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Response of Sodium Pyruvate (CH_3COCO_2Na) on Phycocyanin Production of *Spirulina platensis*

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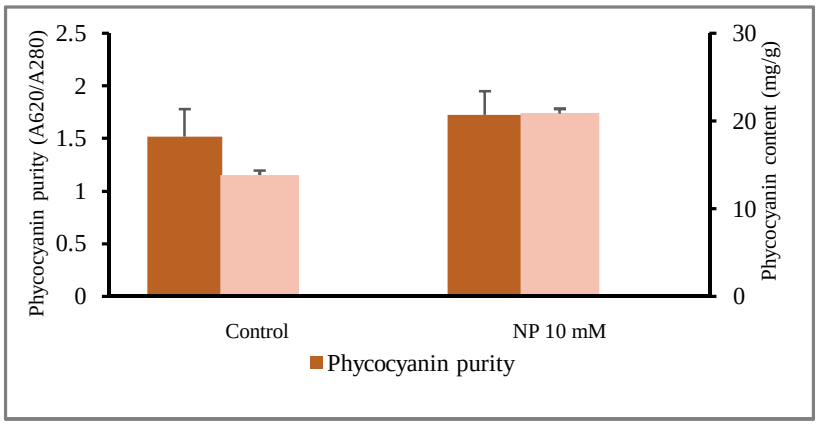
ABSTRACT

Spirulina platensis is one of the microalgae that are often cultivated. The largest essential biopigment compound in Spirulina platensis is phycocyanin. Phycocyanin can function as an antioxidant that can prevent free radicals, cancer cell growth, increase immunity and stamina. Therefore, phycocyanin is a potential therapeutic agent to treat various diseases and maintain a healthy body. This research was conducted to see the effect ofsodium pyruvate (CH_3COCO_2Na) concentration on growth rate and phycocyanin content of Spirulina platensis. The methods used in this study included microalgae growth as measured by cell density method with a wavelength of 680 nm, dry biomass weight using linearity regression formula, phycocyanin concentration and phycocyanin purity by measuring wavelengths of 620 nm and 280 nm. The results showed that the addition of sodium pyruvate concentration had a negative effect on the growth rate of microalgae, but it could increase the phycocyanin concentration and phycocyanin purity.

Graphical Abstract



Keywords: *Spirulina platensis*, Metabolic stress, Sodium Pyruvate, Growth rate, Phycocyanin concentration, Phycocyanin purity.

INTRODUCTION

Spirulina platensis is a type of microalgae that contains high enough protein and many nutrients that are needed by the human body. *Spirulina platensis*contains high carotenoid biopigment essential compounds (beta-carotene, lutein and phycocyanin) which can work as human health nutrients [1]. The largest essential biopigment compound in *Spirulina platensis* is phycocyanin. Phycocyanin can work as an antioxidant that can counteract free radicals, cancer cell growth, increase immunity and stamina [2]. Phycocyanin has been reported to exhibit strong cancer chemopreventive activity and exert anti-cancer effects on various types of cancer cells such as liver cancer and lung cancer [3].

Microalgae can accumulate desired biochemical products such as phycocyanin by changing certain environmental factors, one of which is the provision of nutrients in moderate growth. Pyruvate, glutamate, and succinyl-Coenzyme A are precursors in the pathway of tetrapyrole biosynthetic metabolites such as porphyrin, pycobilin, and chlorophyll that can act as nutrients [4, 5]. The following is a summary of the phycocyanin pathway figure 1.

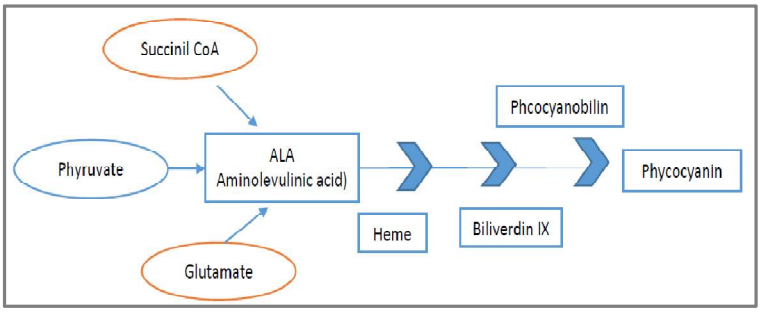


Figure 1. Phycocyanin Biosynthesis Pathway.

