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Research Article

Effects of short-time alkaline pretreatment on growth and photosynthesis efficiency of endemic cyanobacterium *Fischerella* sp. FS 18.

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Abstract

Alkaline pH is one of the most important problems of our aquatic habitat. We used Stigonematalean native cyanobacterium *Fischerella* sp. FS 18 as our model strain, andstudied it under different alkaline pHs (7, 9 and rarely 11) under two different – short and long- time treatments (24 and 96 hours after inoculation). Spectroscopic results showed that both alkalinity and time affected growth rate, phycobilisome and chlorophyll production. Response surface plot analysis of distribution showed that the pH borders between 8.5 to 9 would be critical at 24 hours after inoculation reaching to the highest rates of phycobilisomes. Spectrofluorimetric analysis showed that the highest photosystem I/ photosystem II may be seen at 24 hours at pH9. Photosynthesis-Irradiance curves showed that the highest rate of maximum photosynthesis belonged to pH9 in the short time treatment (24 hours). Increasing the time (96 hours) decreased the maximum amount of oxygen liberation significantly. Moving from the optimum conditions (even slightly) caused a sharp decline in the amount of alpha. The slope of decline was steep until near neutral and longtime condition. Decreasing the time, and at the same time increasing alkalinity (alkaline pH) caused higher activity of photosystems especially photosystem I which lead to higher reductant production and cyclic electron flow operation. Distinct borders of pH (8.5-9) at 24 hours caused considerably high growth and matter production. This was naturally true for phycocyanin, phycoerythrin and the other economically important matters.

Key-Words: Alkalinine pH, Cyanobacteria, Ecophysiology, *Fischerella* sp. FS 18, Time, Ultrastructure

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Introduction

Under natural conditions, cyanobacteria are exposed to the combined influences of several factors, which varied even daily (Safaie *et al*., 2015). Growth, biochemical and physiological characteristics of cyanobacteria are influenced by environmental factors. One of the important factors that affect the abundance of cyanobacteria is the pH of the environment. Cyanobacteria prefer alkaline conditions (Amirlatifi *et al*., 2018). It seems that acidity forms a barrier which cyanobacteria cannot break, although they can penetrate into it (Summerfield *et al*., 2013). The effect of pH on the evolution of cyanobacteria seems basic although there are a lot of questions about different parts of such an effect. For example, the balance of internal and external pH and the role of time in such a homeostasis. Cyanobacteria must be acclimated to environmental fluctuations and adjust their internal systems including photosystem machinery which has a basic role in energy and matter production. As we know the photosynthetic electron transport system in oxygenic photosynthesis converts light energy to biochemical energy and consists of three major large membrane protein complexes, namely, photosystem I (PSI), cytochrome (Cyt) b6f, and photosystem II (PS II) complexes. Alkalinity (alkaline pH) may affect structure and function of all or one (or two) part depending on the strain and other environmental conditions (Ban˜ares-Espan *et al*., 2013; Safaie *et al*., 2015). It seems logical for LHCs and especially PBS systems which may be affected by pH, salinity, irradiance and the others.

Nearly most of our experiments on *Fischerella* sp. FS 18, have been conducted at a relatively fixed time (one or two weeks) without any time course studies. Recently Amirlatifi *et al*. (2018) using below 24 hours' time-course experiments showed that overlaying absorption spectra in salinity treatments cause serious effects on the energy providing and light adjustment systems in such a strain. As mentioned before, just like the photosystems and phycobilisome structure and functions, we have no information about the role of time and time course responses of *Fischerella* sp. FS 18. For the first time, it was shown that short time pre-acclimation causes new and different behaviors in such a strain. In addition, breaking the continuous irradiance into 5 to 10 minutes darkness periods, caused an increase in growth rate, heterocyst frequency, and photosynthesis activity of the strain. Mohammadian Mosaabadi *et al*. (2014) in an incomplete research found that short time (24 hours) cold stress causes deep fluctuations in the physiological and morphological behavior of the strain. Only Shokravi *et al.* (2014) and Amirlatifi *et al*. (2018), studying salinity stress, clearly showed the importance of time (even below 24 hours) on the physiological behavior of the strain using overlay spectroscopy. Except for Vakili (2007) and Amirlatifi

