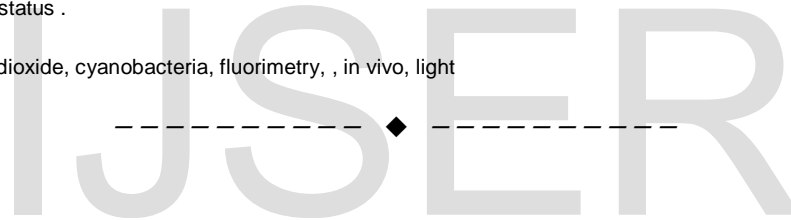


Photobiochemistry of Photosynthetic Pigments of Edaphic Cyanobacterium *Anabaena sp.FS76*, Under the Combination Effect of Irradiance and Carbon Dioxide Concentrations

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Abstract— The agriculture lands , specifically Rice paddies are subjected to sever restrictions seasonally, monthly and even daily, because of placing in flood situations. Habituation of soil cyanobacteria with this ecosystem provides the optimum conditions for growth in this series. The goal of this research is to study the habituation of cyanobacterium *Anabaena SP.FS76* to the simultaneous changes in carbon dioxide and light by using of evaluated fotobiochemical procedures, which is done on the body of living sample and optical shocks, and so their impact is evaluated in this research for the first time. The sample is provided from Golestan state and is placed at specific medium BG.11, then it is studied according to different conditions of light (2,10,50,100 micro mole quanta per square meter per second) ,concentration of carbon dioxide (absolute and relative limitation) ,together with optical shocks at short intervals (15,10,5,0). This study is done by considering the examination of absorption spectrum in vivo and fluorimetry evaluation. Survivorship, growth, chlorophyll content, carotenoid, phycocyanin, allophycocyanin, phycoerythrin, are examined in each of the compound treatments. The results are shown that, the photosynthetic system of cyanobacterium, *Anabaena SP.FS76*, has the ability for rearrangement at short intervals. Light is one of the effective factors in this field. Increasing the light will increase the photosynthetic performance and as a result the phycobiliprotein content will be enhanced. The statistical analysis indicates a significant correlation. Also it should be mentioned that, phycoerythrin pigment has a maximum growth in comparison with other pigments. On the average, phycobiliproteins production reaches to the maximum level at the conditions of relative limitation of co2, light intensity of 100 micro mole quanta per square meter per second and absolute limits of co2, light intensity of 50 micro mole quanta per square meter per second, and at the end a significant difference is shown in comparison with lower light intensities. The condensation mechanism of this sample is so potent and it performs actively in the condition of absolute limits of carbon dioxide. On the whole , by considering the high capabilities of this cyanobacteria, it should be expressed that, it is a suitable sample for practical usage as it can preserve it's duration and contrast with difficult status .

Key words— Anabaena, carbon dioxide, cyanobacteria, fluorimetry, , in vivo, light



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1. INTRODUCTION

Cyanobacterias are the first oxygenic photosynthetic creatures in the earth's surface. Rice paddies are the most important communities which constitute wet soils. (Shokravi and his colleagues, 2003). As rice paddies ecosystems normally have flooding conditions, so they are exposed to light fluctuations and severe restrictions of carbon dioxide, seasonally, monthly, and even daily. Thus rice paddies cyanobacterias should deal with this condition. (Boussiba, 1988). Light is the most important factor which controls the seasonal fluctuations of Fitoplanktons in rice paddies. (Stal, 1995). Changes of light are completely tangible while the rice is being grown. Some of the studies have shown that, the amount of light which is being received by rice paddies after the complete growth of rice, is about one percent of the amount of light, which was received before the growth of the rice. (valiente and leganes, 1989). *Terricolous* cyanobacterias are generally friendly with shadow situation, but this doesn't mean that, these creatures lost the ability to survive and grow in severe conditions of light. (stal, 1995). Optical deterrent in this category of algae rarely happens, even in high intensity of light, and their compatibility with this severe condition can be considered. (stal, 1995).

Also cyanobacterias have specific strategies for the usage of limited amount of light. The existence of phycobilisome mechanism is one of these strategies. (soltani and colleagues, 2006). The role of light in fluidity of photosynthetic pigments and optical systems of cyanobacterias are important characteristics of physiological nature of these creatures. (Tandeau and Marsad, 2003). The phycobilisomic structure with the ability of adaptive optics can affect on cyanobacterias to reach the ability to adapt themselves with limited intensity of light. (valiente and leganes, 1989).