Pyruvate is one of the precursors in the tetrapyrol pathway which directly suppresses the production of phycocyanin [6]. Pyruvate compounds have a structure close to carboxylic acids which can change the tetrapyrol biosynthetic pathway so that it can increase phycocyanin production [7]. Giving metabolic stress to pyruvate is a new thing and has not been studied, so this research needs to be done to determine whether this substance is the optimum precursor in phycocyanin biosynthesis in *Spirulina platensis*

MATERIALS AND METHODS

Microalgae Strains and Cultivation Medium: The microalgae *Spirulina platensis* used in this study was obtained from Biochemistry Laboratory in Andalas University. The medium used in this study was Zarouk's medium. The medium is then stirred until reaching homogeneous solution. Microalgae culture in zarrouk medium was then grown for 4 days with initial cell density reaching ± 0.5 at OD_{680}.

Microalgae Cultivation Conditions and Growth Optimization with Sodium Pyruvate: Lighting on cultivation was carried out with a cycle of 24 h/day with a temperature of 27°C. Then the addition of sodium pyruvate nutrition was carried out at the beginning of cultivation. The variation of sodium pyruvate concentration in the culture medium was 0 (control), 1 mM, 5 mM and 10 mM. Each treatment was measured phycocyanin content and microalgae growth curve or dry biomass weight based on optical density (OD_{680}) and then the optimum conditions were obtained for sodium pyruvate treatment. The experiment was carried out 3 times.

Determining the Growth Rate of Phycocyanin with Dry Weight of Microalgae Biomass: Microalgae dry biomass was determined by weight based on a standard calibration curve. The biomass data obtained was converted into g L^{-1} units. A standard curve is formed and the optical

density value for optimal growth is substituted into the obtained regression equation, where *x* is the weight of dry biomass and *y* is the optical density. Therefore, the dry biomass weight of each isolate can be determined [**8**]. Then the dry weight of the microalgae was calculated using the following regression equation.

$$Y = 0{,}5587x + 0{,}115 \qquad \ldots(1)$$

Determining the Phycocyanin Concentration: For the extraction of phycocyanin, 60 mg of dried biomass for 4 days was suspended in 10 mL of 0.1 M phosphate buffer (pH = 7) and the solution was stored in a refrigerator for 20 h. Then it was extracted using the phosphate buffer [**9**]. Meanwhile, to remove contamination, the solution was centrifuged at 3,000 rpm for 15 min and the supernatant (blue) was collected. The absorption of the pure extract was determined by UV/Vis at wavelengths of 620 and 652 nm [**10**], then the phycocyanin concentration of the extract was calculated using the following formula [**11**].

$$\text{Phycocyanin concentration} = \frac{(OD_{620} - 0.474 \times OD_{652})}{5.34} \quad \ldots(2)$$

Meanwhile, to calculate the ratio of purity of phycocyanin is determined by using the following formula [**12**].

$$\text{Phycocyanin purity ratio} = \frac{A_{620}}{A_{280}} \qquad \ldots(3)$$

Total phycocyanin content per cell weight using equation (4) adopted from [**13**]

$$\text{Phycocyanin content} = \frac{PC_C\, V_e}{M_E} \qquad \ldots(4)$$

Total phycocyanin content per cell weight using equation (4) adopted from [**13**]

RESULTS AND DISCUSSION

ptimization of Sodium Pyruvate concentration on Phycocyanin Production in *Spirulina platensis:* The growth curve of *Spirulina platensis*with different concentration of sodium pyruvate can be seen in (figure 2). The growth graph shows that the growth curve of *Spirulina platensis* with zarrouk medium (control) is still at the highest position by producing dry biomass of 1.783 g L^{-1} at the beginning of the stationary phase. For variations in concentrations of 1 mM, 5 mM and 10 mM, the absorbance values were 1.521 g L^{-1}, 1.198 g L^{-1} and 1.13 g L^{-1}, respectively.

