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Full Length Article

Effect of Glucose Administration on Biomass, β-Carotene and Protein Content of *Dunaliella* sp. under Mixotrophic Cultivation

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Abstract

Dunaliella sp. is a prospective green microalga that can utilize both organic and inorganic carbon simultaneously. This study was aimed to determine the influence of various glucose concentrations on biomass concentration, β-carotene, and protein content of *Dunaliella* sp. under mixotrophic cultivation. Different glucose supplementation of 0.05 g/L, 0.10 g/L, 0.15 g/L, and 0.20 g/L were applied mixotrophically. The culture condition of *Dunaliella* sp. was also performed under photoautotrophic cultivation. The results exhibited that glucose administration significantly influenced the growth, biomass concentration, β-carotene, and protein production of *Dunaliella* sp. ($P < 0.05$). Supplementation of glucose in the mixotrophic culture remarkably improved cell growth, biomass production, β-carotene, and protein content of *Dunaliella* sp. compared to photoautotrophic culture. Increasing glucose concentration from 0.05 to 0.15 g/L increased biomass yield, β-carotene, and protein content of *Dunaliella* sp. The maximum specific growth rate and biomass concentration were produced at the glucose administration of 0.15 g/L with a value of 1.058 per day and 0.896 g/L, respectively. Moreover, supplementation of 0.15 g/L glucose resulted in the highest β-carotene and protein content. The results also noted that nitrate and phosphate consumption was highly related to biomass, β-carotene, and protein content of *Dunaliella* sp. In conclusion, the supplementation of glucose under mixotrophic conditions could improve the biomass, β-carotene, and protein content of *Dunaliella* sp. and could be practically used in mass-scale production.

Keywords: Green microalgae; Growth rate; Mixotrophic; Organic carbon; Photoautotrophic

Introduction

Microalgae have long been applied as a good feed source for animals (Pauw and Persoone 1988; Muller-Feuga *et al.* 2003), an alternative renewable energy source, mainly biodiesel (Hossain *et al.* 2008), and feedstock for industrial application (Singh and Gu 2010). *Dunaliella* sp. is a potential microalga for animal feed and industry because it contains high essential fatty acid, protein, and β-carotene content (Morowvat and Ghasemi 2016). Conventionally, microalgae are cultivated under a photoautotrophic culture where cells conduct the photosynthesis process to generate biomass (Perez-Garcia *et al.* 2011). However, this way of growth produces a small amount of biomass concentration and slow algal growth because a self-shading process that diminishes light penetration into culture media would finally raise the cost of biomass harvesting (Cheirsilp and Torpe 2012).

The heterotrophic or mixotrophic method is an alternative way to overcome the low yield in photoautotrophic cultivation (Perez-Garcia *et al.* 2011; Wang *et al.* 2012). In heterotrophic cultivation, microalgae can utilize organic substrate as the carbon source (Chen 1996; Devi *et al* 2012). However, heterotrophic culture causes photosynthetic activity suppression, therefore, a synergy of autotrophic and heterotrophic (mixotrophic) would be preferable (Marquez *et al.* 1993; Smith *et al.* 2015).

Mixotrophic culture is a feasible method to produce microalga biomass by assimilating both CO_2 and organic carbon concurrently and the process of anabolic and catabolic occurs simultaneously (Kaplan *et al.* 1986; Chojnacka and Marquez-Rocha 2004). Mixotrophic growth reduces the requirement for light and increases the cost efficiency of microalgal biomass production (Lee 2004). Moreover, mixotrophic culture can provide high cell concentration and accumulate photosynthetic pigments (El-Sheekh *et al.* 2012) as well as enhance the protein content of microalgae (Perez-Garcia and Bashan 2015). Dittamart *et al.* (2014) explained that *Scenedesmus* spp. biomass was 17 times higher in mixotrophic culture than in photoautotrophic culture. Besides, mixotrophic cultivation improves biomass productivity of *Chlorella vulgaris* (Abreu *et al.* 2012; Melo *et al.* 2018).

