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Enhancing the Removal of Highly Concentrated CO_2 Through Synergism between Microalgae Consortium and Nutrient Ratio in Photobioreactor

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This research was carried out by developing the carbon capture and storage (CCS) technology to determine the synergism between microalgae consortium and the optimum nutrient ratio as an effort to obtain higher CO_2 removal efficiency and CO_2 utilization efficiency. The microalgae consortium consisting of *Chlorella* sp., *Scenedesmus* sp., and *Ankistrodesmus* sp. have been selected previously as potential candidates for Microbial Carbon Capture and Storage (MCCS) agent and already cultured continuously in PHM (Provasoli Haematococcus Media) artificial medium, in vertical column photobioreactor. Pure CO_2 gas at a high concentration of 10% (v/v) flowed from the bottom of vertical column photobioreactor continuously with optimum flow rate of 5 $L.min^{-1}$. A growth medium (PHM) containing artificial nutrients was flowed continuously at flow rate 7 $L.day^{-1}$ and detention time 3.8 days. Four fluorescent lamps were positioned outside the photobioreactor to obtain light intensity of 4000 lux, set for 16 hours light exposure and 8 hours dark, with operating temperature 30 °C maintained during the study. Three compositional variations of microalgae consortium were used. They are as follows; Ch : Sc : An = 1: 1: 1; Ch : Sc = 1: 1; and Ch : An = 1: 1, where Ch, Sc, and An were *Chlorella* sp., *Scenedesmus obliquus*, and *Ankistrodesmus* sp., respectively. The following variations of nutrient composition were used; C: N: P = 100: 10: 1, C: N: P = 100: 50: 1 and C: N: P = 100: 25: 1. The C, N, and P sources were CO_2 (inorganic), KNO_3, and KH_2PO_4, respectively. This study proves that synergism between the types making up the consortium also determined the ability to utilize inorganic carbon source. Without the presence of *Ankistrodesmus* sp., synergism between *Scenedesmus obliquus* and *Chlorella* sp. showed twice higher CO_2 utilization efficiency in comparison to the synergism between *Ankistrodesmus* sp. and *Chlorella* sp. Increased nitrogen concentration in medium increased the growth of *Chlorella* sp. and *Scenedesmus obliquus* as a consortium, the CO_2 removal efficiency, the CO_2 utilization efficiency and the Carbon Uptake Rate. The nutrient ratios C:N:P of 100:50:1 could increase CO_2 utilization efficiency upto 50% higher than the C:N:P of 100:10:1.

Key words: CO_2 removal, CO_2 utilization, constructed consortium, nutrient ratio, synergism

Untuk memperoleh rasio nutrisi yang optimal sebagai upaya untuk mendapatkan efisiensi removal dan efisiensi pemanfaatan CO_2 yang lebih tinggi, konsorisum miroba yang terdiri dari *Chlorella* sp., *Scenedesmus obliquus* dan *Ankistrodesmus* sp. dipilih sebagai agen potensial untuk menerapkan metode MCCS (*Microbial Carbon Capture and* Storage) dan dikultur dengan sistem kontinyu di dalam fotobioreaktor kolom vertikal yang berisi media PHM (Provasoli Haematococcus Media). Gas CO_2 10% (v/v) dan media PHM dialirkan terus menerus dengan laju alir masing-masing 5 $L.menit^{-1}$ dan 7$L.hari^{-1}$ dengan waktu detensi 3,8 hari. Empat lampu (4000 lux) ditempatkan di luar fotobioreaktor, paparan cahaya selama 16 jam terang dan 8 jam gelap, suhu dipertahankan 30 °C selama penelitian. Tiga variasi komposisi jenis mikroalga yang diuji *Chlorella* sp (Ch) : *Scenedesmus obliquus* (Ch) : *Ankistrodesmus* sp. (An) = 1: 1: 1; Ch: Sc = 1: 1; dan Ch: An = 1: 1. Variasi komposisi nutrien yang digunakan adalah Carbon (C) : Nitrogen (N): Phospor (P) = 100: 10: 1, C: N: P = 100: 50: 1, dan C: N: P = 100: 25: 1. Sumber C, N, dan P masing-masing adalah CO_2 (anorganik), KNO_3, dan KH_2PO_4. Studi ini membuktikan bahwa sinergisme ditentukan oleh kemampuan jenis mikroalga dalam memanfaatkan sumber karbon anorganik. Tanpa kehadiran *Ankistrodesmus* sp., sinergisme antara *Scenedesmus obliquus* dan *Chlorella* sp. menunjukkan efisiensi pemanfaatan CO_2 dua kali lebih tinggi dibandingkan dengan sinergisme antara *Ankistrodesmus* sp. dan *Chlorella* sp. Rasio nutrien C: N: P sebesar 100: 50: 1 dapat meningkatkan efisiensi pemanfaatan CO_2 hingga 50% lebih tinggi dibandingkan rasio 100: 10: 1.

