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# INFLUENCE OF HIGH LIGHT INTENSITY ON THE CELLS OF CYANOBACTERIA *ANABAENA VARIABILIS* SP. ATCC 29413

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**Abstract:** In this article is presented the result of research regardind the effect of high light intensity on the cells of *Anabaena variabilis* sp. ATCC 29413, the main objective is to study the adaptation of photosynthetic apparatus to light stress. Samples were analyzed in the present of herbicide diuron (DCMU) which blocks electron flow from photosystem II and without diuron. During treatment maximum fluorescence and photosystems efficiency are significantly reduced, reaching very low values compared with the blank, as a result of photoinhibition installation. Also by this treatment is shown the importance of the mechanisms by which cells detect the presence of light stress and react accordingly.

**Keywords:** cyanobacteria, *Anabaena* sp., photosynthesis, high light, fluorescence

## Introduction

Study cyanobacteria is of great importance because it can help to develop biofuels as biodiesel and biohydrogen or to better know photosynthesis mechanisms (cyanobacteria are model organisms for studying photosynthesis).

Cyanobacteria are prokaryotic oxygen-evolving photosynthetic organisms which had developed a sophisticated linear electron transport chain with two photochemical reaction systems, PSI and PSII, as early as a few billion years ago cyanobacteria. By endosymbiosis, oxygen-evolving photosynthetic eukaryotes are evolved and chloroplasts of the photosynthetic eukaryotes are derived from the ancestral cyanobacteria engulfed by the eukaryotic cells [GAULT & MARLER, 2009].

The aim of this paper is to study cyanobacterial photosynthesis, study in which chlorophyll fluorescence induced by "flash" is used to elucidate the effect of high light intensity on photosystem II.

Chlorophyll fluorescence may reflect photosynthetic activities in a complex manner. The method discussed in the experiment performed, based on chlorophyll fluorescence induced by "flash" is new in our country.

Research objectives are:

1. Characterization of the growth process based on specific parameters (growth curve, optical density, doubling time,) under the influence of high light;
2. Study of cyanobacterial photosynthesis based on chlorophyll fluorescence induced by "flash";
3. Evaluation the resistance of studied strain under light stress, in order to highlight the suitability of culture growth in open pond.

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## Material and method

Biological material which has been subject of this study is strain *Anabaena variabilis* sp. ATCC 29413, filamentous cyanobacteria that fix aerobic molecular nitrogen and does not requires special growth conditions.

Growth medium used is the BG-11 medium.

For 1000 ml of BG-11 medium were used 10 ml of macronutrients (100 x); 1 ml Trace metal solution (1000 x); 1 ml of each stock solution (1000 x): dipotasic phosphate ($K_2HPO_4$) 175 mM; sodium carbonate ($Na_2CO_3$) 189 mM and ferric ammonium citrate 6 mg / ml; 20 ml of buffer pH, Hepes-1M NaOH (pH 7.5) and double distilled water.

For 1000 ml of macronutrients (1000 x) were used 149.6 g of sodium nitrate ($NaNO_3$), 7.5 g of magnesium sulphate heptahydrate ($MgSO_4$ x $7H_2O$), 3.6 g calcium chloride dihydrate ($CaCl_2$ x $2H_2O$), 0.65 g citric acid and 0.1 g $Na_2$-EDTA.

For 1000 ml Trace metal solution (1000 x) were used: 2.86 g of boric acid ($H_3BO_3$), 1.81 g of manganese chloride tetrahydrate ($MnCl_2$ x $4H_2O$), 0.222 g of zinc sulphate heptahydrate ($ZnSO_4$ x $7H_2O$), 0.391 g of sodium molybdate dihydrate ($Na_2MoO_4$ x $2H_2O$), 0.079 g of copper ph. - sulfate pentahydrate ($CuSO_4$ x $5H_2O$) and 4.947 g of cobalt nitrate hexahydrate (Co ($NO_3$) 2 x $6H_2O$).

BG-11 medium is placed in containers and sterilized by autoclaving at 120˚ C, 20 minutes [ATLAS, 2004].

Treatment was performed in a tank of 10/10 cm, in which was placed a magnet and the tank was placed on a shaker. Culture density was approximately 6 mg chlorophyll, culture volume of 150 ml, 30˚ C temperature and light intensity of 600 μE.

For chlorophyll fluorescence measurements were used 3 ml of culture samples placed in cuvettes with all four sides transparent.

Samples were adjusted to the dark 5 minutes before fluorescence measurement.

Measurements were made in the presence and in absence of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea]. The amount of DCMU added was 1.5 μl.

Curves obtained were recorded using the FluorWin program and reported to a logarithmic time scale and exported to excel. DCMU is a herbicide commonly used in experiments that aims photosynthesis because of the ability to block electron transfer between QA and QB.

