

# Synergistic Effects of Pulse Light Emitting Diode on Growth Rate, Lipid Content and Its Carbon Chain of *Nannochloropsis* sp. in Fatty Acid Methyl Ester Production

Chee Loong Teo, Ani Idris\*, Azrul Nizam Bin Sukiran

Department of Bioprocess Engineering, Faculty of Chemical & Energy Engineering, c/o Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia, 81310, UTM Johor Bahru, Johor, Malaysia  
 ani@cheme.utm.my

Third generation biofuels which is biodiesel from microalgae has its share of problems such as high cultivation costs, expensive dewatering and drying process, lipid extraction and transesterification. *Nannochloropsis* sp. was cultivated indoor using pulse light emitting diodes LED, blue LED and red LED. *Nannochloropsis* sp. was cultivated for 12 d under LED pulsed at a constant frequency between 5 and 100 kHz with various duty cycles (5 – 50 %) by changing the resistance compared with red and blue LED. The number of cells, lipid content and FAME composition were determined using cell counting, Nile red method and gas chromatography respectively. Results revealed that the pulse LED compared to other wavelength LED have a significant effect in the number of cell (pulse: 3.39; blue 2.83; red: 2.63 x 10<sup>7</sup> cell/mL) and exhibited a consistent lipid production stability curve compared with red and blue LED. The biodiesel produced under the pulse LED has the following characteristics: degree of unsaturation (80.41), density (881 kg/m<sup>3</sup>), viscosity (3.96 mm<sup>2</sup>/s), cetane number (61.03), iodine value (97.76), cloud point (7.1 °C), pour point (0.86 °C), CFPP (6.52 °C) and HHV (41.28 MJ/kg).

## 1. Introduction

Petroleum sourced fuels is now widely recognized as unsustainable energy because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment that caused global warming. Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Biodiesel derived from oil crops such as palm and waste cooking oil, vegetable oil, animal fat, bioethanol (Caetano et al., 2013a) and other alcohol is a potential renewable and carbon neutral alternative to petroleum fuels. For the last few decades, fossil fuel depletion and global warming issues have strongly motivated research on fuel production from biomass. Patil et al. (2010) stated that researches have been struggling to explore alternative sustainable and renewable green energy resources around the world such as microalgae (Mata et al., 2013a). Biodiesel is a mixture of fatty acid methyl esters (FAMES) which is produced through transesterification of lipids and is rapidly gaining acceptance by the majority as an alternative fuel for the future (Meng et al., 2009) with some attractive method such as catalytic conversion had been studied (Siow et al., 2016). Currently, indoor cultivation of microalgae for biodiesel production using fluorescent lamp as light source has some drawbacks such as high energy consumption and heat generation. Ward et al. (2013) reported that LED is a well light source for microalgae cultivation due to a number of reasons. Firstly, the design of the LED allows higher conversion efficiency, generate lower heat and longer life-expectancy compared to other artificial light sources (Chen et al., 2011). Secondly, the high intensity generated by LED is an advantage because it can allocate light uniformly and sufficiently to penetrate through the culture in the photobioreactor (Posten, 2009). In many of these studies certain aspects on quality of biodiesel in relation to the type and intensity of LED were not extensively investigated. To improve the microalgae indoor cultivation, the most important factor in designing a successful high density photobioreactor (PBR) is undoubtedly the light source. The expose and the distribution of the light into the culture, as well as the light scattering and equalizing in the culture, must be considered in order to sustain

algal cultures. However, when PBR is supplying continuous light, the photosynthetic photon flux (PPF) cannot be increased to infinity in order to overcome this mutual shading because supplying so much light may cause other problems such as heat and spatial efficiency; which can damage algal metabolism by photoinhibition and may not be economically favourable.

