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OUTDOORS BATCH CULTIVATION OF MARINE MICROALGAE *Nannochloropsis sp* USING PARALLEL GLASS TUBULAR PHOTOBIOREACTOR

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ABSTRACT
Microalgae *Nannochloropsis sp* is autotrophic organism that can utilize atmospheric CO_2 and sunlight for growth via photosynthesis. It is potential sources for biodiesel due to its high lipid content. For mass production, two basic designs of photo-bioreactor are available, i.e. open and enclosed systems. Study was conducted to investigate the growth performance of *Nannochloropsis sp* in outdoor batch culture system using Parallel Enclosed Glass-Tubular Photo-bioreactor (PBR) for 18 days with modified f/2 medium. Nutrient N-P was calculated based on the empiric structure of microalgae cell ($C_1O_{0.48}H_{1.83}N_{0.11}P_{0.01}$) with target of biomass yield was $1gL^{-1}$. Temperature, pH, Optical Density (OD), Dry Cell Weight (DCW), population, lipid content, and fatty acid were observed. Data showed that temperature fluctuated between 22°C and 35°C, pH was stable at 7.0-7.5, OD increased from 0.134 to 0.878, DCW increased 39 times higher than its initial value. At the 18th day, the cell diameter varied in range of 1-9 µm, cell weight increased 5.3 times (3.5 to 18.4 $pg.cell^{-1}$), and lipid content is 35.71%. Fatty acid was composed by saturated fatty acids (92.39%) that covered of lauric (20.30%), myristic (12.69%), palmitic (50.05%), and stearic (9.35%) that was suitable for biodiesel. Unsaturated fatty acid was performed by oleic acid (3.49%).

Key words: CO_2 mitigation, biodiesel, *Nannochloropsis sp*, photo bioreactor

KULTIVASI MIKROALGA *Nannochloropsis sp* SECARA BATCH MENGGUNAKAN PARALLEL GLASS TUBULAR PHOTOBIOREACTOR PADA KONDISI ALAMI

ABSTRAK
Mikroalga *Nannochloropsis sp* adalah organisme autotroph yang dapat memanfaatkan CO_2 udara dan sinar matahari untuk pertumbuhan melalui fotosintesis. Organisme tersebut potensial untuk biodisel karena kadar lipidnya tinggi. Untuk produksi, dikenal dua model fotobioreaktor, yaitu sistem terbuka dan tertutup. Penelitian bertujuan mengetahui pola pertumbuhan *Nannochloropsis sp* di dalam outdoor batch culture system menggunakan Parallel Enclosed Glass-Tubular Photo-bioreactor (PBR) selama 18 hari dengan media f/2 modifikasi. Nutrisi N-P dihitung sesuai rumus empiris sel mikroalga ($C_1O_{0,48}H_{1,83}N_{0,11}P_{0,01}$) dengan target produksi biomasa $1gL^{-1}$. Suhu, pH, OD, DCW, populasi, kadar lipid dan komposisi asam lemak diamati selama penelitian. Data menunjukkan suhu berfluktuasi antara 22-35°C, pH stabil pada 7,0-7,5. OD meningkat dari 0,134 sampai 0,878, DCW meningkat 39 kali. Pada hari ke 18, diameter sel bervariasi antara 1-9 µm, berat sel meningkat 5,3 kali (3,5 ke 18,4 $pg.cell^{-1}$), dan kadar lipid 35,71%. Asam lemak disusun oleh asam lemak jenuh (92,39%), meliputi asam laurat (20,30%), miristat (12,69%), palmitat (50,05%), dan stearit (9,35%) yang cocok untuk biodiesel. Asam lemak tidak jenuh muncul dalam bentuk asam oleat sebesar 3,49%.

