

## Effects of sodium nitrate on the growth and proximate composition of the indigenous marine microalgae *Tetraselmis chuii* (Butcher, 1959)

Sanzib Kumar Barman<sup>1,2</sup> , Helena Khatoon<sup>3</sup> , Mohammad Redwanur Rahman<sup>3</sup> , Sabuj Kanti Mazumder<sup>4</sup> ,  
Shanur Jahedul Hasan<sup>5</sup> 

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### ABSTRACT

Nitrogen is one of the fundamental nutrients for algal growth, underpinning the microalgal biochemical composition. Therefore, this study compared the growth and proximate compositions of *Tetraselmis chuii* (Butcher, 1959), cultured in different nitrate ( $\text{NaNO}_3$ ) concentrations (25, 50, 100, 200 and 500  $\text{mg L}^{-1}$ ). Thus, the cell density, optical density, specific growth rate and division rate of *T. chuii* were measured daily. Furthermore, protein and carbohydrate contents were also determined in the stationary phase. The results showed that *T. chuii* cultivated in a  $\text{NaNO}_3$  concentration of 500  $\text{mg L}^{-1}$  had significantly ( $p < 0.05$ ) higher growth in terms of cell density, biomass, optical density, specific growth rate and division rates compared with other concentrations. Likewise, protein content was also significantly higher under the  $\text{NaNO}_3$  concentration of 500  $\text{mg L}^{-1}$ , whereas significantly ( $p < 0.05$ ) higher carbohydrate content was found at 25  $\text{mg L}^{-1}$   $\text{NaNO}_3$  compared with the other concentrations, showing a contrary trend between protein and carbohydrate concentrations, respectively. Since the primary focus has been on improving the quality of microalgal biomass in order to develop novel processes and products, this is the first study to use higher concentrations of modified nitrate on *T. chuii* isolated from the coastal area of Bangladesh. Thus the indigenous marine *T. chuii* had significantly utilised  $\text{NaNO}_3$  concentrations with higher growth and proximate contents in this study. However, further study is needed on microalgal genetics and metabolic engineering to create a new molecular era of the indigenous marine microalgae isolated from the coastal water of Bengal.

**Keywords:** Nitrogen concentrations, marine microalgae, growth, proximate composition

#### ORCID IDs of the author:

S.K.B. 0000-0002-9011-1067;  
H.K. 0000-0001-5896-0213;  
M.R.R. 0000-0002-2607-8223;  
S.J.H. 0000-0002-8000-0275

<sup>1</sup>Department of Agrifood Production and Environmental Science, Università degli Studi di Firenze (UNIFI), Florence, Italy

<sup>2</sup>Department of Fishery Resources Conservation and Management, Khulna Agricultural University, Khulna-9100, Bangladesh

<sup>3</sup>Department of Aquaculture, Chattogram Veterinary and Animal Sciences University (CVASU)-4225, Bangladesh

<sup>4</sup>Department of Genetics and Fish Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

<sup>5</sup>Bangladesh Fisheries Research Institute, Mymensingh 2201, Bangladesh

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Correspondence:  
Helena Khatoon  
E-mail:  
[helena@vasu.ac.bd](mailto:helena@vasu.ac.bd)

### INTRODUCTION

Microalgae are vital components in the food chain of aquatic ecosystems (Sathasivam et al., 2019). Their fast growth rates and high value of photosynthetic efficacies (Mostafa, 2012) mean that they potentially have better capacities for  $\text{CO}_2$  moderation than terrestrial plants do (Nigam & Singh, 2011). Microalgae are widely used for larval feeding (Khatoon et al., 2013) because of their positive effects on the growth, survival and hatching of aquatic animals. They are also considered potential probiotics in aquaculture because of their antibacterial prop-

erties and effective antioxidant system. With the acceleration of climate change, there is a great demand for the development of alternative energy sources with a reduced environmental impact. Microalgae as a source of biofuel and renewable feedstocks could be part of the solution (Ramanna, Rawat, & Bux, 2017).

Nitrogen is fundamental for all functional and structural proteins, including chlorophylls in microalgae. The availability of nitrogen is crucial for microalgal growth (Cai, Park, & Li, 2013). Studies have shown that nitrogen limitation in the culture medium increases lipid or carbohy-



drate content, but it reduces protein synthesis of microalgal biomass, thus reducing microalgae's cell growth rate (Ho et al., 2014). Thus, the physicochemical structure of marine microalgae depends not only on the species but also on the growing conditions; for instance, temperature, salinity, pH, lighting conditions (intensity and photoperiod), nutrients and medium agitation all play an important role (Khatoon et al., 2014; Bartley et al., 2016).

