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STUDY OF GROWTH AND N, P CONTENT OF MICROALGAE *Chlorella vulgaris* CULTIVATED IN DIFFERENT CULTURE MEDIA AND LIGHT INTENSITY

Dedi Edwin Satriaji*, Muhammad Zainuri, Ita Widowati

Department of Marine Science, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, Semarang 50275, Indonesia

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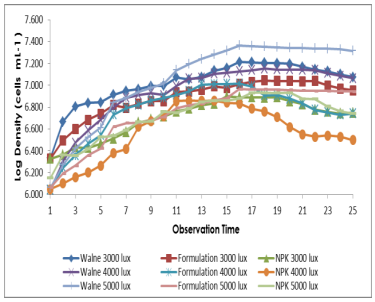
*Corresponding author
dediedwinsatriaji@gmail.com

Graphical abstract



Abstract

Different light intensity and different media types were used to test their effects on algal growth and biochemical composition of cell content to optimize the growth of algal biomass production. Analysis of the results includes growth calculation of *Chlorella vulgaris* obtained from 6 d observations for 12 h a day (06:00 am to 06:00 pm) with 3 h breaks in between observation. Laboratory analysis was done at the end of day 5th to determine the content of N, and P. Density of *C. vulgaris* were influenced by both light intensity and media. Differences media type and light intensity as separated parameters and their interactions significantly ($p < 0.05$) affected on the growth and nitrogen and phosphor content of *C. vulgaris*. The highest density of 2 310 × 10^4 cells · mL^{-1} was on day 4 with walne media resulting in specific growth rate (μ) of 0.43 per day with a light intensity of 5 000 lux, and the lowest density of 725 × 10^4 cells · mL^{-1} was in day 4 with the NPK media and specific growth rate (μ) 0.25 per day with a light intensity of 4 000 lux. The highest nitrogen and phosphorus content (16.12 mg and 28.19 mg) was obtained from walne medium with a light intensity of 5000 lux, and the lowest (3.43 mg and 2.17 mg) was obtained from NPK with a light intensity of 4 000 lux.

Keywords: *Chlorella vulgaris* Beyerinck [Beijerinck], density, light intensity, media, specific growth rate

1.0 INTRODUCTION

One of the challenges that Indonesian people face nowadays was the shortage of food sources as feed to support culture fisheries. Microalgae have a great potential for various applications including the production of compounds for food, feed and aquaculture [1]. *Chlorella vulgaris* Beyerinck [Beijerinck], is a natural resource that can be easily grown in a variety of water conditions. *Chlorella vulgaris* has a high potential value because it contains adequate nutrients in the form of proteins, amino acids, vitamins, and minerals. Considering the richness of the nutrients contained in *Chlorella vulgaris* and its highly extensively application on the food and cosmetic industries, it is necessary to realize the cultivation of *C. vulgaris* by manipulating the medium of life and light intensity.

The culture media has a direct influence on cellular growth, as well as on the biomass composition of microalgae [2]. In this context, microalgae, like other microorganisms, requires sources of carbon, nitrogen, phosphorus and other micronutrients [3], and the nutrients that make up the medium must be dissolved (available) for biochemical uses by the microalgae. Microalgal cell growth rates and lipid content were affected by environmental parameters such as temperature, light intensity and frequency, gas composition, and nutrient level in the culture system. Light quantity and quality determine the amount of energy available to photosynthetic organisms to conduct their metabolic activities. Photosynthesis process is divided into stages of light reaction and a

dark reaction, each of which produce oxygen and carbohydrates. The reaction will be greatly influenced by the intensity of the light, the wavelength of light and nutrients available in the media [4]. Light intensity is also a major factor that determines the formation of carotenoid pigment, if the amount of light available is sufficient to inhibit the synthesis of chlorophyll. Chlorophyll and carotenoids will be synthesized in a balanced manner in the chloroplast. At the moment, the balance is changing as a result of the increase in carotenoids, the plastids structure will change and as a result will degrade chlorophyll but not carotenoids [5]. Studies show that light is important factors which affects the growth of autotrophic microalgae that study also showed *Chlorella* could grow with the intensity range 3 000 lux to 11 000 lux [6] (1 lux = 1 lm m^2). The present study focuses on the growth and content of nitrogen (N), phosphor (P) microalgae *C. vulgaris*, to identify which treatment relevant and efficient of media and light intensity

2.0 EXPERIMENTAL

2.1 *Chlorella vulgaris* Culture

The microalgae *Chlorella vulgaris* was obtained from BBPAP (Brackish Water Aquaculture Research and Development Center) Jepara, Central Java. *Chlorella vulgaris* was cultivated with different culture media and light intensity. Experimental design used was a randomized block with nine culture bottles containing 5 L of sea water with three treatments and three replications. The treatments are Walne Pro-analysed, formulations, and NPK. Each treatment were treated with light densities of 3 000 lux, 4 000 lux, and 5 000 lux. Light intensity was measured with a Lux Meter.

