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Comparison of density, specific growth rate, biomass weight, and doubling time of microalgae *Nannochloropsis* sp. cultivated in Open Raceway Pond and Photobioreactor

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Abstract. Microalgae cultivation can be done in closed system using photobioreactor and open system using raceway pond. Both systems have advantages and disadvantages. The purpose of this research was to compare the density, specific growth rate, biomass weight, and doubling time of microalgae *Nannochloropsis* sp. that was cultivated in open raceway pond and photobioreactor. Microalgae density result in open raceway pond increased from 6.64 x106 cell mL^{-1} in day 0 to 139.89 x 106 cell mL^{-1} in day 10 of observation, while in photobioreactor increased from 10.97 x 10^6 cells mL^{-1} in day 0 of cultivation to 77.11 x 10^6 cells mL^{-1} in day 10. Specific growth rate in open raceway pond were low while in photobioreactor increased exponentially between day 0 and day 1 of observation. Specific growth rate in open raceway pond in the second day was higher than in photobioreactor. Death phase reached faster in photobioreactor compared to open raceway pond. Biomass weight of *Nannochloropsis* sp. in photobioreactor was decreased from 0.2 gr L^{-1} in day 0 to 0.16 gr L^{-1} in day 10, while in open raceway pond they were increased from 0.21 gr L^{-1} in day 0 to 0.33 gr L^{-1} in day 10. Doubling time of *Nannochloropsis* sp. cultivated in open raceway pond (2.27) was faster than in photobioreactor (3.51).
Key Words: exponential phase, death phase, cultivation system, microalgae.

Introduction. Microalgae is a microorganism that can live in freshwater or saltwater. Nowadays, microalgae has been a subject to various research on alternative fuel, such as: biofuel, bioethanol, biohydrogen and biomethane (Kawaroe et al 2010). Microalgae has been suggested as energy source for various reasons: high oil content in several strains, low water consumption and can be reproduced in infertile land (Mascarelli 2009). After the screening of different species, *Nannochloropsis* sp. emerged as a promising candidate for these applications thanks precisely to its high growth rate in a wide range of light irradiations and the ability to accumulate large amounts of lipids (Simionato et al 2011; Simionato et al 2013). Technology in microalgae production can be divided into two major groups: enclosed system and open system. Open system (open raceway pond) is operated outdoor and highly dependent on sunlight for its lighting. Closed system (photobioreactor) can be operated indoor or outdoor, but usually placed outdoor to utilize natural sunlight (Ojamae 2011).

According to Li et al (2008), Pulz (2001), and Richmond (2004) photobioreactor has several advantages: higher microalgae density, higher productivity, prevent contamination, also absorbing direct sunlight and diffused it directly into the medium. But, photobioreactor has also several disadvantages, such as high operational cost (Harun et al 2010; Li et al 2008; Pulz 2001; Richmond 2004), and difficulties in scaled-up due to the light, supply and efficiency. Sunlight intensity that reaches the water surface

will be decreased exponentially as it nears the reactor central area (Luo 2005). Light intensity decreased drastically due to cellular absorbing, scattering and refraction among microorganism cells and cultivation media element (Vincenti & Kruger 1965; Cassano et al 1995). Volume and biomass concentration must be considerably high to cut production cost in large scale photobioreactor. Usually, in a large scale photobioreactor, light penetration inside the reactor will reduced exponentially, thus creating a dark zone in the reactor core and low light intensity area near the reactor surface. Both zone are not conducive for microalgae growth. Excessive light will cause photoinhibition, while light deficiency will cause photolimitation, thus light utilization efficiency is usually low (Pulz & Scheibenbogen 1998).

