Phycobiliproteins production and heavy metals reduction ability of Porphyridium sp

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Abstract. Porphyridium sp is a unicellular red microalga from Rhodophyta class which produces several high value polymers like polysaccharides, polyunsaturated fatty acids and phycobiliproteins. The phycobiliproteins contents are phycerythrins, phycocyanins, and allophycocyanins. Porphyridium sp has the ability to reduce heavy metals pollution in the environment. This research was conducted in order to find out the potential use of Porphyridium sp for phycobiliproteins production and its ability in reducing the heavy metals Fe³⁺ and Co²⁺ in culture media. In this research, Porphyridium sp was cultivated in F2 non-silicate medium. The growth curve of Porphyridium sp was analyzed by cultivation at room temperature for 7 days and the OD 690 was monitored every day. Phycobiliproteins production was evaluated by analyzing the absorbance of the extracts at the wavelengths of 565, 620, and 650 nm. The ability of Porphyridium sp in the reduction of heavy metals Fe³⁺ and Co²⁺ was analyzed by AAS and calculated as bioconcentration factor (BCF). The data showed that the growth of Porphyridium sp in the media containing heavy metals Fe³⁺ and Co²⁺ was faster than in the control. The highest content of phycerythrins, phycocyanins, and allophycocyanins of Porphyridium sp was reached after 4 days of cultivation with the addition of Fe³⁺ at 6.30 ppm. Porphyridium sp reduced Fe³⁺ and Co²⁺ in media with BCF values of 10.5804 and 0.1151, respectively.

1. Introduction

Indonesia is rich in aquatic biological resource such as microalgae or phytoplankton. Phytoplankton growth is rapid and its productivity is high. Phytoplankton or microalgae produce biomass up to 50 times than other higher plants. In addition, microalgae can live in different types of environment even in areas with limited land. Therefore, it does not compete with other plants [1]. Porphyridium sp, the most abundant species of red microalga of Rhodophyta division, has been the subject of intensive study by our group for a number of years. The cells of this red microalga were encapsulated within a cell-wall polysaccharide complex [2]. This strain is unique in building up fluorescent phycobiliproteins, exopolysaccharides, long-chain polyunsaturated fatty acids, carotenoids (zeaxanthin, tocopherol, etc and vitamins during its metabolic processes. Porphyridium purpureum growth process is clearly dependent on culture medium composition. The usual artificial sea water (ASW) culture medium has been proven to be a very convenient support in many algae species growth and production [3].

Red marine microalga Porphyridium sp is a Rhodophyta, a source of valuable phycobiliproteins which assemble into an organized cellular structure, the phycobilisome. They absorb light over a wide range of wavelengths in the visible part of the spectrum, and transfer the excitation energy by less
radiation processes to the reaction in the photosynthetic membranes for conversion to chemical energy. The phycobiliproteins can be divided into three main classes depending on absorption properties: phycocerythrins (PE) max 540–570 nm, phycocyanins (PC) max 610–620 nm, and allophycocyanins (APC) max 650–655 nm. PE is a valuable candidate in the design and characterization of light-sensing elements in biosensors due to its properties that can absorb the light in the range of 540–570 nm. Another interesting application of the phycobiliproteins is their use as natural dyes in foods and cosmetics replacing the synthetic dyes, since the latter are in general toxic, carcinogenic, and therefore unsafe [4]. R-Phycocerythrin has been analyzed for its various physico-chemical properties and the analyses revealed that R-Phycocerythin from Kappaphycus alvarezi has characteristic affinity towards different metal ions, inhibitors, organic solvents, and preservatives at different monochromatic irradiances [5]. The characteristic affinity towards metal ions of microalgae is potential for environmental remediation. Heavy metals are required by microalgae for enzymatic process as trace elements (≥ 3 g/cm³), but they are toxic in high concentration. Remediation by microalgae has advantages since these microalgae can be used as fertilizer after remediation process or as biofuels with low cost, simple and flexible in application, and with low maintenance. Porphyridium has a potential to be used in heavy metals bioremediation. Porphyridium cruentum is promising for bioremediation of heavy metals to overcome environmental pollution [6]. The potential of Porphyridium sp needs to be explored so it can be used for decreasing the heavy metals content in waste water treatment. The objective of this study was to evaluate the phycobiliproteins production of Porphyridium sp and its ability in reducing heavy metals Fe³⁺ and Co²⁺.