Kata kunci: konsorsium terbangun, pemanfaatan CO_2, penyisihan CO_2, rasio nutrien, sinergisme

The global warming issue, mainly caused by carbon dioxide (CO_2) as the major contributor of greenhouse effects, has triggered various efforts to reduce excess amount of CO_2 emitted into the atmosphere. Due to the ability of microalgae to capture CO_2 during the process of photosynthesis, the algae are now being developed into biological or biotechnological Carbon Capture and Storage (CCS) to reduce carbon emissions that would otherwise be

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released into the atmosphere. The carbon capture process works based on the carbon requirement for the process of photosynthesis. Microalgae consortium is a potential producer that requires CO_2 continuously to perform photosynthesis reaction. Compared to various terrestrial plants, microalgae are generally considered photosynthetically more efficient. Chojnacka and Marquez-Rocha (2004) described that there are various types of microalgae based on their metabolism, *i.e.* autotrophs, heterotrophs, mixotrophs, photoheterotrophs, and able to change its metabolism as a form of adaptation or response to changing environmental conditions. Autotroph microalgae in natural environment, that use inorganic CO_2 from the air and the sun as the main energy source, could change into mixotroph that utilize organic compounds and inorganic CO_2 for growth in close system photobioreactor. Beside that, autotroph microalgae able to change into heterotroph as well when only organic CO2 that naturally available to be transformed become another organic matters. It means the microalgae can change its metabolism as a response to changing environmental conditions. Microalgae can utilize inorganic CO_2 from three different sources, *i.e.* fixing CO_2 directly from the atmosphere, taking up CO_2 from industrial waste gas, and taking up CO_2 from dissolved carbonate (Wang *et al.* 2008).

Microalgae growth is strongly influenced by environmental conditions and the availability of nutrients. Carbon ©, nitrogen (N) and phosphorus (P) are macronutrients much needed by microalgae for making proteins. To achieve their optimum growth, source selection and concentration of nutrients must be adapted to the characteristics of strains of microalgae (Westerhoff *et al.* 2010). Many types of microalgae can utilize carbonate ions in the form of Na_2CO_3 and $NaHCO_3$ as sources of C. Other research shows slow algal growth in medium without N (Burkhard *et al.* 1999). Therefore we need extra N to increase the rate of cell proliferation. N sources commonly used for the cultivation of microalgae is in the form of nitrate, ammonia, urea, or a combination of them. Nitrogen can be obtained from KNO_3, $NaNO_3$, or NH_4Cl (Cuaresma *et al.* 2010). Phosphorus is also the base material forming nucleic acids, enzymes, and vitamins that can be obtained from KH_2PO_4, NaH_2PO_4, or Ca_3PO_4, whereas elemental sulfur can be obtained from NH_4SO_4, or $CuSO_4$. The metabolism of these elements; C, N, and P, are related to one another. Phosphate (P) plays a central role in cellular energy transfer. Low concentration has an impact on the balance in C and N assimilation. Potassium (K) plays a role in the metabolism of carbohydrates and also a cofactor for several coenzymes. Potassium can be obtained from KCl, KNO_3, or KH_2PO_4. Iron (Fe) plays a role in the formation of chlorophyll and is an essential component in oxidation. This element can be obtained from $FeCl_3$, $FeSO_4$, or $FeCaH_5O_7$ (Graham and Wilcox 2000). Elements Si and Ca are all ingredients of cell walls or shells. Micro nutrients are also needed to perform various functions in the growth of microalgae. Mn and Zn are necessary for photosynthesis. Mo, Bo, Co are required for the metabolism of nitrogen, while Mn, B, Cu are required to perform other metabolic functions (Westerhoff *et al.* 2010).

