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# The Growth of *Spirulina platensis* in Different Culture Systems Under Greenhouse Condition

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**Abstract:** We aimed in this experiment to compare the growth characteristics of *Spirulina*, which was cultivated in different culture vessels under greenhouse condition. Three types of culture vessels, i.e. transparent jars, polyethylene bags and raceway ponds, were used in the experiment. The jar cultures supported higher cell densities due to their higher culture temperature compared to the others. The dry weight amount in jar cultures was 0.99 g $L^{-1}$ at the end of the experiment, while it was 0.5 g $L^{-1}$ in the others. Specific growth rates were found to be 0.32, 0.21 and 0.20 day-1 in the jar, bag and pond cultures, respectively. The protein levels measured at the end of the experiment were 33.4, 54.5 and 58.3% for the jar, bag and pond cultures, respectively. The reason for the much lower protein amount in jar cultures was interpreted as the depletion of the nitrogen in the culture medium as a result of faster growth and the prolonged steady state. We concluded that the use of small volume cultures would increase the temperature faster, which is the main factor hindering growth especially in the winter period. Moreover, the use of short light-path lengths in addition to the smaller volumes in the cultures would support a higher productivity.

Key Words: *Spirulina*, mass culture, culture vessels, outdoors, greenhouse

## *Spirulina platensis*'in Sera Koşulunda Farklı Kültür Sistemlerinde Büyütülmesi

**Özet:** Bu denemede, bir sera içinde bulunan farklı kültür düzeneklerinde üretilen *Spirulina*'nın büyüme karakteristiklerinin karşılaştırılması amaçlandı. Denemede, şeffaf bidonlar, polietilen torbalar ve raceway tipi havuzlar olmak üzere üç tip kültür düzeneği kullanıldı. Diğerlerine nazaran kültür sıcaklığının daha yüksek olması nedeniyle, bidon kültürlerinde daha yüksek hücre yoğunluğuna ulaşıldı. Deneme sonunda kuru ağırlık miktarı bidon kültürleri için 0.99 g $L^{-1}$ iken diğerlerinde 0.5 g $L^{-1}$ idi. Spesifik büyüme hızları bidon, torba ve havuz kültürleri için sırasıyla 0.32, 0.21 ve 0.20 gün-1 olarak bulundu. Deneme sonunda ölçülen protein miktarları ise bidon, torba ve havuz kültürleri için sırasıyla % 33.4, % 54.5 ve % 58.3 olarak bulundu. Bidon kültürlerindeki protein miktarlarının diğerlerine göre çok daha düşük bulunmasının nedeni, büyümenin daha hızlı olması ve kültürün durgunluk safhasında fazla kalması nedeniyle ortamdaki azotun tüketilmesi şeklinde yorumlandı. Sonuç olarak, küçük hacimli kültür sistemlerinin kullanılması, özellikle kış aylarında büyümeyi engelleyen en önemli faktör olan sıcaklığı arttıracaktır. Ayrıca, kültürlerde küçük hacim yanında kısa ışık yolu uzunluklarının da kullanımı daha yüksek bir üretim sağlayacaktır.

Anahtar Sözcükler: *Spirulina*, yığın kültür, kültür düzenekleri, dış ortam, sera

## Introduction

Microalgae are the organisms capable of producing valuable metabolites, such as pigments, proteins and vitamins for feed additive, pharmaceutical and nutraceutical purposes (1-4). Of all the microalgae, *Spirulina platensis* is the most popular in microalgal biotechnology with respect to its being an easy to grow species and having a simple harvest and drying process. Filamentous microalga *S. platensis* (cyanobacteria) has been produced commercially all over the world due to its high content of protein (up to 70%), pigments (especially the blue pigment phycocyanin), essential fatty acids (e.g., γ-linolenic acid), vitamin $B_{12}$ and minerals (5-8). In addition, it is successfully used in aquaculture and poultry industries as well (9-10).

