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The Effect of Air Injection Rate and Medium Nitrogen Concentration on Cell Biomass and Lipid Content of *Scenedesmus quadricauda* in Flat Plate Photobioreactor

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Abstract

In the present study, the microalgal strain *Scenedesmus quadricauda* was studied in different air injection rates and different medium nitrogen concentrations of Lefebvre-czarda medium by using a flat plate photobioreactor. The result showed that air injection enhanced the biomass concentration, however, high air injection rate did not cause the increasing of growth and biomass concentration. The best air injection rate for saving injection energy among 5 to 65 L/min was 15 L/min. Besides, lipid content was not affected by different air injection rates. Three nitrogen concentrations which are standard, double and triple nitrogen concentrations of Lefebvre-czarda medium were tested. The result of nitrogen effect indicated that the lipid content was enhanced by low medium nitrogen concentration. The highest biomass concentration and lipid content were obtained in standard medium nitrogen concentration of Lefebvre-czarda medium.

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Keywords: Scenedesmus quadricauda, flat plate photobioreactor, Lefebvre-czarda medium, biomass concentration, lipid content;

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1. Introduction

Microalgae are either prokaryotic or eukaryotic photosynthetic microorganisms which can capture carbon dioxide and sunlight to produce the biomass. Besides, they are not only rapid growth but also the ability of living in harsh conditions due to their unicellular or simple multicellular structure [1]. Moreover, biomass growth and lipid accumulation of microalgae are also higher than terrestrial plants and this is an evidence to indicate that microalgae is a potential feedstock for biodiesel [2]. Among them, *Scenedesmus quadricauda* (*S. quadricauda*), a type of fresh water microalgae is a potential lipid producer for biodiesel. Rodolfi et al have investigated lipid production of 30 microalgal strains, and the results showed that the lipid productivity of *S. quadricauda* was relatively high [3]. Additionally, there are few reports about the effect of air injection and medium nitrogen concentration on growth and lipid production of *S. quadricauda*, therefore, *S. quadricauda* was chosen as the subject to investigate the factors that could further increase the growth and lipid production.

Two common systems are used to culture microalgae which include the open system (open raceway pond) and the closed system (photobioreactor). Biomass productivity of photoautotrophic microalgae cultured by photobioreator is higher than raceway pond. The most popular design of photobioreactor are tubular photobioreactor, vertical column and flat plate photobioreactor [4]. Flat plate photobioreactor was used in our study due to their large illumination surface area and smaller light path which could enhance the higher photosynthetic efficiency [5].

The culture mixing and mass transfer in photobioreactor is highly influenced by air injection. Besides, photobioreactor design including geometric design, stirrer, sparger and bubble size also affect the culture mixing and mass transfer [6,7]. Therefore, the optimum air injection rate for sufficient mixing and mass transfer for a flat plate photobioreactor should be determined before the investigation of any other factors.

The biomass and lipid content are the indispensable factors that should be increased in order to satisfy the commercial requirement of biofuel market. On another hand, the biomass and lipid content of microalgae can be easily enhanced by changing the culture medium or cultivation conditions to obtain higher production [8]. The factors which affect the biomass and lipid content of microalgae include: chemical stimulants such as nitrogen and phosphate starvation or salinity stress as well as physical stimulants such as manipulating the pH of medium, temperature, light intensity or photoperiods [2,3,9]. Among these factors, nitrogen starvation is the most suitable technique to enhance the biomass and lipid content [10,11,12].

In this study, *S. quadricauda* was cultured in a flat plate photobioreactor to study the effect of air injection rate and nitrogen concentration. The growth, biomass concentration, and lipid content were used to evaluate the effect of these factors.

