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Original Article

Growth and nutrient profile of *Tetraselmis chuii* under different urea concentrations: implications for sustainable uses

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Abstract
Nitrogen is regarded as one of the most important nutrients for algal cells, having a direct impact on the growth and biochemical contents of microalgae. The goal of this research was to compare the growth and proximate components of *Tetraselmis chuii* cultivated in various urea concentrations as an available source of nitrogen. Results disclosed that *T. chuii* cultivated in urea at 93.4 mg L^{-1} N had higher cell density, biomass, and optical density compared to 11.67 mg L^{-1}, 23.35 mg L^{-1}, 46.7 mg L^{-1}, and 233.5 mg L^{-1} N, respectively. Protein content was highly significant for urea at 233.5 mg L^{-1} N concentration compared to other concentrations. In contrast, higher carbohydrate content was found at 11.67 mg L^{-1} N compared to other concentrations of urea. Thus, the current study found that raising or decreasing urea concentrations had a substantial effect on the growth and proximate composition of indigenous marine *T. chuii*, and the highest biomass was recorded at 0.0145 g L^{-1} (dry biomass) from 93.4 mg L^{-1} N. These findings have implications for the cultivation of microalgae for various applications, including biofuel production, wastewater treatment, mariculture developments, and a sustainable blue economy in Bangladesh.

Keywords: growth; marine microalgae; proximate composition; *Tetraselmis chuii*; urea concentrations

1 | INTRODUCTION

Microalgae are an important component of the food chain in aquatic ecosystems (Sathasivam *et al*. 2019; Hossain *et al*. 2022), with faster growth rates and high photosynthetic efficiency due to their main photosynthetic pigments being chlorophyll (Islam *et al*. 2021). They possess superior CO_2 regulation capabilities compared to land plants (Nigam and Singh 2011). Microalgae are often used as feed during the larval stage and can promote the development, survival, and hatching of aquatic species (Hemaiswarya *et al*. 2011; Hossain *et al*. 2022). Due to their potent antioxidant systems and strong antibacterial properties, they are also considered potential probiotics in aquaculture (Tredici *et al*. 2009). The search for alternative renewable energy sources, such as biofuels, which exert less environmental impact, has gained momentum

amid increasing global warming, rising energy demands, and climate change. In this context, microalgae show great potential as feedstocks for renewable energy (Ramana *et al*. 2017).

Among essential nutrients, nitrogen is considered vital for plant growth. It is fundamental for all functional and structural proteins, including chlorophylls in algal cells (Cai *et al*. 2013). Numerous studies have demonstrated that microalgal biomass can increase in lipid or carbohydrate content when nitrogen in the culture medium is limited, thereby reducing protein synthesis (Ho *et al*. 2014; Barman *et al*. 2021). However, nitrogen limitation can also inhibit microalgal cell proliferation. Different nitrogen sources influence microalgal biomass concentration, and this effect varies among species. Therefore, nitrogen is a critical nutrient for microalgal growth and biochemical composition, making it a key factor in their cultivation for various applications. The physicochemical structure of marine microalgae is affected by multiple factors, including temperature, salinity, pH, lighting conditions (light intensity and photoperiod), nutrients, and medium agitation (Khatoon *et al*. 2014; Bartley *et al*. 2016; Haris *et al*. 2022). Thus, temperature and light intensity are the primary physical parameters, while salinity, pH, and nutritional limits of the culture medium are key chemical factors that can impact microalgae production (Sharma *et al*. 2012; Khatoon *et al*. 2014).

Tetraselmis species are flagellated marine chlorophytes that can tolerate a wide range of temperatures, salinities, and pH values (Haris *et al*. 2022). *Tetraselmis chuii*, known for its rapid growth and high nutritional value, has promising applications in biofuel production, aquaculture, and wastewater treatment (Khatoon *et al*. 2014; Barman *et al*. 2021; Islam *et al*. 2022). Due to its high protein, lipid, essential fatty acid, and sterol content, *T. chuii* has been recognized as a novel food in Europe (Paterson *et al*. 2023; Garciarena *et al*. 2025). Nitrogen availability plays a key role in regulating growth and carbohydrate production (Razaghi *et al*. 2014). While earlier studies have examined the growth and biochemical composition of *T. chuii* (Lu *et al*. 2017; Khatoon *et al*. 2018), little is known about how varying nitrogen concentrations affect indigenous marine strains. Urea serves as a prevalent nitrogen source, presenting opportunities for sustainable large-scale cultivation. Thus, this study investigates the effects of different urea concentrations on *T. chuii* growth and proximate composition, aiming to determine optimal nitrogen levels for enhanced biomass quality and broader sustainable applications (Kim *et al*. 2016a).

