CHAPTER 6

Effects of season and CO_2 supplementation on the cultivation of the microalgal consortium in an outdoor open system for lipid production and CO_2 mitigation

6.1 Introduction

Microalgae with high lipid yields have high potential for use as biodiesel feedstock compared to other oil crops (Kirrolia *et al*., 2013). Some species of microalgae can produce lipids at up to 80% of their dry weight. Lipid yields of 20-50% are common among microalgae (Chisti, 2007). Moreover, biodiesel produced from microalgal lipids were found to have similar properties with standard biodiesel (Huang *et al*., 2010). In recent years, the cultivation of microalgae based on biodiesel production has been intensively investigated in both the lab-scale and in the outdoor scale. For large-scale biodiesel production, the high biomass and lipid yields of microalgae are required (Amin, 2009).

Many research studies conducted on the lab-scale suggested that high CO_2 concentrations could promote microalgal growth and lipid accumulation (Yue and Chen, 2005; Tang *et al.*, 2011; Zheng *et al*., 2012). However, when using the high aeration rate of pure CO_2 for large-scale cultivation, the cost of the cultivation process will increase because pure CO_2 is expensive. Thus, pure CO_2 with a low aeration rate (0.0002 vvm) was used for microalgal cultivation in this study. The MC was cultivated with and without pure CO_2 supplementation in a semi-continuous outdoor open system for lipid production and CO_2 mitigation. However, in the outdoor scale or large-scale cultivation processes, the environmental factors such as light and temperature are considered uncontrolled. The variations of light intensity and temperature are dependent on the season, climate and geographical location, which can affect the cultivation of microalgae (Sheet *et al*., 2014). Thus, experiments were performed during both the wet and cold-dry seasons.

6.2 Materials and methods

The methods applied to study the cultivation of MC using pure CO_2 in an outdoor open system are summarized in Figure 6.1

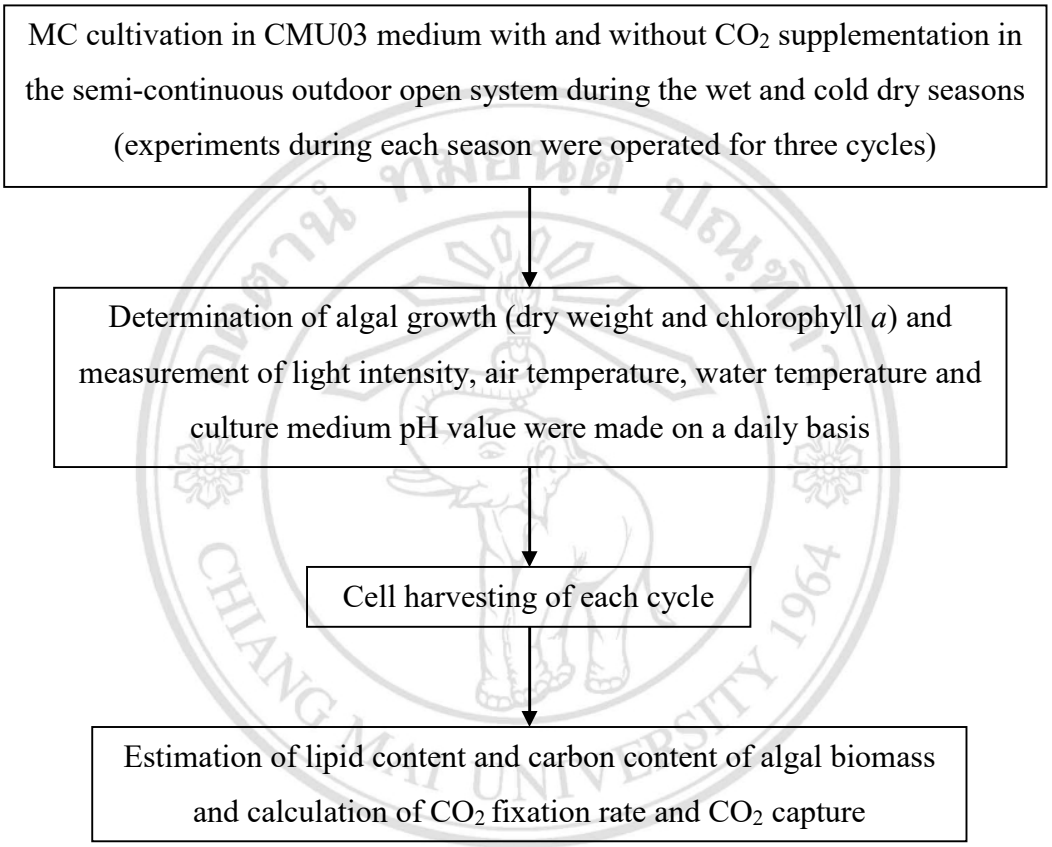


Figure 6.1 Flowchart diagram of microalgal cultivation using pure CO_2 in an outdoor open system

6.2.1 Microalgae and culture

The MC was prepared by the succession of the air borne cultures enriched with the CMU03 medium (Sriphuttra *et al*., 2013) and was 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 47.8% *Acutodesmus dimorphus*, 28% *Chlorella vulgaris*, 13.3% *Monoraphidium contortum*, 4.8% *Nitzschia palea*, 4.3%

Carteria sp., 0.8% *Plantolyngbya limnetica*, 0.5% *Ankistodesmus fusiformis* and 0.5% *Pseudoanabaena galeata*) was incubated in CMU03 medium at ambient temperatures under continuous illumination with fluorescent lamp at 24.3 µmol m^{-2} s^{-1} for two weeks.