High pH and temperature are the key factors for large scale *Spirulina* cultures outdoors. The optimal temperature for *Spirulina* culture is in the range of 35-38 ºC. In addition, *Spirulina* requires relatively high pH values between 9.5-9.8 (4), which effectively inhibit the contamination of most algae in the culture. In this respect, high amounts of sodium bicarbonate must always be present in the culture medium to sustain the high pH, and prevent fluctuations. Zarrouk medium,

which is rich in bicarbonate, has successfully served as a common culture medium in *Spirulina* cultures for years (11).

Different types of culture systems, e.g., open ponds (12), tubular photobioreactors (13), inclined glass panels (14), and the like, are used in the culture of *Spirulina*. However, on a commercial scale, *Spirulina* is mostly produced in raceway type open ponds for various reasons, such as low capital investment and free light energy from the sun (15). Shallow *Spirulina* ponds can be covered by transparent polyethylene nylon to keep the temperature high and to reduce the contamination risk (16).

In our experiment, the cultures were grown in a greenhouse to increase the temperature in three different vessels: open ponds, polyethylene bags and transparent jars. In this respect, we aimed at comparing the growth of *Spirulina* in various culture vessels placed in a greenhouse.

## Materials and Methods

The experimental organism *Spirulina platensis* M2 was obtained from Giuseppe Torzillo, Istituto per lo Studio degli Ecosistemi of Florence, Italy. The cells were grown in drinking water with Zarrouk medium (11), and the macronutrients were added into the culture at half strength. The experiments were carried out in a greenhouse covered by transparent polyethylene sheets. The greenhouse included two identical raceway ponds (8.0 m x 1.5 m), and the cultures in the ponds were circulated by paddle-wheels. The greenhouse, which was East-West oriented, was located in the Dardanos Campus of Çanakkale Onsekiz Mart University. Three types of culture devices were used in the experiment: 20 L cylindrical transparent jars (height: 40 cm, diameter: 27.5 cm), 300 L cylindrical polyethylene bags (height: 140 cm, diameter: 50 cm) and 2500 L polyester covered raceway ponds. The jar and bag cultures were isolated from the ground by styropor plates to prevent the heat exchange. The depth of the pond culture was kept at 25 cm and the flow rate was 30 cm $s^{-1}$. No temperature regulation or additional illumination was provided to the cultures - the cells were merely exposed to outdoor conditions in the greenhouse for 20 days between 19.10.2005 and 07.11.2005. The circulation of the cultures was achieved by paddle-wheels in the ponds and by bubbling air in the transparent jars and polyethylene bags at the rate of 0.5 L $min^{-1}$ L $culture^{-1}$. The temperature, salinity, dissolved oxygen level and pH values were monitored by a YSI 556 MPS multi-probe system. Diurnal alteration of light (PFD) both outdoors and in the greenhouse was measured by a LI-250 light-meter (LiCor) in µmol photon $m^{-2}$ $s^{-1}$.

Firstly, the filaments were grown in the pond and the culture was increased in volume for 10 days. After enough density was attained in the pond, the culture was transferred to the experimental vessels in order to achieve the same cell density and nutrient concentrations in the groups. The initial cell density was 0.12 g $L^{-1}$ in all the groups, corresponding to a mean cell count of 4000 filament $ml^{-1}$. Absorbance values at 680 nm and pigment extracts for chlorophyll *a* analysis were read in a Jasco UV Spectrophotometer. For dry weight (DW) measurements, a 25 ml sample was filtered through pre-dried and pre-weighed GF/C Whatman filter papers in duplicate. The samples were rinsed well with distilled water to remove the chemical load on the biomass caused by the nutrient medium. DWs were calculated in g $L^{-1}$ after the filter papers were dried in an oven at 105 ºC for two hours. Chlorophyll *a* was extracted in methanol, and calculated according to the Bennett and Bogorad (17). Filaments were counted by triplicate samples in a channel type counting chamber. Specific growth rate ($\mu$) and doubling time (d.t.) were calculated as in the following equation:

$$\mu \text{ (cell day}^{-1}) = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \qquad \text{d.t.(day)} = \frac{\ln 2}{\mu} = \frac{0.693}{\mu}$$

$X_2$ and $X_1$ represent the biomass concentrations at the times $t_2$ and $t_1$, respectively.

