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Effect of Urea Addition on *Spirulina platensis* Growth for Production of Lipid and Omega-3 Fatty Acids.

Adelia Dwi Nugraha, Abdi Dharma*, Elida Mardiah, and Marniati Salim.

Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Andalas, Kampus Limau Manis, Padang 25163, West Sumatera, Indonesia.

ABSTRACT

In this study, addition of urea was varied in the growth medium of Spirulina platensis. Effect of urea addition was observed on growth of Spirulina platensis. It was used for production of lipids and omega-3 fatty acids. Medium at concentration of 0.200 g/L of urea gave the best growth of Spirulina platensis in the culture. Growth characteristics showed that the generation time, growth rate, and productivity occurred in culture with 0.200 g/L of urea were 2.767, 0.226, and 0.099, respectively. Biomass of Spirulina platensis was harvested at the exponential phase and the lipid content was extracted method of solvent extraction method. The lipid content of Spirulina platensis biomass was 12.92%. Gas Chromatography Mass Spectroscopy (GC-MS) analysis lipid of Spirulina platensis contained 3.03% of eicosapentaenoic methyl ester as one of the omega-3 fatty acids.

Keywords: Spirulina platensis, microalgae, fatty acids

Corresponding author

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INTRODUCTION

Omega-3 fatty acids has several benefits for human health such as in preventing heart disease, healing artherosclerosis disease and also as anti-aging. Some natural fatty acids which are included in the group of omega-3 fatty acids are linolenic acid, alpha-linolenic acid, eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). These omega-3 fatty acids commonly derived from fish oil, but a lot of indications said that omega-3 fatty acids in fish oil actually come from zooplanktons which consume algae. Therefore, microalgaes are considered as one of the best sources of omega-3 fatty acids [1-4].

Spirulina platensis is a cyanobacterium alkalophilic microalgae commonly found in tropical and subtropical regions in the warm body water with the content of high carbonate or bicarbonate, pH, and salinity. It has the optimal growth at pH 9.0 to 10.0 whereas this pH is the most effective to inhibit other microalgae contamination during the process of cultivation [5].

This study was conducted to see the lipid content, especially omega-3 fatty acids in *Spirulina platensis* grown in media with various concentration of urea. It is known that growth medium of microalgae commonly use sodium nitrate, potassium nitrate, and ammonium nitrate as nitrogen source. The production process of growth media were modified by using urea as a nitrogen source to sort out the cost. It is expected that with this condition, the value of *Spirulina platensis* biomass production still increase. Low production costs are expected to enhance the cultivation of *Spirulina platensis* not only in the laboratory and industry needs but also in the household environment as a family alternative dietary supplement [5].

MATERIALS AND METHODS

Materials

Materials used are growth media (containing: $NaHCO_3$, NaCl, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, vitamin B, urea (PT Pupuk Indonesia Persero Group), and NPP), distilled water, urea, chloroform, methanol, and H_2SO_4. Microalgae *Spirulina platensis* was obtained from BBPBAP (Center of Brackish Water Aquaculture Development), Jepara, Indonesia.

Instrumentations

The equipment used are glassware, filter paper, satin, aquarium pumps, air pipes, UV-Vis Spectrophotometer (Thermo Scientific Genesys 20), analytical balance (Kern ALJ 220-4 NM), a pH meter (Lovibond Senso Direct), centrifuge (Health H-C-12 Centrifuge), magnetic stirrer, ultrasonicator (Ultrasonic Cleaner Model No CD-4800), and gas chromatography (GCMS QP 2010 Ultra Shimizu).

Methods

Modification of *Spirulina* Medium

Growth medium used in this study was *Spirulina* Medium by Schlösserwhich which has been modified by replacing $NaNO_3$ with urea as nitrogen source. Medium were autoclaved and cooled to room temperature before use. In this condition, the pH of medium was 9.36 [5].

Spirulina platensis were inoculated into 100 mL of medium at four different concentrations of urea in the medium: 0.200g/L, 0.800g/L, 1.350g/L, and 1.800g/L. Cultures were kept at room temperature (25-30ºC) under solar light irradiation (light and dark photoperiode). The atmospheric air were aerated into the flask through air pump as the source of CO_2. Microalgae growth rate was observed daily by using spectrophotometer at 560 nm [6-8].

