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# OPTIMISATION OF CULTURE CONDITIONS FOR *SPIRULINA LABYRINTHIFORMIS* . II. LIGHT AND TEMPERATURE

**RENU BHATIA and PUSHPA SRIVASTAVA**
Department of Botany, University of Rajasthan, Jaipur 302 004, India.

Temperatures ranging from 28-35 °C, 30-40 °C and 32-40 °C were provided to the cultures under three different sets of culture conditions i.e. continuous light, alternate light and dark 8:16h and natural day and dark periods in window facing north. The best growth with high cell contents was found under natural day and dark condition between 32-40 °C.

**Keywords** :Growth; Light periods; *Spirulina labyrinthiformis;* Temperature.

## Introduction

Algae often respond to environmental changes by adjusting the concentrations of photosynthetic pigments, nucleic acids and other cellular components. Concomitant with these shifts in cell composition, there have been changes in the specific growth rate[1] and cyanobacteria can not be an exception to this conclusion. Although no single environmental factor was known to regulate the photosynthetic activity of algae, but light and temperature were the most critical ones[2,3]. Some blue-greens give varied growth and pigment responses to changed light conditions[4,5]. Earlier it was found that constant illumination added with high light intensity reduced the pigment and protein content[6-9] in different algae, while lipids have been found to be less influenced by such factors[10]. Increase in chlorophyll-a content was observed in algae grown at low light levels[11,12].

The present study has been taken up with *S. labyrinthiformis* to avail high yield of biomass with improved nutrients under different culture conditions as it is proposed to be utilized for nutritional and pharmaceutical purposes.

## Material and Method

*S. labyrinthiformis*, a Jaipur isolate was raised into unialgal cultures. Various inorganic media were tried and Zarrouk's medium[13] with pH 10.28 proved to be the best[14] which was employed through out this experiment.

The following sets of conditions were experimented upon

Set I Constant light at 28-35°C

Set II Alternate light and dark periods 8:16 h at 28-35°C

Set III Constant light at 30-40°C

Set IV Alternate llight and dark periods 8:16h at 30-40°C

Set V Natural day and dark periods in window facing north at 32-40°C

A pair of test tubes containing 8 ml. of Zarrouk's medium added with 2 ml of freshly growing cultures was subjected to each set of culture condition. One of the tubes was used exclusively for optical density records and the other one was employed for morphological observations. A parallel set of culture flasks of 250 ml capacity containing

100 ml of medium and 20 ml. of culture sample were also subjected to each of the culture set in duplicate, for the analysis of cellular constituents.

Growth of the cultures was measured periodically in term of optical density and chlorophylla-a contents. Data pertaining to colour, morphology and cellular changes in trichomes pertaining to pigments, protein; amino acids, lipids, carbohydrates and nucleic acids were additional parameters taken into account. Observation have been carried out every week over a period of 5 weeks.

**Results and Discussion**

Under continuous illumination at 30-40°C, cultures showed higher growth rates upto 3 weeks (Fig. 1). After that, these cultures became yellowish and ultimately died. Comparatively, low temperature range i.e. 28-35 °C with continuous illumination not only increased the density about 16 fods, at the end of 5th weeks, but the trichomes were also healthy and green in colour. Width under both the sets of culture condition on an average ramained 2.775 μm only.

Under 8h/d light period at 30-40° C, although growth was linearly progressive upto 4th week with the density increase of 10 fold. Thereafter, growth declined and within a week's time, cultures turned white. Cultures at 28-35° C under similar condition continued to grow slowly but steadily and their density also increased about 10 times at the end of the 5th week (Fig. 1). Trichomes were brillient bluish green with healthy appearance. Their average breadth was 2.8 μm. Cultures which received natural day and dark periods showed a density increase of 4 times within a week and then 3 fold increase after 2 weeks. The growth was rapid under this set of culture condition which ended up with about 16.5 times increase of the cultures. The impact of light was temperature dependent as higher temperature range 30-40°C either with continuous illumination or with 8h/d illumination, could not support the growth for 5 weeks.

Cellular components showed variability in different culture conditions. Chlorophyll-a was 1.23% in cultures received continuous light at 28-35°C, while 2.1% in alternate ight and dark condition at the same temperatures. In natural day/dark regime with 32-40 °C, it turned out to be 2.4%. Eppley and Dyer[11] also found more chlorophyll-a in algae grown at low light. The light and dark regimes have been required to complete the photosynthetic cycle and continuous illumination caused photo-oxidation of photosynthetic pigments like chlorophyll-a. Carotenoids on the other hand showed reverse trend, as they were known to provide protection against photo-oxidation[15], they were highest (0.105%) under continuous illumination and were reduced to 0.056% under 8h/d light and 0.064% under natural day and dark cycle. Amongst the phycobiliproteins a major variation was registered under constant light a total of 11.7% was on record with 3.7% phycocyanin, 5.2% allophycocyanin and 2.8% phycoerythrin. Under alternate light and dark regime there were 8.96% with phycocyanin 2.60%, allophycocyanin 4.59% and phycoerythrin 1.77%. Under natural day and dark condition, 9.45% phycobiliproteins had fractions of 2.74% phycocyanin, 4.73% allophycocyanin and 1.98% phycoerythrin.