The aim of this study was determining the composition of microalgae consortium and the optimum nutrient ratios to obtain higher CO_2 removal efficiency.

MATERIALS AND METHODS

Microalgae Consortium and Artificial Growth Medium. The consortium of green microalgae consisting of *Chlorella* sp., *Scenedesmus* sp., and *Ankistrodesmus* sp. was originally isolated from the Bojong Soang wastewater treatment plant, Bandung, Indonesia. The microalga was screened, and then a potential candidate was selected as Microbial Carbon Capture and Storage (MCCS) agent (Rinanti *et al.* 2013). The microalgal cells were cultured in PHM (Phovasoli Haematococcus Media) artificial medium, which was prepared as follows (per litre): 1.0 g KNO_3, 0.2 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g Fe stock consisting of (per litre) 189 g EDTA and 24.4 g $FeCl_3.6H_2O$. All nutrients were dissolved in distilled water containing (per litre) 0.1 mL trace element made of (per 500 mL) 2.05 mg $ZnCl_2$, 30.5 mg H_3BO_3, 2.55 mg $CaCl_2.6H_2O$, 3.0 mg $CuSO_4.5H_2O$, 2.05 mg $MnCl_2.4H_2O$ and 19 mg $(NH_4)_6.Mo_7.O_{24}.4H_2O$ (Provasoli and Pintner 1959).

Cultivation of Microalgae in Vertical Column Photobioreactor. The vertical photobioreactor used in this experiment was made of glass with a capacity of 10 L containing 8 L PHM growth medium and an initial cell density of 10^6 cell.mL^{-1}. CO_2 was injected from the bottom of the column to allow gas mixing with the medium. Sparger was attached at the bottom of the photobioreactor to convert the gas into small bubbles. Air was bubbled at the bottom. Sparging with microbbuble allows thorough mixing, CO_2 mass transfer and also removes O_2 produced during photo-

synthesis. It was a strategy to provide good overall mixing, sufficient supply of CO_2, and efficient removal of O_2. Pure CO_2 gas at 10% (v/v) was flowed from the bottom of vertical column photobioreactor continuously with optimum flow rate 5 $L.min^{-1}$. A growth medium containing artificial nutrients (PHM) was flowed continuously at a flow rate 7$L.day^{-1}$ and the detention time of 3.8 days. Four fluorescent lamps were positioned outside the photo-bioreactor to obtain light intensity of 4000 lux, set for 16 hours light exposure and 8 hours dark with operating temperature of 30 °C maintained during the study (Rinanti *et al.* 2014). Three compositional variations of microalgae consortium were used. They are as follows; Ch : Sc : An = 1: 1: 1; Ch : Sc = 1: 1; and Ch : An = 1: 1, where Ch, Sc, and An were *Chlorella* sp., *Scenedesmus obliquus,* and *Ankistrodesmus* sp., respectively. The following variations of nutrient composition were used; C: N: P = 100: 10: 1, C: N: P = 100: 50: 1 and C: N: P = 100: 25: 1. The C, N, and P sources were CO_2 (inorganic), KNO_3, and KH_2PO_4, respectively.