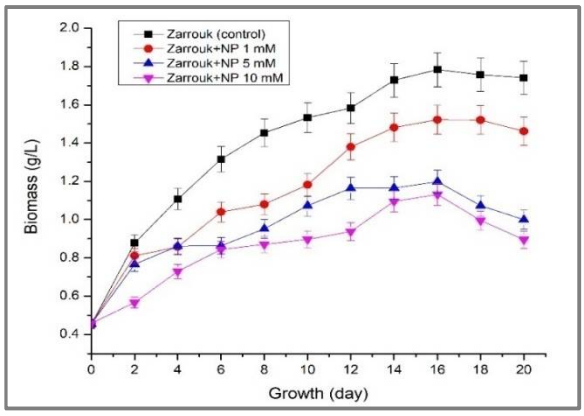


Figure 2. Biomass Growth of*Spirulina platensis* by giving different sodium pyruvate (NP) concentrations

As for the death phase, namely on days 18 and 20, the four variations of sodium pyruvate concentration experienced a significant decrease, namely in the control at 1.741 g L^{-1} and the lowest biomass on that day was obtained at a concentration of 10 mM, namely 0.895 g L^{-1}. A decrease was seen in dry cell biomass in *Spirulina platensis* with every addition of sodium pyruvate, it can be concluded that the more sodium pyruvate added to the growth medium, the smaller the biomass value for *Spirulina platensis* growth.

The graph shown in (figure 2) illustrates that there has been a negative effect of adding sodium pyruvate to dry biomass on the growth of *Spirulina platensis* . This situation can be caused by a stress condition of sodium pyruvate (CH_3COCO_2Na). This trend is in accordance with the findings of previous studies on the impact of increasing salt concentration on biomass production in *Spirulina platensis* [**14**]. After knowing the growth rate using variations of sodium pyruvate, then determining the concentration of phycocyanin which can be seen in the image below.

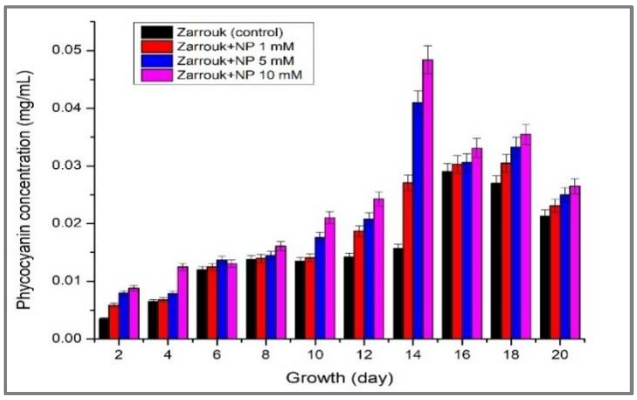


Figure 3. Phycocyanin Production in *Spirulina platensis* with variations of Sodium Pyruvate (NP) concentrations.

The graph above shows an increasing trend during the cultivation process and the peak of phycocyanin production on day 14 was 0.027 mg mL^{-1} for the control and 0.099 mg mL^{-1} for the highest peak at a concentration of 10 mM sodium pyruvate. Then at the concentration variations of 1 mM and 5 mM obtained respectively 0.059 mg mL^{-1} and 0.062 mg mL^{-1}. However, it decreased on day 20 with concentrations of phycocyanin in the final stationary phase of 0.0213 mg mL^{-1} for the control, 0.036 mg mL^{-1} for variations in concentration of 1 mM. Followed by variations in concentrations of 7.5 mM and 10 mM with concentrations of 0.057 mg mL^{-1} and 0.079 mM. After optimization for the best concentration of sodium pyruvate, it was found that the concentration of 10 mM was the best concentration for the growth of phycocyanin productivity in the microalgae *Spirulina platensis* .

Pyruvate is an important substrate in metabolic pathways. Pyruvate can be converted into carbohydrates through gluconeogenesis, into fatty acids or energy through acetyl-CoA, into the amino acid alanine, and into ethanol. Therefore, pyruvate compounds unite several major metabolic processes. Pyruvate is a precursor in the metabolite pathway of tetrapyrole biosynthesis such as porphyrin, pycobilin, and chlorophyll that can act as a nutrient so as to encourage the production of phycocyanin in the tetrapyrrole pathway (Figure 1). So far there have been no relevant studies using sodium pyruvate as a source of metabolic stress.