2.2 Algal Growth Medium

The medium that was used in the cultivation of the microalgae was Walne, formulations, and NPK, compositions of walne medium is as follows Na_2EDTA 45 g L^{-1}, NaNO3 100 g L^{-1}, $NaH_2PO_4.2H_2O$ 20 g L^{-1}, $FeCl_3.6H_2O$ 1.3 g L^{-1}, $MnCl_2.4H_2O$ 0.36 g·L^{-1}, H_3BO_3 33.60 g L^{-1}, Trace Metal Solution consisted of 1 ml from 100 ml solution $CuSO_4.5H_2O$ 2.0 g L^{-1}, $ZnCL_2$ 2.1 g L^{-1}, $CoCl_2.6H_2O$ 2.0 g L^{-1}, $(NH_4)6.Mo_7O_{24}.4H_2O$ 0.9 g L^{-1}, Vitamins in 100 mL, B1 100 g · L^{-1}, B12 5 g L^{-1} [7].

2.3 Growth Evaluation

Cell concentration was determined by cell counting, 1 mL of algal suspension was collected via sampling tube. Cell density was directly counted with a Neubauer hemocytometer and an Olympus light microscope where it represents cell density mL^{-1}. Algal growth was evaluated daily in three replication. Specific growth rate was calculated using Eqs. (1), as follows [8]:

$$\mu = \frac{\ln[X2 - X1]}{t2 - t1} \quad (1)$$

Volumetric biomass productivity, was calculated using Eqs. (2), as follows [9]:

$$N = \frac{(\Sigma N1 + \Sigma N2)}{2} \times \frac{1}{1\ mm \times 0.2\ mm \times 0.1\ mm} \times \frac{1\ mm^3}{10^{-3} ml} \quad (2)$$

where X1 and X2 are the biomass concentration on days t1 and t2, respectively. and N1, N2 is the number of observations.

Growth calculation and specific growth rate of *C. vulgaris* was obtained from 5 d observations for 12 h a day (06:00 am to 06:00 pm) with 3 h breaks in between observation times of 3 h intervals with a total of five observations per day. Laboratory analysis was done at the end of d 6 to determine the content of N, P.

2.4 Statistical Analysis

Cell densities were analyzed using polynomial orthogonal for obtaining the growth model of *C. vulgaris*. Prior to statistical analysis, normality and homogeneity of data were checked by using Shapiro-Wilk and Kolmogorov-Smirnov test, respectively. All data were statistically performed at a maximum significance level of 5 % by two-way analysis of variance (ANOVA) followed by tukey's honest significant difference (HSD) post hoc multicomparison test.

3.0 RESULTS AND DISCUSSION

From the polynomial orthogonal result, all treatment showed the same model of growth, even from different peak density.

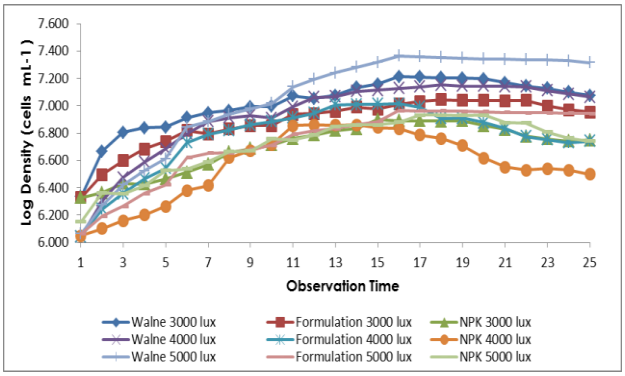


Figure 1 The growth pattern of *C. vulgaris* at three light intensity (3 000 lux, 4 000 lux, 5 000 lux) and media (Walne, Formulation, NPK). Each value is the mean of three samples

Figure 1 shows the highest density from initial density 115 × 10^4 cells · mL^{-1} to (867 to 2 310) × 10^4

cells · mL^{-1} with average 1 589 × 10^4 cells mL^{-1} with (μ) 0.43 per day was obtained in day 4 with the walne medium with a light intensity of 5 000 lux. The lowest density from initial density 112 × 10^4 cells · mL^{-1} to (416 to 725) × 10^4 cells mL^{-1} with average 570 × 10^4 cells mL^{-1} with (μ) 0.25 per day was obtained in day 4 with the medium NPK with a light intensity of 4 000 lux.