Measurement of Biomass Dryweight. To measure the biomass dryweight, the microalgal culture must be dried by evaporating the liquid in the culture. Prior to the evaporation, culture was centrifuged in 100mL tubes at 3,500 rpm for 10 minutes (Weldy and Huesemann 2007). Supernatant was then removed from the tube and the cell pellet was kept. The pellet was then placed in a petri dish that had previously been weighed (x). The sample-containing petri dishes were then kept in the oven set at 105 °C overnight until constant weight (y) was reached. Then, the sample was left in a desiccator for 30 minutes to evaporate any remaining liquid before re-weighed. Biomass (dry weight) was calculated according to the formula from Torzillo *et al.* (1991):

dry weight (X; mg) = y (mg) - x (mg).

Specific growth rate (μ; d^{-1}) was calculated as follows:

$$\mu = \frac{1}{X}\frac{dX}{dt} \quad (1)$$

Measurement of CO_2 Concentration and Determination of CO_2 Removal Efficiency. The CO_2 concentration in the influent gas and effluent gas was measured by Portable Combination Gas Detector RIKEN Model RX-515. Efficiency of CO_2 removal can be calculated by the following formula:

$$\%\ CO_2\ \text{removal efficiency} = \frac{\text{Influent of } CO_2 - \text{Effluent of } CO_2}{\text{Influent of } CO_2} \times 100\% \quad (2)$$

Carbon Uptake Rate. A formula ($CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$) suggested by Grobbelaar (2004) was used to make an expected estimate of the dry biomass yield and carbon uptake rate was determined by using the following equation:

$$\text{Carbon uptake rate} = C \times P \quad (3)$$

where C is the carbon content of the dried cell (g carbon.g $biomass^{-1}$), P is the productivity (g $biomass.L^{-1}d^{-1}$). Results of elemental analysis in our study showed that the carbon content in the mix culture was 67.56%**.**

CO_2 Utilization Efficiency. CO_2 utilization efficiency was determined by using the following equation (Ryu *et al.* 2009):

$$\%\ CO_2\ \text{utilization efficiency} = \frac{\text{carbon content} \times P \times M_{CO_2}}{\text{aeration rate of } CO_2} \times 100\% \quad (4)$$

where C is the carbon content of the dried cell (g carbon.g $biomass^{-1}$), P is the productivity (g $biomass.L^{-1}.h^{-1}$), 44 and 12 are the molecular weights of carbon dioxide and carbon, respectively, and V is the rate of CO_2 supplied to the microalgal culture medium (g $CO_2.L^{-1}.h^{-1}$).

RESULTS

Species Variation of Microalgae Consortium. The three species of microalgae composing the consortium used in this study, *Chlorella* sp., *Scenedesmus obliquus*, and *Ankistrodesmus* sp., seemed to have different physiological characteristics. As mentioned by Chojnacka and Marquez-Rocha (2004), every species of microalgae carries specific physiological properties. Although these three species are capable of living synergistically by utilizing highly concentrated inorganic carbon source (10% v/v) together, synergism between *Chlorella* sp. and *Scenedesmus obliquus* showed better growth rate compared to the synergism between *Chlorella* sp. and *Ankistrodesmus* sp. (Fig 1, Fig 2, and Table 1).

Determination of the Optimum Nutrient Ratios. Previous studies reported about algal cell vitality reduction due to the deficiency of various nutrients. This is related to the loss of the cell's ability to build functional structures associated with the limited amount of nutrients. Hence, the following attempt to improve the CO_2 removal efficiency was done by adding different concentrations of nitrogen to the growth medium (artificial PHM). The CO_2 removal efficiency increased in accordance to the increment of nitrogen addition. Nutrient ratio C : N : P of 100 : 50 : 1 was found to be the optimum ratio and was very sufficient for the