DCMU inhibits electron transfer because compete with the plastochinona for QB binding site, blocking chain irreversibly. So QA can not be reduced by the QB and recombine with redox components of the donor side electron of transport chain.

By adding DCMU to the sample can obtain information about the donor side integrity of the electron transport chain.

At normal light intensity, electron transfer from water to chlorophyll is efficiently done and does not requires artificial electron donors. Photosystem II efficiency is provided by the participation of a protein complex of membrane subunits, which transfer electrons from water across the tilacoid membrane to plastochinone [SIPPOLA, 2000].

Chlorophyll fluorescence decrease is due to increased energy dissipation rate and decreasing photochemical rate, because chlorophyll fluorescence is complementary with photochemistry and heat dissipation [BERCEA, 2008].

If photoinhibition installs photosynthetic capacity reduction occurs through a reduction in the proportion opened reaction centers of photosystem II and the state of tilacoid membranes is high energized [KOBLIŽEK & al. 2001].

Photoinhibition of photosystem II activity involves at least two levels of inactivation. The first level is considered reversible and occurs during approximately one hour without causing injury to photosystem II [LEITSCH & al. 1994].

The second level is supposed to be accompanied by degradation on the reaction centers of photosystem II, this level is oxygen dependent and is reversible by replacing the structural protein [JAHNS & MIEHE, 1996].

The graphic curve form shows the photosystem electron transfer. Samples without DCMU shows the acceptor side of photosystem, and by adding DCMU we can see the donor side of photosystem.

## Results and discussion

The effect of high light on *Anabaena variabilis* sp. ATCC 29413 cells is presented in Fig. 1. Curves obtained are represented by colors corresponding to samples taken at different time intervals (control sample – dark blue, sample at 15 minutes – pink, sample at 30 minutes – yellow, sample at 60 minutes – light blue, sample at 120 minutes – cherry). Panels (a, b, c) give test results with DCMU and panels (d, e, f) give the results of samples without DCMU. Panels (a, d) represent curves without normalization and panels (b, e) represent curves normalized to the same initial amplitude. Panels (c, f) are schematic representation of the evolution of maximum fluorescence values (Fig. 1).

Panel (a) shows the relative fluorescence intensity (with DCMU). Diuron (DCMU) added to the sample increased the amplitude of chlorophyll fluorescence. Maximum amplitude recorded at control sample. Depending of exposure time to high light chlorophyll fluorescence amplitude decreases significantly during the 120 minutes of exposure, compared with control sample.

PARK & al. (1995) said there is reciprocity between light intensity and duration of illumination for photosystem II functionality, indicating that inactivation of photosystem II depends on the total number of absorbed photons rather than the photons absorption rate. Photoinhibition of photosystem II is due to amino acid changes in protein D1 [SCHULZE & CALDWELL, 1995]. Continue photodegradation and resynthesis of D1 protein is called rapid turnover of the D1 protein of photosystem II [MATTO & al. 1984].

Panel (b) shows the normalized fluorescence intensity (with DCMU). Normalization helps to observe the differences between curves. In this case the curves are relatively close re-oxidation rates from the QA samples are similar, which indicates slow recombination of QA with the water oxidation complex.

Panel (c) shows the variation of maximum fluorescence values (fmax) under the action of high light (with DCMU). During treatment there is an inhibition of active reaction centers at a rate of 56%. Decreases of maximum fluorescence leads to decreases in the proportion of active reaction centers. The initial curve slope is steeper in the fact that the first 30 minutes of exposure to high light degradation of the photosystem II is more pronounced. After the 30 minutes of exposure the slope curve is smoother as a result to the initiation of defense mechanisms to accommodate the new conditions. This are mechanisms of regulation of D1 protein synthesis cycle by phosphorylation and dephosphorylation of D1 protein.

Panel (d) shows the relative fluorescence intensity (without DCMU). It can be seen rapid growth in the number of active reaction centers of photosystem II (able to reduce QA) at control sample but after the first 15 minutes of treatment the proportion of active centers decreases reaching values below the control sample.

The maximum amplitude it has been registered at control sample. During the 120 minutes of treatment occurs reduction of photosynthetic activity. This fact results from reduced amplitude of chlorophyll fluorescence. It can be observed a decrease in chlorophyll fluorescence amplitude from sample to which was added DCMU.

Panel (e) shows the normalized fluorescence intensity (without DCMU). Control sample indicates the steeper slope therefore electron transport between QA and QB performs faster compared with samples exposed different times to high light. The intensity of electron transfer is performed in the following order: control sample, sample exposed to 120 minutes (purple curve), the sample exposed to 15 minutes (pink), the sample exposed to 30 minutes (yellow) and the sample exposed to 60 minutes (blue).