Moreover, *T. chuii* production can also help to preserve marine ecosystems by offering an alternative food source for fish and shrimp farms, minimizing overfishing and depletion of wild fish stocks. Farming this species can create job opportunities for local people, particularly women and youth, who can be trained to cultivate and harvest microalgae. Thus, the objectives of this study were also to discuss the prospects of *T. chuii* culture in the sustainable economy of Bangladesh.

2 | METHODOLOGY

2.1 Sample collection, culture, and maintenance

The indigenous marine microalga *T. chuii* strain (CVAS-UAQ02) was obtained from the culture collection of the Department of Aquaculture, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Bangladesh. *T. chuii* was cultured in an erlenmeyer flask containing the conway culture medium at 24 ± 1°C at a light intensity of 150 $\mu Em^{-2}s^{-1}$ using cool fluorescent light. The subculturing was done every two weeks to keep a healthy and good stock by utilizing various amounts of Erlenmeyer flask to prepare a specific stock for the core experiment.

2.2 Preparation of natural seawater and Conway medium

The vacuum pump filtration unit (Millipore) was used to filter seawater collected from the Bay of Bengal with a salinity of 26 ppt using 47 mm Ø Whatman® GF/C™ glass microfiber filter papers and then stored in Nalgene® bottles and stored in a cold, dry area until later usage. Enriching culture media was crucial for increasing algal productivity and so the Conway medium (Tomkins *et al*. 1995) in this aspect was considered. And therefore, the main minerals stock solution (solution A), trace metals solution (solution B), vitamin B (thiamine) and vitamin B_{12} (cyanocobalamin) solutions (solution C) were also prepared accordingly. Then 1 ml of solution A and 0.5 mL of solution B were added per 1000 mL of filtered seawater. The pH was adjusted to pH 8.0 ± 0.2 (Horiba Twin pH Compact pH meter) and autoclaved at 121°C for 15 min (DAC- 60 Autoclave). It was subsequently allowed to cool for 1-2 days. Next, 0.1 mL of vitamin B and vitamin B_{12} were added to the solution (Table 1).

2.3 Modified minerals stock solution preparation

The modified main minerals stock solution was made by different urea concentrations instead of sodium nitrate as an alternative nitrogen source. James (1996) inspired the technique adopted here. As a result, five different treatments were used to prepare the main minerals stock solution shown details in Table 2, respectively. Each solution was stored and tightly-capped in a Schott- Duran® bottle and kept in a refrigerator (Samsung SilverNano) until further use.

2.4 Determination of corresponding nitrogen conc. in the medium

Whatman GF/C glass fiber filters were used to filter the algae suspension. In a porcelain vial, the filtered media

(20 mL) and 2 mL of 0.5 g sodium salicylate in 100 mL of distilled water were evaporated to dryness for about three hours in a water bath set at 80°C. 2 mL of concentrated H_2SO_4 was added to the vial once it had cooled to room temperature. Ten minutes later, 15 mL of distilled water and 15 mL of Seignette-sal solution (400 g of NaOH and 60 g of K-Na-tartarate in one liter of distilled water) were added. A spectrophotometer (Varian Cary 50 Scan, Varian Australia Ltd, Australia) was used to measure the absorbance of the samples at 410 nm after the solution had cooled to room temperature and compared to a blank made with distilled water. A calibration curve created with urea solutions at various concentrations was used to determine the results (Németh 1998).

TABLE 1 Chemical compositions of Conway medium (Tompkins *et al.* 1995).

