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Original Article

ENHANCED SULFATED EXOPOLYSACCHARIDE PRODUCTION IN *PORPHYRIDIUM PURPUREUM* FROM INDONESIA: IMPACT OF CULTURE MEDIUM AND DURATION

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ABSTRACT

Objective: This study investigates the influence of culture medium (Walne and F/2) and culture duration (14 ds and 28 ds) on sulfated Exopolysaccharide (EPS) production in *Porphyridium purpureum* from Indonesia. EPS sulfate has significant biotechnological potential due to its versatile applications, including in nanotechnological applications and optimizing cultivation conditions is essential to maximize yields.

Methods: Cultures of *P. purpureum* were subjected to two distinct culture media, Walne and F/2, and maintained for two different durations, 14 ds and 28 ds. The microalgal biomass was harvested, and sulfated EPS was extracted using established methods. Statistical analysis was employed to assess the significance of differences between the cultural conditions. The novelty of this study lies in the comprehensive examination of the interactive effects of culture medium composition and duration on sulfated EPS production in *P. purpureum*. The findings contribute to our understanding of the dynamic responses of *P. purpureum* to different culture environments.

Results: The results of this research reveal a significant increase in sulfated EPS production in the 28 d culture compared to the 14 d culture.

Conclusion: This outcome underscores the importance of prolonged culture durations for achieving higher yields of sulfated EPS in *P. purpureum*.

Keywords: Duration, Medium, *Porphyridium purpureum*, Sulfated exopolysaccharide

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INTRODUCTION

Microalgae are a diverse assembly of single-celled eukaryotic organisms that develop in a variety of habitats. They are composed of numerous species [1]. With light as their energy source and carbon dioxide as their carbon substrate, they use photosynthesis to produce biomass, which they do ten times more efficiently than terrestrial plants [2]. Microalgae show promise as a renewable feedstock for various applications due to their rapid growth rates [3]. The focus of microalgal production has historically been on energy, but in the present, there is a shift toward biomass production for feed, food, biomedicine, and nutraceuticals [4]. Among these microalgae, *Porphyridium purpureum*, a red microalga, has emerged as a promising candidate due to its unique characteristics and potential for applications in pharmaceuticals, cosmetics, and other industries [5]. Sulfated extracellular polysaccharides, or EPS, are essential substances that microalgae release through their growth and are an integral part of the microalgal life phase [6]. Applications of EPS are numerous and include dietary supplements, cosmetics, and medicines. Outstanding antiviral, anti-inflammatory, antioxidant, immunomodulatory, and anti-tumor properties are displayed by these polysaccharides [7]. Despite its potential, the current production levels of sulfated EPS in *P. purpureum* from Indonesia are suboptimal, limiting its practical utility [8]. This research investigates and overcomes the challenges associated with low productivity, with a specific emphasis on extending the culture duration as a potential solution.

MATERIALS AND METHODS

Microalgae sample

The microalgae sample of *Porphyridium purpureum* originates from the Oceanographic Research Center-BRIN collection. Samples are collected and maintained in the cultural collection at the Biological Research Center-BRIN's Indonesian Culture Collection (InaCC).

Chemical and reagent

Sea water, Culture media (Walne, f/2), 2-Propanol for analysis (Merck, Germany)

Inoculum of microalgae

The culture remains at a constant temperature of 25±0.5 C during all of these stages using a TL lamp daytime with a light period of 12 h dark and 12 h dark. For a period of ten days, microalgae are cultivated in culture media contained in sterilized 40 ml erlenmeyer with glass covers. Subsequently, many microalgae cells were cultivated once more in new growth media within a 250 ml erlenmeyer container. The microalgae subculture is re-established in fresh media in a 1 l sterile glass bottle once it has grown well. The culture was aerated at 100 ml min^{-1} after being transferred to a 1 l glass container. This culture will serve as the research's initial inoculum [9].

