CHAPTER 4

Effect of different CO_2 aeration rates on CO_2 fixation efficiency of microalgal consortium

4.1 Introduction

CO_2 reduction by using microalgae has been extensively researched in a present. So, the CO_2 fixation efficiency of microalgae is very important and it is the main factors for estimated the efficacy of CO_2 reduction. The CO_2 fixation rate of microalgae was associated with the photosynthesize efficiency and cell density of microalgae. Also, CO_2 biofixation efficiency of microalgae depending on microalgal species, nutrients supply, light intensity, temperature, pH, photo-bioreactor type, CO_2 concentration and CO_2 flow rate (Chiu *et al*., 2008; Cheng *et al*., 2013; Li *et al*., 2013).

Aeration rate of CO_2 is a major parameter affecting on CO_2 mass transfer into the photosynthesis system and promote the growth of microalgae. The suitable CO_2 aeration rate has many positive impacts to the microalgal culture, including: (i) supply CO_2 to the culture that can lead to enhanced algal metabolisms; (ii) control of pH that increased by algal growth; and (iii) enhances the internal mixing of bioreactor that helping to evenly distribute nutrients and helping microalgal cells in the culture to receive the light for photosynthesis process (Kumar *et al.*, 2010; Ying *et al*., 2013). Therefore, CO_2 aeration rate is very important and deserve more detailed investigation in the cultivation of microalgae for enhance CO_2 fixation ability and lipid production. In Chapter 3, the microalgal consortium (MC) showed the highest growth rate and CO_2 fixation rate under 30% CO_2 level and 0.2 vvm of aeration rate. So, this work investigated the effect of CO_2 aeration rate on CO_2 fixation of MC. The cultures were aerated with 30% (v/v) CO_2 at different aeration rates. The biomass and lipid content also evaluated.

4.2 Materials and methods

The methods for study effect of different CO_2 aeration rates on CO_2 fixation efficiency of microalgal consortium was summarized in Figure 4.1

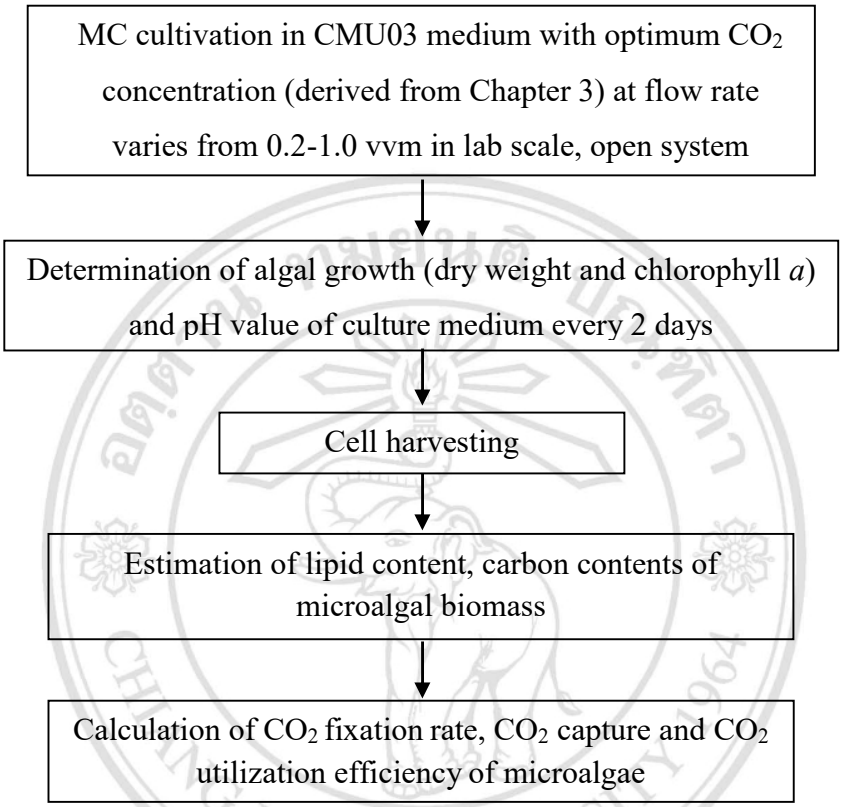


Figure 4.1 Flowchart diagram of microalgal cultivation for studying the effect of different CO_2 aeration rates on CO_2 fixation efficiency of MC

4.2.1 Microalgae and culture

MC was prepared by succession of air borne cultures enriched with CMU03 medium (Sriphuttra *et al*., 2013) and maintained in the same medium at the algal collection of Applied Algal Research Laboratory (AARL), Department of Biology, Faculty of Science, Chiang Mai University. The MC (composed of 35.5% *Monoraphidium contortum*, 26.8% *Chlorella vulgaris*, 19.3% *Acutodesmus dimorphus*, 10% *Carteria* sp., 3.5% *A. pectinatus*, 2.2% *Desmodesmus quadricauda*, 1.5% *A. obliquus* and 1.2% *Planktolyngbya limnetica*) were incubated in CMU03 medium at ambient temperature under continuous illumination.