Open raceway pond also has some advantages and disadvantages. Advantages of raceway pond is easy to be used as the cultivation operating system and building construction (Sanchez et al 2000), and it is also more profitable (Ugwu et al 2008). While its disadvantages are: directly exposed to open environment, evaporation, contamination by algae-eating organisms and less effective use of carbon dioxide caused by evaporation (Chisti 2007). Microalgae productivity can be optimally supported with a research comparing cultivation between photobioreactor and raceway pond, in order to know the most optimal cultivation system for microalgae growth. The objectives of the research were to assess the density cell, specific growth rate, biomass weight, and doubling time of microalgae *Nannochloropsis* sp. cultivated in the photobioreactor and open raceway pond and to determine the best cultivation system.

Material and Method. Research stages consists of making the prototype of photobioreactor and raceway pond, sterilization of tools and materials, cultivation, nutrients (ammonia, nitrite, nitrate and phosphate) and water quality condition analysis, and data processing.

Photobioreactor was constructed using glass material with a design shown in Figure 1. The specification of photobioreactor was using Aquilla water pump with power of electrical voltage of 220-240 V, 50 Hz frequency, 43 watts power, height of spray 2.5 m and maximum water velocity of 2,800 L h^{-1}. This pump was placed in center bottom of the photobioreacor to create circular current inside the photobioreactor. Open raceway pond was built using design by Sanchez et al (2000) (Figure 2). Acrylic plastic was used in constructing this open raceway pond. This pond was placed on top of a table with 1 m height to prevent contamination by soil microorganisms. To protect the pond from rainfall, it was equipped with acrylic roofing. The machine used in this open raceway pond is High Blower with 220-140 V electrical voltage, 50/60 Hz frequency, 100 watts power, WPA model reduction gear type 60.

Nannochloropsis sp. used in the photobioreactor and open raceway pond were previously cultivated in a pond with 200 L capacity for 7 days and 30 ppm urea, 30 ppm amonium sulfat (ZA) and 15 ppm triple super phosphate (TSP) fertilizers were added. Seedling volume are 1/10 of the volume of each system (photobioreactor volume is 350 L and open raceway pond is 260 L).

Data obtained in this research are: cell density, physical and chemical parameters of the medium and biomass weight. These data processed to obtain daily specific growth rate (μ), biomass weight (gr L^{-1}), and doubling time (T_t). *Nannochloropsis* sp. density observed with haemacytometer can be calculated using equation 1:

$$N = \left(\frac{n}{4}\right) \times 10^6 \quad (1)$$

where:
N = microalgae density (cells mL^{-1}), n = amount of microalgae observed.

Daily specific growth rate was calculated using equation 2 (Guillard & Ryhter 1962).

$$\mu = \frac{\ln N_t - \ln N_0}{T_t - T_0} \quad (2)$$

where:
μ = specific growth rate, N_t = microalgae density at t time, N_0 = initial microalgae density, T_o = time of cultivation started, T_t = time of cultivation ended.

Biomass weight was measured using gravimetry method. Weight difference between dry empty filtering paper (put in oven for 30 minutes in 60°C) and paper after filtering *Nannochloropsis* sp. cells, was used as a variable in the following equation (Lin et al 2012) to get the real biomass weight

$$\text{Biomass (gr L}^{-1}) = (A-B)\frac{1000}{mL\ sample} \quad (3)$$

where:
A = weight of paper after filtering, B = weight of paper before filtering.

Doubling time is a period of time needed for a cell population to double its size (Madigan & Martinko (2006) in Engström 2012) and stated in number of days (Velea et al 2011; Devgoswami et al 2011). Doubling time was calculated using equation for specific growth rate referred to Guillard & Ryhter (1962) in equation 2. T in specific growth rate equation is stated in number of days, and can be converted into doubling time per-day (T_t), by dividing natural logarithm value of 2 (0.6931) with as expressed in equation 4.

