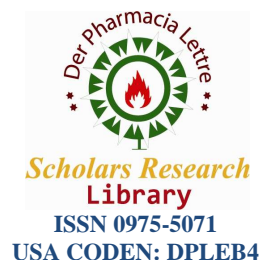




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## Optimization of *Spirulina Platensis* Culture for Antioxidant Production

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### ABSTRACT

*Spirulina platensis* is a unicellular microalgae that has high nutritional value such as proteins, carbohydrates, lipids and carotenoids like  $\beta$ -carotene. The microalgae rapidly grows in Bold Bassal Medium modification and high intensity of light. The aims of this research were to optimize the culture growth and determine antioxidant activity of *S. platensis*. Culture was optimized by adding urea and  $\text{NaHCO}_3$  into the medium and applying dark : light period (12:12 h/h) under solar or fluorescent light (3000 lux). The optimum growth of *Spirulina platensis* was in 0,3 g/L urea, pH 9 at fluoresense light (3000 lux). Antioxidant acivity of various spirulina extract was studied by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals scavenging method. The *Spirulina* radicals scavenging activity of acetone : methanol (1:1) extract was 46,39 %. The acetone:methanol (1:1) extract showed better antioxidant activity than acetone extract. The  $\beta$ -carotene was determined using HPLC in a silica gel column with dichloromethane, acetonitrile, methanol (20:70:10, v/v/v) as a mobile phase. The acetone extract displayed the highest sample area and produced 1.32% of  $\beta$ -carotene out of total biomass.

**Keywords :** *Spirulina platensis*, antioxidant, free radical, HPLC, DPPH

### INTRODUCTION

Free radicals are highly unstable molecules that contain one or more unpaired electrons in atomic or molecular orbitals [1]. Free radicals are also known as reactive oxygen species [ROS] or reactive nitrogen species [RNS]. ROS may lead to stimulation of inflammatory processes, secretion of chemotactic factors, growth factors, proteolytic enzymes, lipoxygenases, and cyclooxygenases, inactivation of antiproteolytic enzymes and activation of oncogenes and transcription factors [2]. Free radicals may come from cigarette smoke, air pollution, and sunlight that can cause "oxidative stress" a process that can trigger cell damage. Oxidative stress is thought to play a role in a variety of diseases including cancer, cardiovascular diseases, diabetes, Alzheimer's disease, Parkinson's disease, and eye diseases such as cataracts and age-related macular degeneration [3-7].

Proteins are the major targets for ROS because of their high overall abundance in biological systems. Since proteins are primarily responsible for most of cell activities, their peroxidative damage by ROS is of particular importance. It has been estimated that proteins can scavenge the majority [50%–75%] of reactive species generated [8]. In order to reduce free radical damage to the human body, synthetic antioxidants are used for industrial processing at the present time. However, the most commonly used synthetic antioxidant has been suspected of being responsible for liver damage and carcinogenesis [2]. This is essential to develop and utilize effective and natural antioxidants that can protect the human body from free radicals and retard the progress of many chronic diseases.

To protect cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals [9].

**These components include:**

1. Nutrient-derived antioxidants like carotenoids including  $\beta$ -carotene, ascorbic acid (vitamin C).
2. Tocopherols and tocotrienols (vitamin E), and other low molecular weight compounds such as glutathione and lipoic acid.
3. Antioxidant enzymes, e.g. superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radicals quenching reactions.
4. Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.
5. Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

Vitamin C, vitamin E, and  $\beta$ -carotene are among the most widely studied dietary antioxidants [10].  $\beta$ -carotene and other carotenoids are believed to provide antioxidant protection to lipid-rich tissues. Research suggests  $\beta$ -carotene may work synergistically with vitamin E [11; 12].  $\beta$ -carotene accounts for 80 % of the carotenoids present in *S. platensis* [13].

The generation of biomass by photosynthetic *S. platensis* cultures depend on environmental factors including nitrogen concentration, light intensity, and pH [14-16]. Urea as a source of nitrogen is cheaper than nitrate [17]. The aim of this study were to assess the effect of nitrogen concentration, intensity of light, and pH of *S. platensis* in culture glasses.

