

Effect of Light Intensity for Optimum Biomass and Lipid Production from *Scenedesmus dimorphus* (Turpin) Kützing

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Abstract. One source of alternative energy substitute for petroleum raw materials is renewable vegetable oils known as biodiesel. Biodiesel can be produced from microalgae, since it was more efficient and environmentally friendly. *Scenedesmus dimorphus* (Turpin) Kützing was developed as a source of biodiesel since it had potential of high lipid production. The aims of this research were to know the rate of growth of *Scenedesmus dimorphus* in different lighting and the optimum light intensity for biomass and lipid production. This research used a completely randomized design consisting of 3 treatments with 3 replications. Treatments in this research were the light intensity, i.e. 7,500, 10,000, and 12,500 lux. *Scenedesmus dimorphus* was grown in Bold's Basal Medium (BBM). Parameters observed in this research were the cell number, biomass and lipid production of *S. dimorphus*. Data were analyzed by ANOVA followed by DMRT 5%. The results showed that the optimum growth rate of *S. dimorphus* was in the intensity of 12,500 lux that was 100.80×10^6 cells.ml⁻¹. The optimum production of biomass and lipids was in treatment 12,500 lux i.e; 1.1407 g.L⁻¹ and 0.2520 g.L⁻¹ (22.28% dry weight).

1. Introduction

Energy is needed in the lives of both humans and other organisms. Energy derived from the natural resources of renewable and non-renewable. Innovation of alternative energy sources start to be developed considering the stock of non-renewable energy sources such as oil decreases. Alternative sources of energy are used as a replacement of petroleum feedstock is biodiesel. The use of biodiesel as a diesel substitute standard will not only help the environment, but also will help increasing energy independence and energy security of the country. Biodiesel can be produced from vegetable oils. Microalgae are one source of oil that can be used for making biodiesel, because microalgae contain a high enough lipid.

Lipids in the cell of microalgae serve as a backup energy source in cells deficient in carbohydrates as an energy source. Another function of lipid microalgae can be utilized as a source of alternative energy as a raw material for biodiesel, biodiesel privilege that comes from microalgae i.e. can be updated (renewable), non-toxic, and can be broken down naturally (biodegradable) [1]. The processes of the biosynthesis of lipids on microalgae require great energy, begin with the process of the formation of carbohydrates then continue with the accumulation of lipids [2]. A microalgae that can be developed as a source of biodiesel is *Scenedesmus dimorphus* (Turpin) Kützing. *S. dimorphus* is a cosmopolitan microalgae, mostly live in the aquatic neighborhood, in fresh water or brackish water. *Scenedesmus dimorphus* act as producers in the ecosystems as oxygen providers [3].



Energy generated in the process of photosynthesis can be used as growth in microalgae, spare food or to defend themselves when the pressure on the environment [4]. The autotroph organisms Microalgae are capable to form organic compounds from inorganic compounds through the process of photosynthesis. The existence of the light determines the curve shape of growth for microalgae that performs photosynthesis. [5] do the culturing microalgae *S. dimorphus* in different variations of the Bold's Basal Medium (BBM) shines with 10,000 lux shows that the highest biomass production are present in 125% of BBM media 0.42 g/l, while the production of lipids found in the highest media 200% of BBM 0.026 g/l (18.49% dry weight).

But in fact, until now, especially in light of microalgae utilization as energy source is still not optimal. From the description above, it needs to be carried out a research on the influence of the light shines towards the acceleration of growth in microalgae biomass and lipids to produce the optimum total.

2. Experimental

2.1 Microalgae strain and cultur medium

A microalga used in this research is the culture of *Scenedesmus dimorphus* (Turpin) Kützing obtained from Laboratory Research Center for Limnology-LIPI Cibinong. Bold's Basal Medium (BBM) was used to grow the pure culture of *S. dimorphus*. The stock medium consisted of (g L⁻¹): NaNO₃ (25.0 g), CaCl₂·2H₂O (2.5 g), MgSO₄·7H₂O (7.5 g), K₂HPO₄ (7.5 g), KH₂PO₄ (17.5 g), NaCl (2.5 g), EDTA (50.0 g), KOH (31.0g), FeSO₄·7H₂O (4.98g), H₂SO₄ (1.0 ml), H₃BO₃ (11.42 g), ZnSO₄·7H₂O (8.82 g), MnCl₂·4H₂O (1.44 g), MoO₃ (0.71 g), CuSO₄·5H₂O (1.57 g).

2.2 Cultivation conditions

The microalgae was cultivated in laboratorium at 28-30°C. Various operating conditions were assayed including light intensities of 7,500 lux, 10,000 lux, and 12,500 lux. The light intensity were measured with a light meter. Temperature and pH of the culture medium were monitored daily.

