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Effect of Temperature on the Growth of Marine Diatom, *Chaetoceros simplex* (Ostenfeld, 1901) with Different Nitrate: Silicate Concentrations

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ABSTRACT

Objective: To study the combined effect of temperature, nitrate and silicate on the growth, of the marine diatom, *Chaetoceros simplex* (Ostenfeld, 1901). **Methods:** Samples were analysed for the effect of temperature, nitrate and silicate on the growth, chlorophyll 'a', protein and carbohydrate contents. Totally fifteen experiments were conducted for 12 days under three different temperature (20, 25 and 29 °C), 68 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ light intensity and at five concentrations of nitrate: silicate proportions (882 $\mu\text{M} [\text{NO}_3^-]$ –106 $\mu\text{M} [\text{SiO}_3^{2-}]$, 1323 $\mu\text{M} [\text{NO}_3^-]$ –159 $\mu\text{M} [\text{SiO}_3^{2-}]$, 1764 $\mu\text{M} [\text{NO}_3^-]$ –212 $\mu\text{M} [\text{SiO}_3^{2-}]$, 2205 $\mu\text{M} [\text{NO}_3^-]$ –265 $\mu\text{M} [\text{SiO}_3^{2-}]$ and 2646 $\mu\text{M} [\text{NO}_3^-]$ –318 $\mu\text{M} [\text{SiO}_3^{2-}]$ respectively). **Result:** The maximum cell density reached 23.5×10^5 cells ml^{-1} with 1764 $\mu\text{M} [\text{NO}_3^-]$ and 212 $\mu\text{M} [\text{SiO}_3^{2-}]$ concentrations at 29 °C, in 10th day of culture. The high chlorophyll 'a' content of 1.57 ± 0.05 pg/cell at 20°C and 2205 $\mu\text{M} [\text{NO}_3^-]$ –265 $\mu\text{M} [\text{SiO}_3^{2-}]$. The high protein content of 4.48 ± 0.17 pg/cell was found in 2205 $\mu\text{M} [\text{NO}_3^-]$ –265 $\mu\text{M} [\text{SiO}_3^{2-}]$ at 25°C. The high carbohydrate contents of 0.78 ± 0.03 pg/cell were found in 1764 $\mu\text{M} [\text{NO}_3^-]$ –212 $\mu\text{M} [\text{SiO}_3^{2-}]$ at the temperature of 25 °C. **Conclusions:** The growth rate was directly proportional to nutrient concentration and temperature whereas chlorophyll 'a' and biochemical concentration was directly proportional to nutrient concentration. Based on the present results, future work on growth optimization with the other physical and nutritional factors will yield noteworthy information on the mass cultivation of *C. simplex* for aquaculture purposes.

1. Introduction

Microalgae contribute a wide range of commercial products from consumables such as feedstocks, essential oils and drugs, to energy resources and means for carbon capture through biofuel production [1–4]. Successful commercial utilization of microalgae has been established in the production of nutritional supplements, antioxidants, cosmetics, natural dyes and polyunsaturated fatty acids (PUFA). Combination of different algal species provides better balanced nutrition and improves animal growth better than a diet composed of only one algal species [5]. Protein and vitamin content is a major factor determining the nutritional value of microalgae. The nutritional quality of food sources mainly depends on many biochemical

constituents such as polyunsaturated fatty acids, vitamins, sterols and carbohydrates [6]. Thus, the characterization of growth parameters such as specific growth rate and biomass production is essential. Moreover, establishing the basic cellular composition (e.g. dry weight, protein, carbohydrate and lipid) allows researchers and applied practices to select appropriate strains and taxa for specific uses. Consequently, there is a continuous need to establish factors that alter these properties.

