Optimization of intermittent CO_2 supply and flocculation recovery for the cultivation of a haptophyte *Isochrysis galbana*

ハプト藻 *Isochrysis galbana* の培養に向けた間欠攪拌による CO2 供給および凝集沈殿回収法の最適化

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SYNOPSIS

The demand for high fucoxanthin and fatty acids content in *Isochrysis galbana* is rising year by year, therefor, the low-cost cultivation and harvesting in large-scale has become an indispensable issue. Microalgal cultivation is influenced by several factors such as solar irradiance, nutrient availability, agitation, and CO_2 supply condition. Continuous aeration for CO_2 supply occupies a considerable portion of total energy consumption for microalgae cultivation. While the intermittently CO_2 supply has a potential to reduce aeration cost, it may cause a carbon starvation and inhibit the grow of microalgae. Microalgal harvesting also a challenge due to its small cell size. There is a method of flocculation that reduces the cost of harvesting algae by simply adding flocculant and adjusting the pH. Therefore, firstly, this study investigated the effects of concentration and frequency of CO_2 supply on productivity of marine microalgae *I. galbana*. Semi-continuous cultivation of *I. galbana* was conducted under different CO_2 concentration and frequency (0.04 -10% CO_2 and continuous, 1/9 minutes of supply; Study 1). Secondly, the effects of using different pH values (between pH 8 and 10) and PO_4 (Ca^{2+}) concentrations (between 0 and 10 mM) on microalgal sedimentation were investigated by evaluating the sedimentation efficiency of microalgae cells (Study 2). As a result, under air supply conditions, the biomass productivity reached 0.17 g L^{-1} d^{-1} with continuous supply(control). The 5% continuous condition showed highest biomass productivity (0.35 g L^{-1} d^{-1}). The productivity was only 0.11 g L^{-1} d^{-1} in continuous CO_2 supply with its concentration of 10%, indicating that a higher DIC concentration also would inhibit the growth of microalgae. On the other hand, the condition with CO_2 concentration of 5% and 10% showed 0.3 and 0.22 g L^{-1} d^{-1} of productivity in intermittent CO_2 supply of 1/9min/min, respectively. These productivities were higher than control. In addition, under air supply conditions, a higher fucoxanthin content was achieved 0.61 mg/g-DW with continuous supply, than 5% and 10% (0.18 and 0.11 mg/g-DW), respectively. However, the fucoxanthin content reached 0.56 mg/g-DW under 5% continuous supply with 1/9 min/min agitation frequency. This result suggests that continuous supply of high concentration of CO_2 inhibited the accumulation of fucoxanthin, but intermittent supply was beneficial to the accumulation of fucoxanthin. On the other hand, Fast and efficient sedimentation occurred (within 30 min) at a high PO_4 concentration (5,10 mM) at pH 10, the sedimentation rate was achieved 40%.

Keywords: CO_2 supply concentration, CO_2 supply frequency, productivity, Fucoxanthin, Harvesting, Flocculation

INTRODUCTION

Isochrysis galbana is one of the promising species known to be an efficient producer of fucoxanthin and fatty acids, and have been widely used in aquaculture for feeding juvenile fish and shrimp to improve growth rate, survival rate and nutritional value. In addition to aquaculture, microalgae with high fucoxanthin and fatty acids contents have the potential to be used in food, cosmetic and pharmaceutical industries [1]. Also, fucoxanthin has anti-oxidant properties that has effects on humans health [2]. However, the cost of making it into a commercial product is quite high, costs of microalgal biomass production in flat panels were estimated on 596 cts € $kg^{-1}DW^{-1}$. [3] In the microalgae production process, cultivation and harvesting account for a large proportion, accounting for about 60% and 20%. of the total cost [4]. These costs include equipment, nutrient supply, labor and power consumption, etc. Equipment, nutrients, and labor are indispensable costs for algae cultivation, so this paper focuses on reducing microalgae cultivation and harvesting power consumption through intermittent CO_2 supply and flocculation.

