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# The Combination of Medium and Method of Cultivation on The Growth Performance of *Porphyridium cruentum*

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**Abstract:** *Porphyridium cruentum* has advantages in pigments and exopolysaccharides, so it has the potential to be developed in the food, pharmaceutical, nutraceutical, and cosmetic industries. *Porphyridium cruentum* cultivation media uses synthetic and indoors, causing high costs. This study aims to determine the growth performance of *Porphyridium cruentum* in different mediums and method of cultivation. The research methods used cultivating *Porphyridium cruentum* for 7 days with four treatments, namely indoors Guillard media (IGM), outdoors Guillard media (OGM), indoors fertilizer media (IFM), and outdoors fertilizer media (OFM. Each treatment had three replications. Growth was identified using an optical density spectrophotometer uv-vis, gravimetrically dried weight of biomass and pH adjustment during 7 days of cultivation. The result shows the best growth performance in IFM, followed by IGM, OFM, and OGM. Fertilizer media is better for the growth of *Porphyridium cruentum* than Guillard media. pH growth *Porphyridium cruentum* during cultivation 7-7.5. Indoor cultivation is better than outdoor cultivation. The highest biomass was IFM, which had the best performance in IFM with OD 0.247, pH 7, and biomass 79.6%.

*Keywords: growth, Porphyridium cruentum, fertilizer, Guillard*

## 1. Introduction

Microalgae is an organism's microscopic producer of oxygen that is critical to the global ecosystem and contributes to the chain of aquatic food. Microalgae efficiently produce biomass. Microalgae can be used for industry food, pharmaceuticals, biofuels, and remediation environments [1]. Microalgae are rich in carbohydrates, fat, protein, dietary fibre, vitamins, and minerals [2]. Microalgae can convert nitrogen, phosphorus, carbon, and heavy metals into the environment as a growth medium [3]. Microalgae are rich in omega 3 and 6, pigments as source antioxidants, and bioactive components such as antibacterial and antiviral, which can increase the immune body [4]. Potentially, a very large microalgae will develop in the future.

The cultivation media influences the production of biomass microalgae [5]. Cultivation media is a key factor in the growth of microalgae. Cultivation media microalgae generally consist of two types, namely synthetic media and alternative media. Synthetic media is developed using chemical ingredients consisting of organic nutrients and non-organic and non-nutrient factors. Organic nutrients include proteins, amino acids, sugars, thiamine, biotin, and riboflavin. Non-organic nutrients consist of salt and trace metals. Non-nutrient factors are from *growth-stimulating hormone*s [6]. Alternative media is another media that can be used for the growth of microalgae, among others, extract husk rice [7], fermentation water hyacinth goitre [8], phosphorus recycle repeat from results side agriculture [9], and fertilizer [10].

Biomass and growth microalgae are also affected by environmental conditions. The environment inside the room is more controlled, especially for lighting and temperature; in contrast, the environment outside the room lighting depends on the sun's light, and the temperature quickly changes the environment. Microalgae *Dunaliella sp., Chaetoceros calcitrans, Isochrysis galbana, Nannochloropsis salina*, and *Tetraselmis tetrahedron*, which grow inside the room and outdoors show that their growth is better inside the room [11]. Spirulina grown indoors and outdoors showed that productivity and growth were better outdoors than indoors, but protein content was better indoors than outdoors [12].

*Porphyridum cruentum* is a microalga that is safe for consumption based on the FDA GRASS classification [13]. *Porphyridium cruentum* is a red microalgae cell measuring 4.86-9.93 µm cultivated using seawater media [14]. *Porphyridium cruentum* has a carbohydrate content of 22.82 % [15], lipids of 38-43.3% with EPA 8- 30 % and DHA 5-14 % [16], protein of 15.96 %, carbohydrates of 48.53 %, lipids of 6.85 %, moisture 8.52 %, inorganic components 20.14 % [17]. *Porphyridium cruentum* contains phycobiliproteins pigments consisting of Allophycocyanin (APC), R-Phycocyanin (R-PC), and

B-Phycoerythrin (B-PE) [18]. B- phycoerythrin pigment is the most considerable pigment content, around 79 % of the total pigment, and has the potential as a natural pigment in drinks [19] and chewing gum [20]. *Porphyridium cruentum* contains sulfated exopolysaccharides, which are used as immunostimulants in shrimp [21], can improve skin conditions [22], antioxidant, anti-inflammatory, immunomodulatory, and can regenerate tissue [23]. Phycoerythrin and exopolysaccharide pigments have the potential to be developed in the food, pharmaceutical, nutraceutical, and cosmetic industries [13]

