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PROCEEDING

ISEE
INTERNATIONAL SEMINAR ON ENVIRONMENTAL ENGINEERING
2013

Advances in Sustainable Environmental Resource Management and Sanitation Technology

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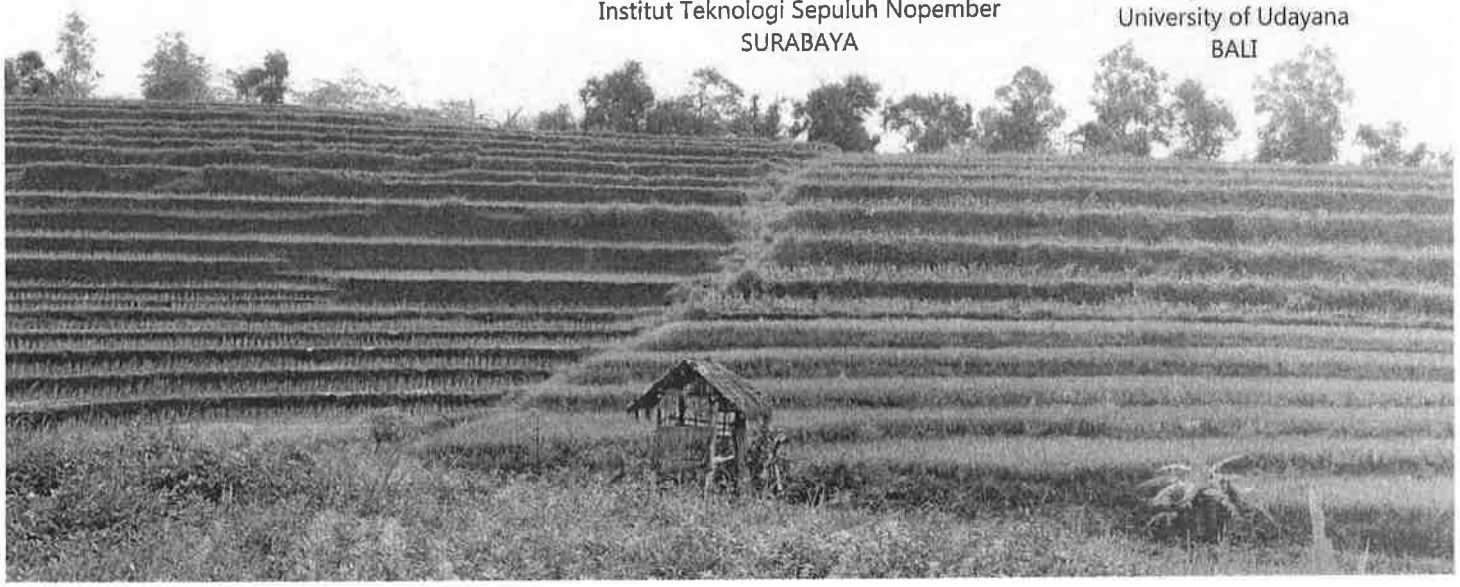
In Collaboration with:

Department of Environmental Engineering
Faculty of Civil Engineering and Planning
Institut Teknologi Sepuluh Nopember
SURABAYA

School of Public Health
Faculty of Medicine
University of Udayana
BALI



Proceeding

The 4th International Seminar of Environmental Engineering 2013

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Institut Teknologi Sepuluh Nopember

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October 2013

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Welcome Speech of Organizing Committee of International Seminar

Om Swastiastu.

and sincere greetings to all.

On behalf of the organizing committee, it is my distinct honor to welcome all of the participant in the 4th International Seminar on Environmental Engineering (ISEE). The ISEE is four-yearly international seminar organized by Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember Surabaya. And for this year, this seminar organized under collaboration of Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember and School of Public Health, Faculty of Medicine, Udayana University.

