Effect on the Electron Transport Chain of Changing Carbon Dioxide Concentration in Some Model Cyanobacteria Species

Original Article

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Abstract

The objective of the present study was to evaluate the adaptation of photosynthetic apparatus and growth of cyanobacteria to 5% concentration of atmospheric CO_2. We investigated the tolerance of several cyanobacteria under conditions of 5% carbon dioxide concentration. Cultures of *Synechocystis* sp. PCC 6803, *Anabaena variabilis* sp. ATCC 29413, *Synechococcus* sp. PCC 7002 and *Cyanothece* sp. ATCC 51142 were grown in liquid culture sparged with CO_2 enriched air. Cell density was determined by optical density (OD) measurements at 580 and 730 nm and the adaptation of photosynthetic apparatus was determined by studying differences from cyanobacteria treated and untreated cells on the steadystate turnover of PS II, as assayed through chlorophyll *a* fluorescence yield decay after flash excitation.

Keywords: cyanobacteria, carbon dioxide concentration, photosynthesis, electron transport, photosystem II.

1. Introduction

Cyanobacteria are considered a phylum of bacteria that performe photosynthesis. They may be responsible for the atmosphere oxygenation because are able to reduce nitrogen and carbon in aerobic conditions [1].

The first component of the photosynthetic electron transfer chain is photosystem II (PSII), located in the thylakoid membranes of cyanobacteria [2, 6]. PS II supplies an electron by oxidation of water to molecular oxygen [7].

The biofixation of CO_2 by cyanobacteria according to research projects is a sustainable strategy because by the bioconversion of carbon dioxide is produced fatty acids, biodiesel and biohydrogen that are high added value products [8, 9, 10].

Some photosynthetic microorganisms can grow in the presence of 0.001% to ~ 40% CO_2 concentration [4].

According to Lee et al., 2006 [5] and Jacob-Lopes et al., 2007 [3], carbonates and bicarbonates are more highly represented for increasing of the carbon dioxide fixation rates.

2. Materials and methods

Biological material which has been subject of this study are strains *Synechocystis* sp. PCC 6803, *Anabaena variabilis* sp. ATCC 29413, *Synechococcus* sp. PCC 7002 and *Cyanothece* sp. ATCC 51142.

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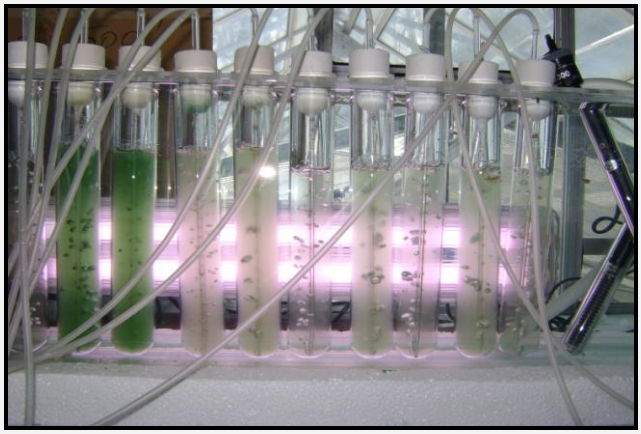


Fig. 1. Carbon dioxide bubbling experiment (original photo)

Cyanobacteria cells were grown at 30°C except *Synechococcus* sp. PCC 7002 which was grown at 38°C in tubes of 300 ml with BG-11 liquid medium. The growth medium was constantly bubbled with 100% air (control cells) and with air containing 5% CO_2 (treated cells). Cultures were illuminated with cool-white fluorescent light (fig. 1).

Cell density measurements

Growth was measured under conditions of 100% air (control samples) and 5% carbon dioxide concentration in air (treated samples) for up to 6 days by taking samples and measuring the absorbance at OD 580 and 730 nm with a spectrophotometer.