The solution for this problem is to supply light in short flashes naturally which acts like heart pulses. The existence of a longer dark period between short flashes light can increase the efficiency of photosynthesis, mainly for high intensity light (Kok, 1956). Flashing light work as pulsed system in PBR was obtained by a sectored, rotating disk to imitate the square wave. Dynamic light regimes in PBRs can be simplified by square-wave light / dark cycles. Matthijs et al. (1996) found that the use of flashing LEDs in indoor algal culture yielded a major gain in energy economy compared to luminescent light sources. Gordon and Polle (2007) advocated a lighting technique using LEDs that pulsed on the order of tens to hundreds of microseconds while also increase the instantaneous photonic flux. However influence of quality and quantity LED used on pulsed system or flashing light on the growth rate and lipid content of species such as *Nannochloropsis* sp. have not been extensively studied since most of the studies have been done based on short term photosynthetic oxygen evolution measurements in which the algae did not have time to acclimate to the light regime imposed (Brindley et al., 2011) or in batch experiments with continuous changes in light regime. Photoacclimation resulted in a change of the specific light absorption coefficient (optical cross section) and determine the photo synthetic efficiency. Thus the aim of this research is to study the effect of different types of LED (blue and red) in the cultivation of *Nannochloropsis* sp. assisted with the pulsed system (exposure light: dark cycles). In addition, the lipids produced from microalgae were then converted to biodiesel.

## 2. Materials and methods

### 2.1 Cultivation of microalgae with LED system and pulse system

*Nannochloropsis* sp. was cultivated in 1000 ml flasks at  $23 \pm 0.5$  °C. pH  $8 \pm 0.2$  under a light intensity of 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  under blue (457 nm), and red (660 nm) LED with a 24:0 light-dark cycle for 12 d with aeration condition as the control growth environment. The light source intensity was measured with a quantum sensor connected to quantum light meter (Light Scout Dual Solar). To ensure reproducibility of results, each research was performed in duplicates. Then, the experiment repeated by adds the frequency modulator (pulse system) in LED system.

### 2.2 Growth analysis

The cell concentration was determined by a direct microscopic count with a 0.1 mm deep Neubauer haemocytometer (9BOECO, Hamburg, Germany) and a light microscope (Olympus CX21, Japan). The specific growth rate was calculated from the Eq(1).

$$\mu = \frac{\ln(N_2 - N_1)}{t_2 - t_1} \quad (1)$$

$N_1$  and  $N_2$  are the cell number concentration at time  $t_2$  and  $t_1$ . The time required to duplicate the cell number division rate ( $k$ ), was calculated according to the Eq(2).

$$k = \frac{\mu}{\ln 2} \quad (2)$$

In order to ensure reproducibility of results, each experiment was performed in duplicates.

### 2.3 Measurement of lipid via Nile Red staining method

The amount of lipid in *Nannochloropsis* sp. was measured rapidly using upgraded Nile red staining method (Chen et al., 2011) by using Perkin Elmer LS - 55 fluorescence spectrophotometer. For this method, 1 mL of sample was added to 50  $\mu\text{L}$  of Nile red (9 - diethylamino - 5H - benzo (a) phenoxazine - 5 - one; Sigma, USA). The excitation and emission wavelengths for the fluorescence in this study were 585 nm and 490 nm respectively.

### 2.4 Biodiesel conversion

Alkali transesterification process was further best method extraction process to prepare adequate samples by using extracted crude oil from biomass via method by Bligh and Dyer (1959). Methanol was mixed with 0.5 g of NaOH and stirred for 20 min at 400 rpm at approximately 65 °C. The ratio of methanol to oil in the mixture was kept to 6:1. The mixture of catalyst and methanol was then poured into the conical flask containing the microalgae oil so as to initiate the transesterification process. The conical flask was stirred continuously for 3 h at 300 rpm and allowed to settle for 16 h in order to achieve 2 separate layers; the

supernatant layer (glycerol) and sediment layer (biodiesel). The biodiesel was separated cautiously from the sediment layer by a flask separator and washed up using 5 % water until the entire methanol is removed. The biodiesel was dried using dryer and kept under running fan for 12 h.

## 2.5 Fatty acid methyl ester analysis

In order to determine the relationship between quality of light and light source on the FAMES composition, the GC analysis was performed on the samples obtained from the optimized conditions. The FAME's obtained from the optimized conditions were separated, identified and analyzed in a gas chromatography (GC) (Agilent Technologies, 7820A) and HP - 88 capillary column (60 m x 0.25 mm x 0.2  $\mu$ m). The carrier gas used is hydrogen and the flow rate used was 40 mL/min. Maximum temperature in the column was kept at 220 °C and both the injector and flame ionization detector (FID) temperature were set at 220 °C. Initial temperature was 220 °C and the back inlet was set at splitless mode. The column initial temperature was set at 80 °C during 2 min; the thermal gradient to 220 °C was at a rate of 13 °C/min, post temperature at 50 °C in 2 min. The quality of the biodiesel were characterised using various properties which were determined using the following models (Table 1).