Microalgae culture

In sterile one-liter glass vials with culture media, four replicates of the microalgae culture were cultivated. The microalgae *P. purpureum* is cultivated using two types of culture medium: f/2 and walne. Following that, 24 lED bulbs were used to provide artificial light to cultures with an initial density of 10^5 cells/ml. The final luminous intensity of the lights was 4000 lux, and the light was white (daylight) with a photoperiod of 12 h light and 12 h dark. Aeration of the cultures was conducted at a rate of 100 ml. min^{-1}. The study maintained a constant salinity range of 28±0.5 ppt [10]. Two distinct conditions were used for the duration of the culture, requiring 14 ds and 28 ds.

Growth measurement

Every 24 h, the density of microalgae cells was measured under a light microscope with an improved neubauer hemocytometer. Using a 1 ml fixed volume pipette, a 1 ml sample was taken and put into the Improved neubauer hemocytometer, which has two count boxes. Microalgae cell count in a single box; the count is subsequently multiplied by the formula to get the number of individual cells/ml:

Number of individual cells per ml = number of individuals in the count box x 50,000

Both boxes were counted, and the sample was taken three times over. The computation's total outcomes are then averaged.

Biomass harvesting

Centrifugation was utilized to harvest microalgae on the last day of cultivation (10,000 rpm, 20 min, 4 °C). After removing the biomass from the supernatant, the mixture is repeatedly centrifuged and cleaned with distilled water until no salt is remaining (as indicated by 0.1 M AgNO3 solution). Before testing, the biomass is dried via lyophilization and kept at -20 °C. After being collected, the supernatant was kept at 4 °C for EPS extraction [12].

EPS extraction

According to the literature, total EPS extraction occurred Coragliotti *et al.* A gel from EPS had been produced by adding a cold 70% isopropanol solution (ratio 1:2) to the centrifuged supernatant. The EPS gel was subsequently separated by centrifugation, which was done for 10 min at 4 °C and 4000 rpm. Three times through the process, the EPS gain is optimized [13].

Statistical analysis

Every cultivation experiment was conducted independently three times; following that, the mean value and standard deviation (± SD) were determined. The data analysis program used was SPSS 26.0 (ANOVA). When $p<0.05$ occurred, mean differences were determined to have statistical significance [12].

RESULTS

Growth characteristics of *Porphyridium purpureum* under different medium

The growth characteristics of *P. purpureum* under walne and f/2 medium was shown in fig. 1. The production of biomassa and EPS in *P. purpureum* under walne and f/2 medium was shown in table 1.

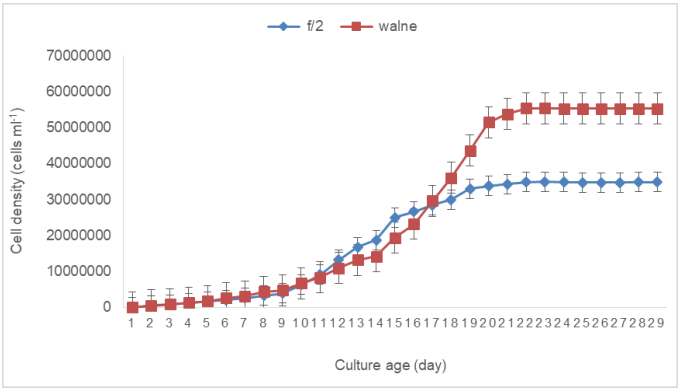

Fig. 1: Growth characteristics *Porphyridium purpureum*. Error bars indicate SD values of three determination.

Table 1: Production of biomassa and EPS in *Porphyridium purpureum* under different medium culture and duration

Type of medium	Biomass weight (g l^{-1})	EPS weight (g l^{-1})
Walne (14 d)	1.12±0.020*	0.16±0.025*
Walne (28 d)	2.56±0.105*	1.28±0.035*
f/2 (14 d)	0.78±0.015*	0.96±0.020*
f/2 (28 d)	1.82±0.075*	1.68±0.085*

The data were presented as the means±standard deviation. (*) denoted significant differences $p<0.05$.