About the condensation mechanism of carbon dioxide in cyanobacterias, the researches have done in this field are less than the researches about unicellular green algae. (shokravi and colleagues 2004). Condensation mechanism is a sensitive system which in one hand, depend on the desired performance of energy generating systems like photosynthesis and respiration, and on the other hand is in direct contact with the acidity of the environment. (Richmond, 1986).

Flooding rice paddies make a kind of balance between carbon dioxide and bicarbonate. Determinant factor of this balance is the acidity of environment. (stal, 1995). The

studies have shown that some the cyanobacterias can induce the collection mechanism of inorganic carbon when it has less CO₂ or when it receives light for 4 to 6 hours. The existence of this system allows to the cyanobacteria to absorb the carbon dioxide of environment with high acidity condition or absorb HCO₃⁻ in low acidity condition. (shokravi and colleagues, 2004). In the absence of advanced condensation mechanism in cyanobacteria, specially in soils and alkaline condition, diazotrophic process faces some problems in the production of some of the collection of amino acids. Because of the sharp decline of alpha ketoglutarate and also reduction in the production of amino acids collection like aspartate and alpha ketoglutarate, which mainly happens because of reduction in the activity of phosphoenolpyruvate carboxylase, are branches of this group (stal, 1995). So the samples which have flexibility in the excitation of this mechanism and also have required resources for energy supply, are capable in the creation of development (Richmond, 1986). As yet in Iran, the complete and independent researches have not done by relying on photobiochemistry about the observations of fluidity of photosynthetic pigments in cyanobacterias. In the reports of Soltani and his colleagues (2005), Strain *Fischerella*'s physiological changes in the inoculation process of carbon dioxide were surveyed at different salinities and relatively limited light intensity (Lux 6000). In the studies of Shokravi and his colleagues, the potential of cyanobacteria as the candidate of biological fertilizer and the problem of light and the role of it in the survival and growth of *terricolous* cyanobacterium were considered. About the optical frequency, there are only reports of Shokravi and his colleagues (2003). In these reports, the reactions of nostocales cyanobacterial and stigonematales to the 12 hours of photo periods were checked. Also in the studies which were done by Shokravi et al 2011 and 2012, Terrestrial cyanobacteria was under the combined effects of carbon dioxide and alkalinity, acidity and some parts of research were also assigned to the photo biochemical studies. In this research, habituation of cyanobacterium *Anabeana SP.FS76* was surveyed under the combined condition of light and density of carbon dioxide.

2. Materials and methods

Soil sampling was done in Golestan State. After the stages of soil cultivation, successive sub cultured colonies were created and finally *Anabaena SP.FS 76* cyanobacterium was

prepared in pure form (stal,1995). The sample was applied to a specific culture BG011, and the cultivation was done in 250 ml flask containing 100 ml of suspension (Soltani et al.,2006). The sample was placed under proper conditions of the acidity of the liquid medium PH=7.2 (Basic condition for growth). After the initial investigation of physiological and passing a life cycle, the sample was prepared for treatment process (stal.1995). for applying treatment at the same time, the sample suspension was inoculated in test tubes, after homogenization with a certain amount of specific environments BG011. It was continuously placed under different conditions of light intensity (2,10,50,100 Quantum micromoles per square meter per second), and also it was adjusted by Luxury device detector at certain distance from the light source. However the various conditions of the carbon dioxide density with no aeration (sever limitation of co2) and with aeration (relative limitation of co2) were considered to compare and select the optimal growth of the sample. The growth was measured on the base of turbidity by using of spectrophotometer with 750 nm wave length (soltani and colleagues 2006). Investigating about the status of chlorophyll pigments (a), carotenoid and phycobiliproteins (phycocyanin, allophycocyanin, phycoerythrin, and mycosporine like amino acid) was done in the visible region of light, with spectroscopic method as in vivo (suda et al.,2002). Also for the first time, to assess and rearrange the systems for photosynthetic sample, the light-induced short term shocks were used in four minutes (15, 10, 5, 0),with comparing the results altogether. The growth of pigments in chlorophyll area and phycocyanin pigments were evaluated as in the vivo and by spectro fluorimetry device in the third day of growth.

