

The Impact of Combined Alkalinity and Time Pretreatments on Light-harvesting System in Terrestrial Cyanobacterium *Fischerella* sp. FS 18 (Oscillatoriales, Cyanophyta)

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Received: 2019-11-10

Revised and accepted: 2021-03-05

Abstract

Possibility of change in the phycobilisome status, photosynthetic pigments, photosynthetic ratios, and photosynthetic parameters of soil cyanobacteria *Fischerella* sp. FS 18 investigated. Neutral and extreme alkaline pH (7, 9), and short time incubation including 20, 40, and 60 minutes treatments. After purification, cyanobacteria were subjected to extreme alkaline treatment for one hour at 20, 40, and 60 minutes intervals. Colorimetric assays of phycocyanin, allophycocyanin, phycoerythrin, chlorophyll) and a comparison of the combined effect of time and alkalinity on photosynthetic ratio performed. Indeed, the photosynthesis-light curves compared with direct measurements. The results showed that the combined treatment of time and alkalinity after 20 minutes of inoculation significantly increased the performance of the photosystem and stability of the phycobilins. While, under the 40 min and both neutral and alkaline treatments, the yield of photosystem II, increased the production of the photosystem I, and significantly the linear fraction of the

photosynthesis-light curve. Although, the needed energy to achieve maximum photosynthesis reduced. Further, the maximum photosynthesis was completely different at 40 min pretreatment and without pretreatment. Furthermore, the results show no specific regularity and trend at 20 and 60 minutes times treatment. Thus, the production of light collecting-antennas is influenced by both time and alkalinity treatments. In consequent, 60 minutes or less treatment times, cause a significant change in the structure and performance of the photosynthetic apparatus. While, alkaline treatments at a short time significantly save energy and enhance photosynthesis.

Keywords: Pretreatment, Time, Cyanobacteria, Alkalinity, *Fischerella* sp. FS 18

Introduction

In this paper, the effect of alkaline pretreatment for 1 hour on *Fischerella* sp. FS 18 studied. Unlike most cyanobacteria, *Fischerella* sp. FS 18 grows better in neutral media (Soltani et al., 2007). Abbasi et

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al. (2019, 2020) studied *Calothrix* sp. FS 56 and *Fischerella* sp. FS 18 and revealed the effect of short-term (24 h) treatments on efficiency and biomass production. Based on the research, cyanobacteria tend to live and reach their optimum growth rate and photosynthesis capacity in alkaline pH conditions. It is noteworthy that the effect of the shorter 24-hour time treatment compared to longer time (96 hours) is more visible (Abbasi et al., 2019). In such conditions, the performance of the phycobilisome and rate of photosynthesis increases. Abbasi et al. (2019), showed that the activity of photosystems, particularly photosystem 1, increase significantly within 24 h under alkaline treatment (Abbasi et al. 2019). This major achievement can apply to both pure and biotechnological mass cultivation. It assumed that there is a possibility of better result in less than 24 hours. Amirlatifi et al. (2018) represented that time treatments less than 24 h (ten, six, four and two hours after inoculation) in different salinity environments (17, 80, and 160 mM) on *Fischerella* sp. FS 18 provide higher growth rate and photosynthesis capacity.

Tang and Vincent (1999) began the investigation of the pretreatments in the cyanobacteria. Their findings on Oscillatorials showed that applying temperature treatments at intervals of 5 °C could completely change the behavior of cyanobacteria. Rosen and Mares, (2016) studied the effect of pretreatments and initial cyanobacterial habituation. They showed that the use of pretreatments can completely change the

expected behavior. The application of time is among the most crucial factors in this fundamental change (Downing, 2014). When quite different results observed in a simple pretreatment, by developing molecular techniques we can use molecular biology analysis in this prokaryotic cyanobacteria.

This study aimed to use alkaline shocks to evaluate the impact on optimization. It is assumed that if the time below one hour can somehow affect the photosynthetic apparatus to enhance its performance, there will be a significant economic savings in the future mass cultivation process of this cyanobacterium. The interval between 24 hours and one hour is considerable, and time reducing will be associated with economic efficiency. In addition, the derived results are useful for a better understanding of environmental cyanobacterial fluidity.

