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Growth and Biochemical Composition of *Spirulina platensis* Geitler in Summer Period Under the Conditions of Çanakkale, Turkey

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In present study the growth of *Spirulina platensis* Geitler in summer period under the conditions of Çanakkale, Turkey, was investigated. *S. platensis* was cultivated in raceway ponds and the cultures were kept in greenhouse. Accordingly, the percentages of chlorophyll *a* and phycocyanin pigments in the biomass were 0.93 ± 0.17 and 14.86 ± 0.99, respectively. Protein accounted for 59.20 ± 2.02 % whereas total lipids were 6.83 % of the dry weights. As a result, the cultivation of *S. platensis* in Çanakkale was successfully carried out. The climate in Canakkale is colder for outdoor cultivation of *S. platensis* compared to the other places where *Spirulina* is commercially produced in open ponds. However, the use of greenhouse was found to be effective to keep the culture temperature higher.

Key Words: *Spirulina platensis*, Open pond, Growth, Biochemical composition.

INTRODUCTION

Microalgae are important organisms producing valuable metabolites such as pigments, proteins and vitamins for various applications[1-4]. *Spirulina platensis* Geitler, a filamentous cyanobacterium, is the major microalgal species cultivated in open ponds all over the world because of its high protein, pigments (especially phycocyanin), fatty acids, vitamins and minerals contents[5-8].

S. platensis can be cultivated in different types of culture systems, *e.g.*, open ponds[9], tubular photobioreactors[10] and inclined glass panel photobiorectors[11]. *S. platensis*, however, is usually cultivated in large-scale raceway type open ponds due to high temperature (35-37 ºC) and pH (8.5-11.0) optima[5], so that the contamination risk of the species in outdoors is low. Low capital investment and free light energy from sun are the other reasons why open ponds are mostly preferred for *Spirulina* spp. cultivation[12]. In addition, taking the *Spirulina* ponds in a greenhouse covered with transparent polyethylene nylon sheets is a common application to keep the temperature high[13].

In this preliminary study, the growth of *S. platensis* under climatic condition of Çanakkale, Turkey, was observed. In addition, the changes in some biochemical parameters such as protein, phycocyanin, chlorophyll *a* and total lipids were examined.

EXPERIMENTAL

The starter culture of *Spirulina platensis* M2 was obtained from Giuseppe Torzillo, Istituto per lo Studio degli Ecosistemi of Florence, Italy. The cells were cultivated in two identical raceway ponds (8.5 m × 1.6 m each), which were in a greenhouse covered with transparent polyethylene sheets, between 19.06.2007 and 25.07.2007. The experiment was carried out in Çanakkale, Turkey and the cells were grown in Zarrouk medium[14]. The depth of the culture in the pond was kept at 10 cm and the flow rate was achieved by paddle-wheels to be 30 cm s^{-1}. Temperature regulation or additional illumination was not provided to the cultures. The cells were filtered through a 60 µm screen to harvest and dried in an oven at 35 ºC. Diurnal changes in temperature and pH were monitored by YSI 556 MPS multi-probe system (YSI Inc., USA). The light intensity in the greenhouse was measured by LI-250 lightmeter (LiCor, USA) in µmol photons m^{-2} s^{-1}. The data were measured every hour between 09:00-17:00 in a day.

Absorbance values, which were measured at 680 nm (Abs 680) and pigment extracts were read in a Jasco UV Spectrophotometer. For dry weight measurements, 10 mL sample was filtered through a pre-dried and pre-weighed GF/C Whatmann filter papers (Whatmann, Maidstone, UK) in duplicate. The samples were rinsed well with distilled water to remove the chemical load on the biomass caused by the nutrient medium. Dry weights were calculated in mg L^{-1} after the filter papers were dried in an oven at 80 ºC overnight. Chlorophyll *a* was extracted in methanol and calculated according to the Bennett and Bogorad[15]. For protein amount, 0.5 g dried *Spirulina* biomass was used and found according to Kjeldahl method[16]. Water soluble phycocyanin pigment was extracted from the biomass by phosphate tampon (pH 7.0) and the supernatant was measured using 615, 652 and 750 nm wavelengths[17]. In order to determine total lipids, Folch method was used[18].

