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EFFECTS OF MICROAEROBIOSIS ON PHOTOSYNTHESIS IN THE CYANOBACTERIUM *SYNECHOCOCCUS* SP. PCC 7002

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SUMMARY. In this study the effect of microaerobiosis on photochemical activity in the cyanobacterium *Synechococcus* sp. PCC 7002 is presented, by measuring chlorophyll fluorescence. Microaerobiosis was achieved by argon bubbling for 120 minutes, with samples being taken in four specific time periods.

In vitro measurements of absorption showed dominancy in chlorophyll a and the presence of carotenoids. Maximum fluorescence Fm decreased after 15 minutes of exposure to argon bubbling. Decline of Fm attests the large number of closed reaction centers, as well as plastoquinone reduction and growth of fluorescence emission. Variable fluorescence, expressing the difference between Fm and F0, showed positive values compared to the control, except after 60 minutes of argon treatment. Maximal quantum yield (Fv/Fm) of PS II photosynthesis was weakly stimulated by the argon treatment, except at 60 minutes of argon effect when negative values were observed. The coefficients of photochemical quenching, qP and qL, were maintained at higher values, except when 30 minute exposure to argon treatment.

By exposure to argon, the Pm signal was high in the first 30 minutes, followed by a significant decrease towards the end of the stress treatment suggesting a decrease in the reduction state of P700 reaction center compared to oxidized state. Moreover, effective PS I photochemical quantum yield Y(I) dropped significantly in the first 30 minute compared to the control. The decrease in quantum yield Y(I) reveals decreased reduction state due to the lack of limitation by the acceptor, respectively, decrease in photochemical energy conversion in PS I. The interchange of oxidative/reduced state of the plastoquinone nuclei was revealed in the kinetics of the chlorophyll fluorescence induction by pulse saturation in control. Fluorescence kinetics showed on the logarithmic scale revealed important modifications in I – P spectrum due to reduction of the plastoquinone nuclei.

Keywords: Cyanobacteria, fluorescence, microaerobiosis, photosynthesis, *Synechococcus*.

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Introduction

Cyanobacteria are among the very few groups of bacteria that can perform oxygenic photosynthesis and their photochemical reactions are similar to those of superior plants (Bryant, 1987). They have three types of antenna systems: chlorophyll antenna system associated with photosystems PS I and PS II of the thylakoid membrane and an external antenna complex comprised of phycobilisomes that are attached to PS II and PS I (Gant, 1975; 1981; Glazer, 1982; 1985; Mondori and Melis, 1986). The components of the phycobilisome are the phycobiliproteins that are responsible for the blue-green pigmentation, that have an absorption spectra of 470-650 nm, while the chlorophyll complex can absorb the light at 430-440 and 670-680 nm. This separation of the absorption bands allows analysis of the relative contribution of phycobiliproteins and chlorophyll a to the action spectrum of PS I and PS II (Wang *et al.*, 1977; Butler, 1978; Diner, 1979).

The main environmental factors affecting the photosynthesis in cyanobacteria are: temperature, light intensity, UV light, drought and salinity, temperature being the major factor that controls this process. By decreasing the temperature the photosynthetic electron transport and carbon fixation is limited and this can induce a reduction in the ability to convert light. The accumulation of light energy leads to the damage of the photosystem PS II and photoinhibition, making it the most thermolabile aspect of the photosynthetic complex (Davison, 1991; Zak and Pakrasi, 2000). Stoichiometry of the photosynthetic apparatus and phycobilisomes is regulated by both light and temperature (enzyme phosphorylation, electron transport and plastoquinone diffusion), although the initial photochemical reactions are independent of temperature (Davison, 1991; Miskiewicz *et al.*, 2002; Murakami, 1997). Photosynthetic acclimatization to low temperatures mimics the mechanism of acclimatization to high light (Campbell *et al.*, 1995).

Cyanobacteria rely on their ability to sense the action of these external factors and to use their ability to adjust morphologically, physiologically and molecularly which give rise to acclimatization to environmental changes (Huner *et al.*, 1996; 1998). Photosynthetic acclimatization is not due to temperature rise or irradiation, but rather is due to the interaction of these factors (Köhler *et al.*, 2005; Miskiewicz *et al.*, 2000; Nicklisch *et al.*, 2008; Wieland and Kühl, 2000). The direct effects of temperature act synergistically with other environmental factors (Robarts and Zohary, 1987; Bhogavalli *et al.*, 2012).

Several studies show that cyanobacteria have a large spectrum of adaptive strategies (Tang and Vincent, 1999): 1. – tend to have a low photosynthetic capacity at decreased temperatures due to depressed Rubisco activity. 2 – decreased number of chlorophylls and pigments in the light-harvesting complex. Low temperatures can lead to a decrease in the chlorophyll levels (Young, 1993), although carotenoid levels can remain intact because of the photoinhibition (Falk *et al.*, 1990; Davison, 1991; Krause, 1993). Carotenoids can act as a protective screen which blocks the harmful effect of high light at these lowered temperatures (Krause, 1993).