2.3 Analytic determinations

2.3.1 Cell number, biomass, and lipid concentration

The cell concentration of culture was determined regularly by measuring optical density at wavelength 680 nm (denoted as OD₆₈₀) using a UV/VIS spectrophotometer and converted into a number of cells with the regression curve. Extraction of lipids was done on the day when the first appropriate exponential growth and density measurement results cells. As many as 50 ml culture tube centrifuge microalgae was centrifuge with the speed of 3,000 rpm for 5 minutes. The pellets then dried with an oven for 24 hours at a temperature of 80°C and weighed as a dry weight. The dry Pellet is suspended with the solvent chemical that consists of 2 ml of distilled water, ion-free 5 ml methanol and 2.5 ml of khloroform and then be homogenized with the help of shaker horizontally for 24 hours. Into the results stirring 2.5 ml water added back destilat free ion and 2.5 ml of khloroform then do a speed 4,000 rpm sentrifuse for 25 minutes. A mixture of lipids and chloroform placed separately from the other materials then be heated so that chloroform can be evaporated, the rest mixture are dried and weighed as lipid weight of lipids. Percentage of lipids can be calculated with the formula [6] :

$$\% \text{ Lipid} = \frac{\text{Lipid Weight}}{\text{Dry Weight Microalgae}} \times 100 \% \quad (1)$$

While the productivity of lipids can be calculated with the formula [2] :

$$\text{Productivity (g/l/days)} = \frac{\text{Lipid Weight / l}}{\text{The Number Of Days}} \quad (2)$$

3. Result and Discussion

3.1 The growth pattern of *S. dimorphus*

Growth curve of *S. dimorphus* (Figure 1) shows a high light intensity effect on the growth. The highest cell numbers appears on the light intensity 12,500 lux with $100.8 \times 10^6 \text{ cell.ml}^{-1}$, and the amount of the lowest cell appears at 10,000 lux light intensity with $65.3 \times 10^6 \text{ cell.ml}^{-1}$. While the culture of 7,500 lux is in the middle with a cell number $70.8 \times 10^6 \text{ cell.ml}^{-1}$. The higher intensity light is used, the higher the microalgae cell growth. The intensity of the light is indispensable in the process of photosynthesis because it relates to the amount of energy received by the microalgae to perform photosynthesis.

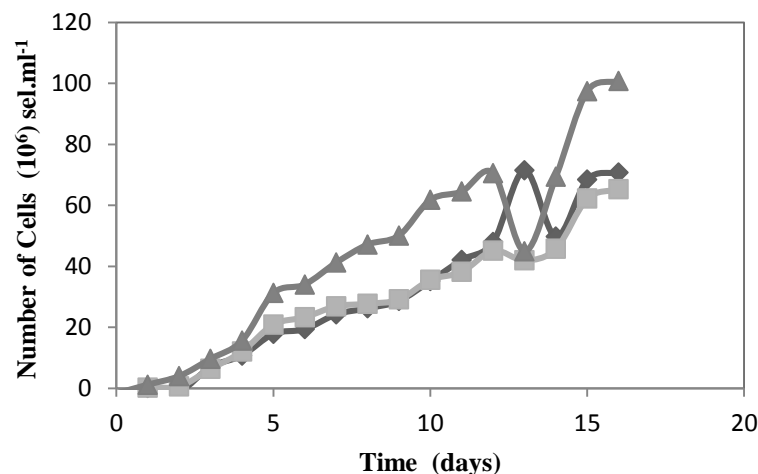
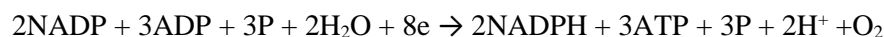


Figure 1. Growth curve of *S. dimorphus* in different light intensity 7,500 lux (—◆—), 10,000 lux (—■—), and 12,500 lux (—▲—)

Light is the main energy source for *S. dimorphus*. If the supply of light is limited, the supply of nutrients will decrease. Thus, *S. dimorphus* growth will be hampered. According to [7], the light is needed in the process of photosynthesis to produce energy (hexoses) and oxygen from carbon dioxide and water. Energy from light is used in the electron transfer process to CO_2 through the reduction of nicotinamide adenine dinucleotide phosphate (NADP) becomes NADPH. Electrons are extracted from the water of the happening in photosystem II and transported through the cycle of Quinones and released photosystem I. Electrons received by the ferredoxin that causes the reduction of NADP become NADPH allowing cell synthesis of molecular components. On the ideal condition, the light reaction in photosynthesis reaction as follows:



NADPH product is then used for the formation of glucose through reactions with the Calvin cycle.

3.2 The production of biomass and Lipid *S. dimorphus*

In the study, every treatment is done by harvesting as many as 50 ml sampling to quantify the dried biomass and weight the dried lipid. Uptake of lipid extraction method of Bligh-Dyer with solvent methanol, chloroform and aqua bides. Harvesting is done at the exponential phase, because in this phase of the microalgae cells grow quickly so that was expected to produce biomass. The biochemical composition of *S. dimorphus* is influenced by a variety of conditions such as nutrients, temperature, and light intensity. In the process of photosynthesis, lipids are formed from carbohydrate and protein. It is because in the third cycle of the substance met the Crab. Lipids are included in the groups of compounds rich in cluster C and H. Fats and oils is a compound that contains lipids.