**Table 1.** Amino acids in *S. labrynthiformis* in different cultural conditions.

| Amino Acid | Continuous light (28-35°C) | Alternate light (28-35°C) | Natural day/dark (32-40°C) |
|---|---|---|---|
| 1. Leucine* | + | + | + |
| 2. Isoleucine*+ | + | + | + |
| 3. Tryptophane* | + | . | + |
| 4. Methionine* | + | - | + |
| 5. Phenyhl alanine* | + | + | + |
| 6. Alanine | + | + | + |
| 7. Tyrosine | + | + | + |
| 8. Serine | + | + | + |
| 9 Glutamic acid | + | + | + |
| 10. Proline | + | + | - |
| 11. Glycine | + | + | + |
| 12. Arginine* | + | + | + |
| 13. Lysine* | - | + | - |
| 14. Cystine | + | - | - |
| 15. Valine* | - | - | + |
| 16. Histidine* | - | + | + |
| 17. Cysteine | - | + | + |
| 18. Threonine* | - | - | + |
| Toal number | 13 | 13 | 15 |

* = Essential amino acids

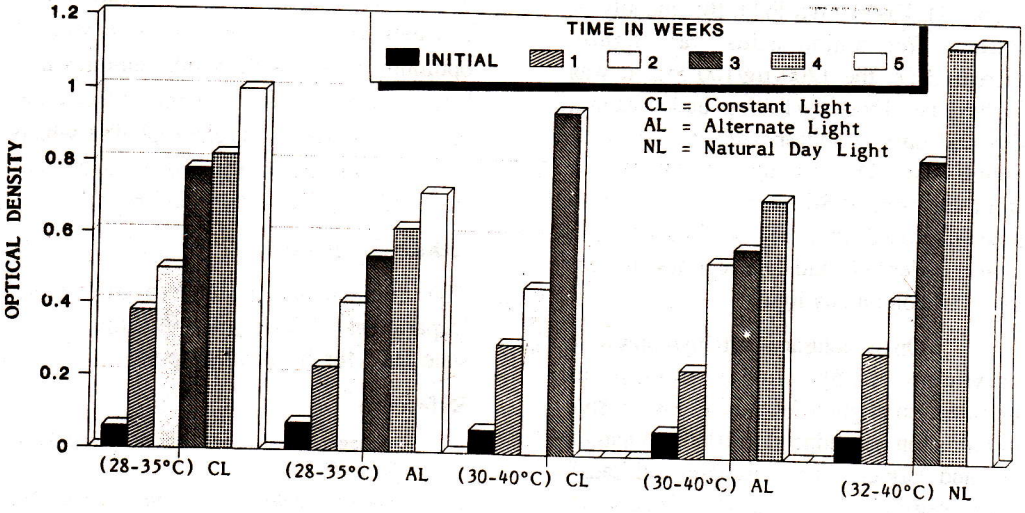


Fig. 1. Growth of *S. labyrinthiformis* under different culture conditions.

Protein percentage has been reduced by the detrimental impacts of the constant illumination. It was only 58%, but was 60% under 8h/d light periods, a maximum of 61% was recorded in the cultures grown under natural day and dark hrs. Low protein percentage in continuous light grown cultures may be attributed to ammonia or nitrate which has been known to be the precursor of protein in unicellular algae and the assimilation of ammonia or nitrate, were greatly influenced by light and dark cycle[6,16]. They found that proteins were more at low light levels. Ohmori *et al*[17], also found increased levels of photogenerated compounds at high light regimes, prevented the assimilation of ammonia into proteins. The same may be attributed to the *S.labyrinthiformis* as well. Similarly the quantity of free amino acids reduced upto 1000 µg/100 mg algae with a total number of 13 amino acids under constant illumination (Table 1). Under 8h/d light, the quantity of thirteen free amino acids was slightly increased i.e. the 1200 µg/100 mg. It was highest i.e. 1500 µg/100mg under natural day and dark condition with a sum of 15 amino acids. This finding drew its support from the work of Shamala *et al*[18], who also noted increased amino acids in *Secenedesmus acutus* under light and dark regimes and *vice versa* in continuous light

On the contrary, carbohydrates were maximum 12.5% under continuous illumination slightly less i.e. 11.8% in 8h/d illumination but reduced to 10.9% in natural day and dark condition. Redaljii and Laus[19] also registered increased levels of carbohydrates, especially, at high light regimes. They proved it by labelling C14 which get incorporated into carbohydrates rather than proteins. Nucleic acids i.e. RNA and DNA were found to be maximum 0.42% and 0.23% respectively, under constant illumination at 28-35$^0$C. These were slightly less 0.38% RNA and 0.18% DNA under 8h/d light period. The least amount amongst the three was recorded in natural day and dark grown cultures i.e. 0.28% RNA and 0.19 DNA. Lipid contents were found to be almost equal under all the sets of conditions with slight increase under constant illumination (8.2%) as cyanobacterial lipids have been suggested to be less influenced by environmental factors[10].

It may be concluded that continuous illumination though instricated the growth rate but inflicted detrimental impacts on chlorophyll-a, proteins and amino acids. Alternate light and dark regime at low temperature were favourable but natural day and dark condition was best, as it yielded optimum growth with healthy cultures and high contents of essential metabolities i.e. protein, amino acids and chlorophyll-a, which stood a witness to the improved nutritional status of *Spirulina labyrinthiformis.*

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