Journal of Engineering Science and Technology
EURECA 2014 Special Issue January (2015) 112 - 121

THE OPTIMIZATION OF LIGHT INTENSITY AND DRYING TEMPERATURE ON LIPID CONTENT OF MICROALGAE *NANNOCHLOROPSIS OCULATA*

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Abstract

The production of sustainable renewable energy is currently being studied extensively globally to determine a suitable feedstock to displace non-renewable fuel. In this context, microalgae are foreseen as a potential source in producing biodiesel. This is due to its high lipid yield and fast growth that does not compete with essential food crops for land. The aim of the present work was to study the effect of different light intensities on the growth rate and drying temperatures with respect to the lipid yield for *Nannochloropsis oculata*. The result showed that at 5000 lux, the microalgae achieved the highest specific growth rate of 0.236 day^{-1} with the shortest generation time of 2.9 days. This was followed by 3000 and 7000 lux which produced lower growth rate. Drying temperature was also found to influence the lipid yield of *Nannochloropsis oculata*. Highest lipid yield comprising of 6.47% were obtained when the cells were dried at 85°C as compared to cells dried at 70°C and 100°C.

Keywords: Biodiesel, Microalgae, Lipid, *Nannochloropsis oculata*, growth rate, drying.

1. Introduction

The world consumption and needs on energy has significantly increased in the past decade. However, the availability of energy sources are insufficient to meet the current demand. At present, fossil fuels are utilized as the main energy source.

Nomenclatures

K'	Specific growth rate, s^{-1}
N	Biomass, gram
t	Time, s
v/v	Volume percentage
W_{DA}	Weight of dried microalgae biomass, gram
W_L	Weight of extracted lipid, gram
$wt\%$	Weight percentage
$Y\%$	Lipid yield

Greek Symbols

μE	Amount of photons emitted (Fig. 1)

Abbreviations

ANOVA	Analysis of Variance
LED	Light-Emitting Diode
rpm	Revolution per minute

The overconsumption of these energy sources will lead to continuous depletion of fossil fuels and are predicted to be exhausted in 70 years [1]. Furthermore, the carbon emission due to combustion of fossil fuel is another major problem that contributes to pollution and global warming. Therefore, vital steps need to be taken in terms of investment and research to explore, develop and optimise the use of alternative and renewable energy. Renewable energy has become very promising since it is clean, environmental friendly, and completely biodegradable. It also produces less or negligible greenhouse gases and pollutants [2].

Biomass is typically referred to materials derived from animals or plants which are used as feedstock to produce biofuel [2]. In recent years, biomass from microalgae was found to be another potential source to produce renewable energy besides energy generated by hydro, wind, solar, geothermal and tidal wave [2]. Microalgae produces lipid through photosynthesis by converting energy received from sun into chemical energy. As the fastest growing plant, microalgae are relatively easy to be cultivated and require less nutrition. It can grow well on land and also utilize waste water as its growth medium. Based on the economic point of view, utilizing algae as one of the second generation crops for biofuel feedstock is more encouraging than utilizing first generation crops such as palm oil. This are due to the high price of first generation crops as they are also used as food source and have a higher demand [3].

Despite these advantages, algae technology is relatively new and various studies are currently being undertaken [2]. The biofuel produced from algae still lacks stability due to its polyunsaturated content. The processing efficiency of biofuel is lower than conventional fossil fuel and also has a higher cost compared to fossil fuels [2]. The final cost of algae biofuel is currently between \$0.42-0.97 per litre in 2012 [4]. These are the setbacks which limits algae biofuel to be at the forefront and displace fossil fuels. In order to produce algae biofuel which are more reliable, further studies should be carried out on various algae species pertaining to its lipid content, optimisation, cultivating condition and extraction methods. In the present study, the optimum light intensities for the growth of

Nannochloropsis oculata and the optimum drying temperature with respect to the microalgal lipid yield were studied.

1.1. Light intensity

Apart from light wavelength, color, source of light, flashing, and phototropic period; light intensity also plays an important growth parameter for algae cells [5, 6]. In general, the range of light intensity is between 15 to 150 μmol of photons $m^{-2}s^{-1}$ or equivalent to 1,000-10,000 lux [5]. However, finding the optimum light intensity is still on-going as different species require different sets of light intensities. Current study on *Nannochloropsis oculata* shows the optimum light intensity varies between the ranges of 2,500 to 7,000 lux. However, the range mention is still wide. Hence, a more precise and accurate value should be obtained since light intensity plays important role on microalgae growth which will affect the photosynthesis process and contribute to the oil content of microalgae.

