Enhancing the production of eicosapentaenoic acid (EPA) from *Nannochloropsis oceanica* CY2 using innovative photobioreactors with optimal light source arrangements

Chun-Yen Chen^a, Yu-Chun Chen^b, Hsiao-Chen Huang^b, Shih-Hsin Ho^c and Jo-Shu Chang^{b,d*}

^aCenter of Bioscience and Biotechnology, National Cheng Kung University, Tainan 701, Taiwan

^bDepartment of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan

^cOrganization of Advanced Science and Technology, Kobe University, Kobe, Japan

^dResearch Center for Energy Technology and Strategy, National Cheng Kung University, Tainan 701, Taiwan

***Correspondence:** Prof. Jo-Shu Chang

E-mail: changjs@mail.ncku.edu.tw

Fax: +886-6-2357146

Abstract

Binary combinations of LEDs with four different colors were used as light sources to identify the effects of multiple wavelengths on the production of eicosapentaenoic acid (EPA) by an isolated microalga *Nannochloropsis oceanica* CY2. Combining LED-Blue and LED-Red could give the highest EPA productivity of 13.24 mg L⁻¹ d⁻¹, which was further enhanced to 14.4 mg L⁻¹ d⁻¹ when using semi-batch operations at a 40% medium replacement ratio. A novel photobioreactor with additional immersed light sources improved light penetration efficiency and led to an 38% (0.170 to 0.235 g L⁻¹ d⁻¹) increase in the microalgae biomass productivity and a 9% decrease in electricity consumption yield of EPA (10.15 to 9.33 kw-h (g EPA)⁻¹) when compared with the control (i.e., without immersed light sources). Operating the immersed LEDs at a flashing-frequency of 9 Hz further lowered the energy consumption yield to 8.87 kw-h (g EPA)⁻¹.

Keywords: EPA; *Nannochloropsis oceanica*; immersed light source; LED; flashing frequency; light wavelength

1. Introduction

Eicosapentaenoic acid (EPA), a kind of omega-3 fatty acid, is a natural substance obtained from fish, unicellular microalgae or seaweeds that is used as a food supplement with many health improvement functions, such as the prevention of cardiovascular diseases, cancers and diabetes ([Connor, 2000](#); [Laviano et al., 2013](#); [Narayan et al., 2006](#)).

EPA is also a major component of phospholipids, which are the main molecules in biological membranes, and therefore they are important for both cell structure and functioning ([Alessandri et al., 2004](#)).

EPA is usually obtained from the oil of marine fish (e.g., salmon, mullet and mackerel). However, fish-based EPA has the drawbacks of an unpleasant taste and pollutant accumulation, and its production is now facing the threat of abrupt declines in worldwide fish stock ([Allen et al., 2013](#)). It is thus of interest to develop more sustainable alternative sources for EPA production, and microalgae are considered as one of the most promising of these ([Adarme-Vega et al., 2012](#)). In contrast to fish-based EPA harvesting processes, such microalgae could be cultivated in well-controlled photobioreactors or closed fermentation systems. Furthermore, as the related microorganisms engage in photosynthesis, they can convert CO₂, water and light energy into chemical energy and biomass through carbon fixation, and so microalgae-based EPA production can be seen as one way to reduce the amount of atmospheric CO₂.

It is known that the light wavelength affects the cell growth and fatty acid composition of microalgae (Kim et al., 2013). Different pigments in microalgae cells with different ranges of absorption peaks assist in the operation of the photosynthetic system. The light absorption ability of photosystem II (PSII) could be improved by red light, while that of photosystem I (PSI) could be improved by blue light (You and Barnett, 2004). A recent study showed that cultivating *Nannochloropsis* sp. with illumination of a specific wavelength could enhance the cell growth and EPA content (Das et al., 2011a). In addition, Guiheneuf et al. (2010) and Huang and Cheung (2011) investigated the relationship between ultraviolet light and microalgae growth, and found that under the stress of UV light microalgae would alter the proportion of unsaturated fatty acids (e.g., EPA, DHA, and ALA), increasing the fluidity of the cell membrane and thus preventing cell damage from high-energy radiation (Huang and Cheung, 2011). Based on the mosaic model, the fluidity of the cell membrane is associated with the fatty acid composition of lipids (and especially that of the unsaturated fatty acids) (Singer and Nicolson, 1972). The aim of the current study is thus to identify the preferred light wavelength for EPA production from microalgae.

One common problem with the cultivation of photoautotrophic microalgae in photobioreactors is the light penetration efficiency. Light intensity attenuates exponentially as it penetrates into the photobioreactor, and as the cell density increases

over, the penetration efficiency becomes even poorer, due to self-shading effects. Therefore, maintaining a constant average light intensity inside the photobioreactor is the key to attaining a high microalgal biomass concentration. Immersed light sources can thus be used as an internal light source to avoid shading effects and improve light distribution. Moreover, the photobioreactor should be designed so that there is a high surface area to volume ratio (S/V ratio), so that a shorter light path length and higher illumination efficiency can be achieved.

