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Research

Efficiency of Microalgae Cultivation Automated System: A Case Study of Green Algae *Chlorella ellipsoidea* TISTR 8260

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ABSTRACT

Microalgae play an important economic role as aquaculture feed. This study aimed to create an automated algae cultivation system with variable light intensity for the culture of *Chlorella ellipsoidea* strain TISTR 8260. The automated cabinet could work continuously for at least 30 days, with the growth rates of microalgae in culture systems with light intensities of 1000 Lux, 3000 Lux, and 5000 Lux peaking on day 14, whereas the fluorescent control showed peak microalgae growth on day 6. On day 30, the biomass harvested from microalgae grown in 1000 Lux, 3000 Lux, 5000 Lux, and fluorescent control was 0.1935 ± 0.151 mg/L, 0.1996 ± 0.220 mg/L, 0.2041 ± 0.159 mg/L, and 0.0674 ± 0.191 mg/L, respectively, which was not significantly different between the groups but significantly higher than the control (*P*-value = 0.05, DF = 3, F(3,36) = 7). The automated algae cabinet with a light intensity of 5000 Lux and a rotation speed of 150 r.p.m produced the maximum biomass, which was three times that produced by a fluorescent light source.

Key words: Aquaculture, growth rate, microalgae, automated cabinet

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INTRODUCTION

Microscopic unicellular green algae play an important role as producers in aquatic ecosystems. Worldwide, 450 species of microalgae have been identified, with *Chlorella* sp. being used in aquaculture. It is fed to aquatic larvae in aquaculture farms. Several studies on *Chlorella* sp. have been published, with the microalgae being used as a feed supplement for the degradation of engine oil and as an adsorbent in wastewater treatment of diverse water sources (Prabakarana *et al*., 2018; Jankovska *et al*., 2000). China was the first country to use *Chlorella* sp. for medicinal and food purposes, whereas Japan uses it as food, condiment, and side dish, as well as in the food industry, medicine, and cosmetics (Dawczynski *et al*., 2007; Muys *et al*., 2019).

Chlorella sp. is a nutrient-dense alga with high protein and fiber content. The protein content of *Chlorella* sp. is 60%, followed by 37% in soybean, 34% in chicken meat, 30% in beef, and 20% in fish meat. The fat content of microalgae (7%) is lower than that of soybeans (18%) and peanuts (48%) (Rasheed *et al*., 2020). *Chlorella* sp. also contains alpha-linolenic acid, which plays a role in lowering cholesterol levels in blood vessels. Furthermore, it has high levels of vitamin B12, vitamin A, beta-carotene, and nucleic acid (Parniakov *et al*., 2018). Aquaculture farmers in Thailand use uncontaminated *Chlorella* sp. of desired quantity and quality as a feed supplement to prevent infection among economically valuable aquatic animals such as fish and shrimp (Seyfabadi *et al*., 2011). Using *Chlorella* sp. from natural water sources may have some limitations, such as the limited supply and contamination with bacteria or viruses, which can contribute to the spread of aquatic animal diseases (Gifford *et al*., 2007; Satthong *et al*., 2019). An automated closed algae incubator system is currently

being developed and engineered to promote the increased production of *Chlorella* sp. (Henshall *et al*., 2017). A pathogen-free microalgae culture tool used to obtain high-quality products at a lower cost of production can be expanded to include commercial production. Nakhon Si Thammarat is a province in southern Thailand that borders the Eastern Sea. Aquaculture entrepreneurs use *Chlorella* sp. in quantities ranging from 1500 to 2000 kilograms per year as a feed supplement for young aquatic animals (Guo *et al*., 2015). This type of microalgae is harvested after 21–30 days (Levin *et al*., 2021). Quality improvement and industrial-scale production of *Chlorella* sp. requires the aseptic production of the microalgae with production control (Carvalho *et al*., 2019a; Bošnjakovic & Sinaga, 2020). The variable climate of southern Thailand is the primary reason for the inability to control production and quality as needed. Therefore, it is necessary and advantageous to develop an intelligent farming system that can control the environment, reduce labor requirements in production, and monitor productivity during algae production (Bialevich *et al*., 2022). An important factor in the development of automated algae rearing is the control of light. Light-emitting diodes (LEDs) are used in energy-saving LED lamps, which are commonly used for light supply in plant-rearing laboratories. These lamps have a long life expectancy and produce immense light energy (80–120 lumens/watt), which makes them potential candidates to replace fluorescent bulbs. More importantly, these lamps have a lower environmental impact than other types of bulbs due to lower heat emission (Amini-Khoeyi *et al*., 2012; Huesemann *et al*., 2017). The rearing of the microalgae requires controlling the light according to the duration and intensity that is suitable for the photosynthesis of *Chlorella* sp., as well as controlling the temperature appropriately throughout the growth period of the microalgae. Moreover, incubators that can control other factors, such as humidity and contamination from external microorganisms, are being developed. Such automated incubators could produce high-quality microalgae and harvest it at appropriate times throughout the year by operating through various control cabinets, facilitating the uninterrupted growth of the microalgae value chain, and creating a continuous circular economy (Mahmood & Ali, 2017; Prabakarana *et al*., 2018). Therefore, this study aimed to build an innovative automated closed-system algae incubator that could control the environment by adjusting the temperature and light intensity and influence the growth of *Chlorella* sp. Closed-system rearing can prevent contamination from various microorganisms while simultaneously accelerating growth and increasing the biomass of *Chlorella* sp.

