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Original Article

Growth performance and production cost of laboratory-scale marine microalgae culture using a light-emitting diode

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Abstract

The effects of a light-emitting diode (LED) were examined and compared to fluorescent light on the growth performance of four marine microalgae, *Nannochloropsis oculata*, *Tetraselmis suecica*, *Chaetoceros calcitrans*, and *Thalassiosira weissflogii* cultured under laboratory conditions for 240 h (10 days). The results showed that the cell densities of *N. oculata* cultured using fluorescent lamps (T1) and cool daylight LED (T2) were higher than warm white LED (T3), and the growth rate of this species using T2 were higher than for T1 and T3. The cell density of the *T. suecica* culture in each treatment was not different, but the growth rate using T1 was higher than for T3. The cell densities and growth rates of *C. calcitrans* and *T. weissflogii* culture in each treatment were not different. In addition, commercial *Chaetoceros* produced using cool daylight LED illumination had a lower production cost compared with production using cool white fluorescent illumination. Therefore, commercial laboratory-scale production of marine microalgae should use a cool daylight LED as the light source in order to reduce the production cost without affecting the growth and quality of microalgae.

Keywords: microalgae, light-emitting diode (LED), growth performance, production cost

1. Introduction

Microalgal cultures are one of the modern biotechnologies and are a promising new source of biodiesel fuel. The cultivation of microalgae has been carried out for more than 40 years with the main microalgal species cultured being *Chlorella* sp. and *Spirulina* sp. for health foods, *Dunaliella salina* for *β*-carotene, and several species for aquaculture (Araújo & Garcia, 2005; Mobin & Alam, 2017). The commercial production of marine microalgae in Thailand opens up the field to different possible products. These products can be split into two major commercial groups commercial: laboratory-scale production and large-scale production (Arkronrat, Deemark, Hengcharoen, Pradubtham, & Oniam, 2014). For example, the green algae *Chlorella*, *Tetraselmis* and *Nannochloropsis*, the flagellate *Isochrysis* and the diatoms *Chaetoceros*, *Skeletonema*, and *Thalassiosira* are commonly used as important live food for crustacean, fish, and bivalve larvae. Therefore, they are produced commercially in Thailand (Arkronrat & Oniam, 2012a, 2012b; Wongrat, 2000). One of these was developed in a cost-effective, laboratory-scale culture system for marine microalgae (Arkronrat & Oniam, 2012c; Arkronrat, Deemark, & Oniam, 2016).

Since, the laboratory-scale production of microalgae is suitable for seed culture, the future development of most microalgae applications will need to have a reliable and economical-scale cultivation process. Generally, the farmers in Thailand obtain microalgae seed stock cultures from either government agencies or private farms. Seed culture is usually produced using a batch culture in 1-L glass bottles in the laboratory but many people are interested in producing microalgae on a commercial scale for business purposes (Arkronrat *et al*., 2014). The success of the production of microalgae depends on many factors. The main factors are those which control growth, such as temperature, nutrients, light, salinity, and pH (Khatoon *et al*., 2014; Sánchez-

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Saavedra & Voltolina, 2006; Sureshkumar, Jasmin, Rahiman, & Mohammed, 2014; Tzovenis, De Pauw, & Sorgeloos, 1997; Zhu, Lee, & Chao, 1997). However, one factor which is more important than those affecting the growth of microalgae is the development of production methods that have the most profit at the lowest cost without affecting the growth and quality of the microalgae. Currently, the production of microalgae at the laboratory scale has a high operation cost. Previous studies found that the main operating cost of commercial, laboratory-scale production of microalgae was for electricity which was 24–30% of the total cost (Arkronrat *et al*., 2014; Arkronrat & Oniam 2012c;).

