The spectrum of light and nutrients required to increase the production of phycocyanin Spirulina platensis

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The spectrum of light and nutrients required to increase the production of phycocyanin *Spirulina platensis*

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Abstract. *Spirulina platensis* is one of the microalgae that have several active ingredients that can be used in fisheries, pharmaceuticals, food additives and cosmetics, and one of them contains a high level of phycocyanin. Different light qualities can be used to increase the production of cyanobacterium phycocyanin. Some of the light spectrum can affect pigment synthesis, and besides that, nutrients are also very important to maintain the quantity, quality and production of *Spirulina platensis* in the biosynthesis of phycbiliprotein (phycocyanin and phycoerythrin). This study aims to determine whether there was an influence from a combination of the color spectrum of light and nutrient composition on the phycocyanin production of *Spirulina platensis*. The research method was experimental. The study used a combination of two light spectra (red and blue) as well as two different nutrients (Walneand technical nutritional modification and MT). Each treatment combination was repeated four times. The results showed that the light spectrum and nutrient composition had an effect on *Spirulina platensis* production. The combination of the nutrient MT and red spectrum resulted in the highest production of phycocyanin and a dry weight of *Spirulina* of 5.1078 mgg^{-1} and 0.6677/0.5 g L^{-1} respectively. This was significantly different (P<0.05) compared to the other treatment combinations.

1. Introduction

Spirulina is a microalgae with excellent nutritional characteristics. Beside its high protein content, there are several compounds and natural pigments therein. Among them are the phycobiliproteins derived from *Spirulina*, phycoerythrin (PE) and allophycocyanin (AP) which can be found in small amounts. The most abundant is phycocyanin (PC), which is a bright blue pigment [1].

The productivity of the *Spirulina platensis* cells is influenced by the availability of both macro and micronutrients (C, N, P, K, S, Mg, Na, Cl, and Ca, Fe, Zn, Cu, Ni, Co, and Se) [2]. Most of the pigments absorb certain wavelengths of light [3]. Meanwhile, some of the light prompts different cyanobacterium phycocyanin production. According to [4]'s research results, Blue Light (45%) is the most suitable condition, with Red Light (29%) and Green Light (25%) showing second and third place for pigment synthesis respectively. The culturing conditions can influence decisively the growth phases of *Spirulina*, causing changes in its composition and increasing or decreasing the proportion of phycobiliproteins, including phycocyanin [1].

The problem that arises is if the use of different light spectrums and different nutrients affects the production of phycocyanin? Which combination of light spectrum and nutrients provides the optimum phycocyanin production?

Therefore, it is necessary to do research into the use of different light spectrums and nutrients for the production of *Spirulina platensis* phycocyanin. The purpose of this study was to determine the effect of different light spectra on phycocyanin production, to determine the different nutrient compositions involved in phycocyanin production and to find the best combination of light spectrum and nutrients associated with the optimum production of phycocyanin *Spirulina platensis*.

2. Research Method

2.1 Place and time

The research study was implemented on March 1st and lasted until March 31st2015 in the Education Laboratory of the Faculty of Fisheries and Marine, Universitas Airlangga in Surabaya, Indonesia.

2.2 Materials and research

The tools needed in for the pure culture of *Spirulina* are 2 TL 40 watt lamps (red and blue) as the sources of light and energy, aerators, aeration hoses, 16 1 liter-size culture bottles, 16 measuring glass, black plastic as a light barrier, pipette drops, volume pipettes, filter paper, funnels, a Sedgewick rafter, lux meters, thermometers, pH meters, refractometers, nitrate and phosphate test kits and microscopes.

The materials used were a pure stock of microalgae *Spirulina platensis*, a fertilizer with medium technical modifications (MT), a control sample of Walne, fresh water, sea water, alcohol 90%, Natiosulfate 20 ppm and 60 ppm of chlorine.

2.3 Research design

This research study used an experimental method. The research design used was a Complete Random Factorial Pattern Design with 2 factors (factor A was the color of the lights with levels a1 and a2 and factor B was the nutrients with levels b1 and b2), so we obtained 2 x 2 treatment combinations: a1b1, a1b2, a2b1, and a2b2. Each treatment was repeated 4 times; 4 x 4 = 16 experiments [5] : Combination fertilizer Walne with a red light (a1b1), combination fertilizer Walne with a blue light(a1b2), combination fertilizer MT with a red light (a2b1) and combination fertilizer MT with a blue light (a2b2).