Light stimulates the photo-oxidation process in microalgae which contributes to its growth. More light provided will result in higher growth rate. However, when the saturation point is reached; any further increase in light intensity will damage the microalgae cells due to photoinhibition which is also affected by temperature. Higher light intensity will generate more heat which will raise the temperature and cause the decline in growth rate. In general, green microalgae will reach its optimum growth rate at light intensity of 15 to 150 μmol of photons $m^{-2}s^{-1}$ or equivalent to 1,000-10,000 lux [5]. Beyond 150 μmol of photons $m^{-2}s^{-1}$ (10,000 lux), the growth rate will start to decline due to cell damage as shown in Fig. 1. High light intensity between the ranges of 8000 to 10,000 lux also has the potential to damage the cell as a slight increase in light intensity or heat accumulation will result in culture collapse. Therefore the optimum range of light intensities falls between 2,500 to 7,000 lux [5]. Based on the finding above, here three different light intensities 3,000, 5,000, and 7,000 lux, were studied with respect to algae growth rate.

Mutual shading is another aspect of light to be considered. As the microalgae grow and replicates, cells will tend to block each other from absorbing the emitted light. Therefore, light intensity should be increased throughout the growth of microalgae. Since high light intensity may damage the cell, flashing light or adjusting the phototropic period may indirectly reduce the light intensity. Flashing light provides a blinking effect at certain frequency, whereas phototropic period is the ratio of day and night for the lighting period [7].

1.2. Drying temperature

Pre-treatment process such as algae drying is usually carried out before lipid extraction. This is carried out to increase the extraction efficiency which depends on drying temperature and period. The optimum extraction efficiency is determined based on the drying temperature and period. Very low temperature or shorter drying period will result in inadequate drying and less moisture removal. On the other hand, extreme high or low temperature drying period has the potential to break down and evaporate the lipid. Thus, lower liquid content were obtained [8]. At present, limited information regarding drying temperature and

drying period are available in open literature. Hence, the effect of drying on extraction efficiency needs to be further studied [9].

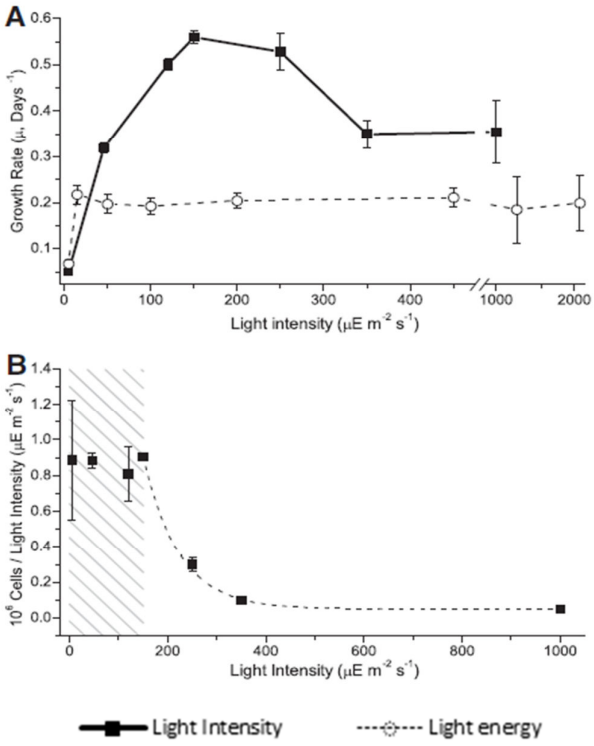

Fig. 1. A) Growth rate of microalgae *Nannochloropsis oculata* under constant lighting at its exponential phase with respect to the light intensity as shown in black solid line. The dotted line represents the light energy available. B) The normalised cell concentration with respect to the light intensity. The shaded area represents constant values of normalised cell concentration [5].