The process of light absorption in cyanobacteria takes place in phycobilisomes and photosystems PS I and PS II (Glazer, 1989; Bryant, 1995; van Thor *et al.*, 1998). The light-harvesting antenna of cyanobacteria does not contain chlorophyll. Phycobilisomes have a central core, mainly composed of the phycobiliproteins: phycoerythrin, phycocyanin and allophycocyanin (Fig. 1). These are polypeptides which contain phycocyanobilin in the form of trimer or hexamer complex. The complex can be bound to the membrane surface of thylakoids (Arteni *et al.*, 2009).

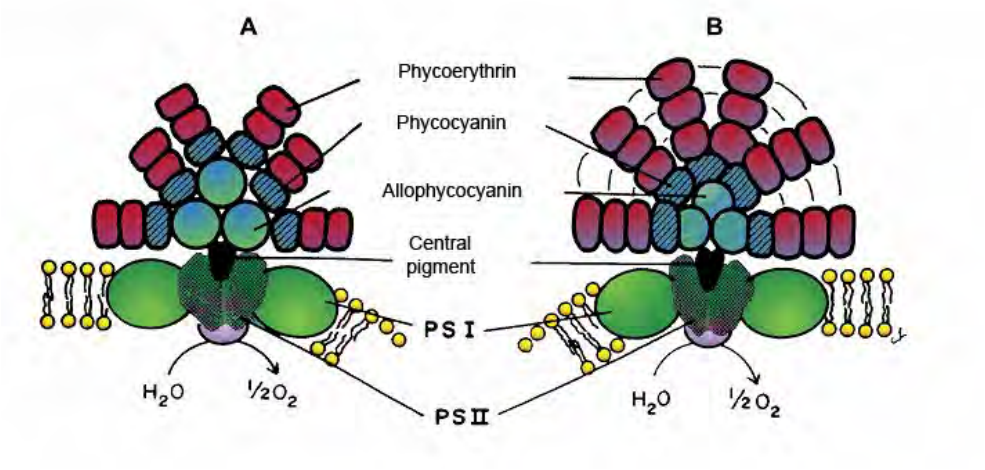

Figure 1. Structural model of a hemidiscoidal phycobilisome (A) and a hemispherical phycobilisome (B) (Gantt, 1986)

Resonance energy transfer from phycocyanin to allophycocyanin is a Förster type energy transfer that has a rate of 45-120 ps. Other investigations targeted the phycobilisome of PS II or PS I (Harnischfeger and Codd, 1978; Mimuro and Fujita, 1978; Kawamura *et al.*, 1979; Redlinger and Gantt, 1982; Ley, 1984), and by measurements of static electricity they concluded values of μs (Holzwarth *et al.*, 1990; van Thor *et al.*, 1998). The central core pigments have a very efficient resonance energy transfer chain with a yield of over 95% (Glazer, 1989). Phycobilins that form the phycobilisomes have a high fluorescence.

The basic aspects of photosynthetic light-harvesting and electron transport can be measured in a fast and non-invasive way by analyzing the chlorophyll fluorescence. The photosynthetic system of cyanobacteria is a link between metabolic processes (iron, nitrogen and carbon fixation), therefore chlorophyll fluorescence signals can provide fast and real-time information about photosynthesis and all phases of acclimatization.

Fluorescence analyses depend on the moment when a pigment absorbs a photon of energy and enters a state of electronic excitation, this is followed by: - photochemical reactions that take place in specific chlorophyll molecules of the photosynthetic reaction centers, the excited electron of the pigment molecule enters the electron transport chain; - heat dissipation that brings bag excited electrons to their initial state by giving off heat; - excited energy transfer to surrounding pigment molecules occurs in the light-harvesting antenna system; - fluorescent emission at a wavelength similar to the photon absorbs initially. These are competing processes, and changes in the fluorescence emission reflect in a corresponding way changes in the competing deexcitation pathways. For every kind of pigment, fluorescence emission levels rely upon concentration of the pigment, intensity of excitation light and production of fluorescence or fluorescence emission efficiency (Campbell *et al.*, 1998). Chlorophyll associated with PS II has a different fluorescence emission from that associated with PS I (Pfündel, 1998). The amount of chlorophyll combined with photosystems depends on the species, and its variability is induced by stress (Riethman and Sherman, 1988; Straus, 1994; Falkowski and Raven, 1997; Boekema *et al.*, 2001).

After illumination, chlorophyll molecules in PS II get in an excited singlet state (Chl a*). The energy resulted from the excited state is transferred to the reaction center for its further utilization in photochemical charge separation and conversion to chemical energy necessary in photosynthesis (photochemical) or can be dissipated as heat (non-photochemical diminution) either it can be reemitted as fluorescence. The sum of these energies is equivalent with the absorbed light energy. These three processes compete with each other, thus fluorescence will be higher when less energy is used in photochemical reactions or this energy is emitted in the form of heat. By measuring the amount of chlorophyll fluorescence, the efficiency of photochemical processes and of non-photochemical quenching can be determined (Krause and Weis, 1991). The wavelength of emitted fluorescence is higher than the wavelength of the absorbed light.

In the dark, all of the reaction centers are open, and the photochemical processes are maximized, while fluorescence emission is very low (F0, fluorescence in the absence of photosynthetic light) (Fig. 2). Illumination with strong light leads to charge separation in the reaction centers, while electrons move toward the first electron acceptor, QA. When QA is reduced, reaction centers are in a closed state, and photochemical processes are in a transitional blocked state. Reaction centers are closed because they are incapable of further electron accepting. Closing of the reaction centers result in a reduced efficiency of photochemical processes and increased fluorescence (van Kooten and Snel, 1990).