6.2.2 Cultivation of MC

The semi-continuous cultivation of MC was constructed in a 60 L plastic tank, which contained 50L working volume of CMU03 medium. The cultures were aerated with two different conditions; CO_2 supplementation (feeding pure CO_2 at an aeration rate of 0.0002 vvm combined with ambient air at an aeration rate of 0.2 vvm and without CO_2 supplementation (feeding only ambient air at an aeration rate of 0.2 vvm). The time interval of CO_2 aeration was 8 h d^{-1}. Both treatments were conducted in triplicate. The microalgal cultivation was performed in an outdoor open system (without light and temperature control) at Boonsom Farm, Mae Wang District, Chiang Mai Province and this experiment was investigated during two seasons: the wet season (July-August, 2013) and the cold-dry season (November-December, 2013). When the growth of microalgae reached to the early stationary phase, 90% of the cultures were harvested. After harvesting, the microalgae cultivation tank was refilled up to its initial volume with the CMU03 medium. The semi-continuous cultures were repeatedly operated for three cycles (4-5 weeks). The microalgal cultivation system is presented schematically in Figure 6.2.

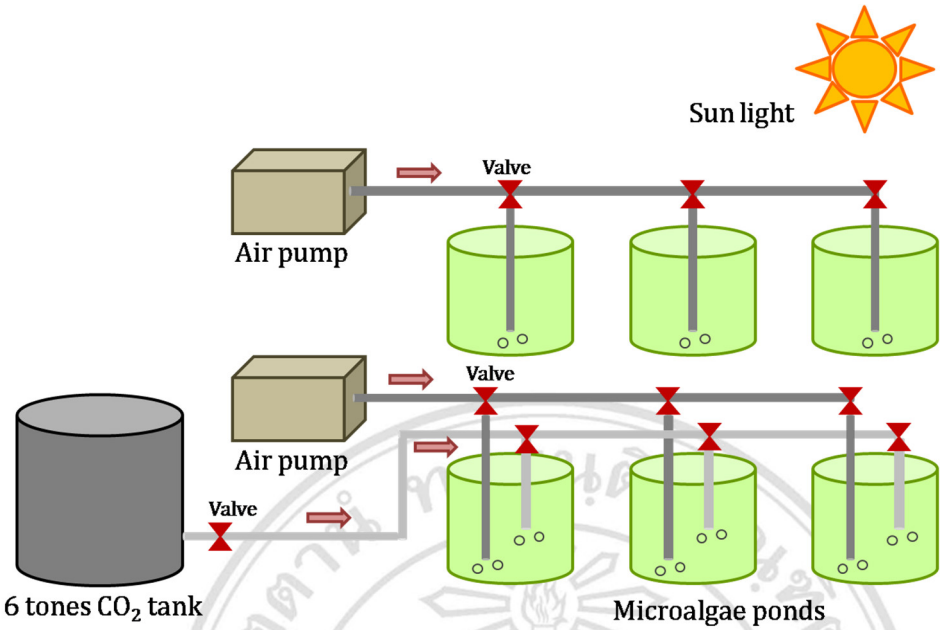


Figure 6.2 Schematic diagram of microalgal cultivation using pure CO_2 in an outdoor open system

6.2.3 Sample collection and analytical determination

The 50 mL of culture samples was collected to determine the microalgal growth and the constant volume of the culture medium was maintained by adding an equivalent volume of new medium to the sample. The growth of microalgae and certain relevant parameters were determined on a daily basis. Light intensity was measured using a Lux meter (Tecpel 530). Air and water temperature were measured using a thermometer. pH value of the culture medium was measured using a pH meter (electrode kit of WTW Company). All parameters were measured 3 times a day (at 10 am, 1 pm and 4 pm).

The dry weight, chlorophyll a content, dominant species, lipid content, carbon contents, CO_2 fixation rate and CO_2 capture rates were analyzed according to the procedures described in Chapter 3.

6.2.4 Lipid analysis

The lipid composition of MC cultivated with and without CO_2 supplementation in the wet season was analyzed using a gas chromatography-mass spectrometer (GC-MS). The samples collected during the wet season

were selected because the MC in this season revealed high biomass and lipid productivity. Each lipid sample was dissolved in chloroform: methanol (2:1). The lipid analysis was carried out using Agilent 7890A/5975C (EI) GC/MSD equipped with a DB5-MS column (30 x 0.25 mm ID x 0.25 μm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL^{-1} min^{-1}. The 0.2 μL of the lipid sample was injected into the column. The injector and detector temperatures were 250°C and 280°C, respectively. The oven temperature was increased from 100°C at 10°C min^{-1} to 250°C and this was maintained for 3 min. The compounds were then identified by comparing the mass spectra with the NIST08 library data.

6.2.5 Statistic analysis

The results are expressed as mean±SD (standard deviation) of the 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 were used to evaluate the differences between the MC cultivated with and without CO_2 supplement. A value of $p<0.05$ was considered statistically significant.

6.3 Results and discussions

6.3.1 Microalgal growth and environmental parameters

In order to study the MC cultivation using pure CO_2 in an outdoor open system for lipid production and CO_2 mitigation, the microalgae was cultivated in a semi-continuous outdoor system with and without CO_2 supplementation. The microalgal cultivations were performed during the wet and cold-dry seasons. The two seasons were selected because they have different light-dark periods (13-11 for the wet season and 11-13 for the cold-dry season). The growth and certain relevant environmental parameters of microalgal cultivation during the wet and cold dry season are shown in Figures 6.3 and 6.4, respectively. The results found that the growth of microalgae in the wet and dry seasons showed the same trend. The dry weight and chlorophyll *a*

content of the MC supplemented with CO_2 seemed higher than of those without CO_2 supplementation.

In the case of the wet season, the biomass concentration of the first cycle of MC with CO_2 supplementation was significantly higher than those of all cycles without CO_2 supplementation (Figure 6.3). The highest dry weight of 0.44 ± 0.01^{a} g L^{-1} was observed in the MC cultured with CO_2 supplementation in the first cycle, while the lowest dry weight of 0.24 ± 0.042^{c} g L^{-1} was observed in the MC cultured without CO_2 supplementation in the third cycle. The results of the chlorophyll *a* content found that there was no statistically significant difference between all cycles of the MC with CO_2 supplementation and the second and third cycles of the MC without CO_2 supplementation. It was also found that the chlorophyll *a* content of the MC with CO_2 supplementation in the first cycle ($1,940.03\pm69.82^{a}$ µg L^{-1}) was significantly higher than of that in the third cycle of the MC without CO_2 supplementation ($1,261.57\pm42.05^{c}$ µg L^{-1}). According to Figure 6.3, the chlorophyll *a* concentration data showed a higher variability than the biomass concentration. This happened because there were a number of factors that have an effect on the concentration of chlorophyll *a* in microalgal cells, such as cell age, light intensity, temperature, nutrient concentrations and trace elements (Dolan *et al*., 1978).