Solution A - macronutrients	
Sodium nitrate	$NaNO_3$ (100.0 gL^{-1})
Sodium orthophosphate	$NaH_2PO_4.4H_2O$ (20.0 gL^{-1})
Sodium EDTA	$C_{10}H_{16}N_2O_8$ (45.0 gL^{-1})
Boric acid	H_3BO_3 (33.4 gL^{-1})
Ferric chloride	$FeCl_3.6H_2O$ (1.3 gL^{-1})
Manganese chloride	$MnCl_2.4H_2O$ (0.36 gL^{-1})
Solution B - trace metals	
Zinc chloride	$ZnCl_2$ (4.2 gL^{-1})
Cobalt chloride	$CoCl_2.6H_2O$ (4.0 gL^{-1})
Copper sulphate	$CuSO_4.5H_2O$ (4.0 gL^{-1})
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}.4H_2O$ (1.8 gL^{-1})
Solution C - vitamins	
Vitamin B_1 (Thiamin)	200 mgL^{-1}
Vitamin B_{12} (Cyanocobalamine)	10 mgL^{-1}

TABLE 2 Different concentrations of urea with the corresponding N concentrations of each treatment.

Treatments	**Concentration (mgL^{-1})**	
	Urea	**Corresponding N**
Treatment 1 (T_1)	25	11.675
Treatment 2 (T_2)	50	23.35
Control treatment 3 (T_3)	100	46.7
Treatment 4 (T_4)	200	93.4
Treatment 5 (T_5)	500	233.5

2.5 Determination of growth curve adopted

The growth curve experiment (Figure 1) was done based on the cell density (cells mL^{-1}) and optical density (450 nm) adopted by Barman *et al*. (2021).

2.6 Experimental design

For the sources of nitrogen (Urea) experiment, fifteen autoclaved 500 mL borosilicate Erlenmeyer flasks were each filled with approximately 100 mL of culture media. Then, 30 mL of *T. chuii* culture was added to each of the flasks. Then, Conway culture medium was added to each flask until the final volume of 300 mL was obtained, in which The cultures were maintained at 24 ± 1°C at a light intensity of 150 µE m^{-2} s^{-1} using cool fluorescent light with 24 h continuous lighting and aerated continuously with natural sterile air using an air pump. The openings of all of the flasks were closed with an autoclaved cotton each with a sterile pipette aeration tube inserted through the cotton. The growth of the cultures was monitored everyday throughout the experiment. Finally, fifteen of *T. chuii* cultures were centrifuged (Hitachi® High- Speed Refrigerated Centrifuge, himac CR 21G-II) two days before it reached the stationary phase (based on the growth curve experiment) to obtain pellets for the main experiment.

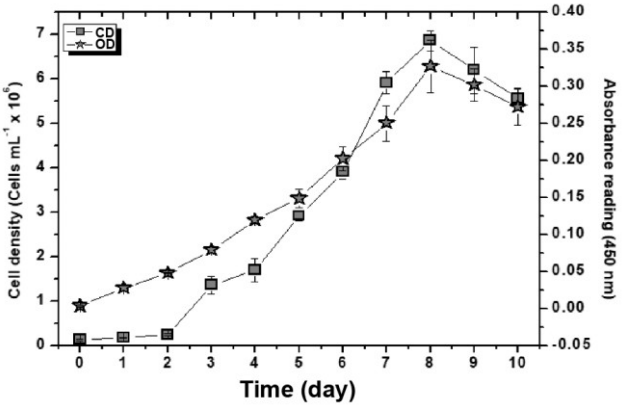

FIGURE 1 Changes (growth curve) in cell density and absorbance reading of *Tetraselmis chuii* cultured on Conway media.

Algal pellets were randomly transferred into fifteen 2 L flasks containing 1.5 L of culture medium having different urea concentrations. Here, fifteen flasks (culture samples) indicated three replicates for each five treatments T_1 (25 mg L^{-1}), T_2 (50 mg L^{-1}), T_3 (100 mg L^{-1}), T_4 (200 mg L^{-1}), and T_5 (500 mg L^{-1}), respectively (after Barman *et al*. 2021). Cell density, biomass, optical density, and specific growth rate were the metrics used to measure the cultures' growth over the course of the experiment, which included daily pH checks (Table 3).

At the end of the experiment, the matured *T. chuii* cells were harvested at the stationary phase (depending on different N concentrations of five treatments), and the cells were harvested by centrifugation at 7000 rpm for 3 min followed by washing twice with sterilized distilled water. Then the collected dried (overnight at 60°C) biomass was kept at –20°C for proximate composition analysis.

2.7 Determination of growth parameters

Microalgae growth was measured in terms of cell density, biomass and optical density.

