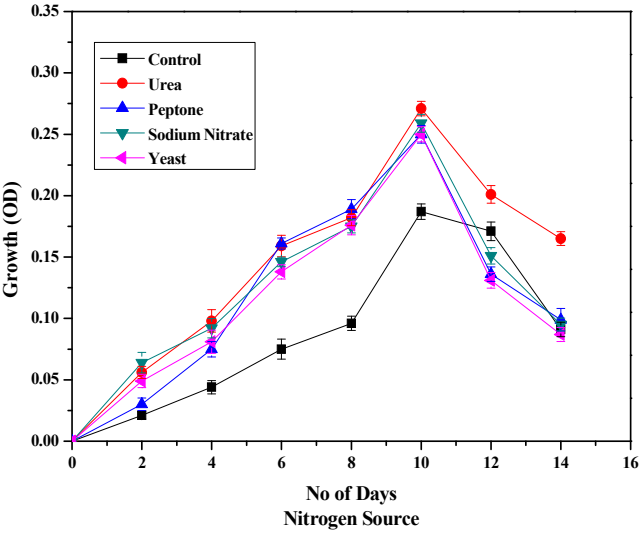

Fig.3. Effect of different nitrogen source on the growth of *N. palea*

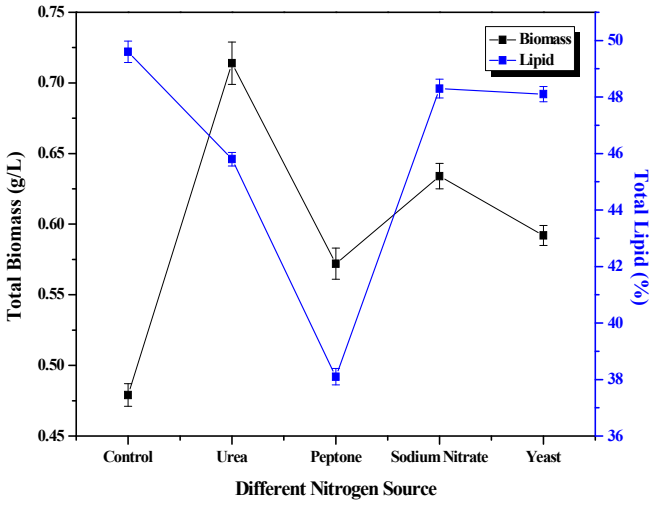

Fig.4. Effect of different nitrogen source on the biomass and lipid content of *N. palea*