Cyanobacteria are basophil organisms (Witton and Potts, 2000; Soltani et al., 2006). Alkaline conditions are a major evolutionary barrier that separates cyanobacteria from other photosynthetic prokaryotic organisms in the response to the environmental factors (Shokravi et al., 2007). The movement of the environment and the transfer of cyanobacteria from alkaline to neutral or acidic conditions have altered the behavior of these organisms and even enabled them to live in acidic conditions (Shokravi et al., 2010). Apparently, grow in acidic conditions require the ability of the photosynthetic apparatus trapping CO₂ and light energy. Therefore, photosynthetic apparatus fluidity is possible even under the most abnormal conditions in

cyanobacteria.

A few studies conducted on the effect of short time treatment (below 60 min) on soil cyanobacteria in Golestan province, Iran (www.Irandoc.ac.ir). Soltani et al., (2011) investigated the effect of salinity on growth and photosynthesis of *Fischerella* sp. FS 18. Shokravi et al. (2014) studied the combined effects of pH and extreme light on growth and pigment status of *Hapalosiphon* sp. FS 56. Rodríguez-Sánchez et al. (2012) and Karseno et al. (2018) revealed the effect of pH on survival, growth, and pigment status of *Mirocheate* sp. FS 101 and *Anabaena* sp. FS 76. Although, some studies do not directly and completely investigate the effect of pH on the physiology and ecophysiology of cyanobacteria (Iranshahi et al., 2013; Amirlatifi et al., 2013). Amirlatifi et al. (2018) studied the combined effects of salinity and carbon dioxide limitation on optical physiology and biochemistry on *Calothrix* sp. FS 56.

Material and methods

The pure culture of *Fischerella* sp. FS 18 obtained from the algae bank of Shahid Beheshti University. Indeed, collection and cultivation technique data provided by Soltani et al. (2009). Soil samples cultured according to the method of soil cyanobacteria (Kaushik, 1987). Then, colonization, isolation, and subsequent cultures of *Fischerella* sp. FS 18 was prepared (Kaushik, 1987). Identification was performed using Desikachary (1959), Prescott (1962), Anagnostidis and Komarek (1990), John et al.

(2003). The Primary culture carried out in BG0-11 solid and liquid medium at 60 $\mu\text{mol}/\text{m}^2$, at 28 °C, and pH of 7.8 (Soltani et al., 2009). After the initial growth of the isolated samples, in the pretreatment condition of pH of 7.9 for 20, 40, and 60 minutes and then transferred to the normal culture medium at pH 9. Additionally, the sample selected as a control without pretreatment. The growth curve plotted based on turbidimetry and dry weight (Léganes et al., 1987). Biochemical analysis including chlorophyll, phycocyanin, phycoerythrin and allophycocyanin were performed in the form of microbes using absorbance spectra (Abbasi et al., 2019). Photosystem ratios and photosynthesis-light parameters evaluated using the Clark model oxygraph according to Soltani et al. (2007). The number and rod part to phycobilisome base calculated according to Amirlatifi et al. (2013) and Shokravi et al. (2014). Indeed, initial evaluation of the pretreatment effect performed by comparing absorbance spectra (Amirlatifi et al., 2018). Then, the statistical analysis performed using SPSS (Version 11). Additionally, data were standard based on Poza-Carrion et al. (2001) using RSP (Version 10) (Ghobadian et al., 2015).

Results

Generally, the effect of short-term treatments (less than one hour), in the photosystem II and phycobilisome, is evident from the comparison of absorbance spectra (Fig. 1). Indeed, there is a uniform order in the two phycobilisome and photosynthetic organelles. This means that the effects of time

and alkalinity treatments were significantly different in treated and untreated phycoerythrin and phycocyanin. In other words, 20 minutes of alkaline treatment changes the phycobilisome contents. In comparison with phycobilisome, the effect of 20 minutes more pronounced in the carotenoids, the situation is different with the phycobilisome and photosystem II. Although, the use of the time of 20 minutes in alkaline conditions has led to a significant increase in light-collecting pigments, however, application of 40 and 60 minutes reduced the production of these pigments. While, 40 and 60-min treatments enhance the performance of the phycobilisome and photosystem II, production and performance of light-collecting antennas around optical systems diminished. In addition, there are unknown peaks in these areas due to the production of light collector pigments, which affected by the time treatment and appear to decrease in concentration and performance after inoculation at 40 and 60

minutes (Fig. 1). Consequently, treatment for 20 minutes increases more than 30-40% production of carotenoid compounds, which is quite acceptable to justify the efficacy of this method for this cyanobacterium.