MATERIALS AND METHODS

Designing an automated algae incubator

Using the 3D Sketch-UP Pro 2021 program instructor (7-day trial, Trimble Navigation, USA) (Javed *et al*., 2021), automated farming was designed for microalgal cultivation (Figure 1a). Briefly, first chose the template and the measurement scale to be used, which was the measurement type mainly used in basic model design, was decimal, and had a precision of 10-4 cm. Angle units were used to define angle sub-units and set the snapping of a given angle. Then, we drew parallel lines along the X-axis using a reference to the direction along the reference axis for all three axes (Figure 1b). Finally, we constructed an automated algae incubator component with the back designed to connect to PVC pipes, allowing steam to flow through the incubator when the temperature inside exceeded the specified temperature. The interior of the automated algae incubator was designed to allow for the installation of LEDs, the temperature controller, a shaker tray, a castor, and a fogger (Figure 1c).

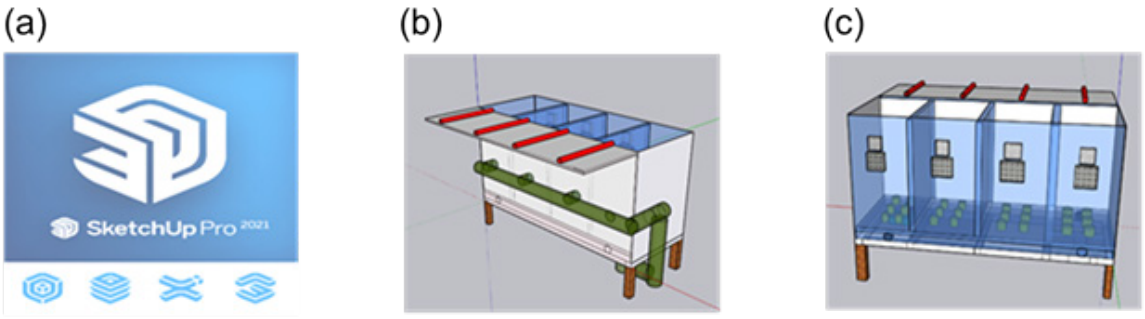

Fig. 1. (a) An automated algae incubator was designed using the 3D Sketch-UP Pro 2021 program (7-day trial). (b) A PVC pipe was attached to the cabinet's back to allow steam from the ultrasonic fogger to pass through. (c) A temperature controller was installed in the front of the cabinet.

Materials for the automated algae incubator

Equipment materials were divided into the following three parts: (1) the structure of the automated algae incubator composed of box steel (2.54 cm length × 2.54 cm width) and plastwood sheets (thickness 0.5 cm length × 0.3 cm width) (Figure 2 a-b), (2) the system structure composed

of the motor (UNITE brand, 250 W, 12 V), a fan (4 cm width × 4 cm length × 1 cm depth) (HLOLMG, model GIOR08025S, 12 V), and an 11.43-cm pulley groove with a belt (SAMMERA, No. M-20) (Figure 2 c–e), and (3) a cabinet control system structure composed of a light-emitting diode lamp (B-1, model Eco, 9 W, 1000 Lumens), an ultrasonic mist sprayer (MIST MAKER, model HC-2410, 24 V), a fan (4 cm width × 4 cm length × 1 cm depth) (HLOLMG, model GIOR08025S, 12 V), a temperature controller (XH-W3001, 220 V), and a control device (XH-W3001, 220 V) (Figure 2 f–j).

