ISSN 0868-854 (Print)
ISSN 2413-5984 (Online). Algologia. 2019, 29(1): 40–58
https://doi.org/10.15407/alg29.01.040

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EFFECTS OF ALKALINITY, EXTREMELY LOW CARBON DIOXIDE CONCENTRATION AND IRRADIANCE ON SPECTRAL PROPERTIES, PHYCOBILISOME, PHOTOSYNTHESIS, PHOTOSYSTEMS AND FUNCTIONAL GROUPS OF THE NATIVE CYANOBACTERIUM *CALOTHRIX* SP. ISC 65

In this research, *Calothrix* sp. ISC 65 was characterized physiologically by the combination of extremely low irradiance (2 $\mu E \cdot m^{-2} \cdot s^{-1}$), different alkalinity (pH 7, 9, 11), and extremely limited carbon dioxide concentration (no aeration, no carbon dioxide enrichment). Spectroscopical analysis showed that pH 9, after 96 hours, caused a significant increase in growth rate, chlorophyll, and phycocyanin production. A lower (pH of 7) caused a decrease of phycobilisome production even after 24 hours. Excitation of the light harvesting complex and the reaction center of photosystems resulted from a pH of 9. Phycocyanin seems to be the main part of phycobilisome but pH 9 caused phycoerythrin and allophycocyanin production excitation in the outer part of the photosynthetic antenna as well. A fluorimetric and photosynthesis-irradiance curve analysis showed that increasing alkalinity (up to pH 9) caused an increase in photosynthesis efficiency and a decrease of non-photochemical fluorescence especially after 96 hours. PSII : PSI ratio increased by increasing alkalinity from pH 7 to 9 and reached the highest level after 96 hours. Surface response plot analysis showed that there is a narrow border line around pH 9 and 96 hours which caused the highest PSII : PSI ratio. FTIR analysis showed that alkalinity caused configuration changes of the functional groups. The difference of the functional group patterns between pH 7 and 11 was significant especially after 24 hours. Differences in asymmetric carbon vibration, lipid stretching and OH bending of the polysaccharides occurred with both pH 9 and 11 treatments. pH 9 caused the most physiological activities in *Calothrix* sp. ISC 65 at extremely limited irradiance and carbon dioxide concentration.

K e y w o r d s: alkalinity, *Calothrix*, cyanobacteria, dissolved inorganic carbon, limited irradiance

A b b r e v i a t i o n s: APC – allophycocyanin, CCM – carbon dioxide concentrating mechanism, DIC – dissolved inorganic carbon, PBS – phycobilisome, PC – phycocyanin, PE – phycoerythrin, PSI, PSII – photosystems I and II

Introduction

Under natural conditions in rice fields and petroleum polluted soils, cyanobacteria are exposed to the combined influences of several factors such as alkalinity, irradiance and dissolved inorganic carbon fluctuations, which may vary even on a daily basis (Shokravi, Soltani, 2011). Growth, biochemical and physiological characteristics of cyanobacteria are influenced by these environmental factors. Furthermore, widely fluctuating environmental parameters, including light level and quality, as well as temperature and mineral nutrient availability, interact to influence growth, molecular resource allocation, and photosynthesis through complex adaptation strategies. For example, Gan et al. (2014) believed that cyanobacteria can alter their total Chl. and PBS content; adjust their PSI to PSII ratio; perform non-photochemical quenching using the orange carotenoid protein; and modify their light-harvesting complexes in response to nutrient stresses (S, N, and Fe limitation).

Light is evidently one of the most important factors that determine the natural distribution of cyanobacteria. As other photosynthetic organisms, cyanobacteria are able to adapt to variations in light intensity; nevertheless, little work has been done in this area (Shokravi, Soltani, 2011). Authors Bacares-Espaca et al. (2013) believed that cyanobacteria capable of forming surface blooms cannot cope as well with high-light stress as well as green algae can. This cyanobacterium acclimated to the light field by changing both its size and the number of its photosynthetic units. In rice fields, light reaching the floodwater varies both daily and over the crop cycle because of the variation in light transmission caused by changes in rice canopy height. In Iran, the drop of sunlight in rice-fields seems relatively harder (for example measurements at Golestan province at the north of Iran) showed a declining rate from 1000 μmol photon·m^{-2}·s^{-1} early in the growth of the crop to 2 and even 0.5 μmol photon·m^{-2}·s^{-1} when the crop was matured, especially on cloudy and rainy days. Therefore, the acclimation of cyanobacteria to such an extremely low light condition seems important and basic (Shokravi and Soltani, 2011).