3. Results

By considering the growth curves at different light intensities , the sample was placed in the high level of growth, in terms of relative limitation of carbon dioxide in the maximum light intensity ,100 micromoles Quantum per square meter per second with little difference from high light intensity about 50 micromole. The results were shown that , from the first days ,the sample had better growth

after inoculation with this light intensity rather than other light conditions. (figure 1). In terms of absolute limits of co2 , the sample in the high intensity of light ,50 micromoles Quantum per square meter per second , was increased and in the first and last days of growth cycle with other light intensities , there are significant differences. (figure 2) (sig<0.05)

In the first days after inoculation, the content of chlorophyll , carotenoid and phycoerythrin pigments had greater increase, in terms of absolute limits of carbon dioxide and limited light treatment, than aeration (relative limitation of co2) , and so this made a significant difference . (sig<0.05). Also it should be mentioned that, the performance of phycobilisome device was weaker in the production of other pigments like phycocyanin and Allophycocyanin. This process had been changed from fifth day after, and by comparing with the increased non-aerated condition , it should be considered that , the production of all pigments substantially increased in the aerated condition ,(relative limitation of carbon dioxide), with high brightness. So at the end significant differences were resulted. (sig<0.05). increasing the light will affect phycobilisome after fifth day of inoculation. (fifth day R=0.66). while, the sample was also growing in the relative and extremist conditions of light, although this growth had been low because of the high ability of sample photosynthetic system, for dealing with restrictions. The performance of photosynthetic system , condensation mechanism of carbon dioxide , the productions of sample phycobilisome device at different intensity of light conditions , and applied factors of aeration and non-aeration at the 11 day of treatment are shown in figure 3. The results of short term light shocks (15,10,5,0,minutes) showed that, this sample is very powerful in the rearrangement of the photosynthetic system at the shortest possible time (figure 4,5). The growth of phycobiliprotein pigments were evaluated by considering as in vivo with excitation of different light treatment in the third day after inoculation by fluorescence device at the wave length of 800-600. All treatments were producing pigments and the light intensity of 10 micro moles Quantum per square meter per second in the relative limitation of co2 within the chlorophyll and phycocyanin pigments, which showed a maximum absorption. (figure 6).

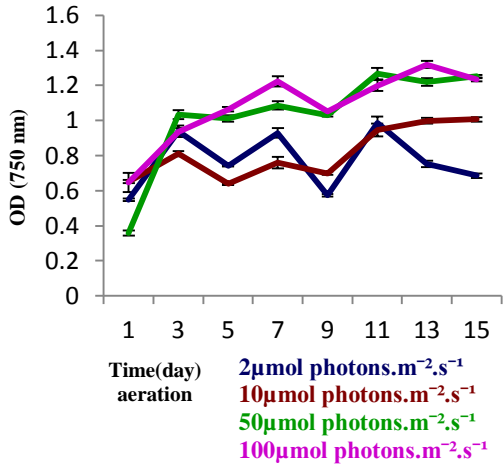
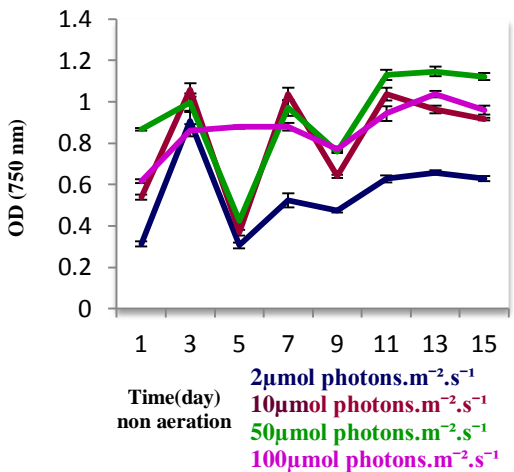
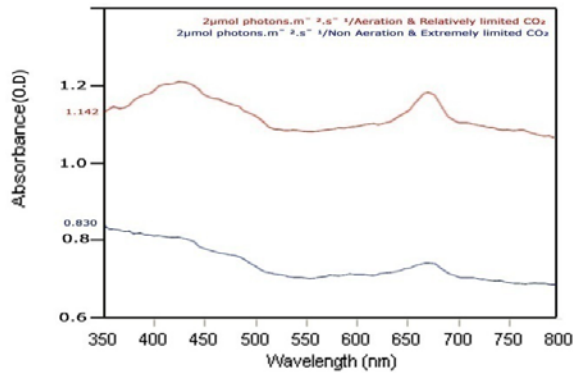