4.2.2 Cultivation of MC

The MC was cultivated in 1 L Erlenmeyer flask with 500 mL working volume of CMU03 medium under ambient temperature and continuous illumination with a fluorescent lamp at 24.3 µmol m^{-2} s^{-1}. The cultures were aerated continuously with 30% (v/v) CO_2 at different aeration rates: 0.2, 0.4, 0.6 and 1.0 vvm. The microalgal cultivation system is presented schematically in Figure 4.2.

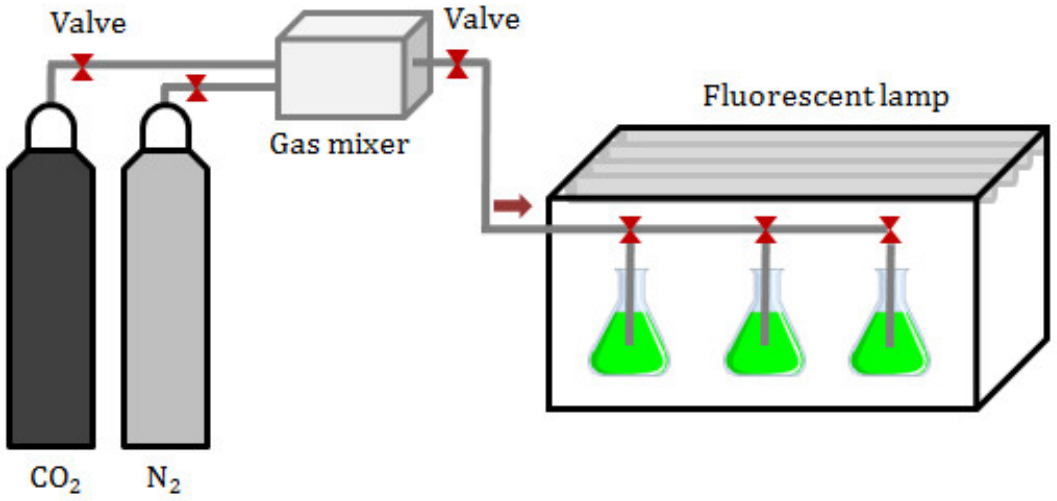


Figure 4.2 Schematic diagram of cultivation of MC under different CO_2 aeration rates

The 50 mL of culture samples was collected to determine the microalgal growth and the constant volume of culture medium was maintained by adding an equivalent volume of new medium to sample. The growth of microalgae and pH value of the culture medium was measured in every 2 days until it reached the early stationary phase. The pH value of the culture medium was measured using a pH meter (electrode kit of WTW Company). The dry weight, chlorophyll *a* content, dominant species, lipid content, carbon contents and CO_2 fixation rate were analyzed as the procedures in Chapter 3.

The CO_2 utilization efficiency was calculated using the following equation (Ryu *et al.*, 2009):

$$CO_2 \text{ utilization efficiency } (\%) = C_C \times P \times \left(\frac{M_{CO_2}/M_C}{V_{CO_2}}\right) \times 100$$

Where C_C is the carbon content (g dw^{-1}), P is the biomass productivity (g L^{-1} d^{-1}), M_{CO_2} and M_C are the molecular weights of CO_2 and elemental carbon, respectively, and V_{CO_2} is the aeration of CO_2 supplied to the culture medium (g CO_2 L^{-1} d^{-1}).

4.2.3 Statistic analysis

The results are expressed as mean±SD (standard deviation) of three replicates. All data were performed by SPSS version 16.0 for Windows. One-way analysis of variance (ANOVA) and least significant difference (LSD) test was used to evaluate differences among the four CO_2 aeration rates. A value of p<0.05 was considered statistically significant.

4.3 Results and discussions

4.3.1 Microalgal growth

The MC was cultivated with 30% v/v CO_2 in different aeration rates. During the cultivation period, the ambient temperature ranged from 23.5 to 35ºC. The effect of CO_2 aeration rates on the biomass concentration of MC is shown in Figure 4.3.