et al. (2018), who selected 24 hours, the other experiments were conducted in the laboratory for one or two weeks and most of the analysis was done between the 3rdand 4th day (72-96 hours) after inoculation. Therefore, we selected this border of time (24 – 96 hours) after inoculation and different pH (alkaline) treatments as the minimum and maximum. This will enable us to compare our results with the other researchers. Over or below such a border of time, we have no documents for comparison.

With regard to what was mentioned above, our aim was studying combination effects of two different time periods (24-96 hours) and alkaline pHs (7, 9, and rarely11) on growth, pigment composition fluctuations, photosystems dynamism, PSII: PSI ratios, phycobilisome operation, photosynthesis-irradiance curves parameters and functional groups alternation of the strain. Since we have no similar experiments regarding such a strain and treatments, we seriously believe that the results may help us to improve our knowledge in both pure and applied aspects that can help with future projects.

Materials and methods

Fischerella sp. FS 18 was isolated inbenthic (epilithic, periphytic) form from Khuzestan Province (Khark Island, South of Iran and near the Persian Gulf) and paddy-fields (Golestan province, North of Iran and near the Caspian Sea). Epilithic forms were collected from bricks and cement blocks which were submerged in waters. The collected samples were cultured by ordinary methods. After colonization and isolation, the cyanobacteria *Fischerella* sp. FS18 was purified and it was made axenic. Molecular identification was done according to Soltani *et al*. (2015). Stock cultures were grown in the $BG11_0$ according to Soltani *et al*. (2015). Culture media were buffered with 2.5 mM HEPES (for pH 7) or 10 mM BTP (for pHs 9 and 11) and adjusted to the pH with HCl or KOH. Cultures were illuminated via different numbers of nets between light source and flasks. Since some earlier works of Safaie *et al*. (2015) showed that this strain contains a relatively powerful carbon dioxide concentration mechanism (CCM) which especially works unidirectional, we avoided carbon dioxide enrichment to the cultures.

Growth measurements, pigment composition, and the PBS situation were analyzed spectroscopically after 24, 48, 72- and 96-hours alkalinity treatments (pHs 7, 9, 11) following Fraser *et al*. (2013). Photosystem ratios and characterisitcs were done spectrofluorimetrically according to Inoue-Kashino *et al*. (2005) and Zorz *et al*. (2015). PSII and PSI analysis were done using Peter *et al*. (2010). Oxygen exchange was measured with a Hansatech O_2 electrode. Pmax and alpha were measured after drawing P-I curves. For statistical analysis, data used were the means and

standard deviations of at least four replicates. Statistical analysis was examined using Response Surface Plot (RSP - My-Designs Ver.7 and 10). One factor and multifactor analysis were done following following Ghobadian *et al*. (2015).

Results

Specific growth rate at pH 7 (0.019 h^{-1}) was lower than pH 9 (0.02 h^{-1}). High alkaline pH caused higher growth rate. This was the same for chlorophyll production. The peak of chlorophyll absorption remained constant at neutral or alkaline conditions with little shift to the red region. The rate of chlorophyll production per cell was not compatible with total chlorophyll production. The amount of PBS production was significantly more at short time alkaline conditions. Increasing time caused decreasing differences in the rates between two conditions. The highest belonged to pH 9 at 24 hours after inoculation (Table 1). The patterns of pigment ratios (chlorophyll, carotenioids, and phycocyanin) seemed the same as the combination of time and pH (Table 2). Alkalinity (alkaline pH) especially after 24 hours caused a noticeable increase in all the ratios. The lowest belonged to neutral conditions at 24 hours. The effect of time was obvious. It seemed that short time alkaline pH causes the highest production of PBS and LHC (Tables1 and 2). Therefore, it must be natural if the photosynthesis efficiency went up under this condition.