Glucose is the most favorable organic carbon source for optimizing microalgal production. It promotes significant respiration and growth rates when compared to other organic carbon substrates (Ogbonna and Tanaka 1996). Boyle and Morgan (2009) explained that glucose has more energy content approximately 2.8 kJ/mole than acetate that produces about 0.8 kJ/mole. Cheah *et al*. (2018) reported that glucose addition under mixotrophic cultivation improved the biomass production of *Chlorella sorokiniana*. The study about the influence of glucose on biomass and β-carotene content of *Dunaliella salina* has been reported by Morowvat and Ghasemi (2016). However, the response of microalgae on the environment and nutrients is species-specific. Therefore, there is a necessity to analyze the biomass, β-carotene, and protein content of *Dunaliella* sp. under mixotrophic culture with the addition of glucose and photoautotrophic culture. We also evaluate the influence of trophic cultures on nitrate and phosphate utilization and their relationship to growth, β-carotene, and protein content of *Dunaliella* sp.

Materials and Methods

Algae cultures and medium

Dunaliella sp. was obtained from the Institute of Brackishwater Aquaculture, Jepara, Indonesia. Walne medium which contains: 100.0 g $NaNO_3$; 20.0 g NaH_2PO_4; 33.6 H_3BO_3; 0.36 $MnCl_2$; 1.3 g $FeCl_2$; and 45.0 g EDTA per liter were used to culture the cells. 1 mL of medium was supplemented to 1,000 mL of sterilized seawater.

Experimental culture conditions

Initially, *Dunaliella* sp. was grown under a photoautotrophic condition in batch mode. A logarithmic phase of *Dunaliella* sp. was utilized as inoculum in both photoautotrophic and mixotrophic treatments. For photoautotrophic culture, aeration was applied to provide CO_2 within the culture. In terms of mixotrophic culture, four different glucose concentrations (0.05, 0.10, 0.15 and 0.20 g/L) were added to the culture. For all treatments, algae cultures were aerated by the air pump with an airflow rate of 1 L/min and were incubated at the temperature of 28°C under constant illumination with a light intensity of 3,000 lux. The inoculum was cultivated into 2.5 L bottles (working volume of 1.5 L) at a salinity of 15 ppt and a pH of 7.5. Initial cell concentration was adjusted at 1 x 10^5 cells/mL. All treatments were carried out in triplicate.

Growth analysis

Neubauer hemocytometer (BOECO, Hamburg, Germany) was applied to count cell concentration. A specific growth rate (μ) was calculated as stated by Fogg and Thake (1987):

$$\mu\ (/\text{day}) = \frac{\ln (x2) - \ln (x1)}{t2 - t1} \quad (1)$$

Biomass analysis

Microalga biomass was determined by using the gravimetric method. A Whatman GF/C Filter Paper was used to filter a 25 mL algal sample. First, distilled water was applied to clean the algal pellet. Then, the sample was dried at 105°C for 2 hours and finally, weighed after cooling in desiccators (Janssen *et al.* 1999).

β-Carotene analysis

β-carotene was analyzed and calculated by a suitable method according to Morowvat and Ghasemi (2016).

$$\beta\text{-carotene } (\mu g/mL) = 25.2 \times A_{450} \quad (2)$$

Protein analysis

Protein was performed according to Lowry's method (1951) and calibrated with standard bovine serum albumin (BSA).

Nitrate and phosphate analysis

Nitrate and phosphate concentrations in the culture were measured by using the spectrophotometric method at 410 and 690 nm, respectively (Boyd 1979). Nitrate and phosphate were analyzed in day 0 and day 4 of culture.

Statistical analysis

One way analysis of variance using S.P.S.S. 20.0 was applied to analyze data among the treatments. A level of significance was tested at 95%.

