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LIST OF PUBLICATIONS

1) Boonma, S., Chaiklangmuang, S., Chaiwongsar, S., Pekkoh, J., Pumas, C., Ungsethaphand, T., Tongsiri, S. and Peerapornpisal, Y. 2015. Enhanced carbon dioxide fixation and bio-oil production of a microalgal consortium. Journal of Clean-Soil, Air, Water, 43(6): 761-766.

2) Boonma, S., Vacharapiyasophon, P., Peerapornpisal, Y., Pekkoh, J. and Pumas, C. 2014. Isolation and cultivation of *Botryococcus braunii Kützing* northern Thailand. Chiang Mai Journal of Science, 41(2): 298- 306.

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Research Article

Enhanced Carbon Dioxide Fixation and Bio-Oil Production of a Microalgal Consortium

In this study, a microalgal consortium was cultivated with different CO_2 supplements: ambient air (0.03% CO_2), 10 and 30% (v/v) CO_2. It was found that the growth rate of the cultures supplemented with 30% CO_2 was the highest among the others. The biomass and lipid productivity for the microalgal consortium with 30% CO_2 were 21.1 and 4.8 $mg\,L^{-1}\,day^{-1}$ (27.6% of dry weight, dw). The ability of CO_2 fixation under 30% CO_2 supplementation was found to be 0.0271 $g\,CO_2\,L^{-1}\,day^{-1}$, which is higher than in the ambient air supplementation. Then, the microalgal consortium was cultivated with exhaust gas (19% CO_2) from a power generator supplied by biogas from chicken manure. It was found that the growth of the microalgae supplemented with exhaust gas was higher than in the ambient air. The biomass and lipid productivity for the microalgal consortium with exhaust gas were 25.82 and 5.2 $mg\,L^{-1}\,day^{-1}$ (16.96% of dw) and the most dominant algal species observed were *Acutodesmus* (*Scenedesmus*) sp., *A. dimorphus* (Turpin) Tsarenko and *Scenedesmus obliquus* (Turpin) Kützing, respectively. Moreover, it could be revealed that the ability of CO_2 fixation under supplementations with exhaust gas increased 1.3-fold compared with ambient air. Therefore, the microalgal consortium has high potential for both CO_2 reduction and bio-oil production, simultaneously.

Keywords: Biofuels; Biomass; Greenhouse gas; Microalgae; Renewable energy

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1 Introduction

Global warming induced by an increase in the concentration of greenhouse gases in the atmosphere is of great global concern [1]. Carbon dioxide (CO_2) is one of the most important contributors in the increase of the greenhouse effect [2]. Among the various strategies for mitigating CO_2 emissions, the biological sequestration of CO_2 using photosynthetic microalgae has been receiving considerable attention, as the microalgae have a higher CO_2 fixation ability than terrestrial plants, and they can convert atmospheric CO_2 into biomass, fatty acids and lipids [3, 4]. Microalgal biomass contains approximately 50% C dry weight (dw). All of this carbon is typically derived from carbon dioxide. It has been estimated that 100 t of algal biomass fixes roughly 183 t of CO_2 [5]. Moreover, many researchers have proved that microalgal lipids could be utilized as a feedstock for biodiesel. The other benefits of microalgae for biofuel production include the fact that it does not require a large area for cultivation, it is easy to culture, it is characterized by rapid growth and it can be grown in water that is considered unsuitable for human consumption. In other words, it can be grown anywhere where there is access to sunlight and where simple nutrients are available, though its growth rate depends also on the availability of the addition of certain specific compounds and appropriate aeration [4, 6].