Table1: Characteristics of the of *Fischerella* sp. FS 18 over 24 and 96 hour pH treatments.

***Fischerella* sp. FS18**	**Time (hour)**	**pH7**	**pH9**
Ln (A 750)	24	-5.41	-3.72
	96	-1.84	-2.25
Ln (A680-A750)	24	-6.30	-6.84
	96	-3.08	0.03
(A680-A750)/A750	24	0.40	0.04
	96	0.28	0.29
Chl (λmax)	24	684	684
	96	684	684
(A630-A750)/(A680-A750)	24	0.09	2.95
	96	1.10	1.06

Table 2: Absorption ratios of the of *Fischerella* sp. FS 18 after 24- and 96-hours pH treatments time course.

	Time (Hours)	**Absorption ratios of *Fischerella* sp. FS18**		
		440/680	**470/680**	**621/680**
pH 7	24	0.99	0.72	0.67
	96	1.32	1.21	1
pH 9	24	1.79	1.68	1.11
	96	1.39	1.26	1.01

It seemed that in the first 24 hours after inoculation (which caused the highest rates of PBS production), passing from pH 8.5 to 9 may be critical. PBS production system seemed sensitive to alkaline pH in short time trials and little change in alkalinity caused high PBS production responses. The border of sensitivity at the highest alkaline pH decreased sharply with the passing of time and reached the narrowest after 96 hours. This pattern was the same regarding the lowest rates. Neutral conditions, especially in the short time trial (24 hours), caused the lowest PBS production ability. The range of sensitivity seemed not so vast and most of the parameters affected in the middle range of time and alkaline pH when the pattern of distribution was nearly regular. Collectively the pattern of distribution was noticeable especially dealing with the applied point of views. Applications of critical times and alkalinity resulted in reaching the lowest and highest PBS production for large-scale cultivation projects (Fig. 1).

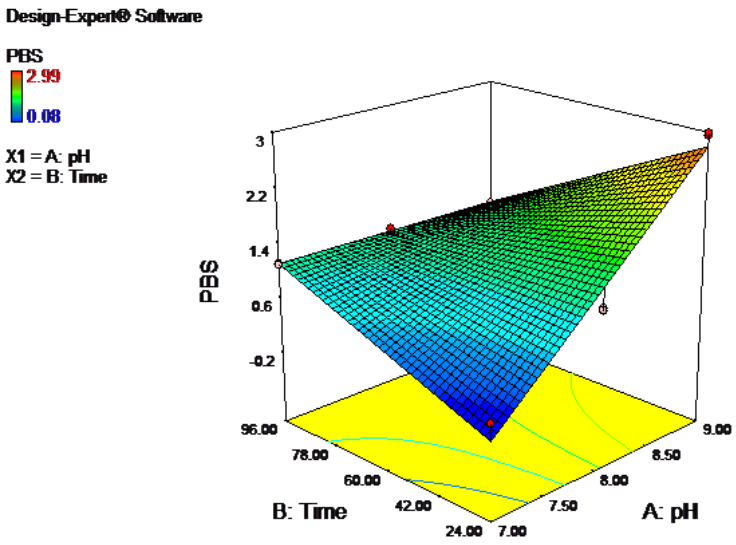

Figure 1: RSP analysis of phycobilisome, pH and time in *Fischerella* sp. FS 18.

Spectrofluorimetric emission spectra (Figs. 2 and 3) of PSII (excited at 440 nm) obviously showed the effect of time on photosystem operations at different pH treatments. Alkaline pH especially after 24 hours caused an increase in PSII activity (although less than PSI- see the followings). The lowest belonged to neutral conditions at 96 hours. It seemed that in the long time alkaline pH treatments, PSII activity was nearly equal to short time neutral conditions. Naturally, alkalinity in the short time trials caused the highest photosynthesis efficiency. Longtime neutral conditions decreased the activity significantly.

Although time and pH affected PSII system, the effects on PSIwere considerably higher (Fig.4).