Cultivation of *Spirulina platensis* with Various Initial Optical Densities

Spirulina platensis were inoculated into 100 mL medium of 0.200g/L urea with three initial cell concentrations measured as optical density (OD550): 0.078, 0.129, and 0.229. Cultures were aerated and kept

at room temperature (25-30ºC) under solar light irradiation (light and dark photoperiode). Their growths were observed daily using a spectrophotometer at 560 nm [6-7].

Lipid Extraction

The culture at optimum growth rate was harvested during its exponential phase. By filtration method and then the biomass was dried. The lipid was extracted by the modified method of Blight and Dyer with the solvent mixture of $CHCl_3$, MeOH and water at ratio 1:2:0.8 (v/v), 50 mL of solvent was used for 1 gram of *Spirulina platensis* biomass. The mixture was stired for 5 hours and ultrasonicated for 8 minutes to enhance the extraction process. Organic phase as crude lipid was separated from water phase, biomass residues was separated from lipid by centrifugation at 4000 rpm for 10 minutes. Remaining solvent was allowed to evaporate overnight at room temperature [9-10].

Fatty Acid Analysis

Lipids were transesterificated by adding methanol and H_2SO_4 as catalyst. Fatty acids methyl ester in upper layer was separated from the glycerol in bottom one. Methyl ester was analyzed by using GC-MS [11].

RESULTS AND DISCUSSION

Growth of *Spirulina platensis* in Different Consentration of Urea

In this study, the consentration of urea used were 0.200 g/L, 0.800 g/L, 1.350 g/L, and 1.800 g/L. The addition and elemition of nitrogen in the medium were used to see the impact for *Spirulina platensis* growth.

The best growth culture was occured in growth medium with 200 g/L concentration of urea (Figure 1). In this growth media, *Spirulina platensis* had a longer life time and highest absorbance value compared to others. The best generation time, growth rate, and productivity of this culture, with value of each were: 2.767, 0.226, and 0.099. The growth of *Spirulina platensis* in medium containing 1.800 g/L urea was poor growth wich highest absorbance only reach at 0.431 and even the death phase begin on the day 9 [12].

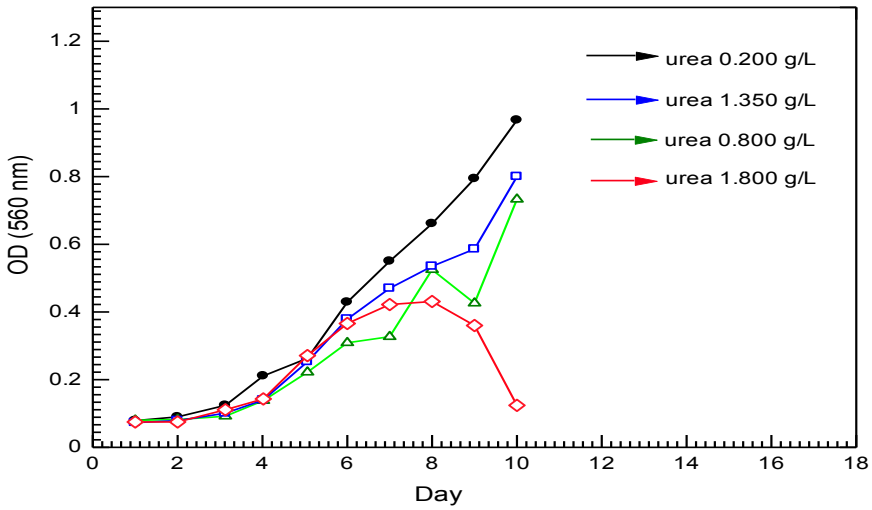

Figure 1: Effect of different concentrations of urea on growth of *Spirulina platensis* for 10 days of cultivation

***Spirulina platensis* with Various Initial Optical Density**

Biomass productivity of *Spirulina platensis*is was directly proportional to the consentration of inoculums shows as optical density (Figure 2). From these data, it can be obtained the generation and the relative growth rate values of various types of microalgae cultures. Generation time was the time required to perform the division cycle.

