



## Phycocyanin Accumulation of *Porphyridium cruentum* Culture at Indoor Tubular Photobioreactor

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**Abstract:** Microalgae are used in aquaculture and various industrial fields such as pharmaceuticals, feed, food, agriculture, and energy. Microalgae is a potential natural food coloring agent as pigments and contain bioactive components such as polyunsaturated fatty acids (PUFA) in their composition. *Porphyridium cruentum* is a red alga with the ability to accumulate valuable pigments biomolecules such as phycocyanin (PE), chlorophyll, and other carotenoids. In this study, *P. cruentum* was cultured for 51 days at the indoor pilot scale tubular photobioreactor (PBR). The highest cell number was  $31.84 \times 10^6$  cells mL<sup>-1</sup> and the highest specific growth rate was determined as 0.80. Total phycobiliprotein and phycocyanin amounts were reached  $0.252 \pm 0.009$  mg mL<sup>-1</sup> and  $0.224 \pm 0.007$  mg mL<sup>-1</sup> at the early exponential phase, respectively.

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## *Porphyridium cruentum*'ün Kapalı Alan Tübüler Fotobiyoreaktör Kültüründe Fikoeritrin Birikimi

### Makale Bilgileri

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### Anahtar Kelimeler

Fikoeritrin,  
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Tübüler fotobiyoreaktör.

**Öz:** Mikroalgler, akuakültür ile birlikte farmasötik, yem, gıda, tarım ve enerji gibi endüstriyel alanlarda kullanılmaktadır. Mikroalg pigmentleri potansiyel gıda renklendirme ajanı olmakla birlikte, yapılarında çoklu doymamış yağ asitleri gibi biyoaktif bileşenler içermektedir. *Porphyridium cruentum* fikoeritrin, klorofil ve karotenoidler gibi değerli pigmentleri biriktirebilme yeteneğine sahip kırmızı bir alg olarak bilinmektedir. Bu çalışmada, *P. cruentum*'ün 51 gün boyunca kapalı alanda pilot ölçekli tübüler fotobiyoreaktörde kültürü yapılmıştır. En yüksek hücre sayısı  $31.84 \times 10^6$  hücre mL<sup>-1</sup> olarak belirlenmiş olup, en yüksek spesifik büyüme oranı 0.80 olarak belirlenmiştir. Toplam fikoeritrin ve fikoeritrin miktarları erken üssel fazda sırasıyla  $0.252 \pm 0.009$  mg mL<sup>-1</sup> ve  $0.224 \pm 0.007$  mg mL<sup>-1</sup>'ye ulaşmıştır.

## 1. Introduction

Microalgae are well-studied microorganisms and have been used for feeding zooplankton, molluscs, and lastly, for human consumption (Kent et al., 2015). In years, the use of these organisms went beyond the previous purposes and started to see as a resource in many different fields such as

bioenergy, bioplastic, pharmaceuticals, drugs, and biofertilizers (Spolaore et al., 2006). As a result, different production systems such as photobioreactors (PBR) and fermenters have been started to be used commonly in recent years (Enzmann et al., 2019). The major role of PBRs on microalgae production has already been explained (Acién et al., 2017). PBRs could be designed in many shapes, such as tubular, flat plates, and columns. Every design may have specific advantages and disadvantages. Also, target species could help to choose the design of PBRs. For instance, it was stated that tubular PBR is more efficient than flat plate PBR for *Nannochloropsis oculata* cultivation (Durmaz and Erbil, 2020). Many methods have also been developed to isolate valuable metabolites from these algae. Recently, research and development on the efficiency of culture systems and methods have become the main objective in microalgae production.