## MATERIALS AND METHODS

### Test Chemical

*S. platensis* was obtained from BBPBAP (Balai Besar Pengembangan Budidaya Air Payau) Company, Indonesia in form living cell. Bold basal medium, sodium bicarbonate, urea, aquadest, ascorbic acid, acetone, 1,1-diphenyl-2-picryl-hydrazyl free radicals, methanol, acetonitrile, dichloromethane.

### Culture Condition

The *S. Platensis* cultures were maintained in 500 mL glass which is containing 300 mL bold basal medium for each. Cultures was stirred by bubling air at room temperature and keep under fluorescent of 3000 lux with photoperiode dark : light (12:12 h/h) and sunlight for 19 days.

Productivity of biomass was calculated by measuring absorbance of cells. The absorbance of cell was determined by using spectrophotometer UV-vis (Genesys 20 Thermoscientific). The growth of the cell was cotrolled once in two days.

The optimum variation of this method was used for the next step. The following variations including the initial absorbance of cells were moderated by using spectrophotometer. The wavelength used was 560 nm. The pH of the cultures were measured by pH meter and continued by adding  $\text{NaHCO}_3$  gradually until gotten pH 8 and 9. Urea was used as a nitrogen source in this process.

### Antioxidant activity

Free radical scavenging activity of different extract using acetone and acetone: methanol (1:1) from *S. platensis* was measured by DPPH method. In brief, 100  $\mu\text{L}$  of DPPH 0.1 mM was added to 100  $\mu\text{L}$  *S. platensis* extract into 48-wells microplate. The concentration of *S. platensis* was variated (500, 250, 125, dan 62.5  $\mu\text{g/mL}$ ). The mixture was incubated using room temperature for 30 min. The absorbance was measured at 517 nm by using spectrophotometer UV-vis (Shimadzu, Japan) [13]. The positive control used ascorbic acid and the experiment was done in triplicate [16].

$$\% \text{ Inhibition} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times 100 \%$$

### HPLC Measurement

The homogeneity and the purity of  $\beta$ -carotene were checked by HPLC (Shimadzu L17844, Japan) reverse phase 25 cm, C18 column with isocratic system consisted methanol: acetonitrile: dichloromethane (10:70:20, v/v/v) at flow rate 1.0 ml/ min and the column temperature was 25°C. The identification of  $\beta$ -carotene was performed by comparing retention time.  $\beta$ -carotene was monitored at 450 nm with a UV-visible detector (Shimadzu, Kyoto, Japan).

**RESULTS AND DISCUSSION**

**The growth of *Spirulina platensis***

The growth of *S. platensis* cell in 300 mL culture using BBM standard under sunlight was showed in figure 1. The other growth curves were the same cultural cells among different condition (figure 2-5). The higher of absorbance of cells was selected for further studies.

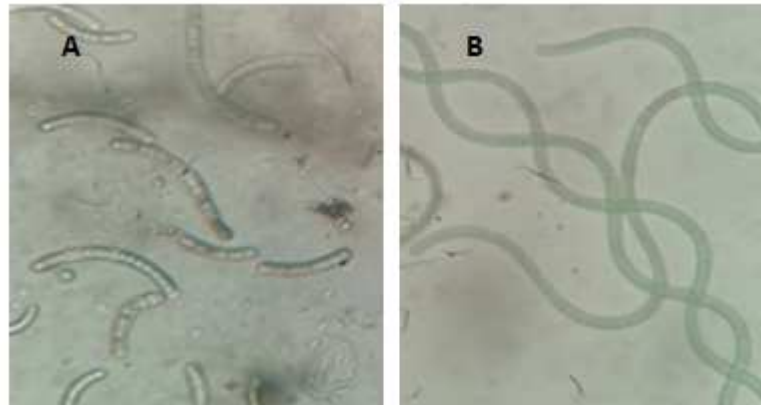


Figure 1. Morphology of *S.platensis* cells; *S.platensis* at second day (A). *S. platensis* at tenth day (B)