Kata kunci: Mitigasi CO_2, biodiesel, *Nannochloropsis sp*, fotobioreaktor

INTRODUCTION

The optimal scenario of atmospheric carbon dioxide (CO_2) mitigation that involves its transition to renewable fuel (biodiesel) by using photosynthetic organisms (microalgae) should be promoted (Christi, 2007). Micro-algae are suitable for biodiesel due to its high photosynthetic efficiency, biomass production, and lipid content (Borowitzka and Moheimani, 2010). It was reported that the most of algal lipids have similar profile to vegetable oil that suitable for biodiesel (Xu et al., 2006). Biodiesel itself is alkyl ester of long-chain fatty acids (triglycerides) that derived of fatty acids by esterification

with methanol or ethanol. It is renewable, biodegradable and nontoxic (Li et al., 2008). Some researchers reported biodiesel is composed by fatty acids with carbon atoms of 12-24 (Xu et al., 2006); 16-20 **(**Christi, 2007); 10-24 (Hu et al., 2008).

The growth and lipid characteristic of microalgae is determined by the species and cultivation parameters. It is important to note that high lipid productivity is a key desirable characteristic in the choice of species to use for biodiesel. Selection of fast growing, productive strains, and adaptable to local climate conditions is a fundamental importance in algal mass culture (Huntley and Redalje, 2007).

Microalgae *Nannochloropsis sp* (Eustigmatophyceae) have been considered as potential sources of renewable energy due to its high lipid content, i.e. 62% (Hu dan Gao, 2006); 36.19-46.67% (Astuti et al., 2008); 28.7% (Gouveia and Oliveira, 2009); 44.5% (Su et al., 2010). Nevertheless, the proper method of cultivation is required to obtain the high yield, both of biomass and lipid. For mass production, two basic designs are available, i.e. open and enclosed systems. It was reported that in open ponds, *Nannochloropsis sp* collapses after a few weeks due to contamination by bacteria and competition by other algal species. The adoption of closed photo-bioreactor is required, since open ponds do not ensure a long-term reliable cultivation (Pulz, 2001).

Enclosed photo bioreactors have received much attention because of its high biomass productivity, low contamination, and minimal evaporation, able to reduce of CO_2 losses, and easier to control (Grobbelaar dan Kurano, 2003; Merchuk et al., 2000). The utilization of various designs of enclosed photo bioreactors could prevent the culture to collapse (Vasudevan and Briggs, 2008). This study was aimed to investigate the growth characters of *Nannochloropsis sp* at outdoor batch cultivation system in parallel glass tubular photo-bioreactors

MATERIAL AND METHOD

Microalgae Standard Culture

The research centre for biotechnology, Indonesian institute of as sciences provided standard cultures of *Nannochloropsis sp*. Cultures were maintained in a modified f/2 medium that used ammonium nitrate (NH_4NO_3) as sole nitrogen sources. Medium was prepared by using sea water (Ancol Jakarta), enriched with $8.83x10^{-4}$M NH_4NO_3; 3.63 x 10^{-5}M $NaH_2PO_4.1H_2O$; 1.07 x 10^{-4}M $Na_2SiO_3.9H_2O$; $1x10^{-5}$M $FeCl_3.6H_2O$; $1x10^{-5}$M $Na_2EDTA.2H_2O$; $4x10^{-8}$M $CuSO_4.5H_2O$; $3x10^{-8}$M $Na_2MoO_4.2\ H_2O$; $8x10^{-8}$M $ZnSO_4.7H_2O$; $5x10^{-8}$M $CoCl_2.6H_2O$; $9x10^{-7}$ M $MnCl_2.\ 4H_2O$; $1x10^{-10}$ M Vitamin B_{12}; $3x10^{-7}$M Thiamine; and $2x10^{-9}$ M of Biotin. Cultures were cultivated in outdoor to adapt the local condition, bubbled with filtered atmospheric air (0.47 μm). Then, cultures with Optical Density (OD) ≈ 1 (680 nm) was used as inoculums in study.