*Tetraselmis* is a genus of rapidly growing flagellated marine chlorophytes that can tolerate a wide variety of physical conditions (Khatoon et al., 2014). *Tetraselmis chuii*, the species of interest in this study, has recently been approved as a novel food in Europe and may play an important role in supplying essential nutrients because of its high protein, lipid, essential fatty acid and sterol contents (Ghezelbash et al., 2008). Thus, *T. chuii* can be economically important, and there is a need to optimise the growth rate for the production of this microalga. At the same time, the availability of nitrogen needs to be assured to accomplish optimum carbohydrate production and a rational growth rate (Razaghi, Godhe, & Albers, 2014). Although growth, biochemical composition and different nutritional aspects of *T. chuii* have been studied (Khatoon et al., 2018), there is little information available on the effects of different nitrogen concentrations on the growth and proximate composition of indigenous marine *Tetraselmis* species isolated from the Bay of Bengal coast of Bangladesh. Some previous studies have considered the effects of different nitrogen concentrations on *T. chuii*, but the present study was the first to be conducted in a tropical environment, so its region is geographically isolated from the regions described in other studies. Most previous studies have been conducted using very low concentrations of nitrogen, but in this study, a higher concentration of nitrogen was used to evaluate the growth and proximate composition of this indigenous species for the first time. This will help to optimise the nitrogen concentration for higher growth of *T. chuii*. In this study, *T. chuii* was considered because it has high nutritive value, enabling its high potential as feed in aquaculture (Kim, Mujtaba, & Lee, 2016). The main interest was to enhance the microalgal biomass quality so that innovative processes and products can be established. The biochemical composition of microalgae can be modified and improved by manipulating nutrient concentrations. Therefore, the objective of this study was to determine the effects of different nitrogen concentrations on the growth and proximate composition of indigenous *T. chuii*.

## MATERIALS AND METHODS

### Sample collection, culture and maintenance

The indigenous marine microalgae *T. chuii* strain (CVASUAQ02) was obtained from the pure stock culture of the Department of Aquaculture, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Bangladesh. Filtered natural seawater was used as the seawater base, and the salinity was set at 26 ppt confirmed by a hand refractometer (Refractometer: Atago ATC- S/ Mill- E Salinity 0 - 100%, Japan). The seawater was autoclaved at 121 °C for 15 minutes (DAC 60 Autoclave). A healthy and good stock of microalgae was maintained by observing its morphological features under the microscope. The health status of the cells was considered based on the three following criteria: the cells had to be oval in shape and green in colour, and they could not be found settled at the bottom

of the flask. To maintain a healthy stock, *T. chuii* was cultured in an Erlenmeyer flask containing Conway medium at  $24 \pm 1$  °C and a light intensity of  $150 \mu\text{E m}^{-2} \text{s}^{-1}$ , maintained using cool fluorescent light. Sub-culturing was done every 2 weeks to prepare the required stock for the main experiment.

### Modified minerals stock solution preparation

A stock solution modified in terms of its main mineral contents with different N concentrations was prepared, with sodium nitrate ( $\text{NaNO}_3$ ) used as the main source of nitrogen. The procedure was adapted from James (1996). Hence, the stock solution was prepared with five different treatments, where  $100 \text{ mg L}^{-1}$  of  $\text{NaNO}_3$  was used as the control treatment (see Table 1). Each solution was tightly capped in a Schott Duran® bottle and stored in a refrigerator (Samsung SilverNano) until further use.

### Determination of growth curve

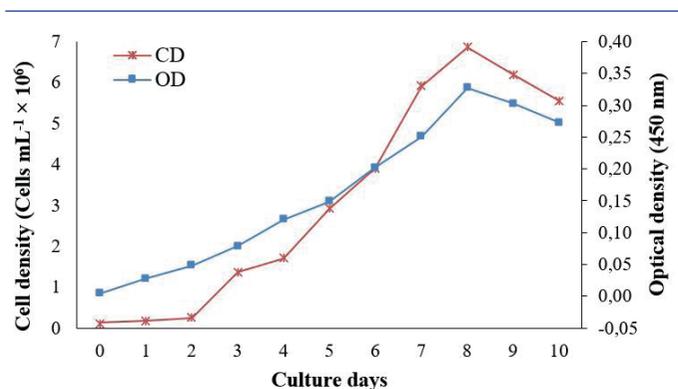
**Table 1.** Different concentrations of nitrate ( $\text{NaNO}_3$ ) with the corresponding N concentrations of each treatment for the study on growth of *Tetraselmis chuii*.

