

## The Effect of Light Intensity and Nutrient Formula on the Growth of *Chlorella ellipsoidea* in Batch Cultivation

Sudarat Theerapisit<sup>1</sup>, Somrak Rodjaroen<sup>2</sup> and Siriluk Sintupachee<sup>1,\*</sup>

<sup>1</sup>Program in Creative Innovation in Science and Technology, Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, Nakhon Si Thammarat 80280, Thailand

<sup>2</sup>Program in Agriculture Innovation in Science and Technology, Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, Nakhon Si Thammarat 80280, Thailand

(\*Corresponding author's e-mail: siriluk\_sint@nstru.ac.th)

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### Abstract

In the aquaculture feed industry, the batch cultivation of the alga *Chlorella ellipsoidea* is frequent because it allows the control of factors contributing to product quality. In this study, we conducted batch-cultivation experiments in a smart algae cabinet with 4 different light intensities, 1,000, 3,000 and 5,000 Lux, as well as a fluorescent lamp (8,000 Lux). The growth curves, specific growth rate and biomass productivity of *C. ellipsoidea* provided with the BG-11, Bold and Chu-13 nutrient formulae were evaluated. The results revealed biomasses of  $1.315 \pm 0.0087$ ,  $1.042 \pm 0.0063$  and  $1.096 \pm 0.0036$  g/L/day and specific growth rates of  $0.979 \pm 0.0067$ ,  $0.7453 \pm 0.0060$  and  $0.7781 \pm 0.0026$  per day for the 3 formulae, BG-11, Bold and Chu-13, respectively. The mean biomasses of *C. ellipsoidea* grown on BG-11 at all light intensities were significantly higher than those for other media ( $p$ -value = 0.0001). With the log phase achieved in 3 days, BG-11 was more suitable for rearing at all tested light intensities, while Chu-13 was only applicable at 5,000 Lux. Using all 3 light intensities, it was possible to reduce the log-phase of algae growth from 7 to 3 days and increase biomass by more than 1.2 times when compared to fluorescent light culture.

**Keywords:** Batch cultivation, Biomass, Chlorella, Growth rate, Light intensity, Media, Microalgae

### Introduction

*Chlorella* sp. is a single-celled green alga common in natural water sources. It is small (2.5 - 3.5  $\mu$ M) and has more chlorophyll than other plants, with 3 - 5 % of weight [1,2]. The photosynthesis process of *Chlorella* sp. can quickly convert carbon dioxide into food. Therefore, it provides protein to rotifer mites and artemia, zooplanktons used in the nursery of young aquatic animals like shrimp, tilapia and gourami [3]. In addition, this green microalga has a high survival rate since it is used as feed in aquaculture ponds [4]. *Chlorella* sp. derived protein powder was created in a less complicated and low-tech manner than plant-based meat [5]. As a result, microalgae, specifically *Chlorella* sp., are believed the future plant and food. There is increased interest in research and utilization of *Chlorella* sp. for a variety of purposes. *Chlorella* sp. has applications in food supplements, animal feed, fertilizers, biofuels, pharmaceuticals, cosmetics and anti-aging sera. It is also used in the aquaculture industry as a protein to promote the growth and health of aquatic animals [6,7].

Currently, there are 2 microalgae cultivation systems, the open and the closed systems [8,9]. In the open system, microalgae are frequently cultivated in ponds with propeller agitators or in those with constantly swirling water. The advantage of this system is that algae use natural solar energy (sunlight), which they transform into large biomasses, yet the growth variables in this system are difficult to control, and there is a high possibility of pathogen contamination [10]. On the other hand, cultivating algae in closed bioreactors allow better operation control, which results in high-quality biomass yield. However, the disadvantages of the closed system include being expensive, the difficulty of increasing the culture volume in large bioreactors, and the higher cost of generating photosynthetic light made from bioplastic materials [11,12].

Hence, the closed-system culturing of *Chlorella* sp. saves space and critically maintains a pathogen-free environment. The growth rate of microalgae is influenced by many factors, including food source, the intensity of light, temperature and humidity. Using liquid nutrients when establishing a closed-batch system and harvesting the final biomass yield makes culturing *Chlorella* sp. more convenient. The quality and

quantity of the used nutrient formula for culturing *Chlorella* sp. are essential factors affecting biomass yield. A typical nutrient formula mainly includes 1 - 10 g/L of a carbon source, such as CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>, an amount of 10 - 2,000 mg/L of a nitrogen source (NO<sub>3</sub><sup>-</sup>, Urea, or N<sub>2</sub>), phosphorus in the form of phosphate or hydrogen phosphate (10 - 500 mg/L), and 200 mg/L of sulfate as a source of sulfur. Inorganic salts of K, Ca, Na and Mg in a concentration range of 0.1 to 100 mg/L should also be included. Additionally, micronutrients, such as Fe, Zn, Mn, Pb and Cd (0.01 - 10 mg/L) and vitamins B, C and E (0.01 - 1,000 g/L) are incorporated to promote the growth of algae. Therefore *Chlorella* sp. under optimal conditions using the appropriate nutrient medium and light intensity enables the control of several culturing conditions, resulting in high-quality biomass yield at the right time of the year [13].