Parallel Glass Tubular Photo-bioreactor (PBR)

Triplicates of Parallel Glass-Tubular Photo-bioreactor (PBR), i.e. PBR 1; PBR 2; and PBR 3 were used for study. The main body of PBR is a clear glass tubular with diameter 4 cm and length 140 cm. Each PBR were consisted of eight glass tubular, constructed horizontally in series on panel, placed side by side with position at 847 m above sea level, latitude 06°52'57.5" SL and longitude 107°36'39.8" EL. Silicone tubes was used for connecting between glass tubular to transfer and distribute cell and nutrients. Total volume of PBR is 15 L approximately, including spaces of silicone tubes. PBR system was designed as presented schematically in Figure 1.

Sterilization of glass tubular of PBR was conducted by soaking with sodium hydroxide (NaOH) 2% for 24 hours, and then flashed-out with boiled water until the residual of chemicals has been removed

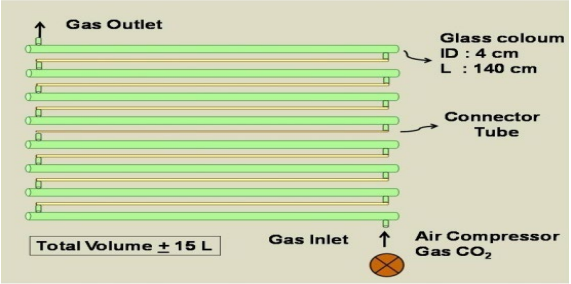

Figure 1. Schematically design of PBR system.

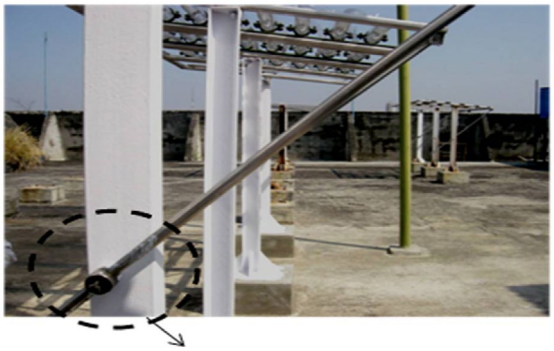

Figure 2. The slope regulator of PBR panel (screw type).

completely (pH ≈ 7). PBR was adjusted facing to north direction with slope of 30° to optimize solar energy absorption. Slope could be changed by turning of screw regulator that equipped on panel as displayed in Figure 2.

Growth Medium and Culture Condition

Modified f/2 medium (without N, P sources) was prepared, boiled and filled into PBR 1; PBR 2; and PBR 3 under aseptic condition using peristaltic pump. Nutrient N and P was prepared in aqueous solution, sterilized, and injected into medium at room temperature via silicone tube connectors. The addition of N and P was calculated based on both of the empiric structure of microalgae cell, i.e. $C_1O_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Christi, 2007) and the target of biomass yield in study, i.e. 1 g. L^{-1}.

In order to minimize variables of experiments, inoculation was carried out aseptically using stock culture (OD_{680nm} = 0.96) in equal concentration (5% v/v), then incubated in outdoor batch culture system as presented in Figure 3. Filtered atmospheric

Figure 3 Outdoors batch culture systems of *Nannochloropsis sp* in PBR.

air was supplied (flow rate ± 1 $L.min^{-1}$) as sole carbon sources, from the bottom part and circulated continuously with peristaltic pump to optimize distribution of nutrients and cells. The cell biomass was harvested at 18th days and then analyzed.

Analytical Methods

Local temperature and pH of culture medium

These parameters were measured due to observe the adaptability capacity of microalgae cell to local environment condition. pH was measured by using pH meter. Meanwhile, temperature of the atmospheric air and the growth media were checked with using thermometer.