The alkalinity of the soils is one of the most important problems in both north and south Iran (Amirlatifi et al., 2013). We have no special information about behaviors of different strains of native *Rivulariaceae* to alkalinity fluctuations other than what (at species level) has been reported for other cyanobacteria. For example, Padhi et al. (2011) have studied the effect of alkalinity on the biomass of different species of *Anabaena* and have shown special behaviors of each species. Authors Shokravi et al. (2010), studying

acclimation of the native cyanobacterium *Haplosiphon* sp. FS 44 to combined effects of carbon dioxide concentration, acidity, and alkalinity showed that differences of growth rates seemed insignificant between acidic and alkaline conditions but carbon dioxide enrichments caused a significant increase in the growth rate. Phycobilisome system of this strain lack phycoerythrin, however it may complete its structure both at the core and the rode at alkaline conditions. However, it seems that most of the cyanobacteria including *Rivulariaceae* prefer neutral to alkaline environments (Poza-Carriyn et al., 2001; Soltani et al., 2006). Preliminary tests showed that the strain of *Calothrix* is similar to another cyanobacterium studied from the northern paddy-fields of Iran, resembled an alkalophile strain. Microscopic observations showed that with pH 5, at the end of the first week, most of the filaments degenerated, and their colors changed to yellow. Irradiance and DIC fluctuations had no effect on this (Abbasi et al., unpublished data). This was compatible with our previous results as well (Soltani et al., 2007; Shokravi et al., 2014; Safaie et al., 2015). Regarding the above, we avoided using acidic conditions in spite of the previous papers (Soltani et al., 2007; Iranshahi et al., 2014; Shokravi et al., 2014).

Fast and high amplitude changes in light happen in the context of a variable environmental Ci concentration, influenced by water temperature, pH, exchange with the atmosphere, photosynthetic and respiratory activities of the plankton and benthos, and import from terrestrial sources (Cole et al., 1994). To maintain a high intracellular Ci concentration across variable environmental Ci concentrations, cyanobacteria can induce powerful carbon concentrating mechanisms (CCM) to actively concentrate sparse environmental Ci into the cell using energy from photosynthesis, and then release internal Ci as CO_2 near Rubisco at concentrations sufficient for efficient assimilation into organic form by the Calvin cycle (Tyler et al., 2004).

The aim of this work was to establish the combined influence of alkalinity and DIC limitation on growth, photosynthesis, and photosynthetic pigmentation, of the new collected, and identified cyanobacterium *Calothrix* sp. strain ISC 65 which for the first time, was isolated from oil polluted soils of Iran (Soltani et al., 2012) and has been recently collected and determined (but not reported) from paddy fields of Iran at different alkalinities, extremely limited DIC, and with irradiance more or less similar to natural rice-field of Golestan province when maturing of the crop takes place (2 μmol photon·m^{-2}·s^{-1}). We focused on extreme representative alkalinity values in Iranian rice fields (pH 7, 9 and rarely 11). In addition, we added time as an important factor, especially for the influence of irradiance. Authors Soltani et al. (2007) showed

that the longtime photosynthesis differed completely at combinations of alkalinity and irradiance after 24 and especially 96 hours.

Materials and Methods

Isolation of strain

Calothrix sp. was isolated for the first time from endaphic and epilithic forms in Khuzestan province (Khark Island, south of Iran and near the Persian Gulf). Recently we collected and identified such a strain from paddy-fields of Golestan Province as well. The complete descriptions of the stations and their geographical and environmental conditions have been reported in Soltani et al. (2012). The collected sampleswere culturedby ordinary methods (Kaushik, 1987). After colonization and isolation, the cyanobacteria *Calothrix* sp. ISC 65 was purified and became axenic (Kaushik, 1987). Morphologically identification and determination were done according to Desikachary (1959), Prescott (1962), Tiffany and Britton (1971), Komárek and Anagnostidis (1989), and John et al. (2003). Molecular identification was done by 16S *r*DNA according to Dezfulian et al. (2010). PCR-identification of *Calothrix* sp. ISC 65 was isolated from south of Iran. NCBI: GU591756.

Stock cultures were grown in N-free medium. BG110 solid medium was used for culturing (materials for BG110 medium: $MgSO_4 \cdot 7H_2O$, 0.3 mM; $CaCl_2 \cdot 2H_2O$, 0.25 mM; $K_2HPO_4 \cdot 3H_2O$, 0.18 mM; $Na_2Mg \cdot EDTA$, 0.003 mM, Citrate ferric 0.02 mM; Acid Citric, 0.029 mM; $Na_2CO_3 \cdot$ 0.188 mM; microelements 1 $mL \cdot L^{-1}$). The pH was then raised to 7.2 by adding of NaOH, and the solution was autoclaved. Purification and the axenic culture method were controlled microscopically (Shokravi, Soltani, 2011).