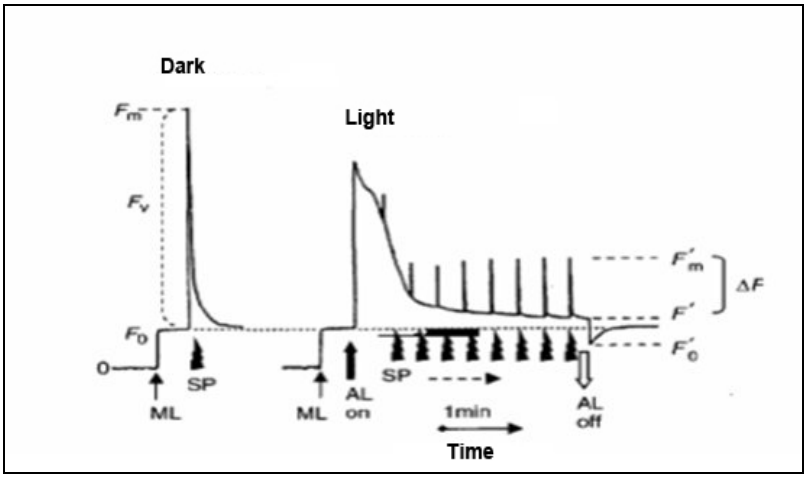

Figure 2. Model of pulse saturation method (adapted from Schreiber, 1986). Minimal and maximal fluorescence, F0 and Fm, measured on samples adapted to dark using modulated measuring light ML and saturation pulse of light SP. If actinic light AC and a series of saturation pluses is used for illumination F´ and F´m can be reached. When illumination is stopped F´0 can be obtained

Because photochemical production is minimal, fluorescence dissipation and production are proportional, and fluorescence production of the closed centers is noted Fm. As fluorescence production becomes proportional with the closed level of PS II, opening of the reaction centers act as fluorescence reducers (quencher), process noted with qP (photochemical reduction) (Genty *et al.*, 1989). qP values between 0-1 indicate the level of QA oxidation.

The difference between Fm (all QA reduced) and F0 (all QA oxidized) is called variable fluorescence Fv. Ratio between Fv/Fm is 0,65-0,80 for the samples adapted to dark and shows the photochemical production in PS II. Productions can vary with irradiation and physiological treatment. When the photosynthetic system is exposed to light the decrease of maximal fluorescence occurs and F´m is obtained. This phenomenon is called non-photochemical quenching NPQ, resulting in energy dissipation as heat. This non-photochemical diminution is the opposite of photochemical reactions and it is considered to be a protection valve against damages caused by excess irradiation.

In cyanobacteria, PS I and phycobilisomes significantly contributes to the fluorescence affecting the Fv/Fm parameter. Phycobiliproteins contribute to fluorescence to by overlapping the emission of chlorophylls influencing F0 (Campbell *et al.*, 1998; Cruz *et al.*, 2005).

There is an inverse correlation between photochemistry and fluorescence emission. Photochemistry and fluorescent photochemical quenching (qP) are maximized when the reaction centers and PS II are open, while fluorescence is weak.

When all reaction centers are closed, qP decreases, and also fluorescence reaches a maximum level. Fluorescence is regulated by the oxidized state of the primary acceptor QA. When QA is oxidized, minimum level of fluorescence is achieved, while fluorescence reaches maximum level when QA is fully reduced (Bissati *et al.*, 2000).

In this study we present the effects of microaerobiosis achieved with argon bubbling, on photochemical activity of photosynthesis in the cyanobacterium *Synechococcus* sp. PCC 7002.

Materials and methods

The wild type *Synechococcus* sp. strain PCC 7002 is maintained in the Collection of Cyanobacteria and Algae of the Institute of Biological Research in Cluj-Napoca, Romania. For this experiment, the cells were photoautotrophically grown in flasks with A^+ medium (Stevens *et al.*, 1973). Light was provided by cool-white fluorescent lamps (250 μmol m^{-2} s^{-1}), while the standard growth temperature was 38°C, this value being maintained with a water bath within ±1°C. The density of the photon flux was measured using a QSPAR Quantum Sensor (Hansatech Instruments Ltd, Norfolk, United Kingdom) light meter while cell growth was monitored by the optical density at 550 nm (OD_{550}) with a model Shimadzu UV-1700 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

Cells were grown for 5 days, until they reached exponential growth phase, and then they were bubbled with argon in otherwise standard conditions for 120 minutes.

For monitoring PS I and PS II activity, chlorophyll fluorescence was measured with a Waltz Dual–100 analyzer. Identification of assimilation pigments was based on the maximum absorption peaks measured with a Jasco V-630 spectrophotometer, and their concentration was determined according to Arnon (1949), Lichtenthaler and Wellburn (1983).