Treatments	$\text{NaNO}_3$ concentration ( $\text{mg L}^{-1}$ )	Corresponding N concentration ( $\text{mg L}^{-1}$ )
Treatment 1 ( $T_1$ )	25	4.125
Treatment 2 ( $T_2$ )	50	8.25
Control 3 ( $T_3$ )	100	16.5
Treatment 4 ( $T_4$ )	200	33.0
Treatment 5 ( $T_5$ )	500	82.5

The growth curve experiment was conducted in sterile 500 mL borosilicate Erlenmeyer flasks, where the *T. chuii* culture volume was 300 mL (270 mL of Conway medium and 30 mL of stock of *T. chuii*). This was done in triplicate. The cultures were maintained at  $24 \pm 1$  °C at a light intensity of  $150 \mu\text{E m}^{-2} \text{s}^{-1}$  using cool fluorescent light for 24 h of continuous lighting and aerated continuously with natural sterile air using an air pump. Growth curves based on cell density ( $\text{cells mL}^{-1}$ ) and optical density (450 nm) measurement were constructed. The highest cell density ( $6.86 \times 10^6$  cells  $\text{mL}^{-1}$ ) and optical density (0.327) was found at Day 8, indicating the stationary phase of the growth curve and the beginning of the death phase (Fig. 1).

### Experimental design

Fifteen autoclaved 500 mL borosilicate Erlenmeyer flasks were used and filled with approximately 100 mL of culture media, 30 mL of *T. chuii* stock culture and 170 mL of Conway medium. The cultures were maintained at  $24 \pm 1$  °C at a light intensity of  $150 \mu\text{E m}^{-2} \text{s}^{-1}$  using cool fluorescent light for 24 h. The cultures were aerated with natural sterile air using an air pump. The flask openings were closed with autoclaved cotton, each with a sterile pipette aeration tube inserted through the cotton into the flask. The growth of the cultures was monitored daily throughout the experiment. Finally, *T. chuii* cultures were centrifuged (Hitachi® High-Speed Refrigerated Centrifuge, himac CR 21G-II) 2 days



**Figure 1.** Growth curve of *Tetraselmis chuii* in terms of cell density (CD) and optical density (OD), cultured in Conway medium.

before reaching the stationary phase (based on the growth curve experiment) to obtain pellets for the main experiment. The pellets were rinsed twice with sterilised distilled water before being transferred randomly into fresh medium. Microalgal pellets were randomly transferred into fifteen flasks with a 2 L capacity, containing 1.5 L of culture medium with different  $\text{NaNO}_3$  concentrations. The growth of the culture was monitored in terms of cell density, biomass, optical density and specific growth rate. The water salinity and pH were maintained at 26 ppt and 7.3–7.5, respectively; nevertheless, the  $\text{CO}_2$  concentration was adjusted through aeration during the experimental period. The experiment was conducted in a controlled condition in the laboratory.

#### Collection of freeze-dried sample

At the end of the experiment, the matured *T. chuii* cells were harvested in the stationary phase (depending on different N concentrations of five treatments) by centrifugation at 7000 rpm for 3 minutes, followed by rinsing twice with sterilised distilled water. Since the highest productivity (cell density and biomass) and matured cells (due to higher nutrition level) were found at the end of the exponential phase, i.e. indicating the stationary phase of *T. chuii* growth (according to the growth curve), microalgae were harvested at this phase (Araujo et al., 2020). The cells were then dried (overnight at 60 °C) and the dried biomass was at -20 °C for proximate composition analysis.