*Porphyridium cruentum* is a red alga that belongs to the Rhodophyta. The microalga has a rounded shape with a lack of cell walls. Its unique red color comes from phycobiliprotein substances. Dominant accessory pigment phycoerythrin is the most valuable biomolecule of *P. cruentum* and can be reached to 82% of the total phycobiliprotein amount (Fuentes et al., 2000). The phycobiliproteins include R-phycoerythrin, allophycoerythrin, and three forms of phycoerythrin. Phycoerythrins, present in the greatest amount, are giving to *Porphyridium* its bloody red color. B-phycoerythrin is present in the more primitive red algae and is found in *Porphyridium*. Phycoerythrin has used for food varied applications such as food colorization, pharmaceuticals, and immunology (Toker, 2019).

*P. cruentum* can naturally accumulate high amounts of pigments, fatty acids, proteins, vitamins, hydrocarbons, polysaccharides, and many other metabolites. However, the biochemical composition of microalgae biomass depends on growth conditions such as PBRs, nutrient environment, temperature, salinity, pH, light. Therefore, it is important to optimize cultural conditions. The aim of this study is to investigate of effects of indoor pilot scale tubular PBR and culture growth phases on the phycobiliprotein accumulation of *P. cruentum*.

## 2. Materials and Methods

Inoculation culture of *Porphyridium cruentum* (UTEX 161) was grown at flasks under constant illumination (Philips Master TLD Super 80 830 & 840 36w) for three weeks. F/2 growth medium (Guillard, 1975) was used in both inoculation cultures and in the pilot scale tubular PBR system.

### 2.1. Photobioreactor

The tubular PBR system can be separated into two parts as the solar receiver (tubular area) and the reservoir tank (Figure 1). The working volume of the system is 140 L and the solar receiver constitutes 107 L of it. Four fluorescent lights (2 x Philips Master TLD Super 80 830 36w & 2 x Philips Master TLD Super 80 840 36w) were placed between the tubes, and four low CRI cool white led lights (100 w) were placed against the system for supporting the illumination. Solar irradiance on the reactor surface was measured as  $250 \text{ mmol m}^{-2} \text{ s}^{-1}$  on-line using a quantum scalar irradiance meter (LI-190 SA, Licor Instruments, Lincoln, NE, USA). System circulation was provided by a submersible water pump placed in the reservoir tank.