Results

To determine the influence of photoautotrophic and mixotrophic culture on biomass, β-carotene, and protein content by *Dunaliella* sp., various concentrations of glucose ranging from 0.05 to 0.20 g/L were added to the basal Walne medium, whereas the photoautotrophic system was conducted with an air pump for the source of inorganic carbon. Both photoautotrophic and mixotrophic cultivations were grown under batch cultivation for 4 days (Fig. 1). The results for specific growth rate, maximum cell concentration, biomass yield, β-carotene, and protein content are exhibited in Table 1. The highest specific growth rate (1.058 ± 0.002 per day), the maximum cell concentration (6.893 x 10^6 cells/mL), and biomass yield (0.896 ± 0.004 g/L) were observed when *Dunaliella* sp. was administered with a glucose concentration of 0.15 g/L. These results were significantly higher compared to 0.05, 0.10, and 0.2 g/L glucose ($P < 0.05$) (Table 1). Increasing glucose administration from 0.05 to 0.15 g/L increased the growth rate and biomass production of *Dunaliella* sp. However, when glucose concentration increased to 0.20 g/L, the growth rate and biomass production dropped about 3.49 and 11.61%, respectively.

Table 1: Specific growth rate, maximum cell concentration, biomass concentration, β carotene, and protein content of *Dunaliella* sp.

Treatment Glucose concentration (g/L)	Specific growth rate (per day)	Maximum cell concentration (x 10^6 cells/mL)	Biomass concentration (g/L)	β-carotene (μg/mL)	Protein (%)
0.00 (Photoautotrophic cultivation)	0.940 ± 0.002[a]	4.287 ± 0.037[a]	0.456 ± 0.004[a]	6.067 ± 0.022[a]	16.990±0.362[a]
0.05	0.980 ± 0.004[b]	5.041 ± 0.072[b]	0.529 ± 0.002[b]	9.765 ± 0.019[b]	21.701±0.276[b]
0.10	1.015 ± 0.005[c]	5.786 ± 0.109[c]	0.681 ± 0.006[c]	11.701 ± 0.134[c]	25.143±0.371[c]
0.15	1.058 ± 0.002[e]	6.893 ± 0.051[e]	0.896 ± 0.004[e]	17.485 ± 0.100[e]	31.762±0.060[e]
0.20	1.021 ± 0.002[d]	5.928 ± 0.046[d]	0.792 ± 0.004[d]	13.630 ± 0.038[d]	27.965±0.147[d]

Means followed by different superscripts are significantly differences ($P < 0.05$)

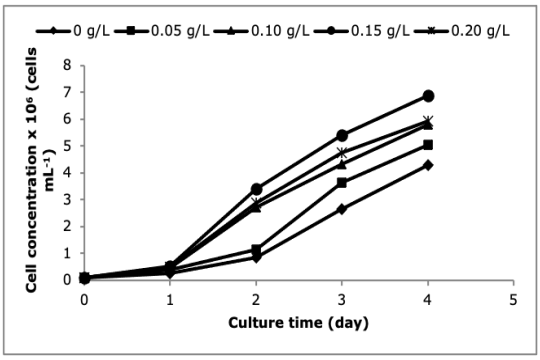

Fig. 1: Cell growth of *Dunaliella* spp. under mixotrophic and photoautotrophic cultivation

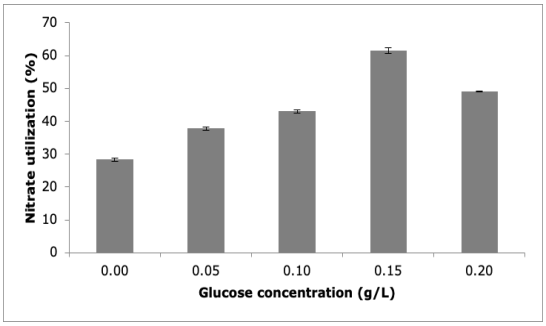

Fig. 2: Nitrate utilization by *Dunaliella* sp. under mixotrophic and photoautotrophic cultivation