Phytoplankton growth and composition may be influenced by both physical parameters such as, light, temperature [7] and nutrients like nitrate, phosphate and silicate [8, 9]. In addition to the macro nutrients nitrate and phosphate proportions that are essential for the growth of all algae, diatoms also depend on the availability of silicic acid ($\text{Si}(\text{OH})_4$) to produce their frustules. Most guidelines on published studies of experiments with phytoplankton carried out between 20 and 30°C [10, 11] and it is suitable for the mass production. Even though phytoplankton photosynthesis influenced by temperature [12] these organisms may modify their pigment composition [13], enzyme activity [12, 14], lipid profile and membrane fluidity [15] with response to

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temperature variation. Several authors [16, 7] have proposed that acclimation of algal and cyanobacterial photosynthesis to low temperature is basically an acclimation to high photosystem II (PSII) excitation pressure.

In contrast to the high number of studies addressing nutrient limitation of phytoplankton growth [17–20] only a reduced number of studies deals with nutrient limitation in marine diatoms [20, 21]. Under changing macronutrient, nitrate and phosphate proportions in controlled incubations, the diatom, *Nitzschia seminae* showed a significant variability in skeletal morphology, as represented by the mean width of a centrally located pseudoseptum in the valve, whereas valvar size and shape were more constant [22]. Silicic acid limitation slows the rate of cell division process but does not affect cellular anabolic functions. In chemostat cultures of *Skeletonema marinoi* (formerly *costatum*) grown under $\text{Si}(\text{OH})_4$ limitation, for instance, lower Si/cell values were correlated with increased N/cell, P/cell and chlorophyll/cell [23]. Thus the present study was carried out to find out the optimal temperature and nutrient concentrations for well growth, photosynthetic pigment, biochemical compositions of diatom, *C. simplex*.

2. Materials and Methods

2.1. Algal culture

The *Cheatocecos simplex* strain was obtained from RGCA (Rajiv Gandhi Centre for Aquaculture), Sirkazhi, Tamilnadu, India. The unialgal culture was maintained with F/2 media.

2.2. Experimental design

The experiments were conducted in 250 ml conical flasks with 100 ml of 2–3 days aged exponentially grown algal cultures. Totally fifteen experiments were conducted for 12 days under three temperature levels (20, 25 and 29°C), 68 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ light intensity and at five different concentrations of nitrate and silicate proportions (882 $\mu\text{M} [\text{NO}_3^-]$ –106 $\mu\text{M} [\text{SiO}_3^{2-}]$, 1323 $\mu\text{M} [\text{NO}_3^-]$ –159 $\mu\text{M} [\text{SiO}_3^{2-}]$, 1764 $\mu\text{M} [\text{NO}_3^-]$ –212 $\mu\text{M} [\text{SiO}_3^{2-}]$, 2205 $\mu\text{M} [\text{NO}_3^-]$ –265 $\mu\text{M} [\text{SiO}_3^{2-}]$ and 2646 $\mu\text{M} [\text{NO}_3^-]$ –318 $\mu\text{M} [\text{SiO}_3^{2-}]$). The cell density (growth) was estimated at a day interval, protein and carbohydrate contents were analysed during the stationary phase of culture (10th day).

2.3. Cell density and Growth rate

Cell density was enumerated with hemocytometer (0.1 mm

depth) under light microscope and expressed as 10^5 cells/ml. The growth rate (μ) was calculated by the formula of OECD [24].

2.4. Laboratory analyses

Chlorophyll analyses were performed by the modified method of Strickland and Parsons [25]. The 5 ml of acetone was added to 2 ml of algal culture and vortexed for one minute and kept at 4°C under dark in refrigerator for 24 h. Then the samples were centrifuged at 5000 rpm for 10 min. The absorbance of supernatants was read at 630, 645 and 660 nm in UV–Vis. Spectrophotometer (Perkin–Elmer Lamda 25). Raw acetone used as blank. Ten milliliter aliquots of algal cultures were collected by centrifugation at $5000 \times g$ for 10 min. Proteins were analyzed in pellets based on the method Lowry et al. [26] using bovine serum albumin (BSA) as a standard after re-suspension of cells in 0.1N NaOH and sonicated for 5 min. Protein content was measured at 660 nm in UV–Vis. Spectrophotometer (Perkin–Elmer Lamda 25). Carbohydrate was estimated in pellets by the phenol–sulphuric acid method [27], using glucose as standard.