Incubation conditions and treatments

Stock cultures were grown in the BG110 culture medium. Temperature was maintained at 30 °C and cultures were incubated under a constant light intensity of 60 μmol photon$\cdot m^{-2} \cdot s^{-1}$ supplied by three fluorescent lamps (Poza-Carrion et al., 2001). Cells in the logarithmic phase of growth were collected from stock cultures and used as inoculate for experiments. Cells from the stock culture were inoculated in 300 mL of BG110 medium in 500 mL Erlenmeyer flasks stoppered with cotton plugs. Cultures were illuminated via different numbers of nets between light source and flasks. Illumination was supplied with 40 W cool white fluorescent tubes to attain a desired low irradiance (2 μmol photon$\cdot m^{-2} \cdot s^{-1}$). Light measurements were made with Licor LI-1000 Datalogger equipped with a quantum sensor. Aliquots were taken and used for determinations when cells adapted to the light regime in

logarithmic phase. Finally, we compared cultures without supplementary aeration or stirring (standing condition, extremely DIC limitation) (Shokravi et al., 2014). Alkalinity treatments were made using NaOH at three ranges (pH 7, 9, and 11) which were prepared in different flasks.

Analytical methods

Growth measurements, pigment composition, and PBS system were analyzed spectroscopically after 24, 48, 72, and 96 hours of alkalinity treatments according to Fraser et al. (2013). Photosystem ratios and characteristics were done spectrofluorimetrically according to Zorz et al. (2015) and Inoue-Kashino et al. (2005). For absorption spectra of intact cells; after harvesting the cells, the pellets were suspended in 3 mL of reaction buffer. This cell suspension was taken for scanning the absorption spectra from 360 nm to 800 nm. The absorption spectra of intact cell suspension were taken using a Hitachi-557 double beam spectrophotometer. At 750 nm the absorption of the cell suspension was adjusted to give approximately the same reading. Room temperature fluorescence emission spectra of the cells were recorded on a Perkin-Elmer LS-5 spectrofluorimeter following Tiwari and Mohanty (1996). PSII : PSI ratio analysis was done by spectrofluorimetry according to Gan et al. (2014), and Amirlatifi et al. (2018). The fluorimetric analysis was operated using Marvizadeh et al. (2013). For FTIR analysis, 1.5 mL from the suspension was centrifuged at 10000 rpm for 10 min. The pellet was dried using lyophilization and the 100 mg were mixed with 1000 mg KBr the disc was prepared and the total fatty acids were evaluated using FTIR (Ray leight-510) (Kiaei et al., 2013).

Statistical analysis

Data are the means and standard deviation of at least four replicates. Statistical analyseswere examined using Designs-Expert Ver.7 and 10. One factor and multifactor RSP analysis were done according to Ghobadian et al. (2015).

Results and Discussion

The growth of *Calothrix* sp. ISC 65 continued at neutral (pH 7) and alkaline (pH 9) conditions in log phase to 96 hours (Table 1). The biomass production rate was influenced by alkalinity under both conditions. The rate of chlorophyll production seemed compatible with the growth at extreme conditions. This was not true for chlorophyll contents per cell and the peak of chlorophyll absorption. Despite this; the effect on PBS (normalized to chlorophyll) seemed more outstanding. DIC limitation could not change the

pattern of biomass production in *Calothrix* sp. ISC 65. Alkaline condition (pH 9) and limited irradiance, caused the maximum biomass production and growth rate. The role of alkalinity was more outstanding than irradiance because pH 7 caused the decline in biomass production under the same conditions of DIC and irradiance. Comparing optical densities at 750 nm after 24 hours showed that cyanobacterium had a better ability for acclimation at pH 9. This is in agreement with Soltani et al. (2007). Results for the response of the toxic cyanobacterium *Dolichospermum* sp. to lowered pH (–0.4 units by adding CO_2) and elevated temperature (+4 °C) in an experimental set-up were examined. Growth rate, microcystin concentration and oxidative stress were measured. The growth rate and intracellular toxin concentration increased significantly as a response to temperature. When *Dolichospermum* was exposed to the combination of elevated temperature and high CO_2/low pH, lipid peroxidation increased and antioxidant levels decreased (Brutemark et al., 2015).

Spectroscopical analysis showed that the effect of alkalinity on phycobilisome production was outstanding especially after 96 hours (Table 1). After 24 hours, the difference between the effect of pH 7 and 9 was not significant. After 96 hours, the high alkaline condition caused excitation of phycobilisome production. This was not true under neutral condition. The production of phycobilisomes depended on the alkalinity under long periods and not for short periods. The role of time seemed more outstanding than DIC and irradiance (Fig. 1).

Table 1

Spectral characteristics of *Calothrix* sp. ISC 65 over 24 and 96 hours alkalinity treatments

Spectral characteristics	Time, hours	pH 7	pH 9
ln (A750)	24	-1.46	-1.78
	96	-1.23	-1.35
ln (A680-A750)	24	-3.78	-3.25
	96	-3.69	-2.24
(A680-A750)/A750	24	0.10	0.23
	96	0.17	0.19
Chl (λ_{max})	24	685	685
	96	686	688
(A630-A750)/(A680-A750)	24	1.21	1.28