$$T_t = 0.6931/\mu \quad (4)$$

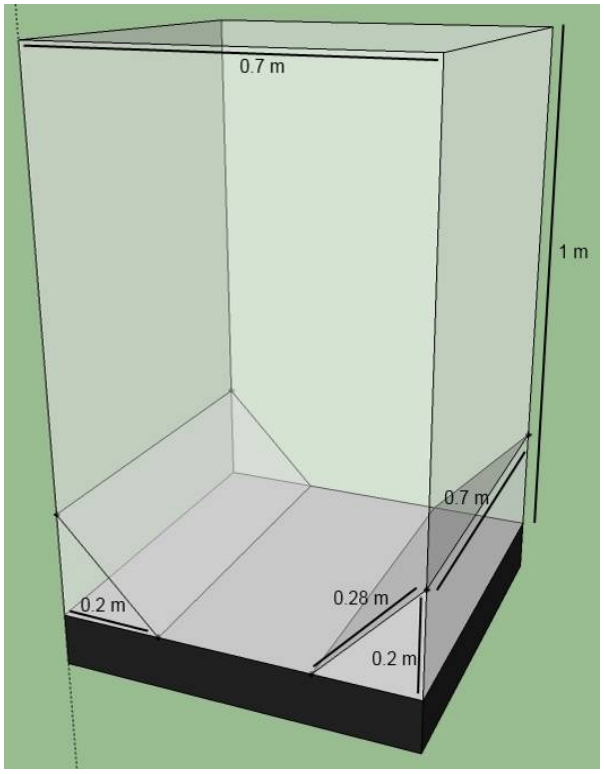

Figure 1. Photobioreactor design.

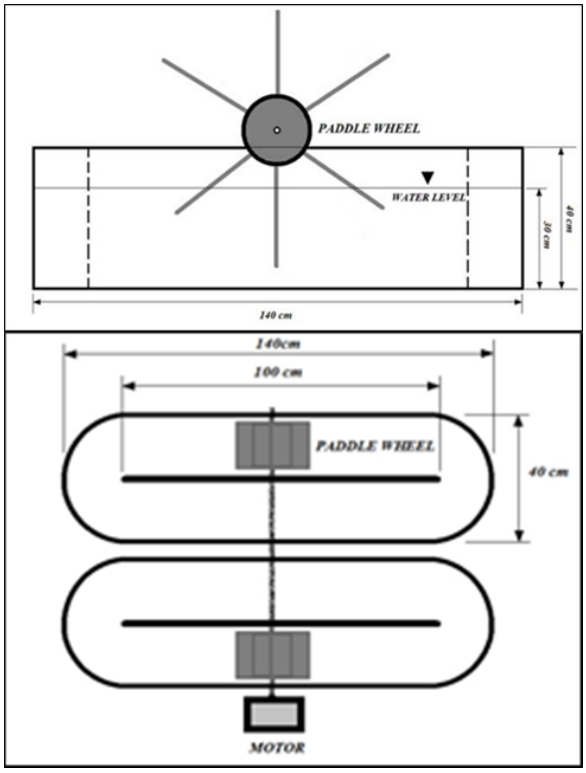

Figure 2. Open raceway pond design.

Nannochloropsis sp. cultivation media to be analyzed first was taken from 200 mL cultivation media and then put into the bottle sample. The sample was put into refrigerator to be chilled after filtered using Whatman filtering paper no. 42. Temperature, salinity and acidity were measured in situ. Ammonia level was analyzed using 4500-NH_3 F (APHA 2005a), nitrite level was analyzed using 4500 NO_2-B (Colorimetric Method) (APHA 2005b). Nitrate level was analyzed using Brucine (APHA 1979). Orthophosphate level was analyzed using 4500 NO_2-B (Colorimetric Method) (APHA 2005b).

This experiment result was statistically analyzed to find out effect of each group differences to the observed parameters. The analysis of variance used in this research was random block design method (Mattjik & Sumertajaya 2006).

$$Yij = a + \tau i + \beta i + \epsilon ij \quad (5)$$

where:
Yij = observation value of treatment-i, group-j, a = population general average,

τi = effect of observed treatment, βj = effect of system treatment,
Єij = experiment error.
The hypothesis are :
H0: β1 = 0 - system difference did not affect the observed responses;
H1: β1 ≠ 0 - system difference affected the observed responses.
If H0 was denied during the testing process which means there is significant difference, Tukey Test will be used further.