## Materials and methods

### Test strain, culture conditions and experimental plan

The *C. ellipsoidea* used in this study was strain TISTR 8260, purchased from the Thailand Institute of Scientific and Technological Research (TISTR). Following an aseptic technique, the microalga stock culture was prepared in 2L-flasks containing the appropriate culture medium at a nutrient-to-alga ratio of 9:1. Cultivation was carried out in a smart algae cabinet for 14 days with a light intensity of 1,000, 3,000, 5,000 Lux, or a fluorescent lamp (8,000 Lux) with shaking at 150 rpm. A completely randomized design (CRD) using 3 different culture formulae, BG-11, Bold and Chu-13, was employed to study the growth of *C. ellipsoidea* strain TISTR 8260. Each experiment was conducted in triplicate. All experiments were carried out in a smart algae incubator with a light interval of 12 h per day for 30 days at 28 ± 1 °C with shaking at 150 rpm. The light source was a T8 LED (B1) bulb, and the intensity was measured with a Mastech MS6610 Digital light meter (China). In a smart algae cabinet (**Figure 1**), the distance between the bulb and the bottom of the flask was set at 40 cm.



**Figure 1** A smart algae chamber with the light intensity.

In this study, 3 nutrient formulae, BG-11, Bold and Chu-13, (Himedia, India) were tested for growing *C. ellipsoidea*. The composition of culture media was as follows: 1) The BG-11 formula contained 15.0 g/L of NaNO<sub>3</sub>, 0.4 g/L of K<sub>2</sub>PHO<sub>4</sub>, 0.75 g/L of MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.36 g/L of CaCl<sub>2</sub>.2H<sub>2</sub>O, 0.06 g/L of citric acid, 0.06 g/L of C<sub>6</sub>H<sub>5</sub> + 4yFexNyO<sub>7</sub>, 0.01 g/L of C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>8</sub>.2H<sub>2</sub>O, 0.02 g/L of Na<sub>2</sub>CO<sub>3</sub> and 1.0 mL of the Trace Metal Mix A5. 2) The Bold formula included NaNO<sub>3</sub> (2.50 g/L), K<sub>2</sub>PHO<sub>4</sub> (0.75 g/L), MgSO<sub>4</sub>.7H<sub>2</sub>O (0.75 g/L), CaCl<sub>2</sub>.2H<sub>2</sub>O (0.25 g/L), NaCl (0.25 g/L), H<sub>3</sub>BO<sub>3</sub> (1.14 g/L), ZnSo<sub>4</sub>.7H<sub>2</sub>O (8.82 g/L), CuSo<sub>4</sub>.5H<sub>2</sub>O (0.71 g/L) and KH<sub>2</sub>PO<sub>4</sub> (1.75 g/L). 3) The Chu-13 formula comprised K<sub>2</sub>PHO<sub>4</sub> (0.04 g/L), MgSO<sub>4</sub>.7H<sub>2</sub>O (0.1 g/L), CaCl<sub>2</sub>.2H<sub>2</sub>O (0.054 g/L), NaNO<sub>3</sub> (0.2 g/L), H<sub>3</sub>BO<sub>3</sub> (2.85 g/L), Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O (0.05 g/L), ZnSo<sub>4</sub>.7H<sub>2</sub>O (0.02 g/L), CoCl<sub>2</sub>.6H<sub>2</sub>O (0.08 g/L), C<sub>6</sub>H<sub>5</sub>+4yFexNyO<sub>7</sub> (0.01 g/L), MnCl<sub>2</sub>.4H<sub>2</sub>O (1.8 g/L) and CuSo<sub>4</sub>.5H<sub>2</sub>O (0.08 g/L). The prepared culture media were sterilized in an autoclave at 121 °C for 15 min and divided into 250-mL aliquots to cultivate the *C. ellipsoidea*. The *C. ellipsoidea* TISTR 8260 strain was inoculated with a medium: Microalga ratio of 9:1 to determine the growth rate and biomass yield.

### Determination of cell number, biomass and specific growth rate

To determine the cell density of *C. ellipsoidea*, 1 mL of the culture was collected, and the optical density (OD<sub>600 nm</sub>) was measured using a spectrophotometer (Peak brand, model C-7100, USA). The biomass and the specific growth rate of the microalga were calculated using Eqs. (1) and (2), respectively [14].

### Equation

$$P = \frac{x_1 - x_0}{t_1 - t_0} \quad (1)$$

$$\mu = \frac{\ln(x_1/x_0)}{t_1 - t_0} \quad (2)$$

where P is the mass productivity (biomass productivity; g/L/day),  $\mu$  is the specific growth rate (per day), and  $X_1$  and  $X_0$  are the alga mass (g/L) at day  $T_1$  and  $T_0$ , respectively.