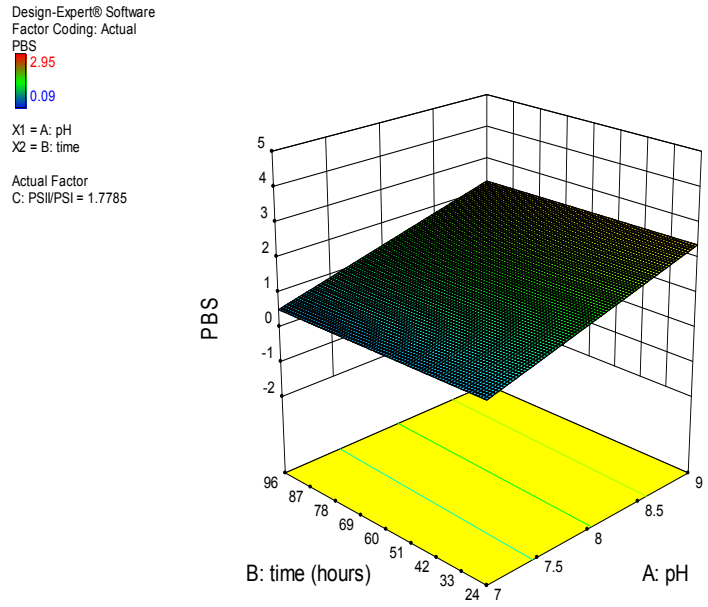

Fig. 1. RSP-analysis of PBS in *Calothrix* sp. ISC 65 at different alkalinities and time treatments

Collectively, we can say, the effect of alkalinity in the growth rate and phycobilisome production in this strain seemed essential and this did not depend on extremely limited DIC and irradiance. This was not true for *Fischerella* sp. FS 18 (Soltani et al., 2007) and *Hapalosiphon* sp. FS 44 (Shokravi et al., 2014) collected from the same region.

Table 2 shows the effect of alkalinity on pigment composition of *Calothrix* sp. ISC 65. Results emphasized that alkalinity treatments caused excitation of both light-harvesting complex and phycobilisome system. Pigmentation is the main phenotypic difference within the similarly sized, planktonic freshwater picocyanobacteria.

Table 2

Absorption ratios of *Calothrix* sp. ISC 65 after 24 and 96 hours alkalinity treatments time course

pH	Absorption ratio of *Calothrix*					
	440/680		480/680		621/680	
7	24	96	24	96	24	96
	1.308	1.370	1.367	1.160	1.031	1.943
9	1.379	1.395	1.422	1.315	1.054	1.945

Red PE-rich picocyanobacteria use phycoerythrin, and green PC-rich picocyanobacteria use phycocyanin as major light-harvesting pigments (Moser et al., 2009). The number of carotenoids and phycocyanin increased under alkaline conditions, especially after 96 hours. This was true for chlorophyll especially in the red region which forms essential parts of the main photosynthetic reaction center and light harvesting system. Phycocyanin contents appeared as the main part of phycobilisome and were more stable during time and alkalinity fluctuations. It was interesting that alkalinity at limited DIC and irradiance treatments excited phyco-biliproteins and carotenoid production. Phycocyanin was the most concentrated under the extreme values of the treatments comparing the other parts of phycobilisome system: phycoerythrin and allophycocyanin. The highest rates of alkalinity, especially after long periods of time, caused the highest rates of phycocyanin production but the difference between short and long time periods was not significant (Table 2).

Synechocystis sp. strain PCC 6803 grows photoatrophically across a broad pH range (Summerfield et al., 2013). The mutant of this strain cannot tolerate pH 7 (Summerfield et al., 2013). In spite of *Calothrix* sp. ISC 65, the sensitivity of growth, pigment production and photosynthetic apparatus in *Fischerella* sp. FS 18 (Soltani et al., 2011) and *Hapalosiphon* sp. FS 44 and 56 (Shokravi et al., 2012, 2014) collected from the same region to extremely limited irradiance was noticeable. In Soltani et al. (2007) the amount of chlorophyll production at relatively limited DIC (aeration condition) was about 11.99 $\mu g \cdot mg\ dw^{-1}$ (at 3 $\mu E \cdot m^{-2} \cdot s^{-1}$) and 8.32 $\mu g \cdot mg\ dw^{-1}$ (at 300$\cdot \mu E \cdot m^{-2} \cdot s^{-1}$). Decreasing irradiance to 2 (instead of 3 $\mu E \cdot m^{-2} \cdot s^{-1}$) and limitation of DIC to non-aeration conditions caused different amounts of chlorophyll production especially under alkaline conditions (pH 9). Safaie et al. (2015) showed that in *Fischerella* sp. the higher amount of chlorophyll production at pH 9 belonged to 2 $\mu E \cdot m^{-2} \cdot s^{-1}$ when DIC was not limited but to 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ when DIC was extremely limited. In the case of *Nostoc* sp. UAM205, it has been reported that the maximum growth rate was at pH 9 and increased with increasing light intensity at this pH (Fernández-Valiente and Leganés, 1989).