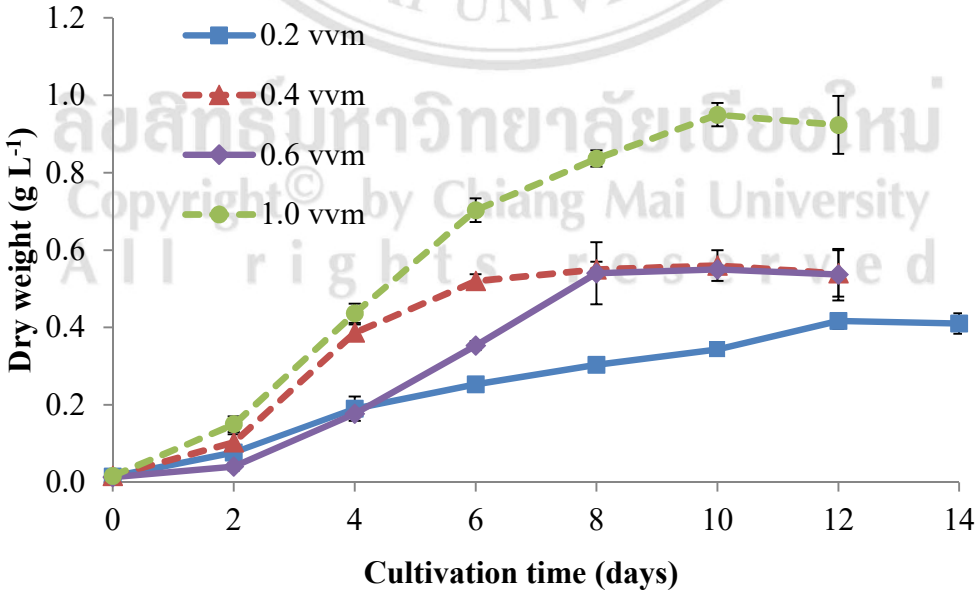


Figure 4.3 Dry weight of the MC under different CO_2 aeration rates

From Figure 4.3, at 1.0 vvm of CO_2 aeration rate, the maximum dry weight of 0.95 ± 0.03^{a} g L^{-1} was obtained after 10 days of cultivation time. While the maximum dry weight of 0.2, 0.4 and 0.6 vvm of CO_2 aeration rates were 0.42 ± 0.021^{c}, 0.56 ± 0.04^{b} and 0.55 ± 0.01^{b} g L^{-1} after 12, 10 and 10 days of culture, respectively. It seems that the growth of MC was enhanced with the increasing of CO_2 aeration rate range from 0.2-1.0 vvm. This result similar to Lee and Lee (2002) who reported that the growth rate of *Chlorella* sp. cultured in the wastewater without the organic carbon source increased after increasing the flow rate of CO_2 from 0.2 to 1.0 vvm.

The result of chlorophyll *a* content was shown in Figure 4.4. It was found that under 0.2, 0.4, 0.6 and 1.0 vvm CO_2 aeration rate, the chlorophyll *a* contents of MC reached to $4,460.61\pm294.46$, $6,497.41\pm818.23$, $7,599.39\pm174.56$ and $12,586.75\pm681.88$ µg L^{-1} on day 12, 8, 8 and 10, respectively. Then, they began to decrease, and they decreased to $4,351.84\pm507.27$, $5,761.38\pm45.08$, $6,031.60\pm420.01$ and $10,762.81\pm653.35$ µg L^{-1} under 0.2, 0.4, 0.6 and 1.0 vvm CO_2 aeration rate in the end of cultivation. It also found that the chlorophyll *a* content of MC under 1.0 vvm CO_2 aeration rate was 2.4, 1.9 and 1.8-folds higher than that under 0.2, 0.4 and 0.6 vvm CO_2 aeration rates, respectively.