In this study, an isolated microalgal strain (identified as *Nannochloropsis oceanica* CY2) ([Chen et al., 2013](#)) was used to produce EPA in a LED-illuminated photobioreactor. Various engineering approaches were applied to promote cell growth and enhance the EPA production of the target isolated microalgal strain. For instance, the optimal combination of light sources with different wavelengths was investigated to improve the biomass and EPA production of *N. oceanica*. Furthermore, semi-batch operation was also used to enhance EPA production to assess the commercial feasibility of the proposed production system. Finally, a novel large-scale photobioreactor was applied to cultivate the microalga. In short, the goal of this work was to determine the optimal light source and operation strategy to enhance microalgae-based EPA production and propose a sustainable and economically feasible method of producing EPA.

2. Material and methods

2.1 The microalgae culture and medium composition

The microalga species used in this work was isolated from water located off the coast of southern Taiwan. The microalga was identified as *Nannochloropsis oceanica* by its morphology as well as by 23S plastid rDNA sequence alignment (Chen et al., 2013). The pure *N. oceanica* CY2 was cultured in sterilized artificial sea water (artificial sea water powder, Red Sea, USA) enriched with (g L⁻¹): NaNO₃, 1.5; K₂HPO₄, 0.03; MgSO₄·7H₂O, 0.075; CaCl₂·2H₂O, 0.036; citric acid, 0.006; Na₂CO₃, 0.02; ammonium ferric citrate, 0.006; EDTA·2Na, 0.001; H₃BO₃, 0.00286; ZnSO₄·7H₂O, 0.00022; MnCl₂·4H₂O, 0.00181; Na₂MoO₄·2H₂O, 0.00039; CuSO₄·5H₂O, 0.000079; and Co(NO₃)₂·6H₂O, 0.000049. The *N. oceanica* was incubated at 28°C for six days under a light intensity of approximately 50 μmol m⁻² s⁻¹ (illuminated by TL5).

2.2 Operation of photobioreactors and culture conditions

The conventional photobioreactor (PBR) was a 1-liter glass-made vessel illuminated with an external light source (14 Watt TL5 tungsten filament lamps; Philips Co., Taipei, Taiwan) mounted on both sides of the PBR. The pre-cultured *N. oceanica* CY2 was inoculated into the photobioreactor at an inoculum size of 65 mg/l. The PBR was operated at 28°C, 300 rpm agitation with CO₂ aeration (2%, 0.1 vvm). During microalgae growth,

liquid samples were collected from the sealed glass vessel with respect to time to determine the biomass concentration, pH, residual nitrogen source (i.e., NO_3^-) concentration and EPA content of the microalgal biomass. The microalga was cultured in the PBR with modified BG-11 medium (Chiu et al., 2009; Yeh and Chang, 2012). To assess the effects of multiple light wavelengths on cell growth and EPA production, four LEDs with different colors (i.e., white, blue, yellow and red) were selected as the light sources and mounted on each side of the PBR at a light intensity $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ to verify whether the combination of multiple wavelengths could enhance the microalgal cell growth and EPA production of *N. oceanica* CY2.

A novel photobioreactor with immersed light sources was also examined for the cultivation of *N. oceanica* CY2. The working volume of the photobioreactor was 10 liters. The inoculum size, aeration condition, and agitation rates were the same as those used for operating the conventional PBR, as described earlier. The immersed light sources were a combination of red and blue LED lights. Moreover, the immersed LED illumination was operated at three different flashing frequencies (i.e., 9 Hz, 8 Hz, and 7 Hz) to reduce electricity consumption and possibly enhance the photosynthetic efficiency of the microalgae. The flashing frequency of the immersed light sources was controlled by a pulse-width modulation (PWM) device.

2.3 Determination of cell concentration and specific growth rate of microalgae

The cell concentration of the microalgae culture in the photobioreactor was determined regularly by optical density measurement at a wavelength of 682 nm (i.e., OD₆₈₂) using a spectrophotometer (model U-2001, Hitachi, Tokyo, Japan) after appropriate dilution with deionized water. The dry cell weight (DCW) of the microalgae biomass was obtained by filtering 10 ml aliquots of culture through a cellulose acetate membrane filter (0.45 µm pore size, 47 mm in diameter). Each loaded filter was dried at 105°C until the weight was invariant. The dry weight of the blank filter was subtracted from that of the loaded filter to obtain the microalgae dry cell weight (DCW). The OD₆₈₂ values were converted to DCW concentration via calibration between OD₆₈₂ and dry cell weight (i.e., 1.0 OD₆₈₂ approximately equals 280-320 mg DCW/L).