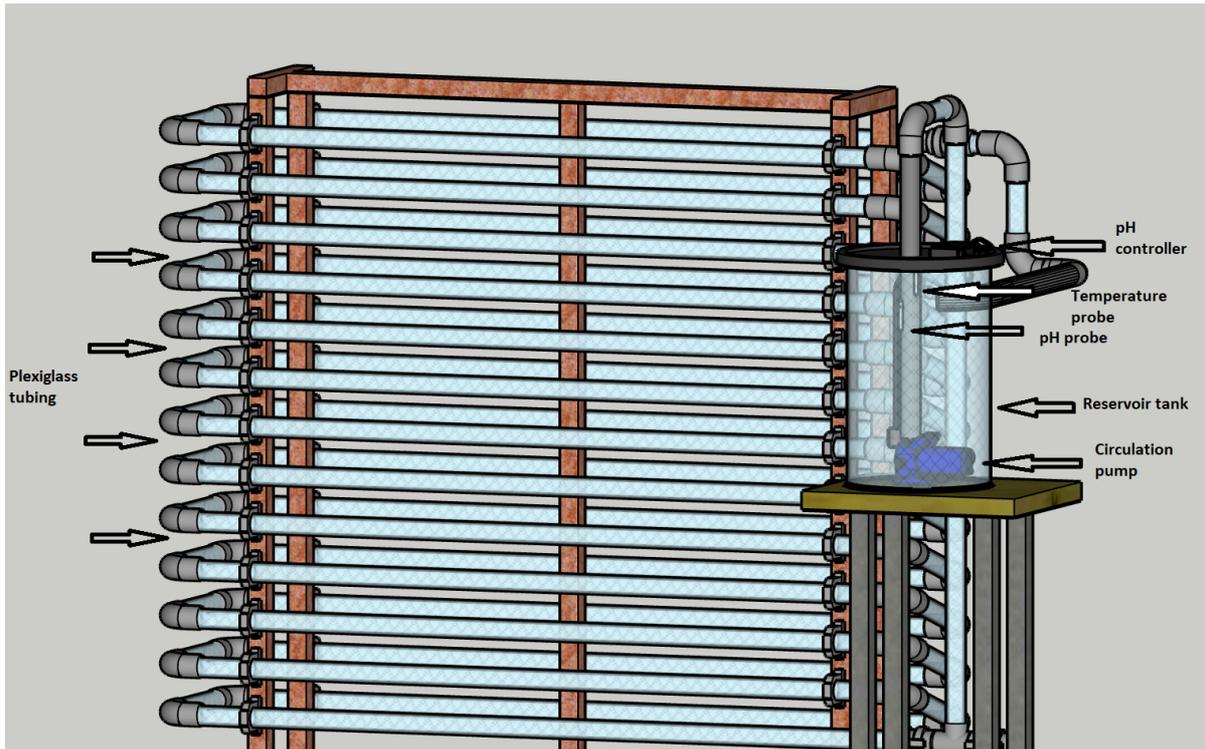


Figure 1. Illustration of the tubular PBR.

Before the inoculation, the tubular PBR was disinfected with sodium hypochlorite and neutralized with sodium thiosulfate. The temperature was arranged with air condition in the room as 20.5-22.5 °C. System pH was controlled with automatically CO<sub>2</sub> injection (JBL CO<sub>2</sub>/pH control 12V) and varied between 7.1-7.8. PBR system was operated semi-continuously.

## 2.2. Analytical Methods

All measurements were performed in triplicate during the study. Samples were taken daily for cell count at Neubauer haemocytometer and for optical density measurement at Hach DR 6000 spectrophotometer. Specific growth rates were calculated by using the equation given below:

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{t - t_0}$$

Where N<sub>t</sub> is cell number at time (t) and N<sub>0</sub> is the beginning cell number at time t<sub>0</sub>.

Total phycobiliprotein (PB), phycoerythrin (PE), R-phycoyanin (R-PC), and Allo-phycoyanin (A-PC) amounts were determined 4 times during the culture period on the day of 14, 24, 34, and 44. Phycobiliprotein amounts were determined spectrophotometrically. Fresh cells were harvested, and phycobiliproteins were extracted with distilled water by sonication. Absorbance values of the samples were read in Hach DR 6000 spectrophotometer at 545 nm, 620 nm, and 650 nm.

Calculations were done according to the formulas 1-4 were given by Gantt & Lipschultz (1974).

$$\text{Phycoerythrin (PE)} = (A_{545\text{nm}} - 0.572(A_{620\text{nm}}) + 0.246(A_{650\text{nm}})) / 5.26 \quad (1)$$

$$\text{R-phycoyanin (R-PC)} = (A_{620\text{nm}} - 0.666(A_{650\text{nm}})) / 3.86 \quad (2)$$

$$\text{Allo-phycoyanin (A-PC)} = (A_{650\text{nm}} - 0.105(A_{620\text{nm}})) / 4.65 \quad (3)$$

$$\text{Total phycobiliprotein (Total PBP)} = (\text{PE}) + (\text{R-PC}) + (\text{A-PC}) \quad (4)$$

### 2.3. Statistical Analysis

Results were analysed by one-way ANOVA with significance at  $P \leq 0.05$ , and Tukey's multiple comparison test was used for the determination of significant differences among means. Statistical procedures were carried out by using IBM SPSS Statistics 23 software.

## 3. Results

### 3.1. Culture Density

The experiment was started with  $0.145 \times 10^6$  cells  $\text{mL}^{-1}$  density and was continued for 51 days (Figure 2). The First five days were recorded as the lag phase. The specific growth rate at the early exponential phase was 0.33 division days<sup>-1</sup> for the first week and the culture cell number was reached  $1.68 \times 10^6$  cells  $\text{mL}^{-1}$  on the 7<sup>th</sup> day. In the early exponential phase of the culture, the specific growth rate was 0.32 division days<sup>-1</sup> and cell numbers were  $9.28 \times 10^6$  cells  $\text{mL}^{-1}$  on the 14<sup>th</sup> day.