The light source or illumination is one of the essential limiting factors for cell growth in microalgal photosynthesis. A light-emitting diode (LED) is considered a good option compared to other lamps due to high energy efficiency and low energy consumption. The improvements in LED equipment in recent years have increased the interest in using this technology for various applications including cultivation of microalgae in photobioreactors and other systems (de Mooil, de Vries, Latsos, Wijffels, & Janssen, 2016; Hahne, Schwarze, Kramer, & Frahm, 2014; Huesemann *et al*., 2017; Koc, Anderson, & Kommareddy, 2013; Schulze, Barreira, Pereira, Perales, & Varela, 2014;). Unfortunately, studies on the commercial laboratory-scale production of microalgae using LED illumination are very limited because farmers rely on fluorescent lamps as the light source at most commercial, laboratory-scale production facilities. Therefore, to make LED illumination ideal for microalgal production at the laboratory scale will require an alternative design to reduce the operation cost and management strategies for the farmers without affecting the growth and quality of the microalgae. To meet these goals, we conducted experiments to cultivate microalgae at the laboratory scale using an LED. The results of the experiments were used to evaluate the effects of the LED on the cell density and growth rate of some commercial marine microalgae (*Nannochloropsis*, *Tetraselmis*, *Chaetoceros*, and *Thalassiosira*). We then considered the impact of the LED on the operation cost compared with fluorescent lamps. The results could lead to applications in the commercial, laboratory-scale production of marine microalgae.

2. Materials and Methods

2.1 Microalgal preparation

The experiment was conducted in the Phytoplankton Laboratory of the Klongwan Fisheries Research Station, Prachuap Khiri Khan Province, Thailand. The original inoculum of the four species of commercial marine microalgae (the green microalgae *Nannochloropsis oculata* and *Tetraselmis suecica*, and the diatoms *Chaetoceros calcitrans* and *Thalassiosira weissflogii*) were obtained from the Prachuap Khiri Khan Coastal Fisheries Research and Development Center, Department of Fisheries.

Microalgae cultures were carried out in 250 mL Erlenmeyer flasks with sterilized sea water adjusted to a salinity of 28 ppt and enriched with liquid medium (Conway medium; Wongrat, 2000) with silicate added only for the diatoms and inoculated at 10% (v/v). The temperature-controlled room was maintained stable at 25±1 ºC under a 12 h:12 h light/dark photoperiod using fluorescent lamps at a light intensity of about 1,000 lux. Microalgae were cultured in Erlenmeyer flasks until the cell density reached approximately 10^6 cells ml^{-1}. Cultures were finally scaled up into 1-L glass bottles and then used for experiment.

2.2 Experimental design and set-up

2.2.1 Experiment 1: Effects of LED (cool daylight and warm white) and fluorescent light sources on cell density and growth rate of marine microalgae

The microalgae were cultured under laboratory conditions using different light sources: 1) cool white fluorescent lamp (T1) (Figure 1A); 2) cool daylight LED (T2) (Figure 1B); and 3) warm white LED (T3) (Figure 1C). The batch cultivation of microalgae was carried out in enriched seawater (28 ppt) in 1-L glass bottles with 1 mL of the Conway medium (silicate added only for the diatoms), grown in a temperature-controlled room (25±1 °C) under 12 h:12 h light/dark photoperiod duration at a light intensity of about 3,000 lux, for 240 h (10 days). All cultures were started with equal amounts of the inoculum (about 1-2×10^5 cell ml^{-1}). The experiment was performed with six replicates and followed a completely randomized design.

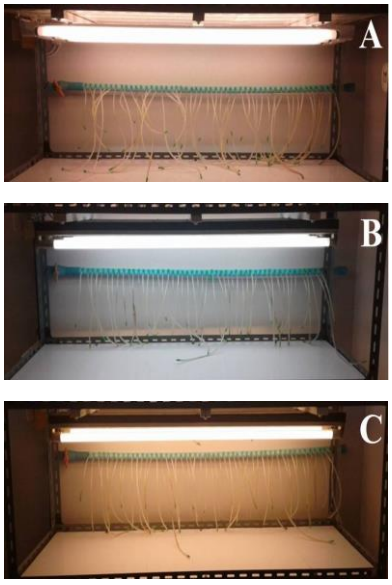

Figure 1. Lighting sources used in the experiments: (A) cool white fluorescent lamp: 1,200 mm, 36W, 4,000 K, 3,250 lm; (B) cool daylight LED: 1,200 mm, 18W, 6,500 K, 1,600 lm; and (C) warm white LED: 1,200 mm, 18W, 3,000 K, 1,600 lm.