2.4 Measurement of light intensity and light wavelength distribution

The light intensity on the reactor surface was measured with a LI-250 Light Meter with an LI-200SA pyranometer sensor (LI-COR, Inc., Lincoln, Nebraska, USA). The light intensity was determined by measuring three different locations on both sides of the reactor and taking an average of these. This light meter gives a unit of µmol photons m⁻² s⁻¹ for the measured light intensity. The light wavelength distribution of different light sources was scanned with an FP-6200 spectrofluorometer (JASCO, Inc., USA). The

results are presented as the relative intensity of light source at different wavelengths.

2.5 Determination of the oil/lipids content

The microalgae cells were harvested from culture broth by centrifugation (5500 rpm for 4 min). The harvested cells were washed twice with deionized water, lyophilized, and weighed. The lipid composition was determined as fatty acid methyl esters (FAMES) through the direct transesterification method (Su et al., 2007). The sample was then analyzed by gas chromatography (GC-2014, Shimadzu, Kyoto, Japan) using a flame ionization detector (FID). Samples were injected into a 100 m long capillary column (SPTM-2560 capillary GC column, Sigma-Aldrich, USA) with an internal diameter of 0.25 mm. Helium was used as the carrier gas, with a flow rate of 1.3 ml/min. The temperatures of the injector and detector were set at 260 and 280°C, respectively. The oven temperature was initially set to 150°C, and was first increased from 150 to 240°C at a rate of 2.5°C/min and then held at 240°C for 20 min.

3. Results and discussion

*3.1 The effects of light wavelength on EPA production from *Nannochloropsis oceanica* CY2*

In this study, LEDs with four different colors (i.e., white, blue, yellow and red) were used to examine whether the multiple wavelengths could enhance the microalgae growth

and EPA production of *N. oceanica* CY2. The effects of different combination of LED light sources on cell growth performance are shown in **Fig. 1a** and **Table 1**. These show that illumination with three binary combinations of LEDs (i.e., Red-Yellow, Blue-Red and Blue-Yellow) led to higher microalgae biomass productivity than that obtained by using the best single wavelength (i.e., LED-Blue) reported in our recent work (Chen et al., 2013). Nevertheless, illumination with a conventional fluorescent light source (TL5) still gave the best cell growth. **Table 1** shows that the maximal biomass productivities of Red-Yellow ($0.263 \text{ g L}^{-1} \text{ d}^{-1}$) and Blue-Red ($0.258 \text{ g L}^{-1} \text{ d}^{-1}$) were 16.7-19.0% higher than the best result obtained from using single wavelength light source (i.e., LED-Blue) (Chen et al., 2013).

The effects of binary combinations of different light sources on microalgae-based EPA production are shown in **Fig. 1b**. The results indicate that using a combination of Blue-Red LEDs could obtain the highest EPA content, 5.13%, followed by using Yellow-White (5.01%), Blue-White (4.87%), Blue-Yellow (4.86%), Yellow-White (4.86%) and Red-Yellow (4.38%). However, there were no significant variations in the EPA content when using those combinations of LEDs. In addition, the highest EPA content obtained from binary LEDs (i.e., 5.13% for Blue-Red,) was slightly lower than the results from using a single wavelength (i.e., LED-Blue, 5.57%) (Chen et al., 2013). However, the microalgae cultivation with multiple wavelengths resulted in higher biomass productivity,

and also led to higher EPA productivity, as indicated in [Table 1](#). The highest EPA productivity ($13.24 \text{ mg L}^{-1} \text{ d}^{-1}$) was obtained when the microalga was cultivated with Blue-Red LED light. This highest EPA productivity obtained from using Blue-Red LED was higher than our previous best result ($11.85 \text{ mg L}^{-1} \text{ d}^{-1}$) obtained from using a conventional TL5 fluorescent lamp ([Chen et al., 2013](#)).

To improve the commercial viability of microalgae-based EPA production, the electricity consumption required to produce a unit of EPA (defined as the electricity consumption yield) must be reduced. Using LEDs as the light sources is an economically feasible method for improving microalgae biomass and EPA production. As shown in [Table 1](#), using Blue-Red as a light source could reduce the electricity consumption yield from $98.79 \text{ kw}\cdot\text{h (g EPA)}^{-1}$ (using conventional TL5 lamp) to $31.94 \text{ kw}\cdot\text{h (g EPA)}^{-1}$. These results clearly indicate that using a binary combination of LED-Blue and LED-Red as the light source could significantly enhance the performance of EPA production of *N. oceanica* CY2, and thus this is a suitable artificial light source for microalgae-based EPA production. Similar findings were also obtained for *Nannochloropsis* sp., as reported in the literature ([Das et al., 2011b](#); [Teo et al., 2014a](#); [Teo et al., 2014b](#)).