The main topic of the 4th ISEE 2013 is Advances in Sustainable Environmental Resource Management and Sanitation Technology. And it's a great pleasure for us to have eight outstanding keynote speakers this year came from Australia, Austria, Japan, Malaysia and Indonesia as well.

This year, about total 160 participants has been registered, and 120 of the participants will present their paper to share their expertise and experience on this two days seminar. We are very pleased to have participants from researchers, lecturers, industrial practitioners, and students that come from different countries include Iran, Jordan, Bangladesh, Philippine, Malaysia and Indonesia.

The 4th ISEE 2013 will be held in two days, 25 and 26 June 2013, in Widyasabha Usadha Theather, Udayana University.

We do hope that all participants will have the constructive discussion during this seminar and finally can give significant contribution to sustainable development in the future.

Thank you...

Om Shanti, Shanti, Shanti Om

Welcome Address from Rector of Udayana University

Prof. Dr. dr. I Made Bakta, SpPD (KHOM)

Rector of Udayana University
Bali

Om Swastiastu.

and sincere greetings to all.

We are very honored to be the host for The 4th International Seminar on Environmental Engineering, that is organized under collaboration between Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember and School of Public Health, Faculty of Medicine, Udayana University.

I would like to take the opportunity to thank Prof. Dr. Tri Yogi Yuwono, Rector of Institut Teknologi Sepuluh Nopember, for collaborating the successful conference and all keynote speakers. I also would like to thank Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember and School of Public Health, Faculty of Medicine, Udayana University, all the organizing and scientific committee members for their contribution to the organization of this seminar. I hope this seminar will prove to be an inspiring and truly transformative experience for you.

As the Rector of Udayana University, we continuously support the initiative from our civitas academica that could enhance our real contribution directly to our people and in the same time, could strengthen our international recognition through collaboration with other universities. This conference is the sort of activities that complies both missions.

Finally, I would like to wish the conference a great success. I would also like to wish all participants an enjoyable visit to Bali.
Thank you.

Om Shanti, Shanti, Shanti Om

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Welcome Speech from Rector of ITS

Prof. Dr. Ir. Tri Yogi Yuwono, DEA

Rector of Institut Teknologi Sepuluh Nopember Surabaya

Assalamualaikum Wr. Wb.,
Dear Distinguished guest, ladies and gentlemen,

It gives me great pleasure to welcome all the speakers, participants and distinguished guests to the 4th International Seminar on Environmental Engineering at Udayana University, Bali. I trust that you will find the conference informative and interesting, and hope that numerous scientific discussions will be deliberated and friendship will bloom as well.

I would like to express our highest appreciation to all keynote speakers who is willing to share experiences in this conference, to our close partner Udayana University, particularly, Prof. Dr. dr. I Made Bakta, SpPD (KHOM) Rector of Udayana University who has given us a chance to collaborate and put his confident to make this conference possible.

I would like to take this opportunity to express my sincere appreciation and gratitude to the organizers of The 4th International Seminar on Environmental Engineering for their commendable effort in organizing and conducting the conference, Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember and School of Public Health, Faculty of Medicine, Udayana University, and also speakers as well as participants for their distinctive role in making this seminar a success.

It is quite fascinating to learn that our colleagues from different universities have similar interests and dedication. We appreciate every effort that has been put down by each of us, with impudence expectation that we could share our knowledge in sustainable environmental resource management and sanitation technology.

It is already known, the environmental issues are often triggered by 'conservative factors' such as increase in population growth and living standard. These factors would lead to imbalance exploitation on natural resources that could eventually raise the number of environmental pollutions in the following years. As a result, the natural resources would be more vulnerable to devastation so that be unable to support the sustainable living. Another common environmental issue in developing countries is lack of adequate sanitation facilities for community. Poor sanitation contributes child deaths from diarrhea. chronic diarrhea can also hinder child development by impeding the absorption of essential nutrients that are critical to the development of the mind, body, and immune system. Therefore managing of environmental resources and creating sanitation infrastructure will very important for our future.