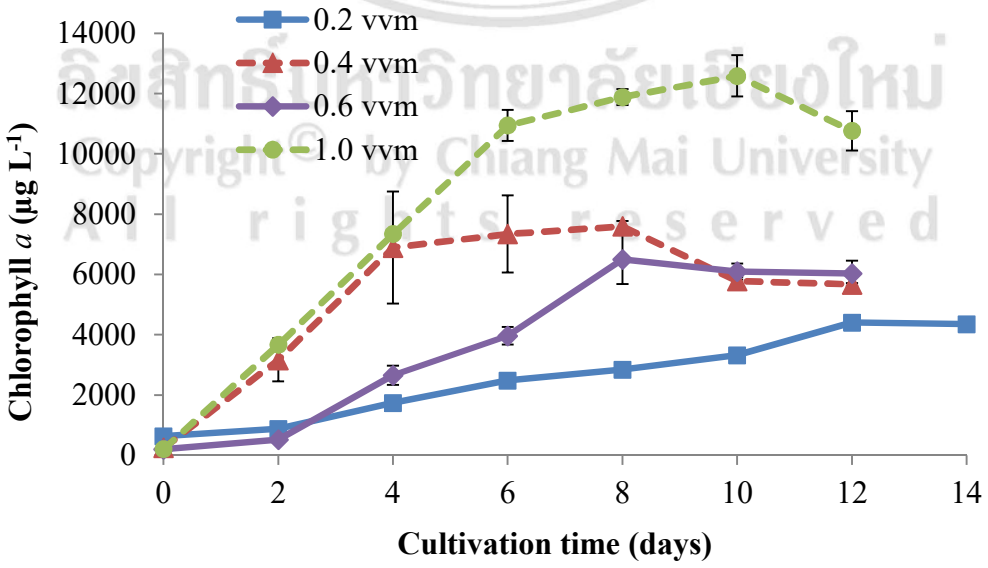


Figure 4.4 Chlorophyll *a* contents of the MC under different CO_2 aeration rates

According to the results of microalgal growth (Figures 4.3 and 4.4), these results indicated that the high CO_2 aeration rate had strong effect on microalgal growth because CO_2 aeration rate plays an important role in promoting microalgal cells to uptake nutrient and promoting light exposure of microalgae for growth (Kumar *et al*., 2010).

4.3.2 Culture pH

Measurement of pH during the cultivation period found that the pH values of culture medium decreased from about 7.0-4.63, 4.41, 5.12 and 4.63 with CO_2 aeration rate at 0.2, 0.4, 0.6 and 1.0 vvm, respectively (Figure 4.5).

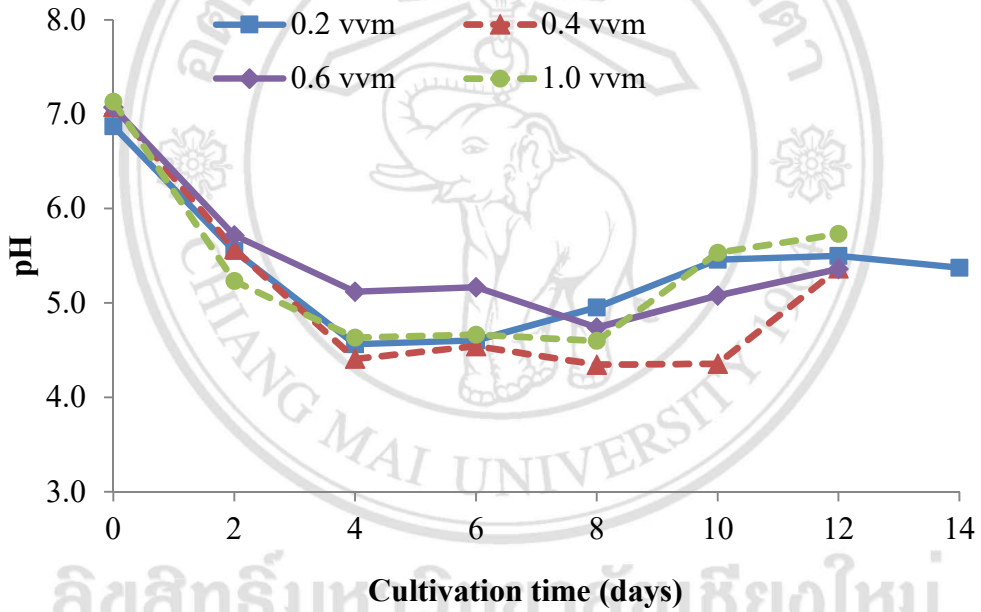


Figure 4.5 Culture medium pH changed under different CO_2 aeration rates

It was observed that under without controlled pH condition, the microalgae in all aeration rate conditions could grow in the lower pH (below pH 5.0). These results indicated that the pH change in medium has no significant effects on the growth of microalgae. Similarly, Sankar *et al.* (2011) reported that the pH shift in medium found not to negatively affect the growth of *Chlorella minutissima*. This alga could grow in medium pH range from 4 to 8 but the maximum growth was observed in pH 6.