Microalgal cultivation is influenced by several factors such as solar irradiance, nutrient availability, agitation, and CO_2 supply condition. Continuous aeration for CO_2 supply occupies a considerable portion of total energy consumption for microalgal cultivation. According to the previous study, the electricity consumption was estimated at 448 KWh/day for microalgae cultivation using ten 3 m^3 tubular photobioreactors [5]. Of this energy consumption, 21% (96 kWh/day) was accounted for by culture agitation to ensure aeration. There is a need for a cost-efficient method for microalgal cultivation. There are two ways to reduce CO_2 cost; adjusting the concentration and supply frequency of CO_2. A previous study [6] showed that CO_2 intermittent supply can achieve the same effect as continuous supply of CO_2. On the other hand, while the intermittently CO_2 supply (agitation) has a potential to reduce aeration cost, it may cause a carbon starvation and sedimentation of cells when the supply frequency is not enough leading to a critical concentration of carbon dioxide. The carotenoid composition in microalgae is also affected by the CO_2 concentration and other environmental factors such as pH, nutrient concentration, light intensity, cell density, aeration, and physiological state of the culture. Therefore, it is possible that the intermittent stirring can have a positive effect on not only the biomass production but also the carotenoid accumulation.

About microalgae harvesting, centrifugation and filtration are the quickest methods for harvesting microalgae. However, these methods require large amounts of energy and have high operating and labor costs. Thus, a cheap and energy-efficient pre-concentration method is required. Flocculation could be an effective method for pre-concentrating microalgae before centrifugation and filtration because simply adding a flocculant can quickly induce microalgal sedimentation [7,8]. It has been known that a consistent, inexpensive, and effective microalgae biomass concentration can be achieved by using a chemical coagulant [8] than a biological coagulant. Chemical coagulants induce coagulation by modifying the electrical charge of the microalgal cells by changing the pH solution and the ions present in the solution. The results of our previous study [9] showed that high pH and cationic conditions can induce flocculation and sedimentation of freshwater algae *Chlorella vulgaris*. Thus, our challenge was to effectively apply this research to marine microalgae *I. galbana* due to the different culture solutions.

Therefore, in this thesis, Study 1 was investigated to check the effects on biomass productivity used semi-continuous cultivation of *I. galbana* under different CO_2 concentration and frequency. Then, in Study 2, the effect of pH and ionic substances on the flocculation and sedimentation in marine microalgae *Isochrysis galbana* was determined.

MATERIALS AND METHODS

Focusing on intermittent CO_2 supply (Study 1) and chemical flocculation (Study 2) method to cultivation and harvesting *Isochrysis galbana.*

Study 1: Optimization of supply frequency and CO_2 concentration in high cell density culture of *Isochrysis galbana*

Microalgae strain

The marine microalga *Isochrysis galbana* UPMC-A009 was isolated from University Putra, Malaysia. The growth medium is Conway medium. The medium was autoclaved at 121 °C for 20 min.

Photobioreactor Setup

The culture system consisted of three parts: (1) a LED light (2) a column reactor with culture medium supplying system, and (3) a thermos-regulator with a water bath to control the temperature (Figure 1). Working volume and light-receiving area in this reactor were 1.2 L and 0.021 m^2, respectively. The LED light was installed along the reactor. The light intensity was confirmed at the surface of the reactor using a quantum sensor (QSPL-2101, Bio spherical Instruments, United States). Carbon dioxide concentration was controlled by a flow meter, and air with rate of 0.2 L min^{-1} was introduced to the reactor through 0.2 µm filter continuously. Sampling was conducted once in a day before the light period, and fresh medium was added until the culture volume reached 1.2 L (simi-continuous experiments). All operations were conducted under sterilized conditions. The DIC, pH, OD_{750}, Dry weight, fatty acid, fucoxanthin was measured.

The effects of the CO_2 concentration and supply frequency on the microalgae growth were investigated to gain an understanding of the growth characteristics of the microalgae. Four different CO_2 concentration (air, 2, 5, and 10 %) and two different supply frequency (continuous and supply CO_2 for 1 minute every 9 minutes (1/9 min)) conditions were applied to the *I. galbana* culture. The temperature was maintained at 25 ± 1◦C and gas was added through a 0.2 µm filter at the flow rate of 0.2 L min^{-1}. The volume of fresh medium supplied for was 1.2 L and the dilution rates of 0.25 d^{-1}. Light intensity of 300 µmol m^{-2} s^{-1} was applied in all dilution rate treatments. The photoperiod was maintained at 12 L:12 D in all experimental conditions. The control was performed at air continuous supply.