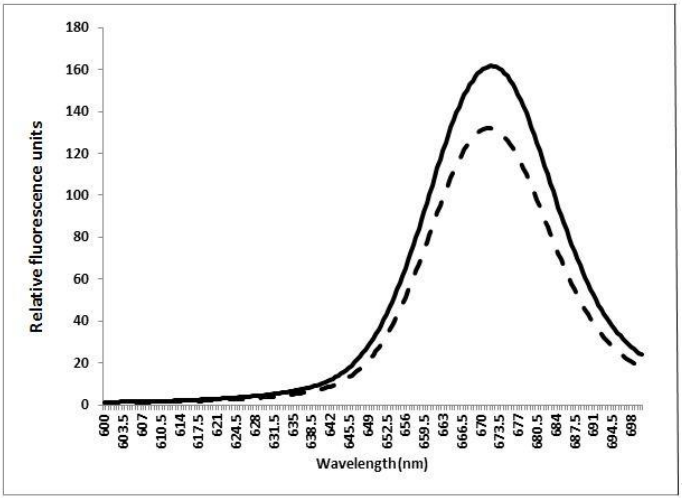

Figure 2: Comparison of Spectrofuorimetric spectra of *Fischerella* sp. FS 18 excited at 440 nm alkaline conditions (dotted line: 96 h., solid line: 24 h).

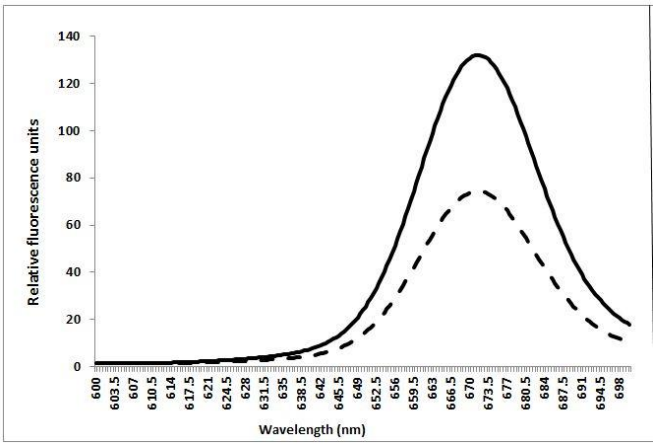

Figure 3: Comparison of Spectrofluorimetric spectra of *Fischerella* sp. FS 18 excited at 440 nm neutral conditions (dotted line: 96 hours, solid line: 24 hours)

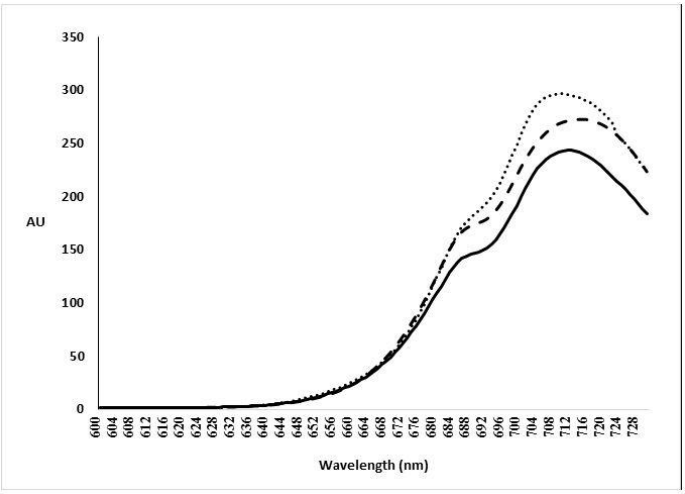

Figure 4: Comparison of Spectrofluorimetric spectra of *Fischerella* sp. FS 18 excited at 440 nm alkaline conditions (solid line: pH 7; dash line: pH11; dotted line pH 9; 24 h).

