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A COMPARATIVE STUDY ON GROWTH OF *SPIRULINA PLATENSIS* AND *DUNALIELLA SALINA* INTERACTED BY DIFFERENT PHOTOPERIOD AND TEMPERATURES.

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The well-known correlation between algae and renewable energy sources and in this fact has brought about a great interest for seeking growth conditions of algae. Light is one of the most important factor for growth and contents of algae. In this study, the effect of different light regimes on the growth rate and chlorophyll were determined and the results were indicated significant varieties in culture at different light intensities. It was observed that maximum growth rate in *Spirulina platensis* (0.077 µ day-^{1}) and *Dunalliella salina* (0.085 µ day-^{1}) was observed at 2500 lux and reduced with increase in intensity. When effect of temperature was studied it was found that the best growth rates for *Spirulina platensis* was at 30ºC and for *Dunalliella salina* at 25 ºC while chlorophyll content in *Spirulina platensis* was found to be maximum at 35ºC and for *Dunalliella salina* at 30 ºC.

Keywords : Chlorophyll content; *Dunalliella salina;* Growth rate; *Spirulina platensis*; Temperature.

Introduction

Algae are informal term for a large, diverse group of eukaryotes that are not necessarily closely related and are thus polyphyletic. Microalgae are very sensitive to changes in their environment. *Spirulina* is a photosynthesizing cyanophyte (blue-green algae) that grows vigorously in strong sunshine under high temperatures and highly alkaline conditions. In the sixteenth century, when the Spanish invaders conquered Mexico, they discovered that the Aztecs living in the Valley of Mexico in the capital Tenochtitlan were collecting a “new food” from the lake [1]. *Spirulina* is, like most cyanobacteria, an obligate photoautotroph, i.e. it cannot grow in the dark on media containing organic carbon compounds. It reduces carbon dioxide in the light and assimilates mainly nitrates. The main assimilation product of *Spirulina* is glycogen. *Spirulina* shows an optimum growth between 28 to 32 °C under laboratory conditions. Outdoors, it seems that an increase in temperature up to 39 °C for a few hours does not harm the blue-green

alga, or its photosynthetic ability. Thermophilic or thermotolerant strains of *Spirulina* can be cultivated at temperatures between 35 and 40 °C. Such a property has the advantage of eliminating microbial mesophilic contaminants. At night, *Spirulina* can tolerate relatively low temperatures. The resistance of *Spirulina* to ultraviolet rays seems to be rather high [2].

Dunaliella species belong to the phylum Chlorophyta, order Volvocales and family Polyblepharidaceae, and are unicellular, photosynthetic and motile biflagellate microalgae morphologically distinguished by the lack of a rigid cell wall[3]. The best-known species of *Dunaliella* are *Dunaliella salina, Dunaliella tertiolecta, Dunaliella primolecta, Dunaliella viridis, Dunaliella bioculata, Dunaliella acidophyla, Dunaliella parva* and *Dunaliella media.* They reproduce by longitudinal division of the motile cell or by fusion of two motile cells to form a zygote.

Materials and methods

Culture Collection of algae- Periodic collections of algal samples (*Spirullina platensis*) were collected at different sites of Jaipur city especially from Jal Mahal and Tal Katora and other algae (*Dunaliella salina*) was collected from Sambhar Lake (Rajasthan). From different water bodies cultures of different algal strains were obtained and were purified and maintained in Basal medium. The cultures were subculture once in every 25 days and cultures of slant were sub cultured once in three months.

Isolation and Purification- By centrifugation method algal samples with very low cell count and in mixed forms were concentrated, while those with high cell counts were diluted with suitable medium. Enrichment and isolations were carried out using enrichment culture media till unialgal forms of species were obtained.

While doing simple enrichment method, the inoculum was prepared by mixing the samples with the selected medium and then serial dilutions were made in test tubes containing similar media. Direct isolations were done by picking up single filament or single cell using sterile Pasture pipettes, which were pulled out to a very thin capillary, using a dissecting microscope. In some cases series of dilutions were made in sterile medium using homogenized cell suspension of natural sample of the algae. Streaking method was used to contaminated cultures which can be made unialgal. In streaking method, a loop full algal suspension is taken and drawn into a long zigzag streak on an algal plate. After incubation, isolated colonies appearing at the tail end of the streak are expected to be unialgal [4].