2.4 Work procedures

2.4.1Tools and materials preparation

The culture equipment to be used was washed thoroughly, rinsed with fresh water and then dried. For the equipment made of heat-resistant glass, it was covered with cotton and gauze and then wrapped in aluminum foil. After that, it was sterilized using an autoclave at 121 ° C for 15 minutes. The heat-resistant equipment was sterilized with a 150 ppm chlorine solution for 24 hours. We then rinsed it with fresh water until clean and until the smell of chlorine was gone.

2.4.2 Culture media preparation

The seawater that will be used for the culture was sterilized using a chlorine solution. The sea water was filtered first using cotton that was placed in a water funnel, before it was sterilized with chlorine 60 ppm for 24 hours and then aerated. The residual chlorine was removed with 20 ppm Na Thiosulfate.

2.4.3 Fertilizer preparation

The fertilizers used as a culture media were the technical medium modification (MT) and Walne medium (control). The liquid fertilizer solutions for nutrients MT and Walne were stored in opaque containers. The liquid fertilizer solutions for the MT and Walne nutrients were then sterilized.

2.4.4 Culture of Spirulina platensis

The microalgae pure stock of *Spirulina platensis* was obtained from the Center for Brackish Water Aquaculture Development (BBPBAP) in Jepara. The growth performance of *Spirulina platensis* was assessed in limited light conditions using a 1-liter bottle incubated at a temperature of 30 ° C illuminated from one side with a fluorescent color lamp in a bright cycle and dark cycle (16 hours: 8 hours). The *Spirulina platensis* culture was done using seawater with a salinity of 15 ppt [6]. The *Spirulina platensis* inoculan which was put into the media had a 10^4 units ml^{-1} density.

2.5 Calculation of phycocyanin

The *Spirulina platensis* cell biomass that was filtered was rinsed with distilled water and dried. The drying of the *Spirulina platensis* was carried out at 40 ° C for 48 hours [6,7]. The dried *Spirulina platensis* cells were then broken down using the sonication method. Phycocyanin extraction was carried out using a 0.1 M Na phosphate buffer (pH 7) with a concentration of 0.04 g of dry *Spirulina platensis* mL^{-1} solvent. It was then shaken at a speed of 100 rpm at 25 ° C. The samples were collected after 24 hours. It was then centrifused, the supernatant was harvested and its optical density was measured using a spectrophotometer at 615 and 652 nm wavelengths [7].

The calculation of the *Spirulina* extraction results based on the concentration, level of purity and the production of phycocyani was done, using several formulas. The phycocyanin concentration (PC, mg mL^{-1}) was calculated using the following formula:

$$PC = \frac{(OD615 - 0.474(OD652)}{5.34} [6], [7]$$

PC : Phycocyanin concentration (mg mL^{-1})
OD615 : Reading the optical density of the sample at a wavelength of 615 nm
OD652 : Reading the optical density of the sample at a wavelength of 652 nm

The calculation of the production of the phycocyanine extraction (mg g^{-1}) was done using the following formula:

$$\text{Phycocyanin} = \frac{PC \times V}{DB} [6]$$

PC :Phycocyanin concentration (mg mL^{-1}),
V : volume (mL) solvent,
DB : dry weight biomass (g).

2.6 Population of Spirulina platensis

During the culturing, the counting of the biomass of *Spirulina* population was carried out by taking samples every 24 hours and counting using the Sedgewick rafter counting cell method. The calculation was done using the following equation:

$$\text{Units/ml} = \frac{C \times 1000\ mm^3}{A \times D \times F}$$

Description:
C = number of organisms calculated
A = Wide, airy viewpoint (3.14 x $(\frac{d}{2})^2$), mm^2
D = depth of fields of view, mm
F = number of fields of view are uncountable.

2.7 Measuring the parameters of the study

The parameters measured in this study included the phycocyanin content of *Spirulina platensis,* the population density, the dry weight of *Spirulina platensis* and water quality.

2.8 Data analysis

The data was analyzed using an ANOVA factorial pattern with an error rate of 5%. If the treatment had a significant effect, then in order to see the treatment with the best results, it was continued with a double comparison of the Honestly Significant Difference [5].