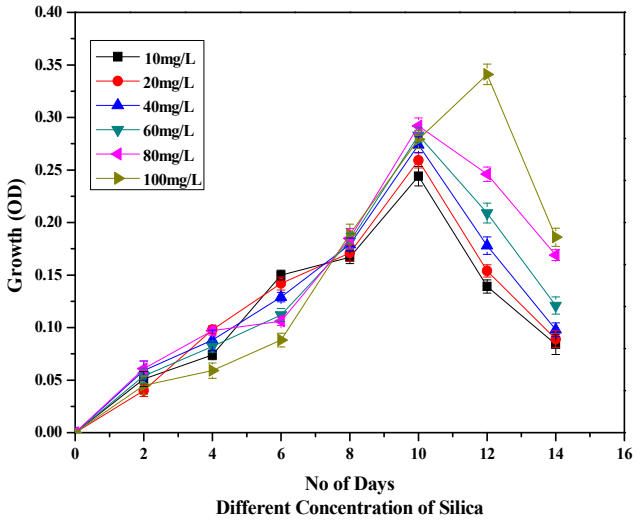

Fig.5. Effect of different Silica concentration on the growth of *N. palea*

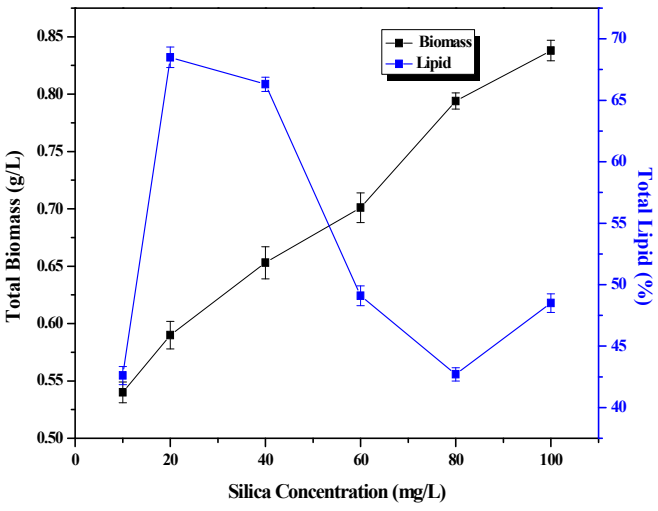

Fig.6. Effect of different Silica concentration on the biomass and lipid content of *N. palea*

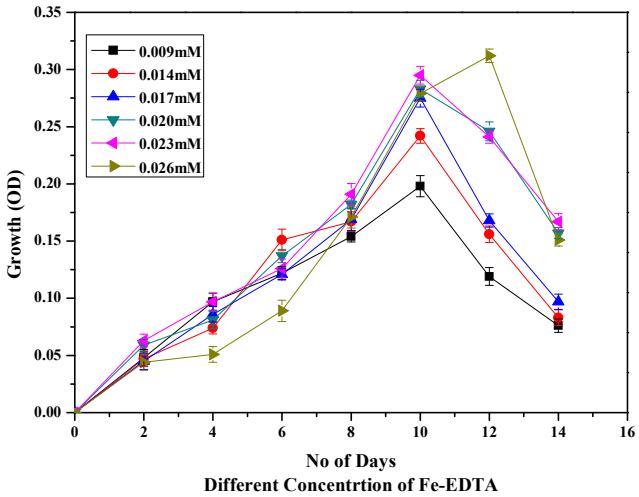

Fig.7. Effect of different iron concentration on the growth of *N. palea*

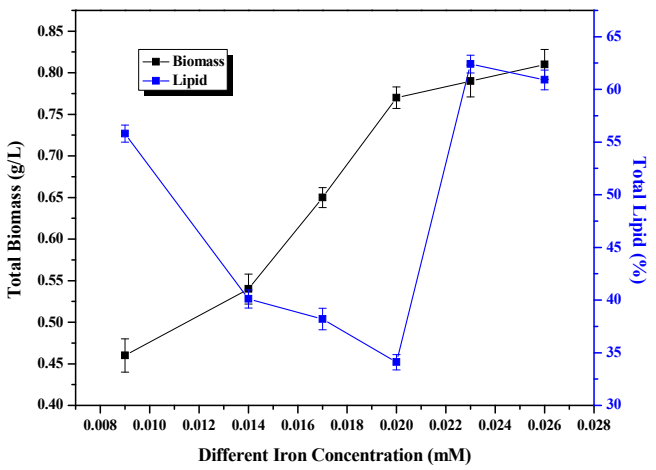

Fig.8. Effect of different iron concentration on the biomass and lipid content of *N. palea*