When CO_2 dissolved in the water it may form H_2CO_3, HCO_3^- and CO_3^{2-}, depending on pH (Moroney and Somanchi, 1999). The equation of CO_2 dissolution can be written as follows:

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^- \longleftrightarrow 2H^+ + CO_3^{2-}$$

In this study, it was observed that after 8 days of cultivation period, the culture pH of all CO_2 aeration rate conditions were increased. This is because microalgae can increase pH by two ways. First, microalgae use HCO_3^- in water as carbon source in the photosynthesis, resulted in reduce of pH changing by CO_2. Another way, microalgae will generate alkalinity from their growth (Brewer and Goldman, 1976; Chen *et al*., 1994). Thus, the pH value of the culture was increased.

4.3.2 Lipid content of MC

The lipid contents of MC show the same trend of microalgal biomass concentration (Table 4.1). The highest lipid content with $24.45+1.03^a$ % of dry weight was found in MC under 1.0 vvm CO_2 aeration rate followed by 0.4, 0.6 and 0.2 vvm with 19.85 ± 1.57^b %, 18.59 ± 0.53^b % and 16.46 ± 0.05^c % of dry weight, respectively. These results indicated that the aeration rate of CO_2 higher than 0.2 vvm can effectively enhance lipid accumulation of microalgal cells. Similar results of Zheng *et al.* (2012) who found that the aeration rate of 5% v/v CO_2 at 0.5 and 1.0 vvm obtained the higher lipid content of *Chlorella vugaris* than that of 0.1 vvm aeration rate. The maximum lipid content was observed at 0.5 vvm with 41 ± 2% of dry weight.

Table 4.1 Lipid contents of MC under different CO_2 aeration rates

CO_2 aeration rate	Lipid content	
	mg L^{-1}	% of dry weight
0.2 vvm	67.50 ± 0.19^c	16.46 ± 0.05^c
0.4 vvm	107.17 ± 8.47^b	19.85 ± 1.57^b
0.6 vvm	100.39 ± 2.87^b	18.59 ± 0.53^b
1.0 vvm	225.78 ± 9.54^a	24.45 ± 1.03^a

Different letters indicate statistical difference ($p< 0.05$)

4.3.3 Biomass and lipid productivity of MC

The comparison of biomass and lipid productivity of the MC under different CO_2 aeration rates are shown in Figure 4.6. The similar trend was observed for both biomass and lipid productivity of the MC. The highest biomass productivity was found at 1.0 vvm aeration rate (75.56±7.09 mg L^{-1} d^{-1}) while the lowest biomass productivity was found at 0.2 vvm aeration rate (28.21±1.56 mg L^{-1} d^{-1}). In the same pattern, the highest lipid productivity was found at 1.0 vvm aeration rate (18.81±0.8 mg L^{-1} d^{-1}) while the lowest lipid productivity was found at 0.2 vvm aeration rate (4.82±0.01 mg L^{-1} d^{-1}). It also found that the both biomass and lipid productivity of the MC had no significant different between 0.4 and 0.6 vvm aeration rates.

