# ENHANCEMENT OF LIPID PRODUCTION OF OLEAGINOUS GREEN MICROALGA, *ANKISTRODESMUS DENSUS*

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## ABSTRACT

Microalgae are recognized as one of the promising non-food-crop-based biomass feedstock for biodiesel production. Since the production of biomass and lipid in outdoor cultures depends on environmental factors, the present study aimed to optimize growth conditions including carbon dioxide ($CO_2$) supplementation, nutrient concentration, and light intensity for high biomass and lipid output of *Ankistrodesmus densus*, a freshwater green microalga from Thailand Institute of Scientific and Technological Research (TISTR) culture collection. The results revealed that supplying 1-20% of $CO_2$ mixed with air to culture medium (BG-11) caused an enhancement of the growth of *A. densus* when compared with air. The maximum biomass and lipid content were respectively, 2.5 g $L^{-1}$ and 31% of dry weight (DW) obtained in the 7$^{th}$ day of cultivation by 1% $CO_2$ feeding. The nutrient deprived condition by decreasing the medium concentration 4 times in comparison to the standard medium resulted in the highest lipid and total fatty acids (TFAs) contents up to 36.6% DW and 26.2% DW, respectively. At this condition, cell accumulated triacylglyceride (TAG) approximately 59% total lipid which represented 1.3 fold higher than its corresponding control. However, the increased lipid and TFAs content seemed to be compensated with the low biomass (1.7 g $L^{-1}$). Moreover, the light intensity is one of the key factors that affect to cell growth and biochemical composition in algae. Cultivating *A. densus* in a helical photobioreactor with various light intensities showed that exposure of culture to 300 µmol photons $m^{-2}$ $s^{-1}$ gave rise to the highest biomass (3.6 g $L^{-1}$) and lipid content (31.0% DW). Therefore, this work paved the way for the improvement of lipid production in *A. densus*.

## INTRODUCTION

High-lipid content microalgae have the potential to be one of the sources of biodiesel, which can replace of fossil diesel. However, achieving high lipid yield greatly depends on growth conditions, including culture system, nutrient supply, and environmental factors. Due to continuous and increasing combustion of fossil carbon, the amount of greenhouse gas $CO_2$ has increased, as a result of global warming. Microalgae have high efficiency of converting $CO_2$ into biomass and other biochemical including oil via photosynthesis than oil crops have. Microalgae can tolerate to various $CO_2$ concentrations, nevertheless most species have low growth rate at high $CO_2$ concentration (Chiu et al., 2009; Tang et al., 2011). Several reports indicated that microalgae fed with $CO_2$ had higher lipid content than that of ambient air (Widjaja et al., 2009; Tang et al., 2011). Utilization of $CO_2$ in flue gas not only alleviates the global climate change, but also can reduce the nutrient cost of microalgal production. The nutrient stress in microalgal cultivation mainly retards the growth and causes the changes in metabolic pathway, especially; starvations of nitrogen and phosphorus induce accumulation of storage lipids (Lombardi and Wangersky, 1991). Light is the most important energy source for photosynthetic organisms. The various light intensities

exhibited the alterations of photosynthetic activity, growth rate and biochemical composition. The saturation intensity yields the maximal growth via the maximal photosynthesis and also different light intensities have been reported to change in the lipid content and fatty acid composition in *Scenesdesmus obliquus* (Ho et. al, 2012). This work aimed to increase the lipid production of *A. densus* by optimization of vital growth conditions including $CO_2$ concentration, nutrient availability and light intensity.

## MATERIALS AND METHODS

### Microorganism and inoculum preparation

Freshwater microalgae *A. densus* was obtained from culture collection of TISTR. The cells were grown in BG-11 medium (Stainer, et al., 1971) at 30 °C and aerated with filter-sterilized ambient air (ca. 0.04% $CO_2$). The illumination was provided by cool-white fluorescent lamps at light intensities of 100 µmol photons $m^{-2}$ $s^{-1}$ with 24 h of continuous light period.