For the cultivation of MC in the cold-dry season, the biomass concentration of microalgae showed the same trend as the MC cultured in the wet season (Figure 6.4). The highest dry weight of 0.47 ± 0.05^{a} g L^{-1} was observed in the MC cultured with CO_2 supplementation in the first cycle while the lowest dry weight of 0.28 ± 0.03^{d} g L^{-1} was observed in the MC cultured without CO_2 supplementation in the third cycle. When compared with the first cycle, chlorophyll *a* content of the MC with CO_2 supplementation was significantly higher. Chlorophyll *a* contents of MC with and without CO_2 supplementation in the first cycle were $2,121.89\pm240.12^{a}$ and $1646.93+183.05^{c}$ µg L^{-1}, respectively.

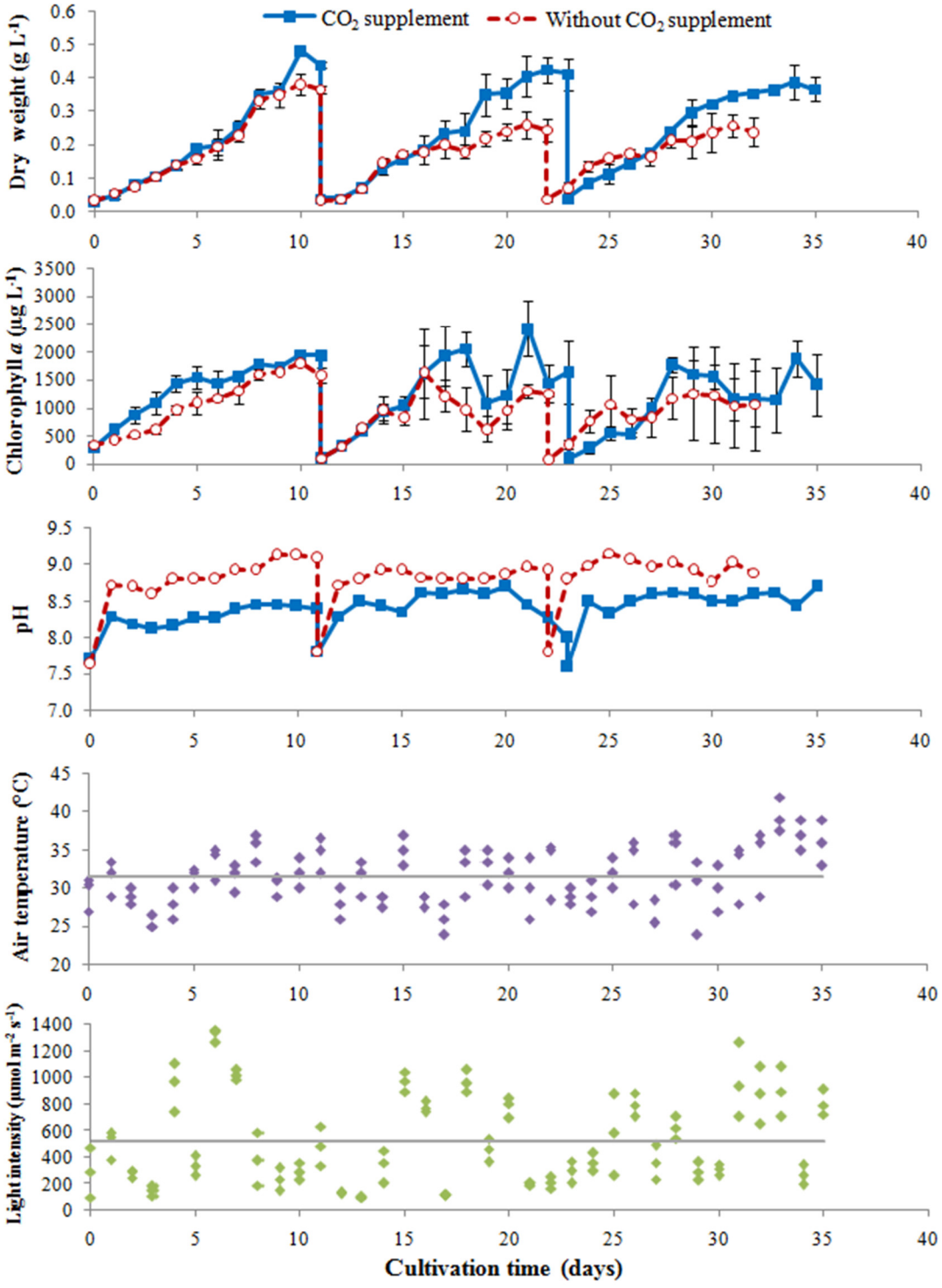


Figure 6.3 Growth and parameters of the MC cultivated in the wet season. The cultures were investigated in semi-continuous cultivation with and without CO_2 supplementation. The semi-continuous cultures were repeatedly investigated for three cycles. Each cycle started when the microalgal growth reached the early stationary phase