#### Determination of growth parameters

Cell density, biomass and optical density were measured to determine microalgal growth. Cell density was determined using a haemocytometer (Hawksley AC1000, UK) according to the method reported by Lavens & Sorgeloos (1996). For determination of biomass, 1 mL of culture aliquoted from each of the flasks was filtered onto the pre-weighed GF/C (Whatman glass microfibre filters combine) glass fibre filters using a vacuum pump filtration unit (Millipore). Then, the filters were dried at 100 °C for 4 h and subsequently cooled in the desiccator (Nalgene) for 15 min. Next, the filter papers were individually weighed (AND, GR-200), and the biomass (dry weight basis) was determined for each sample. Optical density was determined using a spectrophotometer (UV-1601 UV Visible Spectrophotometer, Shimadzu). The absor-

bance readings were taken at a wavelength of 450 nm, considering the culture medium for *T. chuii* as the blanks (Lavens & Sorgeloos, 1996).

#### Determination of specific growth rate (SGR) and division rate

The specific growth rate (SGR,  $\mu \text{ day}^{-1}$ ) of *T. chuii* of different treatment was calculated using the following equation, developed by Clesceri, Greenberg, & Trussel (1989):

$$\text{SGR} = \ln (X_2 - X_1) / t_2 - t_1,$$

where  $X_1$  represents the biomass concentration at the beginning of the selected time interval,  $X_2$  represents the biomass (dry weight basis) concentration at the end of the selected time interval and  $t_2 - t_1$  is the selected time (in days) for the determination of biomass (dry weight) of *T. chuii*.

The division rate ( $\text{day}^{-1}$ ) of the indigenous *T. chuii* was calculated using the following equation (Teo et al., 2014):

$$\text{Division rate} = \text{SGR} / \ln 2$$

#### Determination of proximate compositions

##### Protein determination

For every sample, 5 mg of freeze-dried microalgae was taken to make a 25 mL solution by mixing homogeneously with distilled water. From this 25 mL microalgal solution, 0.5 mL was taken for protein analysis. Prior to the start of the analysis, Reactive 1 (1% NP tartrate) and Reactive 2 (2 g of  $\text{NaCO}_3$  in 100 mL of 0.1 NaOH) were prepared. For protein analysis, 50 mL of Reactive 2 and 1 mL of Reactive 1 were mixed. Then, 0.5 mL of microalgal solution was added with 0.5 mL of 1N NaOH and kept in a 100 °C water bath for 5 minutes. Afterwards, it was cooled in a water bath, and 2.5 mL of the prepared mixed reagent was added 10 minutes after cooling. The mixed solution was added with 0.5 mL of Folin - Ciocalteu reagent and then kept in the dark for 30 minutes. Standard protein concentrations were prepared using bovine serum albumin. The absorbance was measured using a spectrophotometer (Shimadzu UV-1601, Japan) at a wavelength of 750 nm (Lowry et al., 1951).

##### Carbohydrate determination

Freeze-dried microalgae (5 mg) were used to make a 25 mL solution by mixing homogeneously with distilled water. Prior to the start of the analysis, 5% phenol solution and concentrated sulfuric acid were prepared. For carbohydrate analysis, 1 mL of microalgal solution was taken and a 5% phenol solution was added, followed by 5 mL of sulfuric acid. The standard was prepared using glucose. The optical density was measured at 488 nm in a spectrophotometer (Shimadzu UV-1601, Japan; Dubois et al., 1956).

#### Statistical analysis

The results of the growth and proximate compositions were analysed by one-way analysis of variance (ANOVA) considering the nitrate ( $\text{NaNO}_3$ ) concentrations with a significance level of 95% ( $p < 0.05$ ) as well as Tukey multiple comparisons test (where applicable). The graphical presentation of the growth parameters and proximate compositions (% dry weight) with different treatments were analysed by SPSS software and Microsoft Excel. All results were presented as mean  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

### Microalgal growth under different nitrate ( $\text{NaNO}_3$ ) concentrations

The cell density, biomass and optical density of the indigenous marine microalga *T. chuii* cultured in controlled conditions under five different concentrations of  $\text{NaNO}_3$  are shown in Fig. 2. The results showed that *T. chuii* cultured at different  $\text{NaNO}_3$  concentrations stretched their stationary phase at different days, although no significant difference was found between the  $T_4$  (200  $\text{mg L}^{-1}$ ) and  $T_5$  (500  $\text{mg L}^{-1}$ ) treatments. The comparison between  $T_4$  and  $T_5$  was based on the culture days in the stationary phase, in which both treatments indicated the same stationary phase at day 9. In sum, there were no significant differences for biomass. While this finding doesn't support most other studies in which the increasing  $\text{NaNO}_3$  concentrations indicate the increment of biomass production, the biomass productivity of microalgae is highly dependent on the culture media as well as the species and strain of microalgae (Araujo et al., 2020), which might be consistent with the present findings as this new strain is isolated from the Bay of Bengal. Furthermore, the corresponding N concentrations in the growth medium for each treatment were significantly different with respect to the nitrate ( $\text{NaNO}_3$ ) concentrations (Table 1).