3. Results and discussion

3.1 Results

3.1.1 Phycocyanin Spirulina platensis

The measured phycocyanin content of this study ranged from 3.5838 to 5.1078 mg g^{-1}. The data obtained during the study was then analyzed using analysis of variance (ANOVA). In order to find out the differences between all of the treatments, it was necessary to do an honest difference test with a confidence level of 0.05. The results of the test showed significantly different results ($p < 0.05$) and the highest phycocyanin was obtained in treatment a2b1 (nutrient MT combined with a red light) of 5.1078 mg g^{-1} (Table 1).

Table 1. Phycocyanin content of *Spirulina platensis* (mg g^{-1}) with a combination of nutrients and color spectrums in different lights.

Treatment	Average Production of Phycocyanin
a1b1	4.7590^{b}
a1b2	3.0182^{d}
a2b1	5.1078^{a}
a2b2	3.5838^{c}

^{a,b,c,d} The superscript is different in one column to show the real difference ($p < 0.05$), a1b1: Fertilizer Walne with red light spectrum., a1b2: Fertilizer Walne with spectrum blue light., a2b1:Fertilizer MT with red light spectrum., a2b2: Fertilizer MT with spectrum blue light.

3.1.2 Population density of Spirulina platensis

The results of the variance analysis showed that factor a (nutrient type) and factor b (spectrum of light color) were significantly different to the population of *Spirulina platensis*. To find out the difference between the treatments, an Honestly Significant Difference Test (BNJ) was carried out. The data on the population density of *Spirulina platensis* can be seen in Table 2. in treatment a2b1 and a2b2, there was a decrease in the *Spirulina* population on day 8, while treatments a1b1 and a1b2 decreased on day 9.

Table 2. Cell density of *Spirulina platensis* with a combination of nutrients and different light spectrums.

Treatment	Density of *Spirulina platensis* (10^4 unitml^{-1}), Days									
	0	1	2	3	4	5	6	7	8	9
a1b1	1	2.31^{a}	4.23^{a}	4.44^{c}	5.48^{c}	9.37^{b}	10.76^{b}	12.26^{c}	13.38^{a}	10.10
a1b2	1	2.48^{a}	3.08^{c}	4.72^{c}	5.95^{c}	8.21^{c}	9.42^{c}	11.71^{c}	12.57^{b}	9.10
a2b1	1	2.58^{a}	3.27^{c}	5.27^{b}	7.04^{b}	10.18^{a}	12.57^{a}	15.10^{a}	13.41^{a}	-
a2b2	1	2.42^{a}	3.70^{b}	5.94^{a}	7.84^{a}	10.31^{a}	12.08^{a}	13.94^{b}	11.36^{c}	-

^{a,b,c} The superscript differs in one column to show the significant difference ($p < 0.05$).

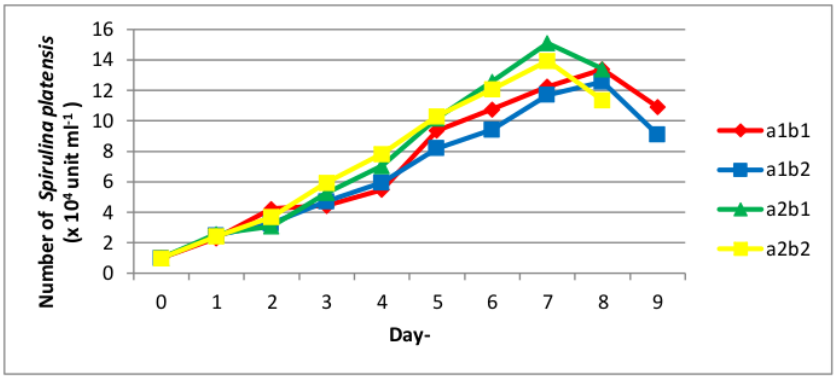

Figure 1. Graph of the average population growth of *Spirulina platensis* (10^4 units/ml). Description: a1b1: Fertilizer Walne with red light.,a1b2: Fertilizer Walne with blue light., a2b1: Fertilizer MT with red light., a2b2: Fertilizer MT with blue light.