Finally, I would like to convey our gratitude to all participants, distinguished guests and presenters that make this seminar a success. Have a nice and pleasant seminar.

Thank you.
Wassalamualaikum Wr. Wb.

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The 4th International Seminar on Environmental Engineering
Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember
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Increasing of Carbon dioxide removal by environmental conditions modification of green microalgae Ankistrodesmus sp. cultivated in photobioreactor

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Abstract

Carbon dioxide sequestration by green algae are receiving increased attention in alleviating the impact of increasing CO_2 in the atmosphere. In this study, a green microalgae, Ankistrodesmus sp., was applied to assess biomass production and CO_2 removal. Microalgae have been cultured in photobioreactors as closed system to sequester carbon dioxide and produce potentially valuable biomaterials. The goal of the study was to utilize Ankistrodemus sp. strain under variable light intensity, light periodism and temperature to remove CO_2. The growth response of Ankistrodesmus sp. was studied under varying concentrations of carbon dioxide (ranging from 0.038% to 7%), temperature (25, 30 and 35°C), light intensity (2500 lux and 4000 lux) and light/darkness cycling (24/0 and 12/12; hour). The results showed that the best growth was at light intensity of 4000 lux during 24 hours continuous artificial lighting and temperature of 30°C. The maximum growth rate (μ) of 0.41 per day was obtained from culture that injected with 5% CO_2 concentration, resulting CO_2 removal efficiency (%) was 23.5, while dry-weight cell mass ($g.L^{-1}$), carbon dioxide transfer rate (CTR; $gCO_2.L^{-1}.h^{-1}$) and carbon dioxide fixation rate (qCO2; h^{-1}) were 7.3, 6040.14 and 867.37, respectively.

Keywords: biomitigation, carbon capture and storage, light intensity, microalgae, photobioreactor

1. Introduction

Increase of carbon dioxide besides methane and oxides of nitrogen in the atmosphere is leading to climate change. These greenhouse gases (GHGs) cause depletion of ozone layer protecting the atmosphere against UV radiation, thereby warming the atmosphere. The average concentration of CO_2 increased from 315 ppm in 1960 to 380 ppm in 2007 (IPCC, 2007). There has been a 35% increase in CO_2 emission worldwide since 1990. Carbon fixation by photoautotrophic microalgae has the potential to diminish the release of CO_2 into the atmosphere and in helping to alleviate the trend toward global warming.

Microalgae response to varying CO_2 concentrations has been widely investigated. The environmental conditions play a determining role in promoting CO_2 fixation and cellular propagation. Basic growth nutrients must be available in order to maintain proper physiological integration of the culture. This can reasonably be overcome using wastewater (P. J. McGinn, *et. al.*, 2011; R. A. Andersen, 2005; A. P. Carvalho *et al.*, 2006).

Temperature can be a determining factor in the selection of the algal species (D.A Caron *et al.*, 1986; Brennan and P. Owend, 2010). However, the diversity in the optimum temperature required to maintain the best growth rates makes it possible to choose the organism with given physical needs (A. Dauta *et al.*, 1990).

Chlorophyll in photosynthetic algae captures light energy, which is used to convert simple molecules (CO_2 and H_2O) into carbohydrates (sugars and starches) with the release of O_2. Microalgae are of particular interest because of their rapid growth rates, tolerance to varying environmental conditions and can also fix greater amounts of CO_2 per land area than higher plants (Brown 1996).

Light condition, especially light intensity, is an important factor because the light energy drives photosynthesis. Typical light intensity requirements of microalgae are relatively low in comparison to higher plants. For example, saturating light intensity of *Chlorella* sp. and *Scenedesmus* sp. is approximately 200 (Hanagata *et al.*, 1992). Microalgae often exhibits photoinhibition under excess light conditions. Photoinhibition is often suspected as the major cause of reducing algal productivity. Ranjbar *et al.* (2008) also reported that the light regime inside a photobioreactor could be improved and a high-density growth was attainable by using an air-lift-type photobioreactor.