Usual sources of CO_2 for microalgae include: (i) atmospheric CO_2; (ii) CO_2 from industrial exhaust gases (e.g., flue gas and flaring gas); and (iii) CO_2 that is chemically fixed in the form of soluble carbonates (e.g., $NaHCO_3$ and Na_2CO_3) [7]. However, industrial exhaust gases can be utilized in ways with clear ecological and economical advantages, even though the presence of high toxic gases and various CO_2 concentrations can be problematic. Thus, screening for microalgae, which are tolerant to high CO_2 concentration levels, has been carried out as an essential step for the CO_2 utilization from flue gases [8]. Several species have been tested under CO_2 concentrations of over 15%, for example, *Chlorococcum littorale* could be grown under 60% CO_2 using the stepwise adaptation technique [9]. Other highly CO_2 tolerant species are *Chlorella* sp. and *Scenedesmus* sp. It is also reported that *Chlorella* sp. could grow under 40% CO_2 conditions while *Scenedesmus* sp. could grow under 80% CO_2 conditions, but the maximum cell mass was observed in 10–20% of the CO_2 concentrations [10]. Interestingly, the red alga, *Cyanidium caldarium* can grow in pure CO_2 [11].

However, the screening and isolation of the suitable mono algal strain was time consuming and not favorable for CO_2 fixation on the industrial scale. A mixed culture community selected by succession of enrichment cultures revealed greater efficiency and accomplishment [12]. Mixed populations (co-culture or consortia) can perform functions that are difficult or even impossible for individual strains

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Abbreviations: Chl-a, chlorophyll a; **dw**, dry weight

or species and they reveal robustness to environmental fluctuations, display stability for the members, possess inability to share metabolites and to weather periods of nutrient limitation, and are resistant to invasion by other species [13]. Nevertheless, using microalgal consortia for CO_2 fixation and lipid production has seldom been reported on. Therefore, in this study, the cultivation of the microalgal consortium was tested under high levels of CO_2 concentrations for enhanced biomass production and CO_2 fixation. Growth, lipid contents and CO_2 fixation of the algal were also evaluated. To test this potential application in the industrial sector, microalgal consortium was cultivated with exhaust gas from a power generator to evaluate the possibility of CO_2 reduction from the atmosphere, as well as biomass and lipid production by microalgae in the industrial process.

2 Materials and methods

2.1 Microalgae and culture

The microalgal consortium used here (composed of 65.7% *Scenedesmus* spp., 25.4% *Micractinium* sp., 3.6% *Dictyosphaerium* sp., 2.7% *Pseudanabaena* sp., 0.8% *Monoraphidium* sp., 1% *Chlamydomonas* sp., 0.4% *Chlorella* sp. and 0.4% *Euglena* sp.) was obtained from the algal collection of the Applied Algal Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Thailand. The microalgal consortium cells were incubated in CMU03 medium [14] at ambient temperature under continuous illumination.

2.2 Cultivation of microalgal consortium

2.2.1 Effect of CO_2 supplementation

The microalgal consortium was cultivated in CMU03 medium with a 500 mL working volume in a closed system. The cultures were aerated with different aerations: ambient air (0.03% CO_2), 10 and 30% CO_2 (v/v), balanced with N_2 at a flow rate of 0.2 vvm, under continuous illumination with a fluorescent lamp at ambient temperature. The algal growth was measured until it reached the early stationary phase. Each treatment was conducted in triplicate.

2.2.2 Cultivation with exhaust gas

The microalgal consortium was cultivated with exhaust gas and compared with ambient air at a flow rate of 0.2 vvm. The exhaust gas (composed of 19% CO_2, 0.2% CH_4, 0.08% CO, 7% O_2, and 73% N_2) was obtained from a power generator supplied by the biogas collected from chicken manure at Hauy Nam Rin's Farm, Lamphun Province, Thailand. The cultures were cultivated in an outdoor open system (without light and temperature control) with a 10 L working volume. Both treatments were conducted in triplicate.

2.3 Determination of microalgal growth

The dry weight of the microalgae was measured using a modified method of Yoo et al. [3]. A known volume of microalgal suspension was filtered through 0.45 μm filter paper under vacuum. After being rinsed with distilled water, the filters were dried at 60°C for 48 h. Algal growth was expressed in terms of dry biomass ($g\,L^{-1}$), which was determined gravimetrically.