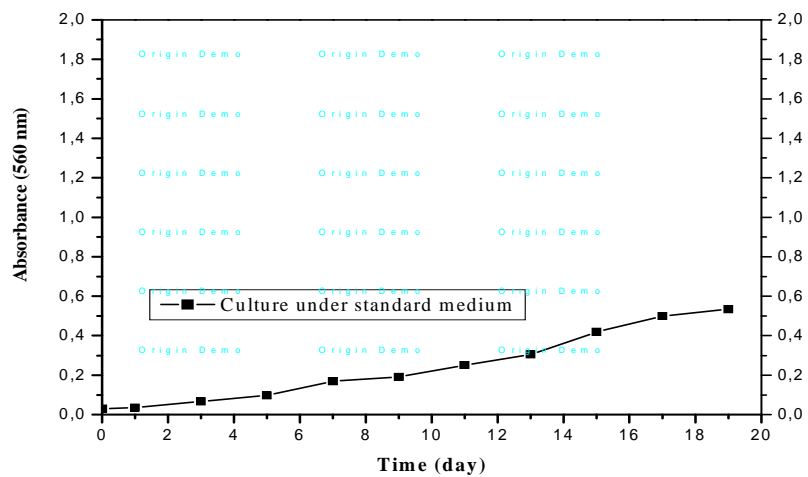


Figure 2. Shows the growth of *S. platensis* cell under sunlight using BBM standard

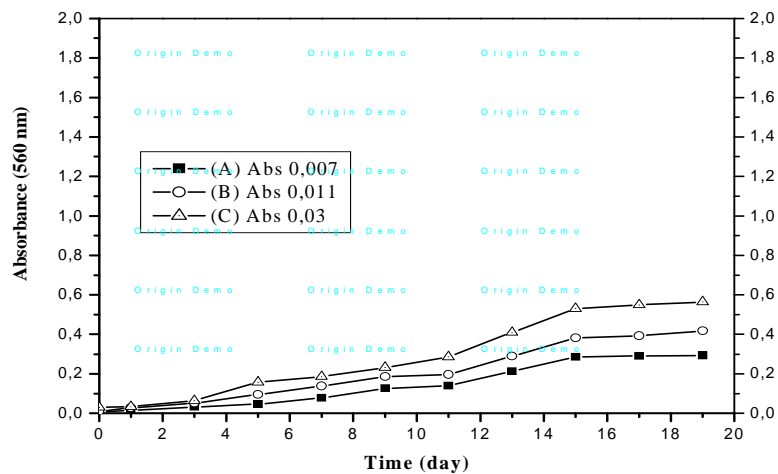
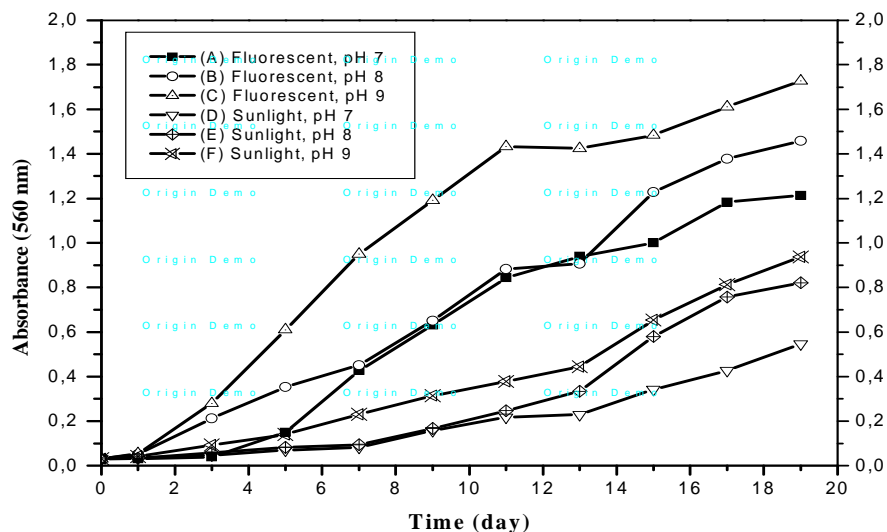
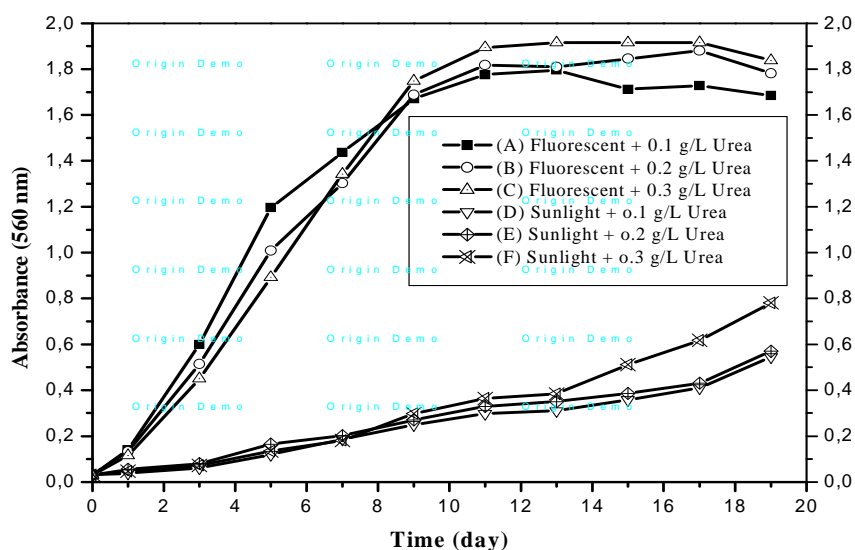


