Behavior of *Nannochloropsis oculata* In Indoor Cultivation: The Effects of Flue Gas Exposure And Illumination Period on The Growth And Lipid Content

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Abstract. Indonesian government launched an energy source substitution from fossil fuel into renewable energy such as biodiesel oil. It is known that microalgae could store high lipid content making it useful for biodiesel production. This research studied the cell growth and lipid content profile of *Nannochloropsis oculata* at various exposure periods of flue gas and illumination. The experiments were done in indoor bioreactor outfitted by a light source and flue gas absorber. Five liters of medium BG-11 was circulated through bioreactor and absorber while the exposure periods of light and flue gas were varied intermittently and/or continuously. *N. oculata* reached the highest cell density of 27.3 mg/L when it was treated at continuous illumination with the absence of flue gas exposure. The highest lipid content of 36.88% was achieved at the cell density of 14.11 mg/L in continuous illumination and 10 minutes per day of flue gas exposure. The CO$_2$ enrichment through flue gas exposure showed an environmental stress toward microalgae growth and drove the lipid content as well as the periodical illumination.

1. Introduction
Indonesian government has developed renewable energy source and made a regulation to use 20% biodiesel in diesel blending fuels. However, the current raw material to produce biodiesel depends strongly on the production of crude palm oil (CPO) which regularly used as an edible oil. It will rise deforesting the land for plantation purpose because it always become the decision to overcome the high demand toward CPO [1]. In consequence, source diversification should be the alternative solution. The microalgae oil is a choice as a potential biodiesel feedstock due to their fatty acids similarity to vegetable oils. Microalgae has also been a good carbon biosequestrian absorbing excess carbon dioxide emission through photosynthetic process. This biological CO$_2$ fixation is deemed attractive as it not only reduce CO$_2$ emission but also it produces useful biomass [2] or starch [3]. Furthermore, harvesting microalgae oil doesn’t depend on the harvesting season like the palm oil. As a result, microalgae could yield higher lipid productivity (12000 L/ha) than most of the utilized oil crop (1190 L/ha) [4].

*Nannochloropsis oculata*, one of marine microalgae, is widely known to utilize light and CO$_2$ efficiently. They stored the product of photosynthesis in the form of protein, carbohydrate and lipid. The lipid content of *N. oculata* was around 31-68% of dry cell weight. This amount is higher than oil palm seeds that only has 25% of total seed weight. Accumulation of lipid in *N. oculata* varies depending on its environmental conditions. Some of the factors are light intensity, dissolved oxygen, CO$_2$ concentration, N$_2$ concentration, temperature, pH, salinity and toxic/heavy metal content [5].
Each of microalgal species will respond differently to luminosity and photoperiod. The optimal range of luminosity for growth is between 76 to 600 mol/m².s, while for the lipid synthesis is 240 mol/m².s [6]. Wahidin et al. [7] showed that luminosity of 100 µmol m²/s and photoperiod of 18 h light: 6 h dark resulted in 6.5 x 10⁷ cells/L and lipid content of 3.31%. Optimal photoperiod provides unique cultivation technique such as dark-light technique in which the light and dark period is set alternately and periodically. The common guidance for the dark-light cycle ratio is 12:12, 14:10, 10:14 and 16:8.

Cell growth favors low level of CO₂ and the rising of its concentration is detrimental for the cell growth. It was reported that the increase of CO₂ from 6%-10% hit the population of the cell by a half. The production of the cell at lower CO₂ concentration was 0.376 g/L.d and 0.15 g/L.d at higher CO₂ concentration [8]. Lipid concentration was also affected by CO₂ concentration. Widjaya et al. [9] reported the increase of lipid content from 0.33% to 3.33% as a result of CO₂ addition to the system.

The effects of flue gas on Chlorella sp. related to the growth and lipid content are well explained. In achieving similar goals, the information regarding the behavior of *N. oculata* using flue gas as CO₂ source must also be perceived. However, no experimental studies have demonstrated the behavior of *N. oculata* using flue gas as CO₂ source in the culture medium. In attempt to satisfy the proposed information gap, we report the growth characterization, biomass and lipid production of *N. oculata* at medium BG-11. The medium was enriched with CO₂ by exposing flue gas to the medium at various contact time. In addition, we also extend the investigation to the effect of illumination period on the behavior of microalgae as mentioned previously.

2. Methods

2.1. Strain of *N. oculata*

The strain of *N. oculata* was come from Laboratory of natural feeding, Jepara, Jawa Tengah, Indonesia. It was cultured in an open pond with salinity of 2.35%, pH of 7.9 and temperature of 25-30 °C.

2.2. Medium BG-11 preparation

The enriched medium for cultivation was medium BG-11. It consisted of 1.5 g NaNO₃, 0.04 g K₂HPO₄.3H₂O, 0.2 g KH₂PO₄.3H₂O, 0.0004 g EDTA, 0.005 g Fe ammonium citric, 0.005 g citric acid, 0.02 g Na₂CO₃ and 1 mL trace element per 1 litre of brackish.