Optical Density (OD) and Dry Cell Weight (DCW)

OD value of culture reflected of its biomass concentration. It was measured turbidity-metrically at 680 nm according to Astuti et al., (2008). At the initial of experiment, several levels of cell concentration of stock culture were prepared in series. Then was measured its OD (680 nm) using spectrophotometer, and its DCW was determined gravimetrically. Then, a standard curve that displayed the correlation between OD with DCW was created. DCW in this experiment was calculated based on that standard curve as presented in Equation 1 (R^2 = 0.989), and expressed in $g.L^{-1}$.

$$DCW = (0.969 \times OD_{680}) - 0.111 \quad \text{..... (1)}$$

Cell population, size, and weight

Cell population was determined microscopically. As much as 2 µL of culture was spotted in object glass and covered with 20x20 mm of cover glass. Immersion oil was added and then observed the population of cell by using Leitz Laborlux D microscope with reproduction ratio 100, and aperture of lens 1.25 mm. Cell population was calculated with considering to the dilution factor and expressed in 10^{10} cells.L^{-1}. Cell size was determined by using micrometer that equipped in microscope system. The average of cell weight was calculated by divided of DCW with cell population, expressed in picogram per cell (pg. $cell^{-1}$), which 1 pg is equivalent to $1x10^{-12}$ g.

Total lipid content

Total lipids content was extracted with chloroform-methanol according to method of Bligh and Dyer (1959). The 15 mL glasses vial that containing of dried algal biomass (M), 2 mL methanol, and 1 mL chloroform was kept for 24 hr at 25°C. The mixture was then agitated in a vortex for 2 min, again added 1 mL of chloroform and mixed in a vortex for 1 min. Afterward, 2 mL of distilled water was added and the mixture was mixed in a vortex for 2 min. Three layers were obtained by centrifugation for 10 min at 2000 rpm, i.e. chloroform fraction (bottom), water (middle), and methanol fraction (upper). The chloroform fraction that contain of lipid compound was sucked using pipette and transferred it into a previously weighed of dried and cleaned vial (W_0). Then, it was dried at oven 105°C for 8 hr, cooled down in desiccator, and weighed at room temperature (W_1). Lipid was calculated by subtracting W_1 from W_0, Lipid content was calculated by divided (W_1-W_0) with M, and expressed in % DCW.

Characterisation of fatty acids

Lipid extract (≈ 0.60 g) was trans-methyl-esterification with BF_3, and then analyzed using Gas Chromatography-Mass Spectroscopy (GCMS) QP5000 that equipped with DB-17 Capillary Column (L 30 m, Ø 0.25 mm). Temperature of injector and detector were maintained at 250 and 300°C, respectively. Temperature was started at 80 °C for 3 min, increased 10 °C.min^{-1} until reached to 260 °C, with final hold time for 10 min. The condition was set with flow gas 1.1 mL.min^{-1}, linear velocity 37.5, and pressure 67.7 kpa. Then, 1 µL of sample was injected with splittless mode. Fatty acids were identified by comparison its retention times of the peaks in GC-Chromatogram to NIST and Wiley Library. The concentration of the fatty acid components was calculated relatively by comparing the peak area (height x wide) of individual of fatty acid to the total peak areas, expressed as the percentage (%) of total fatty acids (Astuti et al., 2008).

RESULT AND DISCUSSION

Temperature and pH of Culture Medium

The growth of microalgae was affected by temperature of the environment, and in outdoor system, it would depend on its local climatic situation. In this study, there was no significant different of temperature in culture medium among the PBR system (PBR 1; PBR 2; PBR 3). During the light day (08.00 to 16.00), the temperature of culture fluctuated in the range of 22-35 °C with the average of 29.6 °C (Figure 4). This level was near with the previous study, i.e. 22.63-34.33°C (Astuti et al., 2008), which was carried out at the same place, but in different period of time. In fact, these ranges were relatively higher than the optimum temperature for growth of *Nannochlorpsis sp*, i.e. 22-27°C (Fabregas et al., 2004). Nevertheless, like other microalgae, *Nannochloropsis sp* has a capability to adapt the local environment, including of the temperature.