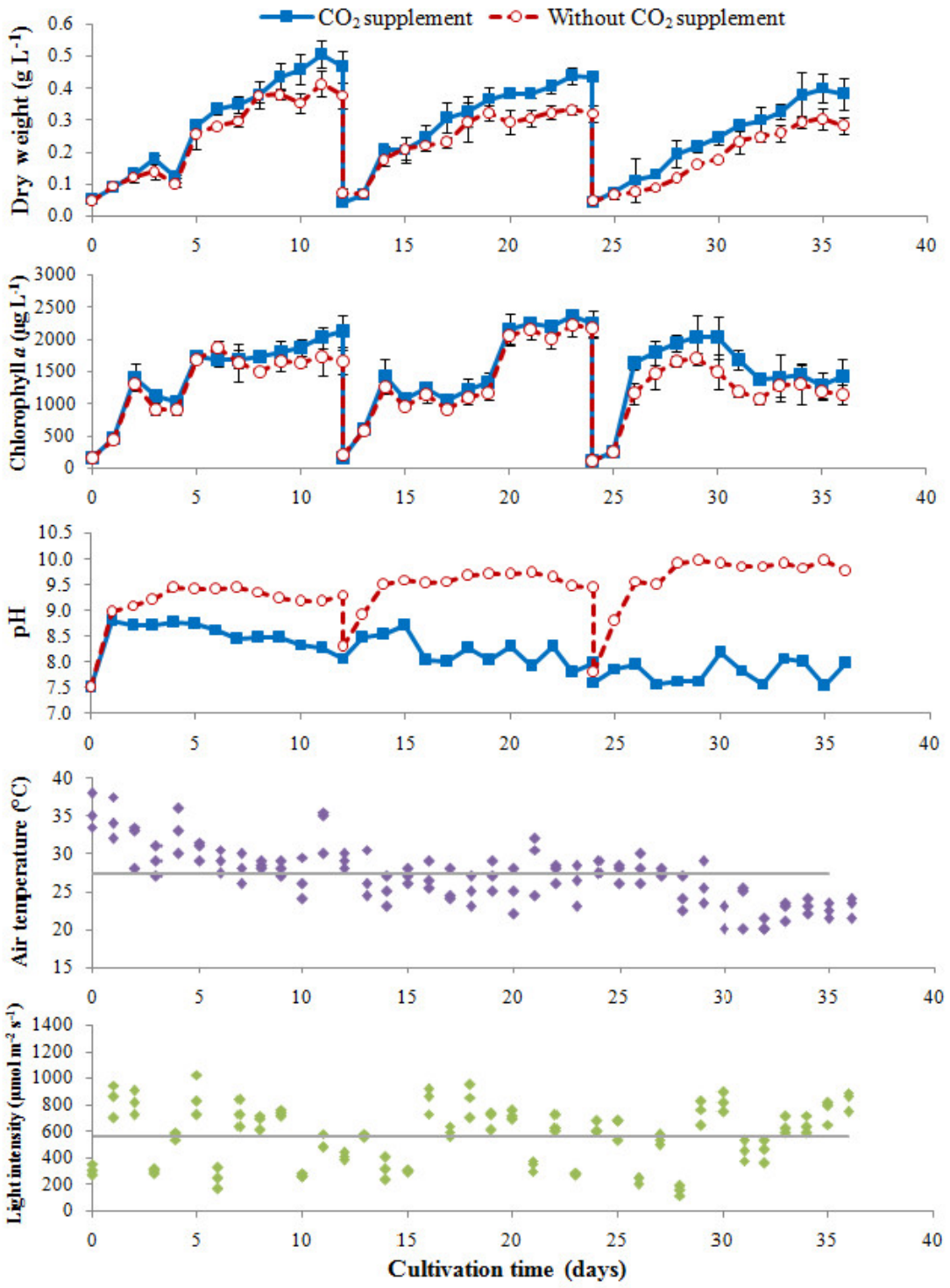


Figure 6.4 Growth and parameters of the MC cultivated in the cold dry season. The cultures were operated under semi-continuous cultivation with and without CO_2 supplementations. The semi-continuous cultures were repeatedly operated for three cycles. Each cycle started when the microalgal growth reached the early stationary phase

Generally, most microalgal species can grow well under neutral pH values (Kumar *et al*., 2010). During the wet season, the maximum and minimum pH of the culture medium were observed to be 7.6 and 8.76, respectively (Figure 6.3), while the maximum and minimum pH values of the culture medium during the cold-dry season was found to be 7.5 and 9.97, respectively (Figure 6.4). These results indicate that a pH value above 7 had no negative effect on MC growth. In all cycles of the semi-continuous cultures, the pH values of the culture medium of MC with CO_2 supplementation were lower than in those without CO_2 supplementation. This is because when CO_2 was dissolved in the culture medium, it will form a weak acid, H_2CO_3 and causes the pH level to decrease (Wu *et al*., 2008). However, the pH value of the culture medium with and without CO_2 supplementation was strongly fluctuated throughout the cultivation period. This happened because the photosynthesis that occurred during the day and the respiration of the microalgae that occurred at night had significant affects on the variation in pH values (Bartley *et al*., 2014).

Temperature is one of the important parameters for microalgal growth. It has significant impacts on growth rate, morphological activity, physiological activity, pigment content, nutrient absorption and the CO_2 fixation of microalgae (Fu *et al*., 2008). In general, the optimal temperature was found to be in the range of between 25 and 30°C. However, the optimal air and water temperature also depended on certain other environmental factors such as light intensity and humidity (Esterhuizen-Londt and Zeelie, 2013). In this study, the average water temperature recorded during the cultivation process in the wet season and cold dry seasons were 31.0 and 25°C, respectively (data not shown). From Figure 6.3, the average air temperature recorded during the cultivation process in the wet season was 31.5$\pm$3.15°C. The maximum temperature was 42°C while the minimum temperature was 24°C. During the cold dry season, the average air temperature was found to be 27.31$\pm$3.5°C. The maximum temperature was 37.5°C while the minimum temperature was 20.5°C (Figure 6.4). These results indicated that variations in temperature had