### Effect of $CO_2$ concentration

Cells of *A. densus* were cultivated in 1 L Erlenmeyer flasks containing 700 mL of BG-11 medium under agitation of ambient air at 1.43 vvm until reaching the logarithmic phase. The $CO_2$ acclimatization step was conducted by growing the cells under various $CO_2$ concentrations; 1%, 5%, 10%, 15% and 20% $CO_2$. Then the logarithmic - $CO_2$ acclimatized cells were cultivated at the same $CO_2$ concentration for 7 days to investigate the effect of $CO_2$ concentration on growth and lipid content.

### Effect of nutrient availability

The cells were cultivated in BG-11 medium under aeration with 1% $CO_2$ at 1.43 vvm until reaching the logarithmic phase for using as inoculum. The cells were grown in 1/2- and 1/4-fold concentrations of BG-11 medium compared to the normal BG-11 medium for 7 days. Growth and lipid content were determined at day 4 and day 7.

### Effect of light intensity

The photobioreactor, helical type (Satorious, PBR2S) was used to conduct this study. The cells were pre-cultured in 1 L Erlenmeyer flasks containing 700 mL of BG-11 medium at 30±2 ºC and 100 µmol photons $m^{-2}$ $s^{-1}$ of continuous light period under agitation of ambient air at 0.5 vvm until reaching the logarithmic phase. The logarithmic cells with the initial $OD_{680}$ at 0.1 with the final working volume of 3 L were transferred to the PBR. The PBR was operated with four light intensities including, 80, 100, 150 and 200 µmol photons $m^{-2}$ $s^{-1}$. The agitation was facilitated by aeration of 1% $CO_2$. Growth and lipid content were daily determined through 7 days.

### Determination of microalgal cell concentration and dry biomass of *A. densus*

The cell concentration was determined daily by measuring optical density at wavelength 680 nm ($OD_{680}$). For dry biomass, cell cultures were collected by filtering through GF/C filter paper (Whatman®). The cell pellet was washed twice with distilled water before drying at 103-105 ºC for 1 h and then the dry weight (DW) was measured until obtaining the constant weight.

### Determination of the total fatty acid, lipid content and lipid profile

Cells were harvested by centrifugation at 10,000 rpm and 10 ºC for 10 min. The cell pellets were washed twice with distilled water, then lyophilized and kept at -20 ºC before further analysis.

Fatty acid methyl esters (FAMEs) were conducted according to method of Lepage and Roy (1984) and were then analyzed by Gas chromatography (GC-17A, Shimadzu) (column size, 60 m × 0.25 mm × 0.25 µm, BPX70 coated cyanopropyl). Temperature of injector and detector was 250 °C and 260 °C, respectively.

Total lipid was extracted from lyophilized cells according to modified method of Bligh and Dyer (1959).

Lipid profile was analyzed by dissolving crude lipid in chloroform. Standard of mono-, di- and triglyceride mix (1787-1AMP, Supelco), free fatty acids (C17:0) and lipid extracts were assayed on thin-layer chromatography (TLC, Silica gel 60 Aluminum sheets 20 × 20 cm, Merck).The mobile phase composed of hexane: diethyl ether: acetic acid (70: 30: 1, v/v) (Touchstone, 1995). The plate was subsequent stained by 0.01% primuline and revealed the lipid under UV light. Individual peaks of lipid class were identified by comparison of their peaks with authentic standards.

## RESULTS AND DISCUSSION

### Effect of $CO_2$ concentrations on biomass and lipid production of *A. densus*

As shown in table 1, an increase in the $CO_2$ concentration stimulated the cell growth resulted in the increase in biomass concentration. Supplying 1% $CO_2$ considerably increased 4 times of biomass concentration (2.45 g $L^{-1}$) compared to that of ambient air (0.64 g $L^{-1}$). The highest biomass concentration was obtained at 1% $CO_2$. It was interesting that the biomass concentration remained constant around 2.4 g $L^{-1}$ with the elevation of $CO_2$ concentration from 1 to 10% $CO_2$. However, it was obviously shown that biomass concentration decreased when the $CO_2$ concentration was increased to 20%. Changes of $CO_2$ concentration from 0.04 up to 20% seemed not to affect the lipid content of *A. densus*. Thus, the lipid contents were approximately 27.2-30.8% DW. Nevertheless, the combination of biomass and lipid content must be taken an account for lipid production which resulted in the highest lipid yield, 0.77 g $L^{-1}$ at 1% $CO_2$.