Results and discussion

The *Synechococcus* sp. PCC 7002 culture had an optical density of OD_{680} = 1.166, given the density of biomass whose pigment components absorb light at 680 nm. *In vivo* absorption of cellular suspension revealed spectral regions of absorption of photosynthetic apparatus components and main absorption peaks (Fig. 3). Carotenoids absorb light in the blue range of spectrum (490 nm), chlorophyll a at 439 nm and in the red range of spectrum at 680 nm. The absorption spectrum of phycobilins is at 631 nm. Summation of phycobiliproteins and chlorophyll a gives rise to the ability of cyanobacteria to efficiently capture light (Mur *et al.*, 1999). *In vitro* measurements of absorption showed a dominancy in chlorophyll a (432, 664 nm) and the presence of carotenoids (481 nm) (Fig. 3 B).

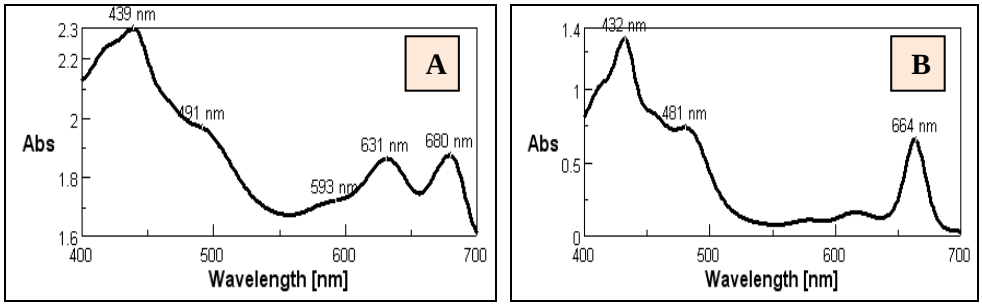

Figure 3. The *in vivo* absorption spectrum (A), and pigment extract spectrum (B) in *Synechococcus* sp. PCC 7002 grown under standard conditions

Assimilatory pigment contents were identified in *Synechococcus* sp. PCC 7002 and they are presented in Table 1. The photosynthetic apparatus in cyanobacteria include chlorophyll *a* (665 nm), which together with different types of carotenoids and phycobiliproteins form the light-harvesting unit. Pigment concentration was determined at the initial control state, before the application of stress treatment.

Composition specificity in assimilation pigments and their concentration is affected by the physiological development of cyanobacteria. Carotenoids can influence the photosynthetic membrane stability having a role in regulation of photosynthetic membrane dynamics (Szalontai *et al.*, 2012).

Table 1.

Assimilating pigments measured in the control sample (mg/l)

	Amount [mg/l]	λ_{nm}
Chlorophyll a	5,714	665
Carotene	0,606	451
Zeaxanthin (+ Cryptoxanthin)	0,451	452
Total carotenoids	1,057	
a/c	5,40	

Evolution of chlorophyll fluorescence parameters in *Synechococcus* sp. PCC 7002 under the effects of argon treatment are presented in Fig. 4. Minimal fluorescence yield F_0, decreased up to 94% after 120 minutes of exposure to argon. Basal fluorescence yield show that the primary acceptor Q_A is in oxidized form, and RC II reaction center are opened, this is a state when photochemical process and use of the excitation energy are maximized, also all redox components of PS II are oxidized. F_0 is the fluorescence emitted by chlorophyll molecules from the antenna before excitation is transferred to the reaction center.

By applying a saturation pulse, fluorescence rises from baseline to maximum value Fm, the primary electron acceptor of PS II becomes fully reduced, also photochemistry is blocked and reaction centers are closed. Maximum fluorescence Fm decreased after 15 minutes of exposure to argon bubbling. Decline of F_m attests the increasing number of closed reaction centers, as well as plastoquinone reduction and growth of fluorescence emission. Variable fluorescence, expressing the difference between Fm and F_0, showed positive values compared to the control, except after 60 minutes of argon treatment. F_0 and Fm are emitted by the chlorophyll molecules from the antenna (Krause and Weis, 1991). All reductions noted for opening and closing of the reaction centers of PS II exhibits a functional imbalance. Decreased Fm yielded low values for variable fluorescence Fv against the control.

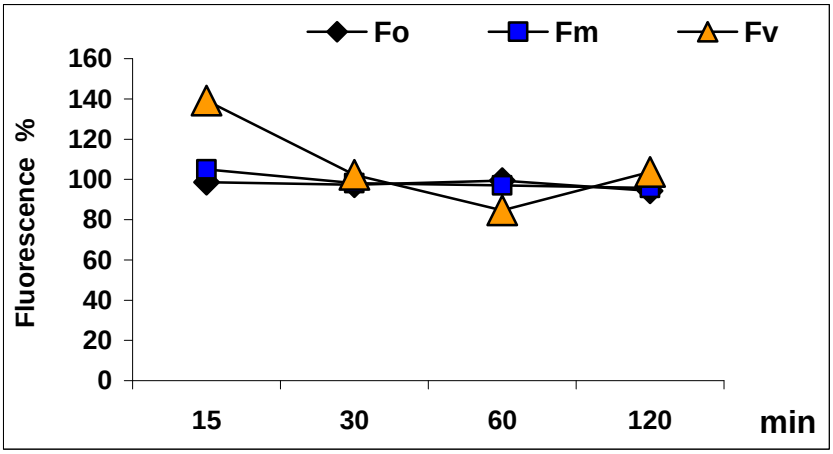

Figure 4. Evolution of chlorophyll fluorescence parameters in *Synechococcus* sp. PCC 7002 under microoxic conditions