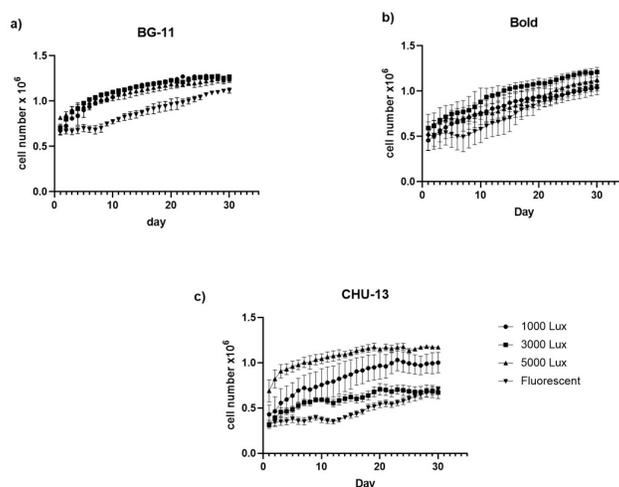
The cell number in 1  $\mu\text{L}$  of *C. ellipsoidea* culture was counted by a hemocytometer using the A-method [15]. A 40 $\times$  magnification using a microscope (Carl Zeiss, model Primo Star, Germany) was used for this purpose. The obtained values were multiplied by 1,000 to calculate the number of cells per 1 mL of culture.

### Statistical analysis

All experiments were conducted 3 times for each sample ( $n = 3$ ). The standard deviation (SD) was calculated, and the differences between groups were determined by the 1-way analysis of variance (ANOVA) followed by Tukey's multiple comparison analysis using the R program. A  $p$ -value of 0.05 at 95 % confidence was considered statistically significant.

### Results and discussion

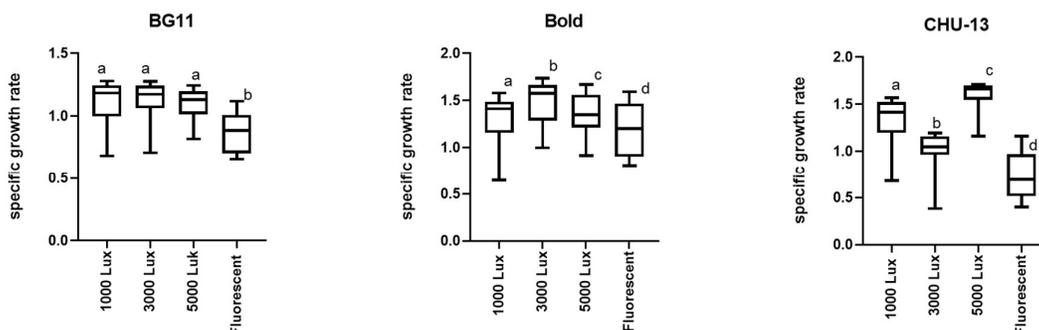
We investigated the influence of 3 culture media, BG-11, Bold and Chu-13, and different light intensities of 1,000, 3,000 and 5,000 Lux, as well as fluorescent light intensity, on the growth of *C. ellipsoidea* strain TISTR 8260. In general, *C. ellipsoidea* grew better in the BG-11 medium at all light intensities compared to the control light intensity from a fluorescent lamp. The growth s-curves of *C. ellipsoidea* cultured in BG-11, Bold and Chu-13 media are shown in **Figure 2**. The log-phase of the *C. ellipsoidea* grown in BG-11 was reached within 3 - 8 days of inoculation, with the growth curves looking similar at the 3 tested light intensities (**Figure 2(a)**). For the Bold and Chu-13 media, the log-phase of *C. ellipsoidea* growth was achieved at 4 - 7 days for all tested light intensities (**Figures 2(b)** and **2(c)**).



**Figure 2** Growth curves of *C. ellipsoidea* strain TISTR 8260 in different culture media: (a) BG-11, (b) Bold, and (c) Chu-13 exposed to 1,000, 3,000, 5,000 Lux and fluorescent light intensities.

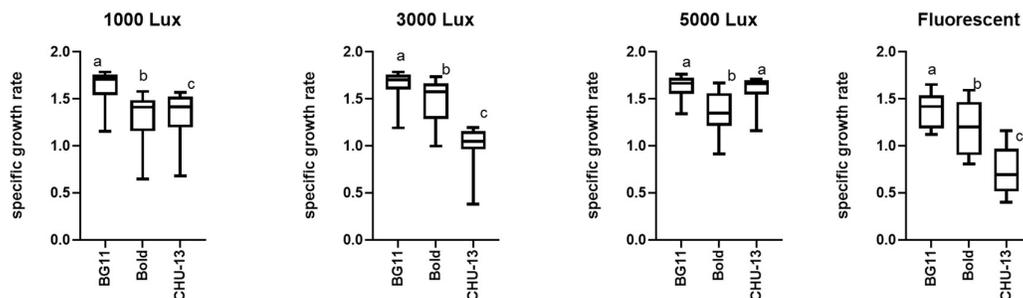
In addition, the average cell numbers of *C. ellipsoidea* grown on BG-11 and exposed to light intensities of 1,000, 3,000 and 5,000 Lux were  $1.107 \pm 0.1745 \times 10^6$ ,  $1.127 \pm 0.1467 \times 10^6$  and  $1.100 \pm 0.122 \times 10^6$  cells/mL, respectively. These cell numbers were significantly higher with a  $p$ -value of 0.0001 (DF = 3,  $F(3, 116) = 18.31$ ) compared to that of the control *C. ellipsoidea* exposed to a fluorescent lamp, which achieved a cell number of  $0.7774 \pm 0.1547 \times 10^6$  cells/mL (**Figure 3(a)**). Meanwhile, the average cell numbers of the Bold cultures in the 3 tested light intensities were  $0.8273 \pm 0.1627 \times 10^6$ ,  $0.9733 \pm$