DISCUSSION

The red microalgae *P. purpureum* from Indonesia was cultivated with two different culture media. Fig. 1 depicts the growth of the microalgae *P. purpureum* in two different types of culture medium: walne and f/2. Walne's medium is a nutrient solution that produces a variety of microalgae, including the red microalga *P. purpureum*. This medium typically has a higher nitrogen content than f2 medium, another prevalent medium for microalgal growth. *P. purpureum* expands rapidly due to the higher nitrogen content of Walne's medium. Nitrogen is required for protein synthesis and overall cellular growth, so its presence in the medium promotes faster biomass production by red microalgae [13].

Based on the results of observations on the growth of the number of cells of the microalgae *P. purpureum* which were cultured for 14 and 28 ds using culture media. Different studies show that the cells of the microalgae *P. purpureum* can grow on all types of culture media used, only growth cells in each medium are different. Media culture walne can be the best choice because it tends to be cheaper and has high nitrogen content nutrients that are good for supporting biomass growth microalgae in general [14, 15]. Table 1 describes the biomass production and EPS of the red algae *P. purpureum*. As *P. purpureum* grows in an f/2 medium with low nitrogen levels, it causes an evident response in the microalga, resulting in increased EPS production. F/2 medium is a popular nutrient solution for growing microalgae, including *P. purpureum*, because it contains a balanced combination of nutrients such as nitrogen, phosphorus, and trace elements [16]. Overall, the increase in EPS production by *P. purpureum* in response to decreased nitrogen levels in f/2 medium represents an adaptive response to nutrient limitation. By synthesizing EPS, *P. purpureum* enhances its resilience and survival in nitrogen-poor environments, ensuring its persistence and productivity under challenging conditions. This adaptive behavior underscores the versatility and resourcefulness of microalgae in coping with dynamic environmental cues [17].

Increasing the duration of time the microalga *P. purpureum* is cultivated for a technique known as two-stage cultivation is a

calculated move that will increase biomass production and the amount of EPS [16]. This method uses a step-by-step procedure that is usually split into two separate stages, each of which is optimized for a particular result. Growth phase optimization is the first step, which is aimed at encouraging *P. purpureum* to grow effectively. In order to promote the alga's photosynthetic activity during this phase, environmental factors, including temperature, light intensity, nutrient availability, and carbon dioxide concentration, are closely monitored. Longer cultivation times enable the microalga to divide their cells more frequently, increasing their cell density and total biomass accumulation. The microalga's total productivity is greatly enhanced by this extended growth phase. The second phase is known as the EPS accumulation phase, in which the cultivation conditions are changed to concentrate on producing EPS after the growth phase. In order to cause *P. purpureum* to devote more resources to the synthesis and secretion of extracellular sulfate polysaccharides, environmental factors are changed to create stress conditions. Stressors can be variations in salinity, light intensity, or nutrient limitation. Because this phase lasts longer, more EPS can accumulate, increasing the total yield of these beneficial bioactive compounds [17].

CONCLUSION

In conclusion, two-stage cultivation of *Porphyridium purpureum* shows promise for lengthening the duration of cultivation and increasing the yield of extracellular polysaccharides sulfated and biomass production. This strategy emphasizes the need for continuous study and development to optimize the process for industrial-scale applications and is in line with the growing interest in microalgae as a sustainable source of valuable bioactive compounds.

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AUTHORS CONTRIBUTIONS

Devi Maulina carried out the experiments and wrote the original paper; Abdul Mun'im conceived and planned the experiments; Asep Bayu verified the analytical methods and contributed to sample preparation; Heri Setiawan supervised the findings of this work and contributed to the interpretation of the results; Diah Radini Noerdjito helped supervise the project. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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