Figure 3. Effect of initial absorbance by using spectrophotometer 560 nm. *S. platensis* in culture glasses under natural sunlight. (A) initial absorbance was 0.007; (B) initial absorbance was 0.011; (C) initial absorbance



**Figure 4.** Effect of initial  $\text{NaHCO}_3$  concentration on *S. platensis* cultivated in glass. (A-C) cultivated under fluorescent light (3000 lux); (A) culture when pH was 7; (B) culture when pH was 8; (C) culture when pH was 9. (D-F) cultivated under sunlight; (D) culture when pH was 7; (E) culture when pH was 8; (F) culture when pH was 9



**Figure 5.** Effect of initial urea concentration on *S. platensis* cultivated in glass. (A-C) cultivated under fluorescent light (3000 lux); (A) culture using 0.1 g/L of urea; (B) culture using 0.2 g/L of urea (C) culture using 0.3 g/L of urea. (D-F) cultivated under sunlight; (D) culture using 0.1 g/L urea; (E) culture using 0.2 g/L of urea (F) culture using 0.3 g/L of urea

Culture of *S. platensis* was grown in modified medium various urea which was each containing nitrogen. After 9 days of cultivation at 3000 lux fluorescence light, the absorbance increased up to 1.7 and 0.25 under sunlight respectively (Fig. 5). Light intensity was an important factor to maximize the conversion of incident light energy to algae biomass. Zainul *et al* reported (2015) light intensity, in order to combination of, from indoors light (sunlight enter to the room) and illumination of room fluorescence light, which intensity region between 774-2000 lux(18), lower than 3000 lux fluorescence light.

#### Antioxidant Test

The *S. platensis* acetone: methanol (1:1) extract of this microalgae showed better antioxidant potential when compare to *S. platensis* acetone extract. Antioxidant activities of various *S. platensis* extract were studied by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals scavenging method as showed in figure 6.