Maintenance of Stock Cultures- The unialgal cultures were grown on agar slant with suitable medium contained in screw capped culture tubes to reduce frequent sub culturing without losing viability. The inoculated slants in 10-15 ml culture tubes with cotton plugs were incubated in a growth room for 7-10 days. The cotton plugs were replaced by pre sterilized, bakelite screw caps provided with rubber liner under aseptic conditions. After sufficient algal growth appears on the agar slants, culture tubes were then transferred to stock culture room under conditions which were just sufficient to keep them viable state. Low temperature (15-20°C) and light intensity (50-200 lux) maintains the culture in viable state for longer duration.

Influence of photoperiods on the growth rate of algal cultures -

Determination of growth and growth rate - Growth was followed through optical

density, fresh weight and dry weight. OD was recorded with the help of Elico CL 160 photo colorimeter (India) at 670 nm. For fresh weight and dry weight 50 ml of cultures was centrifuged with the help of Sartorius 3-18K centrifuge (Germany) every time. The palate was weighed for FW and it was dried at 37 ºC in an oven for overnight for DW. Growth rate was calculated from dry weight by reported equation [5].

μ (divisions/day) = $3.322(\log DW_2 - \log DW_1)/t_2 - t_1$

Effect of temperature on the growth rate and total chlorophyll content of algal cultures

In order to find out optimum culture condition, cultures were subjected to six different combinations of temperature range and illumination, the following conditions were tried for present study:

Set I Cultures receiving Constant light at 18-24^{o}C.

Set II Cultures receiving Constant light at 25-31^{o}C.

Set III Cultures receiving Constant light at 32-38^{o}C.

Three sets of conical flasks containing algal cultures were subjected with each of these culture conditions for estimation. Observations were carried out over a period of five weeks after initial readings.

Results

Effect of photoperiod and temperature on growth rate and total chlorophyll content in S. platensis and D. salina- Light is a major resource for algae and has a complex pattern of spatial and temporal variability in aquatic ecosystems. The culture of microalgae requires a rigorous control of all growth factors: CO_2, O_2 and light. The main factor in mass culture technology of microalgae is optimization of the yield. Light situations effect directly on photosynthesis and the cells growing of microalgae. Algae need in appropriate photoperiod for efficient photosynthesis; it needs light for a photochemical phase to produce (ATP).

It was observed that when different photoperiods were kept there was variation in growth rate in our present investigation. It was observed that in both algal cultures growth rate increased exponentially with increasing light intensities and reduced at 3500 lux intensity. Maximum growth rate (0.077 μ day^{-1}**)** in *S. platensis* and in *D. salina* (0.085μ day^{-1}**)** were observed at 2500 lux intensities and reduced thereafter at 3500 lux intensities.

However it was observe that chlorophyll content was also light dependent. Maximum chlorophyll content in *S. platensis* (1.19%) and in *D. salina* (1.43%) was observed at 2500 lux intensity and they reduced at 3500 lux intensity (**Fig. 1 and 2**). Effect of temperature on total chlorophyll content was also studied. It was observed that in *S. platensis* maximum content was observed at 35ºC while in *D. salina* it was observed at 30ºC. Further increase in temperature reduced the content in *D. salina* **(Fig. 4).**

Temperature is an important element for growing algae. It strongly influences cellular chemical composition, the uptake of nutrients, carbon dioxide fixation, and the growth rates for every species of algae. It is know that the growth rate will increase with the increase in temperature up to its optimum and once it reaches its optimum. Further growth rate will decrease drastically with the increase in temperature.