Table 1. Biomass and lipid productions of *A. densus* grown in various $CO_2$ concentrations (Day 7)

| $CO_2$ concentrations (%) | Biomass concentration (g $L^{-1}$) | Lipid content (% DW) | Lipid yield (g $L^{-1}$) |
|---|---|---|---|
| 0.04 (ambient air) | 0.64±0.13 | 28.9±0.8 | 0.19±0.04 |
| 1 | 2.45±0.24 | 30.8±2.1 | 0.77±0.08 |
| 5 | 2.42±0.23 | 27.2±2.0 | 0.66±0.08 |
| 10 | 2.39±0.30 | 29.8±1.4 | 0.72±0.10 |
| 15 | 2.07±0.26 | 30.3±1.7 | 0.62±0.09 |
| 20 | 1.44±0.49 | 27.2±0.5 | 0.39±0.14 |

Thus, the lipid contents were approximately 27.2-30.8% DW. Nevertheless, the combination of biomass and lipid content must be taken an account for lipid production which resulted in the highest lipid yield, 0.77 g $L^{-1}$ at 1% $CO_2$.

**Effect of nutrient availability on biomass, lipid and TFA production of *A. densus***

As shown in table 2, the biomass concentration increased along with medium concentration and cell ages. After 7 days, the highest biomass concentration of 2.96 g $L^{-1}$ was achieved from control medium whereas the lowest biomass concentration of 1.70 g $L^{-1}$ was obtained from 1/4-fold of the standard medium. There were no difference in lipid contents between cells grown in standard medium and 1/2-fold concentration of the standard medium throughout the cultivation period. Interestingly, reducing the medium concentration to 1/4-fold concentration of the standard one turned to significantly increase in both lipid and TFAs contents. When cells grew in 1/4-fold of the standard medium for 4 days, the lipid content was about 29% DW. In addition, the lipid content reached to the highest amount at 36.6% DW after prolonged cultivation in this condition for 7 days. Moreover, it was found that the less abundant nutrients enhanced the TFAs content. For instance, the TFAs of cell grown under 1/4-fold concentration of the standard medium at both day 4 (18.9% DW) and day 7 (26.2% DW) were higher than those of two media. In addition, the lipid profiles indicated that the 1/4-fold-

Table 2. Biomass, lipid and TFA productions of *A. densus* grown in various concentrations of BG-11 medium

| | Control medium | | | 1/2-fold | | 1/4-fold | |
|---|---|---|---|---|---|---|---|
| | Day 0 | Day 4 | Day 7 | Day 4 | Day 7 | Day 4 | Day 7 |
| Biomass (g $L^{-1}$) | 0.33±0.13 | 1.55±0.09 | 2.96±0.10 | 1.32±0.11 | 2.50±0.08 | 1.10±0.05 | 1.70±0.12 |
| Lipid content (% DW) | 26.1±1.8 | 26.5±3.8 | 30.4±2.8 | 26.8±3.6 | 30.1±2.0 | 29.0±2.5 | 36.6±4.2 |
| Lipid yield (g $L^{-1}$) | 0.01±0.00 | 0.34±0.37 | 0.90±0.06 | 0.34±0.08 | 0.77±0.05 | 0.31±0.03 | 0.62±0.05 |
| TFA (% DW) | 12.8±0.8 | 11.9±0.9 | 14.0±1.0 | 12.9±1.0 | 19.2±1.8 | 18.9±1.1 | 26.2±3.2 |

concentration of the standard medium grown cells accumulated the TAG about 60% of total lipid which were 1.8- (day 4) and 1.3-fold (day 7) greater than that of control (Fig. 1). The result suggested that alteration of nutrient concentration could affect lipid profile in *A. densus* cells. The response to nutrient stress by enhancing TAG in *A. densus* cells was similar to that of others green microalgae such as *Chlorella vulgaris* (Widjaja et al., 2009) and *Monodus subterraneus* (Khozin-Goldberg and Cohen, 2006).