2.7.1 Cell density: Cells were sampled and counted daily

using a haemacytometer (Hawksley AC1000, UK) for all the cultures according to Lavens and Sorgeloos (Lavens and Sorgeloos 1996). The determination of the cell count of the culture aliquots was observed daily during the experimental period to monitor the culture growth and densities. The hemacytometer and its cover slip (Brightline improved Neubauer hemacytometer, 0.1 mm deep chambers, 0.0025 mm^2, Assistant, Germany) were cleaned with Milli- Q water (Millipore Corp.) prior to filling up of the chambers with culture samples. Evenness of cell distribution was checked under low power magnification (4× and 10×) of the microscope (Nikon E600). Cells were counted for both chambers of the hemacytometer under the magnification of 20×. Whenever the cells were actively moving under the microscope view, Lugol's iodine was added to culture aliquots to facilitate counting. It was used for fixation and staining purposes. The formulae to calculate the cells are as the following:

Cell count calculation (cells mL^{-1}) for 5 squares = (Total number of cells counted / 10 × 4) × 10^6.

Where 10 represented the 10 squares of the 2 hemacytometer chambers, and 4×10^6 represented the volume of samples over the small square areas that were equivalent to 0.004 mm^3 (0.2 mm × 0.2 mm × 0.1 mm) expressed in cm^3 (mL).

Cell count calculation (cells mL^{-1}) for 25 squares = (Total number of cells counted / 50 × 4) × 10^6.

Where 50 represented the 50 squares of the 2 hemacytometer chambers, and 4×10^6 represented the volume of samples over the small square areas that were equivalent to 0.004 mm^3 (0.2 mm × 0.2 mm × 0.1 mm) expressed in cm^3 (mL).

TABLE 3 Recorded pH value of the growth medium under different N concentrations of each treatment (average indicates three replications or observations for each treatment).

Day	Urea (average ± SD; mgL^{-1})				
	T_1 (25 mgL^{-1})	T_2 (50 mgL^{-1})	T_3 (100 mgL^{-1})	T_4 (200 mgL^{-1})	T_5 (500 mgL^{-1})
0	7.3 ± 0.001	7.33 ± 0.058	7.3 ± 0.001	7.3 ± 0.001	7.3 ± 0.001
1	7.3 ± 0.001	7.33 ± 0.058	7.3 ± 0.001	7.3 ± 0.001	7.3 ± 0.001
2	7.33 ± 0.058	7.33 ± 0.058	7.3 ± 0.001	7.3 ± 0.001	7.3 ± 0.001
3	7.4 ± 1.1	7.37 ± 0.058	7.33 ± 0.058	7.3 ± 0.001	7.33 ± 0.058
4	7.4 ± 1.1	7.4 ± 1.1	7.4 ± 1.1	7.4 ± 1.1	7.4 ± 1.1
5	7.43 ± 0.058	7.4 ± 1.1	7.4 ± 1.1	7.4 ± 1.1	7.4 ± 1.1
6	7.43 ± 0.058	7.47 ± 0.058	7.4 ± 1.1	7.43 ± 0.058	7.43 ± 0.058
7	-	7.47 ± 0.058	7.5 ± 0.001	7.47 ± 0.058	7.5 ± 0.001
8	-	-	7.5 ± 0.001	7.5 ± 0.001	-
9	-	-	-	7.5 ± 0.001	-

2.7.2 Biomass (dry weight basis): The determination of biomass for each *T. chuii* culture was measured daily as well throughout the experimental period. One millilitre culture aliquot from one of the flasks was filtered using a vacuum pump filtration unit (Millipore) through the preweighed and dried (100°C for 4 h, WTC Binder oven) 47 mm Ø Whatman® GF/C glass microfiber filter papers. Filter papers containing the samples were dried in the oven (WTC Binder) at 100°C for 4 h. After 4 h, the filter papers were taken out from the oven and cooled in the desiccator (Nalgene®) for 15 min. Then, the filter papers were individually weighed (AND, GR-200) and the biomass (dry weight basis) was determined for each sample using the formulae below:

Ds (g) = (Weight of filter paper + sample) – (Weight of empty filter paper)

Biomass (g L^{-1}) dry weight = (Ds / Amount of sample filtered) × 1000

Where the amount of sample filtered was 1 mL.