From the ANOVA result, light intensity and media seemed to have an effect on biomass production of *C. vulgaris*. The variation of light intensities and growth medium as separate parameters and their interactions significantly ($p < 0.05$) affected biomass of *C. vulgaris*. Figure 2 shows that light intensity and media affected biomass of *C. vulgaris*.

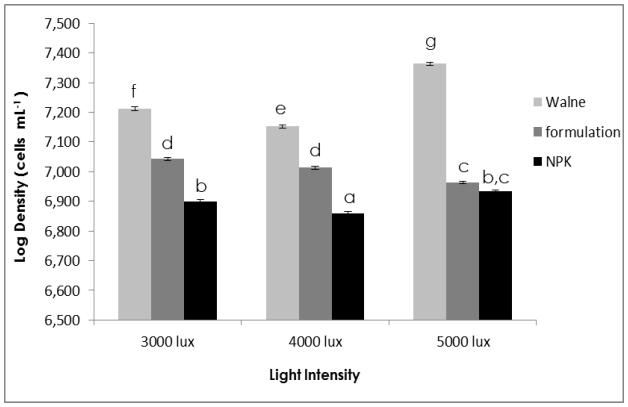


Figure 2 The biomass of *C. vulgaris* at three light intensity (3 000 lux, 4 000 lux, 5 000 lux) and media (Walne, Formulation, NPK). Each value is the mean of three samples

Figure 3 and Figure 4 shows the nitrogen and phosphorus content of *C. vulgaris*. Each light intensity and three different growth medium has maximum nitrogen and phosphorus content.

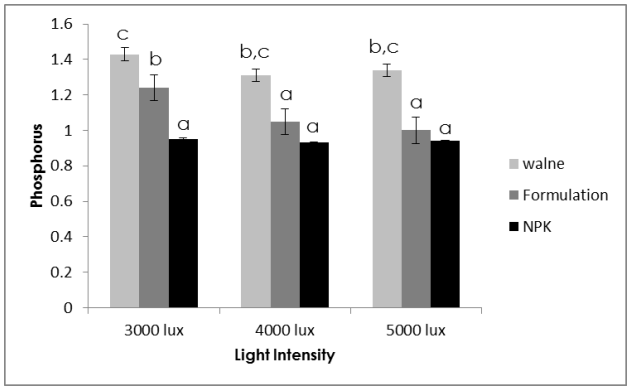


Figure 3 Phosphorus content of *C. vulgaris* at three light intensity (3 000 lux, 4 000 lux, 5 000 lux) and media (Walne, Formulation, NPK). Each value is the mean of three samples

The sample used for analysis of nitrogen and phosphorus as much as 500 mg of total dry weight of the sample was obtained after harvest at the end of culture.

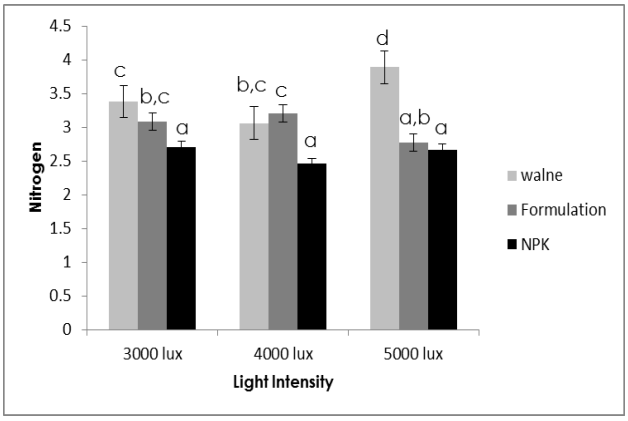


Figure 4 Nitrogen content of *C. vulgaris* at three light intensity (3 000 lux, 4 000 lux, 5 000 lux) and media (Walne, Formulation, NPK). Each value is the mean of three samples

This research also shows that nitrogen and phosphorus content of *C. vulgaris* cultivated in the 5 000 mL medium volume with the light intensity (3 000 lux, 4 000 lux, 5 000 lux) and three medium (Walne, Formulation and NPK) as separate parameters and their interactions significantly ($p < 0.05$) affected nitrogen and phosphorus content of *C. vulgaris*. Overall nitrogen and phosphorus content of all samples are shown in Table 2. The highest nitrogen and phosphorus content of 16.12 mg; 28.19 mg was in walne with a light intensity of 5 000 lux, and the lowest 3.43 mg and 2.17 mg was in NPK with a light intensity of 4 000 lux.

