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Growth of *Spirulina maxima* in Different Physical Conditions

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Abstract: *We designed in this experiment to compare the growth characteristics of Spirulina maxima, which was cultivated in different culture vessels under greenhouse condition. Three types of culture vessels, i.e. transparent jar, polyethylene bags and water tank (greenhouse), were used in the experiment. In comparison to the others, the jar culture supported higher cell densities due to its higher culture temperature. The dry weight amount in jar cultures was 0.91 g/L at the end of the experiment, while it was 0.41 g/L in the others. Growth rates were found to be 0.30, 0.20 and 0.19 day-1 in the jar, bag and water tank cultures, respectively. The protein levels measured at the end of the experiment were 30.2, 50.3 and 56.4 % for the jar, bag and water tank cultures, respectively. We concluded that the use of small volume cultures would increase the temperature faster, which is the main factor hindering the growth.*

Key Words: *Spirulina maxima, culture vessels, polythene bag, transparent jar, greenhouse*

I. 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]. The Spirulina maxima have gained importance and international demand for its high value phytonutrients and pigments, which have applications in health foods, feed, therapeutics and diagnostics. It has been hailed as the ''Food of the future'', besides being considered as an ideal food for astronauts by NASA. It represents the second most important commercial microalga for the production of biomass as health food and animal feed [5-6]. Spirulina known to be richest source of protein and vitamin and can be used to treat children suffering from malnutrition [7]. The major constituents of Spirulina includes protein, vitamin B12, iron, essential amino acids etc., It has been considered as ''Food of the future'' and an ideal food for astronauts by NASA [8]. Cyanobacteria is receiving an increasing interest due to their potential to produce a diverse range of chemicals and biologically active compounds, such as proteins, lipids, vitamins, carotenoid pigments and polysaccharides. For this, Spirulina has to be cultivated in commercial wayn [9].

Different types of culture systems, e.g., open ponds [10], tubular photobioreactors [11], inclined glass panels [12], Standard Zarrouk's media, Modified Zarrouk's media, Gangawater media [13].and the like, are used in the culture of Spirulina. Sodium sesquicarbonate is the raw material (i.e., trona) used for the production of the more refined grades of carbonates/bicarbonates. Trona typically contains high concentrations of silicates and other compounds and is likely one of the least expensive forms of bicarbonate available [14]. 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].

II. Materials And Methods

The experimental organism Spirulina maxima were obtained from Indian Agricultural Research Institute (IARI), Delhi- India. The cells were grown in tap water with Zarrouk medium [17], and the macronutrients were added into the culture at half strength. The experiments were carried out in a out-door water tank covered by transparent polythene sheets. Three types of culture devices were used in the experiment: 20 L cylindrical transparent jars (height: 40 cm, diameter: 28 cm), 300 L cylindrical polythene bags (height: 135 cm, diameter: 55 cm) and 2500 L cemented covered water tank (Greenhouse). The transparent jar and polythene bag cultures were isolated from the roof by styrofoam plates to prevent the heat exchange. The depth of the tank culture was kept at 25 cm and the flow rate was 30 cm/s. The cells were simply exposed to outdoor conditions for 20 days without temperature regulation. The circulation of the cultures was obtained by paddle-wheels in the greenhouse 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 maintained.

Initially, the filaments were grown in the tank and the culture was increased in volume for 12 days. After enough density was attained in the water tank, 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 0.10 g /L in all the groups, corresponding to a mean cell count of 3800 filament ml^{-1}. Absorbance values at 670 nm and pigment extracts for chlorophyll a analysis were read in a UV Spectrophotometer. For dry weight (DW) measurements, a 25 ml sample was filtered through pre-dried and pre weighted 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 after the filter papers were dried in an oven at 40 ℃ for two hours. Filaments were counted by triplicate samples in a channel type counting chamber. Specific growth rate (μ) and doubling time (d.t.) were calculated as in the following equation:

$$\mu \text{ (cell day}^{-1}) = \frac{\ln A_2 - \ln A_1}{t_2 - t_1} \qquad d.t \text{ (day)} = \ln 2/\ \mu = 0.693/\ \mu$$

A_2 and A_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 (28,500 rpm). The crude protein content was calculated by multiplication of the nitrogen (N) amount by the coefficient 6.25.

