Effect of Carbon dioxide (CO2) concentration on Growth of 

*Ulva intestinalis* in Photobioreactor

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Abstract

The alga was cultured with the initial weight of 0.05 g/L in 10 liters of modified Grund’s seawater medium in total volume of 12 liters transparent polyethylene chamber under controlled conditions of 25 ppt seawater and light intensity of 80 µmol m⁻² s⁻¹; and given the aeration by bubbling air with carbon dioxide at different content of 0, 1, 3 and 5% (v/v) with the flow rate at 3 L/min. Each treatment of the experiments was done in three replications for 18 days. The results showed that the growth of *U. intestinalis* had significantly different among the treatment (p<0.05). The final wet weights of the alga were 5.22±0.04, 6.49±0.83, 3.60±0.67, and 0.68±0.16 g/l; respectively. The relative growth rate (RGR) obtained were 2.19±0.04 % g day⁻¹ (for 0 % CO₂), 2.31±0.07 % g day⁻¹ (for 1 %CO₂), 1.98±0.01 % g day⁻¹ (for 3 %CO₂) and 0.81±0.05 % g day⁻¹ (for 5 %CO₂). This study suggested that carbon dioxide supplementary in the cultivation of *U. intestinalis* provides slightly increased in the production; while carbon dioxide exceeded 3 % (V/V) may inhibit the growth of this alga species.

**Keyword:** *Ulva intestinalis*, carbon dioxide, photobioreactor
1. Introduction

*Ulva intestinalis* is the green algae that are tolerant to changing of water conditions. This alga is used as a source of various nutritional food especially minerals and vitamins. This algal specie distributes along the brackish waters, along the tide at sub tidal zone, and also in high nutrients waters. It can live in water with low salinity and resistance to changes in a wide range of salinity (Lobbon and Harrison, 1994) Found in the widely distributed in Asia such as Japan, Korea, India, Indonesia and Thailand etc. The extracts from *Ulva* spp. are used to mixing in food, cosmetic and pharmaceutical industries due to containing of anti-biotic, anti-bacteria, anti-fungi and anti-tumors, etc (Aguilera-Morales et al., 2005). The major water-soluble polysaccharide, ulvan, extracted from the cell wall represents about 8–29% of the algae dry weight was stimulated interest with innovative structure and functional properties (Lahaye and Robic, 2007; Bikker et al. 2016).

In the process of biomass, production must be good, clean and quality to increase the value of alga. Therefore, the one culture method in photobioreactor will increase the biomass productivity. The chamber could control the air, increased carbon dioxide and air flow-rate in the water to accelerate growth and make the product cleaner than in other culture systems. In the current issue of carbon dioxide emissions in to the atmosphere. It is one of the major environmental problems of the world. The concentration of carbon dioxide increased rapidly, resulting in the earth's atmosphere to heat up and cause more problems ensue. This phenomenon is causing global temperatures to rise. This is called the greenhouse effect. A main cause of this phenomenon comes from the burning of fossil fuels such as coal, oil and natural gas for use in the production of energy by about one-third of all carbon dioxide coming from power plants, industrial steel cement factories and petrochemical plants. (Inui et
The solution reduces the amount of carbon dioxide in the atmosphere is divided into three ways: reducing the amount of fuel that produces carbon dioxide, removal of carbon dioxide from combustion from industry and vehicles.

Another method is the use of carbon dioxide by transforming them into compounds that can be utilized in the future, such as methanol, ethanol and acetic acid etc. (Beneman, 1997). *Ulva* as green algae has the ability to absorb carbon dioxide and nitrate, phosphate on growth and generate protein and amino acids in the cell. Suitable for a source of nutrition to aquatic animals and to consumers. From the growth of algae that use these elements as an energy source. It is used as a source of protein and is beneficial to reduce pollution levels in the water that is available. *Ulva* can grow will depend on many factors such as light intensity, temperature, salinity, etc. Each algae species have requirements light and salinity in different quantities. If the right environment to produce high algae, therefore the development and design of the algae in the photobioreactor to increase productivity quality. The photobioreactor can control of the air increased carbon dioxide and air flow-rate in the water to accelerate growth and the product cleaner than in nature.