But results of experiments with *Nostoc* sp. UAM 206 showed that the effect of pH and light intensity depended on the availability of DIC, in such a way that under conditions of DIC limitation growth increased with pH but light conditions had no effect; on the contrary, when DIC was available growth increased with increasing light but not effect of pH was observed (Poza-Carriyn et al., 2001). Therefore, apparently the effects of irradiance and alkalinity significantly depend on the species studied and, on growth conditions (Soltani et al., 2007). It may be species-specific characteristics of

photosynthetic apparatus helping dominance of the strain at DIC concentration tensions, especially at different environmental conditions include light and alkalinity (Deblois et al., 2013). Tyler et al. (2004) showed that *Synechococcus elongatus* cells grown bubbled with air (approximately 370 mmol CO_2 mol) induced a high-affinity CCM with a Km of 14 mmol Ci, which maintained growth rates nearly as high as *S. elongatus* cells grown bubbled with 50,000 mmol CO_2 mol^{-1} air, which had a Km of 281 mmol Ci. Thus, synthesis and maintenance of the CCM required significant investments and rearrangements for cells growing in low-Ci environments, but nonetheless, under steady light and nutrient supplies, low-Ci cells could maintain photosynthesis and growth at levels comparable to high-Ci cells without the same energetic and metabolic constraints of the induced CCM. They hypothesized that the induced CCM in low-Ci cells would, however, constrain the rate and amplitude of light acclimation (Tyler et al., 2004).

In the opposite of *Fischerella* sp. FS 18 (Soltani et al., 2007), the amounts of PE and APC seemed high in *Calothrix* sp. ISC 65. The complete structure of the core and rode parts of PBS, and the ratio between two parts depended on the alkalinity (Table 3). But the effect of alkalinity was not significant. It seemed that under neutral conditions (pH 7), the production of PE was high and even more than PC. It was in dependent on the time. Only high alkalinity for long periods could excite PC production and increase the PC : PE ratio. This was nearly the same for APC. Alkalinity especially for long intervals caused excitation of PC production which seemed compatible with the findings of Amirlatifi et al. (2013) and Iranshahi et al. (2014).

Table 3

Absorption ratios of the rode and core parts of the phycobilisome of *Calothrix* sp. ISC 65 after 24 and 96 hours of alkalinity treatments at specific time intervals

pH	Time, hour	Absorption ratio of *Calothrix*		
		PC/PE	PC/APC	(PC+PE)/APC
7	24	0.87	1.06	2.28
	96	0.78	0.92	2.11
9	24	0.99	1.04	2.10
	96	1.24	1.54	2.27

In Soltani et al. (2007), *Fischerella* sp. FS 18 had no APC at pH 7 and limited carbon dioxide concentration. In Iranshahi et al. (2014), Shokravi et al. (2014), and Safaie et al. (2015), this strain (and *Hapalosiphon* sp. FS 44) had a large amount of APC. This must be related to irradiance and carbon dioxide concentration. In Soltani et al. (2007) there was no limitation of

irradiance and carbon dioxide concentration and this possibly caused new pattern of PBS behaviors. The distribution pattern of PBS (Fig. 1), showed that except for a narrow border around combined pH 9 and 96 hours, the distribution pattern of PBS seemed nearly uniform. We can suggest that PBS production in such a strain is sensitive to the combination of time and alkalinity at pH 9 under DIC and light limitation. From the applied point of view, application of exact amount of alkalinity (pH 9) and time (96 hours) for cultivation of this strain may significantly increase PBS production. This coincides with Abbasi et al. (unpublished data) on *Fischerella* sp.

The sensitivity of pigment production and photosynthesis apparatus to extremely limited irradiance was noticeable. Results (Table 4) showed that although maximum photosynthesis normalized to chlorophyll was higher under an alkaline (pH 9) condition, the degree of adaptation with limited irradiance and consumed energy needed reaching to maximum photosynthesis decreased at extremely high alkalinity (pH 11).

Table 4

Photosynthesis-irradiance curve parameters at different alkalinities in *Calothrix* sp. ISC 65

pH	P_{max} (μmol O_2 mg $chl^{-1} \cdot h^{-1}$)	α	I_k (μmol O_2 mg $chl^{-1} \cdot h^{-1}$)
7	89 ± 8.23	1.3 ± 0.39	76 ± 12.23
9	227.99 ± 37.33	2.85 ± 0.18	48 ± 6.21
11	114.99 ± 17.39	1.35 ± 0.38	82.56 ± 9.11

Studies per biomass (not shown) revealed similar results in that the degree of adaptation to limited irradiances (reaching the highest degree of photosynthesis) was higher under alkaline conditions (pH 9). It seemed that the efficiency of photosynthesis increased with alkalinity.