2.7.3 Optical density: The growth of the cultures was monitored daily throughout the experimental period by measuring the absorbance readings of the culture aliquots using a spectrophotometer (UV-1601 UV- Visible Spectrophotometer SHIMADZU, Japan). The culture medium for *T. chuii* was used as the blanks. The absorbance readings were taken at a wavelength of 450 nm (Lavens and Sorgeloos 1996).

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

The specific growth rate (SGR, μ day^{-1}) of indigenous *T. chuii* was calculated (Table 4) from the equation below:

$SGR = \ln (X_2 / X_1) / t_2 - t_1$

Where, X_1 represented as the biomass concentration at the beginning of the selected time interval, X_2 presented as biomass concentration at the end of the selected time interval and $t_2 - t_1$ was the selected time (in days) for the determination of biomass of *T. chuii*.

Division rate (day^{-1}) of the indigenous *T. chuii* was calculated (Table 4) from the equation below:

Division rate = SGR / ln2

2.9 Determination of proximate compositions

2.9.1 Protein determination: For every sample, 5 mg of

freeze-dried microalgae was taken and made into a 25 mL solution by mixing with distilled water. From the 25 mL of sample prepared, 0.5 mL was taken from each sample for protein analysis. Prior to that, Reactive 1 (1% NP tartrate) and Reactive 2 (2 g of $NaCO_3$ in 100 mL of 0.1 NaOH) were prepared. 50 mL of Reactive 2 and 1 mL of Reactive 1 were mixed. After that, a 0.5 mL sample was added with 0.5 mL of 1N NaOH and it was kept in a 100°C water bath for 5 min. It was cooled in a water bath and 2.5 mL of the prepared mixed reagent was added 10 min after cooling. The mixed solution was put in with 0.5 mL of Folin-Ciocalteau reagent and then kept in the dark places for 30 min. 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).

TABLE 4 Specific growth rate (SGR) and division rate of *Tetraselmis chuii* under different concentrations of urea.

Treatment (mgL^{-1})	SGR (μ day^{-1})	Division rate (day^{-1})
T_1 (25)	0.0397	0.0573
T_2 (50)	0.0411	0.0593
T_3 (100)	0.0590	0.0851
T_4 (200)	0.0689	0.0994
T_5 (500)	0.0517	0.0746

2.9.2 Carbohydrate determination: Five mg sample was taken and made into a 25 mL solution by mixing with distilled water. Prior to analysis, 5% phenol solution and concentrated sulfuric acid were prepared. For analysis, 1 mL was taken from the prepared 25 mL solution 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).

2.10 Data analysis

The growth and proximate compositions were analysed using one-way analysis of variance (ANOVA) at a 95% significance level ($p < 0.05$) as well as Tukey multiple comparisons test (where applicable). The graphical presentation of the growth parameters by Origin V8 software, proximate compositions (% dry weight) with different treatments were analysed by Origin V8 and SPSS software and different tables were made by Microsoft Office 2016, respectively. All findings are shown as averages with standard deviations.

3 | RESULTS

3.1 Microalgae growth

Cell density (cells mL^{-1}), dry weight biomass (g L^{-1}), and optical density (450 nm) of indigenous marine microalga *T. chuii* cultured in controlled conditions in reaction to five varied N concentrations for urea are shown in Figure 2. The study revealed that *T. chuii*, when cultured in varying N concentrations, underwent prolonged stationary phases on different days.