During the growth experiment, microalgal cell samples were collected every 12 h to estimate the cell density (cell ml^{-1}) and growth patterns. Algal cells were fixed with 5% formalin and then counted using a hemocytometer under a compound microscope at 40× magnification. The growth rate of the culture was calculated using the equation

$$K = \ln N_t - \ln N_o/t$$

where K is the growth rate constant (day^{-1}), N_t is the maximum cell count (cell ml^{-1}) at time t, N_o is the initial cell count (cell ml^{-1}) at time 0 and *t* is the time in days (Phatarpekar, Sreepada, Pednekar, & Achuthankutty, 2000).

2.2.2 Experiment 2: Operational cost comparison of microalgal production using LED and fluorescent light sources

The monthly production costs of commercial, laboratory-scale production of the marine microalga *Chaetoceros* were compared for two light sources: 36W cool white fluorescent lamps (1,200 mm, 4,000 K, 3,250 lm) and LED (the type of LED based on the results of Experiment 1). The study was based on data collected from a plankton farm in Phan Thong District, Chonburi Province. *Chaetoceros* seed stock was produced from a batch culture in 1-L glass bottles at 100 L per day. *Chaetoceros* microalga was grown in a temperature-controlled room at 25±1 °C using an 18,000 BTU air conditioning unit under continuous illumination at a light intensity of about 3,000 Lux. Both treatments were aerated using a 1 HP blower.

The microalgal production cost at the laboratory scale was calculated using the following formula

Microalgal production cost = fixed cost + variable cost

where fixed cost = chemical, sea water, public utilities, depreciation, rent of location, labor and other material values, and variable cost = cost of electricity (Arkronrat & Oniam, 2012c).

2.3 Statistical analysis

At the end of the experiments, the statistical significance of the differences of the mean cell density and growth rate among the different treatments in Experiment 1 were examined using one-way ANOVA, and Duncan's multiple range test was applied at the 95% level of confidence. For Experiment 2, the difference between the mean data on operating costs of microalgal production using the LED or the fluorescent lamp as the light source was tested using independent-sample *t*-tests at the 95% level of confidence. All data were analyzed using the IBM SPSS Statistics for Windows software package (Version 21.0; IBM Corp., Armonk, NY. USA).

3. Results

3.1 Effects of LED and fluorescent light sources on cell density and growth rate of marine microalgae

In the green microalgae, the initial cell densities (mean±SD) of *N. oculata* and *T. suecica* were 2.60±0.25 and $1.24\pm0.01\times10^5$ cells ml^{-1}, respectively. The average cell densities of *N. oculata* in T1, T2, and T3 increased rapidly to 34.80–57.03, 38.42–63.42, and 20.70–43.47×10^5 cells ml^{-1}, respectively, at 120–144, 84–108, and 72–96 h cultivation (exponential phase) after which they reached the stationary phase where the average maximum cell densities (mean±SD) were 96.43±11.22, 86.46±4.56, and $68.37\pm12.18\times10^5$ cells ml^{-1}, respectively. Then, the cell densities of *N. oculata* in T1, T2, and T3 decreased considerably until the end of the experimental period. The *T. suecica* culture in T1 and T3 had gradual increases in cell density, without any apparent exponential phase of growth during the experimental period. In contrast, the average cell density of *T. suecica* in T2 increased rapidly to 5.59–9.10×10^5 cells ml^{-1} at 84–144 h cultivation (exponential phase) after which it reached the stationary phase where the average maximum cell density was $12.45\pm3.90\times10^5$ cells ml^{-1}. The cell densities of *T. suecica* in T1 and T3 continued to increase until the end of the experimental period but in T2 there was a gradual decrease in cell density at 192 h cultivation onward (Figure 2). In addition, the average maximum cell densities of *N. oculata* culture in T1 and T2 were not significantly different and were higher than the average maximum cell density of *N. oculata* culture in T3 (ANOVA, P=0.001). The average growth rate of *N. oculata* culture in T2 was significantly higher than the average growth rates of *N. oculata* culture in T1 and T3 (ANOVA, P=0.001). In the *T. suecica* culture, the average maximum cell densities in T1, T2, and T3 were not significantly different (ANOVA, P=0.822), but the average growth rate of *T. suecica* in T1 was higher than in T3 (ANOVA, P=0.013) (Table 1).