**Effect of light intensity on biomass, lipid and TFA production of *A. densus***

*A. densus* cells were grown in the photo-bioreactor, helical type under different light intensities and aerating with 5% $CO_2$ at 0.05 vvm. As shown in table 3, among all light intensities, 80 µmol photons $m^{-2}$ $s^{-1}$ caused the lowest growth rate (0.78 $d^{-1}$) and biomass concentration (0.89 g $L^{-1}$). The optimal light intensity, 300 µmol photons $m^{-2}$ $s^{-1}$ resulted in the highest biomass concentration at 3.62 g $L^{-1}$ and lipid content at 31.0% DW. Consequently, the highest lipid yield was 1.12 g $L^{-1}$.

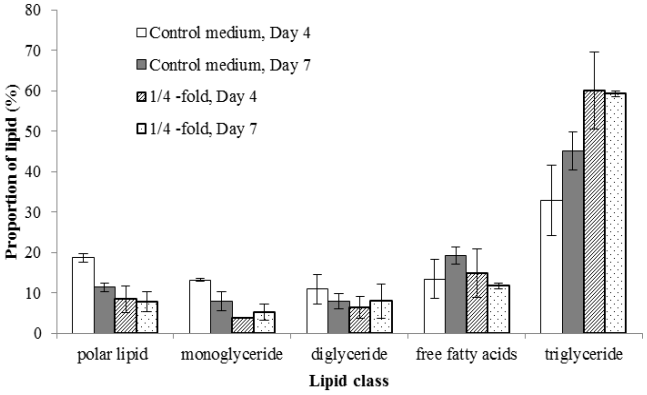


Figure 1. The proportion of lipid of *A. densus* grown control medium and 1/4-fold concentration of the standard medium harvested at day 4 and day 7

Table 3. Specific growth rate, biomass, lipid and TFA productions of *A. densus* grown in various light intensities (day 7)

| | Light intensity (µmol photons $m^{-2}$ $s^{-1}$) | | | |
|---|---|---|---|---|
| | 80 | 100 | 200 | 300 |
| Specific growth rate (µ, $d^{-1}$) | 0.78±0.00 | 0.99±0.04 | 1.09±0.05 | 1.14±0.12 |
| Biomass concentration (g $L^{-1}$) | 0.89±0.01 | 1.87±0.32 | 2.04±0.42 | 3.62±0.23 |
| Lipid (% DW) | 28.9±0.10 | 27.4±0.10 | 29.5±1.10 | 31.0±0.10 |
| Lipid yield (g $L^{-1}$) | 0.26±0.00 | 0.51±0.07 | 0.60±0.12 | 1.12±0.05 |

## CONCLUSION

There are several approaches to enhance the lipid productivity in *A. densus*. This work indicated that (1) the increase amount of inorganic carbon, $CO_2$ enhanced growth of *A. densus* and the cells could tolerate and grow under $CO_2$ concentration up to 20%; however, the maximum biomass concentration and lipid yield were achieved at 1% $CO_2$; (2) cultivation of *A. densus* with nutrient deficiency at 1/4-fold concentration of BG-11 medium enhanced the lipid yield by compensation of loss of biomass and accumulation of lipid content and; (3) the optimal light intensity of *A. densus* for growth and accumulation of lipid was 300 µmol photons $m^{-2}$ $s^{-1}$. Considering use of $CO_2$ and low amount of nutrients provided a direction to reduce the cost of microalgal oil production. Furthermore, response of *A. densus* to high light intensity could be a guideline to manage the appropriate cell concentration in outdoor cultivation.

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