PRODUCTION OF SULFATED EXOPOLYSACCHARIDE FROM *SPIRULINA PLATENSIS* GROWING ON PALM OIL MILL EFFLUENT

Muhamad Maulana Azimatun Nur
Chemical engineering UPN "Veteran" Yogyakarta
Jl. SWK 104 Condongcatur, Sleman, Yogyakarta, 55283, Indonesia

ABSTRACT: Sulfated EPS (sEPS) from *Spirulina platensis* is a potential substance that can be utilized as an antiviral and anti-inflamatory. However, the production of sEPS from *S. platensis* growing on POME has not been explored yet. This research investigated the potency of *S. platensis* cultured on palm oil mill effluent (POME) to produce sEPS under various nutritional and environmental factors. The experiment was done by using factorial design to unravel the interactive effect of POME concentrations, salinity, irradiance, and nitrate addition. Results showed that the highest sEPS was found on low light and low nitrate addition. The interactive effect of POME concentration and nitrate addition significantly influenced sEPS concentration. POME, irradiance, and salinity influence sEPS concentration, while nitrate alone did not influence the concentration. By culturing *S. platensis* on POME medium, the lower production cost of sEPS could be achieved.
Keywords: bioproducts, cultivation, microalgae, wastewater

1 INTRODUCTION

Sulfated exopolysaccharide is well known as a secondary product from microorganism such as bacteria and microalgae [1]. Previous report showed that sulfated exopolysaccharide from *Spirulina platensis* is a potential substance as anticoagulant, anti-human immunodeficiency virus (HIV), and anti-herpes simplex virus type 1 [2]. However, the large scale production from *S. platensis* has not well explored due to the low production [3].

The production of extracellular polymeric substances (EPS) from *S. platensis*, which is associated to sulfated exopolysaccharide, is mainly influenced by low light and ligh salinity [3]. To increase the concentration, low light intensity and high salinity need to be applied to obtain high EPS up to 1.03 g/g biomass. However, the production was still using commercial medium which is costly in the large scale production. Previous research reported the production of sEPS from *Phaeodactylum tricornutum* grown on palm oil mill effluent (POME) which could replace synthesis fertilizer [4]. It is hypotesized that the production cost of sEPS from *S. platensis* could be lowered when using POME as medium growth.

The objective of this research was to investigate the potency of *S. platensis* cultured on POME by varying the environmental and nutritional factors and to understand the interaction effect of the factors to obtain high sEPS concentration by using factorial design.

2 MATERIAL AND METHODS

2.1 Material preparations

POME was collected from a small factory in South Sumatra, Indonesia. POME was obtained from a secondary facultative anaerob-aerobic secondary traditional wastewater treatment. POME contained 1300 mg L^{-1} COD, 140 mg L^{-1} total dissolved nitrogen, and 10 mg L^{-1} dissolved phosphate. POME was filtered using GF/C filter paper to reduce total suspended solid before using as the growth medium.

S. platensis was cultured on Zarrouk medium at 28°C, 50 µmol m^{-2} s^{-1}, for 12:12 h. The culture was adapted to the experimental conditions for at least 2 weeks prior to experimentation.

2.2 Experimental analysis

In the first experiment, *Spirulina platensis* was cultured on Zarrouk medium at 28°C and different light intensities (15 - 800 µmol m^{-2} s^{-1}) and nitrate addition (2.5 g L^{-1} and 0.5 g L^{-1}) in a 100 mL Cellstar culture flask. For the second experiment, a factorial design (Table 1) with four factors was applied to understand the interactive effect, which was consisted of POME concentration, light intensity, salinity, and nitrate addition.

Cellstar culture flask (100 mL) with volume 70 mL was used to culture *S. platensis* at different POME fractions. Sea water, reef salt, and or Milli-Q water was used to adjust the salinity and POME concentrations. pH was adjusted to 9 using 0.5 NaOH. Bicarbonate 500 mg L^{-1} was added to provide inorganic carbon source. The culture flasks were placed in a special chamber equipped with a controlled fluorescence lamp and a waterbath which was set at 28°C. Each day, the flask was shaken manually to mix the culture. The growth was monitored each day using spectrophotometer (Hach, DR2400) at 750 nm. At the end of cultivation, the culture was harvested by using GF/C filter paper. The filtrate was kept to determine sEPS concentration.