Figure 2: comparing the growth (turbidity) at cyanobacterium *Anabaena SP.FS 76* at the conditions of absolute limits of carbon dioxide (non aeration) and different light treatments (2,10,50,100 micro mole quanta per square meter per second)

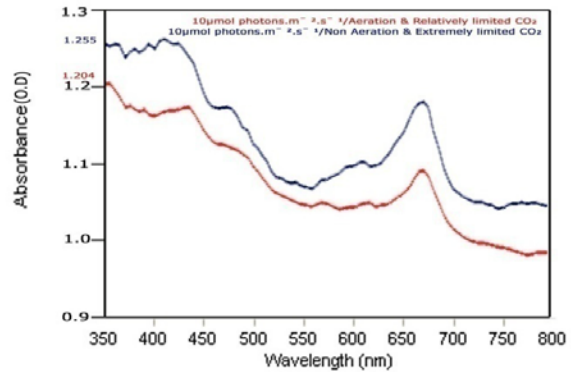
Figure 1: comparing the growth (turbidity) in cyanobacterium *Anabaena SP.FS 76* at the conditions of relative limitation of carbon dioxide (aeration) and different light treatments (2,10,50,100 micro mole quanta per square meter per second).



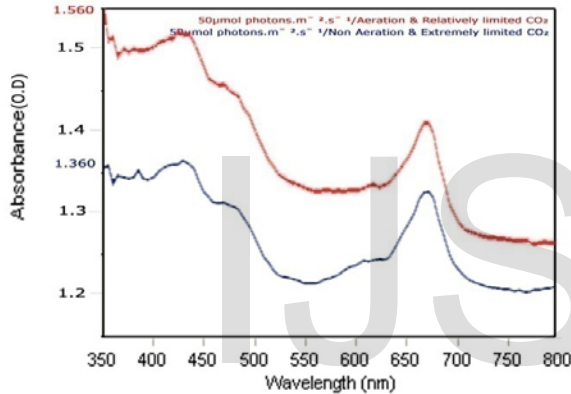
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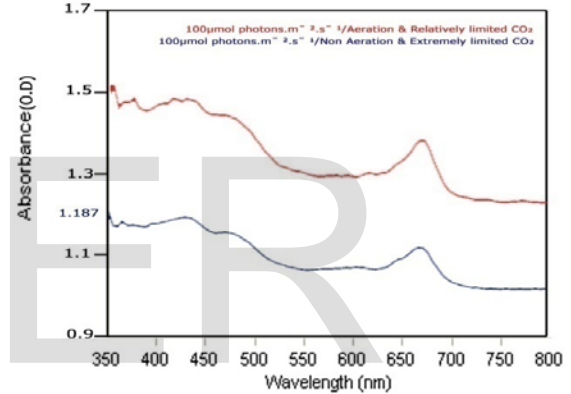
Comparing the conditions of aerated and non aerated in light treatment $2 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$



Comparing the conditions of aerated and non aerated in light treatment $10 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$



Comparing the conditions of aerated and non aerated in light treatment $50 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$



Comparing the conditions of aerated and non aerated in light treatment $100 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$

Figure 3: comparative curves of aeration and non aeration of phycobiliprotein pigments in cyanobacterium *Anabaena SP.FS76* which were divided according to light treatments a) 2 micro mole quanta per square meter per second, b) 10

micro mole quanta per square meter per second, c) 50 micro mole quanta per square meter per second, d) 100 micro mole quanta per square meter per second at eleventh day of treatment

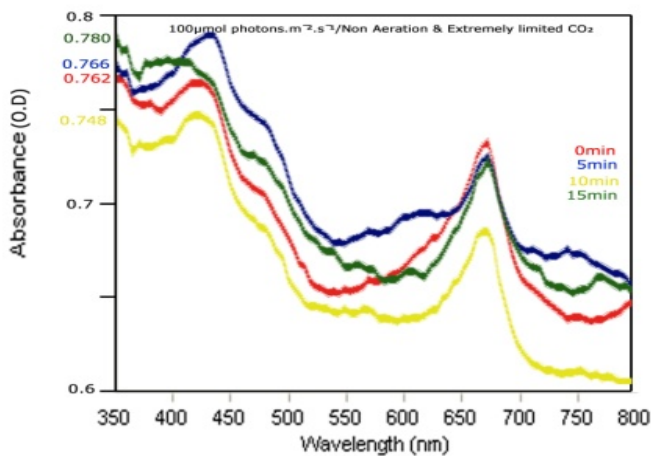
Table 1: comparing the phycobiliprotein pigments in cyanobacterium *Anabaena SP.FS 76* under the light treatments and density of carbon dioxide at eleventh day of treatment