In *S. platensis* , the highest growth rate (0.27±0.02 μ day^{-1}) was observed in cultures at 30°C while there were no significant changes in growth rates in cultures grown at 20 and 25°C. In *D. salina*, the highest growth rate was observed at 25 °C (0.24 ± 0.02 μ day^{-1}) while no

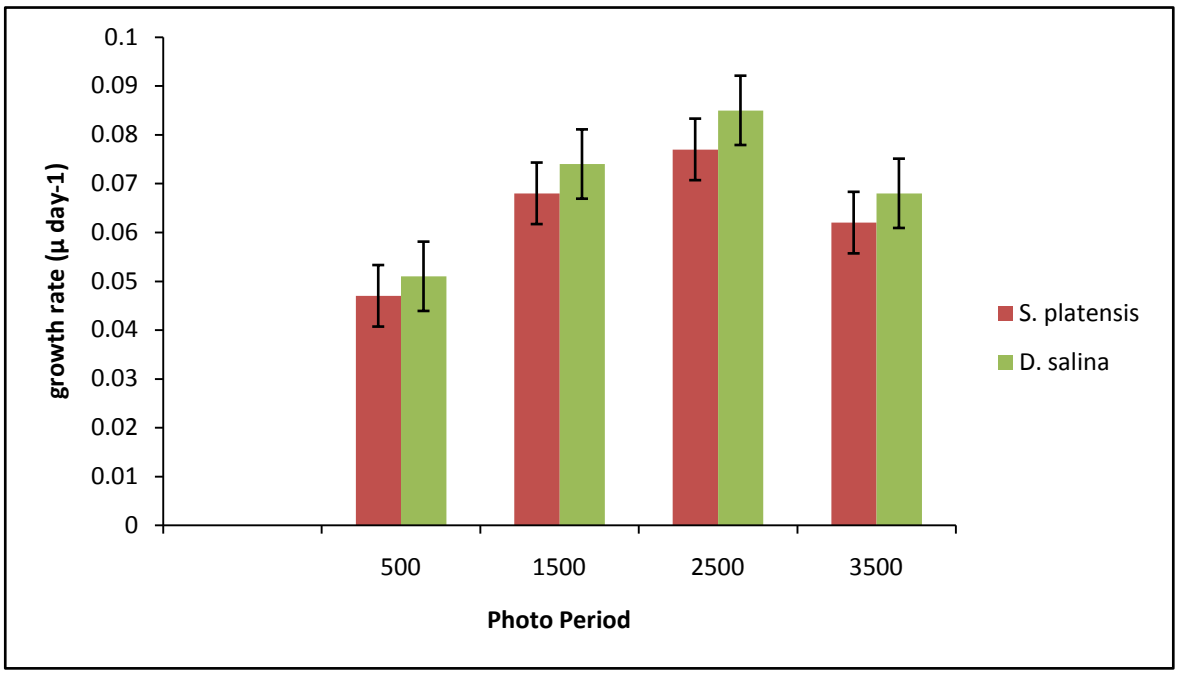

Fig. 1 Effect of photoperiod on growth rate of *S. platensis* and *D. salina*

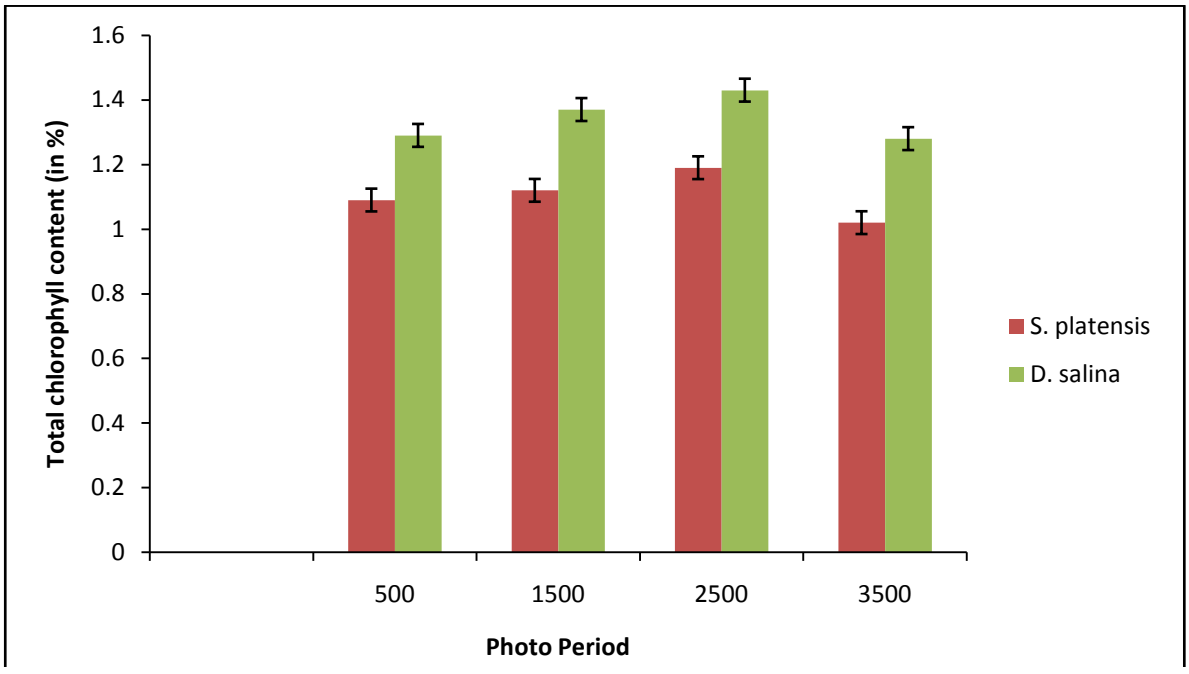

Fig. 2. Effect of photoperiod on Total Chlorophyll content (in %) of *S. Platensis* and *D. Salina*