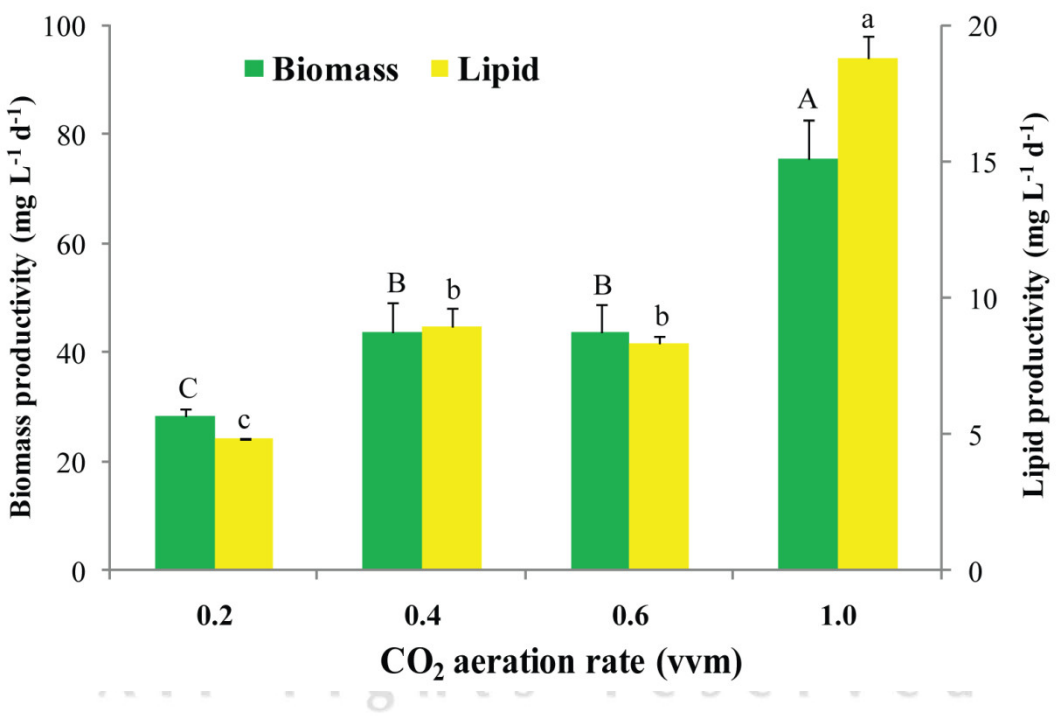


Figure 4.6 Biomass and lipid productivity of the MC under different CO_2 aeration rates. Letters (A, B and C) and (a, b and c) indicated a significant difference (p< 0.05) of biomass and lipid productivity between each condition, respectively

Moreover, the results found that the aeration rate above 0.4 vvm could enhance the biomass and lipid productivity of the MC. Similar results were obtained by Anjos *et al.* (2013) reported that 0.4 and 0.7 vvm aeration rate of

all CO_2 concentrations (2%, 6% and 10% v/v CO_2) showed higher biomass productivity of *Chlorella vugaris* strain P12 than that of the aeration rate below 0.4 vvm. The best biomass productivity was 1.3 g L^{-1} d^{-1} when the alga cultured with 6% CO_2 at aeration rate of 0.4 vvm.

From Figure 4.6, the results proved that the high CO_2 aeration rate related with high CO_2 concentration had significant effect on lipid accumulation of microalgae. This is because microalgae consume CO_2, which supplied under high aeration rate and fix it into the biomass and lipid in the dark reaction of photosynthesis (Longnergan, 2000; Radakovits *et al.*, 2010).

4.3.4 Carbon content, CO_2 fixation rate, CO_2 capture and CO_2 utilization efficiency of MC

Table 4.2 showed the carbon content and CO_2 fixation rate of the MC under different CO_2 aeration. The carbon content under 0.2, 0.4, 0.6 and 1.0 vvm CO_2 aeration rates were 0.4639, 0.4714, 0.4512 and 0.4681 g dw^{-1}, respectively. Normally, CO_2 fixation rate is strongly depends on biomass productivity (Taher *et al*., 2015). The results of CO_2 fixation rate of the MC under different CO_2 aeration rates show that the CO_2 fixation rate was increased when the biomass production of the microalgae increases. The highest CO_2 fixation rate of the MC was obtained from the culture aerated with 1.0 vvm CO_2 flow rate (0.1297 g CO_2 L^{-1} d^{-1}) while the CO_2 fixation rate of MC aerated with 0.2, 0.4 and 0.6 vvm CO_2 flow rate were 0.048, 0.754 and 0.7220 g CO2 L^{-1} d^{-1}, respectively. Ong *et al.* (2010) reported that the CO_2 fixation rates of *Chlorella* sp. wild type and mutant strain MT-7 and MT-15 at 0.5 vvm CO_2 aeration rate were higher than that of 0.25 vvm CO_2 aeration rate. Theologically, for 1 g of produced microalgal dry biomass, about 1.83 g of CO_2 is captured (Bernnan and Owende, 2010). The results of CO_2 capture of MC found that the microalgae under all CO_2 aeration rates could fix 1.65-1.72 g CO_2 for 1 g dry biomass production. The highest CO_2 capture was observed under 0.4 vvm CO_2 aeration rate while the lowest was found at 0.6 vvm because of CO_2 capture ability of microalgae is depends on carbon content in the microalgal biomass (Taher *et al*., 2015).