3.2 Photobioreactor operation strategy for improving microalgal EPA production

To improve the productivity of microalgae-based EPA, the photobioreactor was

operated with the semi-batch strategy under the optimal light source arrangement. This semi-batch approach has been shown to be an effective bioreactor strategy to promote carbohydrate and lutein production from microalgae (Ho et al., 2014; Xie et al., 2013). It is particularly suitable for prolonged operation of microalgae culture to produce target products (such as EPA), the accumulation of which in microalgae does not require an induction period under stress conditions (Takagi et al., 2006; Yeh and Chang, 2011). Therefore, in this study, the *N. oceanica* CY2 strain was cultivated in a photobioreactor that was operated on semi-batch mode at different replacement ratios (i.e., 20%, 40% and 60%) for long-term operation (between six to nine batches) using the optimal conditions identified earlier (namely, modified BG-11 medium; $\text{NaNO}_3 = 1.5 \text{ g L}^{-1}$; light intensity = $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$; light source = combination of LED-blue and LED-Red). Before the semi-batch operation, *N. oceanica* CY2 was first cultivated on batch mode for five days, until the maximum EPA content and EPA productivity were reached (Fig. 2). After that, the operation was changed to semi-batch mode and the operation procedures were repeated several times. Different percentages (i.e., 20%, 40% and 60%) of the culture medium were discharged with the replacement of the same volume of fresh culture medium, which was rapidly added into the photobioreactor to reach a final volume of 1 L. The pH was maintained approximately constant (at ca. 7.1), which is within the favorable pH range of 6.5-8.5 for microalgae growth.

As shown in [Fig. 2](#), the biomass productivity remained consistent during the semi-batch operation; however, the EPA contents were quite different when different medium replacement ratios were employed. [Table 2](#) further summarizes the biomass productivity, EPA content, EPA productivity and energy consumption yield at different medium replacement ratios. Compared with batch cultivation, the average biomass productivity increased by 14-20% when the replacement ratio was set at 40% or 60%, but no significant improvement in biomass productivity was observed when the replacement ratio was low (i.e., 20%) as compared to the batch operation. Since an adaptation period for the growth of microalgae is required after medium replacement, a lower cell growth rate was often observed at the beginning of replacement. As a result, the condition of too frequent replacements (or too low replacement ratio) during semi-batch operation might not be favorable for biomass production. However, in general, when using the semi-batch operation the biomass productivity was similar or slightly higher than that obtained from using batch culture.

[Table 2](#) shows that the average EPA content obtained from semi-batch operation (4.80-4.98%) was similar to that from batch culture (4.93%), except for when using a 60% replacement ratio, which resulted in a lower EPA content at 4.10% when compared with other conditions. Previous studies mentioned that the EPA content of microalgae might be affected by the ratio of different microalgae cells at different growth phases ([Lin et al.](#),

2012; Wan et al., 2013). As the replacement time interval for using a 60% replacement ratio was much longer than that with the other replacement ratios, it is likely that most microalgal cells were in the later growth phase. This may have led to the lower average EPA content for the semi-batch operation at a 60% replacement ratio. Furthermore, the highest average EPA productivity of $14.4 \text{ mg L}^{-1} \text{ d}^{-1}$ was obtained from the semi-batch operation with a 40% replacement ratio due to the higher biomass productivity and more stable EPA content (consistent with that seen in the batch operation) (Fig. 2 and Table 2). In addition, with a 40% replacement ratio the electricity consumption yield of EPA decreased to $29.18 \text{ kw h g}^{-1} \text{ EPA}$, which is also the lowest among all the operation modes examined in this work (Table 2).

3.3 The effects of immersed light source on the photobioreactor performance

Light energy is the sole or major energy source for phototrophic and mixotrophic growth of microalgae. However, along with cell growth the cell concentration gradually increases, leading to the self-shading effect, which inhibits light penetration into the microalgae culture, and thus significantly limits the growth of microalgae cells (Yen and Chiang, 2012). Likewise, in large-scale microalgae cultivation, the length of the light path of large scale PBRs is much longer than that of laboratory-scale PBRs, which also causes insufficient light supply and eventually leads to a slower cell growth rate. Therefore, the

optimal light intensity obtained from lab-scale PBRs usually cannot be directly applied in the large-scale PBRs or other cultivation systems. Due to these difficulties, a novel PBR with an immersed light source was designed to overcome the problems associated with the self-shading effect. **Figure 3** shows that the light distribution in the novel PBR (with immersed light sources) is much better than that seen with the conventional one (with external light sources), as the light intensity of immersed light sources could be closer to the optimal light intensity (I_{opt}), thus providing a more sufficient light supply to support microalgae growth.

The results of microalgae cultivation in the novel PBR are shown in **Fig. 4a**. It can be seen that using the immersed light sources could effectively enhance the microalgae growth, as the biomass concentration in 7 days was 1.6 and 1.0 g L⁻¹, respectively, for the novel and conventional PBR. The maximal biomass productivity of the microalgal culture illuminated with two immersed light sources could thus be improved by 32% compared with the conventional PBR. However, when using four immersed light sources photo-inhibition occurred, and this damaged the microalgal culture at the beginning of cultivation (data not shown). In addition, the time course profile of cell growth (**Fig. 4a**) shows that the cell growth was similar in the first two days. This indicates that the external light sources were sufficient for microalgae growth until the biomass concentration was higher than 0.4 g L⁻¹. Therefore, the immersed light source might be unnecessary at the

beginning of microalgae culture. However, when the biomass concentration is higher than a threshold level (ca. $> 0.4 \text{ g L}^{-1}$ in this study), an additional light supply would be needed. Therefore, the appropriate timing to turn on the immersed light sources should be determined to achieve better growth while keeping electricity consumption to a minimum.