If the treatment condition changes to, extreme alkaline (pH 9) the results will be changed (Fig. 2). However, the 40 min time treatment under neutral pH increases the concentration and performs the phycobilisome and photosystem II. Whereas, 20 and 60 min times treatment at alkaline condition had a decreasing effect (Fig. 2). Additionally, at extreme alkaline and 20 to 60 min treatment, the performance of photosystem, phycobilisome and light-collecting pigment regions reduced in compared with neutral condition. Unlike neutral treatment, the behavior of the light-collecting part follows a pattern that is true for phycobilisome and photosystem II. Whereas, application of 40 minutes treatment in extreme alkaline condition enhances the performance of photo-

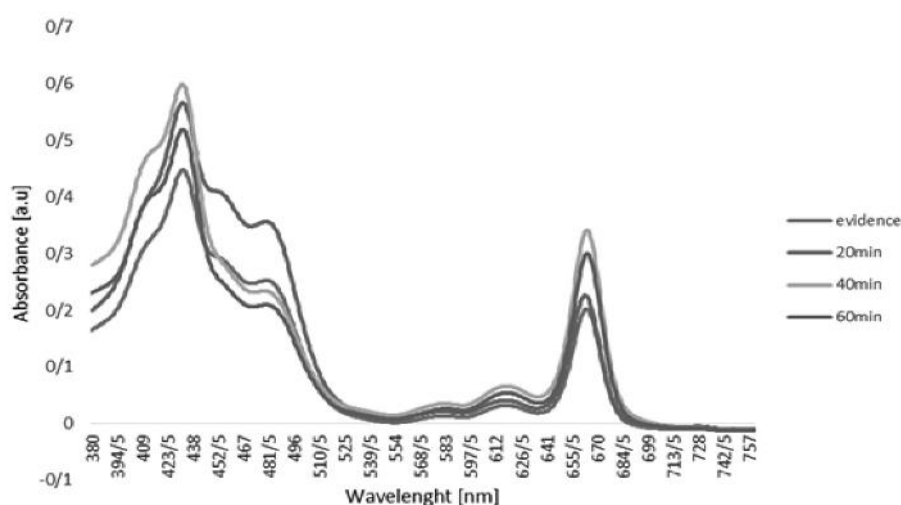


Fig. 1. Comparative study of the absorbance spectrum of *Fischerella* sp. FS 18 at pH 9 after 60, 40, and 20 minutes

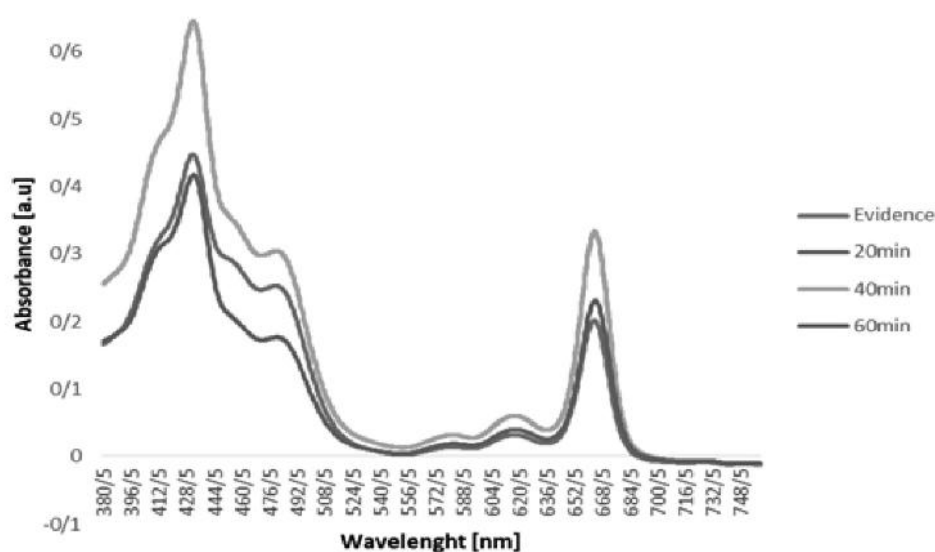


Fig. 2. Absorbance spectrum of different time treatments on *Fischerella* sp.