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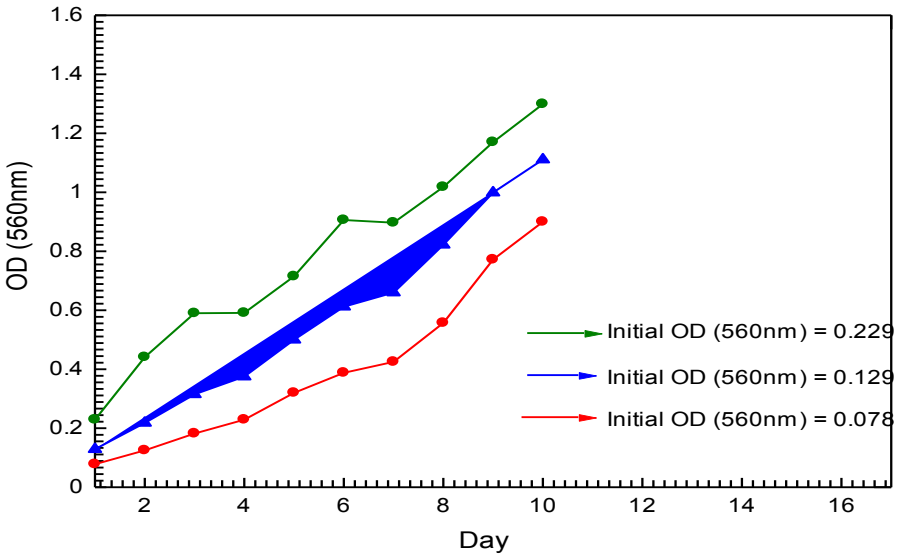

Figure 2: Effect of different initial optical density on growth of *Spirulina platensis* for 10 days of cultivation

Based on the growth characteristics, culture *Spirulina platensis* with 0.078 of initial optical density had the most rapid growth with 2.551 days of generation time. The highest relative growth rate was 0.301 occurs in cultures with 0.129 of initial optical density. Meanwhile, the best productivity was occurred in cultures with 0.229 of initial optical density [13].

Lipid Content

Solvent extraction by Blight and Dyer has been commonly performed to extract the lipids in microalgae. In this process, the difference polarity of the solvent used to separate lipid from the other components. Lipids, as non-polar compound, were distributed into the organic phase, chloroform, which is at the bottom layer. Lipid was extracted from dry biomass of *Spirulina platensis* cultivated in the medium with 200 g/L of urea. It showed that there was 12.92% of lipid contained in *Spirulina platensis* biomass [10].

Fatty Acids Analysis

Lipids were transesterificated to form two layers separating glycerol and fatty acid methyl esters. Fatty acid methyl esters were in the top layer and glycerols were in the bottom. GC-MS analysis showed fatty acid composition of lipid constituent in *Spirulina platensis* biomass. It was found that *Spirulina platensis* contains 3.03% of omega-3 fatty acids in EPA form (Figure 3) from total fatty acid methyl esters, while the hexadecanoic acid and z- 9-octadecenoic acid were dominant fatty acid in the *Spirulina platensis* biomass.

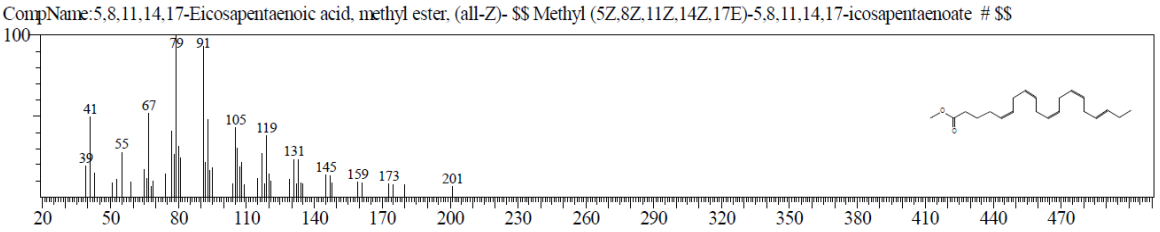

Figure 3: Data analysis of mass sphectroscopy

CONCLUSIONS

From this study, it can be concluded that the suitable modifications medium for the growth of *Spirulina platensis* was with 0.200g/L urea. In this condition, the biomass of microalgae was capable to produce 12.92% of lipids and 3.03% of omega-3 fatty acids in EPA form from total lipid.

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