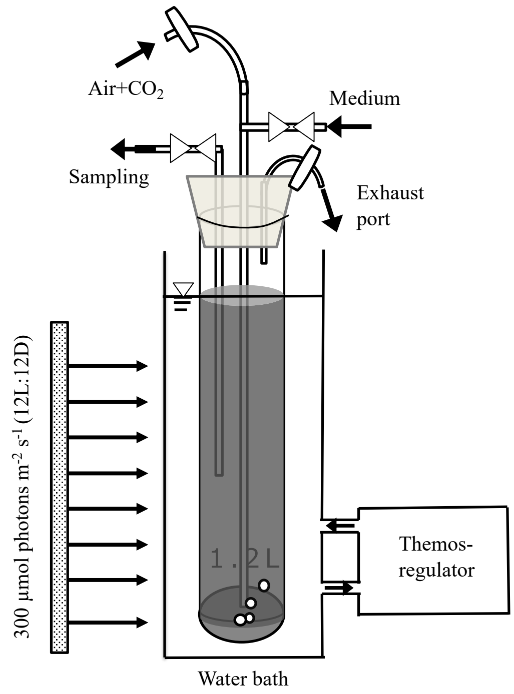

Figure.1. Schematic diagram of the column photobioreactor: inner diameter of 6.0 cm, working volume of 1.2 L. Light was provided to the surface of the water bath at 12L:12D. Filtered air (0.2 µm) was added with air, 2, 5, 10% CO_2 from the bottom of the column reactor at a flow rate of 0.2 L min^{-1}.

Study 2: Recovery of *Isochrysis galbana* by flocculation and sedimentation method

The high cost of harvesting microalgae is a major hurdle for the microalgae industry, harvesting cost accounting for about 20% of the total cost, and an efficient pre-concentration method is required. The flocculation and sedimentation characteristics of freshwater *Chlorella vulgaris* were investigated in our previous research[12]. High pH and high cationic conditions can induce flocculation and sedimentation of microalgae. The mechanism is that calcium ion can react with phosphoric acid (PO_4) at high pH and form large flocs to achieve flocculation and sedimentation effect. But unlike freshwater microalgal flocculation, seawater has a higher calcium content, but low in phosphoric content. The aim of this experiment was to study the flocculation effect of pH and phosphoric acid concentration on *Isochrysis galbana* of marine microalgae.

Effects of the pH and PO_4 concentration on the sedimentation efficiency

The effects of the PO_4 concentration and pH on the sedimentation efficiency were investigated to gain an understanding of the flocculation characteristics of the microalgae. The experiment was performed using Conway medium containing no PO_4 (PO_4-free Conway medium). Na_2HPO_4 was added to aliquots of the Conway medium to give test solutions with PO_4 concentrations of 0, 5 and 10 mM. Tests were performed at pH 8, 9, and 10. The pH was adjusted by adding 2 N NaOH. Tests were performed using 9 conditions, and triplicate tests were performed using each condition. The control was performed at pH 8 with no PO_4 present. The sedimentation efficiency was measured in each test.

The sedimentation efficiency was measured using the procedures described here. The microalgae culture was centrifuged at 1000rpm for 10 min at 25 °C and then diluted with PO_4-free Conway medium to give an optical density at 750 nm (OD_{750}) of 0.2±0.02.

The pH was then adjusted by adding NaOH, and the PO_4 concentration was adjusted by adding Na_2HPO_4. A 250 mL aliquot was then transferred to a modified cylinder and the microalgae sedimentation dynamics were evaluated. The sedimentation rate was determined by measuring the Chl. *a* concentration at different heights (1.0, 6.8, 12.5, 18.2, and 23.2 cm) from the bottom of the cylinder at different times (0, 1, 3, 5, 10, 20, and 30 min) after the experiment started. A hole on the side of the plastic mixing cylinder was made, and then the gap was sealed with a rubber plug. The sampling was done with an injection needle, and author made a mark on the rubber plug to ensure that the needle was inserted in the same position. The sedimentation efficiency was calculated using the equation

$$\text{Sedimentation efficiency (\%)} = \left(1-\frac{B}{A}\right) \times 100$$

where A is the Chl. *a* concentration at time 0 and B is the Chl. *a* concentration in the supernatant after 10, 30 and 60 min.

RESULTS AND DISCUSSION

Study 1: Optimization of supply frequency and CO_2 concentration in high cell density culture of *Isochrysis galbana*

Biomass productivity:

The effects of the CO_2 concentration and supply frequency on the microalgae growth were investigated to gain an understanding of the growth characteristics of the microalgae. Fig. 2 shows the change of pH, DIC concentration and dry weight (DW) over the time. The air continuous condition is control. Air 1/9 and 2% 1/9 condition showed lower growth rate and DW, Maximum DW just achieved 0.3 and 0.26 g/L, respectively. The 2% continuous condition showed similar results as control (DW was 0.71 g/L). Compared with control, 5% CO_2 supply conditions showed higher grows rate and DW. Under 10%,1/9 condition, DW was higher than the control, but the grows rate was low. On the other hand, the Maximum DW was lower in condition of 10% continuous, this suggests that high levels of CO_2 continuous supply may be the reason to inhibit the growth of microalgae. About of the pH, it was increased to around pH 9 after 3 days under condition of air continuous and air 1/9 conditions, and other conditions it decreased to 6 around after 3 days (Fig. 2a). Previous studies have pointed out that high concentration of CO_2 supply will acidify the solution and reduce pH, thus inhibiting the growth of microalgae. However, this study found that low pH may not affect the growth of *I. galbana*, but a acclimation process is needed. Under the condition of 10% continuous, the pH decreased rapidly in the solution and microalgae could not growth. In the case of intermittent CO_2 supply at 10%, microalgae have a process of absorption. This process is probably acclimation. The microalgae can be adapted to low pH after acclimation. Same as pH, the DIC

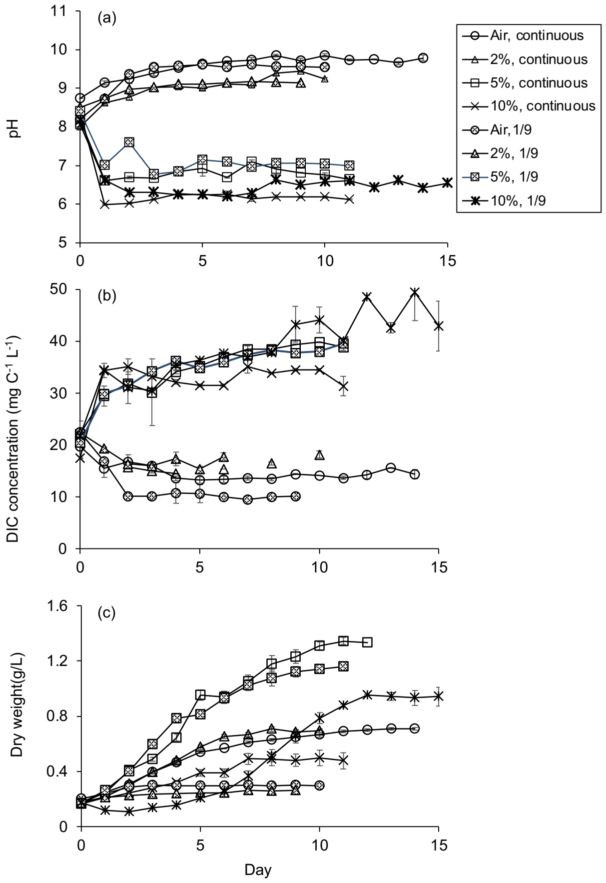

Figure.2. (a) pH, (b) dissolved inorganic carbon concentration and (c) dry weight (g/L) of each CO_2 supply concentration (air, 2%, 5%, 10%) and frequency (continuous, 1/9 min) condition.

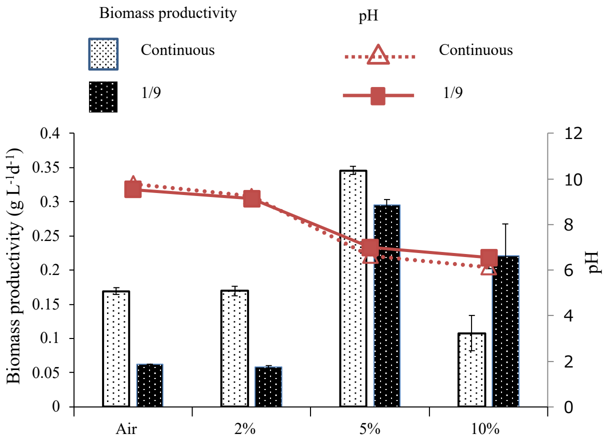

Figure. 3. (a) Biomass productivity and pH in stationary phase of each CO_2 supply concentration (air, 2, 5, 10%) and frequency (continuous, 1/9 min).

content were decreased as well under conditions of air continuous and air 1/9 condition, in other conditions an increase to around 35mg C/L was observed. This is due to the high CO_2 concentration supply (Fig. 2b).