Figure 4b shows the profile of EPA content during the growth of *N. oceanica* CY2. It shows that the EPA content resulting from using the immersed light source was similar or slightly higher than that from using a conventional PBR with an external light source. The EPA content was between 4.2-4.8% before day 5, and gradually decreased as the cultivation time increased. Therefore, the best time to harvest the cells for EPA production would be around day 4. **Table 3** summarizes the performance of microalgal EPA production with different types of light sources and sizes of PBR. The biomass productivity and EPA production efficiency of the 1-L PBR illuminated with external Blue-Red LEDs (no internal light source) is similar to that obtained from a conventional PBR (with TL5 as the external light source). However, the electricity consumption yield for the LED-illuminated PBR (32.94 kW-h per g EPA) is markedly lower than that of TL5-illuminated PBR (49.40 kW-h per g EPA). When the volume of the PBR was increased to 10 L, the biomass productivity and EPA productivity of the LED-illuminated PBR (without an internal light source) was much lower than that obtained from a 1-L PBR. This is obviously due to the insufficient light supply when the working volume was

increased by 10-fold. Nevertheless, although the EPA production rate was slower when the PBR size was 10 times larger, the EPA content (4.49%) was similar to that obtained from a 1-L PBR (4.93%). As a result, the electricity consumption yield of EPA was reduced significantly to 10.15 kW h g⁻¹ EPA for the 10-L PBR illuminated with external LEDs. To improve the EPA production performance, the immersed light sources were added to the 10-L PBR to overcome the self-shading effect of microalgae and provide sufficient light energy for growth. As shown in [Table 3](#), the biomass and EPA productivities improved by 37.5% and 32.5% when two immersed light sources (light intensity = 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were put into the 10-L PBR. Although using additional immersed light sources increased the total electricity consumption, the significantly higher biomass and EPA productivity thus obtained resulted in a lower electricity consumption yield for the 10-L PBR with the immersed light sources (9.33 kW-h per g EPA) than without them (10.15 kW-h per g EPA) ([Table 3](#)). This supports the feasibility of using additional immersed light sources to enhance EPA productivity when the PBR is scaled up.

3.4 Immersed light sources with different flashing-frequencies

The literature shows that applying an appropriate flashing frequency (or light-dark cycle) could enhance the photosynthetic efficiency of microalgae; however, due to a lower total amount of photons penetrating into the photobioreactor, the biomass

productivity might decrease (Janssen et al., 2000; Terry, 1986). In addition, Fabregas et al. (2002) reported that the light-dark cycle could affect the cell composition of microalgae. Moreover, the replacement of a continuous light supply by one with interval periods can reduce energy consumption. There may exist an optimal flashing frequency which could achieve higher photosynthesis efficiency, better cell growth, and lower energy consumption simultaneously. The immersed light sources in this study were thus operated with different flashing frequencies (i.e., 9 Hz, 8 Hz and 7 Hz) to explore the effects on the EPA production performance of *N. oceanica* CY2. **Figure 5** shows that the highest biomass production was obtained with continuous light supply from the immersed light sources. When the flashing-frequency was controlled at 9 Hz, the microalgal cell growth was comparable to that with a continuous light supply, suggesting that this could provide sufficient light energy until the biomass concentration becomes very high. However, the intermittent light at flashing frequencies of 8 Hz and 7 Hz was no longer sufficient for cell growth when the cell concentration was higher than ca. 0.6 g L^{-1} , as the final cell concentrations were significantly lower than that with a continuous light supply (**Fig. 5**).

Table 4 summarizes the EPA production performance with different flashing frequencies. There were no significant differences in maximal EPA content and EPA productivity among the different frequencies. However, a higher flashing frequency led to slightly higher total biomass and EPA production. In terms of the electricity consumption

yield, which takes into account both the positive and negative effects of using an intermittent light supply on EPA production, using a flashing efficiency of 9 Hz had the lowest electricity consumption yield of 8.87 kW-h per gram of EPA produced. (Table 4).

4. Conclusions

Illumination using a binary combination of LED-Blue and LED-Red could enhance the EPA productivity of *N. oceanica* CY2 to 13.24 mg L⁻¹ d⁻¹ and significantly reduced the electricity consumption yield of EPA (ECY) when compared with using single-color LED or conventional TL5 lamps. Using the semi-batch operation could further enhance EPA productivity to 14.4 mg L⁻¹ d⁻¹ with a medium replacement ratio of 40%. The additional immersed light sources (ILS) overcame the self-shading effect in a 10-L photobioreactor and the ECY further decreased to 8.87 kw-h (g EPA)⁻¹ when the ILS were operated with a flashing frequency of 9 Hz.