Drying methods also does affect the lipid content. Microalgae are usually dried using oven, spray drying, freeze drying and sun drying. Sun drying is not effective to remove high moisture content while using spray drying is uneconomical to dry low value products, such as biofuel and protein [10]. Freeze drying is also not applicable for large scale drying. However drying algae using oven is commonly applied due to its economic value and easy maintenance [9-10]. Drying in an oven is often carried out between 65 to 105 °C for 16 to 48 days. This will remove 85 to 95 wt% of water [6].

2. Methodology

Figure 2 summarizes the process of culturing microalgae till the extraction of lipid from the algae.

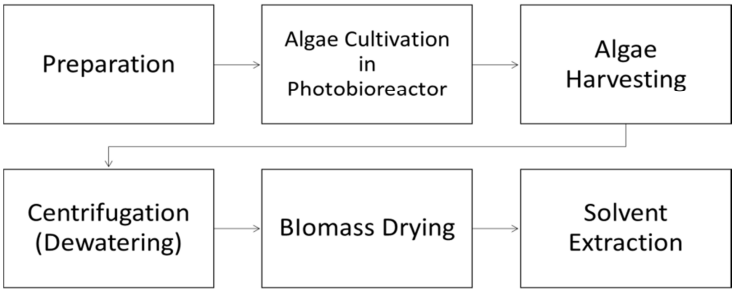

Fig. 2. Process of obtaining lipid from *Nannochloropsis oculata*

2.1. Microalgae

The marine microalgae *Nannochloropsis oculata* as shown in Fig. 3 was classified by Hibberd into six eukaryotic and unicellular species. The microalgal is sphere in shape and has a diameter of 1-4 μm. They are distinctly characterised by the lack of chlorophyll b and c [11]. The microalgae strain was obtained from Algaetech International Sdn Bhd (Kuala Lumpur, Malaysia) in the form of liquid culture.

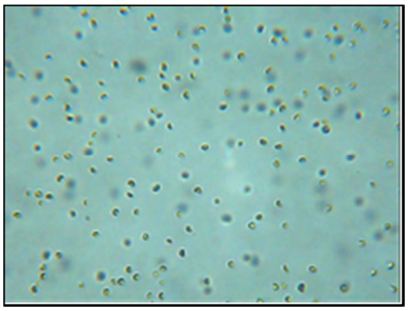

Fig. 3. *Nannochloropsis oculata* (MarineLife Aquarium Society of Michigan, 2013)

2.2. Cultivation

The cultivation of microalgae was carried out in 500 ml photobioreactor. F/2 medium as listed in Table A-C (*Appendix A*) was used as the nutrient source for the microalgae. The volume ratio of microalgae inoculum to the medium was 1:10 with total culture volume of 500 ml. Three different light intensities, 3000, 5000, and 7000 lux were studied in this experiment as shown in Table D (*Appendix A*). Continuous lighting was provided by surrounding white LED bulbs. Phototropic period of 24:0 (light: dark hours) was applied to the culture. The culture temperature was monitored by thermocouple (hi-8314, Hana Instruments) and maintained at 18-25°C by a dynamic cooling fan, whereas the pH was kept at 7-8.5 with salinity of 20-24 gram/litre. The culture was aerated with an air flow rate of 2-4 litre/min. The growth of *Nannochloropsis oculata* was studied by measuring the culture density daily. This procedure was carried out by removing 2ml culture from the photobioreactor which were then centrifuged and measured. The biomass obtained is expressed in gram/ml. The average culture density for two weeks with respect to different light intensities

was then studied. The growth curve and specific growth rate was calculated as shown in equation (1) [12].

$$Growth\ rate, K' = \frac{1}{t_2 - t_1} Ln\left(\frac{N_2}{N_1}\right) \quad (1)$$

where N is the biomass at time t during the exponential phase.

2.3. Harvesting and drying

The cultures were harvested after two weeks at stationary phase by centrifuging at 4000 rpm for 10 minutes using 500 ml capacity centrifuge (Universal-32R, Hettich, Germany). The algae were then dried in an oven (FAC-350, Protech, Malaysia). The algae were dried at 70, 85, and 100°C for 24 hours. Finally, the biomass yield was calculated by dividing the weight of dried biomass by the culture volume.