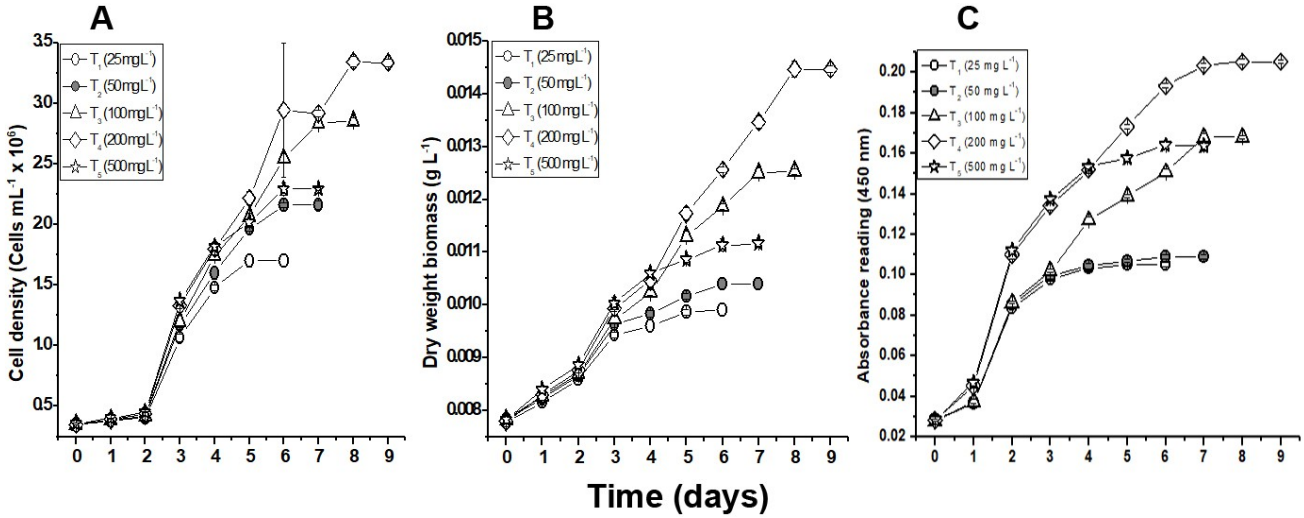

FIGURE 2 Effects of different N concentrations on (A) cell density (cells mL^{-1}), (B) biomass (gL^{-1}), and (C) optical density (450 nm) of *Tetraselmis chuii* under five different treatments of urea (n = 15; values are average ± standard deviation).

The marine microalgae *T. chuii* efficiently utilized organic nitrogen (urea) for the growth and relatively increased growth performance had observed for urea as well. Maximum density had found in T_4 (200 mg L^{-1}) for urea (Figure 2A, Table 5), with the respective cell density of 3.00×10^6 cells mL^{-1} and 3.33×10^6 cells mL^{-1}, which was significant ($p < 0.05$) in respect to control T_3 (100 mgL^{-1}), respectively. *Tetraselmis chuii* showed lower cell density in T_1 (25 mgL^{-1}) comprised of 1.7×10^6 cells mL^{-1} for urea, a significant difference ($p < 0.05$) had found among the treatments, respectively (Figure 2, Table 5). The study findings indicated that the growth performance of *T. chuii* was significantly improved ($p < 0.05$) when the culture media contained higher levels of nitrogen concen-

trations. However, an exception was observed in T_5 (500 mg L^{-1}), where *T. chuii* stopped growing by day 7 with compromised growth performance ($p > 0.05$; Table 5).

The increase of urea concentrations in the culture media ranging from 11.675 to 233.5 mg L^{-1} of N, respectively, significantly ($p < 0.05$) induced increment of biomass production, except for 233.5 mg L^{-1} of N ($p > 0.05$) concentration. The biomass concentration of *T. chuii* at the stationary phase under 11.7 mg L^{-1} N (T_1) were recorded only 0.0099 g L^{-1} (increased by 26% biomass based on previous experiment reported by Barman *et al*. 2021), respectively (Table 5). However, biomass concentrations of the control T_3 (100 mg L^{-1}) for urea (0.0125 g L^{-1} dry biomass), was not significantly ($p > 0.05$) different to each other, which increased about 58% (comprising 16.5 mg L^{-1} N) and 60% (comprising 46.7 mg L^{-1} N) of dried biomass, respectively.

TABLE 5 Mean maximum values of each parameter with standard deviation among different treatments.

Treatments	Values (n = 15; Mean ± SD)				
	T_1 (25 mgL^{-1})	T_2 (50 mgL^{-1})	T_3 (100 mgL^{-1})	T_4 (200 mgL^{-1})	T_5 (500 mgL^{-1})
CD (as 10^6)	1.700 ± 0.628	2.160 ± 0.806	2.827 ± 1.044	3.333 ± 1.198	2.293 ± 0.855
Biomass (gL^{-1})	0.010 ± 0.006	0.010 ± 0.001	0.013 ± 0.002	0.015 ± 0.003	0.011 ± 0.001
OD	0.105 ± 0.033	0.109 ± 0.034	0.168 ± 0.053	0.205 ± 0.066	0.164 ± 0.054

Similar trends were observed when optical density (OD) measurements were made using absorbance readings at 450 nm in which significantly ($p < 0.05$) increased absorbance with increasing N concentrations were recorded (Figure 2C, Table 5). Results also showed a similar trend for the specific growth rate (SGR day^{-1}) and division rate of *T. chuii* which increased with the sufficient nitrogen concentration. The highest SGR and division rate was calculated 0.0689 day^{-1} and 0.0994 day^{-1} (considering 93.4 mg L^{-1} N) concentrations, respectively.