Fig. 2. The equipment material (a) steel box, (b) plastwood sheets, (c) motor, (d) fan, (e) pulley groove, (f) light-emitting diode lamp, (g) ultrasonic mist sprayer, (h) temperature controller, (j) control device.

Cultivation of *Chlorella* sp. in an automated algae incubator

Microalgae species

Chlorella ellipsoidea strain TISTR 8260 was obtained from the Center for Biodiversity, Thailand Institute of Scientific and Technological Research, Pathum Thani Province.

Media

The CHU-13 medium was used to cultivate the *C. ellipsoidea* strain TISTR 8260, according to Carvalho *et al*. (2019b). Briefly, 1 L of the medium containing 0.2 g $NaNO_3$, 0.04 g K_2PHO_4, 0.1 g $MgSO_4.7H_2O$, 0.054 g $CaCl_2.2H_2O$, 0.1 g citric acid, 2.85 g H_3BO_3, 0.05 g $Na_2MoO_4.2H_2O$, 0.02 g $ZnSo_4.7H_2O$, 0.08 g $CoCl_2.6H_2O$, 0.01 g Fe citrate, 1.8 g $MnCl_2.4H_2O$, and 0.08 g $CuSo_4.5H_2O$ was taken in a 2-L flask and sterilized at 121 °C and 15 psi steam pressure for 20 min. Then, the microalgae *C. ellipsoidea* TISTR 8260 were inoculated into the medium in a 250 mL flask at a ratio of 1:15, resulting in an OD_{680} of 0.24.

Culturing the C. ellipsoidea strain TISTR 8260 in the automated algae incubator

The culture medium containing the *C. ellipsoidea* strain TISTR 8260 in a 250 mL flask was incubated in the automated algae incubator at 28 ±1 °C with light intensities of 1000, 3000, and 5000 Lux, while a fluorescent light of intensity 8,000 Lux was used as the control. The photoperiod was for 12 h between 6:00 am and 6:00 pm. The flasks were shaken at 150 r.p.m during the incubation period for 30 days, after which the fresh weight was recorded. The algae were placed in a centrifuge tube and centrifuged at 3000 r.p.m for 15 min (NUVE, model NF 048, Turkey) before being subjected to biomass calculations.

Measurement of the growth rate of the C. ellipsoidea strain TISTR 8260

To determine the cell density, 1 mL of *C. ellipsoidea* strain TISTR 8260 was collected every 2 days, and the optical density (OD_{680}) was measured using a spectrophotometer (Peak model C-7100, USA) to analyze the timeline of the growth of *C. ellipsoidea* strain TISTR 8260 with modifications as outlined in Equation 1 and 2 (Tang *et al*., 2011).

Equation 1:

$$P = \frac{X_1 - X_0}{t_1 - t_0}$$

Equation 2:

$$\mu = \frac{\ln(X_1 - X_0)}{t_1 - t_0}$$

Where P is the Biomass productivity(g/L/day).
μ is the specific growth rate/day.
X_1 and X_0 are the mass (g/L) at day t_1 and t_0.
t is the number of days in algal culture (Number of culture experiments).

Data analysis

All experiments were repeated thrice for each sample (n=3), and the standard deviation (SD) was calculated. The experimental results were then statistically analyzed by the one-way analysis of variance (ANOVA) and Tukey multiple comparison tests using the statistical package R (R Core Team, 2020). *P*-values of < 0.05 indicated statistically different means between groups.

RESULTS AND DISCUSSION

The automated algae incubator

The dimensions of the algal culture intelligence system were 70 cm width × 200 cm length × 50 cm height. The cabinet used for culture was a steel box of dimensions 2.54 cm width × 2.54 cm length (Figure 3). The floor was made of plastwood, the lid was made of 0.3 cm thick plastwood, and the shaking tray was made of 0.5 cm thick plastwood. To control the temperature inside the cabinet, a powerful ultrasonic fogger connected to a fan (4 cm width × 4 cm length × 1 cm length) was installed at the back of the cabinet (Figure 3a). A temperature controller was installed at the front of the cabinet (Figure 3b). A motor connected to a pulley was used to control the rotation of the cabinet. This motor was connected to a fan to release the heat during rotation (Figure 3 c-d). The bottom of the cabinet was equipped with a shaking tray connected to the vibrator by a motor. The motor was connected to the pulley with a belt and a fan so that the shaker could function 24 h a day.