$0.1894 \times 10^6$  and  $0.8579 \pm 0.1655 \times 10^6$  cells/mL, which were also higher ( $p$ -value = 0.0001, DF = 3,  $F(3, 116) = 8.13$ ) than the control ( $0.7481 \pm 0.1972 \times 10^6$  cells/mL) (**Figure 3(b)**). Likewise, the cell counts of the Chu-13 cultures at 1,000, 3,000 and 5,000 Lux were  $0.8350 \pm 0.1718 \times 10^6$ ,  $0.6004 \pm 0.0989 \times 10^6$  and  $1.075 \pm 0.1196 \times 10^6$  cells/mL, respectively. These values were again higher with a  $p$ -value of 0.0001 (DF = 3,  $F(3, 116) = 124$ ) than that of the control, which achieved a cell number of  $0.4716 \pm 0.1208 \times 10^6$  cells/mL (**Figure 3(c)**).



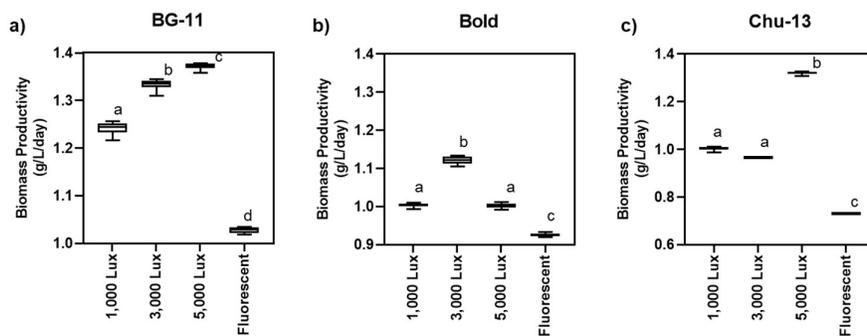
**Figure 3** Mean cell numbers of *C. ellipsoidea* strain TISTR 8260 grown in (a) BG-11, (b) Bold, and (c) Chu-13 at a light intensity of 1,000, 3,000, 5,000 Lux, or fluorescent light intensity control. The alphabets a, b, c and d on the plot represent statistically significant differences.

Comparing the performance of the 3 tested formulae for each light intensity, data revealed that the average cell number in the BG-11 medium was significantly higher than that of the Bold and Chu-13 media for all light intensities, except for the 5,000 Lux, with a  $p$ -value of 0.0001 (DF = 2,  $F(2, 87) = 26.45$ ) (**Figures 4(a) - 4(d)**). At 5,000 Lux, the BG-11 and Chu-13 media produced comparable average cell numbers and were significantly higher than that of the Bold medium with a  $p$ -value of 0.0001 (DF = 2,  $F(2, 87) = 28.30$ ) (**Figure 4(c)**). The cell numbers obtained from the 3 formulae differed at a  $p$ -value of 0.0001 (DF = 2,  $F(2, 87) = 49.95$ ) from the control culture exposed to fluorescent lamp light intensity (**Figure 4(d)**).



**Figure 4** Comparison of the mean cell numbers of *C. ellipsoidea* grown in BG-11, Bold, or Chu-13 media at light intensities of (a) 1,000 Lux, (b) 3,000 Lux, (c) 5,000 Lux, or (d) control fluorescent lamp light intensity. The alphabets a, b and c on the plot represent statistically significant differences.

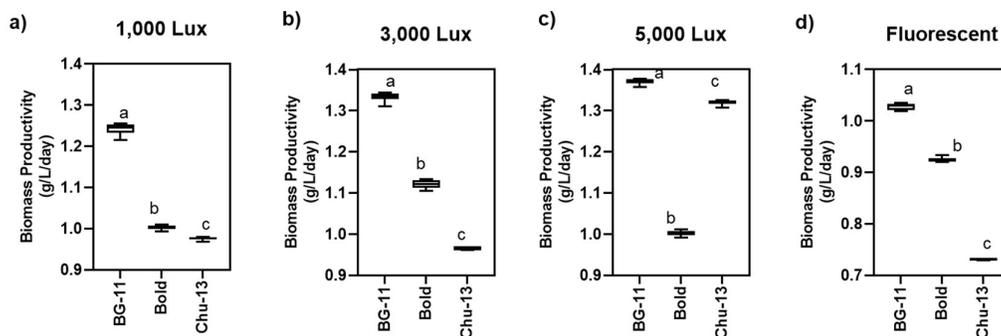
Biomass is an important indicator of microalgae growth as it reflects the amount of nutrients, light and water they can capture. In this study, the mean *C. ellipsoidea* biomass values obtained from growing strain TISTR 8260 in BG-11 medium was significantly higher with a  $p$ -value of 0.0001 (DF = 3,  $F(3, 116) = 9.813$ ) for all light intensities than for the control fluorescent light. The average biomasses were  $1.242 \pm 0.0117$ ,  $1.333 \pm 0.0090$ ,  $1.371 \pm 0.0056$  and  $1.028 \pm 0.0059$  g/L/day at the light intensities of 1,000, 3,000 and 5,000 Lux, as well as the control fluorescent light, respectively (**Figure 5(a)**).