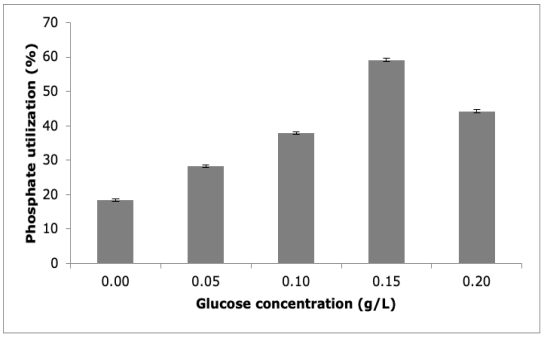

Fig. 3: Phosphate utilization by *Dunaliella* sp. under mixotrophic and photoautotrophic cultivation

There was a significant difference in β-carotene and protein production under different glucose supplementation ($P < 0.05$). Overall, β-carotene content increased from 6.067 ± 0.022 μg/mL (0.05 g/L) to 17.485 ± 0.100 μg/mL and protein content increased from 16.99 ± 0.36 to 31.762 ± 0.06% when microalgae were treated from 0.05 to 0.15 g/L (Table 1). However, when glucose supplementation increased to 0.20 g/L, the β-carotene dropped about 22% and protein content reduced approximately 11.95%.

The supplementation of even the small concentration of glucose (0.05 g/L) enhanced the maximum cell concentration and biomass yield by roughly 1.18 and 1.29 times, respectively, in comparison to the photoautotrophic cultivation. Moreover, the results produced in photoautotrophic cultivation showed a lower amount of β-carotene (6.067 ± 0.022 μg/mL), and protein content (16.99 ± 0.362%) than those observed in 0.05 g/L glucose under mixotrophic cultivation (Table 1).

In this study, sodium nitrate was applied as a source of nitrogen for microalgae growth. The nitrate assimilation by *Dunaliella* sp. under various glucose supplementations and photoautotrophic cultivation was shown in Fig. 2. The provided bar graph shows that increasing glucose concentration from 0.05 g/L to 0.15 g/L led to an increase of nitrate utilization by *Dunaliella* sp. from 37.82 to 61.50%. While the lowest nitrate utilization (28.40%) was observed in photoautotrophic cultivation. This result indicates that mixotrophic cultivation is favorable for nitrate utilization by *Dunaliella* sp.

The phosphate utilization by *Dunaliella* sp. under mixotrophic and photoautotrophic cultivation was exhibited in Fig. 3. The results revealed that the utilization of phosphate was higher in mixotrophic that in photoautotrophic cultivation. Increasing glucose concentration from 0.05 g/L to 0.15 g/L led to an increase of phosphate utilization by *Dunaliella* sp. from 28.22 to 59.26%.

Discussion

Photoautotrophic culture is a conventional method to grow microalgae by utilizing light as a source of energy and carbon dioxide as a source of inorganic carbon (Cheng *et al.* 2013). Nevertheless, some microalgae can improve their growth under a combination of photoautotrophic and heterotrophic conditions that allow the use of organic and inorganic carbon synergistically in the availability of light (Chojnacka and Marquez-Rocha 2004).

In this study, under mixotrophic cultivation, increasing

glucose concentration from 0.05 g/L to 0.15 g/L improved growth rate, biomass concentration, β-carotene, and protein content significantly, however, the growth was inhibited when exposed to the highest glucose supplementation (0.20 g/L). Similarly, Wang *et al.* (2012) observed that cell growth and biomass concentration of *Phaeodactylum tricornutum* enhanced when glucose concentration was enhanced from 0.5 to 1.0 g/L but restrained when the glucose concentration was raised to 2 g/L. Patel *et al.* (2009) reported the higher glucose supplementation from 2 g/L to 10 g/L resulted in higher biomass production from 3.38 ± 0.16 g/L to 4.32 ± 0.32 g/L in *P. tricornutum* without showed a negative influence in higher glucose concentration. Morowvat and Ghasemi (2016) reported that *D. salina* can utilize higher glucose concentration up to 15 g/L. These results prove that the ability of microalgae to utilize organic substrate is strain dependent.