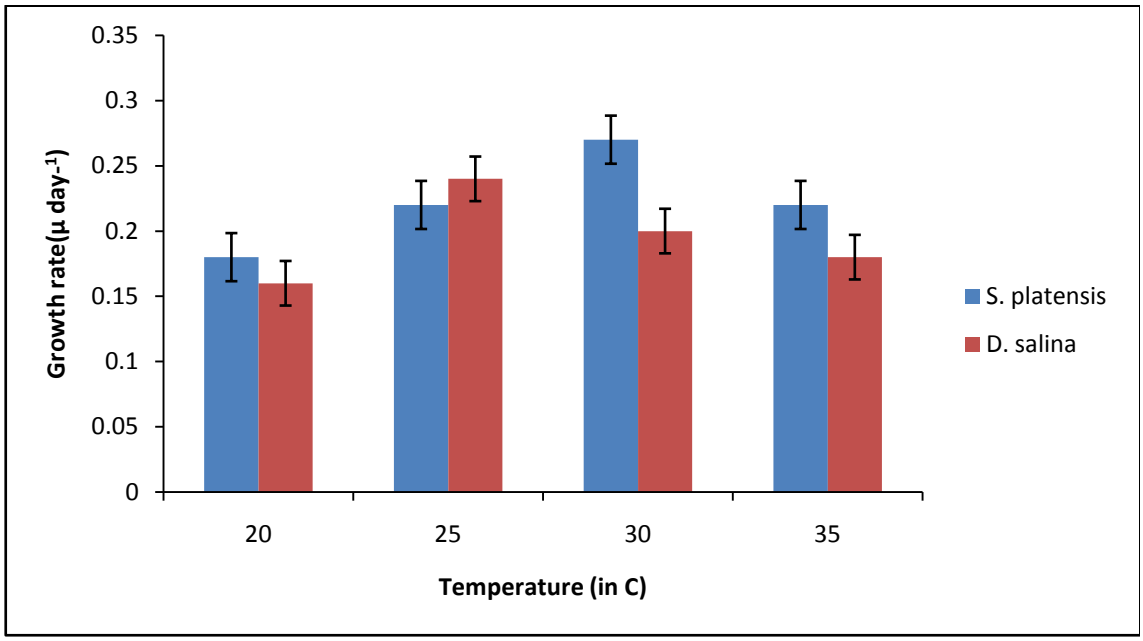

Fig. 3. Effect of temperature on growth rate of *S. platensis* and *D. salina*

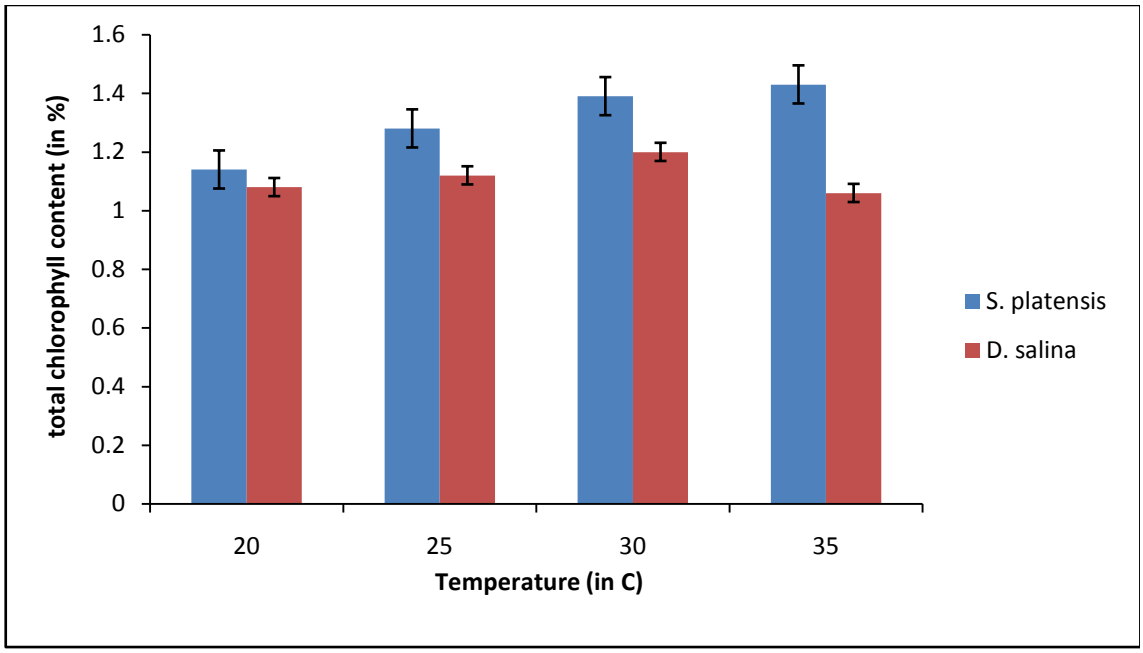

Fig. 4. Effect of temperature on Total chlorophyll content in *S. platensis* and *D. salina*

significant growth rate was observed at 20°C and 30°C. (**Fig. 3**)

Discussion

Algae are unique group of living photosynthetic organisms that are present in every type of environment, such as oceans, ponds, river, lakes on rocks on ice etc. Water covers two-third surface of our earth, and thus the algae covers the greatest area [6]. Now-a-days algae is immensely important in environmental purification because it is inexpensive biomaterial that is used for the removal of oxides of sulphur and nitrogen, heavy metal, waste water, and 50% fixation of carbon dioxide [7]. Various physical factors such as nutrients, light, temperature, pH and salinity effects their biomass and biofuels production[8]. It has been reported that some physical and chemical factors such as, water temperature, salinity, pH and light affect algal growth in aquatic systems. Light regime is discontinuous and the intensity varies daily in nature. Changes in light illuminance, quality and photoperiod bring about varies in their biomass and chemical composition of algae, therefore, showing various adaptations to different environmental conditions[9]. These changes of the light have been shown to bring about differences in biochemical structure and pigment of microalgae[10]. It has been reported that[11] the effects of light, temperature, and nutrition on