Maximal quantum yield (Fv/Fm) of PS II photosynthesis weakly stimulated by the argon treatment, except at 60 minutes of argon effect when negative values were observed compared with the control. Fv/Fm allows the determination of maximum quantum yield (efficiency) of PS II photochemistry, or of the photosynthetic electron transport. Final value recorded was 0.167 that represent 20% of the theoretical value, respectively the proportion of photoinhibited reaction centers. Fv/Fm has a theoretical value of 0.82 and indicates the maximum fraction of photons absorbed and used in photochemical reactions. Values below 0.82 indicate the amount of photoinhibited PS II reaction centers. Maximum production of fluorescence when all the centers are closed represents only 3% of the light absorbed. When the centers are open, fluorescence represents 0,6% (Krause and Weis, 1991).

Effective quantum yield (YII) decreased after 15 minutes of argon treatment (Fig. 5). Y (II) corresponds to the fraction of photochemical converted energy.

Quantum yield of non-regulated energy dissipation Y (NO) dropped, reaching 94% after 15 minutes of stress condition, compared to the control. Theoretically, the quantum yield of non-regulated energy dissipation summarizes the energy dissipation processes that occur in antennas of the photosystems. Because of the decrease in fluorescence emission due to photoinhibition, non-regulated energy dissipation is slightly lower.

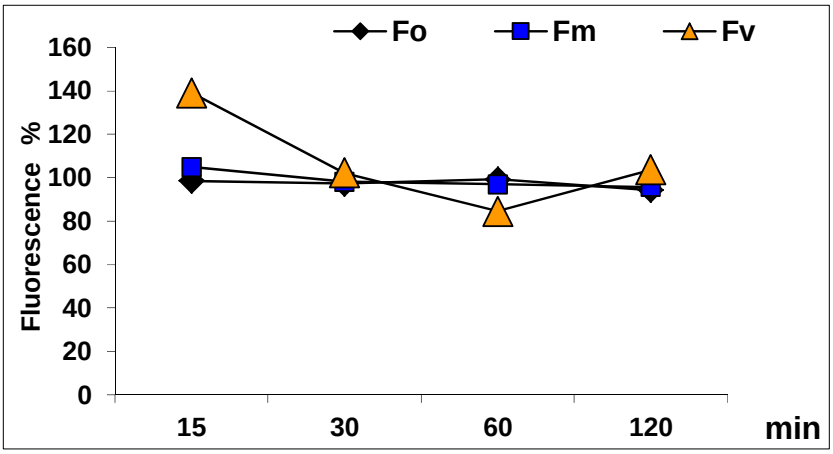

Figure 5. Evolution of quantum yields in PS II in *Synechococcus* sp. PCC 7002 under microoxic conditions

Coefficient of photochemical quenching, qP and qL, were maintained at higher values, except when 30 minute exposure to argon treatment (Fig. 6). qP is a measure of the overall „openness", and the high values observed proves the high open state of the RC II reaction centers, respectively the heavily oxidized state of the primary acceptor Q_A. He coefficient of photochemical quenching qL, is a measure of the fraction of open PS II reaction centers, high values observed in our cyanobacterial suspensions indicate the high amount of opened PS II reaction centers. qP allows the estimation of the oxidized quinone acceptor fraction of opened PS II or PS I reaction centers (Grace and Logan, 1996). High values of qP and qL express an intense photochemical process with decreased fluorescent emission. The fluorescent coefficient of photochemical quenching can be used for estimations of opened PS II reaction centers (oxidized state of Q_A) or closed PS II reaction centers (reduces state of Q_A) (Huner *et al.*, 1998).

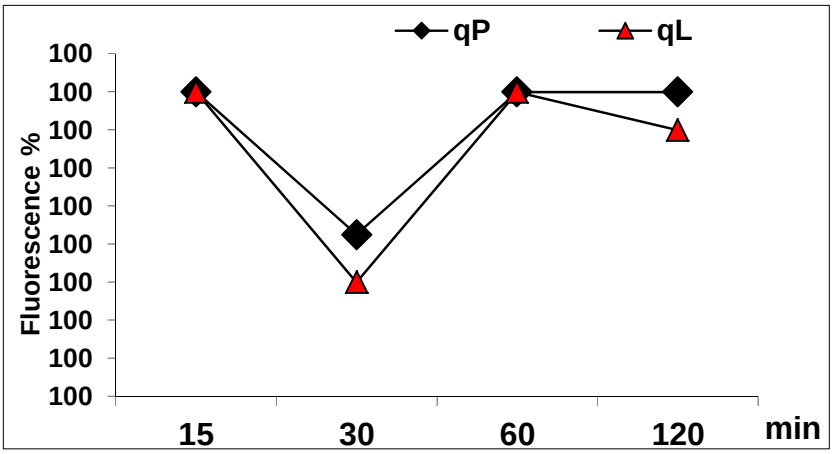

Figure 6. Evolution of photochemical coefficient in *Synechococcus* sp. PCC 7002 under microoxic conditions

Changes in fluorescence yield reflect direct changes in photochemical competing de-excitation paths, in excitation transfer and heat dissipation. When PS II reaction centers are opened and photochemical potential is maximal, fluorescent photochemical quenching is also very high while fluorescence yield is decreased. When reaction centers are closed no photochemical processes are active, photochemical quenching is absent and fluorescence yield is maximal. qP indicates the balance between excitation of PS II centers that are thereby closed, and removing of electrons from PS II by photosynthetic electron transport chain which reopens the centers. This balance in excitation pressure responds to light intensity, temperature and the availability of final acceptors.

