CHAPTER 5

Cultivation of microalgal consortium using exhaust gas for CO_2 mitigation and lipid production

5.1 Introduction

During the Industrial Revolution, the burning of fossil fuel that occurs in industrial processes can generate waste gases or exhaust gases normally consist of CO_2, CO, H_2O, H_2, O_2, N_2, NOx, SOx, etc. (Zevenhoven and Kilpinen, 2001). These gases typically consist of CO_2 and other greenhouse gases which can significantly contribute to the global warming problem. Typically, industrial exhaust gases contain CO_2 levels ranging from 5% to 20% (v/v) depending on the type of fossil fuel that is used in the combustion process. For example, exhaust gases from natural gas combustion contain 5-6% CO_2, while exhaust gases from coal-fired power plants contain 10-20% CO_2 concentration (Ono and Cuello, 2003; Yen *et al*., 2015).

Photosynthetic microalgae are the most productive CO_2 users with higher biomass and lipid yields than of higher oil plants (Kumar *et al.*, 2011). It has been reported that microalgae can fix CO_2 from various sources such as atmospheric CO_2, industrial gases (e.g. flaring gas and exhaust gas) and soluble carbonates (e.g. Na_2CO_3 and $NaHCO_3$) (Znad *et al*., 2012). Thus, biofixation through the use of photosynthetic microalgae is the most promising potential method for CO_2 capture from industrial exhaust gases. In recent years, many researchers have proven that several microalgal species can utilize exhaust gas as carbon sources for their growth. Zeiler *et al*. (1995) suggested that simulated exhaust gas (13.6% CO_2, 5% O_2, 0.015% NO, 0.02% SO_2, balance N_2) could be used as a carbon source for culturing microalgae and research has also found that NO and SO_2 had no negative effect on microalgal growth. Lee *et al*. (2002) reported that the growth rate of *Chlorella* sp. KR-1 cultured with the real exhaust gas from boilers fueled by liquefied natural gas (10% CO_2) was almost equal to that of 10% CO_2-air mixture.

de Godos *et al*., (2010) studied the effects of exhaust gas on the growth characteristics of microalgae. The exhaust gas (7% CO_2) was aerated into a high rate algae pond (HRAP) which contained diluted piggery wastewater. The results found that the microalgae could grow well and had revealed COD and NH_4^+ removal efficiency.

Beside CO_2 mitigation, using exhaust gas for microalgal cultivation has displayed positive effects to biofuel production by microalgae because CO_2 from exhaust gases can be used for biomass and lipid production. Microalgal lipid can be used as a feedstock for biodiesel production (Hossain*et al*., 2008). In this work, the microalgal consortium (MC) was cultivated with the exhaust gas derived from a power generator supplied by biogas from chicken manure in order to study the possibility of CO_2reduction from industrial exhaust gases and bio-oil production from microalgae.

5.2 Materials and methods

The methods used to study microalgal consortium cultivation using exhaust gases for CO_2 mitigation and bio-oil production is presented in Figure 5.1