2. Experimental

2.1. Materials

Porphyridium sp isolate was obtained from Microbiology Laboratory – Research Center for Oceanography, Indonesian Institute of Sciences. The modification of F2 medium was sea water enriched with the following additives (per L): NaNO₃ 0.075 g, Na₂HPO₄ 0.05 g, Na-EDTA 0.00436 g, ZnSO₄·7H₂O 0.022 g, Na₂M₀O₄·H₂O 0.0063 g, CuSO₄·5H₂O 0.0098 g, MnCl₂·4H₂O 0.18 g. The medium was sterilized (120°C, 20 min) prior to use. Cyanocobalamin 1 mg, thiamin HCl 0.2 mg, and biotin 1 mg (per L) were added to the sterile medium. The concentration of FeCl₃·6 H₂O and CoSO₄ were of 3.15 ppm and 6.30 ppm, respectively.

2.2. Methods

2.2.1. Porphyridium sp cultivation. The cultivation of Porphyridium sp was carried out by modified method [7]. The steps of cultivation were as follows: (i) 10 ml stock culture was cultivated in a shaker incubator at 30°C for 7 days. (ii) The culture was then put into 250 ml of medium. Porphyridium sp was cultivated in bottles which were connected to an aeration pump (0.37 MPa) and were exposed to sunlight at room temperature. The experiments were carried out for 7 days. The absorbance of the culture (λ, 690) was monitored every 24 h.

2.2.2. Extraction and estimation of phycobiliproteins. Porphyridium sp culture was extracted and analyzed by the modified method [5]. The analysis of phycobiliproteins content was carried out at the day 4 of culture. The culture was frozen for 3 min and heated to 45°C for 3 min and then homogenized for 1 min. This treatment was carried out four times before being centrifuged at 7000 rpm for 10 min. The amounts of PC, APC, and PE in extracts were calculated based on measurements of the absorbance at 565, 620 and 650 nm using the following equations [5].

\[
\begin{align*}
PC (\text{mg/ml}) &= \frac{OD_{565 \text{ nm}} - 0.7 OD_{520 \text{ nm}}}{7.38} \\
APC (\text{mg/ml}) &= \frac{OD_{520 \text{ nm}} - 0.19 OD_{520 \text{ nm}}}{5.65} \\
PE (\text{mg/ml}) &= \frac{OD_{650 \text{ nm}} - 2.8 [PC] - 1.34 [APC]}{12.7}
\end{align*}
\]
2.2.3. Determination of Fe\(^{3+}\) and Co\(^{2+}\) accumulation. The analysis of Fe\(^{3+}\) and Co\(^{2+}\) in culture media and biomass of *Porphyridium* sp was carried out according to Soeprobowati and Hariyati with modifications [6]. Before analysis, the samples were destructed by 3 ml of concentrated HNO\(_3\) and heated at 50°C until there was no brown smoke. Samples were then cooled, added with 1.5 ml of H\(_2\)O\(_2\), heated again until the volume was about ± 1 ml, and transferred to a 25 ml calibration flask. Analyses of Fe\(^{3+}\) and Co\(^{2+}\) were performed using a Shimadzu AA-7000 Atomic Absorption Spectrometer (AAS) with deuterium background subtraction. The wavelengths used for the analysis of Fe\(^{3+}\) and Co\(^{2+}\) were 248.16 nm and 240.41 nm, respectively.

