



Production of Biodiesel and Growth of *Staurastrum sp.* in Response to CO₂ Induction

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Abstract

The research has been conducted to know the influences of CO₂ induction to cell density and biodiesel production of *Staurastrum* sp microalgae. The experiment design was completely randomized design with ten replications. The treatments comprising of 3 kinds: CO₂ induction, air induction and control (without treatment). At peak population, the highest density for CO₂ induction was 8,895,520 cell.mL⁻¹ at the day of seven, for air induction 5,690,880 cell.mL⁻¹ at the day of eight and control 2,578,045 cell.mL⁻¹ at the day of ten. The highest fresh weight and dry weight was occurred in *Staurastrum* sp culture with treatment CO₂ induction followed by air induction and control. The highest oil production was occurred in *Staurastrum* sp culture with treatment CO₂ induction followed by air induction and control. The highest biodiesel production was occurred in *Staurastrum* sp culture with treatment CO₂ induction at 31.95%-weight followed by air induction at 19.65%-weight and control at 16.52%-weight.

Keywords: Biodiesel, CO₂, Growth, *Staurastrum* sp.

Introduction

Currently, more than 80% of the energy produced globally each year is generated through the combustion of fossil fuels (Sayre, 2010). Industries related to electricity generation, natural gas processing, cement, iron and steel manufacturing, combustion of municipal solid waste are the major contributor of atmospheric CO₂ because of their dependence on carbon sources like coal, oil, natural gas for fulfilling their energy requirement (Kumar et al., 2011).

The use of microalgae for CO₂ sequestration has several advantages: mitigating CO₂, the major source of global warming as well as producing biofuels and other interesting secondary metabolites (Brennan and Owende, 2010). Because microalgal biomass is made up of about 50% carbon, this element is a major nutrient for microalgal biomass growth. Under phototrophic growth conditions, microalgae utilize atmospheric CO₂ as carbon source to synthesize organic compounds (Putt et al., 2011)

Microalgae are found to have higher photosynthetic efficiencies than most photoautotrophic organisms and have 10 – 50 times higher carbon removal efficiencies than other terrestrial plants (Li et al., 2008). Mass cultivation of microalgae has been employed in the production of valuable biochemicals such as astaxanthine, b-carotene, pigments, vitamins, and polyunsaturated fatty acid (Das et al., 2011). Microalgae typically have more than 30% oil yield and possess a great potential as a feedstock of renewable fuels such as biodiesel (Ahmad et al., 2011). Microalgal lipid most accumulated as triglycerides can be transformed to biodiesel (Tang et al., 2011)

The significant amount of fuel demand and the depletion of petroleum diesel, other sources of transportation fuel, such as microalgae are gaining recent attention. A microalga is considered an ideal biodiesel feedstock due to its high growth rate, productivity, and photosynthetic efficiency while its production consumes less energy compared with other feedstock (Lehr and Posten, 2009).

The microalgal cultivation depends on major parameters such as nutrients, carbon dioxide

and light (Soratana and Landis, 2011). Generally, microalgae use CO₂ as the carbon source and light as the energy source for metabolic activity. Green microalgae (*Chlorophyta*) such as species of *Staurastrum* sp has a simple structure which is less than 2 mm in diameter but the high growth rate and contain oils that can be used as biodiesel. Therefore in this study will be used induction gas carbon dioxide (CO₂) to the growth of *Staurastrum* sp microalgae species can be maximized and expected to produce oil that will be converted into biodiesel in large amount. The aims of this research were to determine the biomass, biodiesel production from oil of microalgae *Staurastrum* sp that influenced by carbon dioxide induction.

Materials and Method

The study was conducted from September to December 2010. Microalgae *Staurastrum* sp was derived from the collection of the Laboratory Department of Biology, Faculty of Science and Technology of The State Islamic University Sunan Gunung Djati Bandung, was the result of isolation from the fresh water Cibiru, Bandung. In the experiments, *Staurastrum* sp was cultivated in 500 mL Erlenmeyer flask with 200 ml working volume of Basal Bold medium under 25 ±1°C and 180 μmol m⁻²s⁻² light intensity was measured by a light meter. The initial density of cell in oculum was 10.000 cells.mL⁻¹ and the initial pH was 6.5. A gas distributor provided with flow rates of CO₂ mixed with ambient air was used to prepare CO₂ concentrations of 0.03%. Cultures were aerated continuously with filtered (0.22 μm) mixtures via bubbling from the above of Erlenmeyer flask with an aeration rate of 200 mL.min⁻¹ (i.e. 0.25 vvm, volume gas per volume media per minute).

Calculation of cell density was done periodically every 24 hours for 14 days, using Haemocytometer. For the growth rate calculated by the following equation (Chrismadha et al., 2006):

$$\mu = \frac{\ln(X_t / X_0)}{t}$$

Description: μ = The rate of growth (cell division.day⁻¹), X_t= cell density at time t, X₀ = initial cell density, t = time (days).

The dry cell weight (g.L⁻¹) was measured according to the method as described by Chi et al. (2007). Microalgal cells were harvested by centrifugation (5804R, Eppendorf, Germany) at 8000 rpm for 5 min and washed twice with distilled water. The microalgal pellet was lyophilized drying in a freeze drier (FD-1-50, Boyikang, China) for dry weight measurement.

The total lipids were extracted from microalgal cells using a modified method of Zhu et al (2007). Dry microalgal cells were pulverized in a mortar mixed with liquid nitrogen and extracted using ethanol 99.8%. About 5 mL solvents and 100 mg dry cell were used in each extraction step. The procedure was repeated three times until the total lipids were fully extracted. The solvent phase was transferred by pipette and evaporated in a rotary evaporator under vacuum at 60°C. Then the total lipids were weighed using analytical balance (BS 124S, Sartorius, Germany).