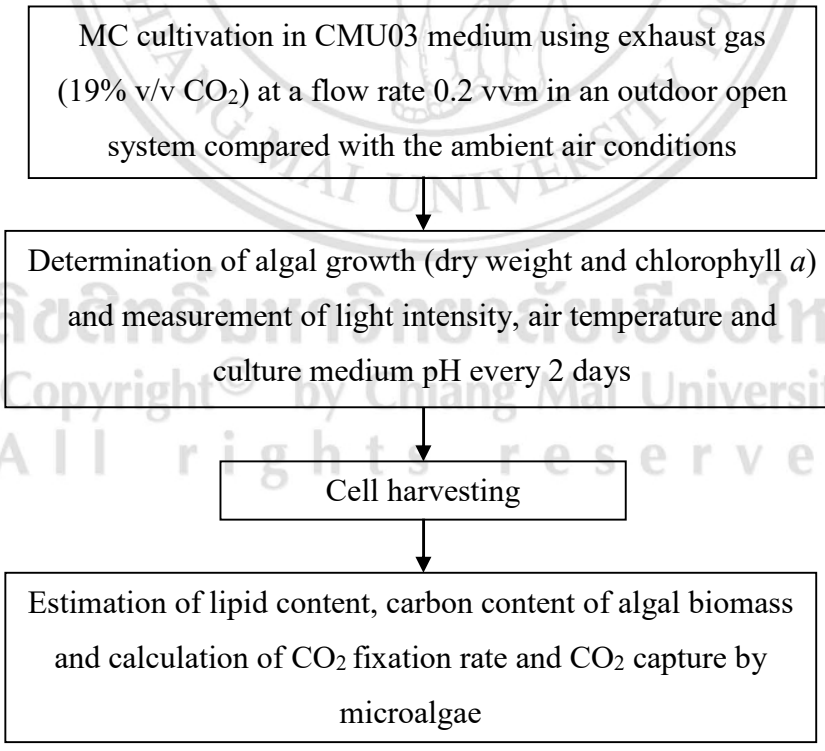


Figure 5.1 Flowchart diagram of microalgal cultivation using exhaust gas for CO_2 mitigation and bio-oil production

5.2.1 Microalgae and culture

The MC was prepared using the succession of air borne cultures enriched with CMU03 medium (Sriphuttra *et al*., 2013) and maintained in the same medium of the algal collection of the Applied Algal Research Laboratory (AARL), Department of Biology, Faculty of Science, Chiang Mai University. The MC (composed 23.7% *Acutodesmus dimorphus*, 42.7% *Micractinium pusillum*, 8.8% *A. acuminatus*, 7.3% *A. pectinatus*, 2.6% *A. obliquus*, 5.7% *Dictyosphaerium granulatum*, 3.8% *Chlamydomonas mirabilis*, 3.5% *Chlorella vulgaris*, 1.0% *Pseudanabaena galeata*, 0.4% *Monoraphidium littorale* and 0.9% *Euglena* sp.) was incubated under ambient temperatures under continuous illumination with a fluorescent lamp at 24.3 μmol m^{-2} s^{-1} for two weeks.

5.2.2 Cultivation of MC

The MC was cultivated with 10 L working volume of CMU03 medium under exhaust gas and compared with ambient air at a flow rate of 0.2 vvm. The time interval of exhaust gas aeration was 8 h d^{-1} (during the daytime). The exhaust gas was obtained from a power generator supplied by the biogas collected from chicken manure at Hauy Nam Rin's Farm, Lamphun Province. The composition of this gas is shown in Table 5.1. The cultures were performed in an outdoor open system (without light and temperature control). Both treatments were conducted in triplicate. The microalgal cultivation system is presented schematically in Figure 5.2.

Table 5.1 Composition of exhaust gas from a power generator

Compound	Vol.% of exhaust gas
CH_4	0.20
CO	0.08
CO_2	19
O_2	7
N_2	73

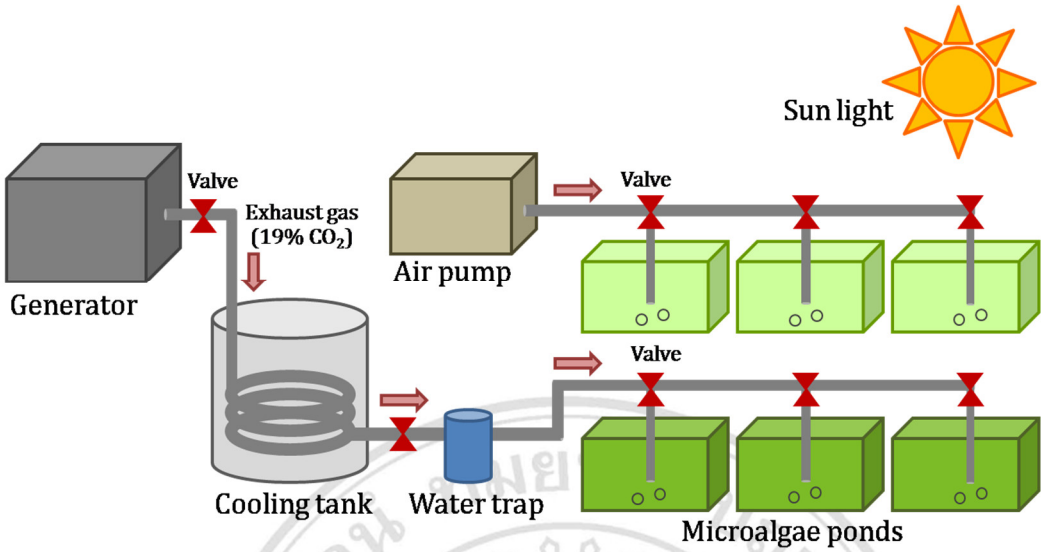


Figure 5.2 Schematic diagram of the cultivation of MC under exhaust gas and ambient air conditions