The chlorophyll a content (Chl-a, $\mu g\,L^{-1}$) was determined according to Wintermans and De Mots [15] and Saijo [16] by using 90% methanol for extraction. The pooled extract was measured spectrophotometrically at 630, 645, 665, and 750 nm, and calculated with the following equation:

$$\text{Chl-a} = \frac{(11.6(A_{665} - A_{750}) - 1.31(A_{645} - A_{750}) - 0.14(A_{630} - A_{750})v)}{Vl} \quad (1)$$

where v is the volume of the extract (mL), V the volume of the sample filtered (L) and l is the path length of the spectrophotometer cuvette (cm).

The biomass productivity (P, $mg\,L^{-1}\,day^{-1}$) was calculated according to the equation [17]:

$$P = \frac{(X_1 - X_0)}{(t_1 - t_0)} \quad (2)$$

where X_0 is the initial biomass ($mg\,L^{-1}$) at the time t_0 (d), X_1 is the final biomass ($mg\,L^{-1}$) at any time t_1 (d).

2.4 Estimation of CO_2 fixation rate

The CO_2 fixation rate was estimated from the carbon content of algal cells and the growth rate, as follows [8]:

$$R_{CO_2} = C_C \mu_L \times \left(\frac{M_{CO_2}}{M_C}\right) \quad (3)$$

where R_{CO_2} and μ_L are the CO_2 fixation rate ($g\,CO_2\,L^{-1}\,day^{-1}$) and the volumetric growth rate ($g\,dw\,L^{-1}\,day^{-1}$), respectively. M_{CO_2} and M_C represented the molecular weight of CO_2 and elemental carbon, respectively. C_C (g carbon per g cell dw) is the average carbon content, measured by a CHNS/O elemental analyzer (PE2400 SeriesII, Perkin Elmer).

2.5 Lipid measurement

Total lipids were extracted using a modified method of Bligh and Dyer [18]. A known weight of dry microalgal biomass was sonicated for 1 h in chloroform/methanol (2:1, v/v). The chloroform layer was collected, and evaporated to complete dryness at room temperature. Lipid contents were measured gravimetrically.

2.6 Microscopic observation

Species and quantities of exponentially growing microalgal cells were observed under a light microscope (Olympus C011) and photographed using an Olympus Normaski microscope. The microalgal species were morphologically identified according to relevant keys, that is, Huber-Pestalozzi [19], Komarek and Anagnostidis [20], and John et al. [21].

2.7 Statistical analysis

The results are expressed as mean ± SD of three replicates. All data were performed by SPSS version 16.0 for Windows, and was examined by one-way ANOVA and paired sample t-test. A value of $p < 0.05$ was considered statistically significant.

3 Results

3.1 Effect of CO_2 supplement

The microalgal consortium was cultivated with different levels of aeration: ambient air (0.03% CO_2), 10 and 30% CO_2 balanced with N_2 with a flow rate of 0.2 vvm. The results showed that the microalgae grew well without any obvious inhibition under all CO_2 concentration levels, even without pH control (ranged from 5 to 8, data not shown). In this study, the growth of the microalgae aerated with 30% CO_2 was the highest with a maximum level of algal biomass at $0.36 \pm 0.07\ g\ L^{-1}$ (Fig. 1A), while under ambient air conditions (0.03% CO_2) and 10% CO_2 aeration level, dry biomass readings were 0.1 ± 0.03 and $0.23 \pm 0.06\ g\ L^{-1}$, respectively. Under ambient air conditions (0.03% CO_2), 10 and 30% CO_2 aeration levels, the Chl-a content reached 1073.04 ± 294.14, 3193.32 ± 540.61, and $2179.67 \pm 674.55\ \mu g\ L^{-1}$, respectively (Fig. 1B). Lipid content readings of the microalgal consortium supplemented with ambient air (0.03% CO_2), 10 and 30% CO_2 aeration levels were 12.96 ± 0.52, 24.55 ± 0.50, and 27.60 ± 1.67% (w/w), respectively (Table 1).

After 18 days of cultivation, according to Fig. 2, the highest biomass and lipid productivity levels were 16.3 and 4.8 $mg\ L^{-1}\ day^{-1}$ recorded with the supplementation of 30% CO_2. It indicated that the microalgae in this condition grew better than under other testing conditions.