The results showed that the production of biomass and lipid affected light intensity (Table 1). Based on the growth comparison, the highest biomass gain on 12,500 lux light intensity. The lowest biomass is at 10,000 lux light intensity. In accordance with the growth of the biomass, the highest number of cells grown on light intensity 12,500 lux, and the amount of the lowest cell in the 10,000 lux. The highest biomass and lipid production on the media culture with lighting 12,500 lux i.e. of 1.1407 g.L⁻¹ and 0.2520 g.L⁻¹ (22.28% dw). It is suspected that *S. dimorphus* can still conduct photosynthesis at 12,500 lux lighting properly, according to the highest cell densities on the growth curve. While the lowest biomass on media culture with 10,000 lux lighting of 0.6160 g.L⁻¹ and 0.1233 g.L⁻¹ (25.46% dw), according the lowest cell density and growth curve.

Table 1. The Production of Biomass and Lipid *S. dimorphus*

Sample	Production of Biomass (g.L ⁻¹)	Production of Lipids (g.L ⁻¹)	Productivity of Lipids (g.L ⁻¹ day ⁻¹)	The Percentage of Lipids (%)
7,500 lux	0.6593 ^a	0.1933 ^{a,b}	0.0276 ^{a,b}	29.17 ^a
10,000 lux	0.6160 ^a	0.1233 ^a	0.0176 ^a	25.46 ^a
12,500 lux	1.1407 ^b	0.2520 ^b	0.0360 ^b	22.28 ^a

Description: The numbers followed the same superscript letters in one column shows not unlike real.

The biosynthesis of lipids are formed from polysaccharides that overhauled through the process of Glycolysis into Acetyl Coenzyme-A, which is prazat for the biosynthesis of fatty acids and glycerol. Fatty acid biosynthesis takes place inside the stoma, whereas the fatty acid oxidation takes place in the mitochondria. Lipid microalgae is generally in the form of glycerol and fatty acids are most numerous on the chains of C₁₆ and at least there are in the chain of C₈, the fatty acids contained in the microalgae including intracellular molecular as there is in the chloroplasts [8]. Lipid *S. dimorphus* is present on the chain of C₁₈ [9].

Triacylglycerol or triglycerides is lipid reserves actively synthesized in the network. The compound is used for the biosynthesis of triglycerides is L-glycerol-3-phosphate and acyl Coenzyme-A compound of fatty acids. Generally L-glycerol-3-phosphate is formed from compounds between the processes of glycolysis, namely dihidroksiaseton phosphate is converted into L-glycerol-3-phosphate by the enzyme glycerol-3-phosphate dehydrogenase with NAD⁺ system attendant or NADH as a Coenzyme. The process of the acylation of the hydroxyl group from L-glycerol-3-phosphate produces lisofosfatidat acid and fosfatidat acid. The reaction is catalized by the gliserolfosfat acyltransferase enzyme. Fatty acid acyl group was transferred from the Coenzyme A-acyl fatty acids to the hydroxyl group of the L-glycerol-3-phosphate gradually. Fosfatidat acid is hydrolyzed by the fostidat fosfatae enzyme to produce diasilgliserol. The last reaction, diasilgliserol reacts with a third molecule of Coenzyme-A acyl fatty acids are then catalyzed by diasilgliserol acyltransferase enzymes to produce triacylglycerols [10].

4. Conclusion

An increasing the intensity of light can increase cells number and lipids production *S. dimorphus*. The most optimum growth of *S. dimorphus* is on media 12,500 lux with cell number 100.8 x 10⁶ sel.ml⁻¹ and produce biomass and lipids each 1.1407 g.L⁻¹ and 0.2520 g.L⁻¹ (22.28% dw).

References

- [1] Campbell M N 2008 *Guelph Eng. J.* **1** 2-7
- [2] Ahmad A L, Mat Yasin N H, Derek C J C, and Lim J K 2011. *Renewable Sustainable Energy Rev.* **15** 584-593
- [3] Stone D 2003 *Biodiversity of Indonesia* (Singapore: Tien Wah Press)
- [4] Khoo H H, Sharratt PN, Das P, Balasubramanian R K, Naraharisetti P K, and Shaik S 2011 *J. Bioresour. Technol.* **102** 5800-5807

- [5] Yogatama R 2015 *Thesis: The Effect of Variation in Media Concentration on Biomass Production and Total Lipid of Scenedesmus dimorphus (Turpin) Kützing as Biodiesel Material* (Surakarta: Courses Of Biology, Science Faculty, Sebelas Maret University)
- [6] Bligh E G and Dyer W J 1959 *Canadian Journal Biochemistry Physiology* **37** 911-917
- [7] Gour R S, Chawla A, Singh H, Chauhan R S, and Kant A 2016 *PloS ONE* **11**(5) e0155321. Doi:10.1371/journal.pone.0155321
- [8] Spolaore P, Joannis-Cassan C, Duran E, Isambert A 2006 *Journal of Bioscience and Bioengineering* **101** 87-96
- [9] Renaud S M V 1994 *Journal of Applies Phycology* **6** 337 – 345
- [10] Wirahadikusumah M 1985 *Biochemistry: Metabolism of Energy, Carbohydrates, and Lipids* (Bandung: ITB press)