Figure 2. *Porphyridium cruentum* culture at tubular PBR (left to right: 1<sup>st</sup> day, 7<sup>th</sup> day, 14<sup>th</sup> day, 24<sup>th</sup> day, 34<sup>th</sup> day, 44<sup>th</sup> day).

However, the specific growth rate was started to decrease at the third week and calculated as an average of 0.15 division days<sup>-1</sup>. After three weeks, the cell density of *P. cruentum* at tubular PBR was reached  $19.88 \times 10^6$  cells  $\text{mL}^{-1}$ , and the average specific growth rate was 0.26 division days<sup>-1</sup>. The highest cell number was determined as  $31.84 \times 10^6$  cells  $\text{mL}^{-1}$  on the day of 28. Both cell count and OD results showed that there were four phases of in culture: 0-5 days was a lag phase, 5-23 days was an exponential phase, 24-34 days were a stationary phase, and 35-51 days were decay phase (Figure 3).

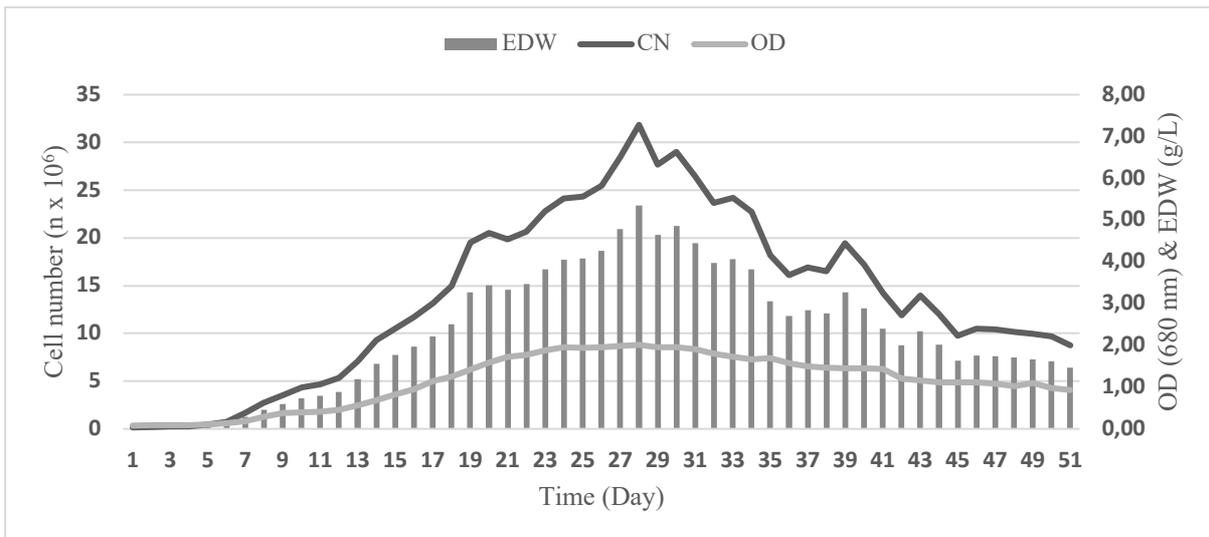


Figure 3. Cell numbers ( $n \times 10^6$ ), optical density (680 nm) and estimated dry weights of the *Porphyridium cruentum* culture in the pilot scale tubular photobioreactor (Estimated dry weights were calculated according to the data given by Razaghi et al., (2014)).

