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The Effect of Different Levels of Lighting on the Content of Proteins and Amino Acids in the *Lyngbya* sp. Algae

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ABSTRACT
The aim of this study was to determine the effect of different lighting levels on the total protein and amino acid content of the isolated *Lyngbya* sp. Algal samples were collected from the Euphrates River in Nassiriyah, southern Iraq. The isolated algae were cultivated under optimal laboratory conditions for two weeks, exposed to varying levels of lighting. After this period, protein and amino acid contents were measured. The results revealed significant differences at a probability level of 0.05. The highest total protein content was observed at a luminance level of 100 (72.17%), while the lowest was at a luminance level of 5 (15.11%). Significant differences in amino acid levels were also noted, depending on the lighting intensity. These findings indicate that lighting levels significantly influence both the protein content and the amino acid profile in the dry matter of the *Lyngbya* sp. samples.

INTRODUCTION

Algae are a diverse group of predominantly aquatic, photosynthetic organisms that belong to the kingdom Protista. They are characterized by their ability to photosynthesize, as they contain chlorophyll pigments of different types and other pigments, such as Carotenoids, Xanthophyll's, Phycobiliproteins in addition to other pigments found in certain genera and types of them **(Yu-Wang *et al*., 2007)**. It also contains important chemical components such as proteins, carbohydrates, fats, vitamins and most of the mineral elements (**Solovchenko *et al*., 2008)**.

From a nutritional point of view, algae are characterized by being rich in protein, sugar and fatty substances, in addition to containing a percentage of salts and vitamins **(Vonshak *et al*., 1996)**. Algae can be used as animal feed for chicken and mice breeding, (**Newsted , 2004**). In addition, they can be used in fish breeding ponds(**Sievonen, 1990**).

Different studies were conducted to study the chemical composition of algae, which included the content of protein, carbohydrates, fatty acids, vitamins, minerals and ash **(Lu**

& Zhang, 2000). For the protein content, it is one of the most important components in the biomass of algae and one of the singnificant factors used in estimating the nutritional value, especially if it is used as a food material **(Ortega *et al*., 2008)**. The abundance, diversity, and concentrations of amino acids is important indicators of the nutritional value of algae. It was found that algae are similar to higher plants as they are able to make all amino acids, while animals are only able to make non-essential amino acids, therefore they get the essential amino acids needed for them through their food **(Mortensen *et al*., 1988)**.

The effect of lighting on the chemical content and nutritional value of algae can be direct, influencing organic compounds such as proteins, carbohydrates, and fats, or indirect, by affecting the process of photosynthesis. The intensity of illumination affected the protein content of cells. **Lionard *et al*. (2008)** stated that when the illumination was increased appropriately, an increase in the concentration of proteins occurred in the cyanobacterium *Scytonema*. **Dybel *et al*. (2006)** studied the effect of four factors, including lighting on proteins. It was found that an increase in the intensity of illumination to more than 2.25 lux led to a deficiency in the synthesis of proteins and consequently a decrease in the alga *Haematococcus* due to the intense illumination of reducing the nitrogen of the medium, that caused a deficiency in one of the important elements involved in the manufacturing of proteins. In addition, **Coombs and Hall (1985)** noted that the protein content in *Oscillatoria* differed according to the intensity of illumination, as the highest content of proteins was recorded at photosynthetically active radiation 50 lux. This was explained on the basis of light stimulation of many important processes, including the process of protein synthesis. Moreover, the effect of light on the content of amino acids in cells is similar to the effect of light on proteins. Most studies have shown that the reason for this is due to the effect of lighting on the nitrogen element entering the composition of amino acids **(Desikachary, 1959)**, while the other section attributed the reason to the discrepancy. The ratio of amino acids to the differences between genera and species led to a clear variation in the proportion and quality of amino acids, but in general, the positively charged amino acids were more affected than others since they contain nitrogen in a greater proportion.

MATERIALS AND METHODS

1. Collection of samples

Algae were collected from various waterways in Al-Tar, Nassiriyah, southern Iraq, using a phytoplankton net with a 20-micrometer mesh. The samples were placed in sterile plastic containers and transported to the laboratory. They were then washed multiple times with distilled water and centrifuged to remove impurities. The samples were examined under a light microscope to identify the isolated alga, and its morphology was determined based on taxonomic references.