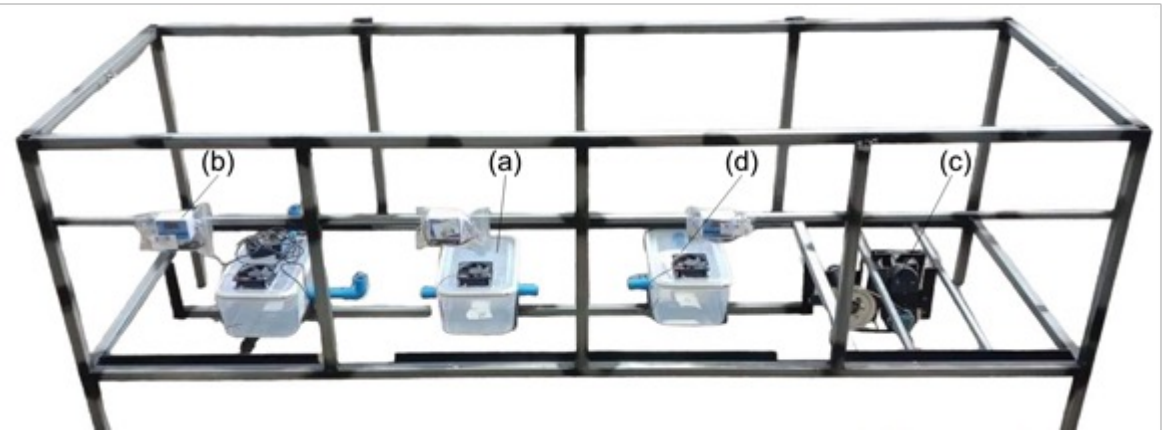

Fig. 3. The automated algae cultivation system cabinet.

Plastwood sheets were used to join the bottom, four sides, and top cover of the cabinet (Fig. 4a). The automated algae culture system cabinet was divided into four chambers based on light intensity levels of 1000 Lux, 3000 Lux, and 5000 Lux, and the fluorescent lamp at 8000 Lux (Figure 4b). The shaking tray had 12 flask chambers of 250 mL each. The flask handle was made of aluminum plates and measured 2.5 cm in width × 26 cm in length. It was attached to the shaker tray and shaken at 150 r.p.m (Figure 4c).

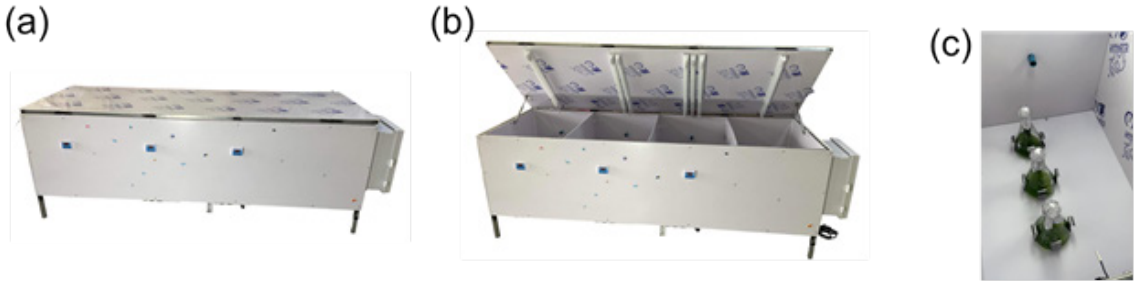

Fig. 4. (a) An automated algae cultivation system cabinet, (b) light intensity distribution in each chamber, (c) placement of flasks in the incubator.

The incubator could function continuously for 24 h, and the shaking cycle could continue throughout the experiment because it used a motor connected to a fan to cool the heat from the motor. A digital timer was installed to automatically turn the lights on and off to maintain a 12-h photoperiod throughout the experiment. An important advantage of the cabinet was that the culture was free of contamination because of the closed-system culturing. Moreover, the LEDs that were chosen to provide light affected the environment the least, owing to the lesser emitted heat and minimal heavy metal content compared to other light bulb types. In contrast, other studies have used the fluorescent bulb as the light source (Koc *et al*., 2010; Matthew *et al*., 2014). An automated algae cultivation system cabinet can manage the factors involved in microalgal growth, such as temperature and light intensity. Although other systems have also managed these factors, the system developed by us does so automatically for at least 30 days without any problems during the culture. The water fogger kept the temperature inside the cabinet stable despite the outside temperature being higher than the setting (Cho *et al*., 2007). The cabinet was prepared for a short circuit by preparing a safety system capable of handling it. The flask size for microalgal rearing could also be adjusted. However, the temperature-maintenance fan was sensitive to humidity and frequently failed to function. The water level of the cabinet was manually adjusted by adding water to the box when the temperature exceeded the setting, which could also be done automatically. The cabinet could also be modified to minimize shaking noise during the experiment.