The maximum CO_2 removal efficiency reached 65% at the third day of the cultivation. The optimal pH and light cycle for the Chlorococcum sp growth was 8.0 and 16:8. The maximum biomass concentration and the CO_2 biofixation rate of light cycle 16:8 were 0.95 g/L and 305 mg/L/d, respectively (Chai *et al.*, 2010).

The aim of the study was to explore capacity of microalgae *Ankistrodesmus* sp. that cultivated in vertical column photobioreactor under variation of temperature, light intensities (lux) and light periodism (light/dark in hour) to get high CO_2 removal efficiency.

2. Material and Methods

2.1 Microorganism and culture condition in photobioreactor

Microalgae *Ankistrodesmus* sp. isolated from Bojong Soang Municipal Waste Water Treatment Plant, Bandung, Indonesia. The culture of *Ankistrodesmus sp* was grown in 10 liter vertical column photobioreactor, filled by 8 litre of sterilized Phovasoli Haematococcus Media (PHM) as growth media, the pH adjusted at 7 ± 0.5. This *in vitro* experiments were carried out in order to find out the best culture condition for growth and CO_2 removal efficiency. The culture growth of environment condition were photoperiod of 24 hours and 12 hours light provided by white fluorescent lamps at a light intensity of 2,500 and 4000 lux and various of temperature i.e 25^0C, 30^0C and 35^0C. Varying light intensities 2,500 and 4,000 lux were provided by adjusting the light with the help of lux meter (Lutron LX-101A). Varying photoperiods 24/0 and 12/12 were make by closed the reactor with barier and turned off the lights. During the process of growth in photobioreactor, 2%, 5% and 7% CO_2 was injected with flow gas rate was 8L.min^{-1}.

2.2. Measurement of growth respond

Dry weight cell biomass was obtained by evaporating the liquid in the cell culture. A total of 100 mL culture tube inserted into centrifuges, and then centrifuged at 3500 rpm for 10 minutes (Weldy and Huesemann, 2007). Supernatant was then removed from the tube pasta until just earned cells. Pasta cells was then put into a petri dish that had previously been weighed (x). Samples were put in the oven with a temperature of 105^0C for one night to get a constant weight (y), and then stored in a desiccator for 30 minutes before re-weighed. Biomass (dry weight) according to Torzillo et al., (1991) calculated by the formula: Dry weight (X; mg) = y (mg) - x (mg). Specific growth rate (μ; d^{-1}) was calculated as follows :

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

2.3. Measurement of CO_2 removal

Concentration of CO_2 in a series of photobioreactor system was measured 2 (two) times a day, using Combination Portable Gas Detector Model RX-515 RIKEN. Measurements were performed to determine changes in the concentration of CO_2 in the gas holder with time, whereas the concentration of CO_2 dissolved in the culture medium was measured once daily by using acidity alkalinity method to know the solubility of CO_2 in the culture medium.

CO_2 removal efficiency is the proportion of the absorbed CO_2 concentration by the photobioreactor system to CO_2 that was supplied.

$$\text{CO2 removal efficiency} = \frac{\text{influent of CO2} - \text{effluent of CO2}}{\text{Influent of CO2}} \times 100\% \quad (2)$$

Carbon Transfer Rate (CTR; $gCO_2.L^{-1}.h^{-1}$) is the amount of CO_2 that is transferred in the medium volume and required by the cell metabolism for a unit of time (Ohtaguchi and Wijanarko, 2003 in Dianursanti, 2012)

$$CTR = \Delta y_{CO_2} \cdot \alpha_{CO_2} \quad (3)$$

Where,
αCO_2 = a constant that contains a fixed number of temperature and pressure, airflow superficial velocity; ΔyCO_2 = the concentration change of CO_2 in and out of the reactor by the incoming CO_2 concentration, multiplied by 100%.