**Figure 5** Mean biomasses from *C. ellipsoidea* strain TISTR 8260 grown in (a) BG-11, (b) Bold, and (c) Chu-13 culture media at 1,000, 3,000 and 5,000, as well as fluorescent light intensity. The alphabets a, b, c and d on the plot represent statistically significant differences.

In the Bold medium, the mean biomasses were larger than the control value for all light intensities;  $p$ -value = 0.0001 (DF = 3,  $F(3, 116) = 5,366$ ). They were  $1.003 \pm 0.0045$ ,  $1.121 \pm 0.0087$ ,  $1.002 \pm 0.0058$  and  $0.925 \pm 0.0038$  g/L/day at the 1,000, 3,000, 5,000 Lux and the control fluorescent light, respectively (**Figure 6(b)**). At the same time, the mean biomass of *C. ellipsoidea* provided with Chu-13 medium was also higher than the control with a  $p$ -value of 0.0001 (DF = 3,  $F(3, 116) = 132,786$ ). Values of  $1.003 \pm 0.0052$ ,  $0.965 \pm 0.0009$ ,  $1.320 \pm 0.0047$  and  $0.731 \pm 0.0013$  g/L/day were recorded at 1,000, 3,000, 5,000 Lux and the control fluorescent light, respectively (**Figure 6(c)**).

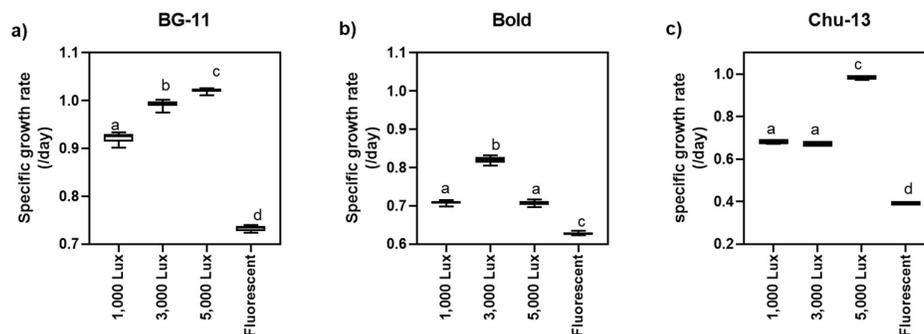
Collectively, *C. ellipsoidea* yielded higher biomasses when grown in BG-11 than in the other nutrient formulae at all tested light intensities. The  $p$ -value was 0.0001, DF = 2, and values  $F(2, 87)$  were 11,294, 19,300, 40,477 and 38,908 at 1,000, 3,000 and 5,000 Lux, as well as the control fluorescent light, respectively (**Figures 6(a) - 6(d)**).



**Figure 6** Mean biomass productivity of *C. ellipsoidea* strain TISTR 8260 provided with BG-11, Bold, or Chu-13 culture media at different light intensities: (a) 1,000 Lux, (b) 3,000 Lux, (c) 5,000 Lux, and (d) fluorescent light. The alphabets a, b and c on the plot represent statistically significant differences.

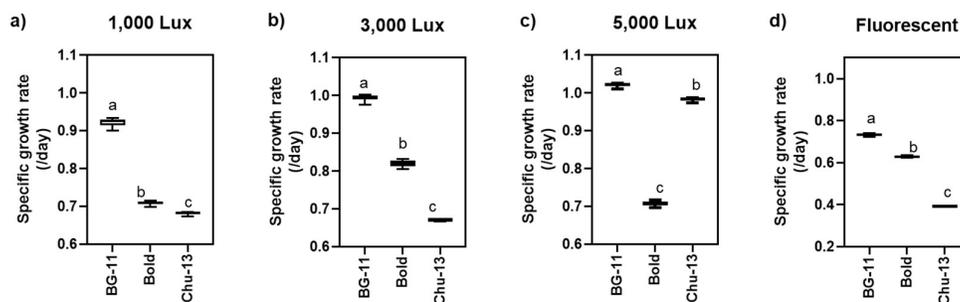
Furthermore, we determined the mean specific growth rates of *C. ellipsoidea* strain TISTR 8260 grown in the 3 tested nutrient formulae. BG-11 medium allowed better *C. ellipsoidea* growth compared to the other tested media. The mean specific growth rates at light intensities of 1,000, 3,000 and 5,000 Lux were significantly higher compared to the control fluorescent light (**Figure 7(a)**) with a  $p$ -value of 0.0001 (DF = 3,  $F(3, 116) = 10,713$ ). They were  $0.922 \pm 0.0095$ ,  $0.993 \pm 0.0067$  and  $1.021 \pm 0.0041$  day, respectively. Unlike BG-11, the growth of *C. ellipsoidea* on the Bold formula was not promoted at 1,000 and 5,000 Lux. The only significant increase in growth rate was observed at 3,000 Lux with a  $p$ -value of 0.0001 (DF = 3,  $F(3, 116) = 5,654$ ) compared to the control light. The average specific growth rate values were  $0.7087 \pm 0.0046$ ,  $0.8199 \pm 0.0077$ ,  $0.7074 \pm 0.005830$  and  $0.6279 \pm 0.0041$  day for 1,000, 3,000, 5,000 Lux and fluorescent light intensity, respectively (**Figure 7(b)**). The mean specific growth rate values of the Chu-13 medium were higher than the control with a  $p$ -value of 0.0001 (DF = 3,  $F(3, 116) = 24,917$ ).