III. Results

Normally the experiment was carried out on clear day. The temperature was recorded from early morning to evening in different three experiments (table 1). The temperature and light variations (table 2) on a clear day characterizing the season are shown in Figure 1 and Figure 2.

Table 1: Diurnal temperature variations on a clear day characterizing the period of the experiment in transparent jar, Water Tank, Polythene Bag

Time (hours)	**Temperature**℃		
	Transparent Jar	Covered Water Tank (Greenhouse)	Polythene Bag (Outdoor)
07:00	17.5	16.0	17.0
09:00	19.2	18.1	19.0
11:00	22.7	21.2	22.1
13:00	28.7	27.1	28.0
15:00	33.8	31.4	33.3
17:00	29.3	27.1	28.9
19:00	25.2	23.9	24.8
21:00	20.3	18.1	20.0

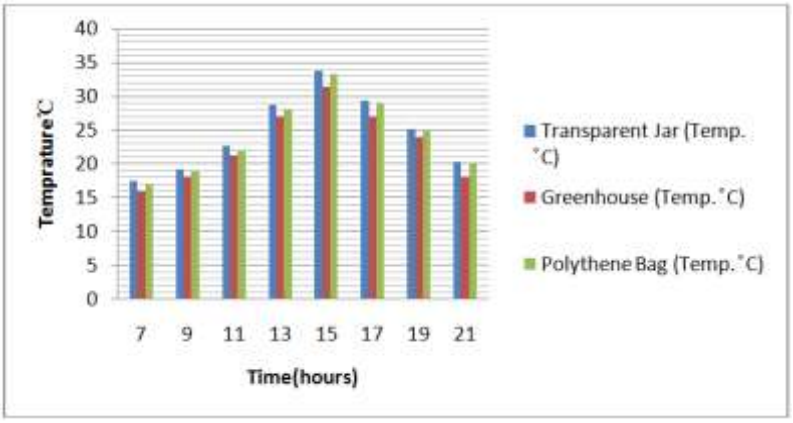

Figure 1: Diurnal temperature variations on a clear day characterizing the period of the experiment in transparent jars, Water Tank, Polythene Bag

Table 2: Diurnal changes in light intensity on a clear day characterizing the period of the experiment in greenhouse (u) and outdoors (Polythene Bag)

Time (hours)	**Light intensity (μmol photon m^{-2} s^{-1})**	
	Covered Water Tank (Greenhouse)	Polythene Bag (Outdoor)
07:00	112	325
09:00	140	360
11:00	324	740
13:00	600	1120
15:00	831	1333
17:00	412	710

19:00	00	5

In this work, all the three experiments were initiated with 0.10 g/L in DW. The increase in dry weight was observed till the 7th day in the polythene bag and water tank, while it increased until the 9th day in the transparent jar. After the cells were limited by light, the growth was almost constant showing a slight increase (Figure 3). While the dry weight values at the end of the experiment were 0.91, 0.41 and 0.42 g/L, the volumetric productivities were 0.80 0.33 and 0.30 g /L for the transparent jar, water tank and polythene bag cultures, respectively. There was no significant change between the water tank and polythene bag cultures in terms of dry weight values.

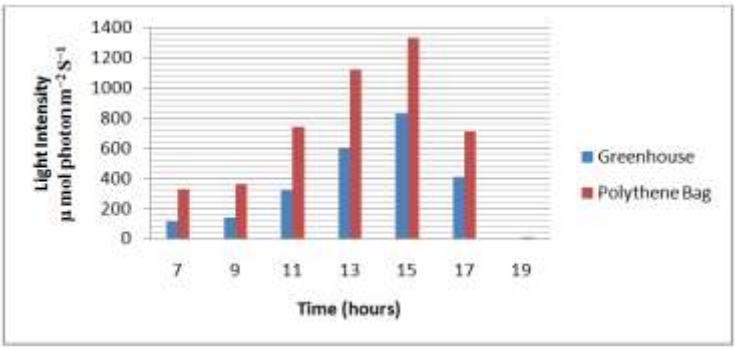

Figure 2: Diurnal changes in light intensity on a clear day characterizing the period of the experiment in greenhouse and outdoors (Polythene Bag)

Table 3: Changes in dry weight amounts of Spirulina maxima cultivated in transparent jar, Polythene Bag (Outdoor) and Covered Water Tank (Greenhouse)