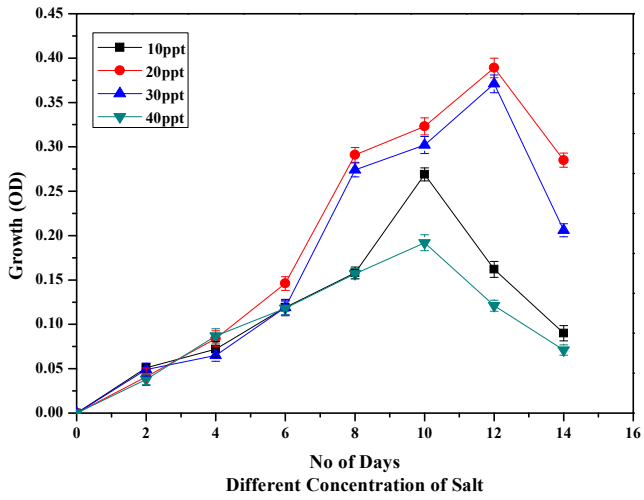

Fig.9. Effect of different salt concentration on the growth of *N. palea*

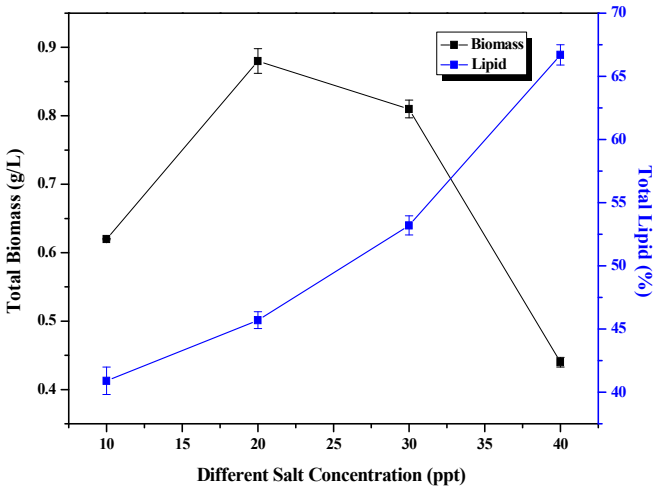

Fig.10. Effect of different salt concentration on the biomass and lipid content of *N. palea*

The biomass and lipid content of *N. palea* (Fig 8) increases according to the increasing concentration of iron (0.009mM to 0.026 mM) whereas maximum biomass was recorded in 0.026 mM (0.81 ± 0.018 g L^{-1} dw) of iron concentration followed by 0.023 mM (0.79 ± 0.019 g L^{-1} dw). Higher percentage of lipid content was also recorded in 0.023 mM (62.4 ± 0.84 %) followed by 0.026 mM (60.9 ± 0.94%)

Effect of different salt concentration on *N. palea*

N. palea showed significantly (P<0.01) highest growth rate (Fig 9) at 20 ppt (OD 0.389 ± 0.011) followed by 30 ppt (OD 0.371 ± 0.01) on 12th day and lowest at 40 ppt (OD 0.192 ± 0.008) on 10th day of incubation. Compared to control, the biomass was maximum at 20 ppt (0.88 ± 0.018 g L^{-1} dw) and minimum at 40 ppt (0.44 ± 0.007 g L^{-1} dw). The lipid content was increased at elevated level of NaCl (from20 ppt to 40 ppt). Maximum lipid content (66.7 ± 0.8%) with low biomass was observed at 40 ppt. Both biomass and lipid content (Fig 10) was found to be optimum at 30ppt concentration of NaCl.

DISCUSSION

Glucose promoted physiological changes in *Chlorella vulgaris*, which strongly affects the metabolic pathways of carbon assimilation, size of the cells, volume densities of storage materials, such as starch and lipids grains and protein, chlorophyll, RNA, and vitamin contents (Martinez and Orus, 1991). De Swaaf *et al.* (2003) found that the lipid synthesis in this *Chlorella vulgaris* occurs in the cytosol. This implies that, during growth with glucose, export of acetyl-CoA from the mitochondrial matrix to the cytosol is required to make it available for lipid synthesis. Similarly glucose promoted the growth, biomass and lipid content of *N. palea* at highest level in this study. Selecting proper nitrogen source for each algal species is important in improving biomass and oil productivity (Li *et al.,* 2008). Nitrate supported increased biomass compared to ammonium in Monoraphidium sp. SB2 (Wu *et al.,* 2013), Yingshen *et al.* (2009) reported that a maximum lipid yield of 0.40 g/l for autotrophic *Scenedesmus dimorphus* from biomass of the 1.8 g/l urea medium, and 5.89 g/l for heterotrophic *Chlorella protothecoides* from biomass of the 2.4 g/l nitrate medium were achieved. In contrast, urea and nitrate were found to be better than ammonia for the growth and lipid accumulation in *Chlorella* sp. and *Neochloris oleoabundans* (Liu *et al.,* 2008; Li *et al.,* 2008; Hsieh and Wu, 2009; Pruvost *et al.,* 2009). Similarly urea and sodium nitrate as nitrogen source increase the growth and biomass, as well as lipid content of *N. palea* . The major stress conditions like limitation of silicon and nitrate, mostly stimulates the lipids accumulation in diatoms. For maximizing the TAG content, the diatoms need to be cultivated in nutrient-deficient media (Samantray *et al.,*