for all light intensities. They were  $0.6811 \pm 0.0033$ ,  $0.6703 \pm 0.0010$ ,  $0.9828 \pm 0.0035$  and  $0.3916 \pm 0.0018$  day at the 3 tested light intensities and the control light, respectively (**Figure 7(c)**).



**Figure 7** Mean specific growth rates of *C. ellipsoidea* strain TISTR 8260 grown on (a) BG-11, (b) Bold, and (c) Chu-13 media with 1,000, 3,000, 5,000 Lux and fluorescent light intensity control. The alphabets a, b, c and d on the plot represent statistically significant differences.

When the specific growth rates of *C. ellipsoidea* provided with either BG-11, Bold, or Chu-13 media were compared for each light intensity, BG-11 was superior to the other media for all tested light intensities with a  $p$ -value of 0.0001,  $DF = 2$ , and  $F(2, 87) = 12,811, 21,901, 41,049$  and  $50,701$  at 1,000, 3,000, 5,000 Lux and control fluorescent light, respectively (**Figures 8(a) - 8(d)**).



**Figure 8** Mean specific growth rates on BG-11, Bold and Chu-13 media of *C. ellipsoidea* strain TISTR 8260 exposed to light intensities of (a) 1,000 Lux, (b) 3,000 Lux, (c) 5,000 Lux, and (d) fluorescent light. The alphabets a, b, c and d on the plot represent statistically significant differences.

Microalgae, including *C. ellipsoidea*, play an important role in the aquaculture industry. Here, we evaluated the growth of *C. ellipsoidea* in 3 different nutrient formulae at different light intensities. The alga was grown at 28 °C in BG-11, Bold, or Chu-13 medium and achieved the log phase within 3 - 8, 4 - 7 and 4 - 7 days, respectively. A previous study on the effect of BG-11 medium on the growth of *Chlorella* sp. reported that the log phase was reached after 2, 3 and 4 days of incubation at temperatures of 15, 25 and 30 °C [16]. In the current study, *C. ellipsoidea* strain TISTR 8260 was cultured in a batch-cultivation system for 30 days to investigate the cell count, biomass and specific growth rate for the aquaculture industry. In contrast, the other studies were conducted using fed-batch cultivation for biodiesel, wastewater treatment and the production of medicines and cosmetics [17]. In the fed-batch cultivation of *C. vulgaris*, rearing for fatty acids, the log phase was reached at 3, 4 and 5 days when cultured in BG-11, Bold and Chu-13 media, respectively, at 28 °C with light intensities of 2,700 and 3,000 Lux [18]. In addition, the *Chlorella* T12 log phase of growth was attained at 4, 4 and 2 days when grown in BG-11, Bold and Chu-13, respectively, at 3,500 Lux of light intensity, while for *C. sorokiniana* PA91 provided with BG-11 at light intensities of 1,000, 3,000 and 5,000 Lux, it was achieved at 3, 6 and 4 days, respectively [19]. Furthermore, in the batch culture of *C. pyrenoidosa* used for biodiesel production, the log phase was reached in BG-11 after 2 days and in Bold after 3 days at 4,000 Lux light intensity [20].

We also determined the algal cell number in different culture media as a growth indicator. The previous studies reported that *C. vulgaris* cultured for 25 days in BG-11 with light intensities of 3,000, 4,000 and 5,000 Lux yielded  $1.729 \times 10^7$ ,  $1.419 \times 10^7$  and  $2.310 \times 10^7$  cells, respectively. In addition, *Chlorella* sp. E1708 grown in Chu-13 medium with a light intensity of 5,000 Lux for 8 days yielded  $3.88 \times 10^6$  cells. Over a period of 5 days, the number of cells at the same light intensity used in the culture system of this study was approximately 10 times smaller than that of *S. arvernesrs* grown in BG-11 and Chu-13 for 18 days at 9 watts (980 Lux) light intensity, yielding  $5 \times 10^6$  and  $4.5 \times 10^6$  cells, respectively. Compared to data in this study, these cell number values were 4 - 5 times higher than those recorded for *C. ellipsoidea* [21]. The same microalga, *C. vulgaris*, used in this investigation was cultured in a bioreactor for wastewater treatment using the BG-11 medium. *C. vulgaris* produced  $2.6 \times 10^6$  cells/mL when grown at  $25 \pm 1$  °C with 12 h of light at 3,500 Lux for 14 days [22]. In another study using *C. vulgaris*, cell numbers of  $4.3 \times 10^6$ ,  $4.6 \times 10^6$ ,  $6.5 \times 10^6$  and  $5.4 \times 10^6$  cells were obtained at red, white, green and blue wavelengths, respectively when *C. vulgaris* was cultured in a 3 L tubular bioreactor for 6 days in Bold medium at 3,000 Lux light intensity [23]. The total number of cells was  $6 \times 10^6$  cells, this data suggests that temperature is a critical factor affecting algal growth, where those algae grown to feed young fish and incubated at 25 °C produced the highest cell number ( $4.486 \times 10^7$  cells) [24].