Results and Discussion. Temperature during observation ranges between 24-30°C (Figure 3a). Temperature observed in photobioreactor ranges between 28-30°C. At the beginning of cultivation, temperature was at 28°C and increased up to 30°C at the end of cultivation. Temperature observed in open raceway pond during cultivation ranges at 24-25°C. At the beginning of cultivation temperature was at 25°C and then decreased to 24°C and on day 5 was at 25°C until the end. The highest temperature observed during cultivation in both systems was 30°C that occurred on day 3 to day 6, day 8, and day 10 in photobioreactor. The lowest temperature observed on days 1-4 in open raceway pond was at 24°C.

Salinity observed during cultivation ranges between 31-42 ‰ (Figure 3b). Salinity during cultivation in photobioreactor ranges between 31-32 ‰. Salinity in photobioreactor at early cultivation day was 31 ‰ then increased to 32 ‰ at the end of cultivation. Salinity observed in open raceway pond ranges from 32-42 ‰. Salinity at the beginning of cultivation in open raceway pond was at 32 ‰ and increased until 42 ‰ at the end of cultivation. The highest salinity was obtained on day 9th and 10th in open raceway pond, which was 42 ‰. Low salinity was at day 0 and 5 in photobioreactor cultivation, which was 31 ‰.

Acidity level (pH) value during cultivation generally ranged between 8.5–10 (Figure 3c). Acidity was observed in photobioreactor ranged from 8.7–10 and at the begining of cultivation in photobioreactor was 8.7 and then slowly increased to 10 on day 5 and then declined and remained at 9.9 on day 6 until the end of cultivation. Acidity values observed in open raceway pond ranges from 8.5 to 9.7 and at the beginning of cultivation in open raceway pond was at 8.5 then increased gradually until it reaches 9.7 at the end of cultivation. Acidity in photobioreactor cultivation reached 10 at day 5 and 6. The highest pH found in day 5 and 6 in photobioreactor cultivation, which was 10. The pH value was lowest at day-0 cultivation in open raceway pond with 8.5.

Ammonia and orthophosphate content decreased from the beginning of cultivation until the 7th day (Figure 3d and 3g). At photobioreactor observations, ammonia levels decreased from 5.66 mg L^{-1} to 3.487 mg L^{-1} at 7th day. Eventhough, it was increased again until 10^{th} day. While at open raceway pond, ammonia levels decreased from 5.39 mg L^{-1} to 1.31 mg L^{-1}. The highest ammonia level was day 0 in photobioreactor, as much as 5.66 mg L^{-1}. The lowest value was at day 10 in open raceway pond, as much as 1.31 mg L^{-1}. The observation in photobioreactor cultivation, orthophosphate decreased from 0.219 mg L^{-1} to 0.154 mg L^{-1}. While in open raceway pond, an increase of 0.242 mg L^{-1} to 0.723 mg L^{-1} was observed.

Nitrite and nitrate content increased from the beginning of cultivation until the end (Figure 3e and 3f). The observations in photobioreactor cultivation, nitrite levels increased from 0.012 mg L^{-1} to 0.165 mg L^{-1}. The observations in open raceway pond cultivation, nitrite levels increased from 0.007 mg L^{-1} to 0.245 mg L^{-1}. Nitrite levels increased significantly on day 4 to day 7 in open raceway pond cultivation. The observations in photobioreactor cultivation, nitrate levels increased from 0.010 mg L^{-1} to 0.285 mg L^{-1}. While in open raceway pond, nitrate levels increased from 0.061 mg L^{-1} to 0.306 mg L^{-1}.