2.5. Statistical analysis

All the data were analysed and graphs plotted with MS–Excel 2007 version. One-way ANOVA was performed to study the statistical difference between temperature and nutrient concentrations by SPSS 16.0 software.

3. Results

The *Cheatocecos simplex* tested in this study showed sensitive response to temperature, nitrate and silicate proportions. The growth of *C. simplex* was highly influenced by temperature. In low nitrate, silicate proportions and temperature (20 °C) showed slower and lower growth than other treatments. The maximum cell density of 18.1×10^5 cells ml^{-1} and 17.51×10^5 cells ml^{-1} was reached at 20 °C in 10th day under 1764 $\mu\text{M} [\text{NO}_3^-]$ –212 $\mu\text{M} [\text{SiO}_3^{2-}]$ and 1323 $\mu\text{M} [\text{NO}_3^-]$ –159 $\mu\text{M} [\text{SiO}_3^{2-}]$ proportions respectively (Fig. 1). Whereas at 25 and 29 °C, the maximum cell density reached 18.89×10^5 and 23.5×10^5 cells ml^{-1} with 1764 $\mu\text{M} [\text{NO}_3^-]$ and 212 $\mu\text{M} [\text{SiO}_3^{2-}]$ concentrations respectively in 10th day of culture (Figs. 2 and 3). The 1764 $\mu\text{M} [\text{NO}_3^-]$ and 212 $\mu\text{M} [\text{SiO}_3^{2-}]$ treatment was showed high cell densities followed by 2205 $\mu\text{M} [\text{NO}_3^-]$ –265 $\mu\text{M} [\text{SiO}_3^{2-}]$ and 1323 $\mu\text{M} [\text{NO}_3^-]$ –159 $\mu\text{M} [\text{SiO}_3^{2-}]$ concentrations. Based on ANOVA results, the nutrient concentrations were not influenced on cell

Table 1.

ANOVA results* within different temperatures and nutrient concentrations

Parameter	Between nutrient concentrations			Between temperatures				
	20 °C	25 °C	29 °C	882 $\mu\text{M} \text{N}$ –106 $\mu\text{M} \text{Si}$	1323 $\mu\text{M} \text{N}$ –159 $\mu\text{M} \text{Si}$	1764 $\mu\text{M} \text{N}$ –212 $\mu\text{M} \text{Si}$	2205 $\mu\text{M} \text{N}$ –265 $\mu\text{M} \text{Si}$	2646 $\mu\text{M} \text{N}$ –318 $\mu\text{M} \text{Si}$
Cell density	0	0.11	0.006	0.082	0.267	0.019	0.002	0.004
Chlorophyll a	0	0.031	0.008	0.005	0.024	0.082	0.023	0.115
Protein	0.002	0.004	0.002	0.011	0.079	0.428	0.235	0.502
Carbohydrate	0.005	0	0.003	0.04	0.005	0.009	0.187	0.016

* The values ≤ 0.05 are significantly different ($P \leq 0.05$)

density under 25 °C at 10th day of culture. Temperature was not affected the cell density of *C. simplex* with slightly increased concentration of N: Si than regular F/2 media. The cell density values were significantly different between temperatures except 1323 μM [NO₃⁻]-159 μM [SiO₃²⁻] (Table 1). The growth curves of Figs. 1, 2 and 3 showed that the increase in temperature increase growth rate, reduce the age of culture. The Fig. 4 showing high chlorophyll 'a'

content of 1.57 ± 0.05 pg/cell at 20°C and 2205 μM [NO₃⁻]-265 μM [SiO₃²⁻] and it showed that the low temperature and high nutrients enhance its content. Among the nutrient concentrations, 2205 μM [NO₃⁻]-265 μM [SiO₃²⁻] produce high content of chlorophyll 'a' followed by 1764 μM [NO₃⁻]-212 μM [SiO₃²⁻]. The Fig. 5 showing protein and carbohydrate contents of *C. simplex* cultured with different temperature, nitrate and silicate propositions at 10th day of culture. The