3.1.3 Dry weight of Spirulina platensis

The dry weight of *Spirulina platensis* obtained from this study ranged from 0.4150 to 0.6677/0.5 gL^{-1}. The data obtained during the study was then analyzed using analysis of variance (ANOVA). In order to find out the differences between all of the treatments, it was necessary to do an honest difference test with a confidence level of 0.05. The full statistical test results can be seen in Table 3. The results of the statistical analysis showed significantly different results and the highest dry weight of *Spirulina platensis* was achieved in a2b1 treatment (nutrient MT and red light spectrum) with 0.6677/0.5 gL^{-1}. This was significantly different from the other treatments ($p < 0.05$).

Table 3. The dry weight of *Spirulina platensis* (in 0.5 L^{-1}) with a combination of nutrients and color spectrums with different lights

Treatment	Dry Weight (in 0.5L^{-1}) g
a1b1	0.4787^{b}
a1b2	0.4150^{c}
a2b1	0.6677^{a}
a2b2	0.5135^{b}

Description: the superscript is different in one column to show the real difference ($p < 0.05$), a1b1: Fertilizer Walne with red light., a1b2: Fertilizer Walne with blue light., a2b1: Fertilizer MT with red light., a2b2: Fertilizer MT with blue light.

3.1.4 Water quality

The water quality and light intensity during the study were measured as supporting data. For seven days, the culture of the water quality and light intensity was measured every 24 hours. The range of water quality and light intensity can be seen in Table 4.

Table 4. Range of water quality and light intensity during the period of the *Spirulina* culture

Parameter	Range
pH	7-9

Temperature (°C)	30-34
Salinity (ppt)	30-32
Nitrate (mg/l)	12.5– 100
Phosphat (mg/l)	0.25– 2
Intensity (Lux)	4232-4257

3.2 Discussion

The results showed that the use of the red spectrum against the production of *Spirulina platensis* phycocyanin was different from the use of the blue spectrum. The highest phycocyanin content was found in the use of the red spectrum compared to the use of the blue spectrum. These things indicate that more disorders can increase the production of phycocyanin. Radiation rays between 380 and 750 nm are enough energy to help the chemistry to absorb th molecules, such as those occurring along the photosynthetic pathway in microalgae [3,8]. Therefore, visible light is the main source of autotrophic microalgae energy to produce organic compounds in photosynthesis. The red light spectrum at a wavelength of 700 nm was supported by the statement by [1] that high absorption occurs in visible light and that phycocyanin pigments absorb the main yellow and orange light which results in high absorbance across the spectrum. Phycocyanin production, which is higher when the red filtered light is used, adapts to the narrow range of light provided by the filter.

The maximum red spectrum absorbed will produce energy which is also optimal in that it can synthesize ATP. The ATP results from photosystem I will be assisted by otons from a wavelength of 700 nm (red spectrum) to produce NADPH[9]. The formed NADPH plays a role in the synthesis process of phycocyanin; it is thought that this causes an increase in the content of phycocyanin *Spirulina platensis*.

The culture growth rates in the MT media were higher than in the Walne media on the same day. This is thought to be the source of the nitrogen used - (NH_4) 2CO (urea) and (NH_4) $2SO_4$ (ZA) quickly ssolves in water so then it is quickly utilized by the microalgae for metabolic processes [10]. Nitrogen is needed for the synthesis of amino acids, which form proteins and other cellular components such as phycocyanin. This does not have a significant effect on biomass [11].

Culture growth rates in the MT media were higher than in the Walne media on the same day. This was thought to be due to the source of phosphorus in the MT media, which was more quickly utilized by the microalgae for metabolic processes. The source of the MT phosphate media was Na_2HPO_4, and lne's medium was $NaH_2PO_4.2H_2O$. Phosphorus deficiency causes the algae cells to decrease the protein content followed by the degradation of various cell components associated with protein synthesis including phycocyanin.

4 Conclu on

From the research that has been carried out, it can be concluded that the use of the light color spectrum influences the production of phycocyanin *Spirulina*. The nutrient composition has an effect on the production of phycocyanin. There was an interaction between the nutrients and the different color spectra on the production of phycocyanin *Spirulina*. The combination of nutrient MT and the red spectrum produced the highest amount of phycocyanin by 5.1078 mg g^{-1}.

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