2. Materials and methods

2.1 Selection and preparation gremling cluster of *U. intestinalis* (Hiraoka and Oka, 2008)

Green macroalgae species, *Ulva intestinalis* were collected from Pattani bay. Cleaned with sea water remove all epiphytes and cut with scalpel into 3 cm in size to stimulate the spores into a gremling cluster. Culture in the round bottle flask volume 250 ml of 30 pieces and used 25 ppt of salinity water with food Modified Grund Medium (MGM) when spores
stick to the bottle wall. Scraping and filter with sieve so that the spores don’t clump together.

Cultured spores in the round bottom flask until the length was 0.5-1.0 cm

2.2 Photobioreactor design

The reactor made of polyethylene terephthalate bottle with lid top. The bottom has a valve connected to the air and carbon dioxide. The total volume of cultivation reactor was 12 L. Air bubble mixing was provided from the bottom and air out in the above. Provide fluorescent light bulbs as a light source. (Figure 1) Biomass harvesting was done from the above by opening the cover and filtering the biomass through the sieve (1x1 mm).

![Figure 1 Schematic of photobioreactor for cultivation of Ulva intestinalis](image)
2.3 Study air flow-rate on growth of *U. intestinalis* culture in photobioreactor

The effect of flow rate 1.0, 2.0, 3.0 and 4.0 L/min with special tube at the bottom and was feed up in three replications of the 10 L photobioreactor. The photobioreactor was set up in the indoor at temperature of about 25°C. The algal samples were cultured using the MGM medium under light intensity of 80 µmol /m²/s. Total fresh weight of the thalli was determined every 2 days interval. The optimal air flow was selected to use for further treatment of carbon dioxide concentration. The optimal air flow was selected for further treatment of carbon dioxide concentrations.

2.4 Study carbon dioxide concentration on growth of *U. intestinalis* culture in photobioreactor

The seedling with the size of 1-2 cm long with 0.05 g/L was cultured in 10 L of photobioreactor. The rate of aeration was 3 L/min. compare the carbon dioxide in 4 levels: 0, 1, 3, and 5% (V/V) or 0, 30, 90, 150 ml/min. Culture at temperature 25°C, light intensity 80 µmol/m²/s and 25 ppt of seawater with MGM (Ruangchuay *et al.*, 2012). Fresh weight of biomass, length, and color space of *U. intestinalis* were determined every two week interval, then brought back to the photobioreactor, and cultured until stationary phase.

2.5 Lab color space

CIE is a system developed by the Commission International de L'Eclairage (CIE) is a numerical system that is independent of the visibility of the reader. The tool is the HunterLab MiniScan EZ 4500L Spectrophotometers measure the color space to find correlations with chlorophyll by bring the sample to a scan with MiniScan EZ 4500L and record the L*a*b* value. Calculate color meaning with the hexadecimal color code program.
2.6 Chlorophyll

Sample of *U. intestinalis* (0.5 g) was placed in a 50 ml test tube, add 15 ml of acetone (90%), wrapped with aluminum foil for protect external light and kept in the refrigerator overnight. Then, grind thoroughly with the homogenizer for 5 minutes and centrifuged at 5,000 rpm for 10 minutes and 5°C. Total chlorophyll extracted from the seaweed was measured by using spectrophotometer at wavelength of 750, 664, 647, 630, 510 and 480 nm. and calculated according to the method of Parson *et al.*, (1984)

