

Effect of Light/Dark cycle on Biomass and Lipid Productivity by *Chlorella pyrenoidosa* using Palm Oil Mill Effluent (POME)

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Palm oil mill effluent (POME) is one of the waste water with highest organic content that could increase the formation of algal bloom. However, this can be taken to advantage in terms of extracting lipids from the microalgae which can be used as feedstock for biodiesel production. The light regimes submitted to the algal cultures are considered to be an important factor for determining the productivity and yield of photosynthetic reactions. The aim of this study was to evaluate the effect of photo period on the biomass and lipid production by locally isolated microalgae, *Chlorella pyrenoidosa* in photo bioreactor (PBR) system. The cultures were grown at temperatures of 24 °C - 26°C and supplied with light density of 150 mol m⁻²s⁻¹. The light cycles evaluated were 24:0, 16:8 and 8:16 (day: night), respectively. The highest amount of biomass 39.41 g/L. and 42 mg/L.d of lipid productivity were obtained under continuous illumination.

Keywords: *Chlorella pyrenoidosa*; lipid productivity; photoperiod; Palm Oil Mill Effluent

Introduction

Microalgae have become the focus of attention for biomass production as early as 1950s¹. Many studies have shown that microalgae are able to remove nitrogen and phosphorus from wastewater and microalgae have a great capacity of photosynthesis than terrestrial plants, which raises the possibility that they can effectively utilize CO₂². The Photosynthesis can be divided into light reaction and dark reaction which occur in algae and contribute for fixing CO₂ and incorporation into carbohydrates and lipids^{3,4}.

The average lipid content of algae varies between 1 % ~ 70 %, but can reach 90 % of dry weight under certain conditions⁵. However, only few reports are available on the effects of the duration of day and night cycles for growing algae in terms of higher lipid production. Hence, a comparison of different photoperiods is necessary in order to determine the most efficient light regimes for higher lipid production which would create algae as feed stock for biogas, biodiesel production and also for nutrient removal from waste water such as POME. This could greatly contribute as an alternative resource to some of the problems such as diminishing of petroleum resources.

Materials & Methods

Chlorella pyrenoidosa and culture medium

Chlorella pyrenoidosa was isolated from a pond located at POME treatment plant, Kulai Besar, Johor, Malaysia. The cultures were cultivated and maintained in a 10-fold diluted POME provided with cultural conditions of 24°C- 26 °C, with a 8h : 16h L:D lighting scheme and pH ranging from 6.5-7.5 with light intensity of 150 mol m⁻² s⁻¹.

Palm Oil Mill Effluent (POME) as substrate

The experiment was carried out using Palm Oil Mill Effluent as substrate for growing algae and was collected from Kulai Besar, Johor, Malaysia and the POME was diluted ten times to reduce the shading effects on the growth of microalgae.

Laboratory-scale photo bioreactor system (PBR)

The laboratory-scale PBR system consisted of double jacket vessel with a working volume of 3L. The rotating speed of agitator was fixed at ~ 60 rpm. Two Master Flex peristaltic pumps were used - one reactor tank with POME from the influent tank and another to draw off the effluent from the reactor tank. The temperature between 24°C-26 °C was controlled through a water bath and the operational sequence of SBR system and movement of all mechanical devices including the mixer and air pump, was controlled by a programmable logic

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controller (PLC) (Jenco, US). DO, pH and temperature in the reactor tank were continuously monitored using respective electrodes (6309PDT, Jenco, US), connected to corresponding transmitters (Eutech, Singapore) and the signals processed by the Jenco computer programme. The experiments were carried out in batch operation, followed with temperature at 24 °C -26 °C, and photon flux density of 150 mol m⁻² s⁻¹. The light cycles evaluated were 8:16, 16:8 and 24:0 (light:dark), respectively. The cell concentration and the carbon-fixation rate were monitored every 12 h during the growth phase of the microalgae.