Table I: Factorial design experiment

Run	POME (%)	Salinity (ppt)	Light Intensity (µmol m^{-2} s^{-1})	Nitrate addition (mg L^{-1})
1	50	15	50	0
2	50	15	50	100
3	50	30	50	0
4	50	30	50	100
5	100	15	50	0
6	100	15	50	100
7	100	30	50	0
8	100	30	50	100
9	50	15	200	0
10	50	15	200	100
11	50	30	200	0
12	50	30	200	100
13	100	15	200	0

14	100	15	200	100
15	100	30	200	0
16	100	30	200	100

2.3 Analysis

2.3.1 Biomass analysis

The biomass of *S. platensis* was collected at the end of cultivation (7 days) by using gravimetric method. GF/C filter paper was used to filter the culture. The filters were washed with 0.5 M bicarbonate. The filter was dried at 85 °C until reached a constant weight.

2.3.2 SEPS production

Sulfated EPS was analysed using alcian blue solution 8GX (Sigma Aldrich) as mentioned previously [4]. A 1 mL of cell-free culture filtrate was mixed with 4 mL 0.5 M acetic acid and 500 µL Alcian Blue 8GX (1 mg mL−1 in acetic acid 0.5M, pH 2.5), then the mixture was vortexed at 2000 rpm for 10 s, and incubated overnight at room temperature. The sample was centrifuged at 5000 rpm for 10 min to remove the debris. The supernatant was read at 610 nm using Hach DR2400. A standard curve was created by using dextran sulfate as the reference.

2.3.3 Statistics

Minitab 18 (trial version) was used to generate and calculate the factorial design. A turkey post hoc test was used to analyse the significance with value of 0.05. First and second experiments were done in three replicates (n=3).

3 RESULT AND DISCUSSIONS

3.1 Effect of light and nitrate addition

S. platensis was cultured on different light intensities and nitrate addition using Zarrouk medium as shown in Fig 1. Result showed that the increasing of light intensity reduce the sEPS content per dry biomass (g g^{-1}).

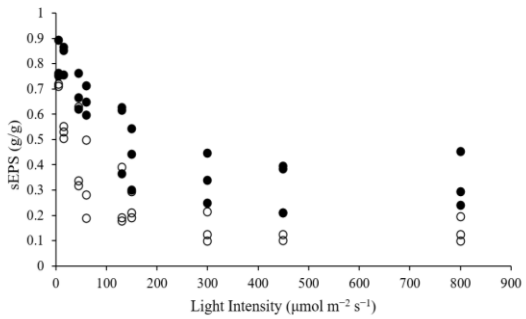

Figure 1: Effect of light intensity and nitrate on sEPS content. White dot indicates 2.5 g L^{-1} nitrate addition, black dot indicates 0.5 g L^{-1} nitrate addition.

The area of light intensity was divided into three phase. Low light phase (15-150 µmol m^{-2} s^{-1}), medium light phase (150-250 µmol m^{-2} s^{-1}), and high light phase (250-800 µmol m^{-2} s^{-1}). The highest sEPS content was found on low light phase (P<0.05). The increasing of light intensity from low to medium phase was significantly lowering the sEPS content (P<0.05). For nitrate addition, high nitrate concentration reduced sEPS content for all light phases. Different nitrate concentrations significantly influenced sEPS content (P<0.05). This result is in agreement to previous researcher who mentioned that low light intensity increased EPS content of *S. platensis* [3]. Soanen [5] reported that EPS content of *Porphyridium marinum* increased at low nitrogen to phosphorus ratio, compared to the higher one. This might be due to the disruption of *S. platensis* growth which is inhibited at low light and low nitrate concentration. S. platensis accumulates more sEPS to protect the cell damage from unvaforable environmental and nutritional conditions. This result is also in agreement with Fig 2. which resulted the increasing of biomass production of *S. platensis* could lower sEPS content. The highest sEPS content (0.90 g g^{-1}) was found on 0.12 g L^{-1} biomass production which was cultured from first experiment.

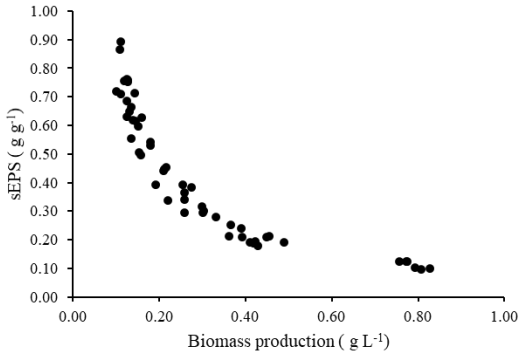

Figure 2: Relationship of sEPS content on biomass production. Data was reordered from the first experiment.