Table 2 shows an increase of carbon content in algal biomass, which enhanced microalgal CO_2 fixation. The carbon content of the microalgal consortium supplemented with 30% CO_2 was found to be highest at $0.4529\ g\ dw^{-1}$, while CO_2 fixation was recorded at $0.0271\ g\ CO_2\ L^{-1}\ day^{-1}$.

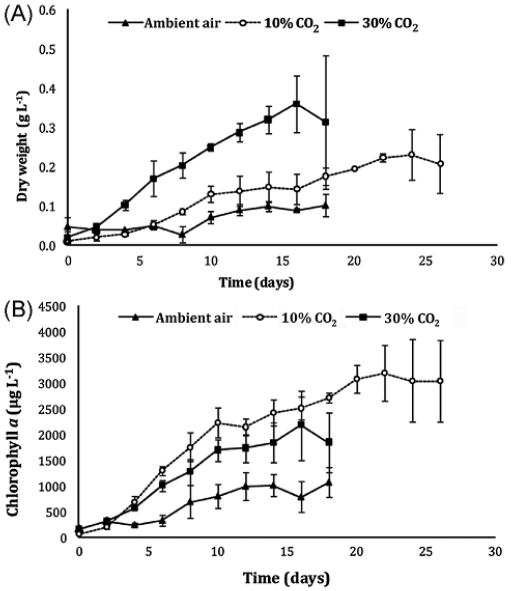

Figure 1. Dry weight (A) and Chl-a contents (B) of the microalgal consortium under different aeration levels.

Table 1. Lipid content of microalgal consortium under different aeration levels

Aeration	Lipid content	
	$mg\ L^{-1}$	% of dry weight
Ambient air	13.17 ± 0.53^c	12.96 ± 0.52^c
10% CO_2	50.95 ± 1.03^b	24.55 ± 0.50^b
30% CO_2	86.49 ± 2.11^a	27.60 ± 1.68^a

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

Table 2. Volumetric growth rate, carbon content, and CO_2 fixation rate of the microalgal consortium under different aeration levels

Aeration	μ ($g\ dw\ L^{-1}\ day^{-1}$)	Carbon content ($g\ dw^{-1}$)	CO_2 fixation rate ($g\ CO_2\ L^{-1}\ day^{-1}$)
Ambient air	0.0031	0.4000	0.0045
10% CO_2	0.0068	0.4303	0.0107
30% CO_2	0.0163	0.4529	0.0271

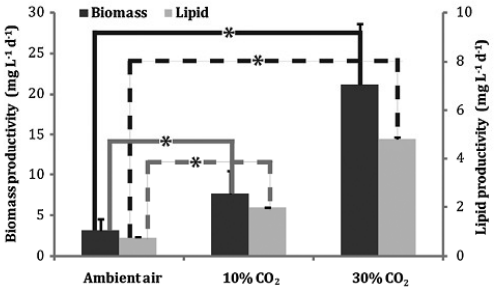

Figure 2. Biomass and lipid productivity of the microalgal consortium under different aeration levels. * indicated a significant difference ($p < 0.05$) between each conditions.

3.2 Cultivation with exhaust gas

The microalgal consortium was cultivated with ambient air and exhaust gas (19% CO_2) from a power generator supplying biogas from chicken manure. The maximum dry weight for microalgae with the exhaust gas and ambient air conditions were observed at 0.25 ± 0.04 and $0.20 \pm 0.01\ g\ L^{-1}$, respectively (Fig. 3A). Under the exhaust gas conditions the chlorophyll *a* content also reached its peak on day 8 of cultivation at $1492.5 \pm 582\ \mu g\ L^{-1}$, while the sample cultivated with ambient air conditions reached its peak on 7th day of the cultivation at $1141.99 \pm 359.0\ \mu g\ L^{-1}$ (Fig. 3B). Lipid content was 16.96 ± 2.29 and 15.70 ± 1.44% (w/w) with exhaust gas and ambient air conditions, respectively (Table 3).