5.2.3 Sample collecting and analytical determinations

The 50 mL of culture samples was collected to determine 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 the microalgae and some parameters were measured every 2 days. Light intensity was measured using a Lux meter[1] (Tecpel 530). Air and water temperature were measured using a thermometer. The pH value of the culture medium was measured using a pH meter (electrode kit of WTW Company). After 8 days of cultivation, the microalgal cells were then harvested. The dry weight, chlorophyll *a* content, dominant species, lipid content, carbon contents, CO_2 fixation rate and CO_2 capture were analyzed according to the procedures described in Chapter 3.

[1]The unit conversions from Lux to µmol m^{-2} s^{-1} can be obtained by multiply the Lux by 0.0185 (for sunlight) and 0.0135 (for cool white fluorescent lamps). For example, the sunlight intensity is 10,000 Lux or 185 µmol m^{-2} s^{-1} (10,000*0.0185) (Thimijan and Heins, 1983).

5.2.4 Statistical analysis

The results are expressed as mean±SD (standard deviation) of three replicates. All data were performed by SPSS version 16.0 for Windows. The paired sample t-test was used to evaluate the differences between exhaust gas and ambient air conditions. A value of $p<0.05$ was considered statistically significant.

5.3 Results and discussion

5.3.1 Microalgal growth

To study the influence of exhaust gas on microalgal cultivation, the growth of MC was determined when it was aerated with exhaust gas and ambient air. The exhaust gas was acquired from a power generator supplied by the biogas collected from chicken manure and consisted of 19% CO_2, 0.2% CH_4, 0.08 CO, 7% O_2 and 73% N_2. This gas composition was selected for this study because it approximated the concentration of CO_2 in a typical industrial exhaust gas sample but some compositions were different from normal exhaust gas (e.g. NOx and SOx have not found in this study). The cultures were performed in an outdoor open system (without light and temperature control). During the cultivation period, the ambient temperature ranged from 28.5 to 35.5ºC and light intensity ranged from 186.9-536.5 μmol m^{-2} s^{-1}. After 8 days of cultivation, the maximum dry weight of MC cultured with exhaust gas and under ambient air conditions were observed at 0.25 ± 0.04^{a} and 0.20 ± 0.01^{b} g L^{-1}, respectively (Figure 5.3). The chlorophyll *a* content also correlated with the dry weight (Figure 5.4). The maximum chlorophyll *a* content of MC cultured with exhaust gas was $1{,}492.5\pm582^{a}$ μg L^{-1} on the 8th day of cultivation, while the maximum value of MC cultured under ambient conditions was 1141.99 ± 359.0^{b} μg L^{-1} on the 7th day of cultivation. Moreover, it was found that the cultures in this experiment were cultivated during a short period (8 days). This was because after this period, the growth of microalgae was dramatically decreased and reached the death phase.