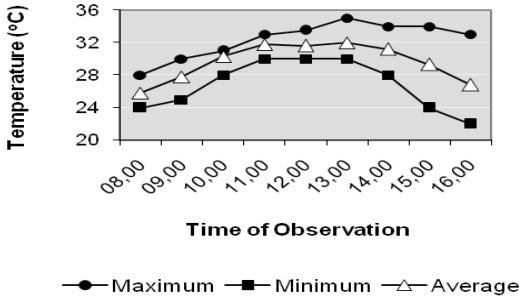

Figure 4. The average temperature of culture medium of *Nannochloropsis sp* in PBR.

Borowitzka and Moheimani (2010) reported the optimum climatic condition for high productivity of algae is the area with high annual average sunshine and high temperatures. Based on the local climate condition, it would be expected that Indonesia is suitable for mass production of microalgae. In Indonesia, sunlight could be emerged almost at the whole of year with the average of sunlight is 6.1-9.7 $hr.day^{-1}$. Minimum temperature is in the range of 23-24°C, maximum is 30-33°C, or the average is 27-29°C.

Microalgae can grow autotrophically or heterotrophically, with a wide range of tolerance to different temperature, salinity, pH and nutrient availabilities (Hu et al., 2008; Brennan and Owende, 2010). Nevertheless, most algae species are sensitive to free NH_3 (Azov and Goldman, 1982). The use of ammonia requires much more careful control of algae culture than when nitrate is used as N source, especially at higher temperatures. In medium, ammonium nitrate (NH_4NO_3) would be dissociated to ammonium (NH_4^+) and nitrate ion (NO_3^-) as presented in Equation 2 (Sawyer et al., 1994).

$$NH_4NO_3 \longrightarrow NH_4NO_3 + NO_3^- \quad \text{..... (2)}$$

As photosynthesis organisms, micro-algae can utilize both of NH_4^+ and NO_3^- as sources of nitrogen for cell synthesis by using CO_2 and solar energy via biochemical reaction (Equation 3, 4). NH_4^+ ion is the chemical form of nitrogen that most readily taken up and assimilated by micro-algae. The previous studies indicated that when NH_4NO_3 is employed in the medium, NH_4^+ ions are preferentially assimilated and NO_3^- ions are utilized only after the former have been exhausted (Proctor, 1957).

$$NH_3^- + NO_2 \xrightarrow[\text{Chlorophyll}]{\text{Solar energy}} \text{Cell} \quad \text{..... (3)}$$

$$NH_4^- + NO_2 \xrightarrow[\text{Chlorophyll}]{\text{Solar energy}} \text{Cell} \quad \text{..... (4)}$$

Figure 5 showed that pH of *Nanno-chloropsis sp* was not different among the units of PBR. It was stable at 7.5 until of 14th days of cultivation. Afterward, pH was decreased to 7.0 at all of PBR tested. The previous study indicated that *Chlorella sp* and *Scenedesmus sp* has the same pattern, the pH at the initial growth was 7.0-7.1 and at the 5th day of cultivation decreased to 5.2-5.6 (Proctor, 1957). The biochemical process of algae metabolism itself might cause the decreasing of pH. It was investigated that during the dark hours, algae produce rather than consume of carbon dioxide. The respiratory processes in the darkness would be exceeding than the photosynthesis processes. Consequently, the concentration of carbon dioxide in the PBR system become higher than the normal process and tends to reduce the pH value of the culture (Sawyer et al., 1994).

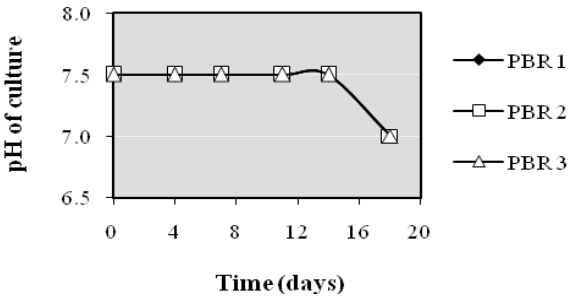

Figure 5. Change of pH level of culture medium of *Nannochloropsis sp*.