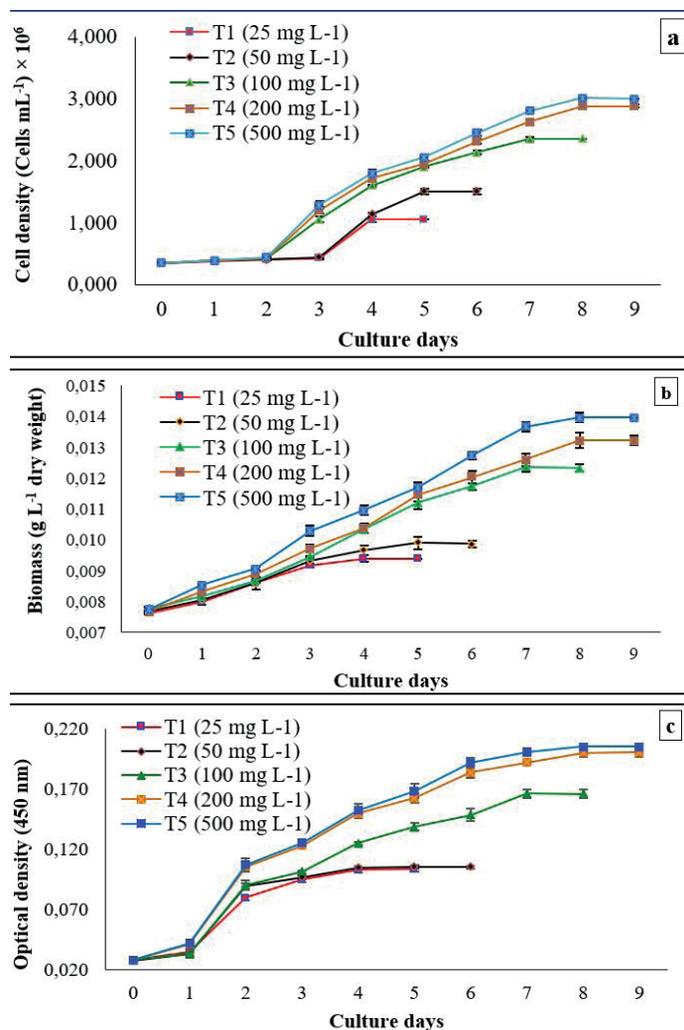
Nutrient convenience has a significant impact on the growth and biochemical composition of marine microalgae (Xia et al., 2013). Moreover, the plentiful supply of nutrients (nitrogen, phosphorus, potassium nitrate, etc.) is the principle of attaining high growth rates in microalgal cells (Xia et al., 2013). The growth rate declines and ceases only when the metabolic requirements do not match the supplied nutrients. There are also other important factors (temperature, light, salinity, pH, etc.) that might distress the growth and biochemical compositions of microalgae (Yeh & Chang, 2012). Microalgae growth and biomass are influenced by light and photoperiod during culture (Ahmad et al., 2020; Yusof et al., 2021). Furthermore, the length of light and dark (24:0h) exposure until the saturation point, at which the maximum photosynthetic rate is reached, may influence cellular contents such as chlorophyll and antioxidants (Darvehei, Bahri, & Moheimani, 2018; Yusof et al., 2021). However, in this study, sodium nitrate, a source of nitrogen, was considered one of the most crucial nutrients for microalgal cell growth because its concentrations in the growth medium significantly affected the growth rate of algal cells (Wu & Miao, 2014), as well as the biochemical compositions of microalgae (Kim, Mujtaba, & Lee, 2016).