Since this experiment is a semi-continuous culture, so the biomass productivity results based on the stationary phase were shown in Figure 3. Air and 2% conditions showed low biomass productivity and 5%conditions showed the highest biomass productivity. The pH for all conditions hovered between 6 and 10. Previous study showed that the optimum pH range for microalgae culture is 8-9. All the conditions are around this range, but biomass productivity is very different. For example, under the conditions of air and 2%, the pH is in this range, but the biomass productivity is very low. On the other hand, under the conditions of 5%, the pH is a little lower than this range, but the biomass productivity was very high. From this result, pH was probably not suppression factor of biomass productivity. DIC content was low under air and 2% conditions, so the microalgae cannot growth very well. On the other hand, DIC values of 5% and 10% are both very high, However, productivity is quite different. In general, carbon source have the different form about CO_2, HCO_3 or CO_3. Although the total content of DIC is the same, the existing forms of CO_2 that are different CO_2, HCO_3 or CO_3 content under different pH. However, no previous study has shown which form is better for microalgae growth. The previous shows the relative proportion of CO_2, HCO^{3-} and CO_3^{2-} of total dissolved inorganic carbon (DIC) under a range of pH values. In this study, for example, taking the continuous supply condition, the pH values at 5% and 10% were 6.63 and 6.12, respectively. The content of HCO_3 was about 75 and 42%, respectively. In 5% condition, the HCO_3 content is significantly higher than 10% condition. It can be concluded that microalgae growth enhances by HCO_3 form of carbon sources. Also, carbon sources in the form of CO_2 maybe inhibit microalgal growth.

Pigment and Lipid production

Figure 4 showed the results of pigment for fucoxanthin content. In continuous condition, the higher of CO_2 supply concentration, the lower of fucoxanthin content. On the other hand, fucoxanthin accumulates was the best under 5% 1/9 intermittent supply condition. This may be due to differences frequency of CO_2 supply. Previous studies have shown that low light is better for fucoxanthin accumulation, but under the continuous condition, microalgae can provide sufficient and uniform contact with light, so they cannot accumulate fucoxanthin well. This indicated that the accumulation of fucoxanthin could be promoted by intermittent supply under appropriate CO_2 conditions. In the results for fatty acids, the control (Air continuous) presented the best fatty acid content and there were no significant differences in fatty acids between conditions. This indicates that CO_2 concentration and frequency have almost no effect on fatty acid accumulation

Study 2: Recovery of *Isochrysis galbana* by flocculation and sedimentation method

The result showed that high sedimentation efficiency (95%) occurred at a high Ca^{2+} concentration (5 mM) at pH 9 and 11. The sedimentation efficiency of the control (No Ca-pH 7) was only 3.9% after 30 min of sedimentation. In the marine microalgae *I. galbana*, the sedimentation efficiency achieved 40% under high PO_4 concentration (10 mM) and pH 10, that was higher than control condition (No PO_4-pH 8). It can be known from the above results that the sedimentation efficiency in seawater is lower than in fresh water. This may be due to the smaller size of *I.galbana* than freshwater microalgal *C. vulgaris*. On the other hand, at high pH, reactions between Ca^{2+} and phosphate and carbonates give flocs [10]. Calcium phosphate usually has a positive surface charge, which allows negatively charged microalgae cells to adhere to the calcium phosphate surfaces. Since there are more anions in seawater, a higher pH may be required to induce microalgal flocculation and sedimentation.

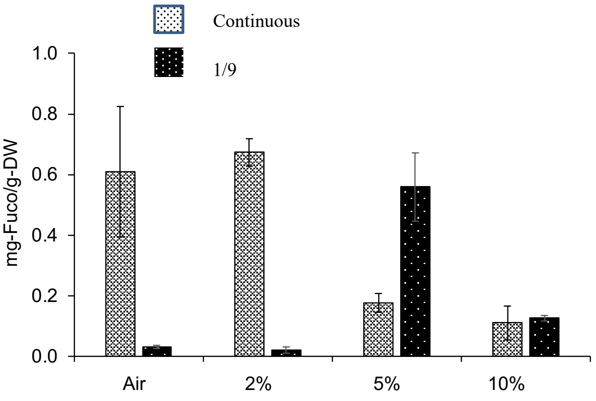

Figure. 4. Fucoxanthin content of each CO_2 supply concentration (air, 2, 5, 10%) and frequency (continuous, 1/9 min) condition.

SUMMARY

1) In order to reduce the cost of microalgae cultivation as well as recovery, this PhD thesis firstly uses intermittent cultivation method to cultivate microalgae and secondly uses chemical flocculation method to recover it.
2) The results show that the intermittent culture can not only improve the microalgal production but also the pigment content.
3) Through our study, we can reduce the cost of microalgae culture and can expect to put it into practical application on a large scale.
4) For environmental considerations, recycling of the flocculated supernatant for microalgae culture can be attempted in the future.

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