Data were analyzed first by one way analysis of variance (ANOVA) and then Duncan's Multiple Range Test (DMRT) for pair wise comparison was used at the 5% significance level. (Gomez and Gomez, 1995).

Results and Discussion

Table 1: Average Growth of *Staurastrum* sp number of Cells in Single Culture for 14 Days

Days	Treatment		
	Control (T0)	Air Induction (T1)	CO ₂ Induction (T2)
1	10.000	10.000	10.000
2	122.000	267.000	545.820
3	505.452	809.785	1.045.930
4	1.020.665	1.200.450	2.455.332
5	1.225.200	2.675.433	4.875.525

6	1.554.445	3.102.344	6.905.572
7	1.690.035	4.470.650	8.895.520 *)
8	1.766.500	5.690.880*)	8.539.912
9	2.190.090	3.985.453	8.005.735
10	2.578.045*)	2.977.053	7.015.250
11	2.485.900	2.554.350	6.900.875
12	2.205.785	2.443.263	6.565.003
13	1.996.700	2.065.651	6.205.359
14	1.595.245	1.885.753	5.980.943

Description: (*) = peak population

Table 1 illustrates the average number of microalgal cells in all treatments showed an increased growth. When compared with the culture of *Staurastrum* sp without treatment (control) all the existing treatments of the air and CO₂ induction showed substantial growth in the number of cells. On the second day all treatments including the control showed a substantial increase. It shows that *Staurastrum* sp cells do not require a longer adaptation to various environmental factors for its growth.

reached the peak on the 10th day with the number of cells as much as 2,578,045 cells.mL⁻¹. The growth of cells number in the treatment of air induction reached peak population on the 8th day by the cells number as much as 5,690,880 cells.mL⁻¹. While the number of cells in the treatment of CO₂ induction reached peak population on the 7th day by the cells number of 8,895,520 cells.mL⁻¹. Attainment of peak population more rapid in treatment of CO₂ induction allows cells carry out photosynthesis resulting in more biomass. Nutrient availability in a medium does not support the growth of cells number. After reaching the peak population, the growth of cell number immediately decreased.

Beginning with that form graphs of exponential growth, the growth of *Staurastrum* sp number of cells in single culture without treatment (control)

Table 2: Average Fresh and Dry Weight Biomass of Microalgal *Staurastrum* sp. at Peak Population of Mass Culture

Treatment	Fresh Weight (gram)	Dry Weight (gram)
T0 = Control	130,72 (a)	12,65 (a)
T1 = Air Induction	182,67 (b)	18,04 (b)
T2 = CO ₂ Induction	238,85 (c)	24,56 (c)

Value in a column followed by a common letter are not significantly different at the 5% level by DMRT.

After harvesting in mass culture both dry weight and fresh weight showed a marked improvement on the basis of statistical tests on the 95% confidence interval of the control. The largest of fresh weight achieved by CO₂ induction treatment followed by air induction and the lowest in control. Moreover the highest dry weight achieved by CO₂ induction treatment. Dry weight in all treatments showed more than

10% of fresh weight, except in control of less than 10%.

Biomass was greater in treatment, especially induction of CO₂ in line with the number of cells of *Staurastrum* sp. Carbon dioxide was induced into the medium has been exploited by *Staurastrum* sp cells for growth and reproduction.

Table 3: Average Oil Yield (%) and Water Content (%) from *Staurastrum* sp. Solvent Extraction with Ethanol (99.8%)

Treatment	Oil Products (%-weight)	Water content (%) (hydroalcoholic phase)
T0 (Control)	20,72 (a)	29,34 (a)
T1 (Air Induction)	24,55 (b)	32,15 (b)
T2 (CO ₂ Induction)	35,36 (c)	38,64 (c)

Value in a column followed by a common letter are not significantly different at the 5% level by DMRT.

Solvent extraction using ethanol 99.8% was very effective because it can produce high enough oil more than 20%. Higher amount of oil obtained in the CO₂ induction treatment of 35.36%- weight.

Oil extracted with ethanol 99.8% gives a bit of green colour. This suggests extracted *Staurastrum* sp chlorophyll by ethanol.

According to Fajardo et al. (2007), the water content in hydro alcoholic phase by 40% of oil provides optimal results. The induction of the CO₂ content of the water treatment reached 38.64%, resulting in the highest oil that is 35.36%-weight.

Table 4: Average Biodiesel (%) and Comparison of Results of Oil (%)

Treatment	Biodiesel (%-weight)	Comparison of results of oil (%)
T0 (control)	16,52 (a)	79,73
T1 (air induction)	19,65 (b)	80,04
T2 (CO ₂ induction)	31,95 (c)	90,36

Value in a column followed by a common letter are not significantly different at the 5% level by DMRT.

The resulting biodiesel is quite high with a value of more than 70% of oil produced. The production of biodiesel is the highest achieved by induction of the CO₂ treatment for 90.36% of oil revenues. The amount of biodiesel produced in the culture of *Staurastrum* sp treated no different air induction control which is about 80% of the oil.

Conclusion

The population peak in CO₂ induction treatment achieved on the 7th day of 8,895,520 cells.mL⁻¹, air induction treatment achieved on the 8th day of 5,690,880 cells.mL⁻¹. And control achieved on the 10th day of 2,578,045 cells.mL⁻¹. Dry weight in all treatments showed more than 10% of fresh weight, except in control. The induction of CO₂ treatment can produce the highest oil 35.36%-weight meanwhile the highest production of biodiesel was achieved by induction of the CO₂ treatment for 90.36% from oil production.

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