Panel (f) shows the variation of maximum fluorescence values (fmax) under the action of high light treatment (without DCMU). In this case, at measuring time the maximum proportion of active centers was recorded at cotrol sample. As time of exposure cultures to high light increases, a decreased in number of active centers occurs, so fluorescence decreases. In these conditions reaction centers of photosystem II are gradually closed during exposure time lowering the photochemical efficiency of photosystem II. During treatment the reaction centers were inhibited at a rate of 52%.

## Conclusions

1. Exposure to high light causes a decrease in maximum fluorescence after the first 15 minutes of exposure.
2. By adding diuron (DCMU) to the cell suspension it has been recorded an increase in amplitude compared to samples without DCMU. It can be seen more rapid growth of the fluorescence and higher amplitudes, suggesting the photosystem II efficiency in energy photons absorption.
3. Maximum fluorescence during treatment was significantly reduced to the samples with DCMU and without DCMU, reaching values lower than those of control sample. In these conditions the photochemical efficiency of photosystem II and electron transport chain efficiency have been reduced.

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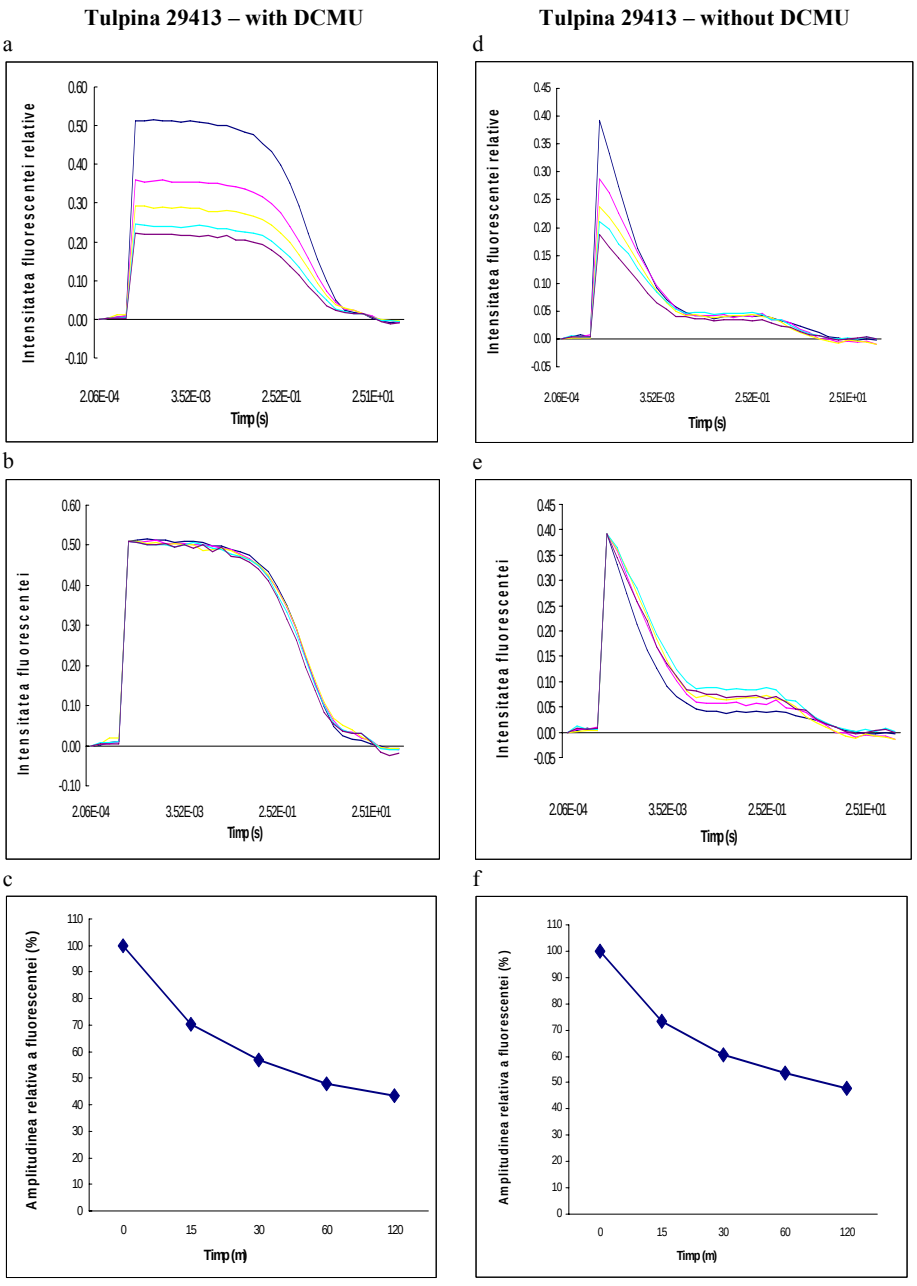


**Fig. 1.** Effect of high light intensity on cells of *Anabaena variabilis* sp.