Zarrinmehr et al. (2020) studied the effects of different nitrogen concentrations (0, 36, 72, 144 and 288  $\text{mg L}^{-1}$ ) on the growth and biochemical composition of *Isochrysis galbana*. Their study revealed that diminishing concentrations of nitrogen reduce the cell growth and biomass production of *I. galbana*. In the present study, the lowest concentration of  $\text{NaNO}_3$  (25  $\text{mg L}^{-1}$ ) showed comparatively very low cell growth; however, the highest cell density ( $3.00 \times 10^6$  cells  $\text{mL}^{-1}$ ) was recorded with the maximum concentration of  $\text{NaNO}_3$  (500  $\text{mg L}^{-1}$ ). These results are consistent with Huang et al. (2013), who also reported that the increment of nitrogen supply significantly increased the growth rates of three microalgae species, namely, *Tetraselmis subcordiformis*

(Wille) Butcher, 1959; *Nannochloropsis oculata* (Droop) Hibberd, 1981; and *Pavlova viridis* Tseng, Jiaofu, & Zhefu, 1992. These results were also consistent with the optical density measurements of the present study. Studies also reported that some species-*Scenedesmus acutus*, *Chlorella vulgaris*, *Nannochloropsis* sp. and *Nannochloropsis oleoabundans*- grow well in nitrogen-deficient environments by exploiting their intracellular nitrogen reserves, such as pigment-protein molecules (Gu et al., 2015); this result also supports the present findings (i.e. growing cell concentration under low concentrations of nitrogen). However, the studies of Araujo et al. (2020) have found the highest productivity at low  $\text{NaNO}_3$  concentrations for the sole microalgae *T. chuii*, which doesn't support the present study findings. This is because of very low concentrations (25 to 75  $\text{mg L}^{-1}$ ) of  $\text{NaNO}_3$  have been conducted in their study, but in this study, a higher concentration of nitrogen was used to evaluate the growth and proximate composition of this indigenous species for the first time. Thus, the aim of this study will help to optimise the nitrogen concentration for higher growth of *T. chuii*.

The marine microalgae *T. chuii* efficiently utilised nitrate ( $\text{NaNO}_3$ ) for growth and the present results showed that the increased N concentrations in the culture media significantly enhanced the growth performance. However, the maximum density was found in  $T_5$  (500  $\text{mg L}^{-1}$ ) treatment (Fig. 2a) with a respective cell density of  $3.00 \times 10^6$  cells  $\text{mL}^{-1}$ ; lower cell density was observed in  $T_1$  (25  $\text{mg L}^{-1}$ ) at  $1.05 \times 10^6$  cells  $\text{mL}^{-1}$ . There were significant differences among all the treatments of  $\text{NaNO}_3$  concentrations when compared with the control treatment  $T_3$  (100  $\text{mg L}^{-1}$ ; Fig. 2a). The increase of nitrate ( $\text{NaNO}_3$ ) concentrations in the culture media, ranging from 4.125 to 82.5  $\text{mg L}^{-1}$  of N (N concentrations ranges indicated the same as nitrate concentrations based on different  $\text{NaNO}_3$  concentrations in the medium), significantly induced the increment of biomass production. The biomass concentration of *T. chuii* in the stationary phase under 4.1  $\text{mg L}^{-1}$  N ( $T_1$ ) was only 0.0094  $\text{g L}^{-1}$  (23% increase of biomass), whereas the maximum biomass concentration (in dry weight) was 0.0140  $\text{g L}^{-1}$  in the stationary phase (81% increase of biomass) under 82.5  $\text{mg L}^{-1}$  N ( $T_5$ ), representing a significant difference between the two results (Fig. 2b). Moreover, there was a significant difference in biomass production among all the treatments compared with the control treatment  $T_3$  (0.0123  $\text{g L}^{-1}$  of dry biomass), which exhibited about a 58% increase (comprising 16.5  $\text{mg L}^{-1}$  N) of dried biomass. A similar trend was noted for optical density (OD), for which higher absorbance was recorded with increasing N concentrations for nitrate ( $\text{NaNO}_3$ ; Fig. 2c).