To further evaluate the performance of the tested culture media, it was important to determine the biomasses and the specific growth rates of *C. ellipsoidea*. Data revealed that BG-11 enabled the highest biomass and specific growth rate values compared to the other culture media. In a published report, the *C. pyrenoidosa* fed-batch cultivation in BG-11 at light intensities of 2,000, 4,000, 6,000 and 8,000 Lux yielded biomass values of 0.368, 0.441, 0.486 and 0.516 g/L/day and specific growth rates of 0.278, 0.319, 0.360 and 0.447 per day, respectively [25]. Similarly, *C. vulgaris* grown in BG-11 and Bold media produced biomasses of 1.300 and 0.630 g/L/day, respectively [26]. The *Chlorella* T12 strain growth rates were 0.420, 0.830 and 0.380 per day, and its biomasses were 0.16, 0.20 and 0.15 g/L/day when grown in BG-11, Bold and Chu-13, respectively, at a light intensity of 3,500 Lux after 14 days at 25 °C [27]. Moreover, *C. vulgaris* provided with BG-11, Bold and Chu-13 media for 17 days produced biomasses of 1.005, 0.944 and 1.07 g/L/day at 3,000 Lux, while *C. minutissima* yielded biomasses of  $850 \pm 0.21$ ,  $730 \pm 0.42$  and  $970 \pm 0.21$  mg/L at 2,500 Lux for 30 days, which were higher than those obtained in this study [28]. The specific growth rates of *Chlorella* sp. grown on BG-11, Bold and Chu-13 media were 0.289/day, 0.196/day, and 0.196/day, respectively, at a light intensity of 3,500 Lux [29]. Furthermore, the growth rate of *C. vulgaris* culture grown in BG-11 with a light intensity of 5,000 Lux at 25 °C was 0.28/day, which was 5 - 6 times higher than the specific growth rate from rearing in this study. Among cultures grown at 15, 20 and 30 °C, those cultivated at 25 °C had the highest growth rate [30].

## Conclusions

In this study, we evaluated the growth of *Chlorella ellipsoidea* strain TISTR 8260 in 3 different culture media, BG-11, Bold and Chu-13, at different light intensities. Among the 3 media, BG-11 was the best in enhancing the growth of *C. ellipsoidea*. All growth indicators, including cell number, biomass and specific growth rate, were significantly higher for *C. ellipsoidea* cultures in BG-11 than in the other media. Moreover, BG-11 promoted the *C. ellipsoidea* growth at all tested light intensities, 1,000, 3,000 and 5,000 Lux, compared to the control fluorescent light. The findings of this study suggest that BG-11 is a suitable medium for batch cultivation of *C. ellipsoidea* at different light intensities. These microalgae will be stimulated in a subsequent experiment by using ultrasonic sound to accelerate the log phase in accordance with the conditions discovered in this study in order to promote biomass.