The loss of photoinhibition was correlated with the redox state of the primary acceptor Q_A. Relative redox state of Q_A *in vivo* can be estimated by 1-qP parameters (excitation pressure) and Fv/Fm (quantum yield) (Öquist *et al.*, 1993). As in cyanobacteria phycobilisomes provide the greatest capacity to capture light, production of photosynthesis rely on the energy transfer efficiency (Foguel *et al.*, 1992).

In PS I, the Pm signal, as well as Fm, represents the maximal change in P_{700} reaction center, equivalent with differences between oxidized and reduces P_{700}. Total reduction can be achieved only in dark, while total oxidation is obtained after brief illumination, when PS I is limited. Increasing in Pm signal depends on the total chlorophyll content of P_{700} reaction center, and decrease is due to limitation in P_{700} reaction center acceptors. P_{700} values varies between 0 (totally oxidized P_{700} centers) and 1 (totally reduced P_{700} centers, in standard dark state).

By exposure to argon Pm signal was stimulated in the first 30 minutes, followed by a significant decrease towards the end of the stress treatment suggesting a decrease in the reduction state of P_{700} reaction center compared to oxidized state (Fig.7). Maximum oxidation can be reached at illumination with intense light, before the electrons leave PS II to re-reduce $P_{700.}$

Effective PS I photochemical quantum yield Y(I) dropped significantly in the first 30 minute compared to the control. The decrease in quantum yield Y(I) reveals decreased reduction state due to the lack of limitation by the acceptor, respectively, decrease in photochemical energy conversion in PS I. Y(I) expresses the quantum yield of photochemical energy conversion in PS I and it is complementary to the non-photochemical quantum yield of energy conversion. The non-photochemical quantum yield of PS I, Y(ND) increased compared to the control. Y(ND) defines the quantum yield non-photochemical energy conversion in PS I due to limitation of electron donors, and represents the fraction of overall P_{700} oxidized in a given state ($P_{700}^{+}{}_{A}$). Oxidized P_{700} reaction centers transform the quantitatively absorbed excitation energy in heat. Limitation due to donors is increased by the trans-thylakoid proton gradient (photosynthetic control of the cytochrome complex b_6f as well as the down regulation of PS I) and damages caused in PS II.

PS I contribution to the fluorescence is low: 5% at 720 nm and 1-2% at 685 nm. Closed PS I centers do not contribute to Fv. This can be explained by the relative stability of P_{700} in oxidized state.

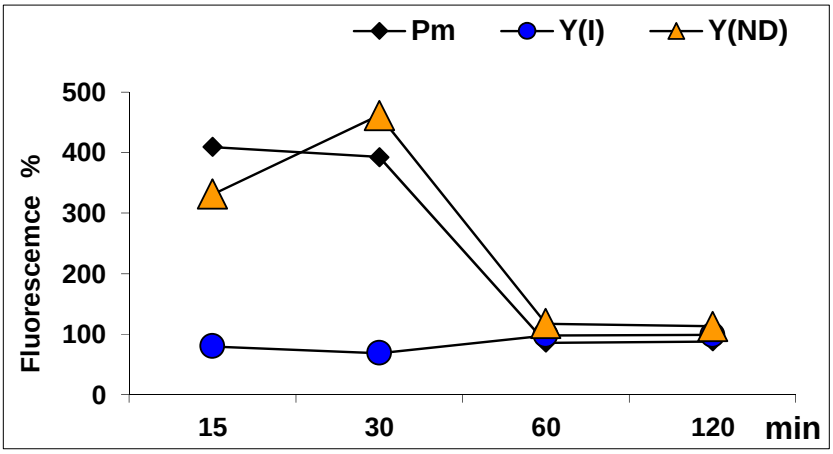

Figure 7. Photochemical activity of photosystem PS I under microoxic conditions in *Synechococcus* sp. PCC 7002

To induce photosynthetic activity pulse amplitude modulation technique (PAM measurements) was used (Schreiber, 2004). Fluorescence transition follows the O-J-I-P sequence, a polyphasic curve of the initial fluorescence F_0 and maximum fluorescence F_m, these can be visualized on a logarithmic scale (Schreiber and Neubauer 1987; Strasser *et al*., 1997; Srivastava *et al.*, 1999). The intermediate J-I level seems to relate to the heterogeneity of plastoquinone volume and refers to the thermal phase of the photosynthetic process. P is reached when all plastoquinone molecules are reduced to PQH_2. Additional DCMU result in curve of O-J. This curve is similar to the kinetics of O-I_1 –I_2 – P obtained by Neubauer and Schreiber (1987).