The harvest regime was performed according to the increase in cell number. Forty-six liters of culture was harvested at the exponential phase, and mean cell density was calculated as  $9.92 \times 10^6$  cells  $\text{mL}^{-1}$  for this period. Thirty liters of *P. cruentum* were harvested at the stationary phase, and the mean cell density was  $27.18 \times 10^6$  cells  $\text{mL}^{-1}$ . Finally, harvested culture amount was 40 liters with  $16.95 \times 10^6$  cells  $\text{mL}^{-1}$  mean cell density at the decay phase, and then the whole system was harvested at the end of the culture.

### 3.2. Phycobiliproteins

Total PB, PE, R-PC, and A-PC amounts were calculated at different culture phases (Table 1). According to the results, the highest amounts of phycobiliproteins were found at the early stationary phase. The maximum PE amount per mL was  $0.224 \pm 0.007$  mg at that culture phase. However, the highest PE/PB ratio was found as  $0.92 \pm 0.013$  at the late stationary phase. Also, at the decay phase decrease in PE and R-PC amounts was found while the A-PC amount was increased.

Table 1. Total PB, PE, R-PC and A-PC amounts of *Porphyridium cruentum* (mg/mL) at different culture phases (Superscript letters show the statistical difference)

Phase	Total PB (mg $\text{mL}^{-1}$ )	PE (mg $\text{mL}^{-1}$ )	R-PC (mg $\text{mL}^{-1}$ )	A-PC (mg $\text{mL}^{-1}$ )	PE/Total PB
Early Exponential	$0.053 \pm 0.003^a$	$0.045 \pm 0.002^a$	$0.004 \pm 0.001^a$	$0.004 \pm 0.001^a$	$0.85 \pm 0.030^a$
Early Stationary	$0.252 \pm 0.009^b$	$0.224 \pm 0.007^b$	$0.014 \pm 0.003^b$	$0.013 \pm 0.001^b$	$0.89 \pm 0.006^{ab}$
Late Stationary	$0.187 \pm 0.006^c$	$0.172 \pm 0.006^c$	$0.009 \pm 0.002^{ab}$	$0.005 \pm 0.001^a$	$0.92 \pm 0.013^b$
Decay	$0.119 \pm 0.007^d$	$0.102 \pm 0.004^d$	$0.008 \pm 0.003^{ab}$	$0.008 \pm 0.000^c$	$0.86 \pm 0.018^a$

Mean  $\pm$  standard deviation. Different superscript uppercase letters show the significant differences in each parameter between culture phases ( $P < 0.05$ ).

A statistically significant difference was detected between Total PB and PE amounts at different culture phases. However, R-PC differed only between early exponential and early stationary phases. Statistical difference in A-PC amounts was found at early stationary and decay phases.

The highest total PB and PE per cell were determined on the day of 24 as  $10.42$  pg and  $9.29 \pm 0.28$  pg, respectively. However, the highest concentrations of R-PC ( $0.7 \pm 0.09$  pg  $\text{cell}^{-1}$ ) and A-PC ( $0.69 \pm 0.02$  pg  $\text{cell}^{-1}$ ) were determined on the day of 44 (Figure 4).