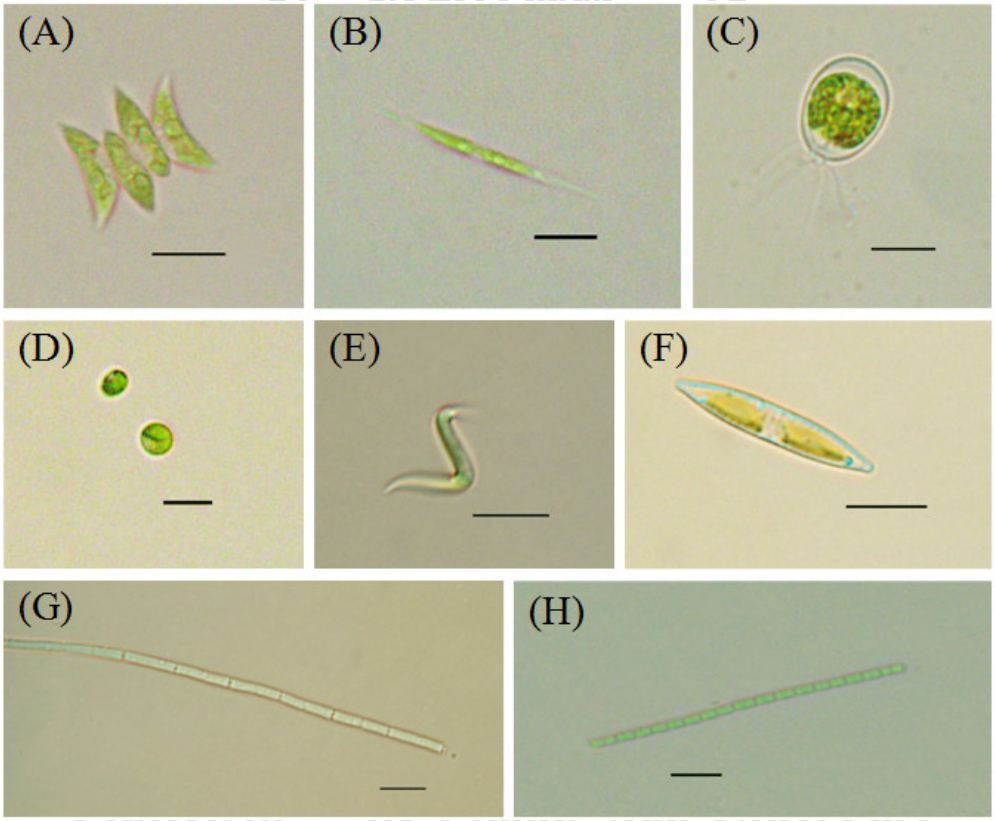
no negative impact on microalgal growth. When the temperature was not controlled, the MC could grow well and tolerate temperatures above the optimum. Similar results were obtained by Béchet *et al*. (2013) who found that the high level of biomass productivity of *Chlorella sorokiniana* was achieved under conditions without controlling the temperature, in which the operating temperature was raised up to 41°C.

Light intensity is an important environmental factor for the growth and CO_2 fixation of microalgae. Light is the energy source for the photosynthetic production of microalgae. In outdoor cultivation, the light intensity is an uncontrollable factor. Normally, the light intensity of natural sunlight can exceed 1600 μmol m^{-2} s^{-1} (Wang *et al*., 2015). Microalgae cannot utilize all the supplied sunlight because they cannot absorb all photons of light. Too high light intensity can inhibit the photosynthesis of microalgae (Ren, 2014). In this experiment, the variations of light intensity for both seasons are shown in Figures 6.3 and 6.4. The averages of light intensity of sunlight in the wet and cold-dry seasons were 513.91$\pm$323.64 and 557.96$\pm$212.91 μmol m^{-2} s^{-1}, respectively. The maximum and minimum light intensity was observed to be 1,354.2 and 92.5 μmol m^{-2} s^{-1}, respectively, for the wet season, and 1,024.9 and 116.55 μmol m^{-2} s^{-1}, respectively, for the cold-dry season. These results found that the MC cells could grow without photo-inhibition under a broader range of light intensity of between 90 and 1300 μmol m^{-2} s^{-1}.

6.3.2 Species composition of MC

The dominant microalgal species of the MC with and without CO_2 supplementation in the wet and cold dry seasons are shown in Figure 6.5. The common algal species found in both seasons were the green microalgae species. The identified microalgal species included *Acutodesmus dimorphus*, *Ankistodesmus fusiformis*, *Carteria* sp., *Chlorella vulgaris*, *Monoraphidium contortum*, *Nitzschia palea, Planktolyngbya limnetica* and *Pseudanabaena galeata*. During the semi-continuous cultivation period in wet season, the population of microalgal community was dramatically changed. The most

abundant microalgal species in term of biovolume at the end of first cycle observed were *C. vulgaris* followed by *A. dimorphus* and *N. palea* while at the end of third cycle, the most abundant species were *A. dimorphus* followed by *N. palea* and *C. vulgaris* (Figure 6.6A). During the semi-continuous cultivation period in the cold dry season, the population of the microalgal community had slightly changed. The most abundant microalgal species in term of biovolume at the end of the second and third cycles observed were *A. dimorphus* followed by *N. palea* and *C. vulgaris* (Figure 6.6B).



Scale bar = 10 µm

Figure 6.5 Dominant species of the MC with and without CO_2 supplementation in the wet and cold dry seasons

(A) *Acutodesmus dimorphus,* (B) *Ankistodesmus fusiformis,* (C) *Carteria* sp., (D) *Chlorella vulgaris,* (E) *Monoraphidium contortum*, (F) *Nitzschia palea,* (G) *Planktolyngbya limnetica* and (H) *Pseudanabaena galeata*