The Effect of Best Concentration on Phycocyanin Production in *Spirulina platensis:* After obtaining the best concentration of 10 mM sodium pyruvate substrate, microalgae cultivation was carried out again with a concentration to see if there was an effect on growth rate and phycocyanin production in *Spirulina platensis*microalgae. The growth curve of *Spirulina platensis* that has been cultivated on zarrouk media with the addition of sodium pyruvate can be seen in the image below.

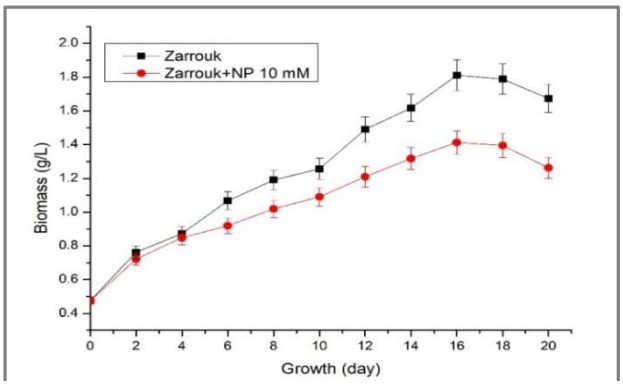
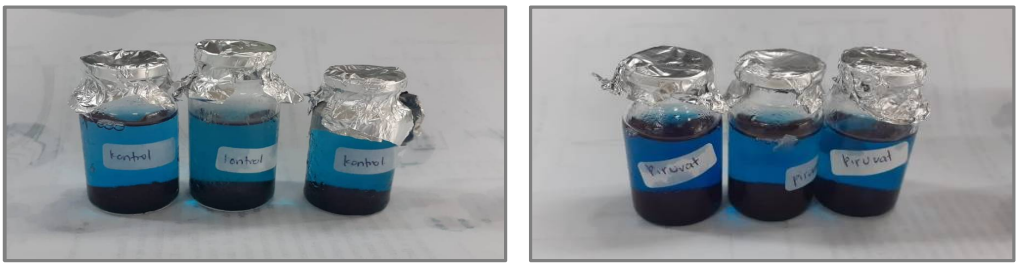


Figure 4. Differences in Biomass Growth of *Spirulina platensis* with 10 Mm sodium pyruvate (NP) and without treatment (control).

From the growth curve obtained, there are differences between the control and microalgae that were given the effect of sodium pyruvate (NP). From the graph, it can be seen that the growth curve of *Spirulina platensis* with zarrouk medium (control) and NP is in the highest position by producing dry cell weight or dry biomass weight of microalgae of 1.811 g L^{-1} and 1.412 g L^{-1} at the beginning, respectively. Stationary phase. As for the stationary phase, both variations were on days 16 to 18. Then the dry biomass decreased in the death phase on day 20 with values of 1.674 g $L^{-1}$and 1.263 g L^{-1}. With a decrease in the value of biomass in each addition of substrate, it can be concluded that the more substrate additions to the growth medium, the smaller the absorbance value on the growth of *Spirulina platensis.*

The phycocyanin pigment contained in *Spirulina platensis* was then extracted. Phosphate buffers have polar properties that are suitable for extracting phycocyanin pigments under stable conditions, namely in the pH range of 4.0 to 9.0 [**15**] so that they are able to extract higher amounts of phycocyanin than other solvents. Then phycocyanin will be stable when isolated at low temperature conditions (<50 Celsius) because chromoproteins (and polypeptides) are sensitive to temperature, so that cell damage is not followed by a denaturation process [**16**]. The following is the extraction result from phycocyanin treated with sodium pyruvate and control.



(a) **(b)**

Figure 5. Results of Phycocyanin extraction on *Spirulina platensis*(a) without treatment (control) and (b) administration of (NP) 10 Mm.