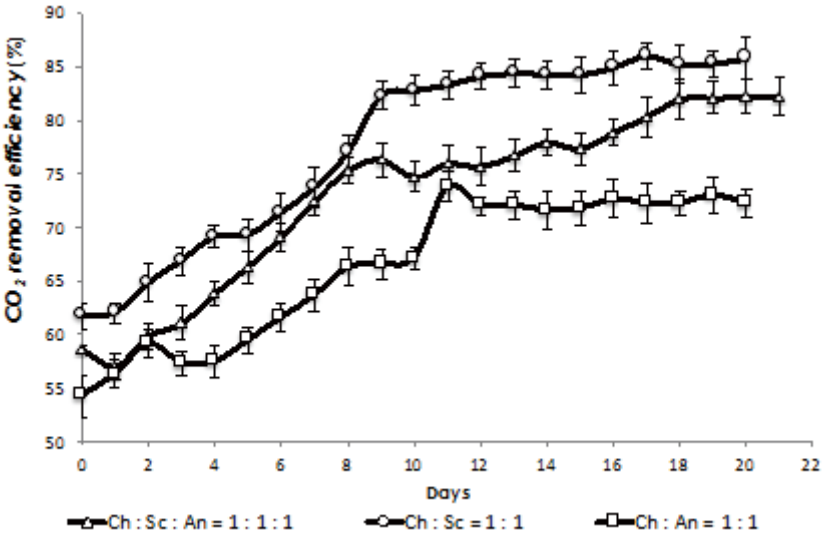

Fig 1 Profile of CO_2 removal efficiency (%) as a function of different species composition in microalgae consortium. (Ch = *Chlorella* sp., Sc = *Scenedesmus obliquus*, An = *Ankistrodesmus* sp.).

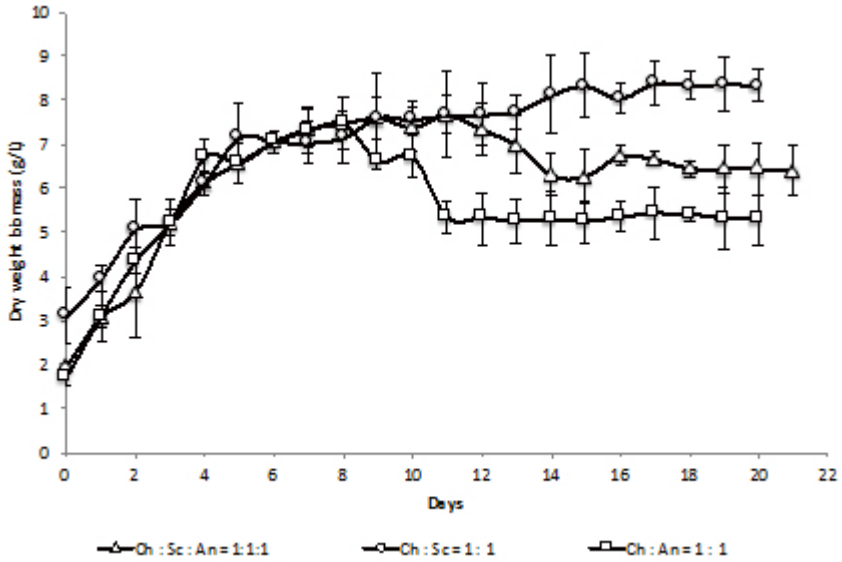

Fig 2 Profile of biomass dry weight (g/L) as a function of different species composition in microalgae consortium. (Ch = *Chlorella* sp.; Sc = *Scenedesmus obliquus*; An = *Ankistrodesmus* sp.).