In the diatoms, the initial cell densities of *Chaetoceros calcitrans* and *Thalassiosira weissflogii* were 2.56±0.45 and $2.51\pm0.46\times10^5$ cells ml^{-1}, respectively. The average cell densities of *C. calcitrans* in T1, T2, and T3 increased rapidly to 8.43–25.67, 6.22–20.92, and 7.94–21.92×10^5 cells ml^{-1}, respectively, at 72–120, 60–96, and 60–96 h cultivation (exponential phase) after which the stationary phase was reached where the average maximum cell densities were 42.75±2.50, 42.16±5.10, and $38.00\pm5.73\times10^5$ cells ml^{-1}, respectively. Then, the cell density of *C. calcitrans* in each treatment decreased considerably at 144 h cultivation onward until the end of the experimental period. There was a similar growth pattern in the *T. weissflogii* culture, where the average cell densities in T1, T2, and T3 increased rapidly to 11.73–26.28, 8.91–23.58, and 13.38–28.25×10^5 cells ml^{-1}, respectively, at 60–108 h cultivation (exponential phase) after which the stationary phase was reached where the average maximum cell densities were 37.25±4.20, 39.20±8.88, and $37.56\pm4.27\times10^5$ cells ml^{-1}, respectively. The cell density of *T. weissflogii* in each treatment gradually decreased at 192 h cultivation onwards (Figure 2). The maximum cell densities and growth rates of *C. calcitrans* cultures in T1, T2, and T3 were not significantly different (ANOVA, P= 0.191 and P= 0.205, respectively). Also, the maximum cell densities (ANOVA, P= 0.844) and growth rates (ANOVA, P= 0.994) of *T. weissflogii* culture in all treatments were not significantly different (Table 1).

Thus, the results of the experiments demonstrated that cool daylight and warm white LED light did not affect the cell densities and growth rates of the diatoms *C. calcitrans* and *T. weissflogii*, but warm white LED light affected the growth performances of the green microalgae *N. oculata* and *T. suecica*.