$$\alpha_{CO_2} = \frac{U_g \cdot A \cdot M_{CO_2} \cdot P}{V_{med} \cdot R \cdot T} \quad (4)$$

Where, Ug = superficial gas velocity i.e discharge gas fed per reactor cross-sectional area ($m.h^{-1}$); A = surface area of the photobioreactor facing or exposed to light (m^2); MCO_2 = relative molecular mass of CO_2 (44 mol); P = operating pressure (1 atm);V = volume of medium (L); R = Rydberg constant (0.08205 L.atm/mol.K); T = operating temperature (^0K).

Carbon fixation rate as specific CO_2 transfer rate (qCO2; $gCO_2.g\ cell^{-1}.h^{-1}$) is the rate of CO_2 that is transferred in a medium volume due to the activity of biological life within a unit of time.

$$q_{CO_2} = \frac{\Delta y_{CO_2} \cdot \alpha_{CO_2}}{X} \quad (5)$$

Where,
X = cell dry weight per unit volume (gL^{-1}).

3. Result and Discussion

3.1. Dry weigh biomass as growth response

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Biomass productivity of *Ankistrodesmus* sp. was evaluated at various temperatures, light intensity 2500 lux, periodism 24 hour light, during 12 days. Growth analysis of cultures grown at different temperatures showed significant difference ($P < 0.05$) in growth pattern.

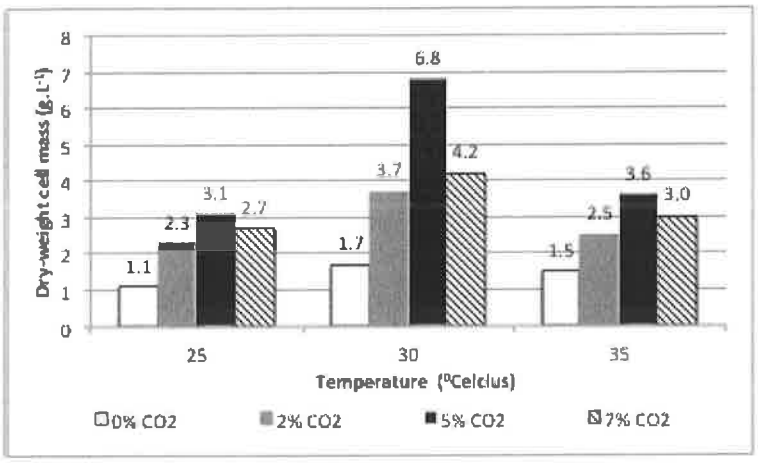

Figure 1. Effect of different temperature on dry weight ($g.L^{-1}$) of *Ankistrodesmus* sp

Maximum biomass concentration (as dry weight) i.e. 6.8 $g.L^{-1}$ was occured in culture that supplied with 5% CO_2, at temperature 30℃ while i.e. 3.6 $g.L^{-1}$ was found at temperature 35℃ (Fig. 1). Ankistrodemus sp able to growth in a wide range of temperature from 25℃ to 40℃ tolerantly, although at 40℃, the growth was almost negligible (data not recorded). The average growth rate (μ) i.e. 0.150 was observed at 30℃, while further increase in temperature there was reduction in growth rate. Growth rate in every various concentration of CO_2 supplied at 30℃ showed almost 2 fold than at 25℃ (Table 1). It means that temperature optimum of the culture was 30^0C. The next experiment will be done at conditions temperature of 30^0C, 4000 lux light intensity with periodism 12 hour light and 12 hour dark. Since microalgal-CO2 fixation involves photoautotrophic growth of cells, the CO2 fixation capability of specific species should positively correlate with their cell growth rate and light utilization efficiency (Jacob-Lopes et al., 2009a,b). Moreover, microalgal photosynthesis efficiency declines with increasing temperature, since CO2 solubility is significantly reduced (Pulz, 2001).