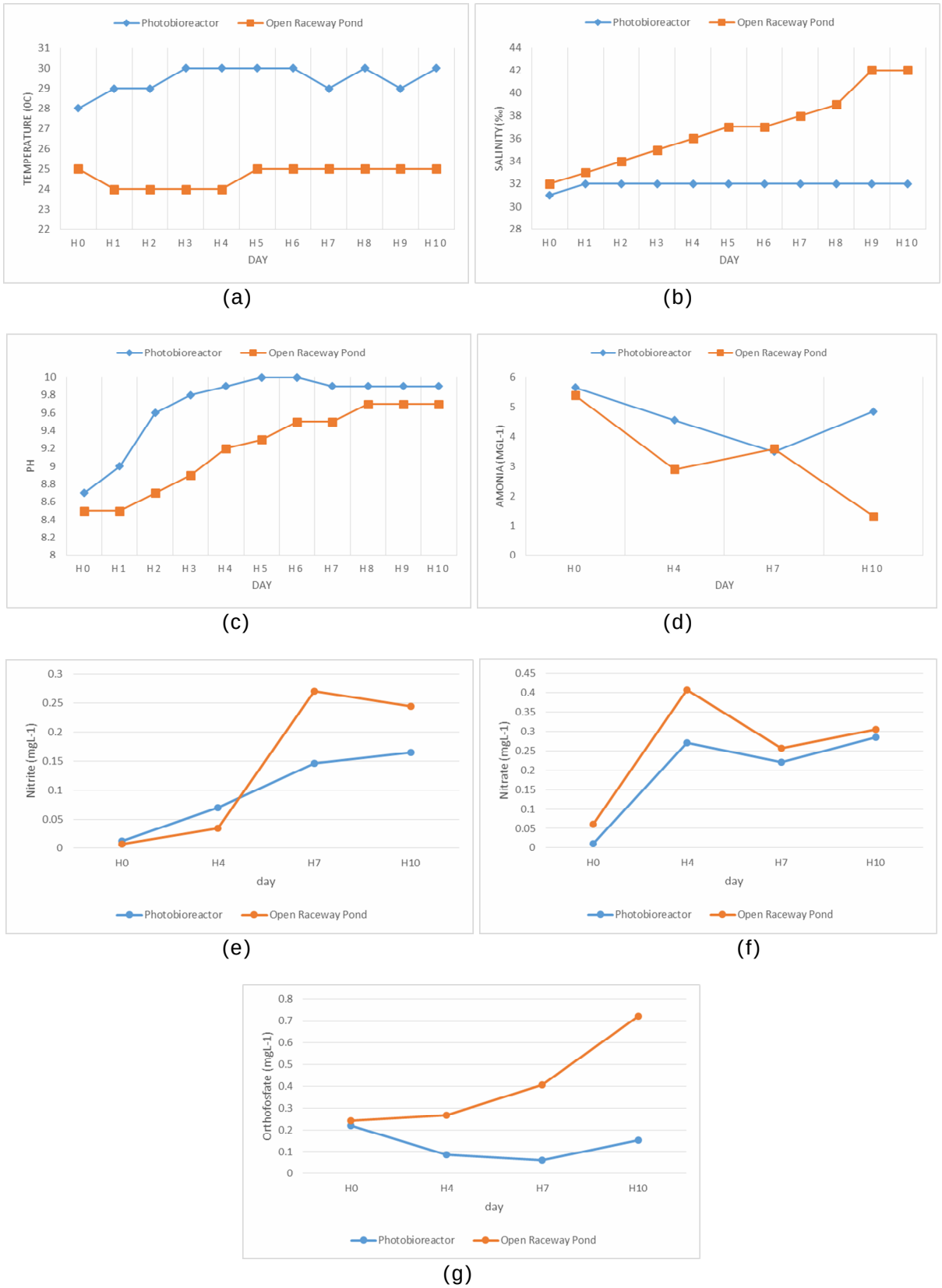

Figure 3a-3g. The quality condition of water as cultivation medium.