As shown in Fig. 4, the biomass and lipid productivity of the culture with exhaust gas was higher than that with the ambient air by approximately 1.2-fold. Moreover, when using exhaust gas, it was found that the carbon content of the microalgae increased and it could lead to an increase of the CO_2 fixation rate by 1.3-fold ($0.0431\ g\ CO_2\ L^{-1}\ day^{-1}$), when compared with the ambient air aeration (Table 4).

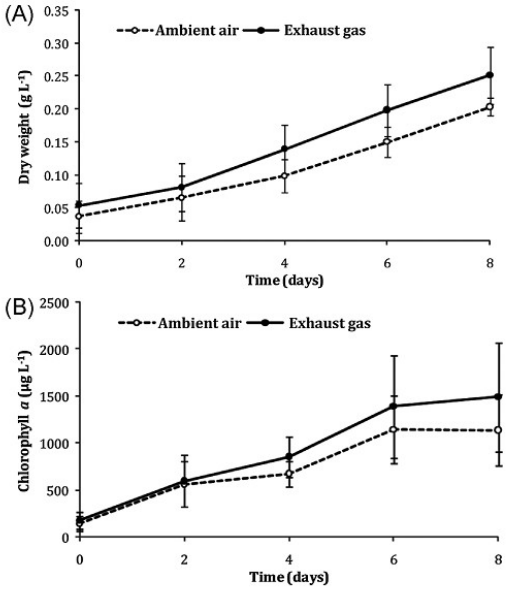

Figure 3. Dry weight (A) and Chl-a contents (B) of the microalgal consortium under exhaust gas conditions compared with ambient air conditions.

Table 3. Lipid content of the microalgal consortium under exhaust gas conditions compared with ambient air conditions

Aeration	Lipid content	
	mg L^{-1}	% of dry weight
Ambient air	31.62 ± 2.11^{b}	15.70 ± 1.44^{b}
Exhaust gas	41.83 ± 6.38^{a}	16.96 ± 2.29^{a}

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

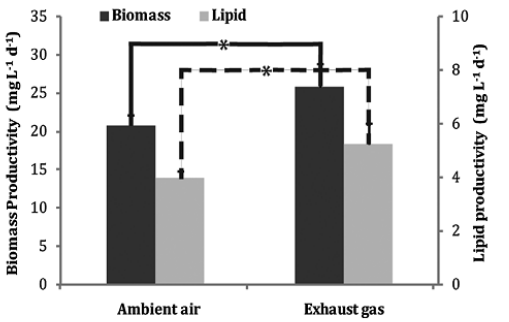

Figure 4. Biomass and lipid productivity of the microalgal consortium under exhaust gas conditions compared with ambient air conditions. * Indicated a significant difference ($p < 0.05$) between exhaust gas conditions and ambient air conditions.

Table 4. Volumetric growth rate, carbon content, and CO_2 fixation rate of the microalgal consortium under exhaust gas conditions compared with ambient air conditions

Aeration	μ (g dw L^{-1} day^{-1})	Carbon content (g dw^{-1})	CO_2 fixation rate (g CO_2 L^{-1} day^{-1})
Ambient air	0.0208	0.4414	0.0337
Exhaust gas	0.0258	0.4551	0.0431

The dominant species of the microalgal consortium in the cultivation stage with ambient air and exhaust gas were investigated. The common algal species found in both aeration conditions were green microalgae species, such as *Scenedesmus* spp., *Acutodesmus* spp., *Chlorella* sp., *Chlamydomonas* sp., *Dictyosphaerium* sp., *Micractinium* sp., *Monoraphidium* sp., and *Euglena* sp. (Fig. 5). It was observed that the dominant population of the algal community changed during the cultivation period (Fig. 6). During the cultivation process using exhaust gas, it was clearly seen that the % change of *Acutodesmus* (*Scenedesmus*) sp. was dramatically increased, followed by *A. dimorphus* (Turpin) Tsarenko, *Scenedesmus obliquus* (Turpin) Kützing, and *Dictyosphaerium granulatum*, respectively. Nevertheless, a significant negative %change was found for *Micractinium* sp.