Marine microalgae can utilise inorganic nitrogen for their growth and metabolic activities and increase their biomass concentrations in conjunction with the nitrogen-enriched condition (Rizwan et al., 2017). The present study findings also revealed that the increment of nitrogen ( $\text{NaNO}_3$ ) supply in the medium significantly increased biomass production of *T. chuii*, but the contrary result was found for decreased concentrations of nitrogen. This finding was subsequently supported by Zarrinmehr et al. (2020), who also reported that the diminishing concentrations of nitrogen decreased the biomass production of *I. galbana*, whereas opposite results were found for increased nitrogen concentra-



**Figure 2.** Effects of different NaNO<sub>3</sub> concentrations on the growth of *Tetraselmis chuii* in terms of (a) cell density (cells mL<sup>-1</sup>), (b) biomass (g L<sup>-1</sup>) and (c) optical density (450 nm; values are mean ± standard deviation).

tions in the medium. Those findings were also consistent with the results reported by El-Kassas (2013), who stated that the cell density and biomass production of *Picochlorum* sp. decreased under the deficient condition of the major macro-nutrients, such as nitrogen or phosphorus, compared with the control treatments. Another important factor is mixing of water, which can also significantly affect the biomass production of microalgal cells. It is important to obtain the proper light absorptions in the culture medium to achieve appropriate growth and biomass production.

It is noteworthy that the culture volume of the present study was 1.5 L in each 2 L of the conical flask supplied with aeration of pure air (comprising 0.03% CO<sub>2</sub>), resulting in a low volume of surface area. Thus, the small surface area was supposed to bring about the poor mixing rate and low light penetration in the medium, resulting in low biomass production. Mohsenpour & Willoughby (2016) also reported that increased concentrations of CO<sub>2</sub> in the air stream enhanced the CO<sub>2</sub> fixation rate in *Chlorella vulgaris*, and the biomass concentrations increased with 5% CO<sub>2</sub> aeration

compared with the pure air (comprising 0.03% CO<sub>2</sub>) concentration, which varied in the present study findings because of the small volume of surface area and low percentage of CO<sub>2</sub> concentrations through aeration. The research by Uggetti et al. (2018) also proved that the addition of CO<sub>2</sub> through aeration induced the rise of biomass concentrations by between 66% and 100%, in which proper mixing was achieved.

The present study findings showed a comparable trend for the SGR (μ day<sup>-1</sup>) and division rate of *T. chuii*, which increased with the increasing nitrogen concentrations (Table 2). The highest SGR (0.0664 μ day<sup>-1</sup>) and division rate (0.0958 day<sup>-1</sup>) were measured for treatment T<sub>5</sub> (500 mg L<sup>-1</sup>) with a NaNO<sub>3</sub> concentration of 82.5 mg L<sup>-1</sup> N.

**Table 2.** Specific growth rate (SGR) and division rate of *Tetraselmis chuii* under different concentrations of NaNO<sub>3</sub>. Values are Mean ± SD (standard deviation).

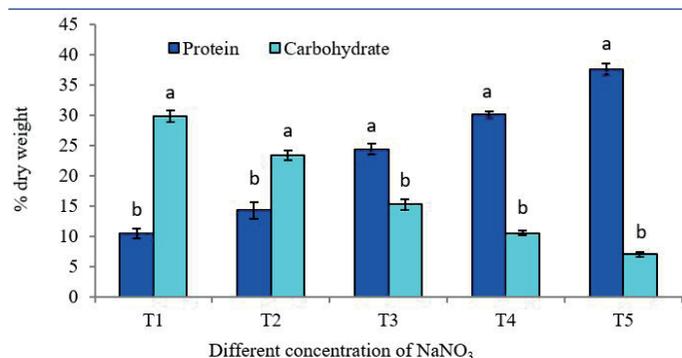
Treatment	SGR μ day <sup>-1</sup>	Division rate day <sup>-1</sup>
T <sub>1</sub>	0.0425±0.0002	0.0613±0.0001
T <sub>2</sub>	0.0419±0.0001	0.0604±0.0003
T <sub>3</sub>	0.0569±0.0003	0.0821±0.0002
T <sub>4</sub>	0.0599±0.0002	0.0864±0.0001
T <sub>5</sub>	0.0664±0.0001	0.0958±0.0001

Zhu et al. (2014) demonstrated that the specific growth rate of *Chlorella zofingiensis* was 0.48 μ day<sup>-1</sup> under low concentrations of nitrogen in the medium, and this condition increased the SGR to 1.02 μ day<sup>-1</sup> during nitrogen depletion. The present study findings were also consistent with Zarrinmehr et al. (2020), who reported that *I. galbana* can grow well in a nitrogen-enriched medium rather than a nitrogen-deficient condition.