2.7 Data measurement and analysis

The data are reported as the mean±sd. The total data were analyses by one-way ANOVA to compare between the treatments. Turk’ys test was performed at p < 0.05 significance level. The weekly growth rate was calculated as the Relative Growth Rate (RGR) using the formula of Lobban and Harrison (1994) as follows: Relative Growth Rate (% day⁻¹) = [(lnWₜ - lnW₀)/t] x100, Where Wₜ=the final weight (g), W₀ = the initial weight (g) and t= the time interval (days). Percentage of increased biomass was calculated by increased biomass (%) = [(Wₜ –Wᵢ )/Wᵢ ]x100, where Wₜ, Wᵢ and t are the final weight, initial weight (g) and culture time (day) respectively. The percentage of increased length was determined by increased length (%) = [(final length- initial length)/initial length] x100

3. Results and discussion

3.1 Effect of air flow-rate on growth of *U. intestinalis* in photobioreactor

This study revealed that growth of *Ulva intestinalis* was increased when the flow rate of the aeration increased to 3 L/min after 16-day cultivation. Nevertheless, the algal growth was decreased when the flow rate increased to 4 L/min (Table 1). The alga cultured at the flow rate of 3 L/min had significantly higher wet weight (6.99±0.19 g/L) and thallus length
(14.20±3.03 cm) than those cultured at 1, 2 and 4 L/min. The highest daily growth rate (DGR) was 15.18±3.11 % /day, obtained from the alga cultured at 3 L/min, while the lowest DGR was 7.33±4.43 % /day at flow rate of 4 L/min. In addition, the relative growth rate (RGR) had a maximum value of 2.65±0.02 % /day. Thalli of the alga were damaged and eventually torn off by strong aeration, when the algal samples were cultured at flow rate of 4 L/min.

**Table 1** Growth rate of *Ulva intestinalis* cultured in photobioreactor under different air flow-rate at day 16th.

| Air flow rate (L/min) | Weight (g/L) | Length (cm) | Daily growth rate (g/day) | Relative growth rate (% g/day) |
|-----------------------|--------------|-------------|----------------------------|-------------------------------|
| 1                     | 4.25±0.32a   | 10.73±2.83a | 7.42±4.66a                 | 2.33±0.05a                   |
| 2                     | 5.39±0.38b   | 11.68±2.85a | 12.87±1.86b                | 2.49±0.05a                   |
| 3                     | 6.99±0.19c   | 14.20±3.03c | 15.18±3.11b                | 2.65±0.02a                   |
| 4                     | 4.75±0.33a   | 8.55±1.81b  | 7.33±4.43a                 | 2.40±0.04a                   |

*Different letters indicate significant difference (p<0.05)*
Figure 2 Growth rate of *Ulva intestinalis* cultured under different air flow-rate in photobioreactor for 16 days.

3.2 Effect of carbon dioxide on growth of *Ulva intestinalis* in photobioreactor

The 18 days cultivation in the photobioreactor showed significantly different growth rate of *U. intestinalis* (p<0.05) at different concentrations of carbon dioxide treatment (Table 2). The algal wet weights had values of 5.22±0.04, 6.49±0.82, 3.60±0.67, 0.68±0.16 g/L for 0,
1, 3, and 5 % CO₂, respectively. This study showed higher algal growth than those of other treatment of carbon dioxide concentrations when feeding with 1% CO₂; given a maximum value of daily growth rate (DGR) of 35.77±4.57 % /day, with a minimum value of 3.49±0.90 % /day at 5% CO₂. In addition, the relative growth rate (RGR) was 2.31±0.07 % g /day when feeding with 1% CO₂ concentration. The RGR was not significantly different compared to CO₂ free in the control treatment (2.19±0.04 % g /day). Our study also showed a significant effect of CO₂ concentrations (p<0.05) on the thallus length. However, there were not significantly different (p>0.05) in every treatment (Figure 3). This was similar to Conitz et al. (2001) and Zhang et al. (2006) which reported carbon dioxide concentrations had an effect on cell growth of Porphyra torta. Another study reported CO₂ effects on growth of Ulva conglobata thalli; which the growth rate of the alga cultured under normal Ci (inorganic carbon dioxide) conditions showed relative growth rate (RGR) of 7 % /day higher than the thalli cultured at Ci-starved seawater (Zou, 2014). This study revealed that carbon dioxide is an important factor controlling photosynthesis and growth of the seaweeds. However, high concentration of carbon dioxide feeding can cause an inhibition of growth of the U. intestinalis and concentration of the chlorophyll of the alga.
**Table 2** Growth rate of *Ulva intestinalis* cultured in photobioreactor under different CO₂ concentration at day 18th.