We could not observe photoinhibition even at extremely high light intensities (more than 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$) at pH 9. But treatment with both pH 7 and 11, caused photoinhibition below 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$. This was the same as relatively "limited and no limited" DIC concentration conditions at different pH levels, irradiances and even nitrogen sources (Soltani et al., 2007, 2009). Moser et al. (2009) studied freshwater picocyanobacteria acclimation to irradiances from low (6 $\mu E \cdot m^{-2} \cdot s^{-1}$) to high (1500 $\mu E \cdot m^{-2} \cdot s^{-1}$), which showed photoinhibition at high irradiance. The photosynthetic parameters varied widely, both among the light acclimation treatments of each strain and between the strains; P_{max} of the LL (low light) culture from 4 to 19 times higher than P_{max} of the PC-rich cultures acclimated to LL. The initial slope of

the P/E curve was highest and the saturation light intensity (Ik) was lowest in the LL culture of BO8801. All cultures had significantly higher cell-specific chlorophyll content in the LL than in the ML (mid-light intensity) treatments. However, we can say alkalinity, up to pH 9, caused the maximum photosynthesis of *Calothrix* sp. ISC 65, besides the higher quantum yield and shade-adapted capacity and was another proof for increasing photosynthesis and carbon dioxide concentration mechanism activity under this condition. Alkalinity at the higher (pH 11) not only caused lower amounts of oxygen liberation but also caused sensitivity to photoinhibition. It seemed that under limitation of carbon dioxide concentration and irradiance, pH 9 treatments caused the resistance of the photosynthetic apparatus against damages caused by high number of irradiances.

In our study a light intensity of 2 μmol photon·m^{-2}·s^{-1} was used, which is lower than the one used by Lu and Vonshak (1999, 2002), Poza-Carrion et al. (2001), Dhiab et al. (2007), Soltani et al. (2005), Soltani et al. (2007), and many other papers. Strain *Nostoc* sp. UAM 205 and 206 have been characterized at extremely limited carbon dioxide concentration but 60 μmol photon·m^{-2}·s^{-1} (Fernandez-Valiente, Leganes, 1989; Zeng, Vonshak, 1998; Poza-Carrion et al., 2001) showed that at a higher light intensity, growing cells had lower photosynthesis activity after photoinhibition under salinity stress, compared with cells growing under lower light intensity condition (Dhiab et al., 2007). However, the carbon dioxide conditions were not considered in this research. The size of phycobilisomes and the relationship between PSII and PSI (Yamanaka, Glazer, 1981; Poza-Carriyn et al., 2001; Soltani et al., 2006) supported this but not completely. However, it was obvious that the highest amount of PSII : PSI ratio and phycobilisome size may be seen at pH 9. The high PSI : PSII (low PSII : PSI) ratio in cyanobacteria caused the higher efficiency of energy transfer from PSII to plastoquinone and then to PSI. In cyanobacteria there is usually more PSI for each PSII. For example results revealed 2.3 in *Synechococcus* sp. and 2.5 for *Synechocystis* sp. before iron starvation but decreased to 0.4 (*Synechococcus* sp.) and 1.1 (*Synechocystis* sp.) after iron depletion (Gan et al., 2014). Ogawa, Sonoike (2016) studying photosystem ratios in *Synechocystis* sp. PCC 6803, at nitrogen deficiency, emphasized that photosystem stoichiometry was more or less constant regardless of the change in growth media. PSI : PSII fluctuated in this strain from nearly 3.5 to 4.5.

In *Calothrix* sp. FS 65, the highest rate of PSII : PSI, or the lowest PSI : PSII, resulted under pH 9 after 96 hours (Table 5). Fluorimetric analysis (Table 5) seemed compatible with photosynthesis efficiency (as a whole) and photosystem ratios (as a special basic factor). A neutral condition

caused a sharp decline in PSII : PSI ratio and a decreased energy transfer photochemically. Collectively, neutrality caused a decrease of energy in photosynthesis and increased fluorescence. The relative fluorescence of PSI chlorophyll (FPSI) of the mutant strains of *Synechocystis* PCC 6803 under alkaline conditions (pH 8.2) was significantly lower than that of the wild-type when normalized at 685 nm (Wang et al., 2008). We measured for pH 11 (data not shown) and the results were nearly the same but just like P-I curve parameters, it seemed that neutrality (pH 7) caused more inefficiency comparing extreme alkalinity (pH 11). The ratio of photosystems in this strain was obviously less than *Fischerella* spp. (Soltani et al., 2007; Safaie et al., 2015) *Hapalosiphon* spp. and *Nostoc* spp. (Shokravi et al., 2012, 2014; Kiaei et al., 2013; Iranshahi et al., 2014).