As for protein determination, the Kjeldahl method was used. After filtration, samples were dried and grounded by an IKA grounder (28,000 rpm). The crude protein content was calculated by multiplication of the nitrogen (N) amount by the coefficient 6.25.

## Results

The experiment was, in general, conducted on clear days. Although the temperature outdoors was reduced by the northeast wind, a characteristic wind in the area, from 22 ± 2.3 ºC to 15 ± 3.4 ºC after day 12, it remained almost the same in the greenhouse due to penetrating sunbeams. The temperature and light variations on a clear day characterizing the season are shown in Figure 1 and Figure 2.

In the experiment, all the groups were initiated with 0.12 g $L^{-1}$ in DW. The fast increase in DW continued until the 6$^{th}$ day in the bag and pond, while it increased until the 8$^{th}$ day in the jar. After the cells were limited by light, the growth was almost constant showing a slight increase (Figure 3). While the DW values at the end of the experiment were 0.99, 0.52 and 0.49 g $L^{-1}$, the volumetric productivities were 0.87, 0.40 and 0.37 g $L^{-1}$ for the jar, pond and bag cultures, respectively. No significant change was observed between the pond and bag cultures in terms of DW values ($P < 0.05$). Changes in the chlorophyll *a* molecule in the groups, which is another important growth parameter, are shown in Figure 4. Jar and bag cultures increased in chlorophyll until days 11 and 14, reaching the maximum levels of 5.71 and 4.33 mg $L^{-1}$, respectively, and then slightly decreased. However, the pond culture showed a constant increase, indicating that the pond culture grew actively in the whole experiment period.

Similarly, the absorbance readings at 680 nm indicated the same pattern as the DW measurements (not shown). In addition, % Transmittance of light, which has a negative correlation with absorbance, showed that the

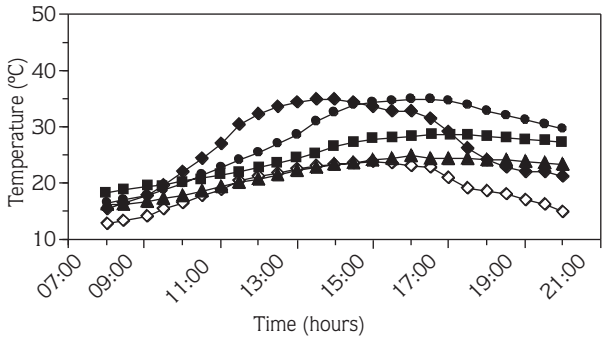


Figure 1. Diurnal temperature variations on a clear day characterizing the period of the experiment in transparent jars (●), PE bags (■), raceway ponds (▲), greenhouse (◆), and outdoors (◇).

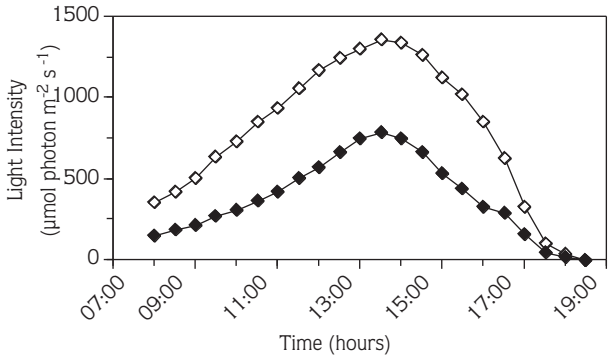


Figure 2. Diurnal changes in light intensity on a clear day characterizing the period of the experiment in greenhouse (◆) and outdoors (◇).

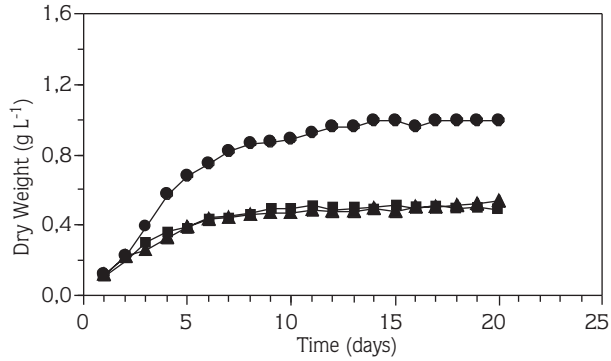


Figure 3. Changes in dry weight amounts of *Spirulina platensis* cultivated in transparent jars (●), PE bags (■) and raceway ponds (▲).