Pigment	Absorption maxima	2	10	50	100
MAA	300-400	-	+	+	+
Chlorophyll a	430,450	+	+	+	+
Carotenoid	480	+	+	+	+
Phycocyanin	620	-	+	+	+
Allophycocyanin	650	-	-	-	+
phycoerythrin	680	+	+	+	+

Non Aeration, Mycosporine like amino acid(MAA), intensity of light($\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$)

Pigment	Absorption maxima	2	10	50	100
MAA	300-400	+	+	+	+
Chlorophyll a	430,450	+	+	+	+
Carotenoid	480	+	+	+	+
Phycocyanin	620	-	+	+	+
Allophycocyanin	650	-	-	-	+
phycoerythrin	680	+	+	+	+

Aeration, Mycosporine like amino acid(MAA), intensity of light($\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$)



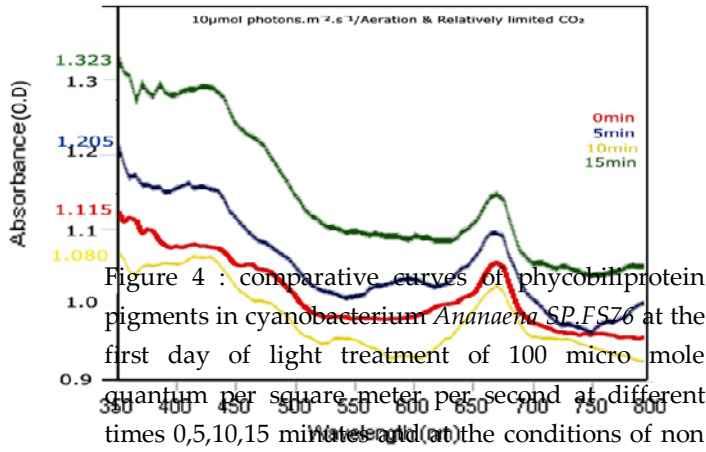


Figure 4 : comparative curves of phycobiliprotein pigments in cyanobacterium *Anabaena SP.FS76* at the first day of light treatment of 100 micro mole quantum per square meter per second at different times 0,5,10,15 minutes and at the conditions of non aeration and absolute limitation of carbon dioxide

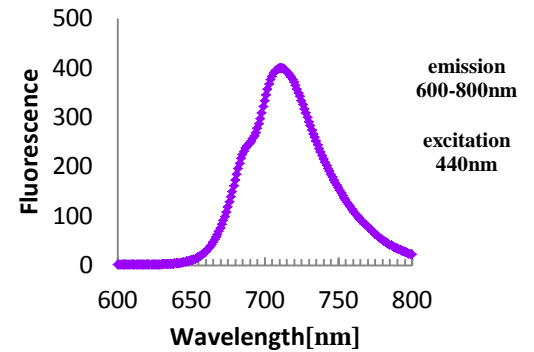


Figure 6: fluorescence spectroscopy curve of the *Anabaena SP.FS76* sample under the light treatment of 10 micro mole quantum per square meter per second and in the condition of aeration and relative limitation of carbon dioxide.

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Figure 5: comparative curves of phycobiliprotein pigments in cyanobacterium *Anabaena SP.FS76* at eleventh day of light treatment of 10 micro mole quantum per square meter per second at different times of 0,5,10, 15 minutes and at the conditions of aeration and relative limitation of carbon dioxide.