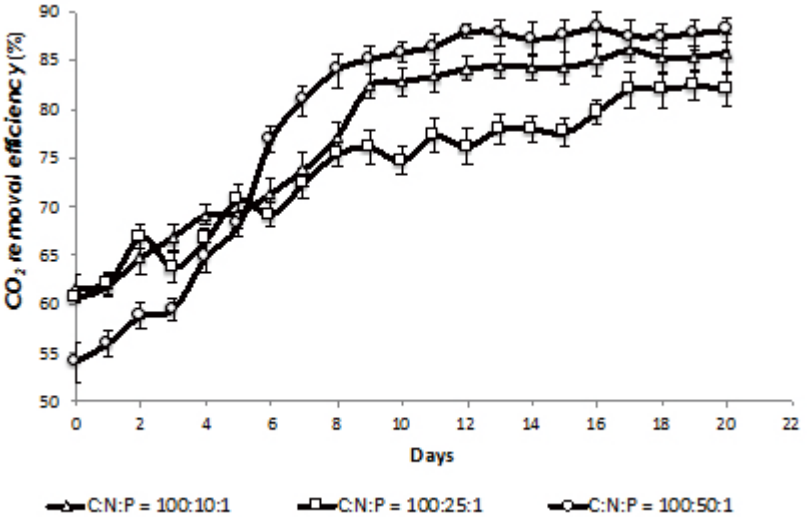

Fig 3 Profile of biomass dry weight (g/L) as a function of different species composition in microalgae consortium. (Ch = *Chlorella* sp.; Sc = *Scenedesmus obliquus*; An = *Ankistrodesmus* sp.).

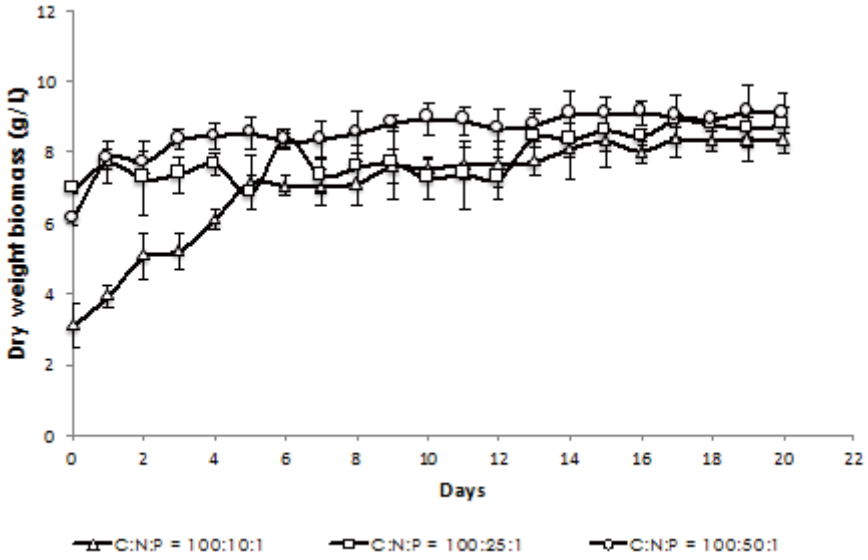

Fig 4 Profile of biomass dry weight (gL^{-1}) as a function of various nutrients composition. (C = carbon, N = nitrogen, P = phosphor).

Table 1 The effects of species variation in microalgae consortium and nutrient ratios towards CO_2 removal and utilization efficiency, biomass dry weight and carbon uptake rate.

Species variation in microalgae consortium*)			Nutrient ratios**)		
CO_2 removal efficiency (%)	Ch : Sc : An = 1 : 1 : 1	80.34	CO_2 removal efficiency (%)	C : N : P = 100 : 10 : 1	84.33
	Ch : Sc = 1 : 1	84.33		C : N : P = 100 : 25: 1	86.03
	Ch : An = 1 : 1	72.33		C : N : P = 100 : 50 : 1	87.63
Biomass dry weight (gL^{-1})	Ch : Sc : An = 1 : 1 : 1	6.52	Biomass dry weight (gL^{-1})	C : N : P = 100 : 10 : 1	8.01
	Ch : Sc = 1 : 1	8.01		C : N : P = 100 : 25: 1	8.36
	Ch : An = 1 : 1	5.32		C : N : P = 100 : 50 : 1	8.86
CO_2 utilization efficiency (%)	Ch : Sc : An = 1 : 1 : 1	3.70	CO_2 utilization efficiency (%)	C : N : P = 100 : 10 : 1	4.68
	Ch : Sc = 1 : 1	4.68		C : N : P = 100 : 25 : 1	4.94
	Ch : An = 1 : 1	2.86		C : N : P = 100 : 50 : 1	5.22
Carbon uptake rate (g Carbon $L^{-1}day^{-1}$)	Ch : Sc : An = 1 : 1 : 1	1.13	Carbon uptake rate (g Carbon $L^{-1}day^{-1}$)	C : N : P = 100 : 10 : 1	1.40
	Ch : Sc = 1 : 1	1.40		C : N : P = 100 : 25: 1	1.51
	Ch : An = 1 : 1	0.87		C : N : P = 100 : 50 : 1	1.59