growth and pigment accumulation of three *Dunaliella salina* strains isolated from saline soil. They reported that that 22 or 25°C were most suitable for *D. salina* growth which is also supported by various researchers[12]. Same scenario were observed in our present investigation thus justifies our study.

In the present research the observation of effects of different photoperiods on *D. salina* indicated a different scenario. In addition to that, in higher light the algae synthesize smaller photosynthetic units, most probably to prevent photo damage, however, in low light larger photosynthetic units are found probably to aid light harvesting [13].

It has been elucidated that [14] reported an increase in biomass content and in chlorophyll content at elevated carbon dioxide (6%) and optimum temperature (30°C). Lipids would increase from 5.9 to 14.7% when the temperature decreased from 30°C to 25°C; at temperatures over 38°C oleic acid, a monounsaturated omega-9 fatty acid, production increased[15]. Here also we observed that in *S. platensis* total chlorophyll content increased with increase in temperature while in *D. salina* it reduced thereafter. Under heat stress or heat shock the algal protein content will decrease and will produce abscisic acid (ABA), a stress hormone[16]. If the stress hormone is produced, it is considered a key factor in controlling downstream responses such as growth and gene expressions. When raising the temperature above 40C^{o}, *Chlorella vulgaris* was less resistant to acidic pH than when it was grown at 35C^{o} or less temperatures [17].