The efficiency of an automated algae incubator

Effect of light intensity on the growth rate of C. ellipsoidea strain TISTR 8260

The experiments revealed that *C. ellipsoidea* strain TISTR 8260 grew in a sigmoid-curve pattern. The growth rate peaked on day 14 of the experiment and gradually declined until day 30 (Figure 5). One-way ANOVA and Tukey multiple comparison tests revealed no statistically significant difference in cultures grown at the three light intensities of 1000 Lux, 3000 Lux, and 5000 Lux, although the culture grown in fluorescent light had a significantly lower growth rate.

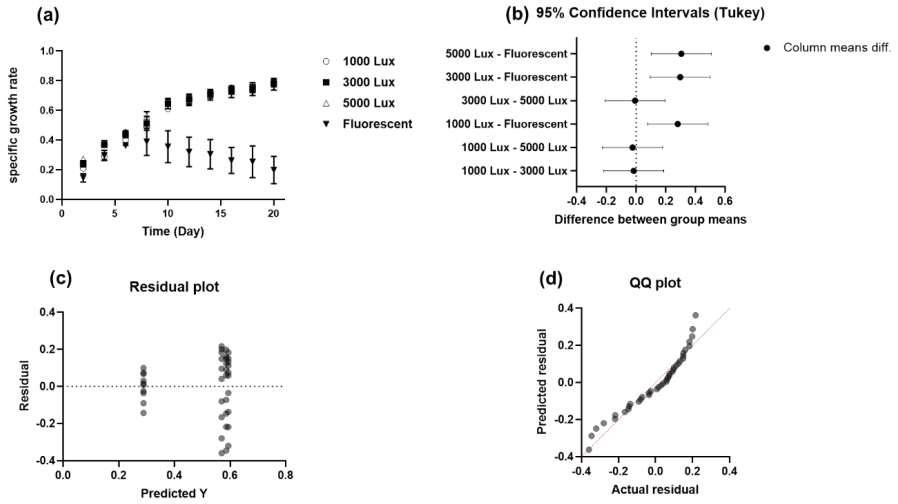

Fig. 5. The growth rate of *C. ellipsoidea* strain TISTR 8260 cultured in an automated algae incubator with light intensities of 1000 Lux, 3000 Lux, and 5000 Lux, and fluorescent light as the control.

Chrorella ellipsoidea strain TISTR 8260 cultured at all three light intensities showed the highest growth rate on day 14 of the experiment, compared to *Chlorella* sp. cultured for lipid production in biodiesel, which showed the highest growth rate on days 15 or 16 (Chinnasamy *et al*., 2009; Lara *et al*., 2021). The light source for plant artificial culturing systems is typically white light and a range of lights specific to photosynthetic pigments. However, our study focused on light intensities and discovered a significant relationship between the intensity of white light and the specific light intensity of the LED (Sabzalian *et al*., 2014). The best photoperiod for culturing *C. vulgaris* was 12 h of light and 12 h of darkness, which yielded the highest growth rate on day 13, whereas, in our study, the maximum growth rate was observed on day 14 (Doucha *et al*., 2005; Madiha *et al*., 2013).

In this study, the fluorescent light system showed the highest growth rate of *C. ellipsoidea* strain TISTR 8260 on day 8, outpacing the other three intensity systems. The growth rate of *C. ellipsoidea* strain TISTR 8260 in the fluorescent light system was 20 times lower than that in the three-intensity system, with the culture cycle appearing to be 20 days. The culture cycle observed in this study is aligned with that demonstrated by other studies for this genus, ranging from 20–21 days (Chandra *et al*., 2019).

Effect of light intensity on the biomass of C. ellipsoidea strain TISTR 8260

The mean biomass of *C. ellipsoidea* strain TISTR 8260 from cultures grown under 1000 Lux, 3000 Lux, 5000 Lux, and fluorescent light was 0.1935±0.151 mg/L, 0.1996 ± 0.220 mg/L, 0.2041 ± 0.159 mg/L, and 0.0674 ± 0.191 mg/L, respectively. According to one-way ANOVA (DF = 3, F (3,36) = 7) and Tukey multiple comparison tests, the biomass obtained from the microalgal cultures at the three light intensities was significantly different (*P*-value < 0.05) (Table 1).