2.3. Starter preparation

Starter for this culture was developed in three steps. Each of them used medium BG-11 with working volume of 10, 100 and 1000 mL. The Culture was continuously aerated for 7 days. The operating temperature and pH were 30 °C and 7.3 respectively. The light intensity used was 3000 lux with 12:12 light : dark cycle.

2.4. Microalgal lipid production

The cultivation of microalgae was done at 5 litre squared photobioreactor (figure 2). The cultivation was treated with two different conditions. The first condition was photoperiod variation compromising continuous and periodical light cycle (16 hours of light and 8 hours of dark). The second variation was flue gas fixation compromising 0 minutes, 10 minutes and 30 minutes exposure. During fixation process, the reactor was continuously exposed to the light. The cultivation was lasted for 15 days for every variable tested.
2.5. Analysis
Dry cell weight was measured by weighing the dried cell directly. Fifty millilitres sample taken from the reactor was centrifuged at 6000 rpm for 15 minutes. The supernatant was drained, the pellet was ovened at 80 °C for 24 hours and weighed. The lipid was extracted from the dried cell using solvent mixture of chloroform and methanol (2:1 v/v) for an hour. The extracted liquid was then centrifuged to separate the supernatant and solvent. The remaining solvent was evaporated and the remaining oil was weighed.

3. Results and discussion
The proximate analysis and lipid content of N. oculata before treatment were approached by analysis results of N. oculata from other open pond of Situbondo, Jawa Timur, Indonesia [10]. Its moisture, volatile matter, fix carbon, ash and lipid content were 3.99%, 67.45%, 8.08%, 24.47% and 11.44% respectively.

3.1. The effect of illumination method to the growth and lipid content of cells
The effects of illumination method were investigated for 15 days and described in Figure 2. The cell grows since the initial day and reaches maximum at the eleventh day of cultivation before decreasing significantly.

N. oculata is seemed to be more comfortable growing at continuous illumination than periodical illumination. It could be observed by how long of the slow growth (lag phase) at early incubation, i.e. 6 days at periodical illumination and 3 days at continuous illumination. Prolonged lag phase may also be happened due to low and near saturation light intensity [7]. Under low intensity, photosynthesis efficiency is also low and therefore the growth is inhibited. Meanwhile, at near saturation intensity, the light induces photo inhibition destructing the devices of photosynthesis and reducing the photosynthesis efficiency.
Figure 2 shows that cell population is higher at continuous illumination than periodical illumination. The highest population is 20.5 mg/L and 27.3 mg/L for periodical and continuous illumination respectively. This result is consistent with the report from Wahidin et al. [7] using *Nannochloropsis* sp. and Amini Khoeyi et al. [11] using *Chlorella vulgaris*. They founded that an increase illumination period will have a favorable effect on biomass production so long that the light is still under light saturation limit.

The lipid accumulation in microalgae increases at stress environment such as growing under nutrient deficiency or limited light exposure. According to the Figure 2, the lipid content of *N. oculata* at continuous illumination is almost constant at 17% - 23% (w/w). In the other hand, at periodical illumination, cell accumulates more lipid than the continuous one gathering with the highest content of 27.25% (w/w). It is the evidence that periodical illumination is a form of environmental stressed to the cell body. This data is in accordance with the report from Liu et al. [12] that the total lipid content of algae reached the highest level at the dark condition. In addition, Cheirsilp and Torpee [13] reported that in the process of low light intensities, microalgae tend to use the synthesized energy to accumulate it in the lipid form.

![Image of temperature and salinity profiles](image-url)

**Figure 3.** The profile of medium temperature (a) and medium salinity (b). (●: (16L: 8D); ■: continuous illumination)

The profiles of temperature during cultivation regardless of the photoperiod variation are unsteady (Figure 3(a)). The temperature of the medium is observed to vary from 27 °C – 30 °C and it is greatly affected by the ambient temperature. Renaud et al. [14] investigated that the optimal temperature for microalgae growth was at 25 °C – 35 °C, while Pahija et al. [15] reported similar tend to grow between temperature 22 °C and 27 °C for microalgae *Chlorococcum*. At current research, it shows that using ambient air alone to control medium temperature is sufficient to meet the appropriate temperature.

The salinity of the medium is increased continuously and starts to be steady at the eighth day (Figure 3(b)). The initial value is 2.3% and ended at 3.0% by the end of process at continuous illumination and 1.8% to 2.3% at periodical illumination. It was previously investigated that the medium salinity affects the lipid accumulation [16]. Campenni et al. [17] reported that 20 g/L NaCl produced higher lipid content than 10 g/L and 30 g/L NaCl.