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## References

- [1] P Feng, Z Deng, L Fan and Z Hu. Lipid accumulation and growth characteristics of *Chlorella zofingiensis* under different nitrate and phosphate concentrations. *J. Biosci. Bioeng.* 2012; **114**, 405-10.
- [2] S Otleş and R Pire. Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. *J. AOAC Int.* 2001; **84**, 1708-14.
- [3] W Zhao, M Duan, X Zhang and T Tan. A mild extraction and separation procedure of polysaccharide, lipid, chlorophyll and protein from *Chlorella* spp. *Renew. Energ.* 2018; **118**, 701-8.
- [4] LBSD Moraes, RFB Santos, GFG Junior, GCP Mota, DMDM Dantas, RDS Bezerra and AO Gálvez. Microalgae for feeding of penaeid shrimp larvae: An overview. *Aquaculture Int.* 2022; **30**, 1295-313.
- [5] MA Hesni, A Hedayati, A Qadermarzi, M Pouladi, S Zangiabadi and N Naqshbandi. Using *Chlorella vulgaris* and iron oxide nanoparticles in a designed bioreactor for aquaculture effluents purification. *Aquacultural Eng.* 2020; **90**, 102069.
- [6] W Sawaengsak, T Silalertruksa, A Bangviwat and SH Gheewala. Life cycle cost of biodiesel production from microalgae in Thailand. *Energ. Sustain. Dev.* 2014; **18**, 67-74.
- [7] DDW Tsai, R Ramaraj and PH Chen. Growth condition study of algae function in ecosystem for CO<sub>2</sub> bio-fixation. *J. Photochem. Photobiol. B Biol.* 2012; **107**, 27-34.
- [8] C Yeesang and B Cheirsilp. Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. *Bioresource Tech.* 2011; **102**, 3034-40.
- [9] EW Becker. Micro-algae as a source of protein. *Biotechnol. Adv.* 2007; **25**, 207-10.
- [10] A Jehlee, P Khongkliang and S O-Thong. Biogas production from *Chlorella* sp. TISTR 8411 biomass cultivated on biogas effluent of seafood processing wastewater. *Energ. Procedia* 2017; **138**, 853-7.
- [11] G Dragone. Challenges and opportunities to increase economic feasibility and sustainability of mixotrophic cultivation of green microalgae of the genus *Chlorella*. *Renew. Sustain. Energ. Rev.* 2022; **160**, 112284.
- [12] S Liang, X Liu, F Chen and Z Chen. Current microalgal health food R & D activities in China. *Hydrobiologia* 2004; **512**, 45-48.
- [13] A Widjaja, CC Chien and YH Ju. Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. *J. Taiwan Inst. Chem. Eng.* 2009; **40**, 13-20.
- [14] D Tang, W Han, P Li, X Miao and J Zhong. CO<sub>2</sub> biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO<sub>2</sub> levels. *Bioresource Tech.* 2011; **102**, 3071-6.
- [15] Y Park, KW Je, K Lee, SE Jung and TJ Choi. Growth promotion of *Chlorella ellipsoidea* by co-inoculation with *Brevundimonas* sp. isolated from the microalga. *Hydrobiologia* 2008; **598**, 219-28.
- [16] SH Cho, SC Ji, SB Hur, J Bae, IS Park and YC Song. Optimum temperature and salinity conditions for growth of green algae *Chlorella ellipsoidea* and *Nannochloris oculata*. *Fish. Sci.* 2007; **73**, 1050-6.
- [17] GL Bhalamurugan, O Valerie and L Mark. Valuable bioproducts obtained from microalgal biomass and their commercial applications: A review. *Environ. Eng. Res.* 2018; **23**, 229-41.
- [18] CK Wayne, CS Reen, PL Show, YY Jiun, LT Chuan and C Jo-Shu. Effects of water culture medium, cultivation systems and growth modes for microalgae cultivation: A review. *J. Taiwan Inst. Chem. Eng.* 2018; **91**, 332-44.
- [19] P Asadi, HA Rad and F Qaderi. Lipid and biodiesel production by cultivation isolated strain *Chlorella sorokiniana* pa.91 and *Chlorella vulgaris* in dairy wastewater treatment plant effluents. *J. Environ. Health Sci. Eng.* 2020; **18**, 573-85.
- [20] CF Yang, ZY Ding and KC Zhang. Growth of *Chlorella pyrenoidosa* in wastewater from cassava ethanol fermentation. *World J. Microbiol. Biotechnol.* 2008; **24**, 2919-25.
- [21] A Baidya, T Akter, MR Islam, AKMA Shah, MA Hossain, MA Salam and SI Paul. Effect of different wavelengths of LED light on the growth, chlorophyll,  $\beta$ -carotene content and proximate composition of *Chlorella ellipsoidea*. *Heliyon* 2021; **7**, e08525.
- [22] M Khalaji, SA Hosseini, R Ghorbani, N Agh, H Rezaei, M Kornaros and E Koutra. Treatment of dairy wastewater by microalgae *Chlorella vulgaris* for biofuels production. *Biomass Convers. Biorefinery* 2023; **13**, 3259-65.
- [23] MB Mangesh and S Felix. Effect of light wavelengths on biomass production and pigment enhancement of *Chlorella vulgaris* in indoor system. *Res. J. Biotechnol.* 2019; **14**, 111-7.

- [24] HS Yun, YS Kim and HS Yoon. Characterization of *Chlorella sorokiniana* and *Chlorella vulgaris* fatty acid components under a wide range of light intensity and growth temperature for their use as biological resources. *Heliyon* 2020; **6**, e04447.
- [25] TJ Gunawan, Y Ikhwan, F Restuhadi and U Pato. Effect of light intensity and photoperiod on growth of *Chlorella pyrenoidosa* and CO<sub>2</sub> Biofixation. *In: Proceedings of the 2<sup>nd</sup> International Conference on Energy, Environmental and Information System, Semarang, Indonesia. 2018.*
- [26] M Alariqi W Long and BI Musah. Algae biomass for biofuel production; yield analysis of *Chlorella Vulgaris* and *Scenedesmus Meyen* in different culture media. *In: Proceedings of the 12<sup>th</sup> International Conference on Environment Science and Engineering, Beijing, China. 2023, p. 144-50.*
- [27] K Chankhong, W Chotigeat and J Iewkittayakorn. Effects of culture medium on growth kinetics and fatty acid composition of *Chlorella* sp. T12. *Songklanakarin J. Sci. Tech.* 2018; **40**, 1098-104.
- [28] R Chandra, Amit and UK Ghosh. Effects of various abiotic factors on biomass growth and lipid yield of *Chlorella minutissima* for sustainable biodiesel production. *Environ. Sci. Pollut. Res.* 2019; **26**, 3848-61.
- [29] S Sathong, K Saego, P Kitrunloadjanaporn, N Nuttavut, S Amornsamankul and W Triampo. Modeling the effects of light sources on the growth of algae. *Adv. Difference Equat.* 2019; **2019**, 170.
- [30] P Nawkarkar, AK Singh, MZ Abdin and S Kumar. Life cycle assessment of *Chlorella* species producing biodiesel and remediating wastewater. *J. Biosci.* 2019; **44**, 89.