5. Acknowledgements

The authors gratefully acknowledge the financial support provided for this study by the Core Research for Evolutional Science and Technology (CREST) of the Promoting Globalization on Strategic Basic Research Programs of the Japan Science and Technology Agency (JST). Support from the Top University Project of NCKU and Taiwan's Ministry

of Science and Technology under grant numbers MOST 104-3113-E-006-003, NSC 101-2221-E-006-209-MY3, MOST 103-2622-E-011-021 -CC2 and NSC 101-2221-E-006 -094 -MY3 is also gratefully acknowledged.

References

1. Adarme-Vega, T.C., Lim, D.K.Y., Timmins, M., Vernen, F., Li, Y., Schenk, P.M. 2012. Microalgal biofactories: A promising approach towards sustainable omega-3 fatty acid production. *Microbial Cell Factories*, 11.
2. Alessandri, J.-M., Guesnet, P., Vancassel, S., Astorg, P., Denis, I., Langelier, B., Aid, S., Poumes-Ballihaut, C., Champeil-Potokar, G., Lavialle, M. 2004. Polyunsaturated fatty acids in the central nervous system: evolution of concepts and nutritional implications throughout life. *Reproduction Nutrition Development*, 44(6), 509-538.
3. Allen, M.S., Ahrens, R.N.M., Hansen, M.J., Arlinghaus, R. 2013. Dynamic angling effort influences the value of minimum-length limits to prevent recruitment overfishing. *Fisheries Manag Ecol*, 20(2-3), 247-257.
4. Chen, C.-Y., Chen, Y.-C., Huang, H.-C., Huang, C.-C., Lee, W.-L., Chang, J.-S. 2013. Engineering strategies for enhancing the production of eicosapentaenoic acid (EPA) from an isolated microalga *Nannochloropsis oceanica* CY2. *Bioresour. Technol.*, 147(0), 160-167.
5. Chiu, S.-Y., Kao, C.-Y., Tsai, M.-T., Ong, S.-C., Chen, C.-H., Lin, C.-S. 2009. Lipid accumulation and CO(2) utilization of *Nannochloropsis oculata* in response to CO(2) aeration. *Bioresour. Technol.*, 100(2), 833-838.
6. Connor, W.E. 2000. Importance of n-3 fatty acids in health and disease. *Am J Clin Nutr*, 71(1), 171s-175s.
7. Das, P., Lei, W., Aziz, S.S., Obbard, J.P. 2011a. Enhanced algae growth in both phototrophic and mixotrophic culture under blue light. *Bioresour. Technol*, 102(4), 3883-3887.
8. Das, P., Lei, W., Aziz, S.S., Obbard, J.P. 2011b. Enhanced algae growth in both phototrophic and mixotrophic culture under blue light. *Bioresour. Technol.*, 102(4),

3883-3887.

9. Fabregas, J., Maseda, A., Dominguez, A., Ferreira, M., Otero, A. 2002. Changes in the cell composition of the marine microalga, *Nannochloropsis gaditana*, during a light : dark cycle. *Biotechnol Lett*, 24(20), 1699-1703.
10. Guiheneuf, F., Fouqueray, M., Mimouni, V., Ulmann, L., Jacquette, B., Tremblin, G. 2010. Effect of UV stress on the fatty acid and lipid class composition in two marine microalgae *Pavlova lutheri* (Pavlovophyceae) and *Odontella aurita* (Bacillariophyceae). *J. Appl. Phycol.*, 22(5), 629-638.
11. Ho, S.-H., Chan, M.-C., Liu, C.-C., Chen, C.-Y., Lee, W.-L., Lee, D.-J., Chang, J.-S. 2014. Enhancing lutein productivity of an indigenous microalga *Scenedesmus obliquus* FSP-3 using light-related strategies. *Bioresour. Technol.*, 152, 275-282.
12. Huang, J.J.H., Cheung, P.C.K. 2011. +UVA treatment increases the degree of unsaturation in microalgal fatty acids and total carotenoid content in *Nitzschia closterium* (Bacillariophyceae) and *Isochrysis zhangjiangensis* (Chrysophyceae). *Food Chem*, 129(3), 783-791.
13. Janssen, M., Janssen, M., de Winter, M., Tramper, J., Mur, L.R., Snel, J., Wijffels, R.H. 2000. Efficiency of light utilization of *Chlamydomonas reinhardtii* under medium-duration light/dark cycles. *J Biotechnol*, 78(2), 123-137.
14. Kim, T.H., Lee, Y., Han, S.H., Hwang, S.J. 2013. The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using *Scenedesmus* sp. for wastewater treatment. *Bioresource Technol*, 130, 75-80.
15. Laviano, A., Rianda, S., Molfino, A., Rossi Fanelli, F. 2013. Omega-3 fatty acids in cancer. *Current opinion in clinical nutrition and metabolic care*, 16(2), 156-161.
16. Lin, Q., Gu, N., Li, G., Lin, J., Huang, L., Tan, L. 2012. Effects of inorganic carbon concentration on carbon formation, nitrate utilization, biomass and oil accumulation of *Nannochloropsis oculata* CS 179. *Bioresour. Technol.*, 111(0), 353-359.