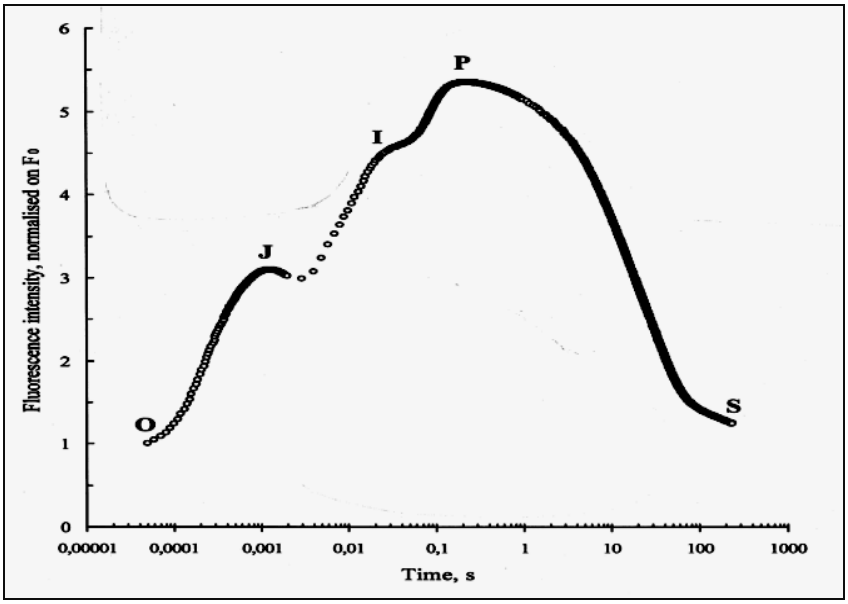

Figure 8. Transition of chlorophyll fluorescence in samples adapted to microoxic conditions and excited with red light of 650 nm (Strasser *et al.*, 1995)

The kinetics of the chlorophyll fluorescence induction by pulse saturation in control and at the end of stress treatment are presented for PS II and PS I in Fig. 9. Fluorescence yield is related to the photochemical activity of PS II. The kinetics of fluorescence variation from F_0 to maximum Fm displays the accumulation of reduced Q_A in reaction centers. Fluorescence kinetics showed on the logarithmic scale revealed important modifications in I – P spectrum due to reduction of the plastoquinone nuclei (Fig. 9, A, C, E, G, I). Changes in the induction curve are shown in Fig. 9, B, D, F, H, J. The length of fluorescence and its amplitude is proportional to the number of antenna molecules (Krause and Weis, 1991).