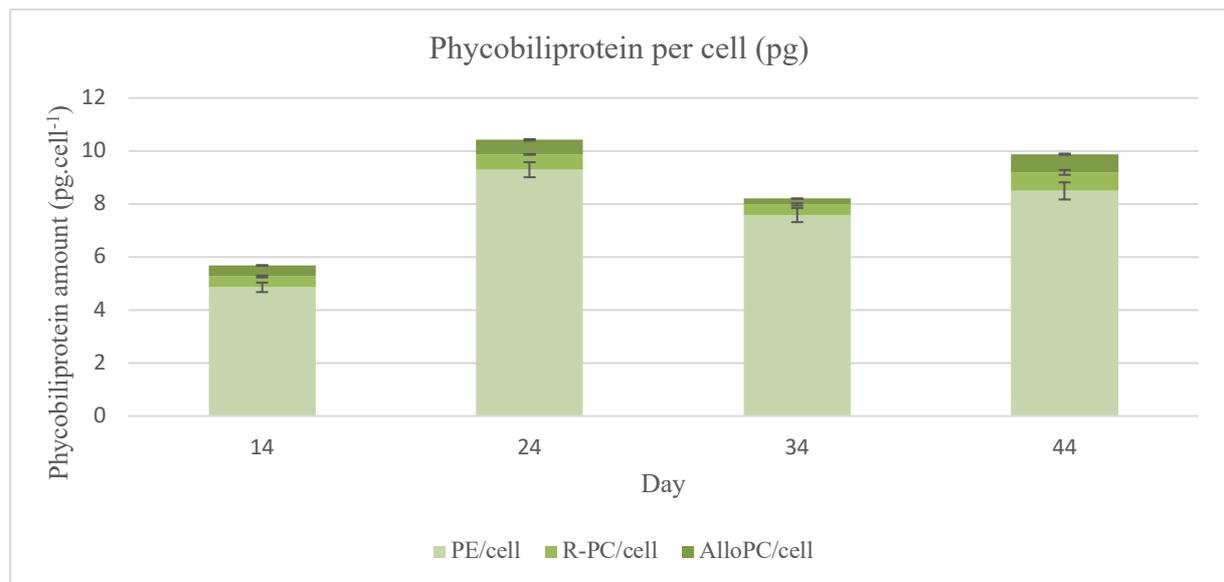


Figure 4. PB (PE+R-PC+A-PC), PE, R-PC, and A-PC amounts per cell of *Porphyridium cruentum* at different culture phases.

According to cellular phycobiliprotein and harvest quantities at culture phases, the highest amount of phycobiliprotein and phycoerythrin were collected as 7.59 g and 6.88 g at the stationary phase, respectively. The total harvested phycobiliprotein amount was calculated as 31.68 g, and phycoerythrin was 27.57 g during the 51 days of the experiment (Table 2).

Table 2. Harvested PB and PE amounts of *P. cruentum*

Harvest Time	PB pg cell <sup>-1</sup>	PE pg cell <sup>-1</sup>	Harvest Quantity (L)	Mean Cell Number (L)	Total (g)	PB Total (g)	PE
<i>Exponential</i>	5.68	4.85	46	9.92 x 10 <sup>9</sup>	2.59	2.21	
<i>Stationary</i> ( <i>early &amp; late</i> )	9.31	8.44	30	27.18 x 10 <sup>9</sup>	7.59	6.88	
<i>Decay</i>	9.88	8.49	40	16.95 x 10 <sup>9</sup>	6.70	5.76	
<i>Final</i>	9.88	8.49	140	10.70 x 10 <sup>9</sup>	14.8	12.72	
<i>Total</i>					31.68	27.57	

#### 4. Discussion

The pilot scale tubular PBR system had a significant effect on the growth rate in the culture of *P. cruentum*. This microalga can be easily grown and obtain maximum biomass using the pilot scale tubular PBR system indoor and on artificial light illumination. This biomass yield is comparable to typical biomass concentrations achieved in other PBRs. In a study, *P. purpureum* was cultured in a greenhouse at BioFence bioreactor and stated that maximum cell number was reached to 14.3 x 10<sup>6</sup> cells mL<sup>-1</sup> (Fuentes-Grünwald et al., 2015). In a flat plate bioreactor, 13.8 x 10<sup>6</sup> cells mL<sup>-1</sup> was found as the maximum cell number by Liqin et al., (2008). In another study conducted with tubular PBR located in an aquaculture facility, *P. cruentum* was reached to maximum cell density as 26.2 x 10<sup>6</sup> (Durmaz et al., 2017). In this study, maximum cell density was reached 31.84 x 10<sup>6</sup> cells mL<sup>-1</sup>. In comparison with the studies mentioned above, this study was achieved the highest cell density. However, it was started to decrease after reaching the maximum density.