4 Discussion

4.1 Effect of CO_2 supplement

The CO_2 concentration in the aeration of the culture media is considered one of the main factors in affecting microalgal growth [22]. In this study, the microalgae could grow well under CO_2 concentrations ranging from 0.03 to 30%. Similar results were also found for *S. obliquus* SJTU-3 and *Chlorella pyrenoidosa* SJTU-2 when the CO_2 concentration level increased from 0.03 to 50%. These results revealed the highest values of maximum biomass concentration at about 1.8 and 1.5 g L^{-1} of *S. obliquus* SJTU-3 and *C. pyrenoidosa* SJTU-2, respectively, at 10% CO_2 concentration [17]. In this study, the maximum dry cell weight was observed at 30% CO_2. These results provided evidence that the CO_2 tolerant microalgae in the mixed microalgal community were enriched and selected by the high CO_2 concentration.

In Fig. 1B, it was found that the Chl-a content of the 10% CO_2 concentration level was higher compared to the 30% CO_2 concentration level. These results indicated that Chl-a is not related to algal biomass. Boyer et al. [23] reported that Chl-a is relatively easy to measure compared to algal biomass. One serious weakness of the use of Chl-a is the great variability of the cellular chlorophyll content (0.1–9.7% of fresh algal weight) depending on the algal species. A great variability in individual cases can be expected, either seasonally or on an annual basis due to species composition, light conditions and nutrient availability.

This study showed that the microalgae grew well without any obvious inhibition under all CO_2 concentration levels, even without pH control. Normally, when CO_2 is aerated into water, it will form carbonic acid (H_2CO_3) and the pH value will be decrease. However, microalgae can increase pH by two ways. Firstly 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

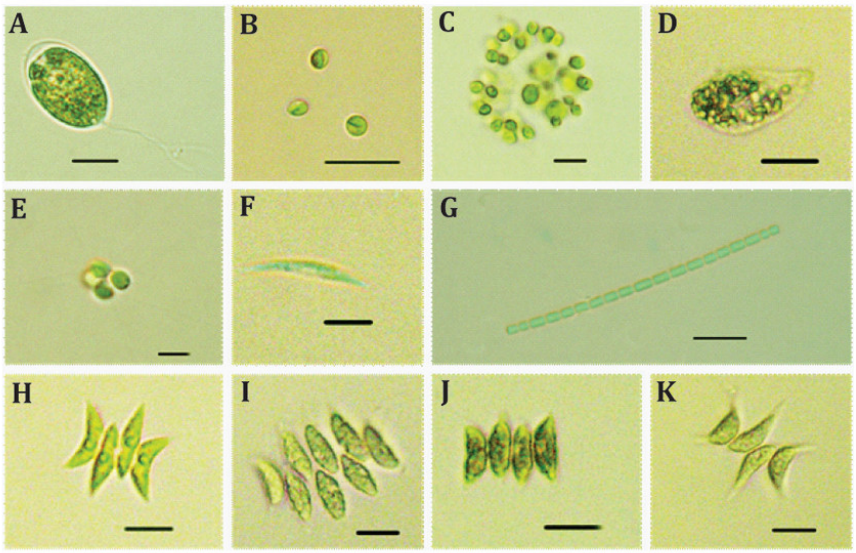

Figure 5. Dominant species of the microalgal consortium under exhaust gas conditions and ambient air conditions. (A) *Chlamydomonas* sp., (B) *Chlorella* sp., (C) *Dictyosphaerium granulatum* Hindák, (D) *Euglena* sp., (E) *Micractinium* sp., (F) *Monoraphidium littorale* Hindák, (G) *Pseudanabaena* sp., (H) *Acutodesmus (Scenedesmus) dimorphus* (Turpin) Tsarenkom, (I) *Scenedesmus acuminatus* (Lagerheim) Chodat, (J) *S. obliguus* (Turpin) Kützing, (K) *Acutodesmus* (*Scenedesmus*) sp.; scale bar = 10 μm.

generate alkalinity from their growth [24]. Thus, pH changing by CO_2 was mitigated.