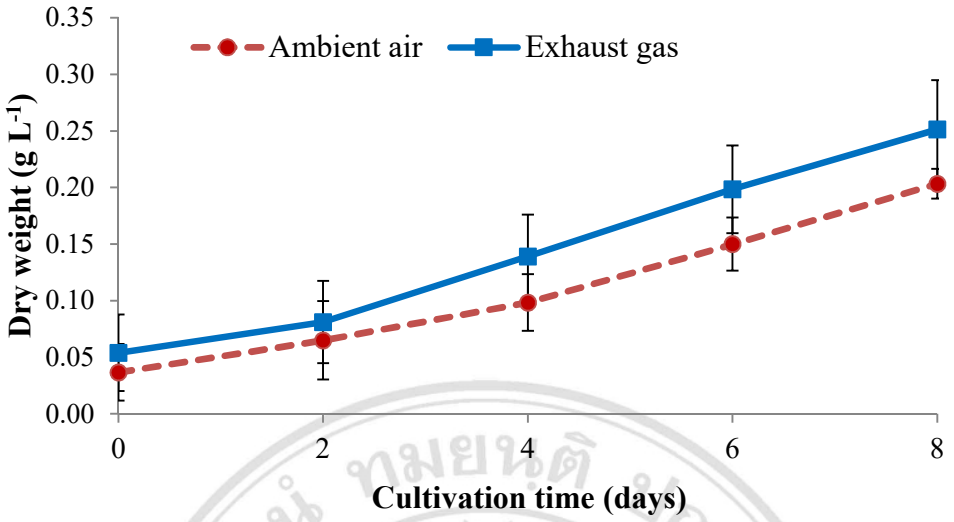


Figure 5.3 Dry weight of the MC under exhaust gas condition compared with ambient air condition

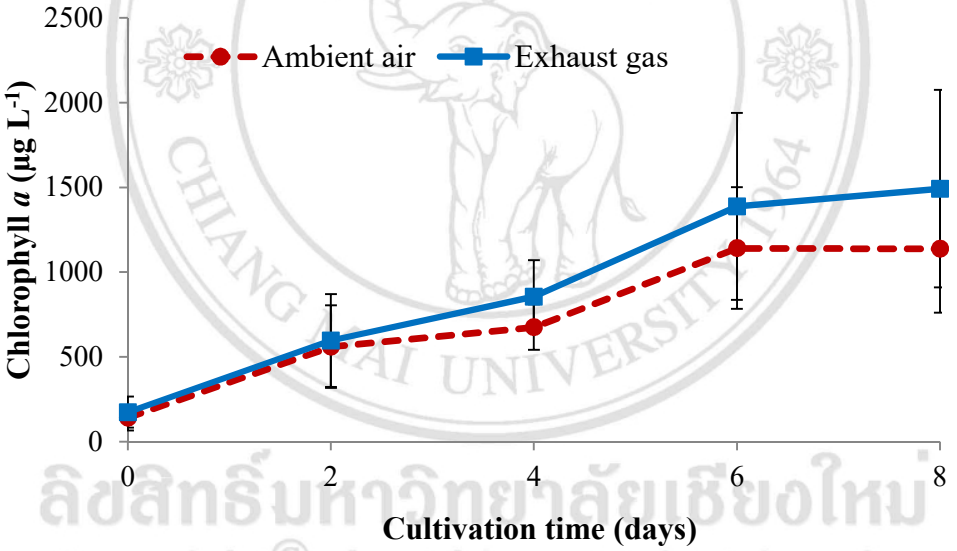


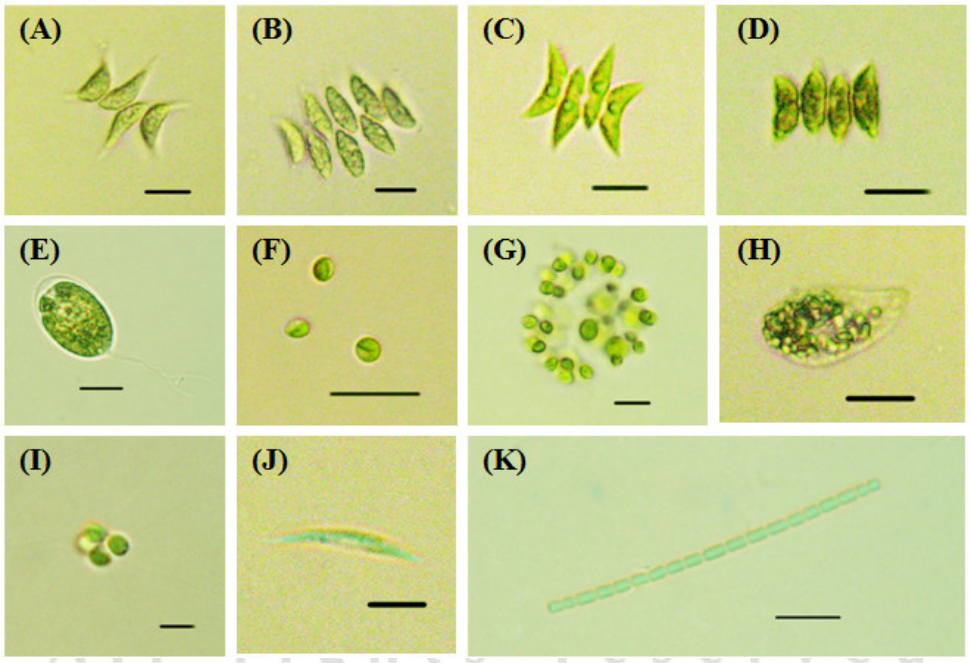
Figure 5.4 Chlorophyll *a* content of the MC under exhaust gas condition compared with ambient air condition