The experiment involved growing a culture in a conical flask where 2 L of the flask could have a maximum volume of 1.5 L of the culture. The culture was aerated with pure air, which contained only 0.03% CO_2, meaning that the air supply was not very conducive to cellular respiration and metabolic activities. This resulted in poor mixing of nutrients and low light penetration, leading to low dry biomass production.

3.2 Effects of different nitrogen sources and concentrations on protein and carbohydrate content

The varying levels of nitrogen in the growth medium of *T. chuii* had a significant ($p < 0.05$) effect on protein and carbohydrate content (Figure 3). The protein and carbohydrate content showed a significant ($p < 0.05$) changes of contrary trends for urea, in respect to sufficient or deficient N concentrations, respectively. The increased N concentrations in the culture medium significantly ($p < 0.05$) induced increment of protein content (% dry weight) and the highest protein content 45.24% dry weight (comprising 233.5 mg L^{-1} N) were recorded from T_5 (500 mg L^{-1}), respectively, was significant ($p < 0.05$) to each other. In contrast, the carbohydrate content (% dry weight) had revealed the highest concentration 28.03% dry weight (comprising 11.7 mg L^{-1} N) in T_1 (25 mg L^{-1}), respectively, was not significantly ($p > 0.05$) different to each other.

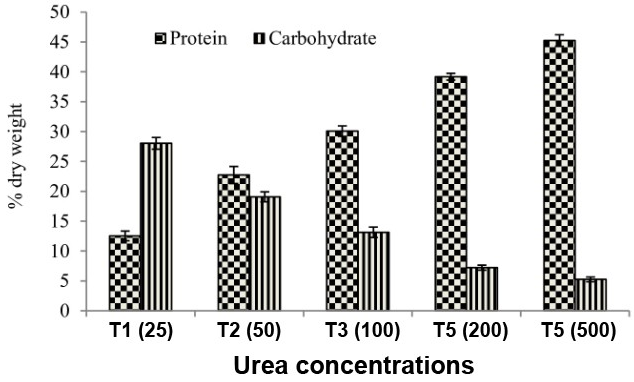

FIGURE 3 Proximate composition (% dry weight) of *Tetraselmis chuii* under different concentrations of urea (values are average ± standard deviation; n = 15).

4 | DISCUSSION

The amount of nitrogen in the growth medium significantly influences algal growth rate, as this nutrient is essential for cell proliferation (Wu and Miao 2014) and the biochemical composition of microalgae (Kim *et al*. 2016a). The present study revealed that *T. chuii* under a low nitrogen concentration of 11.7 mg L^{-1} N showed the lowest cell density, whereas, conversely, increasing N concentrations led to higher cell densities, with the highest density (3.33×10^6 cells mL^{-1}) recorded at 93.4 mg L^{-1} N. Additionally, there was a strong correlation between this data and the optical density readings taken during the experiment. The lowest specific growth rate was observed in a similar trial without nitrogen supply, and growth rates increased markedly with higher N availability for three species: *Tetraselmis subcordiformis*, *Nannochloropsis oculata*, and *Pavlova viridis* (Huang *et al*. 2013). Another study also reported that some species- *Scenedesmus acutus*, *Chlorella vulgaris*, *Nannochloropsis* sp., and *Nannochloropsis oleoabundans*- were able to grow effectively

in nitrogen-deficient environments by utilising their intracellular nitrogen reserves, such as pigment-protein molecules (Gu *et al*. 2015), which also supports the present findings- that is, the growth of cell concentrations under low nitrogen conditions.