4. Discussion

The results of multifaceted factors on cyanobacterium *Anabaena SP.FS67* were shown that, in the first days after inoculation and in the relative comparing of the extremist limitation status of carbon dioxide with relative limitation

of it, the performance of phycobilisome system was better and pigment increase had considerable growth, with the view of containing chlorophyll, carotenoid pigments and phycoerythrin, while the amount of light was 50 micro mole quanta per square meter per second. But the production of pigments had been increased after

entrancing to the logarithmic stage, enhancing the performance of photosynthetic system, activation of the condensation mechanism of carbon dioxide in the high intensity of light as 100 micro mole quanta per square meter per second, and relative limitation of CO₂. Increasing of carotenoid in limited and extremist intensity of light was caused by combined effects of active condensation mechanism of carbon dioxide and light. (Shokravi and colleagues 2009). By considering the evaluation of Shokravi and Sateei 2004, the increase in the carotenoid content under the effect of light intensity up to 5000 Lux had been reported. The results of increase in the production of chlorophyll, carotenoid, and phycobiliprotein under the effect of light factors in *Anabaena* species and in other different species were in accordance with researches of Suda and colleagues, Tang and Vincent, Staal & Stal, Marri zade and colleagues (2013), Six and colleagues, Shokravi and Soltani (2010). In the studies of Shokravi and colleagues (2003), the effect of light intensity in the shape of photo periods of 12 hours was evaluated according to the morphological changes of *Fischerella ambigua*. Enhancement in the production of pigments occurred in the area of high and relative high light condition with the application of aeration. Although the sample grew in the extremist limitation of light (2 micro mole quanta per square meter per second), even thus this growth was low. At the same time some studies were reported about the duration of *Fischerella* species of cyanobacteria at 2 micro mole quanta per square meter per second of light by Soltani and colleagues in 2005. Phycoerythrin had meaningful increase in the optimum condition and in limited area, which showed the capability of photosynthesis system with operative condition.

The phycobilisome system preserved its performance in both relative and absolute limitation of carbon dioxide, however in the high intensity of light it had more considerable performance. The central part of sample phycobilisome system consisted of Allophycocyanin pigments and the marginal part of it contained phycoerythrin. The potent condensation mechanism of sample in the condition of absolute and relative limitation could provide the essential carbonic sources for different processes as the photosynthetic processes. (Shokravi and colleagues 2003). In some strains of stigmatalgae the increase of carotenoid was reported in the lowest intensity under the effect of changes in carbon dioxide content. (Sadani and colleagues 2010). The photosynthetic system provided the essential energy for condensation mechanism of carbon dioxide. It was recognized that, in applying optical shocks at short time intervals, the sample was so capable at the rearrangement of photosynthetic

system. This system was activated for receiving the light in the shortest possible time. This was considered as the performance of this system. Frequency of light in some of the cyanobacteria, like *Agardh. lyngbya SP.FS33*, made a tangible reduction in growth process, however in the light frequency of 2 and 4 hours, the sample preserved its duration. (Shokravi and Sateei 2004, Shokravi and colleagues 2009). In the results of fluorescence spectroscopy of Peter and colleagues, photosynthetic pigments, specially phycocyanin showed the maximum pigment growth in the absorption area of 710-660 nanometer with *Spirulina platensis* cyanobacteria spectroscopy, which was matched with this research. The study of Hader & Donkor about the fluorimetry of photosynthetic pigments of two sample, *Nodularia harveyana* and *Phormidium uncinatum*, at different time factors 0h for *Phormidium uncinatum*, 2h later for *Nodularia harveyana*, between 750-625 region at 645nm was shown a pigment increase which was not matched with this research.

5. The final result

The photosynthetic system of *Anabaena SP.FS 76* cyanobacterium had the ability for rearrangement at short time intervals. Light was one of the effective factors in this field. With increasing the intensity of light, the photosynthetic performance would also be enhanced and as a result the phycobiliproteins content would be grown. The limitation of carbon dioxide was another effective factor in the pigment production. In the condition of severe limitation, the condensation mechanism of carbon dioxide would be operated actively.

By considering the studies of different light intensities, on the average, the production of phycobiliproteins was at the maximum level in the condition of relative limitation of carbon dioxide with light intensity of 100 micro mole quanta per square meter per second and in the absolute limits of carbon dioxide with light intensity of 50 micro mole quanta per square meter per second. In the first stages of growth and habituation with different factors, the production process was the same in both condition of absolute and relative limitation of carbon dioxide which showed the consistency of phycobilisomes into the mentioned changes. Then the production process would be enhanced from extremist limitation of carbon dioxide to relative limitation of it, and it would be consistent at the high intensity of light. Preserving the survivorship, growth and the capability for contrasting with evaluated difficult status were considered in this sample.

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