2. Identification and purification of algae

2.1. Isolation and identification of algae

To isolate and diagnose the algal used in the study, two methods were used for this farm. The first was using filtration. The filtration method involves filtering a volume of 50-100cm^3 with milipores filter papers with a diameter of 0.45 micrometers. The papers were then placed in a small volume of distilled water (5-10cm^3) and examined with a light microscope using an Olympus "Japan" light microscope. The second method involved using a centrifuge (Hitachi, Japan) to isolate the algae. Samples were thoroughly mixed, and 20cm³ of each sample was centrifuged at 3000rpm for 20 minutes. The sediment was then collected, and 1cm³ of distilled water was added. Microscopic slides were prepared from this mixture and examined under a light microscope to obtain a unialgal culture.

The method by **Stein (1973)**, which relies on plating and spreading techniques using a culture medium, was also employed. Additionally, the solid dilution method was used, where a series of dilutions were prepared to achieve a unialgal culture. The algae were identified based on their taxonomic significance, following references such as **Desikachary (1959)**, **Prescot (1975)** and **Bourrely (1980)**.

2.2. Algae culture

After isolating and identifying the algae, the species was transferred from the liquid culture medium, in which it was located using a sterile pipette or from the solid medium using a sterile inoculant (Loop) to a number of sterile glass flasks with a volume of 100cm^3 containing 70cm^3 of sterile culture medium. The mouths of the flasks were closed with clean cotton and incubated at a temperature of 2± 25^{0}C and lighting of 150 lux in the growth cabin with a lighting system of 8:16 lighting: darkness, taking into account the continuous shaking of the samples until the desired growth was obtained **(Tomaselli *et al*., 1981)**.

2.3. Purification of algae

The culture was confirmed to be free of germs by using the method described by **Weidman *et al*. (1984)**. In it, the algal isolates were washed using sterile distilled water, then centrifuged using a centrifuge at a speed of 3000rpm for a period ranging from 50-90 seconds. The filtrate was discarded, and the sediment was taken and mixed again with sterile distilled water. This process was repeated 12 times. In order to ensure purity, they were planted on the nutrient agar medium (Nutrient agar) as described in **Stein (1973)**, where they were incubated at a temperature of 37^{0}C for 18 hours in the culture cabinet. This process was repeated until the culture was confirmed to be free of germs by observing the surface of the nutrient free of any growth. Thus, we obtained pure isolates (Axenic cultures).

Lyngbya was phenotypically identified by preparing temporary glass slides and examining under light microscope to determine its morphological characteristics based on taxonomic references. Sonicator apparatus was used to isolate different microorganism bacteria and fungi attached to the alga, and thus obtaining a pure isolated alga Axenic culture **(Desikachary, 1959)**.

3. Effect of lighting on chemical content

The sterile algal samples were incubated in an incubator, and the effects of different light intensities (measured in lux: 5, 15, 25, 50, and 100) were studied. After adjusting the light intensity using a WTW Klux meter, the samples were placed in a growth cabinet and incubated until the chemical tests were conducted **(Jones & Galloway, 1979)**.

4. Total protein content

Protein was determined by using the method of **Cosper (1982)** through reading the protein concentration at 280nm wavelength for the highest absorption of proteins and the absorbance reading at 260nm wavelength for the highest absorption of nucleic acids. The following equation was then applied to calculate protein concentration:.

Protein concentration = $1.55*A_{280} - 0.77 * A_{260}$

5. Amino acid content

The amino acids were estimated by taking 1gm of dried algae and mixing it with 10cm of absolute ethyl alcohol and leaving for 24 hours on a vibrator at a temperature of 25 Celsius, then separating the precipitate from the filtrate by centrifuging at 6000 cycles/ minutes for 15 minutes. Afterward, the filtrate containing amino acids was collected and estimated using the Technicon Amino Acid Analyzer (TsM1) (HI9142 "Hanna") described in the study of **Kalita and Tytlianov (2003)**. Subsequently, the essential amino acid index was calculated according to the following equation, as assessed in the study of **Lu *et al*. (1999)**:

$$EAAI = \sqrt[n]{\frac{100a}{r^a} * \frac{100b}{r^b} * \frac{100n}{r^n}}$$

6. Statistical analysis

Statistical analysis of the results was performed by one-way ANOVA using SPSS ver. 23. Significant differences ($P< 0.05$) among the concentrations were analyzed by Duncan test.

RESULTS AND DISCUSSION

1. Total protein content

Proteins occupied the main part in the chemical content of algae, as it is noted from Table (1) that the highest protein content was at the illumination of 100 lux reaching 72.17% dry weight, and the lowest content was recorded at the illumination level of 5 lux, reaching 15.11% dry weight. Additionally, it was noticed that there were significant differences at the test level ($P<0.05$).