17. Narayan, B., Miyashita, K., Hosakawa, M. 2006. Physiological effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—A Review. *Food Reviews International*, 22(3), 291-307.
18. Singer, S.J., Nicolson, G.L. 1972. Fluid mosaic model of structure of cell membranes. *Science*, 175(4023), 720-&.
19. Su, C.H., Giridhar, R., Chen, C.W., Wu, W.T. 2007. A novel approach for medium formulation for growth of a microalga using motile intensity. *Bioresour. Technol.*, 98(16), 3012-3016.
20. Takagi, M., Karseno, Yoshida, T. 2006. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. *J Biosci Bioeng*, 101(3), 223-226.
21. Teo, C.L., Atta, M., Bukhari, A., Taisir, M., Yusuf, A.M., Idris, A. 2014a. Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. *Bioresour. Technol.*, 162(0), 38-44.
22. Teo, C.L., Idris, A., Zain, N.A.M., Taisir, M. 2014b. Synergistic effect of optimizing light-emitting diode illumination quality and intensity to manipulate composition of fatty acid methyl esters from *Nannochloropsis* sp. *Bioresour. Technol.*, 173(0), 284-290.
23. Terry, K.L. 1986. Photosynthesis in modulated light - quantitative dependence of photosynthetic enhancement on flashing rate. *Biotechnol Bioeng*, 28(7), 988-995.
24. Wan, C., Bai, F.-W., Zhao, X.-Q. 2013. Effects of nitrogen concentration and media replacement on cell growth and lipid production of oleaginous marine microalga *Nannochloropsis oceanica* DUT01. *Biochem. Eng. J.*, 78(0), 32-38.
25. Xie, Y., Ho, S.-H., Chen, C.-N.N., Chen, C.-Y., Ng, I.-S., Jing, K.-J., Chang, J.-S., Lu, Y. 2013. Phototrophic cultivation of a thermo-tolerant *Desmodesmus* sp. for lutein production: Effects of nitrate concentration, light intensity and fed-batch

- operation. *Bioresour. Technol.*, 144, 435-444.
26. Yeh, K.-L., Chang, J.-S. 2011. Nitrogen starvation strategies and photobioreactor design for enhancing lipid production of a newly isolated microalga *Chlorella vulgaris* ESP-31: Implications for biofuels. *Biotechnology Journal*, 6(11), 1358-1366.
 27. Yeh, K.L., Chang, J.S. 2012. Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris* ESP-31. *Bioresour. Technol.*, 105(0), 120-127.
 28. Yen, H.W., Chiang, W.C. 2012. Effects of mutual shading, pressurization and oxygen partial pressure on the autotrophical cultivation of *Scenedesmus obliquus*. *J Taiwan Inst Chem E*, 43(6), 820-824.
 29. You, T., Barnett, S.M. 2004. Effect of light quality on production of extracellular polysaccharides and growth rate of *Porphyridium cruentum*. *Biochem. Eng. J.*, 19(3), 251-258.

FIGURE LEGENDS

Figure 1 The time-course profiles of (a) microalgal cell growth; (b) EPA content with different combinations of light sources. PBR type = batch with external light source; light intensity = $150 \mu\text{mol m}^{-2} \text{s}^{-1}$

Figure 2 The time-course profile of microalgal cell growth and EPA content with different replacement ratios (a) 20.0%, (b) 40.0% and (c) 60.0%. PBR type = semi-batch with binary combination of blue and red LEDs (external); light intensity = $150 \mu\text{mol m}^{-2} \text{s}^{-1}$

Figure 3 Illustration of the average light intensity distribution in the photobioreactor with immersed light source (LED-Blue/LED-Red): (a) ideal distribution; (b) real distribution of the conventional PBR; (c) real distribution of the novel PBR with an immersed light source. The arrow represents the location of the immersed light source. Light intensity = $150 \mu\text{mol m}^{-2} \text{s}^{-1}$; Φ_{eq} is the length of the light path from the photobioreactor surface to the point of interest in the microalgae culture medium.

Figure 4 The time-course profiles of (a) microalgal cell growth; (b) EPA content with immersed light sources. PBR type = batch; light source: TL5 (external) with or without immersed LED-Blue/LED-Red; light intensity = $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ for each light source.

Figure 5 The time-course profile of (a) microalgal cell growth; (b) EPA content cultivated in novel PBR with immersed light sources controlled at different flashing frequency. PBR type=batch; light source: TL5 (external) with immersed LED-Blue/LED-Red; light intensity = $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ for each light source.