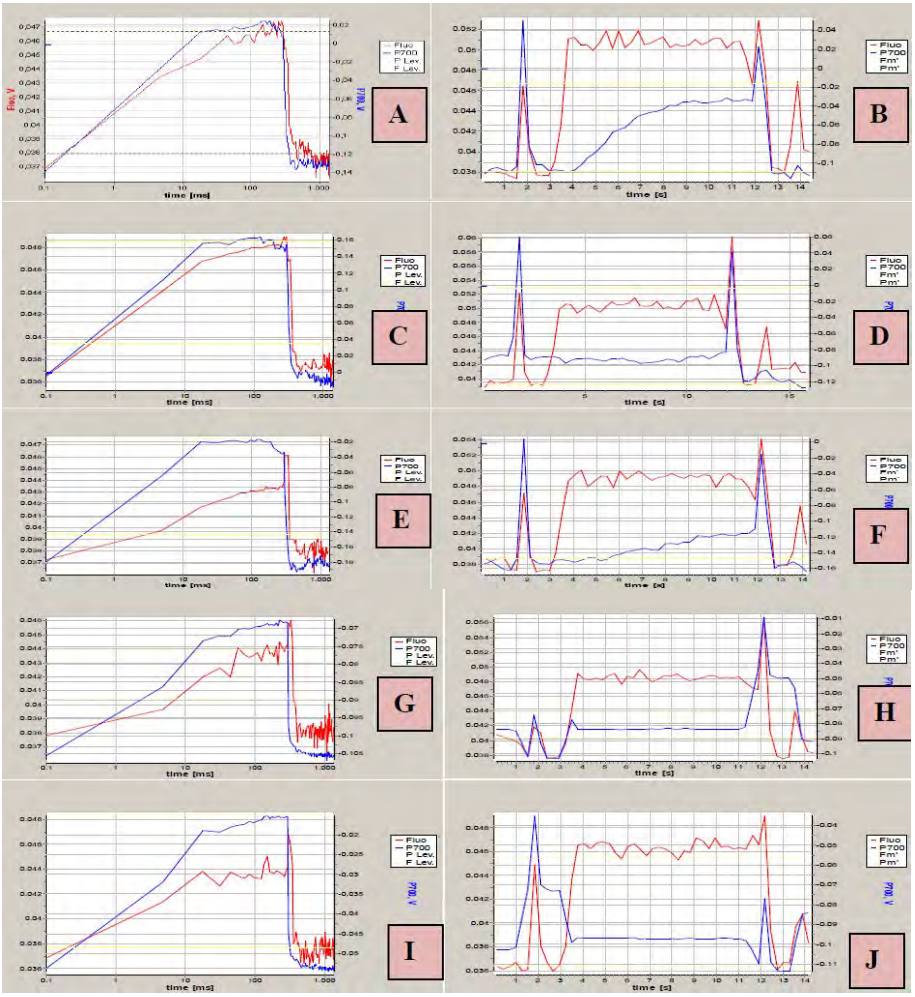

Figure 9. Chlorophyll fluorescence in standard conditions and under microaerobiosis using the saturation-pulse method: A – logarithmic kinetics of the fluorescence in the control; B – fluorescence induction curve in control; C – fluorescence logarithmic kinetics of the sample after 15 minutes of microaerobiosis; D – fluorescence induction curve of the sample after 15 minutes of microaerobiosis; E - fluorescence logarithmic kinetics of the sample after 30 minutes of microaerobiosis; F - fluorescence induction curve of the sample after 30 minutes of microaerobiosis; G - fluorescence logarithmic kinetics of the sample after 60 minutes of microaerobiosis; H - fluorescence induction curve of the sample after 60 minutes of microaerobiosis; I - fluorescence logarithmic kinetics of the sample after 120 minutes of microaerobiosis; J - fluorescence induction curve of the sample after 120 minutes of microaerobiosis. Red – PS II fluorescence; Blue – PS I fluorescence

Conclusions

The cyanobacterium *Synechococcus* sp. PCC 7002 displays a specific composition and concentration of assimilatory pigments, according to the physiological condition of the cells.

For PS II under argon treatment, minimal fluorescence F_0 and maximal fluorescence F_m decreased. Fluorescence yield shows that the primary acceptor Q_A is in oxidized state, and RC II reaction centers are opened, photochemical processes and excitation energy harvesting are maximal, and all redox component of PS II are reduced. Maximum fluorescence shows that primary electron acceptor (Q_A) of PS II becomes totally reduced, photochemical reactions are blocked and reaction centers are closed. Decrease of Fm parameter represents an increase in closed reaction centers, respectively activation of plastoquinone reduction processes and increase of fluorescence emission.

Variable fluorescence, expresses the difference between Fm and F_0, showed positive values compared to the control, except after 60 minutes of argon treatment. All decrease in opening and closing of PS II reaction centers exhibit a functional imbalance.

Maximal quantum yield (Fv/Fm) of PS II photosynthesis weakly stimulated by the argon treatment, except at 60 minutes of argon effect when negative values were observed compared with the control. Fv/Fm allows the determination of maximum quantum yield (efficiency) of PS II photochemistry, or of the photosynthetic electron transport. Final value recorded was 0,167 that represent 20% of the theoretical value, respectively the proportion of photoinhibited reaction centers. Fv/Fm has a theoretical value of 0.82 and indicates the maximum fraction of photons absorbed and used in photochemical reactions. Values below 0.82 indicate the amount of photoinhibited PS II reaction centers. Effective quantum yield (YII) decreased after 15 minutes of argon treatment. Y(II) corresponds to the fraction of photochemical converted energy. Quantum yield of non-regulated energy dissipation Y(NO) dropped, reaching 94% after 15 minutes of stress condition, compared to the control. Theoretically, the quantum yield of non-regulated energy dissipation summarizes the energy dissipation processes that occurs in antennas of the photosystems. Because of the decrease in fluorescence emission due to photoinhibition, non-regulated energy dissipation is slightly lower.

Coefficient of photochemical quenching, qP and qL, were maintained at higher values, except when 30 minute exposure to argon treatment. qP is a measure of the overall „openness", and the high values observed proves the high open state of the RC II reaction centers, respectively the heavily oxidized state of the primary acceptor Q_A. He coefficient of photochemical quenching qL, is a measure of the fraction of open PS II reaction centers, high values measured in our cyanobacterial suspensions indicate the high amount of opened PS II reaction centers. High values of qP and qL express an intense photochemical process with decreased fluorescent emission.

For PS I, by exposure to argon Pm signal was stimulated in the first 30 minutes, followed by a significant decrease towards the end of the stress treatment suggesting a decrease in the reduction state of P_{700} reaction center compared to oxidized state . Maximum oxidation can be reached at illumination with intense light, before the electrons leave PS II to re-reduce $P_{700.}$

Effective PS I photochemical quantum yield, Y(I) dropped significantly in the first 30 minute compared to the control. The decrease in quantum yield Y(I) reveals decreased reduction state due to the lack of limitation by the acceptor, respectively, decrease in photochemical energy conversion in PS I. Y(I) expresses the quantum yield of photochemical energy conversion in PS I and it is complementary to the non-photochemical quantum yield of energy conversion. The non-photochemical quantum yield of PS I, Y(ND) increased compared to the control. Y(ND) defines the quantum yield non-photochemical energy conversion in PS I due to limitation of electron donors, and represents the fraction of overall P_{700} oxidized in a given state ($P_{700}^{+}{}_{A}$). Oxidized P_{700} reaction centers transform the quantitatively absorbed excitation energy in heat. Limitation due to donors is increased by the trans-thylakoid proton gradient (photosynthetic control of the cytochrome complex b_6f as well as the down regulation of PS I) and damages caused in PS II.

Alternation of oxidative/reduced state of the plastoquinone nuclei was revealed in the kinetics of the chlorophyll fluorescence induction by pulse saturation in control. Fluorescence yield is related to the photochemical activity of PS II. The kinetics of fluorescence variation from F_0 to maximum Fm monitors the accumulation of reduced Q_A in reaction centers. Fluorescence kinetics showed on the logarithmic scale revealed important modifications in I – P spectrum due to reduction of the plastoquinone nuclei. The length of fluorescence and its amplitude is proportional to the number of antenna molecules.

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