Table 1. Spesific growth rate (μ) on various temperature

	25^0C	30^0C	35^0C
0% CO_2	0.010	0.022	0.018
2% CO_2	0.080	0.150	0.130
5% CO_2	0.120	0.228	0.190
7% CO_2	0.100	0.210	0.160

Ryu et.al (2009) analyzed the impact of CO_2 concentration in vertical tubular reactors without controlling the temperature of the system. According to their findings, a maximum cell concentration of 2.02 g/l was found at 5% CO_2 and the minimum cell concentration of 1.16 g/l was found at 0.5% CO_2 mixed in air. They suggested keeping the CO_2 concentration lower than 5% because higher CO_2 concentrations may inhibit microalgae growth.

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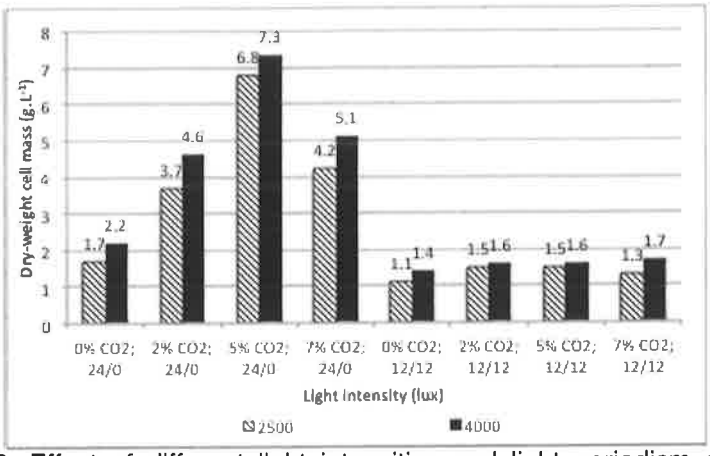

Figure 2. Effect of different light intensities and light periodism on biomass concentration (dry weight $g.L^{-1}$) of *Ankistrodesmus* sp

Although it was not showed different value signicantly, but at experimental irradiances 4,000 lux were found to be the best conditions for biomass production compared with 2500 lux light intensity. Periodism light/dark condition of 24/0 hours showed very good condition for growing of *Ankistrodemus* sp compared with 12/12 hours light condition, i.e the culture showed increased of dry-biomass over than 2 fold (Fig. 2). Moreover, the highest dry-weight biomass 0.73 $g.L^{-1}$ and growth rate 0.409 was found in culture that supplied with 5% CO_2, at 4000 lux light intensity. This value was almost 3.5 fold compared with 0% CO_2. However, reduction in growth rate was observed on further increase in concentration of CO_2 supplied (7% CO_2) (Table 2).

Table 2. Spesific growth rate (μ) on different light intensities and light periodism

Light intensities (lux)	2500		4000	
Light Periodism (light/dark; hour)	12/12	24/0	12/12	24/0
0% CO_2	0.005	0.022	0.008	0.004
2% CO_2	0.008	0.150	0.018	0.294
5% CO_2	0.009	0.228	0.022	0.409
7% CO_2	0.006	0.210	0.019	0.377

Actually, microalgae could grow either in the absence or presence of light. In the absence of light some algae could grow heterotrophically using reduced carbon skeletons, such as glucose, as a substrate. In this mode of growth, the growth rate sometimes much higher than in the presence of light as photosynthetically or photoautotrophically.

It is crucial to select the optimum cell concentration or biomass production as growth response for the efficient CO2 sequestration. Below the optimum cell concentration, not all the light energy is captured by the cells while at above the optimum cell concentration, a larger proportion of the cell are in the dark due to self-shading (Zhang et al., 2001).