It is possible to relate the increase in the amount of proteins to the effect of light on the growth of the algae, as the increase in growth is accompanied by an increase in the basic organic compounds inside the algae's bodies, of which the most important are proteins. This finding is in agreement with that of **Grima *et al*. (1999)**, who addressed the effect of lighting on the growth and chemical content of genera of blue-green algae, as the results showed that the growth of these genera increased with the increase in light density and the increase in chemical content and biomass at the level of illumination of 95 lux. Some researchers suggested that the increase in protein content with higher light intensity may be due to the effect of lighting on nitrogen availability, a crucial element in the synthesis of amino acids and proteins. Notably, studies by **Dortch (1982)** and **Al-Badri and Al-Ebady (2020)** supported this view. They observed that changes in light intensity, whether an increase or decrease, led to a reduction in nitrogen levels in the medium, resulting in a deficiency of this element, which in turn affected the synthesis of amino acids and proteins.

2. Amino acid content

The use of different illumination intensities significantly impacted the levels of essential amino acids in *S. platensis*. As shown in Table (2), the highest lysine content, 3.5% dry weight, was recorded at an illumination intensity of 50lux, whereas lysine was absent at 100lux. Methionine also showed its highest content, 1.6% dry weight, at 50lux, with no detectable amount at 100lux. Similarly, cysteine reached its highest content of 0.8% dry weight at 50lux. Proline had the highest content at 2.9% dry weight at 50lux, but this amino acid was absent at 100lux.

The essential amino acid index also varied with illumination levels, showing a clear trend. The highest index value of 0.949 was recorded at 50lux, while the lowest index value of 0.634 was observed at 5lux

The effect of amino acids, according to the present results, is similar to that of proteins with the light factor. An increase in the content of the studied algae of amino acids in general and essential ones in particular, such as lysine, methionine, tryptophan and histidine, was observed when the intensity of lighting was increased within a limited range. The reason was attributed to the same reasons that explained the increase in proteins, which is the effect of light on the availability of nitrogen, as the high lighting led to the reduction of this element, which leads to a lack of amino acids. Notably, nitrogen is an essential element in the synthesis of amino acids.

The index of essential amino acids (EAAI) in our current study was relatively high at the intensity of illumination of 50lux. The reason may be due to its proportion to the high content of proteins and amino acids, and from observing the value of the evidence of essential amino acids, it was found that the higher its value, the more important the algae from the nutritional point of view.

Table 1. Effect of different levels of light intensity on protein content and protein digestion rate as % of dry weight in the studied alga

Lighting	5	15	25	50	100
Protein content % dry weight	15.11	50.30	61.19	65.70	72.17

Table 2. Effect of different levels of illumination on the content of amino acids (%) in the studied alga

Amino acid	**Lighting**				
	5	**15**	**25**	**50**	**100**
Lysine	0.2	0.9	2.7	3.5	-
Methionine	0.1	0.2	1.1	1.6	-
Lucien	1.1	2.0	5.1	6.5	4.1
Isoleucine	0.5	1.8	4.0	4.7	2.3
Phenylalanine	0.2	1.1	3.3	4.0	2.0
Threonine	-	1.0	3.9	4.0	1.9
Tryptophan	-	0.2	0.6	0.8	0.5
Arginine	0.3	1.8	4.0	4.9	2.8
Cysteine	-	0.4	0.5	0.8	-

Tyrosine	0.2	0.9	2.8	3.2	1.3
Glycine	0.3	1.3	3.7	4.4	2.7
Aspartic acid	0.5	2.5	6.1	7.4	4.6
Glutamic	1.7	0.43	7.8	8.7	4.4
Proline	0.2	0.9	2.2	2.9	-
Serein	-	0.8	2.0	3.6	1.5
valen	0.3	-	3.0	3.8	1.6
Histidine	-	-	1.0	1.2	0.6
EAAI	0.634	0.811	0.899	0.949	0.772

CONCLUSION

It was deduced that, lighting intensity has a significant effect on the total protein and amino acid content of the algae under study. A direct relationship was observed between lighting levels, total protein content, and the rate of amino acids in *Lyngbya* sp.

REFERENCES

Al–Badri, S. H. A. and AL – Ebady, A. R.A.(2020).Effect of uv-b radiation on content of pigments and mineral elements in *Cladophora graminea* and *Spirogyra deadaleoides* Algae. Indian Journal of Ecology., 47 Special Issue (12): 221-224.

Bourrely, P. (1980). Les algaes deuu douce , initiation ala systematique , Soc. Naur. Edit. Boubee, Paris, (517 , cited by Venkataraman and Becker , 1985) .

Bouterfas, R. ; Belkoura, M. and Dauta, A. (2002). Light and temperature effects on the growth rate of three freshwater algae isolated from a eutrophic lake. Hydrobiol., 489 : 207-217 .