## 3. Results and Discussions

### 3.1 Effect of pulsed LED light on *Nannochloropsis* sp. growth

In order to understand how pulsed LED could affect the growth of *Nannochloropsis* sp., two other light condition were used; i) red (660 nm), ii) blue. The pulsed LED light exhibits maximum cell concentration of *Nannochloropsis* sp. which occurs on day 12 (Figure 1). It was proven that photosynthesis of marine microalgae not only depends on continuous light sources but it will tend to growth in pulsed system or light/dark regime. Pulsed LED light created a higher photosynthetic photon flux that affects photon easily to penetrate into the culture compared to continuous white lamp. In this case, both of light conditions have same light intensities. Cell concentration for red and blue light were approximately the same during the 2 d period but pulse LED light ( $0.71 \times 10^7$  cell / mL) exhibited higher cell on concentration compared to red and blue on day 2 . During the 5 d period, pulsed LED and blue has almost the same cell concentration but on day 6 to 8, microalgae on pulsed light and blue light exhibited higher cell concentration compared to *Nannochloropsis* sp. cultivated on red light. Blue tend to produce higher growth compared to red, it is because blue has a low intensity compared to red that damaged the cell because of high intensity. With the low light intensities, blue LED allows the light intensity to penetrated deep into the culture that enhanced the doubling of microalgae and growth cell increased faster (Teo et al., 2014). But during the day 10, cell concentration of blue light ( $2.75 \times 10^7$  cell / mL) was lower compared to pulsed LED light ( $3.32 \times 10^7$  cell / mL) (Figure 1). Based on the Figure 1, pulsed LED light has the higher peak compare to other light conditions. It shows that pulsed LED creates an alternative way that can improvise the growth rate of marine microalgae *Nannochloropsis* sp. The influence of light/dark cycle that was provided by pulsed LED system produced a higher photosynthetic efficiency of microalgae. When algal cells were illuminated by a short flash the rate of oxygen producing and carbon dioxide uptake is maximized similar as those under continuous light. In addition, the pulsed LED light systems enhanced the growth rate of microalgae that increased enzymatic reaction that tend to increase gene expression of cells (Ruyters, 1984). Increased the uptake of carbon dioxide and increased the oxygen rate production subsequently increase the cell concentration (Figure 1). In economic context, the application of flashing light or pulsed lights to the production of algal biomass are more beneficial compared to continuous light illumination with efficient power usage in light illumination. The fluorescent intensities of *Nannochloropsis* sp. which indicates the lipid content under different light conditions is illustrated in Figure 2. An increment in fluorescent intensity was observed for the microalgae throughout the 12 d period. The pulsed LED light showed a constant increase compared to the others. The peak value of pulsed LED light is 248 a.u.at days 12. Although, the fluorescence intensity of the pulsed LED light was lower compared to the red light indicating that lipids produced under red LED were higher, the pulse LED lipid production during the overall cultivation more stable than Blue LED and red LED. Marine microalgae have two specific enzymes that have specific functions in biomass growth and lipid metabolism (Teo et al., 2014). The division times of *Nannochloropsis* sp. at different LED light source with constant light intensity. *Nannochloropsis* sp grows best in pulse LED under light intensity of 100  $\mu$ mol/(m<sup>2</sup>s) with division rate of (4.0395 /d) compared with red LED (3.8953 /d) and blue LED (3.9818 /d).

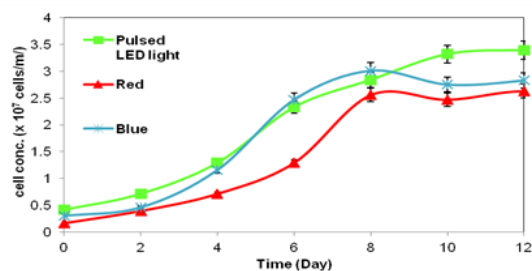


Figure 1: The growth curve in different source of light on *Nannochloropsis sp.*

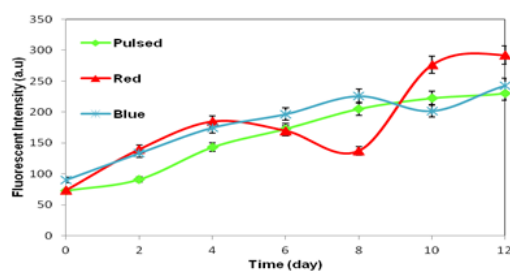


Figure 2: Nile red fluorescence intensity versus over time (Day) of *Nannochloropsis sp.*