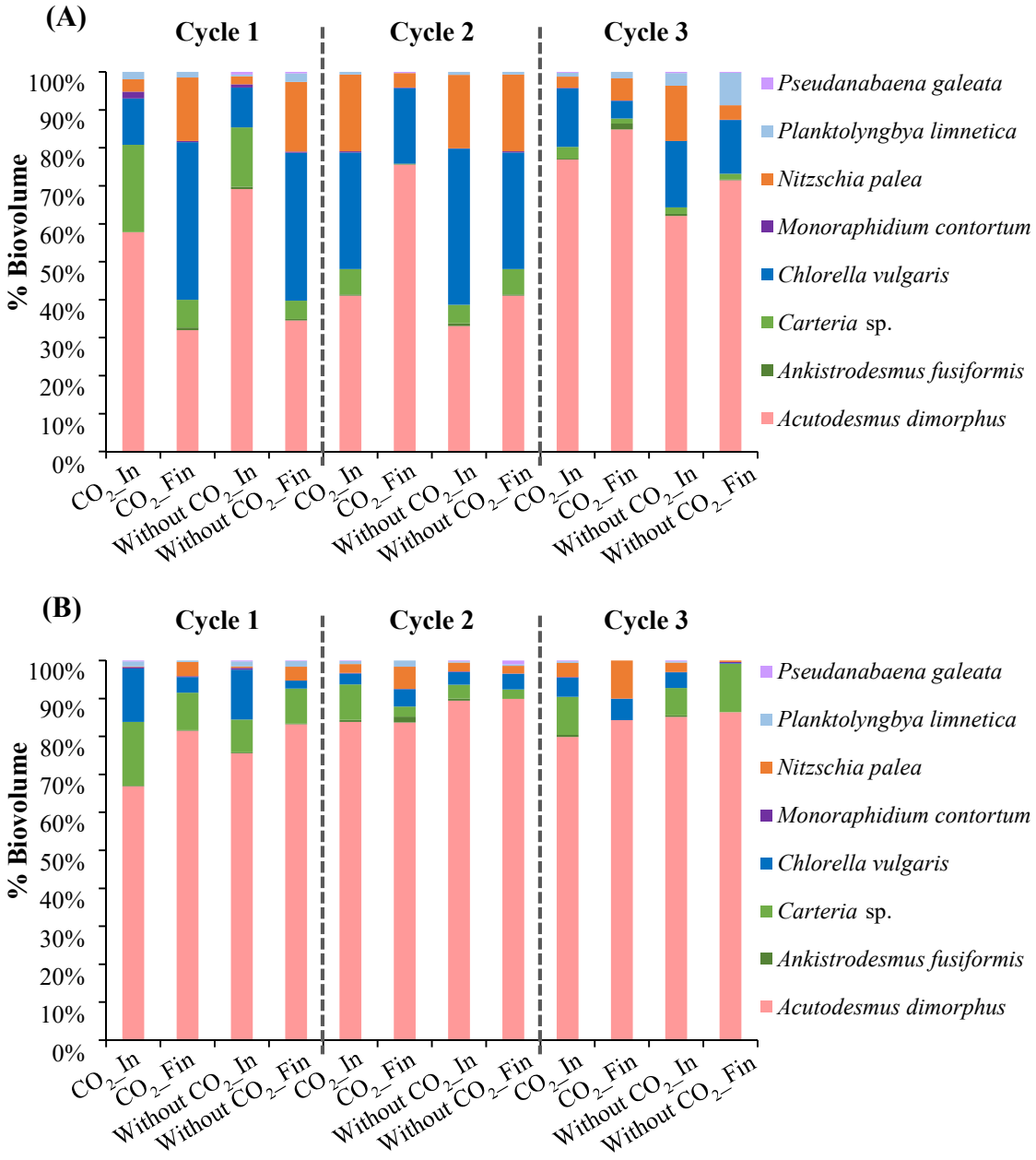


Figure 6.6 % Biovolume of microalgal species in MC cultured with and without CO_2 supplementation in (A) the wet season and (B) the cold-dry season
In = Initial (first day), Fin = Final (last day)

According to the results of the study of the species composition changes in Figure 6.6, when using CO_2 to cultivate MC in both the wet and cold dry seasons, the most abundant microalgal species found under these conditions were *A. dimorphus*, *C. vulgaris* and *N. palea*. This indicated that the utilization of CO_2 for the cultivation of MC had a significant effect on the

microalgal species composition in the culture. All three species, *A. dimorphus*, *C. vulgaris* and *N. palea* were reported to have high lipid content. Mata *et al*. (2010); Hassan *et al*. (2012) and Velichkova *et al.* (2013) reported that the lipid contents of *Chlorella* sp.*, N. palea* and *Scenedesmus dimorphus* were found in a range of 10-58%, 40-60% and 19-34% of the dry biomass, respectively. Furthermore, it was also reported that all three species could tolerate high CO_2 concentrations (Hanagata *et al.,* 1992; Hanhua *et al*., 2008). In this study, it was suggested that the cultivation of algae with CO_2 supplementation could promote the suitable species selection for biofuel production.

6.3.3 Biomass and lipid production of MC

The biomass concentration, biomass productivity, lipid content and lipid productivity levels of the MC with and without CO_2 supplementation in the wet and cold-dry seasons are shown in Table 6.1. The biomass productivity of MC with CO_2 supplementation ranged from 27.39-41.3 g L^{-1} d^{-1}, for the wet season and 28.39-33.25 g L^{-1} d^{-1}, for the cold-dry season. During the cultivation process of both the wet and cold dry seasons, the biomass productivity levels of the MC with CO_2 supplementation in first cycle were significantly higher than of all those cycles without CO_2 supplementation. Next, the lipid contents of the MC samples were analyzed. It was found that the lipid content levels of the MC cultivated with CO_2 supplementation in both the wet and cold dry seasons were higher than of those without CO_2 supplementation. In the wet season, the lipid content levels of the MC cultivated with and without CO_2 supplementation ranged from 16.75-18.18% and 7.92-11.39% of the dry weight, respectively. During the cold dry season, the lipid content levels of the MC cultivated with and without CO_2 supplementation ranged from 16.56-17.32% and 10.72-11.14% of the dry weight, respectively. However, it found that the lipid contents of MC with CO_2 supplementation in wet season seemed similar to the MC in cold dry season but the biovolume ratio of dominant species of both seasons were different. The most microalgal species in term of biovolume in wet season

was *Chlorella vulgaris* while the most microalgal species in cold dry season was *Acutodesmus dimorphus.* This happened because two microalgal strain had similar lipid content (Mata *et al*. 2010; Velichkova *et al.* 2013).