Table 1 The biomass growth and EPA production performance of *N. oceanica* CY2 with different light sources (light intensity of each light source was fixed at 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$)

Light sources	Biomass productivity ($\text{g L}^{-1} \text{d}^{-1}$)	EPA content (% w/w)	EPA productivity ($\text{mg L}^{-1} \text{d}^{-1}$)	Energy consumption yield (kw-h (g EPA)^{-1})	Reference
TL5	0.254 ± 0.013	4.31 ± 0.35	11.5 ± 0.51	98.79 ± 4.89	(Chen et al., 2013)
LED-blue	0.221 ± 0.014	5.57 ± 0.33	12.29 ± 0.79	32.3 ± 1.65	(Chen et al., 2013)
LED- yellow	0.238 ± 0.019	3.78 ± 0.28	9.01 ± 0.54	43.8 ± 2.31	(Chen et al., 2013)
LED-red	0.216 ± 0.015	5.41 ± 0.41	11.68 ± 0.64	34.2 ± 1.45	(Chen et al., 2013)
LED-Blue-Red	0.258 ± 0.013	5.13 ± 0.32	13.24 ± 0.48	31.94 ± 1.85	This study
LED-Blue-Yellow	0.247 ± 0.011	4.86 ± 0.35	11.0 ± 0.70	37.33 ± 1.97	This study
LED-Blue-White	0.221 ± 0.010	4.87 ± 0.35	10.1 ± 0.51	36.25 ± 1.91	This study
LED-Red- Yellow	0.263 ± 0.022	4.38 ± 0.39	11.9 ± 0.60	34.31 ± 1.86	This study
LED-Red-White	0.238 ± 0.016	4.86 ± 0.42	11.3 ± 0.62	35.51 ± 1.98	This study
LED-Yellow-White	0.216 ± 0.014	5.01 ± 0.48	10.2 ± 0.61	40.19 ± 2.51	This study

Table 2 Performance of semi-batch culture with different broth replacement ratios with regard to biomass productivity, EPA content, EPA productivity and energy consumption yield of *N. oceanica* CY2 (Light source: LED-Blue/LED-Red; light intensity = 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$)

Replacement ratio (% v/v)	Biomass productivity (g L ⁻¹ d ⁻¹)	EPA content (% w/w)	EPA productivity (mg L ⁻¹ d ⁻¹)	Energy consumption yield (kW-h (g EPA) ⁻¹)
Batch	0.254 ± 0.013	4.93 ± 0.35	12.5 ± 0.68	32.94 ± 1.95
20	0.264 ± 0.021	4.80 ± 0.42	12.7 ± 1.87	35.03 ± 2.75
40	0.289 ± 0.025	4.98 ± 0.47	14.4 ± 1.32	29.18 ± 2.56
60	0.308 ± 0.029	4.10 ± 0.39	12.6 ± 1.16	31.42 ± 2.57

Table 3 The performance of microalgal EPA production with different light sources and photobioreactors

External light source	Immersed light source	PBR Working volume (L)	Biomass productivity (g L ⁻¹ d ⁻¹)	EPA content (% w/w)	EPA productivity (mg L ⁻¹ d ⁻¹)	Electricity consumption yield (kW-h (g EPA) ⁻¹)
TL5 [*]	None	1	0.266 ± 0.013	4.31 ± 0.22	11.5 ± 0.58	49.40 ± 2.47
LED-Blue/LED-Red [*]	None	1	0.254 ± 0.013	4.93 ± 0.25	12.5 ± 0.63	32.94 ± 1.65
LED-Blue/LED-Red [*]	None	10	0.170 ± 0.009	4.49 ± 0.23	7.7 ± 0.39	10.15 ± 0.51
LED-Blue/LED-Red [*]	LED-Blue/LED-Red [*] (2 set)	10	0.234 ± 0.012	4.35 ± 0.22	10.2 ± 0.51	9.33 ± 0.47

^{*}Light intensity = 150 μmol m⁻² s⁻¹

Table 4 The performance of microalgal EPA production with an immersed light source (LED-blue and LED-red) operated with different flashing frequencies (light intensity = 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$)

Flashing frequency (Hz)	Biomass productivity ($\text{g L}^{-1} \text{d}^{-1}$)	EPA content (% w/w)	EPA productivity ($\text{mg L}^{-1} \text{d}^{-1}$)	Energy consumption yield (kW-h (g EPA)^{-1})
Continuous	0.234 ± 0.012	4.35 ± 0.22	10.2 ± 0.51	9.33 ± 0.47
9	0.231 ± 0.012	4.61 ± 0.23	10.7 ± 0.54	8.87 ± 0.44
8	0.219 ± 0.011	4.17 ± 0.21	9.1 ± 0.46	10.19 ± 0.51
7	0.200 ± 0.011	4.05 ± 0.20	8.1 ± 0.41	11.3 ± 0.57

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5