2010). *N. palea* grown at nitrogen starvation showed higher lipid content than that grown under sodium nitrate. Silica plays a major role in the growth of diatoms and cellular lipid metabolism. In *Cylindrotheca fusiformis*, DNA replication is dependent on the concentration of silica (Darley and Volcani, 1969). Peipei *et al. (*2014) compared the growth rate of silicon starved P. tricornutum with that of normal cultured cells under different culture conditions. From the results of pigment analysis, photosynthesis measurement, lipid analysis, and proteomic analysis, he found that the presence of silicon plays an important role in the growth of P. tricornutum. The results of Moll *et al.* (2014) indicate that increasing the silica concentration will increase cell numbers, which is vital for improving algal biodiesel productivity in terms of increased biomass. Here the higher concentration of 30 to 100mg/L of silica concentration is essential for the growth as well as biomass of *N. palea*

Holmes (1966) suggested that cell division was inhibited due to lack of silicate in the medium. Hence there was a decreased growth rate of *N. palea* in limited supply of silica. Under silicon-depleted conditions of *Cyclotella cryptica*, higher levels of neutral lipids as well as higher proportions of saturated and monounsaturated fatty acids were obtained in comparison with silicon-replete cells. The total lipid was found to increase from 27.6% to 54.1% in *Cyclotella cryptica* under silicon starvation. In the present study, lipid yield was higher at low silica concentrations such as 20 mg/L (68.5% in *N. palea*) as reported earlier (Roessler, 1988). In *Nannochloropsis oculata,* the biomass and lipid gradually increased with increased concentration of EDTA (Dou *et al.,* 2013). Similarly maximum biomass and lipid content of *N. palea* was recorded at high iron concentration. The marine diatom*Amphiprora paludosa* synthesized 65.64% lipid under 0.026mM of Fe-EDTA (Mohan *et al.,* 2012). Iron limitation affects the growth of *N. palea* . Iron-limited conditions are thought to alter cell physiology by reducing cell volume, chlorophyll content, and photosynthetic activity, and thus appear to impact cellular accumulation more than lipid accumulation. Specifically in Phaeodactylum tricornutum, the following enzymes were down-regulated during iron-starvation: β-carbonic anhydrase, phosphoribulokinase (PRK), two RuBisCO enzymes and a HCO3 − transporter, likely resulting in decreased carbon fixation and cellular growth (Allen *et al.,* 2008). Increase in biomass production was reported in *B.braunii* at low salinity level of 17 to 34 mM. However, the growth rate was significantly reduced at higher concentration of NaCl. Similarly the increase in biomass was obtained in present study from 10ppt to 30ppt of NaCl concentration. The increase in lipid content at higher NaCl concentration maybe due to adaptation under stress conditions which helps in accumulation of lipid (Dujjanutat and Kaewkannetra, 2011). Similar result was reported in *N. palea* at high NaCl concentration. The effect of NaCl on *N. palea* was in accordance with Prakash *et al.* (2013). They found that though the biomass yield followed a negative trend with increasing concentration of NaCl in the growth medium, the overall lipid accumulation is significantly increased. This indicates that saline stress can favors lipid overproduction in these freshwater species.

Conclusion

In present study, silica and Fe-EDTA significantly induce maximum growth, biomass as well as lipid content of *N. palea* . All the tested nutrient factors played important role in biomass and lipid production. Hence it is possible to increase the biomass and lipid content of *N. palea* by optimizing the nutrient compositions (carbon source, nitrogen source, silica concentration, iron concentration and salt concentration) of Csi medium

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