![Image of pH profiles](image-url)

**Figure 4.** The profile of pH during day and night at (a) continuous illumination condition and (b) periodical illumination condition (16L: 8D). (---●---: pH during the night; ---■---: pH during the day)
The pH level on the medium is affected by the availability of CO₂ for photosynthesis and the nutrient medium. It is crucial to maintain pH at appropriate values because complete culture collapse may occur due to extreme pH. At current investigation, the value of pH during cultivation is monitored (figure 4) at day and night. pH during the day is mostly higher than the night. At both illumination period, the average pH value is 8.12 and 7.99 during the day and the night respectively. According to the paper from Bartley et al. [18], the pH ±8 was the most optimum values for the growth of Nannochloropsis sp. Day/night pH fluctuation driven by photosynthesis and respiration rate. Photosynthesis reaction and the use of CO₂ raise pH level. In the other hands, respiration reverses this process and lowers pH level.

3.2. The effect of flue gas exposure to the growth and lipid content of cells

The photosynthetic metabolism utilizes light and CO₂ for growth and organic substance production. The aim of contacting flue gas was to enrich CO₂ in the medium and observed its effect on N. oculata growth and lipid content. Figure 5(a) shows the effect of flue gas exposure on the growth of microalgae. For 0 minute exposure, the highest population is 27.3 mg/L then followed by 23.55 mg/L for 30 minutes and the lowest is 14.1 mg/L for 10 minutes. It shows that CO₂ enrichment through flue gas exposure degrades the microalgae growth, while at increasing flue gas exposure from 10 to 30 minutes) seems to favor cell growth. The similar phenomenon of flue gas exposure was also found by Razzak et al. [19]. They observed the rise of total cell weight when the carbon dioxide concentration was increased from 4% to 8%.

The gas exposure gave a stress environment for N. oculata. This claim can be confirmed directly from Figure 5(b) describing the lipid content profile. Microalga prefers to store the energy in the form of lipid rather to utilize it to grow under stressed condition. Based on the observation, the lowest total cell weight which is 10 minutes flue gas exposures can accumulate higher lipid content. Microalgae at 10 minutes gas exposure stored 23.3% - 36.88% lipid and at 0 minutes stored 18.69% - 23.08%. While at 30 minutes, the lipid content was 10.58% - 18.26%. de Castro Araújo and García [20] reported that Chaetoceros cf. wighamii assimilated extra carbon mainly to protein synthesis. This phenomenon was also occurred at Phaeodactylum tricornutum [21]. However, Chu et al. [22] observed increasing in lipids and carbohydrates at protein expenses in Nitzschia inconspicua, when culture was enriched with 5% (v/v) of carbon dioxide.

The temperature during the process vary from 26 °C – 31 °C (Figure 6(a)). The temperature at this process is greatly affected by the environment temperature with almost no impact from metabolic activity. Although it is controlled by the ambient temperature, no significant changing is observed at lipid content and algae growth. It shows that the ambient temperature already give the appropriate temperature for the growth of N. oculata.

The salinity is also increased during the cultivation (Figure 6(b)). It shows that during 10 minutes gas exposure, the salinity changes from 1.8% to 2.3%, while during 30 minutes exposure, it changes from 2.1% to 2.8%. While at the absence of gas fixation the salinity ranging from 2.3% to 3.0%. The reported investigation shows salinity below normal sea water that averagely has salinity of 3.5%. It simply shows that N. oculata can live under brackish water (low salinity) even though it is categorized as marine microalgae.

![Figure 5](image-url) The cell population (a) and lipid content (b) of Nannochloropsis oculata in different flue gas exposure period. (●: 30 minutes exposure; ▲: 10 minutes exposure; ■: 0 minutes exposure)
Figure 6. Temperature (a) and Salinity change (b) of medium during the process. (●: 30 minutes exposure; ▲: 10 minutes exposure; ■: 0 minutes exposure)

Figure 7. The profile of pH during day and night at flue gas exposure of 0 minutes (a), 10 minutes (b) and 30 minutes (c). (---●---: pH during the night; —■—: pH during the day).

The pH values of culture medium (Figure 7(a), (b) and (c)) showed a similar trend with the effect of periodical illumination. It showed that pH during the day was mostly higher than pH during the night. At variable 0 and 10 minutes exposure, the pH trend showed varied greatly through the day while the cell at 30 minutes gas exposure showed almost steady values. This condition may be occurred when the concentration of carbon dioxide in the media is enough to supply the cell to run photosynthetic continuously. It seems by exposing the media to the flue gas for 30 minutes gave enough carbon sources for the N. oculata. While at 0 and 10 minutes variable, it shows clear alteration between respiration and photosynthesis process. By this finding we can infer that the metabolism of N. oculata at phototrophic system are highly depend on the existence of nutrient and light.

4. Conclusion
The effect of illumination was investigated in lighting continuously and periodically (16L:8D). The illumination supported cell growth, while periodical illumination stimulated lipid accumulation. The cell population during continuous illumination (27.3 mg/L) was higher than that at periodical illumination (20.5 mg/L). At periodical illumination, the cell had a higher lipid content (27.25%) than at continuous illumination (17%-23%). Meanwhile, The exposure of flue gas inhibited the cell growth but increased lipid content. In this experiment, the highest cell population was acquired at 0 minutes exposure, i.e. 27.3 mg/L and the highest lipid content of 36.88% was reached by 10 minutes exposure in continuous illumination.

5. References
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