According to the results of lipid productivity, it was clearly seen that the supplementation of CO_2 in the MC cultivation could enhance the lipid productivity of microalgae. The lipid productivity levels of the MC cultivated with CO_2 supplementation in both the wet and cold dry seasons were higher than of those without CO_2 supplementation. The lipid productivity of the MC with CO_2 supplementation ranged from 5.57-7.37 g L^{-1} d^{-1}, respectively, for the wet season and 5.49-6.44 g L^{-1} d^{-1}, respectively, for the cold dry season. From table 6.1, when compared with the cold-dry season, the mean averages of the biomass concentration, biomass productivity, lipid content and lipid productivity of the MC with CO_2 supplementation in the wet season had no significant difference. This indicated that the season had no significant effect on the growth and lipid accumulation of microalgae.

Table 6.1 Biomass concentration, biomass productivity, lipid content and lipid productivity of the MC cultivated in the wet and cold dry seasons

Season	Aeration	Culture cycle	Biomass concentration ($g\ L^{-1}$)	Biomass productivity ($g\ L^{-1}\ d^{-1}$)	Lipid content (% of dry weight)	Lipid productivity ($g\ L^{-1}\ d^{-1}$)
Wet	CO_2	1	0.44 ± 0.01^{A}	41.30 ± 0.50^{A}	16.75 ± 0.96^{A}	7.37 ± 0.42^{A}
	supplement	2	0.41 ± 0.05^{AB}	31.22 ± 3.96^{B}	16.98 ± 1.66^{A}	5.85 ± 0.57^{B}
		3	0.37 ± 0.03^{B}	27.39 ± 2.08^{BC}	18.18 ± 1.53^{A}	5.57 ± 0.47^{B}
		Mean	**0.41±0.04**	**33.0±7.19**	**17.30±0.77**	**6.26±0.97**
	Without CO_2	1	0.37 ± 0.01^{B}	33.13 ± 0.81^{BD}	10.34 ± 0.95^{B}	3.79 ± 0.35^{C}
	supplement	2	0.25 ± 0.03^{C}	19.12 ± 3.09^{E}	7.92 ± 0.33^{B}	1.76 ± 0.07^{D}
		3	0.24 ± 0.04^{C}	20.07 ± 4.94^{E}	11.39 ± 4.47^{B}	2.72 ± 1.07^{C}
		Mean	**0.28±0.07**	**24.11±7.83**	**9.88±1.78**	**2.76±1.01**
Dry	CO_2	1	0.47 ± 0.05^{a}	33.25 ± 2.92^{a}	16.56 ± 0.98^{a}	6.44 ± 0.38^{a}
	supplement	2	0.44 ± 0.01^{ab}	32.72 ± 0.59^{ab}	17.32 ± 1.33^{a}	6.30 ± 0.48^{a}
		3	0.38 ± 0.05^{bc}	28.39 ± 4.10^{bc}	17.20 ± 1.49^{a}	5.49 ± 0.48^{b}
		Mean	**0.43±0.04**	**31.45+2.67**	**17.02±0.44**	**6.08±0.51**
	Without CO_2	1	0.38 ± 0.04^{bc}	27.39 ± 3.63^{bc}	11.14 ± 0.53^{b}	3.50 ± 0.17^{c}
	supplement	2	0.32 ± 0.03^{cd}	20.72 ± 3.22^{d}	10.88 ± 1.84^{b}	2.90 ± 0.49^{cd}
		3	0.28 ± 0.03^{d}	19.72 ± 2.52^{d}	10.72 ± 0.79^{b}	2.53 ± 0.19^{d}
		Mean	**0.33±0.50**	**22.61±4.17**	**10.91±0.21**	**2.98±0.49**

Letters (A, B and C) and (a, b and c) indicated significant differences ($p<0.05$) between the MC with and without CO_2 supplement in the wet and cold dry seasons, respectively

6.3.4 Microalgal lipid composition

The chemical compounds of the extracted lipids from the MC cultivated with and without CO_2 supplementation during the wet season were analyzed using GC-MS. The samples from the wet season were selected because the MC in this season had high biomass and lipid productivity levels. The GC-MS chromatograms of lipid compositions from the MC cultivated with and without CO_2 supplementation are shown in Figures 6.7 and 6.8. The chemical compounds of the lipids from the MC are shown in Table 6.2. The compounds studied in both conditions with and without CO_2 supplementation were consistent with C11-C23 chain length. The identified compounds of lipid from MC with CO_2 supplementation were alcohol (9.6%), carboxylic acids (65.94%), esters (15.18%), hydrocarbons (2.79%), ketone (0.82) and phenol (1.7%), while in the without CO_2 supplementation were amide (1.68%), alcohol (6.46%), carboxylic acids (67.92%), esters (7.09%), hydrocarbons (11.97%), ketone (0.6%) and phenol (0.7%).

Table 6.2 Chemical compounds of lipids from MC cultivated with and without CO_2 supplementation in the wet season

Compounds (MS similarity >90%)	Retention time (min)	Formula	%Area	
			With CO_2	Without CO_2
Amide			-	**1.68**
9-Octadecanamide	17.041	$C_{18}H_{37}NO$	-	1.68
Alcohol			**9.60**	**6.46**
3, 7, 11, 15-Tetramethylhexadec-2-en-1-ol	10.774	$C_{20}H_{40}O$	9.60	6.46
Hydrocarbon			**2.79**	**11.97**
n-Heptadecane	10.305	$C_{17}H_{36}$	1.85	1.52
2, 6, 10-trimethyl, 14-ethylene-14- pentadecane	11.774	$C_{20}H_{38}$	0.94	7.62
cis-9-Tricosene	10.542	$C_{23}H_{46}$	-	2.83

Table 6.2 (continued)