References

1. Sasson A 1997**,** *Micro Biotechnologies: Recent Developments and Prospects for Developing Countries*. BIOTEC Publication 1/2542. pp. 11–31. Place de Fontenoy, Paris. France. United Nations Educational,Scientific and Cultural Organization (UNESCO).
2. Richmond AE 1986**,** Microalgae. Vol. 4, Issue 4. *CRC Critical Reviews in Biotechnology*.pp. 349–438.Boca Raton, Florida, USA.
3. Ben-Amotz A, Gressel J and Avron M 1987, Massive accumulation of phytoene induced by norflurazon in *Dunaliella bardawil* (Chlorophyceae) prevents

recovery from photoinhibition. *J. Phycol*. **23** 176–181.
4. Pringsheim EG 1946, The biphasic or soil-water culture method for growing algae and flagellata. *J. Ecol*. **33** 193—204.
5. Guillard RRL 1973, Division rates. In, Stein (ed), Handbook of Phycological Methods,V. 1, Cambridge University Press, Cambridge, pp. 289-312.
6. Khola G and Ghazala B 2012, Biodiesel production from algae. *Pak. J. Bot* **44**(1) 379-381.
7. Ogbonna JC, Yoshizawa H and Tanaka H 2000, Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. *J. Appl. Phycol*. **12** 277-284.
8. Bartley ML, Boeing WJ, Daniel D, Dungan B N and Schaub T 2016, Optimization of environmental parameters for *Nannochloropsis* salina growth and lipid content using the response surface method and invading organisms. *J. App. Phycol*. **28** 15-24.
9. Katalay S, Bayacıoglu M, Cakal Arslan O, Parlak H, Karaaslan MA 2012,Phytotoxicity of Water and Sediment from Nif Brook (Izmir, Turkey) on green algae *Desmodesmus* (=Scenedesmus*) subspicatus*. Ekoloji **21** (83) 25-31.
10. Richmond A 2004, Biological principles of mass cultivation. In Richmond A (ed), Handbook of Microalgal Mass, CRC Press, Culture. Biotechnology and Applied Phycology, Blackwell Publishing Company, Oxford. pp. 566
11. Wu Z, Promchup D, Zhao P, Juntawong N and Chunhong Ma 2016, The Effects of Light, Temperature, and Nutrition on Growth and Pigment Accumulation of Three *Dunaliella salina* Strains Isolated from Saline Soil. Jundishapur *J. Microbiol*.. **9**(1) e26732
12. Imamoglu E, Demirel Z and Dalay MC 2014, Evaluation of culture conditions of locally isolated *Dunaliella salina* strain EgeMacc-024. *Biochem. Eng. J* .**92** 22–27.
13. Wijanarko AD, Witarto AB and Soemantojo RW 2004, Effect of photoperiodicity on CO_2 fixation by *Chlorella vulgaris* Buitenzorg in bubble column photobioreactor for food supplement production. *Makara. Seri. Teknologi*. **8** 35-43.
14. Chinnasamy Senthil, Balasubramanian R, Ashish B and Keshav C. Das 2009, Biomass Production Potential of a Wastewater Alga *Chlorella vulgaris* ARC 1 under Elevated Levels of CO2 and Temperature. *Int .J. Mol. Sci*. **10**(2) 518–532.
15. Converti A, Alessandro A. Casazza, Erika Y Ortiz, Patrizia Perego and Marco Del Borghi. 2009, Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris*for biodiesel production. *Chem. Eng. Process.* **48** 1146–1151.
16. Bajguz A and Hayat S 2009, Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiol. Biochem*. **47** 1–8.
17. Mayo AW 1997, Effects of temperature and pH on the kinetic growth of unialga *Chlorella vulgaris* cultures containing bacteria. *Water. Env. Res.* **69**(1) 64- 72.