Table 1: Determination of biodiesel properties via a variety of models

FAME property	Correlations	References
Degree of unsaturation (DU)	$DU = \text{wt \% of monounsaturated fatty acid} + 2(\text{wt \% of polyunsaturated fatty acid})$	Ramos et al. (2009)
Density 15 °C (kg/m <sup>3</sup> )	$p_B = \sum X_i p_i$ $p_i = 851.471 + \frac{250.718 \text{ db} + 280.899 - 92.180 (m-1)}{1.214 + n}$	Lapuerta et al. (2010)
Kinematic viscosity 40 °C (mm <sup>2</sup> /s)	$\ln v_B = \sum X_i \ln v_i$ $\ln v_{c12.0-c20.0} = -2.177 - 0.202n + \frac{403.66}{T} + \frac{109.77n}{T}$ $\ln v_{18:1} = -5.03 + \frac{2051.5}{T}$ $\ln v_{18:2} = -4.51 + \frac{1822.5}{T}$ $\ln v_{18:3} = -4.018 + \frac{1685.5}{T}$	Krisnangkura et al. (2006)
Cetane number	$CN_B = \sum X_i CN_i$ $CN_i = -21.157 + (7.965 - 1.785 \text{ db} + 0.235 \text{ db}^2)n - 0.099n^2$	Lapuerta et al. (2009)
Iodine number	$IV_{mixture} = \sum 100 \times \frac{A_f \times 253.81 \times \text{db}}{MW_f}$ $IV = 100 \times \frac{253.81 \times \text{db}}{MW_f}$	Knothe (2002)
Cloud point (CP) (°C)	$CP = 0.526(\text{PAME}) - 4.992$ $0 < \text{PAME} < 45$	Sarin et al. (2009)
Pour point (PP) (°C)	$PP = 0.571(\text{PAME}) - 12.240$ $0 < \text{PAME} < 45$	Sarin et al. (2009)
Cold filter plugging point (CFPP) (°C)	$CFPP = 8.9243 \times \text{LCSF}(A) - 19.325$	Ramos et al. (2009)
Higher heating value (HHV) (MJ/kg)	$\text{LCSF}(A) = \text{MP}_{c18} \times \text{C18}(\text{wt.}\%) + \text{MP}_{c20} \times \text{C20}(\text{wt.}\%)$ $\text{HHV} = 0.4625v + 39.45$	Demirbas (2008)

$A_f$ : amount in (%) of a fatty compound in a mixture

$\text{db}$ : number of double bonds in the acid molecule.

$m$ : number of carbon atoms in the original alcohol used for the transesterification process.

$n$ : number of carbon atoms in the original fatty acid.

$T$ : absolute temperature in Kelvin.

$\text{PAME}$ : content of palmitic acid methyl ester.

$\text{LCSF}(A)$ : long chain saturated factor (A).

$\text{MP}$ : melting point.

$\text{wt.}\%$ : composition of saturated fatty acids.

### 3.2 Analysis of microalgae biodiesel at difference LED light source

The composition of fatty acid methyl esters also known as biodiesel produced from lipids in *Nannochloropsis sp.* cultivated under different LED source is in the range of C14 to C20 (Teo et al., 2014). The extracted crude oils were converted to fatty acid methyl esters and their compositions of FAMES were analyzed by gas chromatography. FAMES produced under pulse LED contains very small amounts of C14, absence of C20:0 and C18:3 but high content of C16:0, C18:1n9c, C18:2n6c and C18:2n6t which accounted for 81 % of the total methyl esters. These results basically illustrates that the type of LED light source influenced the metabolism related to lipid formation. Forjan et al. (2011) have reported that additional exposure to 320-400 nm of light wavelength can change the composition of lipid. Table 2 shows the comparison of a variety of biodiesel properties among different LED light sources. The fatty acid methyl esters as a feedstock for biodiesel production produced under pulse LED was better than red LED and blue LED due to high proportion of saturated and mounsaturated methyl esters (more than 77.98 %). High proportion of saturated and mounsaturated carbon chain provide better cold flow properties without losses of stability of oxidation and maintaining the number of cetane at a reasonable level (Knothe, 2009). The physical quality of biofuel is very much dependent on the crude oil composition. The degree of unsaturation (DU) very much influenced the amount of saturated, monounsaturated and polyunsaturated fatty acid methyl esters' present. The