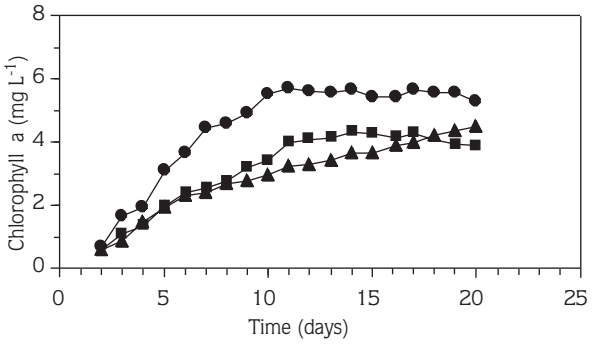


Figure 4. Changes in chlorophyll *a* amounts of *Spirulina platensis* cultivated in transparent jars (●), PE bags (■) and raceway ponds (▲).

light passing through the culture was significantly reduced on the 6th day for the jar culture, and on the 10th day for the bag and pond cultures. The corresponding DW amounts at the points on which the light transmittance started to lessen were 755, 499 and 475 mg $L^{-1}$ for the jar, bag and pond cultures, respectively (Figure 5).

The specific growth rates ($\mu$), doubling times (*d.t.*), and protein amounts on DW basis, average temperature (T), dissolved oxygen (DO) and pH values are given in Table 1. The highest total protein content was found in the pond culture. The mean DO values in the cultures increased every day with the increase in the biomass. While the oxygen production in the jar culture was higher than in the others until the 8th day, when the increase in growth ended, it started to decrease in the following days, especially when compared to the bag culture. The DO levels in the cultures were found to be 16.4, 25.7 and 19.7 mg $L^{-1}$ at the end of the experiment for pond, bag and jar cultures, respectively. The mean pH values of the cultures increased every day as well, and the values were found to be 9.9, 9.9 and 11.2 at the end of the experiment for pond, bag and jar cultures, respectively. In addition, the color of the jar cultures turned to green from blue-green, which is characteristic for *Spirulina*, after day 13.

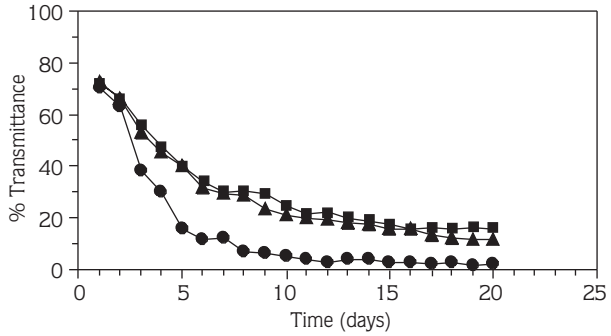


Figure 5. Changes in % Transmittance readings of *Spirulina platensis* cultivated in transparent jars (●), PE bags (■) and raceway ponds (▲).

## Discussion

Diurnal temperature and light intensity cycles are given in Figure 1 and Figure 2. The light intensity of the sun was low in the season when the experiments were carried out. While the midday light intensity outdoors is as high as 2000 µmol photon $m^{-2}$ $sn^{-1}$ in the summer period when sunbeams enter the earth most directly, it was just 1355 µmol photon $m^{-2}$ $sn^{-1}$ at the end of October. This is one of the most important reasons explaining the lower productivity of *Spirulina* when compared with that reported in open ponds (18). The other major factor responsible for the low productivity was the below-optimum temperature. As stated by many authors, the optimum temperature of *Spirulina* is in the range of 35-40 ºC (4, 11). As can be seen by the comparison of Figure 1 and Figure 3, the biomass increase strongly correlated with the temperature of the culture.