RESULTS AND DISCUSSION

The experiment started with a biomass concentration of 720 mg/L and the culture was harvested 4 times during the experiment by the increase of biomass (Fig. 1). In the maintenance of algal mass cultures, determination of biomass concentration is one of the most important routine procedures. In this respect, the spectrophotometric measurement at 680 nm (Abs 680) instead of dry weight measurement may be beneficial to save time[19], which is especially important for commercial plants. The relationship between dry weight and Abs 680 showed that there is a strong correlation (Fig. 2).

Temperature and pH are the key factors for a healthy *Spirulina* culture. The temperature below 15 ºC inhibits the growth of *S. platensis*[20]. In addition, *S. platensis* is an organism naturally grows in alkaline waters. A decline in pH may cause the culture to be contaminated by other opportunist algae or bacteria. In the culture, minimum and maximum temperatures were 23.6 and 33.8 ºC, while pH values were 8.94 and 10.72, respectively. With this respect, no *Chlorella* sp. (Chlorophyceae)

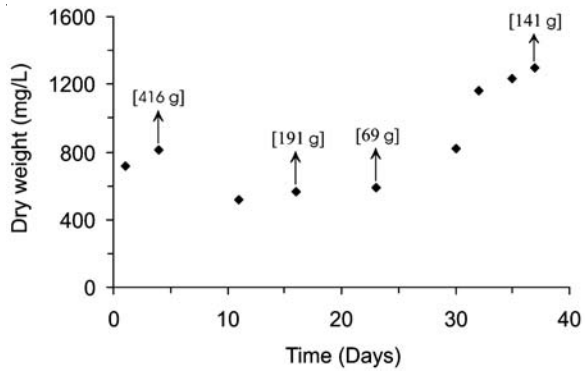

Fig. 1. Biomass concentration of *S. platensis* cultivated in raceway ponds (Arrows indicate the harvest time and amount in the brackets)

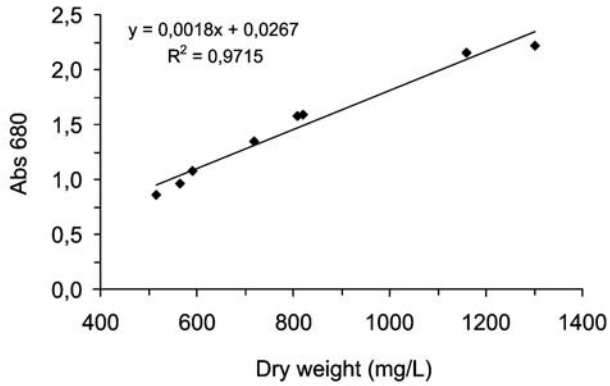

Fig. 2. Relationship between dry weight and Abs 680

contamination, which is the main contaminant of *Spirulina* cultures[21], was observed in microscopic examinations. As for light intensity, minimum and maximum values varied between 571 and 1416 µmol photons m^{-2} s^{-1}, respectively. The length of the filaments was measured 136.39 ± 30.14 µm.

The changes in amounts of chlorophyll *a* and phycocyanin pigments were shown in Table-1. Accordingly, the percentages of these pigments in the biomass were 0.93 ± 0.17 and 14.86 ± 0.99, respectively. The mean protein percentage was 59.20 ± 2.02 (Table-1). The protein content of the biomass in the experiment was higher than that found by Jimenez *et al.*[22]. Protein amount may be affected by some culture conditions such as temperature[23] and light[24]. The protein content of the biomass in present experiment was found reasonable since the production was performed in open ponds outdoors and temperature was not controlled. In general, cyanobacteria are typically poor organisms in fatty acids and may accumulate the lipids between 6-13 % of dry weight[5]. Total lipids in present experiment constituted 6.83 % of dry weight.

TABLE-1
CHANGES IN BIOCHEMICAL PARAMETERS OF *S. platensis*

Parameters	Minimum	Maximum	Mean ± Standard devation
Chlorophyll a (mg/L)	3.86	9.06	6.28 ± 1.71
Phycocyanin (mg/L)	81.27	191.27	112.86 ± 39.94
Protein (%)	57.85	61.52	59.20 ± 2.02

In conclusion, the cultivation of *S. platensis* was successfully carried out during 37 d representing summer period in Çanakkale, Turkey. Although Çanakkale has a colder climate compared to the other places where commercial open pond cultures are performed, the use of greenhouse was effective to increase the culture temperature.

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