The temperature of culture medium in photobioreactor was higher compared to open raceway pond (Figure 3a). This is because photobioreactor is an enclosed mass cultivation system (Behrens 2005; Ojamae 2011; Luo 2005) and prevented any heat transfer between photobioreactor and its surrounding. Open raceway pond is an open

cultivation system where regulation or heat transfer between cultivation medium and its surrounding occurred (Quinn 2011; Luo 2005). Salinity in the open raceway pond (Figure 3b) is higher than photobioreactor. This is probably because open raceway pond is an open system, directly exposed to its surrounding (Quinn 2011; Luo 2005) where evaporation of cultivation medium can increase salinity (Quinn 2011; Borowitzka 2005).

Acidity (pH) increased during cultivation in photobioreactor and open raceway pond (Figure 3c). This is probably caused by *Nannochloropsis* sp. photosynthesis process that consumed CO_2 within the water. That drop of carbon dioxide level caused acidity level increase (Kawaroe et al 2010).

Ammonia level during cultivation showed a declining trend (Figure 3d). High ammonia level in the initial stage is probably because of urea was added. The level then decreased during the cultivation, probably because of ammonification process that turned ammonia into nitrite and nitrate. This is an accordance with Effendi (2003) statement that ammonia level would decrease to partial pressure from increasing pH level. The increase of ammonia level is probably caused by decomposed dead *Nannochloropsis* sp. thus increase the level of organic N within the water and with ammonification process, ammonia level in the culture medium increased. This result is in accordance with a statement from Effendi (2003), that dead and decomposed vegetation and animals is one of organic nutrient sources in the water. The decrease of ammonia level in open raceway pond during cultivation is higher than photobioreactor. This is probably affected by how the system works. In an open system, evaporation is more common when compared to enclosed system. Evaporation is one of the factors of ammonia reduction in the water.

Nitrite existence was unstable due to oxygen supply in the water. As shown in Figure 4e, nitrite level within the culture medium became unstable. It signals that both systems are able to supply oxygen to cultivation medium. Nitrite is a transition form between ammonia and nitrate during the nitrification process. During the experiment, from day-0 to day-4 where ammonification process occurred, marked by nitrite level increase in the medium culture. From day-7 to day-10, where nitrification occurred, was marked by decrease of nitrite level in the cultivation medium and turned into nitrate.

Nitrate level (Figure 3f) observed in day-0 was probably originated from seawater and microalgae seedling. Nitrite level in day-4 has increased, probably because of nitrification process in both systems. Nitrate level, then decreased in day-4 to day-7, probably caused by nitrate was utilized as nutrient by microalgae to grow. Nitrate is the main form of nitrogen in the water and acts as main nutrient source for plant and algae growth (Effendi 2003). Nitrate level increased from day-7 to day-10. This is because of nitrite that was previously formed was turned into nitrate by nitrification process that occurred.

The increase of orthophospate level (Figure 3g) in open raceway pond is probably caused by TSP fertilizer used was not dissolved entirely, still in pallet form, thus its dissolving process was slow. According to Effendi (2003) orthophospate level decrease is caused by orthophospate utilization by microalgae as an essential nutrient for growth. Orthophospate also acts as inhibitor factor for plant and algae. According to Boney (1989), excessive phosphor supply along with nitrogen can cause algae blooming, in this case is accommodate microalgae growth within the medium culture.

The amount of *Nannochloropsis* sp. cells in photobioreactor decreased from day-7 to day-8, and then increased from day-8 to day-9. It is probably because during the decrease many cells died and experienced lysis, thus nutrients secreted to the water was utilized by the remaining cells. This is in accordance with Wei et al (2003) that stated during lysis, cell contents was released into the medium that contributed to organic substrate and utilized by the living microorganism cells.

By comparing between two systems of cultivation, it can be seen that microalgae cells growth in raceway pond system more steady (not up and down) than photobioreactor (Table 1). This can be seen in the fewer growth phase of raceway pond system (phase lag, exponential and stationary) than photobioreactor system (phase lag, exponential, decline, death, and rise again). This indicates the stability of water conditions where microalgae cell grows.