degree of unsaturation under pulse LED is lower than blue LED but higher than red LED reflecting the FAMES produced by pulse system was better than the red LED and stability of oxidation was better than blue LED. Therefore, the FAMES, produced under pulse LED system have both good ignition and oxidation stability properties. As shown in Table 2, the density and viscosity of FAMES produced under pulse LED is similar to that produced under blue LED but different with red LED because the saturated proportions of pulse LED and blue LED are similar. Generally, cetane number is related to the combustion quality and ignition delay, where higher cetane number (CN) values reflect better ignition property improving engine motor efficiency and reducing nitrogen oxides (NO<sub>x</sub>) in exhaust emissions (Ramos et al., 2009). Lower iodine value (IV) values reflects better lubricating properties. IV value is a predication of total unsaturation of a biodiesel and is proportional to DU: higher DU represents higher IV (Knothe, 2002). In Table 2, pulse LED produces cetane number close to that of blue LED which means the combustion quality is quite similar. However the IV of FAMES produced by pulse LED was lower than blue LED which indicated that the lubricating properties are better than blue LED.

Table 2: Comparison of the various biodiesel properties from different LED light sources

FAME component	Red	Blue	Pulse
Saturated	54.10	37.98	41.61
Monounsaturated	28.23	27.64	36.37
Polyunsaturated	17.67	34.38	22.02
Degree of unsaturation	63.57	96.4	80.41
Density	875	881	881
Viscosity	3.93	3.95	3.96
Cetane number	69.42	61.33	61.03
Iodine Value	54.62	99.59	97.76
Cloud point	0.30	2.90	7.10
Pour point	-6.48	-3.65	0.86
CFPP	48.67	34.32	6.52
Higher heating value	41.27	41.28	41.28

Knothe (2009) suggested that each country should specify certain temperature limits for different times of the year, depending on climate conditions. The results illustrated in Table 2, pulse LED's cold filter plugging point (CFPP) is 6.52, blue LED's CFPP is 34.32 and red LED's CFPP is 48.67. The lower value of pulse LED CFPP compared with other LED light source is due to low percentage of C18. The CFPP value is an indicator for the lowest temperature that a fuel will give trouble free flow in fuel systems. When a substance is in low temperature condition, highest melting point (MP) components tend to nucleate, grow and form solid crystals. Cloud point (CP) is defined as the particular temperature at which the crystals become visible and form a cloudy suspension. While, the lowest temperature at which the biodiesel flows or can be pumped is defined as pour point (PP). Both pour point and cloud point should be closely monitored by the user to ensure trouble-free operation especially in cold climates (Martha et al., 2013). Pulse LED produced FAMES with high cloud point and pour point which means it is more suitable for regions without seasons and surrounding temperature is more than 7 °C. Higher heating value (HHV) is a measure of the energy produced when the fuel is burned completely, which also determines the suitability of biodiesel as an alternative diesel fuels. The HHV value of biodiesel produced from pulse LED, red LED and blue LED were around 41.27-41.28 MJ/kg which fulfill the requirements of U.S. and European biodiesel standards which include a specification for heating value in EN 14213 (biodiesel for heating purpose) a minimum of 35 MJ/kg (Martha et al., 2013).

#### 4. Conclusion

The pulse LED light system has an effect on growth rate and lipid content of marine microalgae *Nannochloropsis* sp. The high growth rate (2.8 d<sup>-1</sup>) and stable lipid production of marine microalgae under pulse LED system conditions compared with other LED light sources were proven. Besides, the degree of unsaturation, density, viscosity, cetane number, iodine value, cloud point, pour point, CFPP and HHV produced under pulse LED system from marine microalgae showed that the quality of biodiesel is attracting and promising.

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