2. Methodology

2.1. Strain and starter culture

The *S. quadricauda* strain (50 ml, 7 days old) was purchased from Algaetech International Sdn. Bhd. (Technology Park Malaysia, Kuala Lumpur, Malaysia). The starter culture was conducted in order to increase the microalgal cells and prepare an uniform inoculum for next experiments. The cultivation conditions of starter culture were; 5 L of working volume, Lefebvre-czarda (LC) medium [13], 10000 Lux of light intensity, 25 °C of temperature, 2 L/min of air injection rate, and 10 days of cultivation time. The cells in starter culture were recovered by centrifugation (Hermle Z206A, 41.8 km/s^2 for 5 min), then resuspended into 1.0 L of fresh LC medium. The one liter microalgal suspension was stored under the condition of 5 ^{0}C and darkness as the stock of inoculum for all later experiments.

2.2. Experimental setup

Preculture and experimental culture were conducted consecutively, and microalgal cells in preculture were inoculated for experimental cultures. Preculture and experimental cultures were conducted in the same flat plate photobioreactor as shown in Fig. 1. The cultivation conditions of preculture and main culture which are shown in Table 1 were similar to each others, except for the investigation factors.

In experimental cultures, the effects of different air injection rates and medium nitrogen concentrations were carried out. Different aeration rates which included 5, 15, 25, 35, 45, 55, and 65 L/min were supplied into the culture of *S. quadricauda* through an air distributor by using atmospheric air (0.04% CO_2). Standard nitrogen concentration in LC medium (1N-LC) was maintained at these aeration rates.

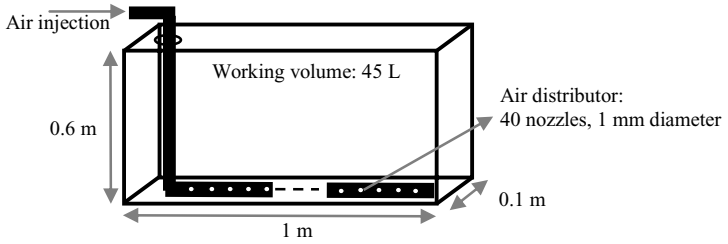

Fig. 1. The design of flat plate photobioreactor.

In order to investigate the effects of medium nitrogen concentration, nitrogen concentration in LC medium was modified. Meanwhile, all other nutrient concentrations were remained constantly as the standard LC medium. The aeration rate was setted at 15 L/min. The nitrogen source in LC medium are $Ca(NO_3).4H_2O$ and KNO_3, 40 mg/L and 100 mg/L respectively, which is referred as 1N-LC (18 mg nitrogen/L). Additionally, 80 mg/L of $Ca(NO_3).4H_2O$ and 200 mg/L of KNO_3 as well as 120 mg/L of $Ca(NO_3).4H_2O$ and 300 mg/L of KNO_3 are referred as 2N-LC (36 mg nitrogen/L) and 3N-LC (54 mg nitrogen/L).

Table 1. Cultivation conditions of experimental cultures

Cultivation condition		Value
Constant condition	Photobioreactor	Flat plate photobioreactor (45 L)
	Medium	Lefebvre-czarda
	Medium nitrogen concentration	1N-LC
	Light intensity	10000 Lux
	Temperature	25 °C
	Air injection (0.04 vol% CO_2)	15 L/min
	Initial biomass concentration	0.049-0.053 g/L
Investigating condition	Air injection rate	5, 15, 25, 35, 45, 55, and 65 L/min
	Medium nitrogen concentration	1, 2, and 3N-LC

2.3. Growth characterization

Optical density at 688 nm (OD_{688}) was monitored daily by a UV/Vis Spectrophotometer (Perkin Elmer Lambda 25 UV/Vis Spectrophotometer). On the final day of cultivation, the correlation equation between dried biomass concentration and OD_{688} was established. The daily dried biomass concentration was determined by the calculation of the daily optical density and the correlation equation [14]. The correlation equation between dried biomass concentration and the OD_{688} is equation (1).

$$\text{Biomass concentration (g/L)} = 0.2732\ OD_{688} + 0.0024 \quad (1)$$

In the establishment of the correlation equation between dried biomass concentration and the OD_{688}, dried biomass concentration was determined by the following description. Microalgal cells in 20.0 ml of culture medium were recovered by centrifuge (Hermle Z206A; 41.8 km/s^2 for 5 min). The supernatant was discarded, while the cell

pellet was washed with distilled water, recovered by centrifugation once, and then dried at 105 °C for 24 hours. The dried mass of biomass was evaluated by an analytical balance [15].