The color difference in the extraction can be seen in (Figure 5)**,** the extraction with the addition of 10 mM sodium pyruvate tends to have a dark blue color while the untreated extraction looks like light blue. This could be due to the more added stress, the greater the binding of phycocyanin molecules to the *Spirulina platensis* protein [**17**]. After extraction, the concentration of the two extractions was determined. The following graph shows the differences in the results of phycocyanin concentrations.
From the graph above, it can be seen the effect of NP substances on the growth of phycocyanin production. From the overall graph given, it can be seen that the increase during the cultivation process and the highest phycocyanin production on day 16 was 0.083 mg mL^{-1} for the control. This was followed by the addition of 10 mM sodium pyruvate concentration variations of 0.171 mg mL^{-1}

each. These data prove that pyruvate is a precursor that can alter the biosynthetic pathway of tetrapyrrole, thereby triggering the accumulation of phycocyanin as a result of metabolic stress. The following table describes the summary of the effect of sodium pyruvate on the accumulation of phycocyanin production.

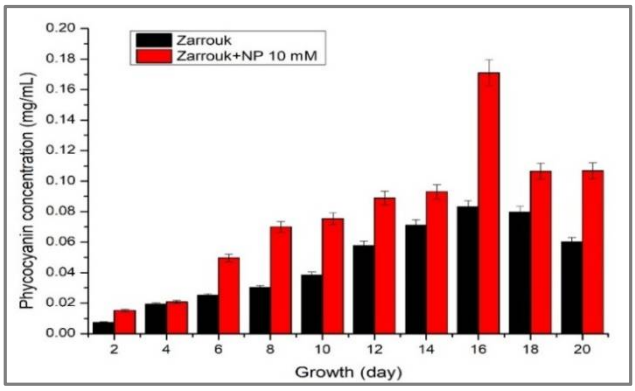


Figure 6 . Phycocyanin Production of*Spirulina platensis*
with administration of (NP) 10 Mm and without treatment (control)

.Table 1. Effect of sodium pyruvate on the accumulation of phycocyanin

Treatment	Phycocyanin concentration ($mg.mL^{-1}$)	Phycocyanin content ($mg.g^{-1}$ of cell weight)	Phycocyanin purity (OD_{620}/OD_{280})
Control	0.083 ± 0.003	13.856 ± 0.507	1.518 ± 0.266
Phyruvate	0.171 ± 0.004	20.905± 0.470	1.727 ± 0.225

*Phycocyanin observations were made from the highest value data on day 18

The concentration of phycocyanin produced in the control and pyruvate can be seen in (Table 1) which is 0.083 ± 0.003 mg mL^{-1} and 0.171 ± 0.004 mg mL^{-1}, while the total phycocyanin pyruvate increases by 1.727 ± 0.225. The purity of phycocyanin was determined by the ratio between the absorbance of the sample of the pigment at 620 nm (A_{620}) i.e. the amount of pigment, and at 280 nm (A_{280}) i.e. the absorbance of the amino acids present in the protein in solution. Samples of phycocyanin with an A_{620}/A_{280} ratio greater than 0.7 were considered food grade. Samples with an A_{620}/A_{280} ratio of 3.9 are considered reactive values, while values above 4.0 are considered to be of analytical grade [**18**]. In this study, phycocyanin acted as a food grade, which was 1.727 ± 0.225 for pyruvate and 1.518 ± 0.266 for the control.

APPLICATION

Phycocyanin production can be applied to a larger scale. Phycocyanin can be a potential agent to be marketed because it contains various benefits such as anti-cancer, anti-oxidant and immune drugs. The purity of phycocyanin in this study acts as a food grade or food with high nutritional value.

CONCLUSION

The administration of sodium pyruvate has a negative effect on the growth of phycocyanin, the more sodium pyruvate (NP) metabolic stress is given, the slower and smaller. The concentrations of phycocyanin produced were 0.083 ± 0.003 mg mL^{-1} and 1.518 ± 0.266 mg mL^{-1}, while the total pyruvate phycocyanin increased from 13,856 ± 0.507 to 20,905 ± 0.470. The level of purity of phycocyanin increased as nutrients were added to the culture medium, namely 1.727 ± 0.225 for pyruvate and 1.518 ± 0.266 for control.

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