The degrees of sensitivity of PSI to treatments were completely more than PSII and it seemed that the real target of the treatments would be PSI. The effect of time was less compared to PSII. Although there were significant differences between the best condition (pH 9, 24 h) and the others (for example pH9 and 96 hours),the best operation of PSI belonged to short time alkaline conditions which was compatible with the pigment composition and situation of LHCs and RCs. Both PSI and PSI systems were affected structurally by treatments but the shifts were more outstanding in PSI components (Figs. 2 to 4). Table 3 shows the highest PSI /PSII or the lowest PSII / PSI in the short time trials under alkaline conditions. In this special treatment, PSI/ PSII reached close to 2 which would be related to high efficiency of capturing energy. After 72 hours this efficiency decreased but was yet outstanding (1.8).

Table 3: Photosystem ratios of the of *Fischerella* sp. FS 18 after 24 and 96hour pH treatments.

pH	Time (hours)	PSII/PSI	PSI/PSII
7	24	0.53	1.48
	96	0.61	1.62
9	24	0.41	2.07
	96	0.48	1.87

Table 4, shows the main parameters of P-I curves in neutral and alkaline conditions. It seemed that the highest Pmax belonged to pH9 after the short time (24 hours) treatments. It reached about 525 µmol O_2 mg chl^{-1} h^{-1}, which shows relatively high photosynthesis activation. Increasing time (96 hours) significantly decreased the maximum amount of oxygen liberation. This was the same for neutral conditions as well. The amount of irradiance necessary to reach Pmax decreased with increasing pH under short time conditions. Neutral conditions especially for a long time caused much higher amounts of energy and clearly showed the efficiency of photosynthesis differences. This pattern seemed the same for low irradiance adaptation ability as well.

Table 4: Photosynthesis – Irradiance curves parameters of *Fischerella* sp. FS 18 after 24 and 96 hour pH treatments.

pH	Time (hours)	Pmax µmol O_2 mg chl^{-1} h^{-1}	α	I_k µE m^{-2} s^{-1}
7	24	265.9±10.4	1.4±0.1	189.9
	96	375.3±12.8	1.7±0.1	279.6
9	24	525.5±10.2	4.9±0.8	55
	96	409.1±28.4	2.4±0.5	161.2

In *Fischerella* sp. FS 18, P-I curves analysis and distribution of the parameters (Table 4), revealed that the highest rate of alpha was completely dependent on the exact time situation. Moving from the optimum conditions (even less than two hours) caused a sharp decline in α. The slope of decline was hard until nearly neutral and longtime combination conditions. Reaching the borders of exact neutral and longtime caused a severe decline of α (reaching the lowest rates). We can say that, the ability of such a strain to reach the best condition of quantum efficiency and dark-adapted form of life seems hard depending on the condition and flexibility of the system is not outstanding (as opposed to the lower degrees which cause outstanding versatility and flexibility).

Discussion

Collectively both pH and time affected growth, PBS, and chlorophyll production. Only the absorption peak of the chlorophyll was not affected by both (separately or in combination). These patterns were not completely compatible with Amirlatifi *et al*. (2018) who worked under the same conditions on such a strain but with salinity treatments. It means that the mode of action seems different between salinity and pH at the same time. We have no certainty about such a pattern dealing with pH and time especially in such a strain. However according to Amirlatifi *et al*. (2018), the importance of time was more than salinity in this strain. Only 5 to 10 hours treatment caused deep effects on physiological behavior. This is the sole research which seems directly similar to our work. In Anabaena variabilis ATCC 29413, and *Synechococcus* sp.6301, the result of irradiances on absorption spectra from 30 min to 3 hours was more or less the same (Fraser *et al*.,2013). Zuo *et al*. (2014) studying the effect of carbon on *Chlamydomonas reinhadtii*, suggested that degradation of photosynthetic pigments had initiated before 12 hours. In the presence of 50 mM Na2CO3, the absorbance peak disappeared after 1 hour, while a new peak reappeared at 416 nm after 2 hours. They emphasized that combination of carbon concentration and time causes noticeable effects on the pigment production in such a strain.