The growth curve was established by plotting the relationship between daily dried biomass concentration in logarithmic scale and cultivation time. Then, the growth with different conditions was evaluated.

2.4. Determination of biomass concentration and lipid content

Approximately, 1.5 g of dry biomass was mixed well with 150 mL of distilled water. The cell suspension was disrupted by microwave oven (Pensonic PMW-20A, medium power setting, 100 ^{0}C and 2450 MHz, for 5 min). Then, the disrupted cell sample was dried at 105 ^{0}C for 24 h for dry mass measurement, and extracted by soxhlet using mixture of chloroform and methanol (2:1, v/v) as extraction solvent. Extracted biomass was dried again at 105 ^{0}C for dry mass measurement. The lipid content was determined by the comparison between the dry mass before and after extraction [16].

$$C_{lipid} = \frac{m_{lipid}}{m_{extraction}} \times 100\% \quad (2)$$

C_{lipid}: lipid content in dry cell biomass (wt%)
m_{lipid}: mass of extracted lipid (g)
$m_{extraction}$: mass of dried cell biomass for cell extraction (g)

The lipid yield was calculated by equation equation (3).

$$Y_{lipid} = M_f \times C_{lipid} \times 1000 \quad (3)$$

Y_{lipid}: lipid yield on the day t (mg/L)
M_t: biomass concentration on the day t (g/L)
C_{lipid}: lipid content in dry cell biomass (wt%)

3. Result and discussion

3.1. Effect of different air injection rate

The experimental cultures were sampled every 24-h interval and observed continously for 8 days. It is evident from Fig. 2 that insignificant difference was found with air injection rates from 15 to 65 L/min. While the growth with 5 L/min was very low compared to higher air injection rate. Similar growth curves were observed in air injection rates from 15 to 65 L/min. Whereby, the log phase continued for 5 days and was followed by stationary phase.

Additionally, Fig. 2 also shows that the growth on the first day of cultivation was remarkable. This remarkable growth indicates that lag phase did not appear in these cultures and the growth started with exponential phase. An interesting finding is that the growth of *S. quadricauda* cells began with exponential phase instead of lag phase in the present study. The reason could be due to the cultivation procedure in which the microalgal cells from preculture on the end of log phase were transferred directly into the experimental culture medium with less changes in cultivation condition. Therefore, the growing cells did not need to adapt to the new culture condition. The present study is in agreement with the previous report, in which the growth of *Nannochloropsis oculata* went directly to exponential phase by using continuous culture procedure [15].

Lag phase in growth curve is observed when microalgal cells are adapting the new culture condition. The period of adaption depends on the inoculum and the new culture condition. *Chlorella* sp. was reported that with static (non-aerated) and aerated cultivation, the period of adaption was different and a long adaption time was observed on static cultivation [14]. Lag phase also appeared when *Nannochloropsis salina* adapted to a new cultivation

condition in which the lag phase was observed in the cultivation of high CO_2 concentration (5vol%) and high $NaNO_3$ concentration (1.5 and 2 g/L $NaNO_3$) [17].

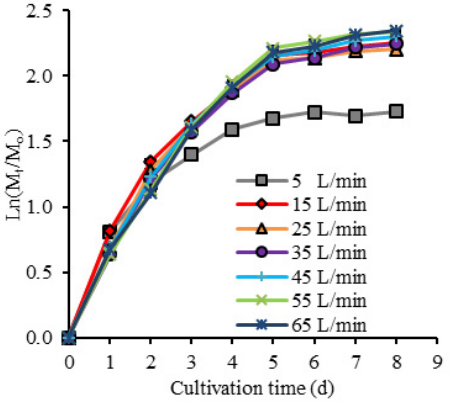

Fig. 2. Growth curves of *S. quadricauda* at different aeration rates in flat plate photobioreactor.