Compounds (MS similarity >90%)	Retention time (min)	Formula	%Area	
			With CO_2	Without CO_2
Carboxylic acid			**65.94**	**67.92**
Myristic acid	10.947	$C_{14}H_{28}O_2$	1.36	0.85
cis-9-Hexadecanoic acid	12.860	$C_{16}H_{28}O_2$	3.80	2.70
Palmitic acid	13.119	$C_{16}H_{32}O_2$	26.97	23.17
Methyl 4, 7,10,13,-hexadecatetraenoate	12.249	$C_{17}H_{26}O_2$	1.43	0.90
Oleic acid	14.804	$C_{18}H_{34}O_2$	29.98	28.37
Stearic acid	14.966	$C_{18}H_{36}O_2$	2.40	2.26
2, 3-Dihydroxylpropyl (9E)-9-octadecenoate	17.208	$C_{21}H_{40}O_4$	-	9.67
Ester			**15.18**	**7.09**
Palmitic acid mthyl ester	12.676	$C_{17}H_{34}O_2$	5.30	2.69
8, 11-Octadecadienoic acid, methyl ester	14.313	$C_{19}H_{34}O_2$	1.68	0.70
Oleic acid methyl ester	14.372	$C_{19}H_{36}O_2$	7.54	3.70
Stearic acid methyl ester	14.605	$C_{19}H_{38}O_2$	0.66	-
Ketone			**0.82**	**0.60**
2(4H)-Benzofuranone, 5, 6, 7, 7a-tetrahydro-6-hydroxy-4, 4, 7a-trimethyl (Loliolide)	11.228	$C_{11}H_{16}O_3$	0.82	0.60
Phenol			**1.07**	**0.70**
2, 4-bis(1, 1-Dimethylethyl)-phenol	8.133	$C_{14}H_{22}O$	1.07	0.70
Unknown			**4.60**	**3.58**
sum			**100**	**100**

From above table, it can be clearly seen that more than 65% of the compounds observed under both conditions with and without CO_2 supplementation were carboxylic acid. The majority of carboxylic acid studied were found to be

saturated and unsaturated fatty acids including myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1). These fatty acids are the common compounds that can be found in plant oils and animal fats, and serve as the substrate for biodiesel production (Khan *et al*., 2009). It was also found that the supplementation of CO_2 in the culture had a positive effect on microalgal lipid composition. The percentages of palmitic acid and oleic acid recorded from the MC under CO_2 supplementation were higher than of those without CO_2 supplementation. These results suggested that CO_2 supplementation could promote the degree of fatty acids found in microalgae. Similar results were found in *Botryococcus buanii*. This alga was cultured with exhaust gas (5.5% CO_2), the percentages of fatty acids, palmitic acid and oleic acid were higher than of those recorded under the ambient air conditions (Yoo *et al*., 2010). CO_2 supplementation not only enhanced the degree of fatty acids acquired from the microalgae, it could enhance the degree of fatty acid methyl esters (FAMEs) acquired from the microalgae. The percentages of some FAMEs such as palmitic acid methyl esters and oleic acid methyl esters from MC under CO_2 supplementation conditions were higher than those without CO_2 supplementation. FAMEs are the one of the fatty acid esters used as a type of biodiesel (Hena *et al*., 2015)

6.3.5 Carbon content, CO_2 fixation rate and CO_2 capture of MC

Table 6.3 shows the levels of carbon content, CO_2 fixation rate and CO_2 capture of the MC with and without CO_2 supplementation recorded during the wet and cold dry seasons.

Table 6.3 Carbon content, CO_2 fixation rate and CO_2 capture of the MC with and without CO_2 supplementation in the wet and cold dry seasons

Season	Aeration	Culture cycle	Carbon content (g dw^{-1})	CO_2 fixation rate (g CO_2 L^{-1} d^{-1})	CO_2 capture (gCO_2 dw^{-1})
Wet	CO_2	1	0.4147	0.0621	1.5206
	supplement	2	0.4111	0.0471	1.5074
		3	0.4110	0.0413	1.5070
		Mean	**0.4123±0.002**	**0.0502±0.011**	**1.5117±0.01**
	Without CO_2	1	0.3224	0.0392	1.1821
	supplement	2	0.3065	0.0215	1.1238
		3	0.3338	0.0246	1.2239
		Mean	**0.3209±0.01**	**0.0284±0.01**	**1.1766±0.05**
Dry	CO_2	1	0.4395	0.0557	1.6115
	supplement	2	0.4438	0.0532	1.6273
		3	0.4055	0.0422	1.4868
		Mean	**0.4296±0.02**	**0.0504±0.01**	**1.5752±0.08**
	Without CO_2	1	0.3920	0.0394	1.4373
	supplement	2	0.3997	0.0304	1.4656
		3	0.3627	0.0262	1.3299
		Mean	**0.3848±0.02**	**0.0320±0.01**	**1.4109±0.07**

It was found that the all values of MC cultivated with CO_2 supplementation in both the wet and cold dry seasons were higher than of those without CO_2 supplementation. In the case of the wet season, the mean averages of the carbon contents of the MC recorded with and without CO_2 supplementation were 0.4123±0.002 and 0.3209±0.01 g dw^{-1}, respectively. The CO_2 fixation rates of the MC with and without CO_2 supplementation were 0.0502±0.01 and 0.0284+0.01g CO_2 L^{-1} d^{-1}, respectively. The CO_2 capture rates of the MC with and without CO_2 supplementation were 1.5117±0.01 and 1.1766±0.05 g CO_2 dw^{-1}, respectively. In the case of the dry season, the mean averages of the carbon content of the MC with and without CO_2 supplementation were 0.4296±0.021 and 0.3483±0.02 g dw^{-1}, respectively. The CO_2 fixation rates of the MC with and without CO_2 supplementation were 0.0504±0.007 and

0.032+0.007g CO_2 $L^{-1}d^{-1}$, respectively. The CO_2 capture rates of the MC with and without CO_2 supplementation were 1.5752±0.077 and 1.4109±0.071 g CO_2 dw^{-1}, respectively. These results illustrated that the MC could fix CO_2 and convert it into biomass and lipids in the cells, thus these microalgae could be used for CO_2 mitigation and biodiesel production. According to a comparison between the wet and cold dry seasons, the carbon content, CO_2 fixation rate and CO_2 capture of the MC with CO_2 supplementation showed no significantly differences. This indicated that the microalgae could utilize CO_2 under variable pH values, temperatures and light intensity levels during the wet and cold-dry seasons.