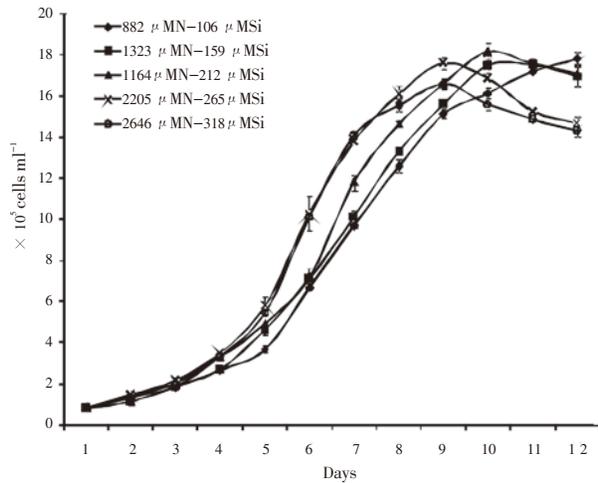


Figure 1. Effect of Nitrate: Silicate concentrations on the growth of *C. simplex* under 20°C

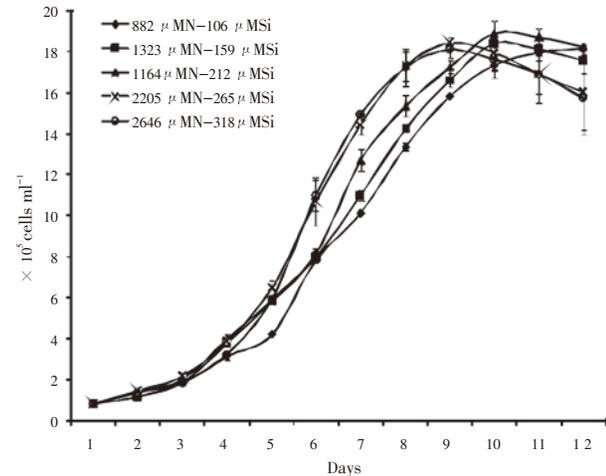


Figure 2. Effect of Nitrate: Silicate concentrations on the growth of *C. simplex* under 25°C

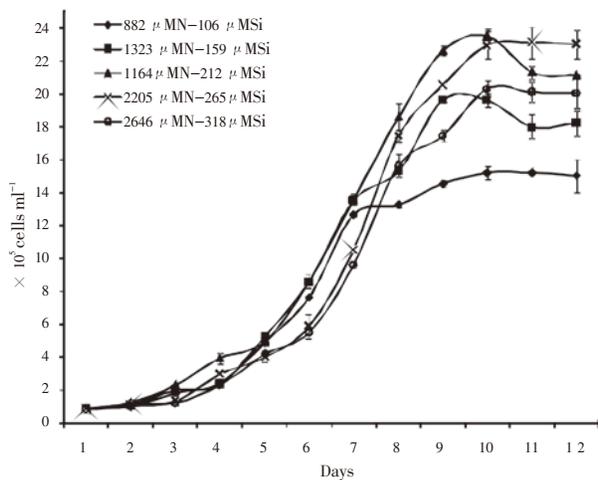


Figure 3. Effect of Nitrate: Silicate concentrations on the growth of *C. simplex* under 29°C