The 5th day on cultivation period was the time that the growth entered stationary phase. Therefore, the cultivation should be harvested on the 5th day. Besides, biomass concentration and lipid content on the 5th day were analyzed and evaluated. The effect of aeration rate on biomass concentration and lipid content on the 5th day are shown in Fig. 3. Increase in air injection rate from 5 to 15 L/min increased the final biomass concentration from 0.230 to 0.382 g/L. However, air injection rate above 15 L/min only resulted in insignificant changing in final biomass concentration. Additionally, lipid content was independent and have similar value in varing air injection rate from 5 to 65 L/min. The lipid yield in the present study are 26, 44, 42, 40, 42, 46, and 44 mg/L corresponding with the air injection rate at 5, 15, 25, 35, 45, 55, and 65 L/min, respectively.

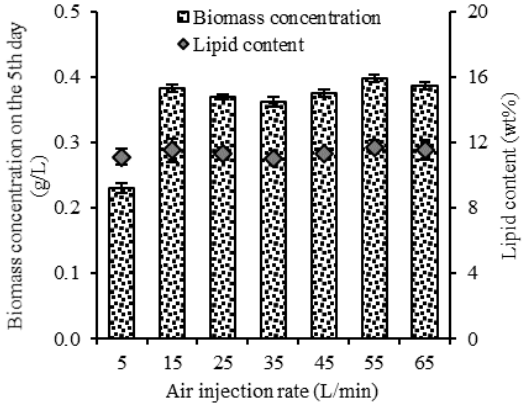

Fig. 3. Biomass concentration and lipid content on the 5th day at different aeration rates in flat plate photobioreactor.

The idea to increase growth and biomass concentration by aeration has been well established. The report on *Chlorella* sp. showed that the biomass concentration increased significantly by high air injection rate at 0.2 L/min (0.2 vvm, 0.03 vol% CO_2) [14]. The present study is consistent with the study on *Dunaiella tertiolecta*, whereby the investigation on eight air injection rates (0-28 L/min or 0.00-6.51 vvm, 0.03 vol% CO_2) showed that higher air injection rate increased the biomass concentration of microalgae [18].

In the present study, it is interesting to note that increase in biomass concentration was insignificant at very high air injection rate (from 15 to 65 L/min). This finding is supported by the previous study on *Isochrysis galbana* that a significant increase in biomass concentration was observed by the increasing of air injection rate from 0.3 to 2.4

L/min (0.5 to 4.0 vvm, 0.03 vol% CO_2). However, at higher air injection rates (from 2.4 to 3.8 L/min or 4.0 to 6.25 vvm, 0.03 vol% CO_2), the increase in biomass concentration was insignificant [19]. This finding can be due to the demand of culture mixing and mass transfer that are supplied by aeration [20]. Higher air injection rate provides sufficient culture mixing and mass-transfer of the essential growth factors and microalgal cells such as nutrient in culture medium, CO_2 capture and O_2 release. This sufficient culture mixing and mass-transfer can lead to the increase of microalgal growth. However, additional supply of air injection rate beyond the requirement of mixing and mass-transfer may not increase the growth of microalgae.

In the present study, the respond of microalgal growth with high air injection rate was the insignificant increase in biomass concentration with high air injection rate. However, different respond of microalgal growth with high air injection rate was also reported in several researches. The reduction of biomass concentration was highlighted when microalgal cells were cultured with high air injection rate. The excess mixing by high air injection rate may lead to hydrodynamic stress that negatively affected the microalgal growth [21–23].

In the view of energy efficiency, the energy utilized for production must be less than the produced energy. Therefore, the reduction of energy utilization in production process is very important [24]. In the present study, beyond 15 L/min did not cause significant increase of growth and biomass concentration. Therefore, 15 L/min is the best air injection rate for growing *S. quadricauda* by using flat plate photobioreactor in term of saving injection energy. This result corroborates the idea in previous report, in which *Scenedesmus* sp. was cultured with low power input by reducing the air injection rates but without significant decrease in biomass concentration [25].