FS 18 at pH 9 after 60, 40, and 20 minutes

system II, phycobilisome, and light-collecting antennas. By increasing or decreasing time for 20 min (20 and 60 min treatments), the cyanobacterial photosynthetic apparatus exhibits contradictory behaviors. Indeed, extreme alkaline and treatment for 40 min significantly increases the efficiency of the photosynthetic ratio resulting in the growth and biomass production. However, this increase is far more than the neutral pH and the sensitivity to time in alkaline conditions will increase dramatically.

The photosynthetic ratio compared under optimal conditions based on the absorption spectra without treatment (Table 1). The result from Table 1 is in consistency with the results of the absorbance spectra in Figures 1 and 2. As mentioned before, treatment for 40 min in alkaline in neutral conditions enhances significantly (more than 20%) the photosystem I to II ratio, which confirms the

results of the absorbance spectrum. It seems that 40 min treatment in alkaline and neutral conditions (especially alkaline conditions) increases the production of photosystem I ratio and the efficiency of energy transfer from photosystem II to I and reduces the energy loss. Naturally, this reduction in energy loss attributed to increase the performance of the set of photosynthetic ratio, which is the best way to measure directly photosynthesis ratio (Table 2). While, prolonged treatment appears to enhance the performance of the photosynthetic apparatus, this difference is not enough to minimize the role of short time effects. Further, it is still not possible to say whether there is a critical time to enhance photosynthetic ratios under 1, 24, and 96 hours. At present, considering the results for ranges below one hour and above 24 hours treatment in neutral and alkaline conditions perform the highest the photosynthetic yield.

Table 1. Photosystem ratios of *Fischerella* sp. FS 18 after 40 minutes and without treatment. The numbers in the parenthesis reported by Abbasi et al., (2019) at 24 and 96 hours treatment

pH	Time (min)	PSI/PSII
7	40	1.23 (1.48)
	0	1.04 (1.62)
9	40	1.56 (2.07)
	0	1.13(1.87)

Table 2. Photosynthesis-Irradiance curves parameters of *Fischerella* sp. FS 18 after 40 minutes and without treatment. The numbers in the parenthesis reported by Abbasi et al. (2019) at 24 and 96 hours treatment

pH	Time (min)	Pmax $\mu\text{mol O}_2 \text{ mg chl}^{-1} \text{h}^{-1}$	A	I _k $\mu\text{E.m}^{-2}.\text{s}^{-1}$
7	40	224.9±12.4 (265)	1.3±0.1 (1.4)	157.9 (190)
	0	125.3±12.8 (375)	1.1±0.2 (1.7)	229.3 (280)
9	40	322.5±15.2 (525)	3.9±0.9 (4.9)	44 (55)
	0	155.1±18.3 (409)	1.9±0.3 (2.4)	171.2 (161)

From this point of view, there is sensitivity to time. It seems that sometimes are crucial, higher, and lower than that will reduce photosynthetic ratios and energy transfer efficiency between photosystems.

Table 2 presents the result of direct measurements of photosynthesis-light curves. Furthermore, it is expected that the highest amount of released oxygen is related to extreme alkaline conditions at 40 min. It is noteworthy that there is a two-fold difference (two hundred percent) between the oxygen released under alkaline conditions and no treatment. In addition, a 40 min treatment increases significantly the oxygen produc-

tion rate from 150 to 320 micromoles per milliliter of chlorophyll per hour. Indeed, the 200% increase in the shadow or linear portion of the photosynthesis-light curve, under the 40-min treatment, is in consistent with the oxygen release rate. While, the 40 min treatment appears to increase the ability to live in limited light conditions, significantly increase the photosynthetic oxygen. Although, the problem of self-shading and the existence in underlying and low-light conditions in mass cultures discussed, this ability survives cyanobacteria. Indeed, increasing the time up to 24 hours increases both capabilities. Furthermore, treatment for