3.2 Interaction effect of POME, salinity, light intensity, and nitrate

From the factorial design calculation, it is revealed that sEPS concentration of *S. platensis* was significantly influenced by the interaction of POME and nitrate addition (p<0.05).

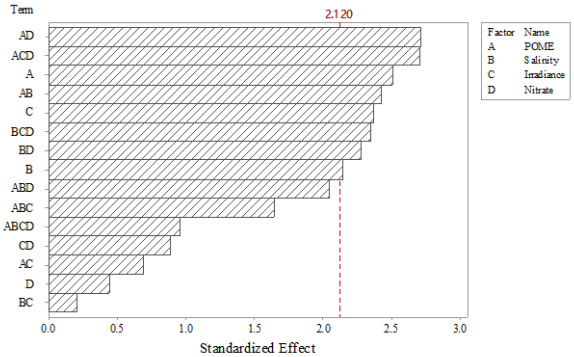

Figure 3: Pareto chart showing the effects of the parameters on sEPS concentration (mg L^{-1}) of *S. platensis*. The vertical line indicates the significance of the effects at 95% confidence level.

All the single factors influenced sEPS concentration except for nitrate alone (Fig 3). The interaction of irradiance and nitrate, the interaction of salinity and nitrate, and the interaction of salinity and irradiance has no significant effect on sEPS concentration. In this experiment, the increasing of POME concentration reduced sEPS concentration, but the biomass was

increased. This is due to the effect of nutrient availability in POME. When higher POME and higher nitrate were supplemented into the culture, the growth and biomass production of *S. platensis* became higher, sEPS production became proportional to the biomass production [4]. The increasing of light intensity from 50 to 150 μmol m^{-2} s^{-1} increased the biomass production as well as sEPS concentration. This indicated that the production of sEPS (mg L^{-1}) is influenced by the biomass production (g L^{-1}) as well as sEPS content (g g^{-1}). Further experiment needs to be done to optimize the sEPS concentration of *S. platensis* cultured on POME medium.

4 CONCLUSION

This finding reported the potency of *S. platensis* grown on POME medium to produce sEPS. High Light intensity and high nitrate concentration significantly reduced sEPS content. The interaction of POME and nitrate influenced sEPS concentration, while the interaction of irradiance and nitrate, the interaction of salinity and nitrate, and the interaction of salinity and irradiance has no significant effect on sEPS concentration.

5 ACKNOWLEDGEMENTS

This work was supported by Lembaga Pengelola Dana Pendidikan, Ministry of Fund, Republik Indonesia.

6 REFERENCES

[1] N.M. Mestechkina, and V. D. Shcherbukhin, Sulfated polysaccharides and their anticoagulant activity: A review, Applied Biochemistry and Microbiology 46, no. 3 (2010), pag. 267-273.

[2] K. Hayashi, T. Hayashi, and I. Kojima, A natural sulfated polysaccharide, calcium spirulan, isolated from Spirulina platensis: in vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities, AIDS research and human retroviruses 12, no. 15 (1996), pag. 1463-1471.

[3] C. Imene, M. Hamdi, A. Doumandji, A. HadjSadok, H. Ben Ouada, M. Nasri, and M. Jridi. Enhancement of extracellular polymeric substances (EPS) production in Spirulina (Arthrospira sp.) by two-step cultivation process and partial characterization of their polysaccharidic moiety- International journal of biological macromolecules 105 (2017), pag. 1412-1420.

[4] N. Muhamad Maulana Azimatun, M. K. Swaminathan, P. Boelen, and A. G. J. Buma, Sulfated exopolysaccharide production and nutrient removal by the marine diatom Phaeodactylum tricornutum growing on palm oil mill effluent, Journal of Applied Phycology 31, no. 4 (2019), pag. 2335-2348.

[5] S. Nastasia, E. Da Silva, C. Gardarin, P. Michaud, and C. Laroche, Improvement of exopolysaccharide production by Porphyridium marinum, Bioresource technology 213 (2016) pag. 231-238.