The reduction of heavy metals was calculated, as well as *Porphyridium* sp population. Bioconcentration Factor (BCF) was calculated to determine the accumulation of heavy metals in the *Porphyridium* sp. BCF is a comparison between chemical concentrations in the organism and concentration in the environment or media [8].

\[
\text{BCF} = \frac{C_{\text{org}}}{C_{\text{media}}}
\]

where:
- \(C_{\text{org}}\): heavy metals concentration in *Porphyridium* sp
- \(C_{\text{media}}\): heavy metals concentration in the culture media (after cultivation)

3. Results and Discussion

*Porphyridium* sp was cultivated for 7 days by using F2 non silicate medium and its growth was monitored every day. Data presented in Figure 1 and Figure 2 show that the growth of *Porphyridium* sp increased between day 2 and 3 in all curves. The growth of *Porphyridium* sp continued and the maximum of OD was at day 4. Then on day 5 a decline of microalgae populations *Porphyridium* sp started and continued until day 7.

![Figure 1](image_url)

**Figure 1.** The effect of Fe\(^{3+}\) on the growth of *Porphyridium* sp.

Figure 1 shows that the concentration of Fe\(^{3+}\) influenced the growth of *Porphyridium* sp. The maximum OD (690 nm) was reached after 4 days of cultivation. This effect was also occurred when the medium contained Co\(^{2+}\). *P. cruentum* tolerated a high concentration of heavy metal, concentration of 1 mg/L Cu had induced *P. cruentum* population growth. The life cycle of *Porphyrydium* tended to go a day forward in the higher heavy metal concentrations, but with a lower population [6].

The red microalga *Porphyridium* sp produces several high value polymers like polysaccharides, polysaturated fatty acids and phycobiliproteins which act as photosynthetic accessory pigments [9]. In this study, the phycobiliproteins content of *Porphyridium* sp was analyzed as well as its correlation with the heavy metals Fe\(^{3+}\) and Co\(^{3+}\) addition.
Figure 2. The effect of Co$^{2+}$ on the growth of *Porphyridium* sp.

Figure 3 shows that with the addition of 3.15 ppm of FeCl$_3$ to the *Porphyridium* sp culture, the phycobiliproteins production was reduced. However, when the concentration of FeCl$_3$ was increased until 6.30 ppm, phycoerythrin (PE) content increased to 17.25%. This perhaps because the biomass was not homogeneous due to the aeration system in the cultivation process did not work well. Pugalendren *et al* [5] studied the addition of 0.1-10 mM FeSO$_4$ to the macroalgae *Kappaphycus alvarezii*, which resulted in the activity of R-PE (R-phycoerythrin) was decreased.

The influence of CoSO$_4$ addition to the *Porphyridium* sp culture was also investigated in regard to phycobiliproteins production. Figure 4 shows that the addition of CoSO$_4$ to *Porphyridium* sp increased phycobiliproteins production. PE production increased to almost ten times. The effect of cobalt on growth, pigment, and photosynthesis in unicellular green alga *Monoraphidium minutum* and *Nitzschia perminuta* was studied by El Sheekh *et al* [10]. Growth and pigment content were slightly increased at low concentration. The application of 0.5 and 1.5 ppm Co$^{2+}$ increased chlorophyll *a* content by 4 and 12%, respectively. In this study, Co$^{2+}$ at 3.15 ppm increased phycoerythrin (PC) and phycoerythrin (PE) contents. Co$^{2+}$ is a trace element that is important for pigment production such as chlorophyll *a* and phycoerythrin.
The use of microalgae for bioremediation has been studied by many researchers. *Porphyridium* had a potential to be used in heavy metals bioremediation. *P. cruentum* was qualified for bioremediation due to the absence of toxic production, was easily cultured, had the ability to grow in extremes of salinity, pH, and temperature, rapid growth in defined media, ability to achieve a high population, and was easily harvested [11]. Bioaccumulation of Fe$^{3+}$ and Co$^{2+}$ by *Porphyridium* sp has been studied and the data was presented on Table 1.