#### Effects of different nitrate (NaNO<sub>3</sub>) concentrations on protein and carbohydrate content

The different nitrate (NaNO<sub>3</sub>) concentrations in the growth medium of *T. chuii* showed significant effects on protein and carbohydrate content (Fig. 3) in some treatments. The protein and carbohydrate contents exhibited significant changes of contrary trends in relation to their sufficient and deficient N concentrations for NaNO<sub>3</sub>, respectively (Fig. 3). The increased N concentrations in the culture medium significantly induced the increment of protein content (% dry weight). The highest protein content of 37.57% dry weight (comprising 82.5 mg L<sup>-1</sup> N) was recorded from T<sub>5</sub> (500 mg L<sup>-1</sup>), and this result was significantly different from that of the control treatment T<sub>3</sub>. In contrast, the carbohydrate content (% dry weight) revealed the highest concentration, comprising 29.76% dry weight (corresponding concentration of 4.1 mg L<sup>-1</sup> N) in T<sub>1</sub> (25 mg L<sup>-1</sup>) for nitrate (NaNO<sub>3</sub>), which was also significantly different with T<sub>4</sub> and T<sub>5</sub>, and the control treatment T<sub>3</sub> (Fig. 3).

The availability of major nutrients has a significant effect on the proximate composition of microalgal cells (Xia et al., 2013). Microalgae generate both lipid and proteins when the carbon:nitrogen ratio is balanced, i.e. when there is a lot of nitrogen, that increases the net nitrogen consumption. But, when nitrogen is



**Figure 3.** Proximate composition (% dry weight) of *T. chuii* under different sodium nitrate ( $\text{NaNO}_3$ ) concentrations (values are mean  $\pm$  standard deviation). Letters indicate the significant levels of protein and carbohydrate among the different treatments.

scarce, microalgae produce fewer nitrogen-containing molecules and store lipids, resulting in a reduction in nitrogen consumption (Araujo et al., 2020). However, the suitable N concentrations for microalgal growth and proximate composition differ from species to species (Kim, Mujtaba, & Lee, 2016). The present study findings revealed that the increased nitrogen concentrations in the growth medium significantly induced the protein content up to 37.57% (dry weight), while the opposite trend was found for carbohydrate concentrations. This finding was consistent with Kim, Mujtaba, & Lee (2016), who also reported this trend for marine chlorophyte *Tetraselmis* sp., in which carbohydrate production increased up to 55% and protein concentrations gradually decreased under nitrogen-deficient conditions. The findings of this study proved that microalgae (*T. chuii*) cause protein accumulation in N-sufficient culture conditions, while carbohydrate accumulation occurs in N-deficient conditions, which was also described by Kim, Mujtaba, & Lee (2016). Guihéneuf & Stengel (2015) also stated that the cellular carbohydrate concentrations of *Porphyridium purpureum* increased up to 40% during the N-deficient logarithmic phase. A similar study conducted by Pancha et al. (2014) demonstrated that different nitrate concentrations, ranging from 247 to 0 mg L<sup>-1</sup>, decreased the protein percentage of *Scenedesmus* sp. from 47.75% to 16.87%. Since nitrogen is the most important pioneer for protein synthesis, nitrogen deficiency is a common physiological response in microalgae to decreasing protein content (Zarrinmehr et al., 2020; Araujo et al., 2020). Although nitrogen deficiency is effective, easy and cost effective for biofuel production, it has some effects on cell physiology, such as decreases in proteins and chlorophyll, and it influences photosynthesis ability, resulting in the decreased growth rates (Ördög et al., 2012).

## CONCLUSION

The indigenous (isolated from the Bay of Bengal) marine microalgae *T. chuii* was found to utilise sodium nitrate efficiently. Though most previous studies have been conducted using very low concentrations of nitrogen, the current study was conducted with higher concentrations of nitrogen to evaluate the growth and proximate composition of this indigenous species for the first

time. It is noteworthy that the present study findings will help to enhance the nitrogen concentration for higher growth of indigenous *T. chuii* as well as to enhance both the protein and lipid content, directing possible future research efforts. In addition, future studies can include experiments on ways to improve the quality and quantity of protein and lipids in microalgae, especially those with commercial advantages. Furthermore, extensive research in the field of microalga genetics and metabolic engineering are necessary to determine methods of producing biofuel in continuous, economic and sustainable ways.

**Conflict of interest:** The author has declared no conflict of interest.

**Ethics committee approval:** The research has complied with all regional, national, and institutional ethical clearance and been approved.

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