Nutrient deficiency, especially nitrogen (N), may affect the cultures in various ways. In N-sufficient growth mediums, protein production is supported, while carbohydrate synthesis is limited. In contrast, carbohydrate synthesis increases and protein production drops in N-deficient mediums (19). The drop in the protein content of the jars was interpreted as the depletion of the nitrogen in the culture medium (Table 1).

Table 1. Changes in specific growth rates (µ), doubling time (d.t.), protein, temperature, pH and dissolved oxygen (DO) in different cultivation vessels during the experiment.

| Groups | µ (div. $d^{-1}$) | d.t. ($d^{-1}$) | Protein (% DW) | T ( ºC) | | | pH | | DO (mg $L^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Min | Max | Avg | Min | Max | Min | Max |
| Jars | 0.32 | 2.15 | 33.4 | 16.1 | 35.1 | 29.1 | 9.3 | 11.2 | 10.3 | 19.7 |
| Bags | 0.21 | 3.34 | 54.5 | 17.4 | 28.6 | 24.7 | 9.1 | 9.9 | 10.2 | 25.7 |
| Ponds | 0.20 | 3.39 | 58.3 | 15.0 | 26.5 | 21.4 | 9.0 | 9.9 | 10.0 | 16.4 |

Several factors contributed simultaneously to N-deficiency in the jar culture, i.e., the fast growth of the cells, the higher biomass concentration, and the addition of $NaNO_3$ at half strength (1.25 g $L^{-1}$) in the medium. In addition, the pre-cultivation of the cells for 10 days in the pond caused the N level to decrease below 1.25 g $L^{-1}$ at the beginning of the experiment.

The other significant effect of the N-deficiency on *Spirulina* cultures related to color change. The color of the jar cultures turned to green after day 13 from the blue-green that is characteristic for cyanobacteria. This phenomenon supports the depletion of N in the medium of jar cultures. When N is exhausted in the medium, phycocyanin is used as the N source, which is responsible for the characteristic color in the blue-green algae, and the color turns to green (5, 20). The constant increase of the chlorophyll molecule, even at the end of the experiment, indicated that the pond culture was N-sufficient, while the others tended to decline (Figure 4).

The pH of the culture is another growth parameter that needs to be strictly monitored. The highest pH values achieved in the experiment were 9.9, 9.9 and 11.2 for the pond, bag and jar cultures. Although *Spirulina* is an organism living in alkaline media, values above 10.3 were shown to be harmful for the culture (21). In this respect, the pH of the jar culture was much above the optimal. Therefore, the addition of $CO_2$ into the highly productive small volume cultures, in which high biomass concentrations are reached, would be beneficial in keeping the pH in the optimum range.

When all the parameters were evaluated, the jar cultures showed the best growth with respect to the $\mu$ and the end biomass concentration achieved. However, the culture became N-deficient due to the factors stated above. Although the jar culture was N-deficient, the pond and bag cultures were mainly limited by temperature, and the average temperature was higher in the smaller volumes (Table 1). As for the bag culture, the cells increased very quickly until the $6^{th}$ day, and then slowed down. Although the temperature was higher than in the pond culture, the biomass concentration was the same. In our opinion, the cells in the bag culture were limited by the light due to the greater diameter compared to the others (50 cm), and the growth was reduced.

## Conclusion

As can be seen from the data, growth was affected by both the temperature and light in the experiment. However, the effect of temperature was superior to light in the experimental period. In this respect, the use of small volume cultures would increase the temperature faster in greenhouse conditions, which is the main factor hindering growth especially in the winter period. Moreover, the use of short light-path lengths in addition to the small culture volumes would support a higher productivity. In addition, $CO_2$ must be added into the culture to maintain optimum pH due to the fast growth of the biomass at elevated temperatures.

Another important point was the use of the macronutrients in the experiment at half strength. Some of the commercial microalgae production plants use the Zarrouk medium in the ponds at half strength to reduce the costs of nutrient. In our experiment, the reduction of the macronutrients in pond and bag cultures had no significant effect on growth in the experimental period, but it did not work with the jar culture, which was highly productive compared to the others in the experiment. In this regard, the level of the nutrients, especially $NaNO_3$, must be strictly controlled in highly productive systems, and added in the medium at full concentration.

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