Table 1. The effect of light intensity on the biomass of *C. ellipsoidea* strain TISTR 8260

Light intensity (Lux)	Biomass (mg/L/day)
1000	0.006495 ± 0.151[a]
3000	0.006652 ± 0.220[a]
5000	0.006802 ± 0.159[a]
Fluorescent (8,000) as control	0.002245 ± 0.191[b]

a b Statistically significant differences were observed at $P = 0.05$.

As the algal biomass from the automated algae cabinet was normally distributed (boxplot, Figure 6a), Tukey's method was used to compare the pairwise means, which demonstrated that each pair showed a statistically significant difference in the mean biomass based on the light intensity (Figure 6b). These results indicate that the data were approximately normally distributed (Figure 6c) and that the difference was distributed at random around the zero point (Figure 6d).

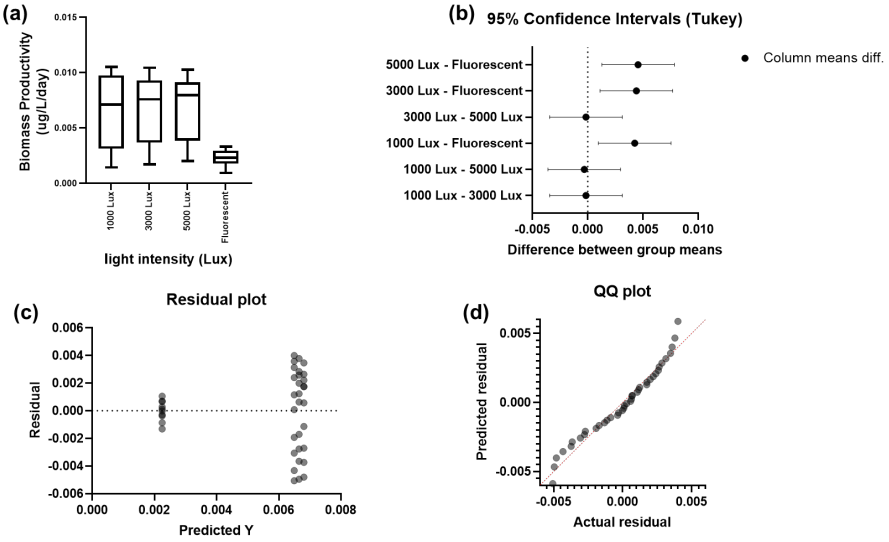

Fig. 6. The analysis of data from a randomized study comparing biomass levels with light intensity. (a) boxplots were used to depict the average biomass level as a function of light intensity, (b) the fitted linear confidence interval, (c) the residual value distribution, and (d) the value of the remainder of the approximation equation.

The highest biomass was observed in the 5000 Lux system and was roughly three times higher

than that in the fluorescent light system. The biomass yield obtained from the three intensities and the control was approximately 10 times higher than that reported in other studies (Vani et al., 2011). The temperature in the algae aquaculture tank was set at 28 °C to match the regional average and mimic the natural conditions of the algae. Furthermore, the shaking conditions for the growth cycle and distribution of light to the culture were set to 150 rpm. Under these conditions, the biomass of the Chlorella sp. culture was 1.8 g/L at harvest (Mandotra et al., 2014; Nguyen Thuy et al., 2019). In contrast, conditions of 110 r.p.m and 27 °C yielded 1.45 g/L of biomass, whereas 160 r.p.m and 24 °C yielded 1.0 g/L of biomass (Yasmeen *et al*., 2017). Thus, the microalgae grown in our tanks yielded higher biomass but under the same conditions as before.

CONCLUSION

The automated algae incubator developed in this study can be used to aseptically grow *C. ellipsoidea* strain TISTR 8260 at various light intensities. Temperature, humidity, and shaking speed are factors that can be adjusted to promote growth. The automated algae incubator can operate uninterrupted for 30 days. The growth rates of *C. ellipsoidea* strain TISTR 8260 in CHU-13 medium with varying light intensities showed no statistically significant differences between the groups. The highest biomass of *C. ellipsoidea* strain TISTR 8260 was recorded in the 5000 Lux light intensity system, which was three times greater than that in the fluorescent system.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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