Kinetic parameters

The specific growth rate (μ) was determined by the following equation:

$$\mu = \frac{1}{t} \ln (X_m / X_0) \quad \dots (1)$$

Where: X_m = concentration of biomass at the end of batch run

X_0 = concentration of biomass at the beginning of batch run,

t = duration of batch run (hour, day)

The biomass productivity was calculated by the equation:

$$(X - X_0) / t \quad \dots (2)$$

Where: X = concentration of biomass at the end of batch run

X_0 = concentration of biomass at the beginning of a batch run,

t = duration of the batch run (hour, day)

The lipid productivity was calculated by the equation:

$$P_{\text{lipid}} = Cl/t \quad \dots (3)$$

Where: Cl = concentration of lipid at the end of batch run

t = duration of run (hour, day)

Analytical methods

Quantification of biomass and other parameters

To determine the biomass of microalgae, 4.7cm Whatman GF/C glass fiber filters were dried at 90 °C for 4h, placed in a dessicator to cool to room temperature and weighed. Then the biomass was determined by filtering 10mL of culture by passing through pre weighed

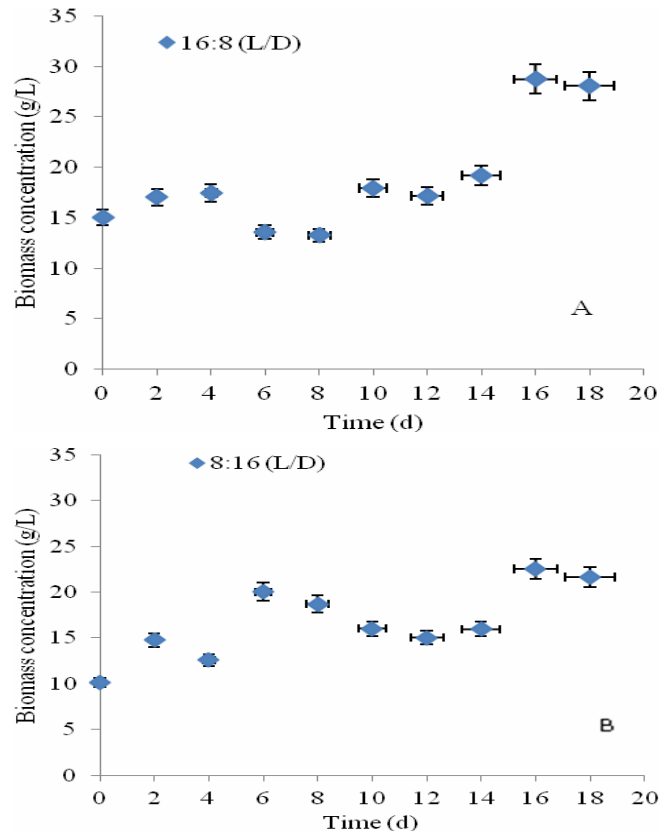


Fig. 10 Growth curve of *Chlorella pyrenoidosa* under different light duration (a) 16:18 L/D ratio (b) 8:16 L/D ratio

filters, dried and weighed as mentioned above. The components of the cells of *Chlorella pyrenoidosa* were determined as suggested by ⁶.

Measurement of lipid content - Nile Red method

The quantification of the cellular neutral lipid was carried out with fluorescent spectrophotometer (HITACHI F-4500) based on the method of ⁶. The cultures were mixed with Nile Red solution stored in 0.1mg.mL⁻¹ in acetone for 7min before analyzing. The relative fluorescence of Nile Red for lipid was obtained after subtraction of auto-fluorescence of algal cells and self-fluorescence of Nile Red from the gross reading at 580nm wavelength.

Results and Discussion

Growth curve of *Chlorella pyrenoidosa* for different photoperiod

The growth curve of *Chlorella pyrenoidosa* shows lack of adaptation phase during continuous illumination. The exponential phase occurred at 2nd day and stationary phase was observed at 14th day. The maximum biomass obtained was 39g/l. The different cell growth profiles for *Chlorella pyrenoidosa*, with different light cycles can be seen as a function of the duration of the light