The present study also found that when microalgae were cultured mixotrophically, cell growth, biomass concentration, β-carotene, and protein content were higher than microalgae cultured in photoautotrophic cultivation. This phenomenon is conforming to Liu *et al.* (2009) who noted that the cultivation of *P. tricornutum* under the mixotrophic condition on 100 m*M* glucose addition resulted in higher biomass (0.555 ± 0.01 g/L) than in photoautotrophic cultivation 0.460 ± 0.003 g/L. This result is also similar to the reports for *C. vulgaris* (Liang *et al.* 2009) and *Chlorella* spp. PCH10 (Wang *et al.* 2016), where mixotrophic cultivation had higher biomass concentration than autotrophic cultivation. Morowvat and Ghasemi (2016) demonstrated that β-carotene content under a mixotrophic system increased 1.95 times than in photoautotrophic. Besides, the protein production in *D. salina* improved when the cells grown in mixotrophic culture (Kadkhodaei *et al.* 2015). Smith *et al.* (2015) explained that increased cell growth and biomass production in mixotrophic culture because the metabolic component of respiration and photosynthesis could work simultaneously, allowing an endogenic carbondioxide and oxygen source, and finally diminishes growth limitation.

Comparing Table 1 with Fig. 2, it can be seen that nitrogen utilization was higher under mixotrophic culture than under photoautotrophic culture. Moreover, when the nitrate utilization of *Dunaliella* sp. is high, its corresponding biomass concentration, β-carotene, and protein content are high. Kim *et al.* (2013) noted that the nutrient utilization of *C. sorokiniana* in photoautotrophic culture is lower than in mixotrophic culture. Wang *et al.* (2016) explained that the higher nitrate was utilized by *C. vulgaris* PCH10, the higher biomass concentration was produced. Nitrate is a key nutrient to support growth and is mainly converted into microalga protein (Hein *et al.* 1995). Singh *et al.* (2019) explained that nitrate is an essential factor in improving the intracellular accumulation of carotenoids in green microalgae.

The effect of trophic conditions on phosphorus utilization is also reported in this study. The result showed that the higher *Dunaliella* sp. assimilate phosphate resulted in the higher growth, β-carotene, and protein content of algae cells. Moreover, *Dunaliella* sp. cultured mixotrophically with the addition of glucose utilized higher phosphate compared to *Dunaliella* sp. grown photoautotrophically. Wang *et al.* (2016) explained that phosphorus has a great effect on the growth of microalgae, and it plays an essential role in energy transfer, metabolic control, and activation of the protein. A deficiency of phosphorus can influence the reduction of growth, pigment, and protein content of microalgae.

Conclusion

Supplementation of glucose under mixotrophic culture significantly affects the growth, biomass, β-carotene, and protein content of *Dunaliella* sp. Mixotrophic cultivation of *Dunaliella* sp. led to higher biomass, β-carotene, and protein content than photoautotrophic cultivation. We proposed that cell growth, biomass concentration, and protein content were related to nitrate and phosphate utilization of microalgae.

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Author Contributions

Muhammad Fakhri, Prive Widya Antika, and Nasrullah Bai Arifin planned and conducted the experiments, Muhammad Fakhri and Arning Wilujeng Ekawati analyzed the results, Muhammad Fakhri wrote the manuscript, Ating Yuniarti and Anik Martinah Hariati edited and reviewed the manuscript and Prive Widya Antika and Nasrullah Bai Arifin statistically evaluated the data.

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