Time (Days)	**Dry Weight (g/L)**		
	Transparent Jars	Polythene Bag (Outdoor)	Covered Water Tank (Greenhouse)
1	0.10	0.10	0.10
5	0.65	0.38	0.36
10	0.91	0.42	0.41
15	1.2	0.44	0.46
20	1.1	0.51	0.50

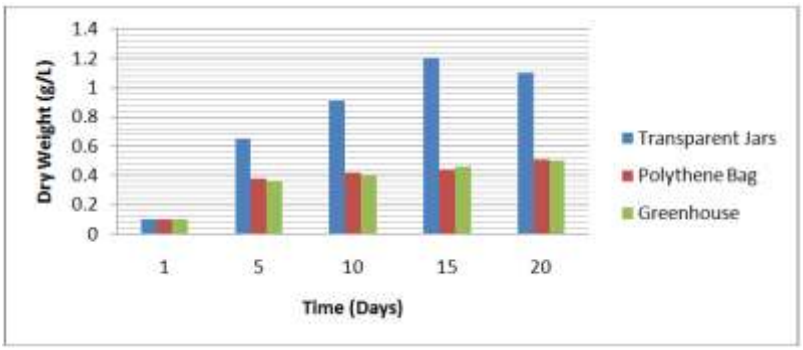

Figure 3: Changes in dry weight amounts of Spirulina maxima cultivated in transparent jars, Polythene Bag (Outdoor) and Covered Water Tank (Greenhouse)

The specific growth rates (µ), doubling times (d.t.), and protein amounts on dry weight basis, average temperature (T), dissolved oxygen (DO) and pH values are given in Table 4. The highest total protein content was found in the green house. 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 15.8, 26.2 and 20.0 mg L-1 at the end of the experiment for Green house, polythene bag and transparent jar cultures, respectively. The mean pH values of the cultures increased every day as well, and the values were found to be 9.7, 9.8 and 10.9 at the end of the experiment for Green house, polythene bag and transparent jar cultures, respectively. In addition, the color of the jar cultures turned to green from blue-green, which is characteristic for Spirulina, after day 12.

Table 4: Changes in specific growth rates (μ), doubling time (d.t.), protein, temperature, pH and dissolved oxygen (DO) in different cultivation vessels during the experiment.

Experiments	Growth rates (μ)	Doubling time (d.t.)	Protein (% DW)	T (˚C)		pH		DO (mg L-1)	
				Min	Max	Min	Max	Min	Max
Transparent Jar	0.30	2.10	30.2	17.5	33.8	9.0	10.9	10.3	20.0
Polythene Bag	0.20	3.15	50.3	17.0	33.3	9.1	9.8	10.1	26.2
Greenhouse	0.19	3.40	56.4	16.0	31.4	9.1	9.7	10.1	15.8

IV. Discussion

Temperature and light intensities were recorded during the research work experiment are given in Figure 1 and Figure 2. In the season when the experiments were carried out the light intensity of the sun was low. 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 1333 μmol photon m-2 sn-1 in the starting of March. The below-optimum temperature was the other major factor which was responsible for the low productivity. As can be seen by the comparison of Figure 1 and Figure 3, the biomass increase strongly correlated with the temperature of the culture.

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) in the medium. In addition, the pre-cultivation of the cells for 10 days in the water tank caused the N level to decrease below 1.25 g /L at the beginning of the experiment.

The pH of the culture is another growth parameter that needs to be strictly monitored. The highest pH values achieved in the experiment were 9.7, 9.8 and 10.9 for Green house, polythene bag and transparent jar cultures. Although Spirulina is an organism living in alkaline media, values above 10.9 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. According to us, the cells in the polythene bag culture were limited by the light due to the greater diameter compared to the others and the growth was reduced.

V. Conclusion

Growth was affected by both the temperature and light in the experiment. However, the effect of temperature affected more the growth of spirulina in comparison of light in our experiment. The use of small volume cultures would increase the temperature faster in greenhouse conditions, which is the main factor hindering growth. In addition, the use of short light-path lengths to the small culture volumes would support a higher productivity. Moreover, 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 water tank at half strength to reduce the costs of nutrient. In our experiment, the reduction of the macronutrients in water tank and polythene bag cultures had no significant effect on growth in the experimental period, but it did not work with the transparent jar culture, which was highly productive compared to the others in the experiment.

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