Table 5

Fluorimetry and Photosystem ratio analysis of *Calothrix* sp. ISC 65 after 24 and 96 hours of alkalinity treatments

pH	Absorption ratio of *Calothrix*					
	Fv/Fm		Fv'/Fm'		PSII : PSI	
	24	96	24	96	24	96
7	0.424	0.577	0.678	0.467	1.031	0.82
9	0.491	0.683	0.586	0.521	1.054	0.64

From applied aspects, increasing time (96 hours) and meanwhile increasing alkalinity (pH 9) caused the highest ratio of PSII : PSI (Fig. 2). We could increase the system's efficiency simply by using a combination of pH 9 and 96-hour treatments. This may be the useful result for large-scale cultivation in limited light and DIC concentration. PSI and naturally reductant production and cyclic electron flow may increase considerably more. For special situations which had to increase the activity of cyclic flow or reductant pool of the strain, we can increase the time under alkalinity conditions. This will provide more water photolysis ability and electron transfer (besides energy) from PSII to Cyt.b6f. This coincides with Zorz et al. (2015) who suggested that the abundance of Cyt b6f and PSI (besides the relatively low level of PSII and Rubisco) are consistent with the increase in cyclical electron flow around PSI in *Prochlorococcus* sp. MIT 9313.

FTIR spectrum comparison (Figs 1 and 2) showed the effect of alkalinity on functional group productions at different times. It was interesting that both (alkalinity and time) caused configurational pattern changes, but the influence of time was more obvious.

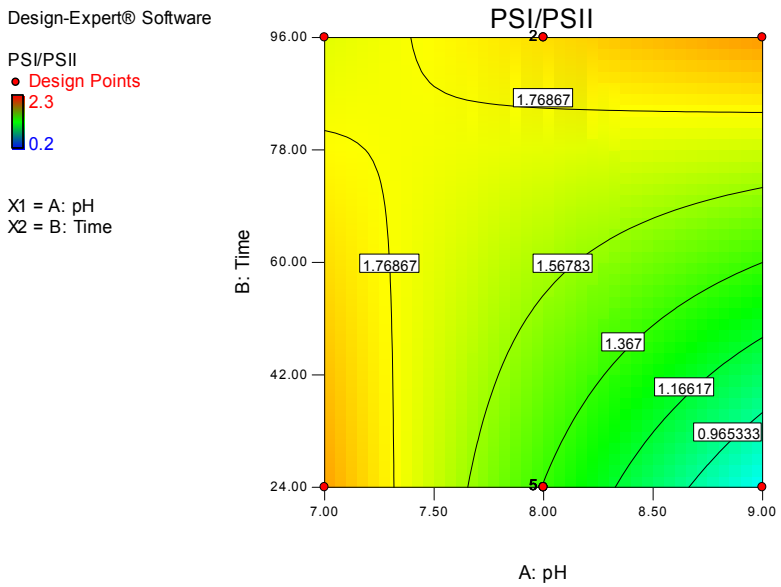

Fig. 2. RSP analysis of photosystem ratios in *Calothrix* sp. ISC 65 at different alkalinities and time treatments

Different height of peaks revealed that changes in chemical functional groups were caused by the combination of alkalinity and time. The bands belong to Amide II groups and OH stretching for proteins and carbohydrates showed different length of peaks after 96 hours. pH 11 caused the most outstanding configuration changes. We showed differences around asymmetric carbon vibration, lipid stretching and OH bending of the polysaccharides at different alkalinities as well. Most these differences were revealed under pH 11. At this time, we cannot discuss the changes at fingerprint borders, although the effect of alkalinity at this region seems important. The combinations of extremely low irradiance, carbon dioxide concentration, and alkalinity fluctuations produced different patterns in functional groups, especially with carbohydrates and proteins in this strain in a short amount of time. Differences appeared at the fingerprint regions after a long amount of time.

FTIR analysis is rarely used in stress physiology (Figs 3 and 4). It is most common in the papers on lipid biochemistry and profile especially for biofuel application purposes. It's importance in the taxonomy of algae has been suggested (Ratledge, Wilkinson, 1988; Cohen et al., 1995; Kenne, van der Merwe, 2013; Borah et al., 2016) but further research is needed in the taxonomy of cyanobacteria. Borah et al. (2016) believed that ATR-FTIR is not a strong tool in chemotaxonomy of cyanobacteria. Bajwa and Bishnoi

(2015), studying the effect of salinity on the overproduction of lipids in *Chlorella pyrenoidosa*, used FTIR analysis, and showed that 5 to 25 mM salinity caused increases in lipid range (from 10 to 45%). They also suggested that FTIR results showed high amounts of lipids, carbohydrates, and nucleic acid contents in such a strain. Besides the strain, results seemed to confirm our results.