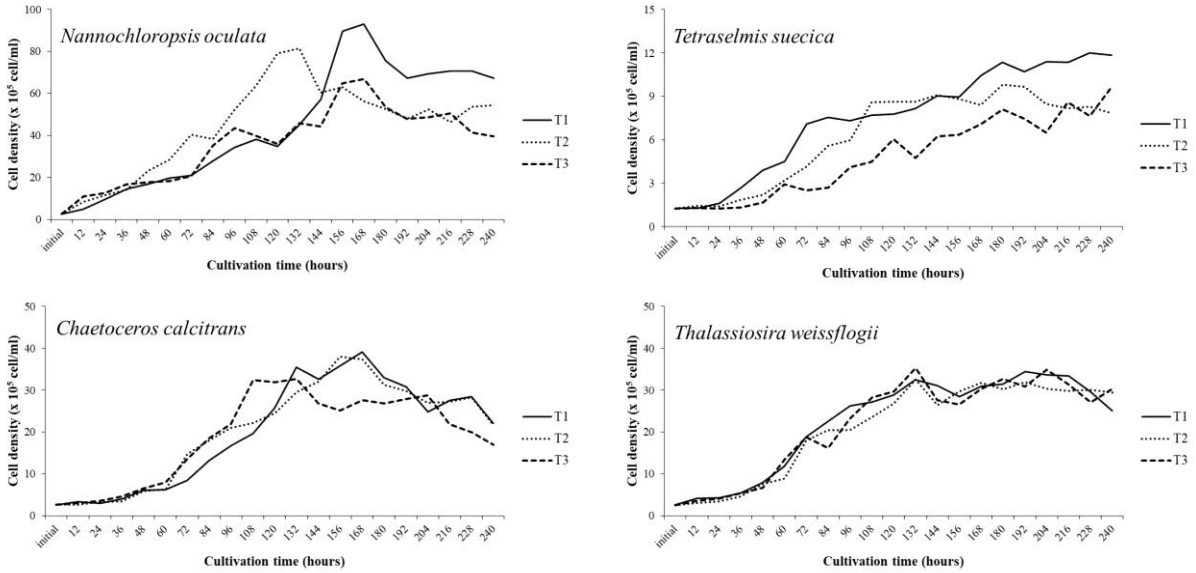

Figure 2. Growth patterns of marine microalgae *Nannochloropsis oculata*, *Tetraselmis suecica*, *Chaetoceros calcitrans*, and *Thalassiosira weissflogii* cultured under laboratory-scale conditions using different light sources: cool white fluorescent lamps (T1), cool daylight LED (T2), and warm white LED (T3), n=6.

Table 1. Maximum cell density ($\times 10^5$ cells ml^{-1}) and growth rate (day^{-1}) of marine microalgae cultured under laboratory-scale conditions using different light sources.

Items	Light source			P-value
	Cool white fluorescent (T1)	Cool daylight LED (T2)	Warm white LED (T3)	
Nannochloropsis oculata				
Maximum cell density	96.43±11.22[a]	86.46±4.56[a]	68.37±12.18[b]	0.001
Growth rate	0.53±0.02[b]	0.63±0.06[a]	0.46±0.07[b]	0.001
Tetraselmis suecica				
Maximum cell density	13.58±9.91[a]	12.45±3.90[a]	11.30±2.14[a]	0.822
Growth rate	0.73±0.22[a]	0.60±0.12[ab]	0.43±0.06[b]	0.013
Chaetoceros calcitrans				
Maximum cell density	42.75±2.50[a]	42.16±5.10[a]	38.00±5.73[a]	0.191
Growth rate	0.36±0.09[a]	0.40±0.04[a]	0.47±0.13[a]	0.205
Thalassiosira weissflogii				
Maximum cell density	37.25±4.20[a]	39.20±8.88[a]	37.56±4.27[a]	0.844
Growth rate	0.38±0.09[a]	0.37±0.08[a]	0.37±0.13[a]	0.994

Note: Data in the same row with different, lowercase superscripts are significantly different (P<0.05).

3.2 Operation cost comparison of microalgal production using LED and fluorescent light sources

Based on the results of Experiment 1, we adopted cool daylight LED as the light source for the commercial laboratory-scale production of *Chaetoceros*. The fixed and variable costs for *Chaetoceros* culture at the laboratory-scale with 100 L/day production capacity are shown in Table 2. Under the LED and fluorescent illuminations, the estimated compositions of the electricity operating costs were 19.4–20.8% and 25.5–26.5% of the total costs, respectively. In addition, the average production costs of the *Chaetoceros* culture with LED and fluorescent illumination were 26,934.6±152.1 and 29,067.3±93.6 Thai baht (THB)/month or 897.8±5.0 and 968.9±3.1 THB/day, respectively. We found that the microalgal production cost with cool daylight LED illumination had a significantly lower cost compared with production using cool white fluorescent illumination (*t*-test, P=0.000) (Table 2). This indicated that an alternative option for the commercial, laboratory-scale production of marine microalgae is the cool daylight LED to reduce production costs and achieve a better profit margin.

4. Discussion

Light conditions are the main factors affecting phytoplankton physiology. One important fact was the quality and quantity of light can cause important variations in the growth rate and metabolism and in the chemical and pigment

Table 2. Laboratory-scale production costs of marine microalgae *Chaetoceros* culture using LED and fluorescent light sources at 100 L/day production capacity.