In this study, the MC exposed to exhaust gas revealed a higher growth rate than that of the ambient air. The biomass concentration and chlorophyll *a* content of the microalgae were increased when exhaust gas was used in the cultivation of microalgae. These results indicated that MC could utilize CO_2 from exhaust gas as a carbon source for their growth. Moreover, it was also observed that the other compounds, which were CH_4 and CO and were

present in the exhaust gas, had no negative effect on microalgal growth. This result was similar to Doucha *et al*. (2005), who found that the microalga *Chlorella* sp. could be grown well when it was cultivated with the industrial exhaust gas (6-8% CO_2) that was contaminated with other gases.

5.3.2 Species composition of MC

The dominant species of the microalgal consortium in the cultivation stage with both ambient air and exhaust gas were investigated. The common algal species found under both aeration conditions were green microalgae species, such as *Acutodesmus* spp., *Chlorella vulgaris*, *Chlamydomonas mirabilis*, *Dictyosphaerium granulatum*, *Micractinium pusillum*, *Monoraphidium littorale* and *Euglena* sp. (Figure 5.5).



scale bar = 10 µm

Figure 5.5 Dominant species of the MC under exhaust gas and ambient air conditions; (A) *Acutodesmus pectinatus*, (B) *A. acuminatus,* (C) *A. dimorphus,* (D) *A. obliguus*, (E) *Chlamydomonas mirabilis*, (F) *Chlorella vulgaris*, (G) *Dictyosphaerium granulatum*, (H) *Euglena* sp., (I) *Micractinium pusillum*, (J) *Monoraphidium littorale* and (K) *Pseudanabaena galeata*

The percentages of biovolume ratio of the dominant species recorded under exhaust gas conditions and ambient air conditions are shown in Figure 5.6. It was observed that the dominant population of the algal community changed during the cultivation period. Under the ambient air condition, the most dominant species in term of biovolume observed were *Acutodesmus pectinatus* followed by *A. dimorphus, A. acuminatus* and *Chlamydomonas mirabilis* while under the exhaust gas condition, the dominant microalgal species observed were *A. dimorphus* followed by *A. pectinatus, A. obliguus*, and *C. mirabilis*. During the cultivation process using exhaust gas, it was clearly seen that the % biovolume change of *A. obliguus* between the end and the early stage of the cultivation period was dramatically increased (4.6-fold) and followed by *A. pectinatus* (2.85-fold) and *A. dimorphus* (2.14-fold), respectively. Nevertheless, a significant negative % change was found for *Micractinium* sp. (6.3-fold loss).