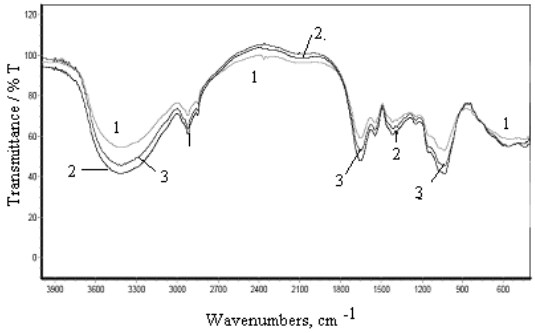

FIG. 3. FTIR analysis of *Calothrix* sp. ISC 65 at different alkalinities after 24-hours treatments (*1* − pH 7; *2* − pH 9; *3* − pH 11)

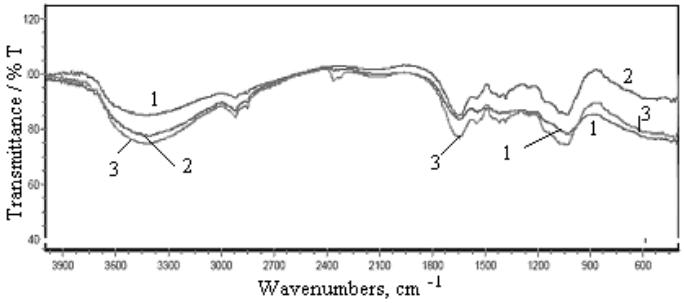

Fig. 4. FTIR analysis of *Calothrix* sp. ISC 65 at different alkalinities after 96-hours treatments (*1* − pH 7; *2* − pH 9; *3* − pH 11)

Kiaei et al. (2013), for the first time, characterized four strain of native Iranian cyanobacteria using FTIR analysis for evaluation of lipids in biofuel projects.They studied the effect of organic and inorganic nitrogen nutrition in lipid (especially fatty acid profiles) production of four cyanobacteria but selected *Synechococcus* as a model strain. They concluded that treatments of *Synechococcus* with different concentration of nitrate changed the profile of fatty acids and amino acids. Peaks of the FTIR shifted and changed their length at different concentrations of nitrogen sources especially the nitrate form.

The authors would like to thank Professor Neda Soltani (Shahid Beheshti University, Iran), Dr. Ali Ebadi, Mr. Mohammad Aiineh, Dr. Davood Beig Nejad, and Mrs. Malliheh Rasaie (Islamic Azad University, Gorgan), for their kind collaboration in theoretical and laboratory studies.

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Поступила 23.11.2017
Подписал в печать А.И. Божков

ISSN 0868-854 (Print)
ISSN 2413-5984 (Online). Algologia. 2019, 29(1): 40–58
https://doi.org/10.15407/alg29.01.040

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ВЛИЯНИЕ ЩЕЛОЧНОСТИ, СВЕРХНИЗКОЙ КОНЦЕНТРАЦИИ ДИОКСИДА УГЛЕРОДА И ИНТЕНСИВНОСТИ ИЗЛУЧЕНИЯ НА СПЕКТРАЛЬНЫЕ СВОЙСТВА, ФИТОБИЛИСОМЫ, ФОТОСИНТЕЗ, ФОТОСИСТЕМЫ И ФУНКЦИОНАЛЬНЫЕ ГРУППЫ НАТИВНОЙ ЦИАНОБАКТЕРИИ *CALOTHRIX* SP. ISC 65

Исследован физиологический ответ штамма *Calothrix* sp. ISC 65 на культивирование в условиях сверхнизкой освещенности (2 $\mu E \cdot м^{-2} \cdot с^{-1}$) при различных значениях pH (7, 9, 11) и низкой концентрации углекислого газа (без аэрации и обогащения углекислым газом). Спектроскопический анализ показал, что через 96 ч культивирования при pH 9 значительно увеличивается скорость роста исследуемого штамма и выработка им хлорофилла и фикоцианина. Снижение pH до нормального

(7) вызывало уменьшение продукции фикобилисомы уже через 24 ч, при pH 9 – возбуждение светособирающего комплекса и реакционного центра фотосистем. Фикоцианин, по-видимому, являлся основным элементом фикобилисомы, но при pH 9 увеличивалось продуцирование фикоэритрина и аллофикоцианина в качестве внешней части фотосинтетической антенны. Флуориметрический анализ и анализ кривых фотосинтеза и освещенности показали, что повышение щелочности до pH 9 (не выше 11) вызывает повышение эффективности фотосинтеза и снижение нефотохимической флуоресценции, особенно через 96 ч. Соотношение ФС II: ФС I увеличивалось при возрастании щелочности от pH 7 до 9 и достигало наивысшего уровня через 96 ч. Анализ RSP показал, что вокруг pH 9 и 96 ч существует узкая граница с самыми высокими показателями соотношения ФС II : ФС I. По данным инфракрасной спектроскопии с преобразованием Фурье (ИК-Фурье), щелочные условия вызывали изменения конфигурации функциональных групп. Разница в структуре функциональных групп между pH 7 и 11 была совершенно очевидной, особенно через 24 ч. Различия между асимметричной вибрацией углерода, растяжением липидов и изгибанием OH полисахаридов отмечались при pH 9 и pH 11. В целом, при крайне ограниченной освещенности и концентрации углекислого газа щелочность pH 9 вызывала у *Calothrix* sp. ISC 65 наибольшую физиологическую активность.

Ключевые слова: *Calothrix*, цианобактерии, щелочность, растворенный неорганический углерод, ограниченная освещенность