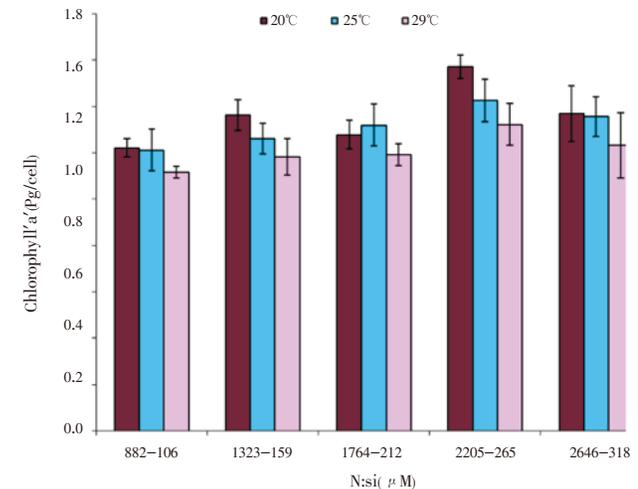


Figure 4. Effect of Temperature, Nitrate: Silicate concentrations on the Chlorophyll 'a' content of *C. simplex*

high protein content of 4.48 ± 0.17 pg/cell was found in 2205 μM [NO₃⁻]-265 μM [SiO₃²⁻] at 25°C and at 20°C, the content was 4.32 ± 0.17 pg/cell. The protein content of 4.19 ± 0.27 pg/cell was observed in 1764 μM [NO₃⁻]-212 μM [SiO₃²⁻] at 25°C and the low protein contents were observed in lower N:Si concentration at all the three temperature levels. The high carbohydrate contents of 0.78 ± 0.03, 0.68 ± 0.05 and 0.64 ± 0.04 pg/cell were found in 1764 μM [NO₃⁻]-212 μM [SiO₃²⁻] at the temperature of 25, 20 and 29°C respectively. The chlorophyll a, protein and carbohydrate content of *C. simplex* were significantly influenced by temperature as well as nutrient concentrations.

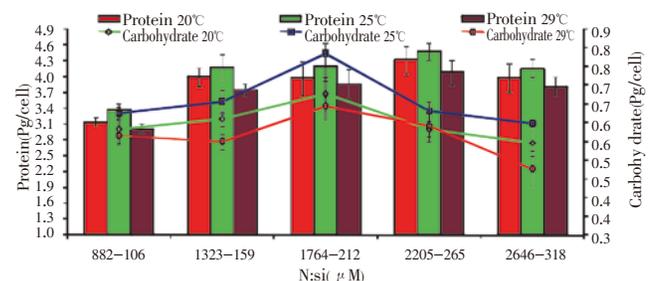


Figure 5. Effect of Temperature and Nitrate: Silicate concentrations on the Protein and Carbohydrate contents of *C. simplex*

4. Discussion

The results clearly showed the influence of temperature, nitrate and silicate proportions on the growth, chlorophyll 'a', protein and carbohydrate contents of *C. simplex*. The growth curves showed that growth was limited after 3–5 days at all the temperature levels under nitrate and silicate treatments. The growth was limited only after 5 days at 20°C and 3 days at 29°C. This indicated that the increase of temperature leads to fast growth and cause reduced generation time of culture. Similarly, growth of *Chaetoceros debilis* was more influenced by silicate availability [28]. Under low silicate conditions, iron and light availability had no effect on growth and there was no significant difference in doubling time among the low silicate treatments [28]. Cucchiari et al. [29] reported that the cultures of *Fibrocapsa japonica* reached cell densities that were roughly 65% lower when grown in 200 mM nitrate and 7.3 mM phosphate, compared to the densities reached in F/2 medium. Otero and Fabregas [30] achieved $6.14 \pm 0.48 \times 10^6$ and $9.49 \pm 0.48 \times 10^6$ cell ml⁻¹ in early stationary phase at 2 and 4 mM nitrate respectively for *Tetraselmis suecica* in the 80 ml culture units. Study on *Heterosigma akashiwo* from the USA, Herndon and Cochlan [31] showed that cultures supplemented with urea had growth rates equal to those grown with nitrate and slightly lower than those supplemented with ammonium. De la Cruz et al. [32] reported that the growth rate of *Rhodomonas* sp. was directly correlated with light intensity and nutrient concentration, but when the concentration of nutrients decreased significantly, growth and maximum cell density were correlated with nutrient availability independently of the irradiance level during the exponential phase. Fogg and Thake [33] suggested that the death phase in batch culture is a result of the exhaustion of nutrients, and this was observed in treatments with low nutrient concentrations. Pathi et al. [34] obtained maximum growth of *Chaetoceros curvisetus* at 3 mM NaNO₃ and declined slightly at 6 mM NaNO₃.