Table 4.2 Carbon content, CO_2 fixation rate and CO_2 capture of the MC under different CO_2 aeration rates

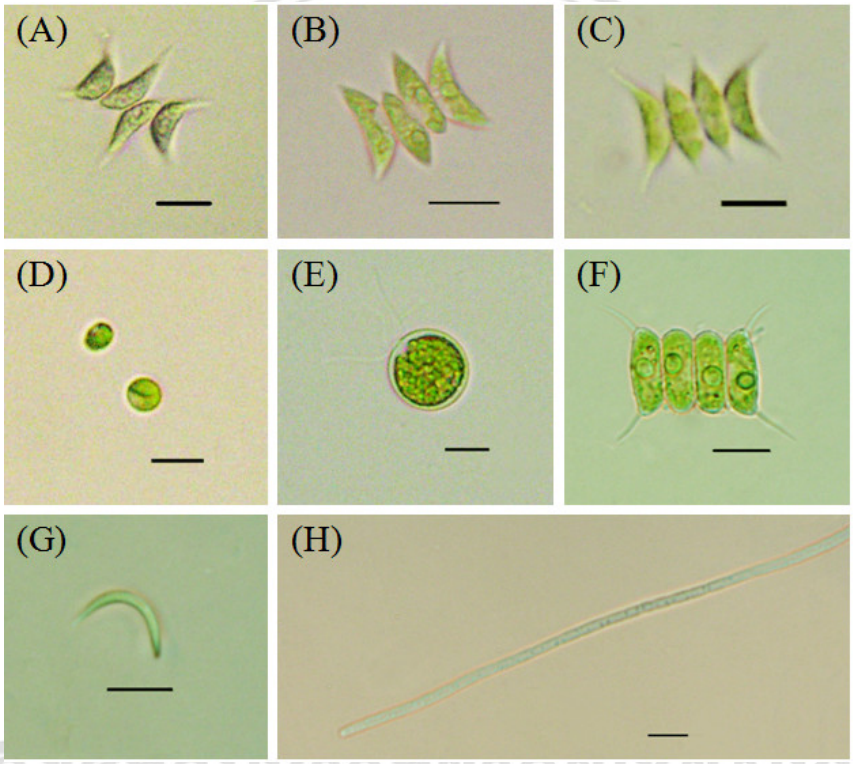
CO_2 aeration rate (vvm)	Carbon content (g dw^{-1})	CO_2 fixation rate (g CO_2 L^{-1} d^{-1})	CO_2 capture (g CO_2 dw^{-1})	CO_2 utilization efficiency (%)
0.2	0.4639	0.0480	1.7010	0.0278
0.4	0.4714	0.0754	1.7285	0.0230
0.6	0.4512	0.0722	1.6544	0.0148
1.0	0.4681	0.1297	1.7164	0.0165

As shown in Table 4.2, the CO_2 utilization efficiency of the aeration rate at 1.0 vvm was found lower than the aeration rate at 0.2 and 0.4 vvm. These results were similar with Ryu *et al.* (2009) reported that high aeration rate (0.4 vvm) has lower CO_2 utilization efficiency than that of low aeration rate (0.1 vvm) while the biomass concentration of *Chlorella* sp. AG10002 was increased with the increasing of CO_2 aeration rate.

However, in order to optimize the aeration conditions, the utilization efficiency of the supplied CO_2 gas and biomass productivity of algae are necessary to be considered (Zhang *et al.*, 2002). In this study, the CO_2 utilization efficiency of the MC under a 1.0 vvm CO_2 aeration rate was lower than of that at a 0.2 vvm CO_2 aeration rate but the biomass and lipid productivity were higher than that of 0.2 vvm CO_2 aeration rate. This is because when CO_2 was supplied with a high aeration rate, the dissolution rate of the CO_2 might prove to be faster than the growth rate of the microalgae. Some of the CO_2 can be released from the water surface into the atmosphere and this can be lead to a low CO_2 utilization efficiency (Raksasak *et al.*, 2012). Using the optimum bubble size of CO_2 and bioreactor development can be solved this problem. Ryu *et al.* (2009) reported that when using the large bubble size of CO_2, the CO_2 mass transfer was decreased because of the interfacial area between gas and liquid decrease. They found that decreasing of the CO_2 bubble size (from 61 to 31 mm) could lead to increase the cell concentration of *Chlorella* sp. AG10002. The highest cell concentration was obtained at the bubble size of 31 mm.