Li et al., (2019) stated that nitrate concentrations might affect the B-phycoerythrin (B-PE) production of *P. purpureum*. According to the study, 17.6 mM concentration of KNO<sub>3</sub> was provided the highest B-PE production as 0.193±0.002 g L<sup>-1</sup> (Li et al., 2019). NaNO<sub>3</sub> was used as a nitrate source in this study with the standard F/2 medium concentration (8.82 mM) which was given by Guillard, (1975). On day 24, the B-PE amount was found as 0.224±0.007 g L<sup>-1</sup>.

Wang et al. (2007) reported that 112 mg l<sup>-1</sup> PE was found as the highest amount at 100 mL liquid volume with a 20% inoculation rate, 6.5 pH and below 4500 lux. In this study, pH was controlled with CO<sub>2</sub> injection and was held around 7.5 and light intensity was higher (9000 lux) when the culture was reached its highest PE amount per liter. However, it was reported that amounts of polysaccharides also increase with the light intensity (Wang et al., 2007), which may cause precipitations of polysaccharides with cells in the culture system. As a result, light intensity, pH, inoculation rate and liquid volume factors were found to be effective on PE production of *P. cruentum*.

Xu et al. (2020), described that decreasing temperature and light intensity under high nitrogen conditions results in higher PE content. According to the study, the highest PE content of *P. purpureum* was 229±11 mg L<sup>-1</sup> (Xu et al., 2020). A similar result (224±7 mg L<sup>-1</sup>) was found in our study without any induction. However, it is seen that temperature and light induction might provide higher PE content, especially if the culture induced between harvest intervals.

82% was the highest PE/PBP ratio of a study on *P. cruentum* culture in tubular PBR (Fuentes et al., 2000). In the present study, the maximum ratio was found as 92%. However, PE/PBP ratio was ranged between 80 and 92% at different culture phases. It can be assumed that the stationary phase is the most suitable for phycoerythrin production in considering accumulation per cell and culture density. The differences might be occurred because of variation of the growth medium and the light between phases.

The effects of nitrogen source and irradiance on *P. cruentum* pigment are already been investigated. It is stated that the use of NO<sub>3</sub> as the nitrogen source under 200 μmol photon m<sup>-2</sup> s<sup>-1</sup> irradiance was yielded 1 pg cell<sup>-1</sup> PE and 2 pg cell<sup>-1</sup> total phycobiliprotein content (del Pilar Sánchez-

Saavedra et al., 2018). Li et al. (2020) showed that a low C/N (0.96) ratio was yielded higher PE content per cell, which was reached more than 30 pg cell<sup>-1</sup>. PE amount of *P. cruentum* per cell was varied between 4.85-9.29 pg and the total phycobiliprotein per cell amount was reached more than 10 pg in this study. The wide difference of the phycoerythrin content per cell between studies might be the result of major variations on culture media, light sources, and culture systems. It is clear that phycoerythrin content per cell is highly variable depending on the culture conditions.

A great deal of work has been devoted to the genus *Porphyridium*, which grows naturally in a diverse spectrum of habitats. They have chlorophyll *a*, but also have large amounts of the pigments phycocyanin and phycoerythrin, which give them their distinctive red, brown, and purple colors. Phycoerythrin, extracted from the microalga *P. cruentum*, is gaining momentum for its application in the food industry as a red pigment (Sudhakar et al., 2015). Phycobiliprotein produced from Rhodophyte *Porphyridium* is commercially available and is widely used as colorants in candy bars, sweets, cold drinks, chewing gums, dairy products, and so on (Spolaore et al., 2006). High prices of phycobiliproteins in the global market make it difficult to use in industrial applications commonly. It is shown that high amounts of phycobiliproteins per cell, also high PE/PB ratio was found in this study. Through this, phycobiliprotein production may spread with further investigations on the optimization of culture. Consequently, it is thought that efforts to increase production efficiency should continue in order to eliminate this obstacle.

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