**Table 1.** Fe$^{3+}$ and Co$^{2+}$ content (ppm) in media and biomass of *Porphyridium* sp (4 days cultivation, room temperature).

| Concentration of heavy metals in media (ppm) | Fe$^{3+}$ (ppm), after cultivation | Co$^{2+}$ (ppm), after cultivation |
|---------------------------------------------|-----------------------------------|-----------------------------------|
|                                             | Media    | Biomass | Media   | Biomass |
| FeCl$_3$, 0                                 | 0.5421   | 0.4404  |         |         |
| FeCl$_3$, 6.30                              | 0.6203   | 6.5630  |         |         |
| CoSO$_4$, 0                                 |         | -0.0061 | -0.0147 |         |
| CoSO$_4$, 6.30                              | 3.8700   | 0.4454  |         |         |

The data in Table 1 shows that for the control culture (Co:0 ppm), the Co$^{2+}$ contents in the medium and biomass were negative. Meanwhile, Fe$^{3+}$ contents in the medium and biomass (Fe:0 ppm) were positive. We predicted that *Porphyridium* sp culture contained Fe$^{3+}$. By the addition of 6.30 ppm of FeCl$_3$, Fe$^{3+}$ content in biomass was much higher than in medium. After added by 6.30 ppm of CoSO$_4$, Co$^{2+}$ content in biomass was lower than in medium. This assumed that *Porphyridium* sp culture could absorb Fe$^{3+}$ optimally. In order to assess the accumulation properties of the used *Porphyridium* sp, bioconcentration factor (BCF) was determined, indicating the ratio of heavy metal concentration in biomass versus heavy metal concentration in medium. BCF values of Fe$^{3+}$ and Co$^{2+}$ in *Porphyridium* sp are represented in Figure 5.

![Figure 4](image.png)
Figure 5. BCF (bioconcentration factor) value of Fe$^{3+}$ and Co$^{2+}$ in *Porphyridium* sp (4 days cultivation, room temperature).

The data show that BCF value of culture sample which contained 6.30 ppm Fe$^{3+}$ (10.58) was much higher than the culture contained Co$^{2+}$ (0.1151). It was shown from this study that *Porphyridium* sp successfully demonstrated the absorption and removal of Fe$^{3+}$ from the medium. This was related to the heavy metal release rate was relatively lower than its absorption. Heavy metals absorption occurred in 2 ways i.e. heavy metal ionic change with cell wall caption or development of covalent bound between heavy metals with active ionic cell wall. *P. cruentum* cell wall consisted of organic protein, polysaccharide, alginate acid and urinate acid which were able to bind heavy metals [12].

There was a correlation between phycobiliproteins production and the accumulation of heavy metals (BCF) by *Porphyridium* sp; if phycobiliproteins produced were low, the BCF also had a low value. Phycoerythrin content of the culture which contained 6.30 ppm Fe$^{3+}$ was higher compared to the culture contained Co$^{2+}$ and this also occurred to BCF value of the same culture sample. This assumed that Fe$^{3+}$ stimulated the photosynthetic process of microalgae. BCF was calculated as the homeostatic ratio of heavy metal concentration on the *P. cruentum* to heavy metal concentration on the media. Microalgae had a protection mechanism against heavy metals by development of heavy metals complex with cellular protein without changing its activity [12].

4. Conclusions
The growth of *Porphyridium* sp was studied and evaluated, as well as its phycobiliprotein production and heavy metals Fe$^{3+}$ and Co$^{2+}$ reduction ability. The addition of Fe$^{3+}$ (6.30 ppm) and Co$^{2+}$ (6.30 ppm) increased the phycobiliproteins (PC, APC, PE) production. BCF value of culture sample which contained 6.30 ppm Fe$^{3+}$ (10.58) was much higher than the culture contained Co$^{2+}$ (0.1151). *Porphyridium* sp successfully demonstrated the absorption and removal of Fe$^{3+}$.

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