4.3.5 Species composition of MC

The dominant species of MC was observed and identified by using light microscope and the relevant keys. It found that most common microalgal species of all conditions were green microalgae (Figure 4.7). The microalgal species observed were *Acutodesmus pectinatus*, *A. dimorphus*, *A. obliquus, Carteria* sp., *Chlorella vulgaris*, *Desmodesmus quadricauda*, *Monoraphidium contortum* and *Planktolyngbya limnetica.*



Scale bar = 10 µm

Figure 4.7 Dominant species of MC under different CO_2 aeration rates; (A) *Acutodesmus pectinatus*, (B) *A. dimorphus*, (C) *A. obliquus*, (D) *Chlorella vulgaris*, (E) *Carteria* sp., (F) *Desmodesmus quadricauda*, (G) *Monoraphidium contortum* and (H) *Planktolyngbya limnetica*

Figure 4.8 show biovolume ratio of microalgal species in MC under different CO_2 aeration rates in the early and the end of cultivation. The most algal species of all CO_2 aeration rate conditions observed in the early cultivation was *Carteria* sp. and followed by *Acutodesmus dimorphus, Chlorella vulgaris* and *Desmodesmus quadricauda*. During the cultivation period, it

found that the biovolume ratio of dominant microalgal species was changed. At 0.2 vvm CO_2 aeration rate, the % biovolume of dominant species observed in the end of cultivation was *A. dimorphus* and followed by *D. quadricauda* and *C. vulgaris*. While at 0.4, 0.6 and 1.0 vvm CO_2 aeration rate, the % biovolumes of dominant species observed in the end of cultivation were *C. vulgaris* followed by *A. dimorphus* and *Carteria* sp. These indicated that CO_2 aeration rate had strongly effect on microalgal species change in the culture, the suitable strain was selected by the concentration of CO_2 supplied with various aeration rate. However, during the cultivation with CO_2 aeration rate range from 0.2-1.0 vvm, the dominant algal species observed were two green microalgae, the CO_2 tolerant strain, *A. dimorphus* and *chlorella* sp. which reported very high biomass and lipid content and appropiate for use as biodiesel feedstock (Goswami *et al*., 2011; Wu *et al*., 2012).

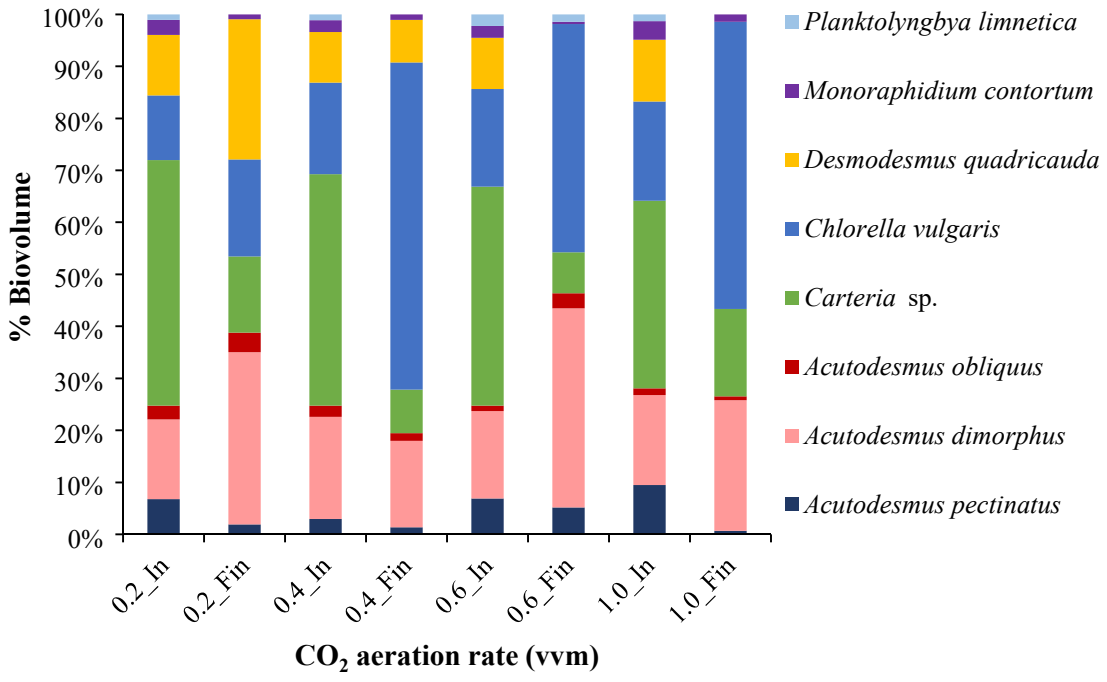


Figure 4.8 % Biovolume of microalgal species in MC cultured under different CO_2 aeration rates; In = Initial (first day), Fin = Final (last day)