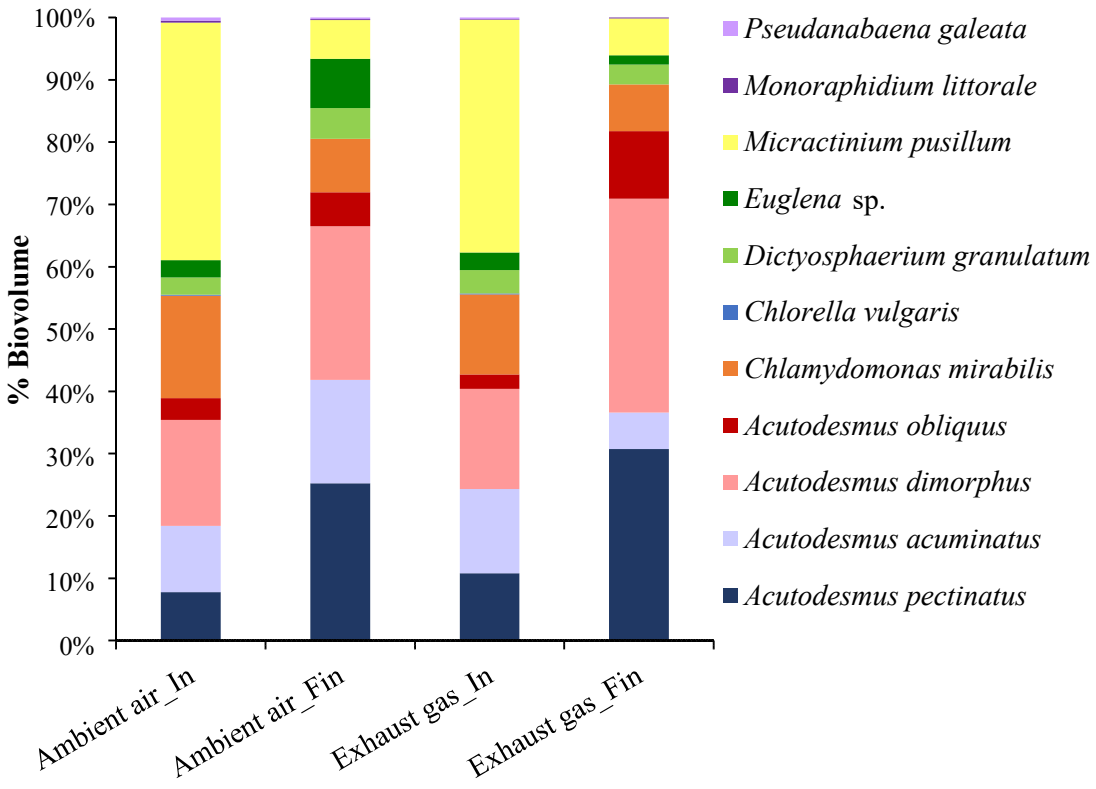


Figure 5.6 % Biovolume of microalgal species in MC cultured under exhaust gas and ambient air conditions; In = Initial (first day), Fin = Final (last day)

In this study, during the cultivation process with exhaust gas, the common algal species observed were *Acutodesmus* spp., which revealed very high biomass and lipid contents (Parker, 2010). It was indicated that variations in the algal community were affected by CO_2 cultivation. Salih (2011) reported that *Scenedesmus* sp. is one of the species that is highly tolerant to CO_2. This strain could grow under 80% CO_2 conditions, while the maximum cell mass was observed in 10-20% CO_2 concentration levels. Moreover, Guruvaiah and Lee (2014) reported that *Scenedesmus* sp., isolated from the power plant habitat had a lipid content of 15 % of its dry weight when cultivated with simulated exhaust gas containing 2% CO_2. These results indicate that the genus *Acutodesmus* sp. has great potential for CO_2 mitigation, environmental tolerance and biodiesel production.

5.3.3 Lipid content of MC

The lipid contents of the MC under exhaust gas and ambient air conditions are shown in Table 5.2. The lipid contents of MC under the exhaust gas conditions were 48.83±6.38 mg $L^{-1}$and 16.96±2.29% of the dry weight, while the lipid contents of MC under the ambient air conditions were 31.62±2.11 mg L^{-1} and 15.70±1.44% of the dry weight. These results indicated that the lipid content of MC recorded under exhaust gas conditions seemed higher than of that recorded under the ambient air conditions. Xia *et al*. (2013) also found that the lipid content of *Chlorella sorokiniana* CS01 cultured with simulated exhaust gas in a range of 5-15% CO_2 was higher than of that using only the ambient air aeration method (0.02-0.03% CO_2).

Table 5.2 Lipid contents of the MC under exhaust gas condition compared with the ambient air condition

Aeration	Lipid content	
	mg L^{-1}	% of dry weight
Ambient air	31.62±2.11	15.70±1.44
Exhaust gas	48.83±6.38*	16.96±2.29*

*indicates a significant difference ($p< 0.05$) between each condition

5.3.4 Biomass and lipid productivity of MC

The values of both the biomass and lipid productivity of the culture with the exhaust gas were higher than of that with the ambient air by approximately 1.2-fold (Figure 5.7). It is indicated that the CO_2 in the exhaust gas was a significant factor for biomass and lipid production. Theologically, CO_2 was absorbed and fixed by the photosynthetic activity (Calvin cycle), and thus enhanced biomass and lipid production (Radakovits *et al.*, 2010; Xia *et al.*, 2013). Guruvaiah and Lee (2014) reported that *Scenedesmus* sp. could up take CO_2 from a wide variety of exhaust gases from coal burning power plants and had higher growth rates and lipid accumulation when compared with the control (non exhaust gas aeration).