The biomass and growth of *Porphyridum cruentum* are influenced by the cultivation media and environmental factors such as temperature, pH, and light intensity [16]. The biomass and growth of *Porphyridium cruentum* are greatly influenced by the nitrogen and phosphorus content in the cultivation media. Nitrogen plays a role in the photosynthesis process and increases the amount of microalgae biomass. Phosphorus functions to carry out energy transfer activities required by microalgae cells [24]. Commonly used media in microalgae cultivation are Guillard F/2 media [16], Johnson media [15], and Walne media [25]. Synthetic media are expensive [7], so alternative uses of cheaper media are needed. Research on alternative media for *Porphyridium cruentum* cultivation, including urea fertilizer media, has been carried out.

The more urea fertilizer is given, the better the growth is compared to the control without urea fertilizer [26]. *Porphyridium cruentum* is generally grown indoors, with outdoor cultivation required for long-term development. Therefore, it is necessary to research alternative media that are cheaper and easier to use, such as fertilizer media, and develop cultivation indoors and outdoors. This study aims to determine the best media and environmental conditions for the growth and biomass of *Porphyridium cruentum.*

## 2. Material and Methods

### *2.1 Materials*

Materials used were microalgae *Porphyridium cruentum,* Guillard media, and fertilizer media. Guillard media can we see in Table 1 and Fertilizer media in Table 2. The tools used in this study were 3L glass jars (Kedaung), UV (Rockwood), pH meter(Swuwu), and Whatman filter paper no. 42, neon lights (Philips), aerators (AP09), hoses (Figo), UV Vis spectrophotometers (SP-UV1000DLAB), ovens (Mamert), scales, measuring cups, tissues (NICE), aluminum foil, label paper, markers, spatulas, 10-100 µm micropipettes (DLAB), bulbs (D & N), lux meters (AS803), Erlenmeyer flasks (Pyrex) and beakers (Pyrex), glass.

Table 1 Guillard Media

| Solution | Material | Quantity |
|---|---|---|
| Solution 1 | $Na_2HPO_4.H_2O$ | 1 g |
| | $NaNO_3$ | 8,415 g |
| | aquades | 100 mL |
| Solution 2 | $FeCl_2.6H_2O$ | 0,145 g |
| | aquades | 100 mL |
| Solution 3 | NaEDTA | 1 g |
| | aquades | 100 mL |
| Solution 4 | vitamine B Neurobion 5000 | 1,5 mL |
| | aquades | 100 mL |
| Trace metal 1 | $CuSO_4.5H_2O$ | 1,95 g |
| | aquades | 100 mL |
| Trace metal 2 | ZnSO4.7H2O | 4,4 g |
| | aquades | 100 mL |
| Trace metal 3 | $NaMoO_4.2H_2O$ | 1,26 g |
| | aquades | 100 mL |
| Trace metal | $CoCl_2.6H2O$ | 2 g |
| | aquades | 100 mL |
| Trace metal 5 | $MnCl_2.4H2O$ | 3,6 g |
| | Aquades | 100 mL |

Table 2 Fertilizer Media

| Solution | Material | Quantity |
|---|---|---|
| Solution 1 | 0,3 g/L urea | 1 L |
| | 0,02 g/LNPK | 1 L |
| | 0,006 g/L TSP | 1 L |
| Solution 2 | vitamine B Neurobion 5000 | 1,5 mL |
| | aquades | 100 mL |
| Trace metal 1 | $CuSO_4.5H_2O$ | 1,95 g |
| | aquades | 100 mL |
| Trace metal 2 | ZnSO4.7H2O | 4,4 g |
| | aquades | 100 mL |
| Trace metal 3 | $NaMoO_4.2H_2O$ | 1,26 g |
| | aquades | 100 mL |
| Trace metal 4 | $CoCl_2.6H2O$ | 2 g |
| | aquades | 100 mL |
| Trace metal 5 | $MnCl_2.4H2O$ | 3,6 g |
| | Aquades | 100 mL |

### 2.2. *Methods*

#### 2.2.1. *Sample collection and preparation Porphyridium cruentum* from BBPBAP Jepara.

Microalgae stock refreshment was carried out under aseptic conditions at room temperature under fluorescent lamp illumination (± 2000 lux) with aeration nonstop. Furthermore, cultivation was carried out in 3 L glass jars. Cultivation was carried out using four treatments:

1. Indoor fertilizer media (IFM)
2. Outdoor fertilizer media (OFM)
3. Indoor Guillard media (IGM)
4. Outdoor Guillard Media (OGM)

Cultivation was carried out for 7 days. During cultivation, 10 ml samples were taken daily for OD

measurement using a UV Vis spectrophotometer at a wavelength of 760 nm. pH measurements were carried out daily using a pH meter on the cultivation medium. *Porphyridium cruentum* cultures were taken every day from day 1 to day 6 and filtered using Whatman paper. The wet Whatman paper was weighed and dried in an oven for 6 hours. The dry Whatman paper was then weighed, and the results were recorded. The results of the OD measurements were then processed in the form of graphs, while the OD and dry biomass measurements were displayed descriptively in the table.

## 3. Results and Discussion

*Porphyridium cruentum* is a red microalga with a cultivation media pH of 7. It was cultivated for 7 days on Guillard media and fertilizer under indoor and outdoor conditions. Figure 1 shows the growth of *Porphyridium cruentum* microalgae in different media and environmental conditions.

Growth *Porphyridium cruentum* was highest in indoor fertilizer media (IFM), followed by indoor Guillard media (IGM), outdoor fertilizer media (OFM), and outdoor Guillard media (OGM). Growth *Porphyridium cruentum* on indoor fertilizer media (IFM) had a lag phase on day 1 and a log phase from day 2 to 7. IFM had the highest OD value of 0.246. Growth *Porphyridium cruentum* on indoor Guillard media (IGM) has an OD value of 0.161. IGM had no lag phase but directly log phase from day 1 to 5, then decreased on day 6 and returned on day 7. Growth *Porphyridium cruentum* on outdoor fertilizer media (OFM) increased until day 4 and fluctuated from day 5 to 7. OFM had a lag phase on day 1 and a log phase on days 2 to 4, then decreased on days 5-7. The value of OD of OFM was 0.107. Growth *Porphyridium cruentum* on outdoor Guillard media (OGM) slowly increased until day 5 and decreased until day 7. OD of OGM was 0.056.

Growth of *Porphyridium cruentum* on OFM, OGM, and IFM media has a lag and log phase, whereas OFM only has a log phase. OFM only has a log phase because the initial stock media comes from Guillard media indoors, so there is no change in the growth media. OGM has a lag phase because the microalgae are treated outdoors, so adjustments to the environment are needed. IFM and OFM have a lag phase because the initial stock and cultivation media are different, so the microalgae need time to adapt. The growth phase of microalgae consists of a lag phase, log phase, stationary phase, and death phase. The lag phase is the adaptation phase of microalgae cells due to differences in environmental conditions. The log phase is the cell division phase, where cells increase. The stationary phase is the constant growth phase of microalgae cells. The death phase is the growth of microalgae cells that decreases and dies [27]

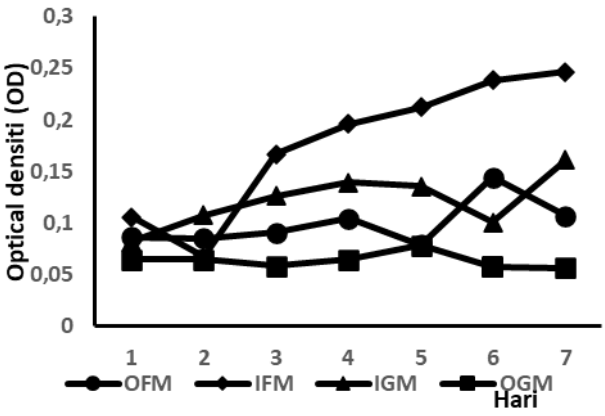


Figure 1. Growth curve *Porphyridium cruentum*

Growth *Porphyridium cruentum* was better indoors than outdoors. It is caused by indoor conditions being more controlled by aspect lighting and temperature than outdoor conditions. Temperature and lighting outdoors are very dependent on the condition of the weather. Temperature cultivation outdoors causes stress on cells microalgae, so their growth becomes disturbed. The lighting outdoors is only from the sun so that when the afternoon lighting is high; however, when the evening is not lighting. High lighting during the day hinders cell microalgae growth. Microalgae are greatly influenced by the environment, including light intensity, temperature, salinity, pH, and aeration [28].