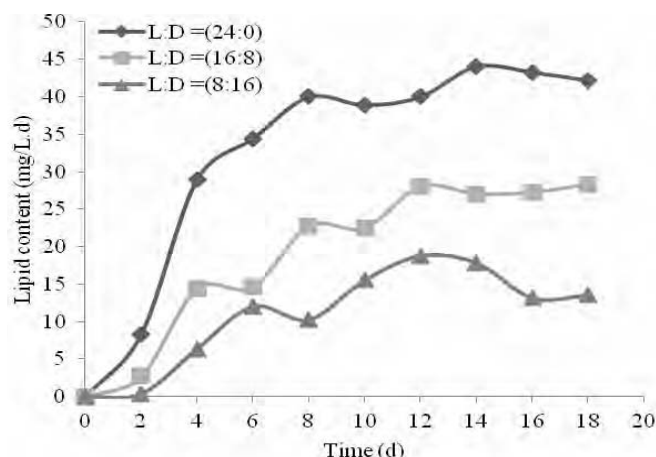


Fig. 26 Lipid content of *Chlorella pyrenoidosa* under different light duration

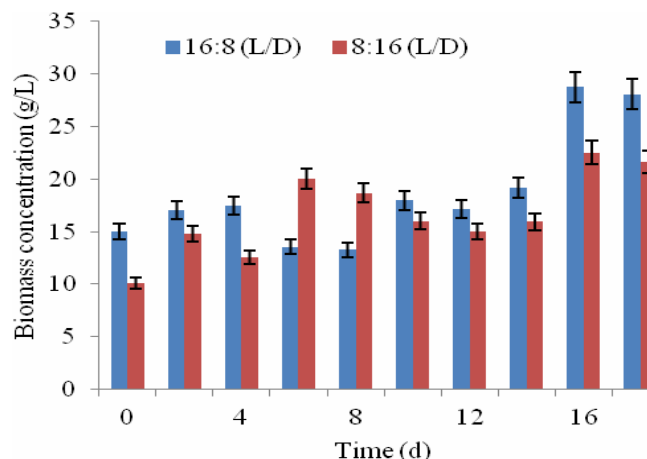


Fig. 36 Comparison between biomass concentrations of *Chlorella pyrenoidosa* under different light duration

Table 16 The kinetic characterisation of lipid and biomass productivity by *Chlorella pyrenoidosa* under different light/dark cycles

Photoperiod (L/D)	P_x (mg/L.d)	X_{max} (g/L)
Continuous	0.018±0.014	39.41±0.726
16/8	0.017±0.010	28.74±0.347
8/16	0.010±0.010	22.53±0.482

periods (Fig. 1). The cultures grown under a photoperiod of 16:8 (day: night) was found similar to the growth under 8:16 (day: night) condition.

Maximum amount of lipid content was achieved at the end of cultivation for three light and dark cycles (Fig. 2). Among the three light and dark cycles, the highest lipid content was achieved when *Chlorella pyrenoidosa* grown under continuous light (24:0) at 44.9 mg/L CDW, then followed by 16:8; 8:16 with value of 28.75 mg/L CDW and 18.53 mg/L CDW, respectively. The lipid as shown in (Fig. 2) clearly shows that photo light duration gives a major influence on resulting of lipid content as well as on growth rates and photosynthetic activity as mentioned before. Studies have also shown that both light cycle and mixing turbulence influenced the photosynthetic activity as well as growth rate of microalgae in a photo bioreactor^{7,8}. Photo bioreactor design are equally important to obtain high biomass productivity for lipid production^{9,10}.

The results are more obvious in (Table. 1), which presents the kinetic characterisation of the lipid and biomass productivity by *Chlorella pyrenoidosa*, under different light cycles evaluated. From the analysis of variance (ANOVA) and Tukey's test ($p < 0.05$), highest biomass productivity (Table. 1) was obtained with

continuous supply of light energy (24:0). These results showed that light energy is crucial for *Chlorella pyrenoidosa* to sustain cell growth^{11,12}. For the other photoperiods evaluated, all the values for specific growth rate differed statistically ($p < 0.05$). There were differences in maximum cell concentration for (16:8) and (8:16) light period as shown in (Fig. 3), suggesting that higher dark period will affect the rate of photosynthetic metabolism. It was suspected that the loss of biomass under intermittent illumination was most probably due to photorespiration.

In addition, the availability of light in a typical photo bioreactor is another factor which determines cell. High cell accounts for mutual shadings, also limit the light intensity within the reactor^{13,14}. Both higher specific light absorption together and lower specific growth rate most probably contribute to under intermittent illumination. A similar study conducted by⁵ revealed that *C. reinhardtii* increased its chlorophyll-content under light/dark cycles; the amount of chlorophyll-*a* content was doubled under intermittent illumination in comparison to continuous illumination.

Conclusion

After altering the light: dark period ratio algae produced more lipid content at higher light/dark time ratio when compared to lower time ratio using POME and the produced lipid from algae may be useful as feedstock for biofuel production.

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