Expenditure	THB/month	THB/day
Fixed cost		
Chemical for Conway medium	920	
Sea water	600	
Public utilities (not electricity)	1,200	
Depreciation	300	
Rent of location	8,000	
Other material	1,500	
Labor	9,000	
Total fixed cost	21,520	717
Variable cost (electricity cost, Min.–Max.)		
LED illumination	5,267–5,571	176–186
Fluorescent illumination	7,451–7,638	248–255
Total production cost (Min.–Max.)		
LED illumination	26,787–27,091	893–903
Fluorescent illumination	28,971–29,158	966–971
Average electricity cost (mean±SD)		
LED illumination	5,414.6±152.1[a]	180.4±5.0[a]
Fluorescent illumination	7,547.3±93.6[b]	251.5±3.1[b]
Average production cost (mean±SD)		
LED illumination	26,934.6±152.1[a]	897.8±5.0[a]
Fluorescent illumination	29,067.3±93.6[b]	968.9±3.1[b]

Note: Data are presented as mean±SD in the same column and different superscripts indicate significant differences ($P<0.05$).
Abbreviation: LED, light emitting diode; THB, Thai baht; SD, standard deviation.

composition of microalgae cultures (Arkronrat & Oniam, 2012a; Blanken, Cuaresma, Wijffels, & Janssen, 2013; Khoeyi, Seyfabadi, & Ramezanpour, 2012; Mercado *et al*., 2004; Mohammadi, Arabian, & Khalilzadeh, 2016; Sánchez-Saavedra & Voltolina, 2006; Tzovenis *et al*., 1997). Artificial lighting in microalgal research and production is usually carried out using fluorescence lamps, which have wide emission spectra, including wavelengths with low photosynthetic activity for certain microalgae (Carvalho, Silva, Baptista, & Malcata, 2011; Sánchez-Saavedra & Voltolina, 2006). Alternatively, an LED is a long-lasting, mercury-free, and fast-responding (nanosecond scale) artificial light source emitting nearly monochromatic light at different wavelengths due to solid-state electronics. Hence, an LED can provide not only a more sustainable control of supplemental light during microalgal growth, but the biochemical composition of the biomass can also be adjusted by means of single wavelengths at different light intensities or pulse light frequencies or both (Olle & Viršile, 2013; Yan, Zhang, Luo, & Zheng, 2013; Zhao, Wang, Zhang, Yan, & Zhang, 2013). In the current experiment, the cell densities and growth rates of the diatoms *C. calcitrans* and *T. weissflogii* were not significantly different using cool white fluorescent, cool daylight LED or warm white LED illumination. The warm white LED affected the cultures of marine green microalgae *N. oculata* and *T. suecica*. The growth rates of both cultures of microalgae using warm white LED illumination were lower than the cool white fluorescent illumination. This was possibly because the warm white LED spectra may not be suitable to grow microalgae in an energy efficient manner. A similar result was reported for the warm white light spectrum with other lamp types (e.g., warm white fluorescent lamp at wavelengths 570-620 nm) because a significant proportion of their emission peaks lies outside the major photosynthetic range of wavelengths (420-450 and 630-690 nm). On the other hand, the spectra of cool white and cool day light have wavelengths between 420 and 485 nm (Markager & Vincent, 2001; Yan *et al*., 2013; Zhao *et al*., 2013).