Moreover, this experiment seemed to indicate that the lipid contents increased with the increase of the CO_2 concentration. Similarly, Brewer et al. [25] reported that the lipid content of *Botryococcus braunii* 765 increased when the CO_2 concentration was increased from 2 to 20%.

The carbon content of the microalgal consortium supplemented with 30% CO_2 was highest at 0.4529 g dw^{-1}. This value coincided approximately with the carbon content of *S. obliquus* SJTU-3 and *C. pyrenoidosa* SJTU-2 (about 0.5 g dw^{-1}) when cultivated with 30% (v/v) of CO_2 concentration [17]. In this study, the CO_2 fixation rates of the microalgal consortium at 10 and 30% CO_2 were higher than under the ambient air conditions (0.03% CO_2), the maximum CO_2 fixation rate was 0.0271 g CO_2 L^{-1} day^{-1} in the presence of 30% CO_2. Prabakaran and David [26] obtained a similar result with *Chlorella* sp. UK001, wherein a maximum CO_2 fixation rate of 0.0318 g CO_2 L^{-1} day^{-1} was obtained at 10% CO_2 concentration.

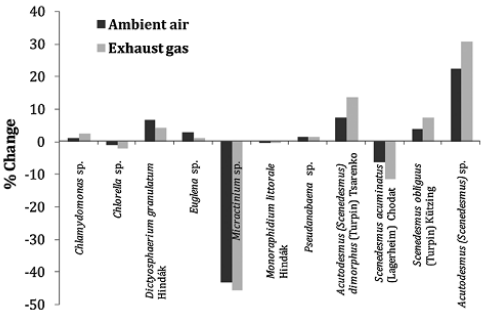

Figure 6. Percentage of species change in the microalgal consortium cultivated under exhaust gas conditions compared with ambient air conditions at the early stage and to the end of the cultivation process.

4.2 Cultivation with exhaust gas

In this study, the microalgal consortium with exhaust gas revealed higher growth rate than the ambient air. This result indicated that the biomass and lipid contents of the microalgae were increased when exhaust gas was used in the same way that they did even when the commercial CO_2 was contaminated with other gases. This result was similar to Chiu et al. [1], who found that the microalgae *Chlorella* sp. MTF-7 could be grown well when it was cultivated with the industrial exhaust gas (25% CO_2) that was contaminated with other gases.

However, it was obvious that the lipid content in exhaust gas condition (19% CO_2) was compared to the 10% CO_2 supplement. It is because both treatments were cultivated in different conditions. In the 10% CO_2 supplement, the microalgae were cultivated in close system of laboratory with continuous illumination and CO_2 was supplied by commercial mixed gases. Whereas, the exhaust gas supplement was conducted in an outdoor open system under ambient condition (without light and temperature control) and this experiment was supplied with the exhaust gas which composed of various gases. Consequently, the growth and lipid productivity in the exhaust gas supplement were affected by those factors.

In this study, during the cultivation process with exhaust gas, the common algal species observed were *Scenedesmus* spp. and its related genus *Acutodesmus* spp., which reported very high biomass and lipid contents [27]. It indicated that variations in the algal community were affected by CO_2 cultivation. Salih [28] 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 concentrations. Moreover, Guruvaiah and Lee [29] reported that *Scenedesmus* sp. that was isolated from the power plant habitat was cultivated with simulated exhaust gas containing 2% CO_2. The lipid content was 15% of its dry weight. These results indicated that the genus *Scenedesmus* sp. has great potential for CO_2 mitigation, environmental tolerance and biodiesel production.

5 Conclusions

This study revealed that a microalgal consortium showed high growth, CO_2 fixation and lipid production rates, when cultivated with high CO_2 concentrations. In addition, it suggested the possibility of using exhaust gas to enhance microalgal consortium cultivation for biodiesel production, without the inhibitory effects of high CO_2. This research showed that microalgal consortium cultivation could be useful for CO_2 reduction in the industrial sector. Moreover, microalgae showed very high potential for renewable energy production and CO_2 sequestration to mitigate the negative impacts of global warming and climate change.

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