Microalgae *Chaetoceros calcitrans*, which is given treatment intensity 1000, 2000, and 2500 lux light, indicates an improvement in the growth cell *Chaetoceros* up to the intensity of 2000 lux light. However, at the intensity of 2500 lux light, the rate of growth cell *Chaetoceros* is hampered [29]. Microalgae *Thalassemia* sp. cultivated with treatment temperatures 18, 21, 23, and 25 ˚C show the best growth at 21 ˚C, followed by 18, 23 and 25 ˚C. The higher temperature at the microalgae of cultivation caused microalgae growth to be hampered [30]. Fertilizer media's OD value is higher indoors or outdoors than Guillard media's, which caused fertilizer media's higher nitrate content than Guillard media.

The nitrogen content influences the rate of growth of microalgae in the medium. The amount of nitrogen content, too high or low, can hinder cell growth [31]. The growth of microalgae using mixed media urea fertilizer was 95% + Wayne was 5% higher than fertilizer media Walne [32]. Urea fertilizer provides 2 times more nitrogen and triggers growth cell microalgae [33]. The higher the urea fertilizer concentration, the more the growth is [34].

Table 3. pH of medium cultivation

| Parameter | Day | Sample | | | |
|---|---|---|---|---|---|
| | | OFM | IFM | IGM | OGM |
| pH | 1 | 7 | 7 | 7 | 7 |
| | 2 | 7 | 7 | 7 | 7 |
| | 3 | 7 | 7 | 7 | 7 |
| | 4 | 7 | 7 | 7 | 7.5 |
| | 5 | 7 | 7 | 7 | 7.5 |
| | 6 | 7 | 7 | 7 | 7.5 |
| | 7 | 7 | 7 | 7 | 7 |

pH is one of the environmental factors that influences microalgae growth. The pH value of cultivation *Porphyridium cruentum* can be seen in Table 3. The pH value of *Porphyridium cruentum* is 7-7.5 during 7 days of cultivation. The OGM pH value of days 4 to 6 increased from 7 to 7.5, and the down return became seven on the seventh day due to the growth cell *Porphyridium cruentum*, which is slow and experienced decline. Decline cell *Porphyridium cruentum* causes death, so that increases pH.

pH of the cultivation medium microalgae influences its growth. Microalgae Cultivated Chlorella *vulgaris* with pH treatment 6, 7, and 8 shows growth highest at pH 8, 7, and 6 [35]. Death microalgae can cause a pH increase due to termination proton absorption and metabolism, resulting in the necessary net proton influx to maintain a higher pH level during growth [36].

Table 4. Dried biomass of *Porphyridium cruentum*

| Parameters | Day | OFM | IFM | IGM | OGM |
|---|---|---|---|---|---|
| Dry biomass per 5ml | 1 | 37.5% | 27.5% | 44.0% | 30.0% |
| | 2 | 63.0% | 79.6% | 56.5% | 46.5% |
| | 3 | 66.7% | 61.7% | 68.0% | 65.0% |
| | 4 | 62.5% | 45.8% | 58.3% | 54.5% |
| | 5 | 48.1% | 69.6% | 36.0% | 38.5% |
| | 6 | 8.9% | 7.0% | 4.8% | 4.3% |

Biomass dry *Porphyridium cruentum*, the value of which is obtained, fluctuates. Biomass dry *Porphyridium cruentum* can see in Table 4. Biomass IGM dried was improved from day 1 to 3. Later, they declined from 4 to 6 days. OGM biomass was improved from day 1 to 3 and so on the decline. OFM biomass was improved from day 1 to 3 and later decreased. IFM biomass was increased on day 2, decreased on days 3 and 4, increased on day 5, and decreased back on day 6. Biomass of microalga followed the growth of microalga. The highest biomass was in IFM media.

Biomass dry *Porphyridium cruentum* influenced by growth. Biomass value dry follows growth *Porphyridium cruentum.* Lighting and nitrogen in the cultivation medium influence biomass microalgae [37]. Nutrients, pH, salinity, lighting, stirring, and culture models influence biomass microalgae [38].

## 4. Conclusion

The best growth performace in IFM, followed by IGM, OFM, and OGM. Fertilizer media is better for the growth of *Porphyridium cruentum* than Guillard media. pH growth *Porphyridium cruentum* during cultivation 7-7.5. Indoor cultivation is better than outdoor cultivation. The highest biomass was IFM. The best performance in IFM with OD 0,247, pH 7, and biomass 79,6%.

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