Optical Density (OD) and Dry Cell Weight (DCW)

The OD value of culture, which repre-sented its biomass concentration were not significantly different among the PBR units (Figure 6). In average, OD_{680nm} value of *Nanno chloropsis sp* was increased linearly with time, i.e. from 0.134 ± 0.002 at the initial to 0.878 ± 0.012 at the 18th day. The increasing of this biomass concentration indicated that microalgae adapted to the average light intensity present in the culture. It was reported (Grobbelaar and Kurano, 2003) that in enclosed PBR batch culture systems, cell population could be progressively became low light acclimated.

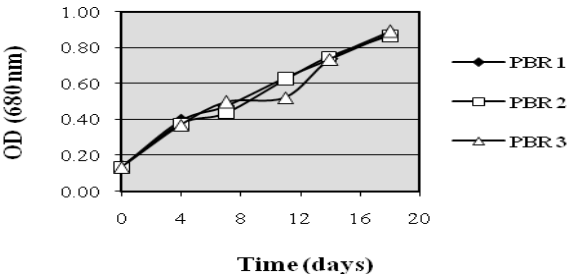

Figure 6. Correlation of OD (680 nm) and age of *Nannochloropsis sp* culture.

It was reported that microalgae could photo-acclimate to various light intensities, both of the spectrum of High Light (HL) or Low Light (LL). Following inoculation of an outdoor batch culture, because of its low density at initial phase, cells receive high average illumination and would characteristically have HL acclimated properties. After a certain time, when culture density increases due to the growth, the average light per cells progressively would become LL acclimated (Grobbelaar and Kurano, 2003).

DCW of *Nannochloropsis sp* was increased by the culture ages. The average of DCW at the 18th was 0.739 ± 0.008 $g.L^{-1}$. Although DCW reached ± 39 times higher compared to its initial concentration (0.019 ± 0.001 $g.L^{-1}$), the production of dry cell was only achieved 73.9% of the target (1g.L-1) as presented in Table 1.

Table 1. DCW in outdoor batch cultivation of *Nannochloropsis sp* using PBR ($g.L^{-1}$)

Culture Age (days)	DCW ($g.L^{-1}$)			
	PBR 1	PBR 2	PBR 3	Average
0	0.021	0.017	0.019	0.019 C 0.001
4	0.271	0.252	0.246	0.256 ± 0.010
7	0.348	0.311	0.371	0.343 ± 0.021
11	0.499	0.493	0.395	0.462 ± 0.045
14	0.603	0.617	0.601	0.607 ± 0.006
18	0.740	0.728	0.750	0.739 ± 0.008

This result is lower than the previous study, which reached DCW in the range of 1.6-2.5 $g.L^{-1}$ (Gouveia and Oliveira, 2009). The low biomass production of *Nannochloropsis sp* in study might be affected by the limited of carbon availability that could be captured for biomass production. In this study, CO_2 as sole carbon source was supplied only by blowing air into the photo-bioreactor. The concentration of CO_2 in the atmospheric air is 0.04%. This condition caused the growth was run unproperly. Microalgae need concentration of carbon dioxide 2-5% in medium (Santos et al., 2010).

$$CO_2 + H_2O \longrightarrow (CH_2O) + O_2 \quad \ldots\ldots\ldots\ldots (5)$$

$$(CH_2O) + O_2 \longrightarrow CO_2 + H_2O \quad \ldots\ldots\ldots\ldots (6)$$

As describes before, microalgae is an autotrophic organism that produced organic matter by capturing carbon dioxide and solar energy in oxygenic photosynthesis process (Equation 5). In the other side, microalgae also carry out respiration, both in light and dark environment. The overall equation of respiration is the reverse of photosynthesis as presented in Equation 6 (Madigan et al., 2000).