*) Ch = *Chlorella* sp.; Sc = *Scenedesmus obliquus*; An = *Ankistrodesmus* sp.)
**) C = CO_2 (inorganic), N = KNO_3, P = KH_2PO_4

growth of *Chlorella* sp and *Scenedesmus obliquus* consortium, consequently the highest CO_2 removal efficiency of 87.63% was obtained when this ratio was applied (Fig 3 and Table 1).

Increased dry weight of the microalgae biomass occured in all tested nutrient ratio variations. In general, the higher the concentration of nitrogen, the higher the dry weight of the biomass produced. Nutrient ratio C : N : P of 100 : 50 : 1 yielded an average biomass dry weight of 8.86 (gL^{-1}) at steady state condition (Fig 4). All variations of nutrient ratio reached steady state starting from day 7 until the end of the study.

DISCUSSION

Species Variation of Microalgae Consortium. Synergism of *Chlorella* sp. and *Scenedesmus obliquus* gave the highest CO_2 removal efficiency (84.33%) and biomass dry weight (8.1 $g.L^{-1}$) compared to the other synergism. Likewise, the synergism of these two green microalgae gave a higher CO_2 utilization efficiency and carbon uptake rate than the synergism of other microalgae species in this study.

Extremely high CO_2 concentration can cause physiological and metabolic changes consisting of stomatal density reduction, low availability of Rubisco enzyme and chlorophyll, also low photorespiration

(Papazi *et al.* 2008). The microalgae species that is able to thrive in a highly concentrated CO_2 environment may still have the genetic ability to photosynthesize (Papazi *et al.* 2008). The species that can adopt transitional state in supporting the photosystem is able to grow well at high CO_2 concentration (Miyachi *et al.* 2003). Microalgae also perform molecular mechanism in response to high CO_2 concentration by changing the photosynthesis mode through Carbon Concentrating Mechanism. Saturated or half-saturated CO_2 concentration can change the characteristics of cellular photosynthesis, for example, the affinity of CO_2 reached 0.5% in *Chlorella kessleri* (Papazi *et al.* 2008).

Composition of microalgae species making up the consortium found in this study indicated that the highest CO_2 utilization efficiency occurred in cultures containing equal amount of *Chlorella* sp. and *Scenedesmus obliquus*, without *Ankistrodesmus* sp.. In the next stage, we tried to find the optimum nutrient ratio to obtain higher CO_2 utilization efficiency by culture containing equal amount of *Chlorella* sp and *Scenedesmus obliquus*.

Determination of the Optimum Nutrient Ratios. A proper and sufficient nutrient ratio remarkably affects the CO_2 removal efficiency. Nonetheless, it was not significantly different from nutrient ratio with C : N : P = 100 : 25 : 1, which produced 86.03% CO_2 removal efficiency. Considering these results, adding higher nitrogen concentration were not expected to increase the CO_2 removal efficiency, and hence was not studied.

Microscopical observation showed that the size of *Chlorella* cells tends to be bigger in the culture with high concentration of CO_2 gas (10% v/v). Therefore, despite the relatively lower cell density in 10% (v/v) CO_2 concentration, a higher biomass dry weight was achieved (Fig 4). It is easily understandable because with adequate supply of carbon, microalgae have greater opportunities to thrive than with limited carbon supply. This result is similar to the studies carried out by Yoo *et al.* (2010) and Chiu *et al.* (2008), where pure 10% CO2 was also streamed into the reactor. Thus, an optimum CO_2 concentration of 10% is needed to gain high productivity of microalgae biomass.