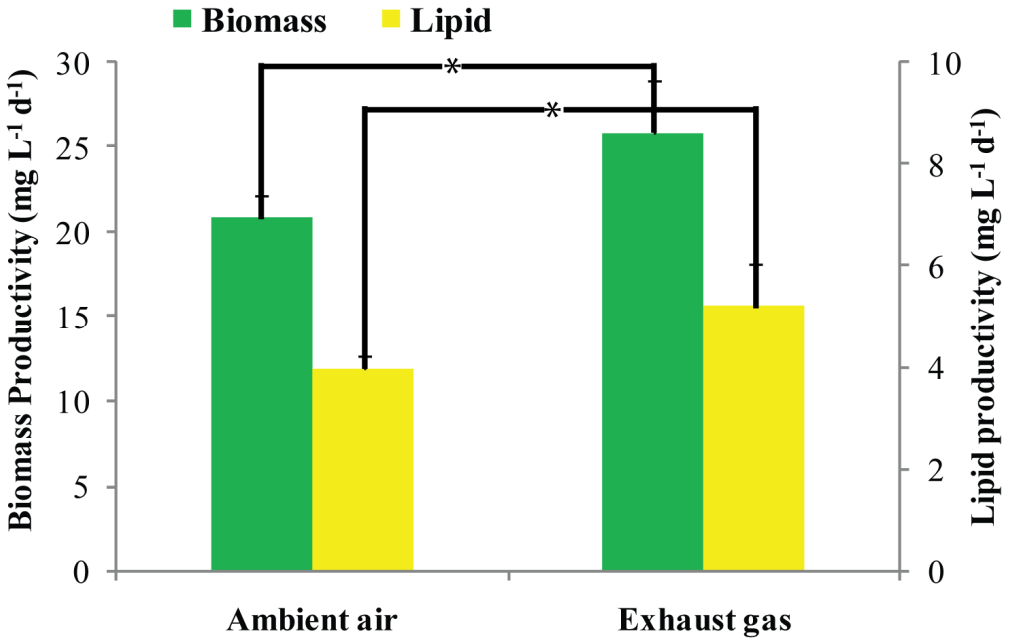


Figure 5.7 Biomass and lipid productivity of the MC under exhaust gas condition compared with ambient air condition. * indicates a significant difference (p<0.05) between each condition

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

The carbon content, CO_2 fixation rate and CO_2 capture of the MC under exhaust gas and ambient air conditions were evaluated. The results of all values are shown in Table 5.3

Table 5.3 Carbon content, CO_2 fixation rate and CO_2 capture of the MC under exhaust gas condition compared with the ambient air condition

Aeration	Carbon content ($g\ dw^{-1}$)	CO_2 fixation rate ($g\ CO_2\ L^{-1}\ d^{-1}$)	CO_2 capture ($g\ CO_2\ dw^{-1}$)
Ambient air	0.4414	0.0337	1.6182
Exhaust gas	0.4551	0.0431	1.7458

As shown in Table 5.3, the carbon content of MC seemed to increase when the exhaust gas was used and it could lead to an increase in the CO_2 fixation rate by 1.3-fold (0.0431 $CO_2\ L^{-1}\ d^{-1}$), when compared with the ambient air condition. These results indicate that CO_2 from the exhaust gas could promote the carbon content and CO_2 fixation ability of microalgae. Yahya *et al*. (2012) found that the CO_2 fixation rate of *Isochrysis* sp. was increased along with an increase in the CO_2 concentration of exhaust gas. The CO_2 fixation ability of microalgae depended on the microalgal species, CO_2 concentration level, nutrient concentration level, temperature, pH value and light intensity. In general, the CO_2 fixation ability is found to be directly proportional to the growth rate of microalgae (Cheng *et al*., 2013). In this study, the results of the CO_2 capture process found that the MC under exhaust gas condition had a higher degree of CO_2 capture than of that under the ambient air condition. The CO_2 capture readings of MC under exhaust gas and ambient air conditions were 1.7458 and 1.6182 g $CO_2\ dw^{-1}$, which were close to the theological value (1.83 g $CO_2\ dw^{-1}$, Chisti, 2007).