3.2. CO_2 removal efficiency

The average CO_2 removal efficiency was 23.50% during 12 days periode of cultivation. The value occurred on culture that supplied with 5% CO_2, at condition of 4000 lux light intensity and 24 hours light. The fact also showed that *Ankistrodesmus* sp was able to growth under elevated CO_2 and utilized up to 7% CO_2 as an inorganic carbon (Fig.3).

Hirata et al. (1996a; 1996b) reported that *Chlorella* sp. UK001 could grow successfully

under 10% CO_2 conditions. It is also reported that *Chlorella* sp. can be grown under 40% CO_2 conditions (Hanagata *et al.*, 1992). Furthermore, Maeda *et al.* (1995) found a strain of *Chlorella sp.* T-1 which could grow under 100% CO_2, although the maximum growth rate occurred under a 10% concentration.

Chlorella vulgaris was cultivated under various light intensities in a gas recycling photobioreactor. The light intensity affected the algal growth and the CO_2 concentration in the exit gas. In the linear growth phase, CO_2 concentration in the exit gas ranged between 4.6% to 6.0% (v/v) when 20% (v/v) CO_2 balanced with 80 % (v/v) N_2 was introduced into the photobioreactor (Y-S. Yun and J. Moon Park, 1997).

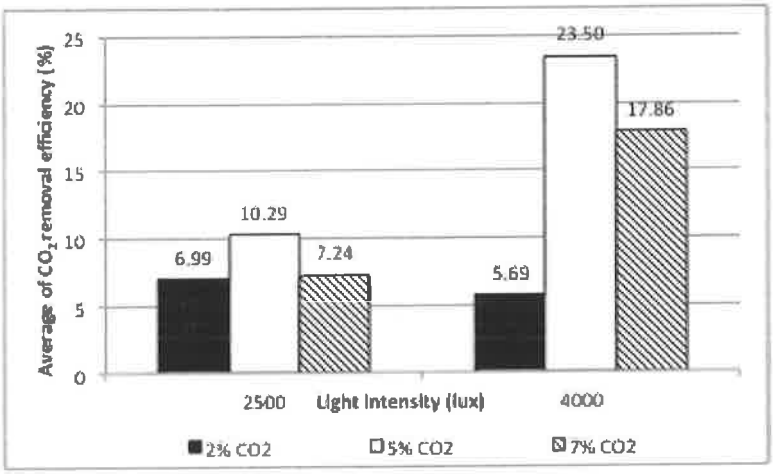

Figure 3. CO_2 removal efficiency of *Ankistrodesmus* sp as a result of light intensities and supplied of CO_2 concentration

Although the CO_2 removal efficiency in our study was not high enough as well as the previous studies from other researcher, but it seem will be increased in next our study if we do optimization in other aspects. CO_2 removal efficiency by microalgae was influenced by several factors, such as CO_2 concentration and flow rate (Ryu et al., 2009), light intensity (Perner-Nochta and Posten, 2007), light fotoperiodism (Lopes *et al.*, 2008), cell density (Jiang *et al.*, 2008), temperature (Chinnasamy *et al.*, 2009), and the type of reactor (Kumar et al., 2010). Ryu et al., (2009) have revealed that the CO_2 removal efficiency decreased with increasing gas flow.

3.3 Carbon dioxide transfer rate (CTR) and Carbon fixation rate (qCO_2)

Fig. 4 and Fig 5. below show the pattern of CTR and qCO_2. Increasing of CTR occurred on culture that supplied with 2% 5% and 7% CO_2, althought only the culture with 5% CO_2 showed CTR increased until days-8 during cultivation periode. It was match with the result of dry-weigh biomass. The longer and higher the CTR could make the higher dry-weight biomass also (Table 3).