In the present study, high chlorophyll 'a' content of 1.57 ± 0.05 pg/cell was observed in low temperature (20°C) and at high nutrient concentrations (2205 μM [NO₃⁻]-265 μM [SiO₃²⁻]). Eriksen and Iversen [35] found that the content of chlorophyll 'a' increased from 0.5 to 1.5 pg. cell⁻¹ during the first 23 h of incubation, but after the nitrate was exhausted, the chlorophyll decreased to 0.3 pg. cell⁻¹. Brzezinski et al. [36] also found the elevated chlorophyll concentrations in the Si treatments relative to the control but at the termination of the experiment, these differences were never statistically significant. Harrison et al. [37] were noticed the lower chlorophyll 'a' per cell in *Skeletonema costatum* (0.14 pg.) under high-nutrient and higher light conditions. Muggli and Harrison [38] reported lower values of chlorophyll 'a' content for *Emiliania huxleyi* (0.11–0.13 pg cell⁻¹). De la Cruz et al. [32] also reported that chlorophyll 'a' content was directly related to cellular density and indirectly to concentration of nutrients. They recorded almost constant chlorophyll 'a' content (1.35 ± 0.15 pg. cell⁻¹) in *Rhodomonas* sp. through the experiment.

The change in the nutrient source can alter the growth rate and cause significant effect on the biochemical characters such as protein, carbohydrate, lipid and amino acid [39]. Pathi et al. [34] reported that the biochemical composition of *Chaetoceros curvisetus* changed with response to varied nitrate and phosphate concentration. The protein contents of 3.8, 9.0, 23, 13.1, 9.7, 83.4 pg. cell⁻¹ of the marine diatoms, *Chaetoceros calcitrans*, *C. gracilis*, *Nitzschia closterium*, *Phaeodactylum tricornutum*, *Skeletonema costatum*, *Thalassiosira pseudonana* and *Tetraselmis chui* respectively were documented by Brown [40]. The protein content in the present study was high (4.48 pg/cell) in high nutrient (2205–265 μM N: Si) concentrations but temperature have less influence on protein contents. It was about similar at 20 and 25°C but reduced at 29°C. Carbohydrates are used as chemical energy reserves in diatoms [41, 42]. An increase in the energy reserves of phytoplankton is usually associated with senescence and spore formation [43, 44]. The carbohydrate content was decreasing with decreasing concentrations of nutrients in the present study and the maximum of 1.57 pg cell⁻¹ was comparable with the reported value of the carbohydrate content of 26.84 ± 0.80 ng/10⁴ cells in *Skeletonema costatum* by Yang et al. [45].

The present study concluded that the growth of marine diatom, *C. simplex* was optimum at 25°C with the proportions of nitrate (1764 μM [NO₃⁻], silicate 212 μM [SiO₃²⁻]) for the maximum cell density and growth rate. Biochemical analysis inferred that chlorophyll 'a', protein and carbohydrate contents were found to be higher for the above said optimum condition. Based on the present results, future work on growth optimization with the other physical and nutritional factors will yield noteworthy information on the mass cultivation of *C. simplex* for aquaculture purposes.

Conflict of interest statement

We declare that we have no conflict of interest.

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