2.4. Lipid extraction

Lipid was extracted using hexane-isopropanol (3:2 v/v) at 25°C for 7 hours. This low toxic solvent mixture is different from other solvents in terms of its ability to extract both neutral and polar lipid out of the microalgae cells [13]. 75 ml of solvent mixture was added to every gram of dried biomass. Filtration was then carried out before transferring the mixture into the separating funnel where the top dark green layer containing lipid and the solvent was taken. The top layer would then be heated up to 60°C in order to vaporise the solvent and obtained the pure lipid [13]. The lipid yield from microalgae was calculated as shown in equation (2).

$$Lipid\ yield, Y\% = \frac{W_L}{W_{DA}} \quad (2)$$

where W_L and W_{DA} were the weights of the extracted lipids and the dried algae biomass, respectively.

2.5. Error analysis

The result and data obtained from the experiment was analysed further by using Analysis of Variance (ANOVA) method which involved error or variance analysis. Fixed effect factorial design comprising one dimensional ANOVA was used as the error analysis model which involved three different levels of responses for one main factor.

3. Results and Discussion

3.1. Effect of light intensity on the microalgae growth rate

The culture would reach the highest and optimum growth at light intensity of 5000 lux, followed by 7000 lux and 3000 lux as depicted in Fig. 4. At 5000 lux, the culture starts to undergo exponential phase on the fourth day. The growth reached the peak at 1.9 gram/litre after three days before declining constantly. At 7000 lux, the culture first underwent lag phase that made the growth lower than 3000 lux culture for the first two days, before surpassing it. The longer lag phase at 7000 lux was due to the culture's difficulty to adapt on the high light intensity at low culture

density. This was due to the very high amount of photons and heat that was absorbed by every cell. Lowest growth rate were observed at 3000 lux. This was due to insufficient light for the photosynthesis process [5]. Moreover, qualitative observation also showed that cells cultured at 5000 lux were darker in colour which represents higher concentration of microalgae. On the other hand, microalgae culture at 7000 lux was found to be darker than the culture at 3000 lux.

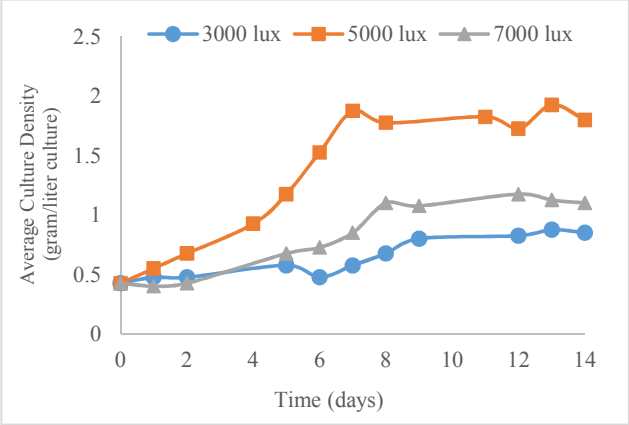

Fig. 4. Growth of microalgae cultures with respect to the different light intensities.

The exponential/log phase during the microalgae growth was determined by observing the steepest gradient on the microalgae growth as shown in Table 1. The highest specific growth rate was achieved at light intensity of 5000 lux. The specific growth rate of 0.236 day^{-1} and 0.340 divisions per day with generation/doubling time of almost 3 days were obtained as shown in Table 2. The optimum light intensity achieved in this study at 5000 lux falls within the optimum range reported in previous studies which were in the range of 2500 to 7000 lux [5]. At 7000 lux, the specific growth rate was 0.208 day^{-1} which was 12% lower than 5000 lux. The microalgae took longer amount of time to double its amount, which would take 3.3 days. This occurs due to the microalgae had reaching the saturation point at which higher light intensity might damage the cell and would result in declining growth. The term to explain this phenomenon was called photo-inhibition whereby high light intensity might generate excess heat and damage the microalgae cells [7]. Lowest specific growth rate and the longest doubling time were observed at 3000 lux. This was caused by insufficient light provided which reduced the efficiency of photosynthesis process.

Table 1. Growth of *Nannochloropsis oculata* at Exponential/Log Phase.

Light Intensity (lux)	Start of Exponential Phase (day)	End of Exponential Phase (day)
3000	6	9
5000	4	7
7000	6	8

Table 2. Growth Parameters Obtained at Different Light Intensities.