3.2. Effect of different nitrogen concentration

The result of nitrogen concentration effect is shown in Fig. 4. The growth curves correspond to previous growth curves in Fig. 2, wherein five days of log phase was followed by stationary phase. Besides, the growth curves at 1 and 2N-LC are similar and higher than the growth curve of 3 N-LC. The result in the present study indicates that supplying nitrogen with high concentration did not result in high microalgal growth.

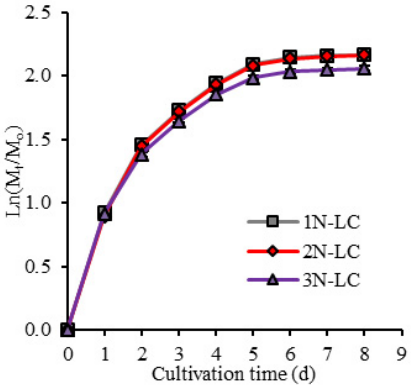

Fig. 4. Growth curves of *S. quadricauda* at different medium nitrogen concentration in flat plate photobioreactor.

The result in Fig. 5 also shows that biomass concentration on the 5th day decreased slightly (from 0.39 to 0.35 g/L) with the increase of nitrogen concentration. Besides, lipid content decreased (from 11.5 to 5.1wt%) with the increasing of medium nitrogen concentration from 1 to 3N-LC. Lipid yield obtained by 1, 2, 3N-LC were 45, 37, 18 mg/L, respectively. This finding implies that lower nitrogen concentration can enhance lipid content. High lipid content due to low nitrogen concentration in culture medium was reported on sevaral microalgal species, mamely *Nannochloropsis* sp. [12], *Nannochloropsis oculata* [3], *Scenedesmus* sp. CCNM 1077 [26], *Chlorella* sp and *Scenedesmus* sp [11].

There is an optimum concentration of nitrogen for microalgal cell growth, higher concentration than the optimum resulted in reduction of cell growth. The report on *Scenedesmus bijugatus* is in agreement with the current finding, wherein the optimum nitrogen concentration was 10 mM (140 mg nitrogen/L), higher nitrogen concentration was

cause the decrease of growth [27]. Similarly, the report on *Scenedesmus* sp. also indicates that the optimum nitrogen concentration among 15.43 to 247 mg nitrogen/L was 123.5 mg nitrogen/L [26].

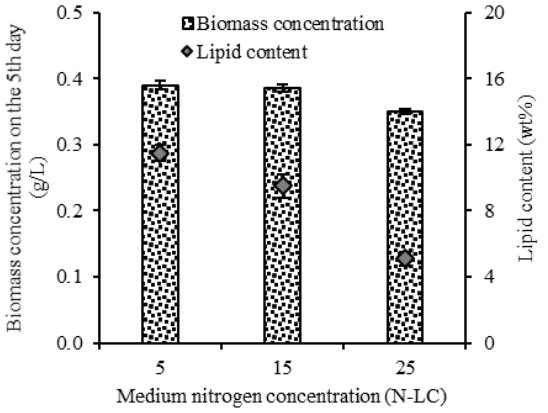

Fig. 5. Biomass concentration and lipid content on the 5th day at different medium nitrogen concentration in flat plate photobioreactor.

4. Conclusion

Direct cultivation from preculture into experimental culture can eliminate lag phase and shorten the cultivation time. Air injection enhanced the growth and biomass concentration compared to no air injection. However, high air injection rate did not cause the increase of growth and biomass concentration. Besides, lipid content was not affected by different air injection rates. The optimum air injection rate for saving injection energy among 5 to 65 L/min was 15 L/min in which the biomass concentration and lipid content were 0.38 g/L and 11.5 wt%, respectively. Additionally, high lipid content was obtained in low medium nitrogen concentration at 1N-LC. Consequently, standard nitrogen concentration in Lefebvre-czarda medium was the appropriate medium nitrogen concentration for high biomass concentration and lipid content of *S. quadricauda.*

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