Table 1
Average *Nannochloropsis* sp. cell density during photobioreactor and open raceway pond cultivation

Day	Photobioreactor		Open raceway pond	
	Cell density ($x10^6$ cell mL^{-1})	Specific growth rate	Cell density ($x10^6$ cell mL^{-1})	Specific growth rate
H0	10.97 ± 4.55	-	6.64 ± 1.61	-
H1	16.42 ± 5.98	0.4	7.99 ± 1.32	0.18
H2	30.83 ± 13.54	0.63	14.10 ± 1.32	0.57
H3	35.81 ± 26.30	0.15	32.02 ± 4.04	0.82
H4	35.94 ± 29.17	0.004	23.71 ± 1.00	-0.3
H5	52.03 ± 20.75	0.37	51.86 ± 7.86	0,78
H6	58.72 ± 21.28	0.12	63.23 ± 8.87	0.2
H7	78.22 ± 18.66	0.29	103.38 ± 14.86	0.49
H8	73.86 ± 23.70	-0.06	111.23 ± 29.85	0.07
H9	75.31 ± 25,13	0.02	129.38 ± 20.40	0.15
H10	77.11 ± 26.06	0.02	139.89 ± 14.69	0.08

Lag phase of *Nannochloropsis* sp. in photobioreactor were from day-0 to day-2, exponential phase from day-2 to day-7, declination phase in day-7 for approximately less than 24 hours, and death phase in day-8. However, from day-8 to day 10, there was an increase of number of cells in photobioreactor cultivation. This was possibly caused by an increase of nutrient (nitrate) supply at that period of time. Lag phase of *Nannochloropsis* sp. in open raceway pond were from day-0 to day-3, exponential phase were from day-3 to day-7, declination phase were from day-7 to day-10, as can be seen from the declining growth rate. Stationary and death phase did not occur during the cultivation in open raceway pond. This was probably because of *Nannochloropsis* sp. growth rate in open raceway were good.

The amount of *Nannochloropsis* sp. cells in photobioreactor decreased from day-7 to day-8 but increased from day-8 to day-9. It is probably during the decrease many cells died and lysis, thus nutrients secreted to the water was utilized by remaining cells. This is in accordance with Wei et al (2003) who stated that during lysis, cell contents was released into the medium that contributed to organic substrate and utilized by the living microorganism.

During the last four days of cultivation, cells density in photobioreactor has decreased, while in open raceway pond the density increased. This is probably caused by stress inflicted by air spray from the water pump machine that resulted in declining cells resistance to air bubble burst at the end of water pump and caused deaths. According to Gudin & Chaumont (1991), shearing stress or stress caused by air bubble can cause growth cells is unbalance, damages and even deaths. While the mixing system in open raceway pond, the water paddle that moves in medium speed, did not incurred further stress to microalgae cells living within. This was becaused *Nannochloropsis* sp. cells in open raceway pond still keep growing to the day-10.

The results above indicated that the physical and chemical parameter conditions greatly affected the aquatic microalgae cell density. High ammonia and temperature of photobioreactor system gave negative impact on the increase of cell density. Vy comparing between two systems it showed that the temperature and ammonia concentration in raceway pond tend to be lower than in photobioreactor since the beginning of cultivation. These conditions resulted at cell density in raceway pond which is higher than photobioreactor. The opposite conditions occurred in salinity, the high salinity in raceway pond actually increase the positive impact of microalgae cell density. Likewise, the condition of nitrite, nitrate, and ortophosphate.

Specific growth rate of *Nannochloropsis* sp. cultivated in photobioreactor increased exponentially during day-0 to day-1, while growth rate of cells in open raceway pond was decrease (Table 1). Growth rate in open raceway pond at next days was higher than growth rate in photobioreactor. It was possibly because of microalgae cells stress of air bubble from water pump. The growth rate in photobioreactor was high in initial stages,

but *Nannochloropsis* sp. cell cultivated in photobioreactor reach death phase more quickly compared to microalgae cultivated in open raceway pond.