Table 1 shows the specific growth rates and maximum cell density, each light intensity and three different growth medium had maximum specific growth rate and density. The highest specific growth rates of 0.43 per day with optimal density 2 310 × 10^4 cells · mL^{-1} was obtained in day 4 walne medium with a light intensity of 5 000 lux, and the lowest specific growth rates of 0.25 per day with optimal density 725 × 10^4 cells mL^{-1} was obtained in day 4 on the NPK medium with a light intensity of 4 000 lux.

Light and medium are major factors that determine the growth and biochemical of *Chlorella vulgaris*. The result on three different culture media and light intensity shows the same graph model, even though that the optimal density was different. It was more likely due to the period of adaptation which was limited in the nutrient quantity. The optimal peak of microalga *C. vulgaris* cultivation could be varied due to the optimal quantity of nutrient, which was provided by the fertilizer and medium [10, 11]. During the mixing process of the fertilizer in the medium, the quantity of nutrient in the earlier process was less than the next period, which could be easier absorbed by the microalgae. Nutrient availability, on the other hand, has a significant impact on the growth and propagation of microalgae as well as on

their lipid and fatty acid composition. In general, when algal growth decreased and there was no requirement for the synthesis of any additional membrane compounds, the cells divert and deposit fatty acids [12–15]. Under nitrogen depletion condition, the growth rate was very low leading to higher lipid productivity [16].

Photosynthetic microalgae photosynthesize assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in this regard intensity. The growth characteristics depend on the light intensities [17]. The compounds that are produced in the light dependent phase (ATP, NADPH) are used in dark cycle to synthesize metabolic molecules essential for growth. Also, it has reported that, some enzymes of the pentose cycle of photosynthesis and CO_2 fixation are inactive during the illumination [18, 19].

Table 1 Maximum cell density and specific growth rate of *Chlorella vulgaris*

	Light Intensity (Lux)	Walne Pro-analysed		Formulations		NPK	
		Maximum cell density	μ	Maximum cell density	μ	Maximum cell density	μ
1	3 000	1729×10^4	0.30	1206×10^4	0.40	894×10^4	0.31
2	4 000	1419×10^4	0.37	1031×10^4	0.32	725×10^4	0.25
3	5 000	2310×10^4	0.43	919×10^4	0.30	856×10^4	0.26

Table 2 Nitrogen and phosphorus content of *Chlorella vulgaris*

	Light Intensity (Lux)	Walne Pro-analysed		Formulations		NPK	
		Phosphorus (mg)	Nitrogen (mg)	Phosphorus (mg)	Nitrogen (mg)	Phosphorus (mg)	Nitrogen (mg)
1	3 000	27.23	13.83	25.04	10.40	2.25	4.05
2	4 000	26.62	12.05	20.97	8.82	2.17	3.43
3	5 000	28.19	16.12	19.01	6.98	2.20	3.94

Media composition had significant effect on both the growth rate and the density of microalgae *C. vulgaris* Microalgae were known to grow more abundantly in nutrient rich (eutrophic) waters leading frequently to algal blooms [20]. A higher light absorption rate would allow for the chloroplast to manufacture increased amounts of usable chemical energy which would result in an increase in algal growth (biomass).

Environmental parameters in this study include temperature, salinity, dissolved oxygen and pH were also observed. Overall, environmental parameters values are as follow: temperature was 27 °C to 29 °C, salinity was 30 ng L^{-1} to 35 ng L^{-1}, dissolved oxygen was 5.4 mg L^{-1} to 6.4 mg·L^{-1}, and between pH 7 to pH 8. The observation of water quality during cultivation showed the optimal range for each variable water quality.

The pattern of biomass growth in this study showed that under low light intensity of 3 000 lux and 4 000 lux, *C. vulgaris* exhibited a slow growth and low biomass, but increased in light intensity of 5 000 lux in walne medium which resulted in highest biomass growth in this study.

4.0 CONCLUSION

This study has identified the effect of media type and light intensity as separate parameters and their interactions significantly ($p < 0.05$) on the growth of *Chlorella vulgaris* and nitrogen and phosphorus content. The highest density of $2\ 310 \times 10^4$ cells mL^{-1} was in day 4 with the walne medium and specific growth rate (μ) 0.43 per day with a light intensity of 5 000 lux, and the lowest density of 725×10^4 cells mL^{-1} was in day 4 with the NPK medium and specific growth rate (μ) 0.25 per day with a light intensity of 4 000 lux. The highest nitrogen and phosphorus content (16.12 mg and 28.19 mg) was in walne with a light intensity of 5 000 lux, and the lowest (3.43 mg and 2.17 mg) was in NPK with a light intensity of 4 000 lux.

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