Zhu *et al.* (2010) explained that the right composition of nutrients is one of the main factors affecting microalgal biomass productivity, in addition to light intensity, temperature, and CO_2 mass transfer into the liquid (Ono and J. Cuello 2006).

The efficiency of CO_2 utilization improves in accordance to the escalation of nitrogen source concentration in the growth medium. The highest CO_2 utilization efficiency of 5.22% occured in the culture with C : N : P ratio of 100 : 50 : 1. Chojnacka and Marquez-Rocha (2004) described that microalgae could perform various types of metabolism, *i.e.* autotroph, heterotroph, mixotroph, and photoheterotroph, and able to perform metabolic alteration as an adaptation measure or response to shifting environmental conditions. During this study, it seemed that the microalgae consortium comprising of *Chlorella* sp and *Scenedesmus obliquus* was mixotroph in conducting photosynthesis as the main energy source, yet it could maximize the utilization of nitrogen, organic compounds, and inorganic CO_2 for its growth.

Adequate nutrient availability for microalgae is a precondition for optimum photosynthesis. Nutrient deficiency will cause interferences with metabolism and production discrepancy in intermediary phase of photosynthesis. However, the correct ratio of carbon, nitrogen, and phosporus as the main nutrients also immensely affects the metabolism, for instance affecting Carbon Uptake Rate. In this study, carbon uptake rate was defined as the absorption rate of CO_2 dissolved in the PHM growth medium by microalgae consortium. When the photosynthesis took place, pure CO_2 gas flow of 10% at 5 L/min flow rate was the primary source of inorganic carbon consumed by microalgae to generate energy. The rate of CO_2 uptake by microalgae cells was allegedly stimulated by an increase of CO_2 content in the media, so that the culture without CO_2 addition absorbed less CO_2, escalating the culture pH, yet had never exceeded value of 8. Stepan *et al.* (2002) stated that media pH ranging from 7.0–8.0 is good enough for laboratory microalgae culture. Accordingly, the media pH was maintained at 7 during the study. Microalgae commonly use nitrate as the primary nitrogen source. Shi *et al.* (2007), Bich *et al.* (1999), and Qing-xue *et al.* (2010) explained that nitrogen compounds are strongly influenced by dissolved oxygen concentration in the water. Nitrogen turns into ammonia (NH_3) at low-level dissolved oxygen and into nitrate (NO_3) at high-level dissolved oxygen. Nonetheless, when the environmental conditions are not favorable, then ammonia or urea can serve as nitrogen source (Sassano 2007; Soletto 2005). The lack of nitrogen content will result in the limited production of proteins needed for new cells (Westerhoff *et al.* 2010).

This study proved that in addition to the specific characteristics of microalgal cells' physiology, synergy between the types making up the consortium also

determine the ability to utilize inorganic carbon source. *Chlorella* sp have the highest synergy to interact together well with the *Scenedesmus obliquus* or *Ankistrodesmus sp*. Without the presence of *Ankistrodesmus* sp, synergism between *Scenedesmus obliquus* and *Chlorella* sp. provides twice higher CO_2 utilization efficiency than the synergism between *Ankistrodesmus* sp and *Chlorella* sp without the presence of *Scenedesmus obliquus*.

Increased nitrogen proved to increase the growth of *Chlorella* sp and *Scenedesmus obliquus* as a consortium in line with the increase of CO2 removal efficiency, CO2 utilization efficiency and carbon uptake rate. The availability of macro nutrients, inorganic carbon, nitrogen, and phosphorus, in the appropriate ratio (C: N: P of 100: 50: 1) may increase CO_2utilization efficiency 50% higher than when the C: N: P ratio was 100: 10 : 1. However, the efficiency was not significantly different from when the C: N: P was 100: 25: 1.

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