Biomass weight of *Nannochloropsis* sp. (Table 2) during cultivation in both systems had different results. There was a decrease of biomass weight in photobioreactor system and increased in open raceway pond system. Biomass weight of *Nannochloropsis* sp. cells in photobioreactor decreased from 0.2 gr L^{-1} in day-0 to 0.16 gr L^{-1} in day-10. While biomass weight in the open raceway pond increased from 0.21 gr L^{-1} in day-0 to 0.33 gr L^{-1} in day-10.

Table 2

Dry weight biomass of *Nannochloropsis* sp.

Day	*Biomass weight(gr L^{-1})*	
	Photobioreactor	*Open raceway pond*
H0	0.20 ± 0.02	0.21 ± 0.03
H1	0.18 ± 0.02	0.19 ± 0.01
H2	0.18 ± 0.03	0.19 ± 0.01
H3	0.18 ± 0.02	0.18 ± 0.01
H4	0.19 ± 0.01	0.21 ± 0.01
H5	0.17 ± 0.05	0.24 ± 0.00
H6	0.19 ± 0.09	0.23 ± 0.04
H7	0.17 ± 0.07	0.25 ± 0.04
H8	0.14 ± 0.04	0.25 ± 0.02
H9	0.15 ± 0.05	0.37 ± 0.09
H10	0.16 ± 0.05	0.33 ± 0.03

The decrease of biomass weight in the photobioreactor was possibly caused by the microalgae cells endured some stress from a strong air spray form the aerator machine and resulted in microalgae biomass growth that was not optimum. This is accordant with the statements from Gudin & Chaumont (1991), Tramper et al (1986) and Chisti (2007) that air spray may cause problems in photobioreactor and air spray can damage cells.

Meanwhile biomass weight of *Nannochloropsis* sp. cells cultivated in open raceway pond increased during cultivation and reached its maximum value in day-9. This was possible because of the speed of water pedal rotated not so high, thus did not damage cell that was cultivated in the pond. Based on statistical analysis, biomass weight from both cultivation systems was significantly different.

Doubling time (Table 3) of *Nannochloropsis* sp. cells cultivated in photobioreactor and open raceway pond were different. Based on doubling time value, open raceway pond was better than photobioreactor cultivation system.

Table 3

Doubling time of *Nannochloropsis* sp.

Doubling time (days)	*Photobioreactor*	*Open raceway pond*
	3.51 ± 0.27	2.27 ± 0.10

Doubling time of *Nannochloropsis* sp. cultivated in open raceway pond was 2.27. This means that it needs 2.27 days to multiply their cell, while doubling time in photobioreactor was 3.51 days, or *Nannochloropsis* sp. cells need 3.51 days to multiply. It was maybe because of aerator machine overwhelming stress, thus *Nannochloropsis* sp. needed more time to multiply. While in raceway pond, such stress did not exist, thus *Nannochloropsis* sp. can grow well and needed less time to multiply.

Conclusions. Microalgae cultivated in open raceway pond system had a better result compared to the photobioreactor. It is evident from microalgae cell density, biomass weight and doubling time research. Conditions of physical and chemical parameters of microalgae cultivation media was very influential on the growth of microalgae cells.

Influential parameters were temperature, ammonia, nitrate, nitrite, and orthoposphate. The growth of microalgae cells in open raceway pond was relatively constant from the beginning and continue to increase until the end of cultivation, while on photobioreactor the cell growth have increased and decreased at certain phase so that the growth phase amount was more than open raceway pond. Specific growth rates in both cultivation systems were not significantly different. Biomass weight and doubling time of *Nannochloropsis* sp. cells in photobioreactor were significantly different from the ones in open raceway pond. This research suggests that the best cultivation system is open raceway pond.

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