The bottleneck of photosynthetic growth of microalgae in outdoor system might be not in the capturing of light energy, but in the converting step in fixed carbon into the structural biomass (Christi, 2007). In this study, the oxygen gas that resulted in photosynthesis might be trapped and accumulated in PBR system. In this high concentration of oxygen, photosynthesis would be blocked and respiration would be stimulated greater than photosynthesis, resulting the decreasing of biomass productivity.

Cell Population, Size, and Weight

The growth of microorganisms, included microalgae was related to the increasing of cells, both in population and size. The average of cell population was increased linearly with the ages of cultures, i.e. from 0.54 to 3.729 ($x10^{10}$ cells. L^{-1}) as in Table 2.

Table 2. Cell population in outdoor batch cultivation of *Nannochloropsis sp* using PBR.

Culture Age (days)	Population (x 10^{10} cell.L^{-1})			
	PBR 1	PBR 2	PBR 3	Average
0	0.55	0.53	0.54	0.540 ± 0.0062
4	1.76	1.67	1.64	1.688 ± 0.0469
7	2.13	1.96	2.24	2.110 ± 0.1031
11	2.86	2.83	2.36	2.685 ± 0.2177
14	3.37	3.43	3.36	3.385 ± 0.0312
18	4.03	3.97	4.08	3.729 ± 0.0365

Cell population increased approximately 6.9 times compared to its initial stage. Based on the microscopic observation, the cultures in the PBR could be kept in sterile condition due to absence of any contaminant organisms in culture medium. The cell diameter and weight of *Nannochloropsis sp* was not different among units of PBR. It was appeared that *Nannochloropsis sp* have various shapes, spherical to slightly ovoid cells.

The cell size was in the range of 1-9 μm in diameter, with the average in PBR 1; PBR 2; and PBR 3 were 4.5; 4.3; and 4.8 μm, respectively. It was bigger than the previous study that has a range of 2-4 μm in diameter (Hoek et al., 1995). The difference of that cell size might be caused by the culture condition itself, such as nutrient availability, temperature, pH, and the culture age. The cell weight of *Nannochloropsis sp* increased with the ages of the culture. There was no significant different of cell weight among the PBR units. It showed that the cell population was increased 6.9 times, meanwhile the cell weight was increased significantly (5.3 times), i.e. from 3.5 to 18.4 pg.cell^{-1} as presented in Table 3.

Table 3. Cell weight of *Nannochloropsis sp* in PBR (pg.cell^{-1})

Culture Age (days)	Cell weight (pg.cell^{-1})			
	PBR 1	PBR 2	PBR 3	Average
0	3.8	3.2	3.5	3.5 ± 0.20
4	15.4	15.1	15.0	15.2 ± 0.15
7	16.3	15.9	16.5	16.3 ± 0.22
11	17.4	17.4	16.7	17.2 ± 0.30
14	17.9	18.0	17.9	17.9 ± 0.03
18	18.4	18.3	18.4	18.4 ± 0.02
Average	14.9	14.7	14.7	14.7 ± 0.02

Total Lipid Content

Data indicated that lipid content of *Nannochloropsis sp* was similar among the PBR units, i.e. 34.42 (PBR 1); 37.16 (PBR 2); and 35.71% (PBR 3). High lipid content in study might be as a response of cell to environmental stresses, which was resulted by the limited carbon supply. As comparing data, the previous studies investigated that lipid content of *Nannochloropsis sp* is 62% (Hu and Gao, 2006); 36.19-46.67% (Astuti et al., 2008); 28.7% (Gouveia and Oliveira, 2009); 44.5% (Su et al., 2010).