Light Intensity (lux)	Specific Growth Rate, K' (day^{-1})	Divisions per Day (div. day^{-1})	Generation/Doubling Time (days)
3000	0.174 ± 0.031	0.251 ± 0.045	4.0 ± 0.557
5000	0.236 ± 0.031	0.340 ± 0.045	2.9 ± 0.557
7000	0.208 ± 0.031	0.301 ± 0.045	3.3 ± 0.557

3.2. Effect of drying temperature on the microalgae lipid yield

The highest microalgae lipid yield was obtained at drying temperature of 85°C as shown in Table 3. At this temperature, the lipid yield reached 6.47%. This was lower than the finding reported by Halim et al. of 25.0% [13]. There were no significant differences on the lipid yield at drying temperature of 70°C and 85°C, but there were significant difference at 100°C. Lipid reduced by 3.53% when dried at 100°C instead of 85°C. This lipid degradation occurred due to extreme high drying temperature which degraded down the lipid structure, thus causing it to be more volatile and easier to vaporise [8]. Therefore, drying temperature lower than 85°C was preferred in order to preserve the microalgae lipid and minimising lipid loss. Moreover, lower drying temperature required less energy.

Table 3. Lipid yield at Different Drying Temperatures

Drying Temperature (°C)	Biomass (milligram)	Lipid Weight (milligram)	Lipid wt% of Biomass
70	170	10	5.88% ± 1.89%
85	170	11	6.47% ± 1.89%
100	170	5	2.94% ± 1.89%

4. Conclusions

The outcome of this study revealed that light intensity has a significant effect on the growth rate. Highest growth rate were achieved when *Nannochloropsis oculata* was cultured at 5000 lux. The specific growth rate of 0.236 day^{-1} with the shortest generation time of 2.9 days were achieved during the exponential phase compared to cells cultured at 7000 and 3000 lux. Drying temperature was also found to affect the lipid yield of *Nannochloropsis oculata*. The optimum drying temperature was at 85°C as it gave the highest lipid yield of 6.47%. Therefore by determining the optimum light intensity and drying temperature, a higher growth rate and lipid can be achieved in the shortest time to be converted into biodiesel.

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Appendix A

Table A. Components in F/2 Medium.

No.	Component	Stock Solution Concentration	Quantity	Molar Concentration in Final Medium
1	NaNO3	75 g/L dH2O	1 mL	8.82 x 10-4 M
2	NaH2PO4.H2O	5 g/L dH2O	1 mL	3.62 x 10-5 M
3	Na2SiO3.9H2O	30 g/L dH2O	1 mL	1.06 x 10-4 M
4	Trace metal solution	(Refer to Table B)	1 mL	
5	Vitamin solution	(Refer to Table C)	1 mL	

Table B. Components in F/Trace Metal Solution.

No.	Component	Stock Solution Concentration	Quantity	Molar Concentration in Final Medium
1	FeCl3.6H2O		3.15 g	1.17 x 10-5 M
2	Na2EDTA.2H2O		4.36 g	1.17 x 10-5 M
3	CuSO4.5H2O	9.8 g/L dH2O	1 mL	3.93 x 10-8 M
4	Na2MoO4 2H2O	6.3 g/L dH2O	1 mL	2.60 x 10-8 M
5	ZnSO4.7H2O	22.0 g/L dH2O	1 mL	7.65 x 10-8 M
6	CoCl2.6H2O	10.0 g/L dH2O	1 mL	4.20 x 10-8 M
7	MnCl2.4H2O	180.0 g/L dH2O	1 mL	9.10 x 10-7 M

Table C. Components in F/2 Vitamin Solution.

No.	Component	Stock Solution Concentration	Quantity	Molar Concentration in Final Medium
1	Thiamine HCl (vit. B1)		200 mL	2.96 x 10-7 M
2	Biotin (vit. H)	1.0 g/L dH2O	1 mL	2.05 x 10-9 M
3	Cyanocobalamin (vit. B12)	1.0 g/L dH2O	1 mL	3.69 x 10-10 M

Table D. Calibration Data between Light Intensity, Current, and Voltage.

Light Intensity	3000 lux	5000 lux	7000 lux
Current	2.15 A	2.36 A	2.45 A
Voltage	10.8 V	11.3 V	14.5 V