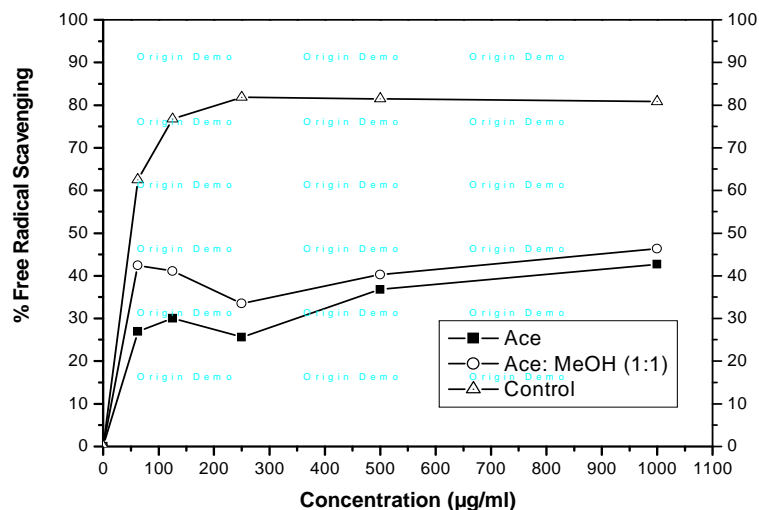


Figure 6. Effect of initial urea concentration on *S.platensis* and antioxidant activities

### HPLC analysis of $\beta$ -carotene

The carotenoid compound especially  $\beta$ -carotene obtained from *S. platensis* produced several major peaks which were separated within 23.08 min (Figure 7.A) and 24.42 min (Figure 7.B) through a C18 column showed the available  $\beta$ -carotene. The detectable of  $\beta$ -carotene and other carotenoid undetectable were showed (Figure 7)

The purity calculated as the percentage of the  $\beta$ -carotene peak area was 2.58 and retention time was 23.08 whereas the acetone extract displayed the highest sample area and produced 1.32% of  $\beta$ -carotene out of total biomass (Figure 7.A).

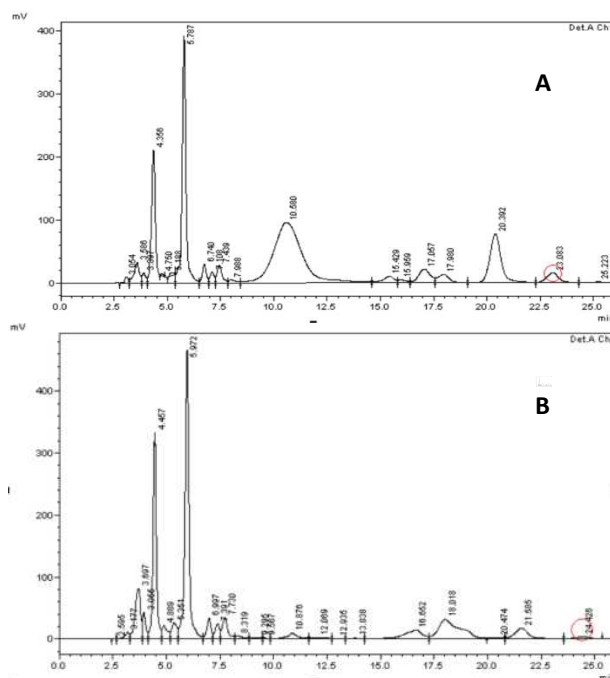


Figure 7. HPLC profile of total carotenoid extract from *S. platensis* extracted using acetone (Figure 7.A) *S. platensis* extracted using acetone: methanol (Figure 7.B)

### CONCLUSION

The carotenoids are widely used as colorants, they also play an important role as a precursors of vitamin A and antioxidant. The optimum growth of *Spirulina platensis* was in 0.3 g/L urea, pH 9 at fluorensense light (3000 lux). Antioxidant activity of various spirulina extract was studied by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals scavenging method. The *Spirulina* radicals scavenging activity of acetone:methanol (1:1) extract was 46.39 %. The

acetone:methanol (1:1) extract showed better antioxidant activity than acetone extract. The  $\beta$ -carotene was determined using HPLC in a silica gel column with dichloromethane, acetonitrile, methanol (20:70:10, v/v/v) as a mobile phase. The acetone extract displayed the highest sample area and produced 1.32% of  $\beta$ -carotene out of total biomass.

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