Fatty Acids Composition

Data analysis using GCMS showed that lipid of *Nannochloropsis sp* was composed of 92.39% saturated fatty acid, 3.49% unsaturated fatty acid, and 4.12% other unidentified compounds. It was obtained, that the saturated fatty acid of *Nannochloropsis sp* was dominated by palmitic acid (50.05%), lauric acid (20.30%), myristic acid (12.69%) and stearic acid (9.35%) as presented in Table 4. Similar data were obtained in the previous researchers, which reported that palmitic acid is a major of fatty acid in lipid of *Nannochloropsis sp* that is of 47.93% (Astuti et al., 2008); 40.44% of total fatty acids (Fabregas et al., 2004).

Saturated fatty acids are a long-chain carboxylic that no double bonds, such as lauric acid ($C_{12}H_{24}O_2$), myristic acid ($C_{14}H_{28}O_2$), palmitic acid ($C_{16}H_{32}O_2$), stearic acid ($C_{18}H_{36}O_2$), and arachidic acid ($C_{20}H_{40}O_2$). Saturated fatty acids are saturated with hydrogen, since double bonds reduce the number of hydrogen on each carbon. Unsaturated fatty acid of *Nannochloropsis sp* in this study was only

Table 4. Fatty acid (FA) composition of *Nannochloropsis sp* in outdoor batch culture system using PBR

Scientific name	Common name	Chemical Formula	C atom: Double bond	Molecule weight	Content (% Total FA)
n-Dodecanoic acid*	Lauric acid	$C_{12}H_{24}O_2$	C12:0	200	20.30
Tetradecanoic acid*	Myristic acid	$C_{14}H_{28}O_2$	C14:0	228	12.69
Hexadecanoic acid*	Palmitic acid	$C_{16}H_{32}O_2$	C16:0	256	50.05
Octadecanoic acid*	Stearic acid	$C_{18}H_{36}O_2$	C18:0	284	9.35
9-Octadecanoic acid**	Oleic acid	$C_{18}H_{34}O_2$	C18:1n9	282	3.49
Saturated FA*					92.39
Unsaturated FA**					3.49
Other compounds					4.12

oleic acid (3.49% of total fatty acids). Oleic acid (C18:1n9) is a mono-unsaturated fatty acid with one of double bond with position at atom C-9.

Xu et al., (2006) reported the utilization of lipid materials depends of its composition and characteristic, such as chain-length, degree of saturation, and proportion of fatty acids. The required characteristics of fatty acid for biodiesel are long-chain, un-branched, no double bond, with carbon atoms 12-24 (Xu et al., 2006); (Christi, 2007); (Hu et al., 2008). Based on this result, it was suggested that lipid of *Nannochloropsis sp* can be used for biodiesel due to its fatty acid profile. Nevertheless, a better method should be found to optimize its biomass productivity, combined with high lipid content and appropriate composition for biodiesel.

The conceptual solution that consists of two processes should be applied to maximiz production of biomass and lipid. The first stage is maintaining constant conditions that favour continuous cell division and prevent contamination. The second stage is exposing cells to nutrient deprivation and other environmental stresses that lead to synthesis lipid (Huntley and Redalje, 2007).

CONCLUSION

Nannochloropsis sp could be cultivated in the outdoor batch system using Parallel Enclosed Glass-Tubular Photo-bioreactor. Performances of three units PBR as growth provider of algal cell were similar, both in biomass production and lipid content. *Nannochloropsis sp* had a thermo tolerant characteristic to local climate condition (22-35^{o}C). DCW and cell population increased 39 and 6.9 times, respectively, higher than initial. Lipid content was 35.71%, averagely. Fatty acids were composed by saturated fatty acids (92.39%) that covering of lauric (20.30%), myristic (12.69%), palmitic (50.05%), and stearic (9.35%) acid P that were suitable for biodiesel. Cultivation method in the outdoor batch system using PBR should be improved to obtain high biomass productivity, high lipid content, and appropriate of fatty acid composition for biodiesel.

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