Table 3. Average of dry-weight cell mas, carbon transfer rate and carbon fixation rate

CO_2 supplied	Dry-weight cell mass ($g.L^{-1}$)	Carbon transfer rate (CTR; $gCO_2.L^{-1}.h^{-1}$)	Carbon fixation rate (qCO_2; h^{-1})
0%	2.2	4	2
2%	4.6	695	175
5%	7.3	6040	867
7%	5.1	2615	515

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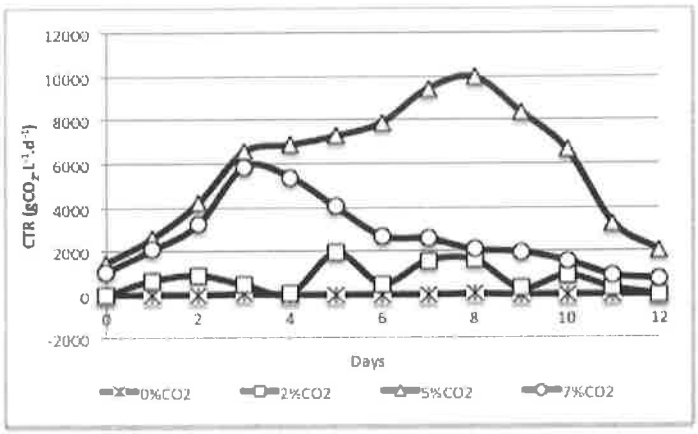

Figure 4. Carbon Transfer Rate of *Ankistrodesmus* sp as a result of variety of CO_2

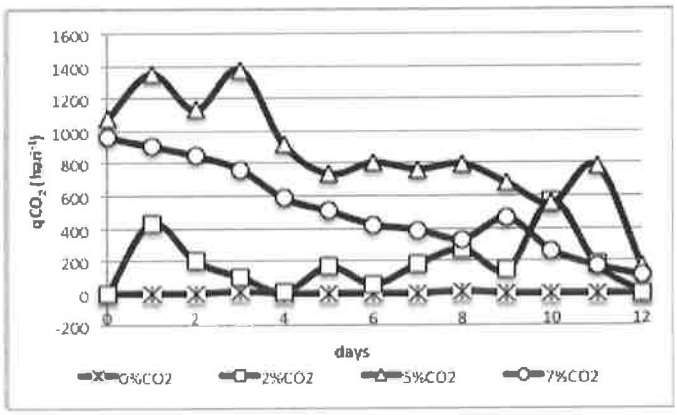

Figure 5. Carbon dioxide Fixation Rate of *Ankistrodesmus* sp as a result of variety of CO_2 concentration supplied

Zak et al. (2001) and Badger & Price (2003) described that the microalgae are capable of using free CO_2 and bicarbonate ions as a source of inorganic carbon during photosynthesis, transporting them across the fine plasmatic membrane where they accumulate in the cell as an inorganic carbon reservoir for photosynthesis. The bicarbonate is converted into CO_2 by the enzyme carbonic anhydrase. These process depend on how much CTR occurred in the photobioreactor and qCO2 in the cell of microalgae.

Average of CTR values obtained from 5% CO_2 condition reached 3-fold higher than the CTR value obtained at 7% conditions. Likewise the average of qCO_2 value obtained from 5% CO_2 condition reached over 1.5-fold higher than the qCO_2 value obtained at 7% conditions. All differences were very significant (Table 3).

4. Conclution

In considering the results of the studies described above, *Ankistrodemus* sp demonstrated optimum capacity to remove CO_2 at 5% CO_2 supplied, althought it was also able to growth under 7% CO2 supplied. CO_2 removal efficiency was increased when it cultivated in 4000 lux of light intensity, periods of light/dark (24/0), and temperature 30^0C. This was evidenced by CO_2 removal efficiency above 3.5 times higher. In addition, carbon transfer

rate also increased. Nevertheless carbon fixation rate decreased significantly. All results were compared with initial condition of 2500 lux, light/dark (12/12) and 30^{0}C. In further research we will do optimization on flow rate of CO_2 gas input to get higher CO_2 removal efficiency.

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