The amount of PC production was significantly more in short time, alkaline pH conditions. Increasing time decreased the rates between the two conditions. This was compatible with spctrofluorometric results. Deblois and Giguère (2013) suggested that the decrease in the amount of chlorophyll a (Chl a) in aquatic algae was normally associated with a decrease in the number of photosystems, while a decrease in accessory pigments (Chl b, c, d, and phycobiliproteins) was associated with a decrease in the size of the light-harvesting complexes (LHC). We did not reach such results, and the disagreement is possibly due to different kinds of organisms and habitats (aquatic and terrestrial).

However, from the applied point of view, the highest rates of carotenoids and phycocyanin were reached when we decreased the time from 96 hours to 24 hours under high alkaline conditions. This will require large amounts of time and expenditure in large scale cultivation projects. Gan *et al*. (2014) showed that *Fischerella thermalis* PCC 7521 was activated by far-red light in the PSI region and even produced more new systems beyond 700 nm irradiance. The high PSI/PSII ratio in cyanobacteria causes the higher efficiency of energy transfer from photosystem II to plastoquinone and then to photosystem I. In cyanobacteria usually there is more PSI for each PSII. For example, 2.3 in *Synechococcus* sp. and 2.5 for *Synechocystis* sp. before iron starvation which decreases to 0.4 (*Synechococcus* sp.) and 1.1 (*Synechocystis* sp.) after iron depletion (Gan *et al*., 2014). In contrast, Kopečná *et al*. (2012) and Ogawa and Sonoike (2016) studying photosystem ratios in *Synechocystis* sp. PCC 6803, under 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.

Applyingthe aspects of decreasing time and at the same time increasing alkalinity caused higher operation of both photosystems. PSI and naturally reductant production and cyclic electron flow may increase considerably more. In special situations when we need to increase the activity of cyclic flow or reductant pool of the strain, we can decrease the time under alkaline conditions especially in the borderline of pHs 8.5-9. This will provide more water photolysis ability and electron transfer (besides energy) from PSII to Cyt.b6f. This is in agreement with Zorz *et al*. (2015) who suggested that the abundance of Cyt b6f and PSI (besides the relatively low level of PSII and Rubisco), were consistent with the increase in cyclic electron flow around photosystem I in *Prochlorococcous* sp. MIT 9313. Collectively this special condition (pH8.5-9 for 24 hours) caused more matter production and growth. This is naturally true for PC, PE and the other economically important matters.

Touloupakis *et al*. (2016), studying high alkaline pH on the growth of *Synechocystis* sp. PCC 6803; suggested that there is a linear negative correlation between increasing pH and light conversion efficiency. Productivity, growth, and biomass yield with light energy declined by more than 30% at pH 11.0 (compared with neutral condition) clearly showing that combination of irradiance and pH, affect photosynthesis rate. This was not true for *Synechocystis* sp. PCC 6803, where in spite of growth and pigment composition, photosynthesis was not affected by pH increase (Kopečná *et al*., 2012; Osundeko *et al*., 2014; Touloupakis *et al*., 2016). Two different patterns revealed that we may have different behavior and the necessity of species-specific characterization.

In *Synechococcus* 6803, generally, when the α value decreased, three possibilities could be considered: (A) a decrease in the relative content of PS II, (B) a decrease in the antenna size of PS II, and (C) a decrease in the water oxidation rate in individual PS II. As discussed above, the observed property in the low Pmax and *I*k in the strain would be attributed to this low efficiency of water oxidation in PS II. The former two possibilities (A and B) could be excluded because the fluorescence emission spectra indicated otherwise.

Collectively we concluded that the highest operation of photosynthesis efficiency would be related to the short time, high alkaline pH conditions. The combination of pH and time may control the efficiency of photosynthesis. From the applied point of view, the highest activity including highest rate of photosynthesis in extremely low light conditions (for example when self-shading or using a low number of light sources) would be compensated by increasing pH and decreasing the time of the treatments. We can use the lowest rate of light if keep the strain for only 24 hours under alkaline conditions. From the other aspect, extremely low light conditions (like paddy –fields and oil-polluted water habitats), in alkaline aquatic habitatsof north and south of Iran, seem suitable for using the strain in fisheries projects.

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