Coombs, J. and Hall, D.O. (1985). Techniques in bio productivity andphotosynthesis. The Pergamon Text book . Press une : 289 .

Cosper, E. (1982). Effects of variations in light intensity on the efficiency of growthof *Skeletonema costatum* (Bacillariophyceae) in a cyclostat Journal of Phycology., 18 : 360-368 .

Desikachary, T. (1959). Cyanophyta .ndian Council of Agricultural Research, New

Delhi, 517 .

Dortch, Q. (1982).Effect of growth conditions on accumulation of internal NO_3 , NH_4, amino acids and protein in 3 marine diatoms. Journal of Experimental Marine Biology and Ecology., 61 : 243-264 .

Dybel, J. ; Tester, P.A. and Litaker, R.W. (2006). Effects of light intensity on

Cylindrospermopsin production in the cyanobacterial HAB species *Cylindrospermopsin raciborskii* .African Journal of Marine Science., 28(2) : 309-312.

Grima, E ; Fernandes, F. ; Camacho, F. and Chisti, Y. (1999). Photo bioreactors :

light regime, mass transfer and scale up. Journal of Biotechnology., 70 : 231-247.

Jones, T.W. and Galloway, R.A. (1979). Effect of light quality and intensity on glycerol content in *Dunaliella tertiolecta* (Chlorophyceae) and the relationship to cell growth / osmoregulation. Journal of Phycology., 15 : 101-106 .

Kalita, T.L. and Tytlianov, E.A. (2003). Effect of temperature and illumination on

growth and reproduction of the green alga *Ulva fenestrate*. Russian Journal of Marine Biology., 29(5) : 316-322 .

Lionard, M. ; Muylaert, K. ; Gansbeke, D.V. and Vyverman, W. (2005). Influence

of changes in salinity and light intensity on growth of phytoplankton Communities from the Schelde river and estuary (Belgium / The Nether lands). Hydrobiology., 540 : 105-115 .

Lu, C.M. ;Torzillo, G. and Vonshak , A. (1999). Kinetic response of photosystem II photochemistry in the cyanobacterium *Spirulina platensis* to high salinity is characterized by two distinct phases. Australian journal of plant physiology., 26 : 283-292 .

Lu, C. and Zhang, J. (2000). Role of light in the response of PSII photochemistry to

salt stress in cyanobacterium *Spirulina platensis*. Journal of Experimental Botany., 51(346) : 911-917 .

Mortensen, S.H. ;Borsheim, K.Y. ; Rainuzzo, J.K. and Knutsen, G. (1988). Fatty

acid and elemental composition of the marine diatom *Chaetoceros gracilis*. Journal of Experimental Marine Biology and Ecology., 122 : 173-185 .

Newsted, J.L. (2004). Effect of light, temperature and pH on the accumulation of phenol by *Selenastrum capricornutum* , a green alga . Ecotoxicology and Environmental Safety., 59 : 237-243 .

Ortega, G.L. ;Snoeijs, P : Robledo, D. ; Freile-Pelegrin, Y. and Pedersen, M. (2008). Growth and pigment composition in the red alga *Halymenia floresii* cultured under different light qualities. Journal of Applied Phycology., 20 : 253-260 .

Prescott, G.(1975). Algae of the western Great lake areas. Ellion C .Brown Co.,pub.Dugugue.Iowa.:977.

Sievonen, K. (1990). Effects of light , temperature , nitrate , orthophosphate and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. Applied and Environmental Microbiology., 56(9) : 2658-2666 .

Solovchenko,A.E.;Khozin – Goldberg, I .; Dioli-Cohen, S. ; Cohen, Z. and Merzlyak, M . N.(2008). Effect of light intensity and nitrogen starvation on growth ، total fatty acids and arachidonic acid in the green microalga *Parietochloris incisa* .Journal of Applied Phycology.,20 : 245 – 251 .

Stein, J.R. (1973). Hand book of phycological methods. Cambridge Unv. Press. Cambridge , U.K .

Tomaselli، L.; Giovannetti ، L. and margheri، M.C. (1981) . on the mechanism of trichome breakage in *Spirulina platensis* and *Spirulina maxima*. Annals of Microbiology، 31:27-33.

Vonshak, A. Kancharaks, N.; Bunnag, B. and Tanticharoen, M. (1996). Role of

light and photosynthesis on the acclimation process of the cyanobacterium *Spirulina platensis* to salinity stress. Journal of Applied Phycology., 8:19- 124.

Yu-Wang, C, ; Chong, C. and Liu, Y.C. (2007). Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. Biochemical Engineering Journal., 37: 21-25 .