Inorganic nitrogen is utilized by marine microalgae to support their growth, metabolic functions, and amplify their biomass levels when exposed to an environment enriched with nitrogen (Cheng and He 2014; Rizwan *et al*. 2017), which also supported by the present study findings. The current study also showed that *T. chuii*'s growth performance was negatively impacted by an increased nitrogen concentration of 233.5 mg L^{-1} N for urea due to nutrient imbalance. It is known that excessively high N concentrations limit other nutrients, vitamins, and trace metals and have a detrimental effect on cell growth (Razaghi *et al*. 2014). Dry biomass of *T. chuii* in the present experiment increased with increasing supplemented N in the medium, but also showed a decreasing trend in biomass production at low N concentrations. The research by El-Kassas (2013) also reported the same thing in which *Picochlorum* sp. cell density and biomass production reduced when the primary macronutrients nitrogen or phosphorus were stressed compared to the control treatments.

Mixing is critical for achieving optimal light concentrations in all microalgae culture media to support appropriate growth and biomass output (Richmond 1988). Mohsenpour and Willoughby (2016) also reported that increased CO_2 concentrations in the air stream enhanced CO_2 fixation in Chlorella vulgaris, and biomass concentrations were higher with 5% CO_2 aeration than with pure air (0.03% CO_2), results supported by the present study.

The suitable N concentrations for growth and proximate composition of microalgal cells differ species to species (Kim *et al*. 2016a). The results of the present study demonstrate that different nitrogen concentrations significantly affected protein content in *T. chuii*, with an increase of up to 45.24% in dry weight observed at higher nitrogen concentrations in the growth medium. However, contrasting trends in carbohydrate concentrations were observed in response to urea. Similar results were reported by Kim *et al*. (2016a), who found that carbohydrate production increased by up to 55% under nitrogen-deficient conditions in *Tetraselmis* cells, whereas protein production decreased steadily. Therefore, it appears that *T. chuii* accumulates protein when grown under nitrogen-sufficient conditions but accumulates carbohydrates when grown under nitrogen-deficient conditions, as noted by Kim *et al*. (2016b).

Cultivation of *T. chuii* in Bangladesh supports a sustainable economy by providing cost-effective and nutritious aquafeed for the growing aquaculture sector. Its ability to absorb and convert surplus nutrients can help mitigate water degradation and eutrophication in coastal zones. Additionally, *T. chuii* cultivation can support the growth of biotechnology industries, promoting the manufacture of biofuels and other value-added products. However, to ensure long-term usage, proper legislation and regulations are necessary.

5 | LIMITATIONS OF THE RESEARCH

Due to financial constraints, lipid concentrations were not examined in this investigation. *Tetraselmis chuii* is crucial to the manufacture of biofuel, however its lipid content is still unclear. Lipid extraction and in-depth analysis will be part of future studies to investigate its potential as a biofuel.

6 | CONCLUSIONS

This study investigated the growth performance of indigenous marine microalga *T. chuii* under different urea concentrations in the culture medium. The marine microalga *T. chuii* were shown to use urea (organic nitrogen) effectively, yet under varying urea concentrations, relative enhanced growth and proximate compositions were detected. The specific growth rate and division rate of *T. chuii* also increased with higher nitrogen concentrations. Additionally, protein and carbohydrate content were influenced by the nitrogen concentration, with protein content increasing with higher nitrogen levels while carbohydrate content showed an opposite trend. Based on the results of this study, it can be concluded that it was a good first step in the study of the marine microalga *T. chuii* strain that was isolated from the Bay of Bengal and for the improvement of our knowledge of the physiological and biological characteristics of *T. chuii*. Cultivating *T. chuii* at different nitrogen concentrations has resulted in encouraging outcomes. Furthermore, their potential for bioremediation of dirty water and CO_2 sequestration can contribute to the long-term viability of Bangladesh's economy. However, due to financial constraints, lipid concentrations were not examined in this investigation. *T. chuii* is crucial to the manufacture of biofuel, however its lipid content is still unclear. We suggest studies on lipid extraction and its in-depth analysis.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

AUTHORS' CONTRIBUTION

Md. Jahid Hossain: Conceptualization, Data Curation, Data Processing, Formal Analysis, Writing of the Original Draft, Validation, Revising and Editing, Sanzib Kumar Barman: Conceptualization, Data Processing, Formal Analysis, Writing of the Original Draft, Validation, Revising and Editing; Helena Khatoon: Conceptualization, Funding Acquisition, Supervision, Resources, Validation, Writing – Review & Editing. Kishor Kumar Tikadar: Data Curation, Formal Analysis; Revising and Editing; Debasish Pandit: Formal Analysis, Data Processing, Revising, Editing; All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data will be made available on request.

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