| CO₂ concentration | Weight (g/L) | Length (cm) | Daily growth rate (g/day) | Relative growth rate (% g/day) |
|-------------------|--------------|-------------|---------------------------|-------------------------------|
| CO₂ free          | 5.22±0.04a   | 14.30±2.06a | 28.72±2.20b               | 2.19±0.04a                   |
| 1 % CO₂           | 6.49±0.82b   | 18.70±2.77b | 35.77±4.57b               | 2.31±0.07a                   |
| 3 % CO₂           | 3.60±0.67c   | 12.46±1.70a | 19.72±3.74c               | 1.98±0.01b                   |
| 5 % CO₂           | 0.68±0.16d   | 5.34±1.14c  | 3.49±0.90d                | 0.81±0.05c                   |

*Different letters indicate significant difference (p<0.05)

**3.3 Chlorophyll and color space correlation**

Chlorophyll content of *Ulva intestinalis*, cultured in photobioreactor with different carbon dioxide concentrations. Each experiments were 370.31±24.01, 380.14±17.75, 330.44±22.70 and 276.55±41.83 mg. for 0, 1, 3, and 5 % CO₂, respectively. Using Hunter Lab Miniscan EZ 4500L to analyze L *, a * and b * data is significantly different (p <0.05) (Table3). From the data, it is found that there is a relationship between the color value and the amount of *Ulva intestinalis* cultivation with different carbon dioxide concentrations, with significant positive relationships with the color values L * and b *(r = 0.664 and 0.228 respectively) and have a negative relationship with a *(r = -2.282)(Figure 4). Whenever the L * and b * color values increase, the number of chlorophyll will increase and when the color value a * decreases, the chlorophyll content will decrease similar Hu et al., (2010) studied on assessment of chlorophyll content based on image color analysis.
Table 3 Chlorophyll content and color space of *Ulva intestinalis* cultured in photobioreactor under different CO$_2$ concentrations at day 18$^{th}$.

| Color space | control   | 1%        | 3%        | 5%        |
|-------------|-----------|-----------|-----------|-----------|
| L*          | 16.79±1.07$^a$ | 20.29±1.86$^b$ | 15.82±0.85$^a$ | 14.56±0.66$^a$ |
| a*          | -4.34±1.11$^a$  | -3.91±0.69$^b$  | -4.54±0.73$^a$  | -7.02±1.33$^a$  |
| b*          | 6.74±1.67$^a$   | 16.33±1.85$^b$   | 5.72±3.64$^a$   | 3.99±3.30$^a$   |
| Color meaning | green | yellow green | green | yellow green |
| Chlorophyll (mg/dw) | 370.31±24.01$^a$ | 380.14±17.75$^a$ | 330.44±22.7$^a$ | 276.55±41.83$^b$ |

*Different letters indicate significant difference (p<0.05)*
Figure 3 Growth rate of *Ulva intestinalis* cultured under different CO$_2$ concentrations in photobioreactor for 18 days.
Figure 4 Relation between chlorophyll content and Lab color space ($L^*, a^*, b^*$) of *Ulva intestinalis* cultured under different CO$_2$ concentrations in photobioreactor for 18 days.

4. Conclusion

*Ulva* spp. grows well when adding the right amount of carbon dioxide and grows better than red algae receiving carbon dioxide. Although carbon dioxide is a food source for algae, but if there is high concentration will inhibit growth as well.

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