40 minutes enhances both the pigmentation efficiency of the photosystem II reaction center and phycobilisomes especially in alkaline conditions. Consequently, by increasing the production rate of photosystem I, energy loss reduces in the transmission path of photosystem II to. While, this capability increases the oxygen production efficiency and the linear fraction of the photosynthetic-light curve, reduces the energy required to reach the maximum photosynthesis. It can be concluded that the combination of time and alkalinity affects the photosynthetic behaviors in *Fischerella* sp. FS. Although, it is not possible to understand which time treatment, significantly increases the efficiency of the photosynthetic apparatus, time in cyanobacterial intelligency and adaptability, interpret specifically. Indeed, it may be possible to adapt the photosynthetic apparatus to tolerate 24 hours light by altering the circadian rhythms (Yen et al., 2004). Generally, Stigonematales such as *Fischerella* are found in the habitats that do not tolerate 24-hour light conditions, and secondly it is not possible to reduce the impact of sub-hour time and its inconsistency. Abbasi et al. (2019) observed the behaviors of *Calothrix* sp. FS 56 after time and salinity treatment that required separate analysis in pigment growth and production. Science-based attitudes here are paradox and researchers inevitably take approaches such as agriculture and biotechnology manipulation that have problems. Particularly, many of these mechanisms are unknown to humans (Abbasi et al., 2019; Amirlatifi et al., 2018).

It stands to know that, the phycobilisomes relatively maintain their structure at less than an hour treatments and the absorption spectra of the rod section presented. Indeed, the effect of time under one hour is less pronounced in the photosystems, and the difference between neutral and alkaline conditions is reduced. Whereas, 40 min treatment in both neutral and alkaline conditions enhances the phycobilisome system, the 20 and 60 min treatments have a similar feature. Another point is that the absorption peaks of the phycocyanin and phycoerythrin change at different times, and it may be associated with the restructuring of the phycobilisome. Generally, 20, 40, and 60 min treatments influence the phycobilisome structure.

Indeed, photosystem ratios in cyanobacteria have been the subject of much attention in recent years. In this regard, if we assume that unlike flowering plants, cyanobacteria do not produce a single photosystem I per two photosystems and at the variable ratio. Naturally, we are facing new processes in terms of energy transfer increasing the amount of photosystem I to II results more efficient transmission of energy from the photosystem II to I (Lambers et al., 2008). If this ratio is one the efficiency of photosystem I will reduce due to mutations and prevent loss of transferred energy into five terms, including higher ratios of photosystem I (Paeizi et al., 2012). Additionally, in *Calothrix* sp. FS 56 at different concentration of salinity and alkaline treatment the ratio of photosystems and energy transfer efficiency increased or decreased. Certainly, other key components,

especially phycobilisome are essential and the benefits of increasing or decreasing the photosystem ratio weakened by the low performance of the phycobilisome. Although, if other parts of the photosystem are healthy, using extreme alkaline and 40 min time treatment can significantly increase the ratio of photosystems I and II. Furthermore, it affects the entry of the electron current into the energy-generating reactions and the redaction by reducing the fluorescence and heat processes.

Concerning the same studies, Soltani et al. (2007), Shokravi et al. (2012, 2014), and Safaie et al. (2015) studied the photosynthetic ratio of 2.5. In this regard, the photosystem ratios in investigated cyanobacteria were below 2. Additionally, this was a factor that justified the possibility of interference with other parts of the photosynthetic system. Further, 40 min treatment, especially in alkaline conditions, increase all respects of the photosystems and release more oxygen in low light and low energy conditions. In addition, indicator of the linear part of the photosynthesis-light curve can be due to the increase in the length of photosynthetic antennae or pigment production. Indeed, by increasing the shading coefficient the range of growth and survival will be increase in the depth of the water (Harati et al., 2009) which is a major advantage in the biotechnology of algae. Further, by reducing the amount of energy needed to achieve maximum photosynthesis, the competition potential of the cyanobacteria will increase compared to other

algae in similar habitats. Furthermore, The specimens are able to prevent the influx of dimming or shading and remove them. Although, competing cyanobacteria with the same strategies in turn, will stabilize it in the bulk, either pure or in combination with other algae, can expand the range of growth in the natural environment. However, in the studies conducted by Boshruye et al. (2007) and Sasani et al. (2009) on Oscillatorials, a disruption reported after using temperature, light, and salinity treatments. Vakili et al. (2007) studied the effect of 10 min periods of illumination on cyanobacteria *Fischerella* sp.

Shokravi et al. (2011) showed the effectiveness of time treatment on chlorophyll and sugar content. As mentioned above, a few studies presented the effect of the combination of alkalinity and short time, below 60 minutes on the soil cyanobacteria. So far, further studies needed based on combination of different time and alkaline treatments to study the impact of treatments on photosynthetic and biomass production in cyanobacteria.

Acknowledgment

The authors sincerely thank Mrs. Ras-saei for her assistance in laboratory analysis and Mr. Ayne for assistance in statistical analysis.

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