The visible light spectrum is divided into six main colors: violet 380-450 nm, blue 450-495 nm, green 495-570nm, yellow 570-590 nm, orange 590-620 nm, and red 620-750 nm. Not all wavelengths are equally absorbed by the main photosynthetic pigments of microalgae (Mitchell & Sosik, 1995). Each microalgae community has different light absorption spectra, reflecting either different adaptation or different growth species assemblages. For example, the major pigments of the marine green microalgae from the genus *Nannochloropsis* are chlorophyll *a* (not chlorophyll *b* or chlorophyll *c*), *β*-carotene, violaxanthin, and vaucheriaxanthin (Lubián *et al*., 2000). The major pigments of the genus *Tetraselmis* are lutein, chlorophyll *b*, neoxanthin, violaxanthin, and chlorophyll *a* (Sansone *et al*., 2017) while the major pigments of the diatoms *Chaetoceros* and *Thalassiosira* are fucoxanthin, diadinoxanthin, diatoxanthin chlorophyll *a*, and chlorophyll *c* (Hou, Huang, Cao, Chen, & Hong, 2007; Jeffrey & Vesk, 1997). Chlorophyll *a* and chlorophyll *b* preferentially absorb blue (450-495 nm) and red (620-750 nm) light, while the other wavelengths are absorbed in different magnitudes by the other pigments. The xanthophyll pigments, such as fucoxanthin and diadinoxanthin, have optimal absorbed light in the ranges of 425-450 and 570-600 nm, repsectively (Fujiki & Taguchi, 2001; Mitchell & Sosik, 1995). Thus, warm white LED did not affect the growth rate of either diatom but affected the growth rate of both green microalgae in the current study and consequently, this study recommends that cool daylight LED is optimal for commercial, laboratory-scale marine microalgae cultures compared to warm white LED.

The laboratory-scale production of microalgae that is suitable for seed culture and the future development of most microalgal applications will need to have a reliable and economically scaled cultivation process. Although the growth pattern and growth rate of microalgae are important measurements of culture methods, factors such as temperature, nutrients, light, and salinity can affect the growth performance of microalgae (Khatoon *et al*., 2014; Sánchez-Saavedra & Voltolina, 2006; Sureshkumar *et al*., 2014; Tzovenis *et al*., 1997; Zhu *et al*., 1997). In the current study, the effects of LED light on microalgal growth can also be examined with reference to the exponential growth phase and growth rate. Furthermore, the use of LED for laboratory-scale microalgae production is essential due to the much lower energy consumption compared to fluorescent lamps. In the current study, the commercial production of *Chaetoceros* at 100 L/day production capacity under cool daylight LED illumination had a significantly lower cost (mean 26,934.6 THB/month) than under cool white fluorescent illumination (mean 29,067.3 THB/month), and had electricity costs of 19.4–20.8% and 25.5–26.5% of the total costs, respectively. This indicated that LED illumination for laboratory-scale microalgal production can reduce the operational cost by about 7.3%. Generally, the commercial, laboratory-scale production of marine microalgae (*Chaetoceros*) has a high operational cost, with the main component being the cost of electricity (24-30% of total cost) because fluorescent lamps are used for the light source (Arkronrat *et al*., 2014; Arkronrat & Oniam 2012c). In another system, the productivity of microalgae can be enhanced by cultivating the culture under LED lamps with a peak emittance of 680 nm which would result in doubling the number of cells produced. Examples include the *Chlorella kessleri* cultured in a closed photobioreactor (Koc *et al*., 2013), *Chlorella vulgaris* and *Euglena gracilis* cultured in disposable bags (Hahne *et al*., 2014), and *Chlorella sorokiniana* and *Picochlorum soloecismus* cultured in indoor raceway ponds (Huesemann *et al*., 2017). For these reasons, cool daylight LED could be a viable alternative for commercial, laboratory-scale production of marine microalgae.

5. Conclusions

Cool daylight LED (1,200 mm, 18W, 6,500 K, 1,600 lm) and warm white LED (1,200 mm, 18W, 3,000 K, 1,600 lm) illuminations did not affect the cell density and growth rate of the diatoms *C. calcitrans* and *T. weissflogii* compared to cool white fluorescent illumination (1,200 mm, 36W, 4,000 K, 3,250 lm). However, the warm white LED light affected the growth performances of marine green microalgae *N. oculata* and *T. suecica* as the growth rates of both microalgae were lower than for cool white fluorescent illumination. In addition, the commercial microalgae produced under cool daylight LED illumination had a significantly lower cost than for production using cool white fluorescent illumination, and the operational cost was reduced by about 7.3%. This study demonstrated that an alternative option for the commercial laboratory-scale production of marine microalgae is cool daylight LED as the light source to reduce production costs and achieve a better profit margin.

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