Variable fluorescence measurements

The fluorescence parameter shows the efficiency of captured energy by open PS II reaction centres. The Chl *a* fluorescence yield from PSII was measured for 6 days with a Double Modulation Fluorometer FL 3500 connected to a computer. Samples containing 3 ml culture were dark-adapted for 5 min and then irradiated with low intensity-modulated light to determine the minimum fluorescence (F_0). The maximum fluorescence (F_m) was measured by a single saturating pulse of white actinic light.

Additionally the maximum fluorescence (Fm) was obtained by closing at least 50% from reaction centers with 1,5 µl DCMU [3-(3,4-diclorofenil)-1,1-dimetiluree, Diuron], which blocks the acceptor side of PSII.

Samples with DCMU provides informations about the electron donor side integrity of PSII and samples without DCMU offers informations about the electron acceptor side integrity of PSII.

3. Results and Discussion

The results presented in this paper shows that 5% carbon dioxide concentration induce different cellular effects in response to the cyanobacteria species used.

Panels with relative fluorescence intensity shows the amplitude differences during treatment with 5% carbon dioxide.

Panels with normalized fluorescence intensity gives us information about the electron transport.

Graphics with maximum fluorescence variation (Fmax) shows the number of active reaction centers of photosystem II under the action of treatment at the time of measurement. Initial amplitude of the fluorescence signal is directly proportional to the photosystem II reaction centers that are able to reduce Q_A. Number of active photosynthetic reaction centers represents difference between the total number of photosynthetic centers of photosystem II and the number of centers that are inhibited.

Growth curve graphics shows conditions in which studied cyanobacteria species perform better and register higher growth rates.

Figures 2; 3; 4; 5 - panels A, C (with DCMU) shows the relative fluorescence intensity.

The highest amplitude is observed at treated samples compared to the control samples at *Synechocystis* sp. PCC 6803, *Anabaena variabilis* sp. ATCC 29413, *Synechococcus* sp. PCC 7002 but at *Cyanothece* sp. ATCC 51142 the highest amplitude is observed at control sample compared to the treated sample.

In the cyanobacteria *Synechocystis* sp. PCC 6803 the highest amplitude for treated sample is observed in the third day of treatment and for control sample is observed in the fifth day; in *Anabaena variabilis* sp. ATCC 29413 the highest amplitude for treated sample is observed in the third and the fourth day of treatment and for control sample is observed in the fifth and the sixth day; in *Synechococcus* sp. PCC 7002 the highest amplitude for treated sample is observed in the the fourth day of treatment and for control sample is observed in the sixth day; in *Cyanothece* sp. ATCC 51142 the highest amplitude for treated sample is observed in the the second day of treatment and for control sample is observed in the sixth day. This means that in those days the potential photochemical efficiency of donor side of PSII occurred with a better performance.

DCMU caused an increase of amplitude compared to samples without DCMU.

Figures 2; 3; 4; 5 - panels B, D (with DCMU) shows the normalized fluorescence intensity, the inhibition by DCMU of linear electron transport and also provides information about the reoxidation rate of QA^-. Therefore it can be seen that the reoxidation rates of various samples are relatively close, indicating the slow recombination of QA^- with donor side components of PS II.

In the cyanobacteria *Synechocystis* sp. PCC 6803 at treated sample QA^- reoxidate with Tyr^+ (tyrosine molecule) in day four and in the rest of the days reoxidate with water reoxidation complex but at control sample QA^- reoxidate with Tyr^+ in day six and in the rest of the days reoxidate with water reoxidation complex.

In *Anabaena variabilis* sp. ATCC 29413 at treated sample QA^- reoxidate with water reoxidation complex in day six and in the rest of the days reoxidate with Tyr^+ and at control sample QA^- reoxidate with water reoxidation complex in the first day and in the rest of the days reoxidate with Tyr^+.

Reoxidation rates of QA^- in cyanobacteria *Synechococcus* sp. PCC 7002 and *Cyanothece* sp. ATCC 51142 are relatively close there are no significantly changes during treatment by days and between treated and untreated samples.

During this cyclic pathway from donor side of PSII, electron transport occurs faster when QA^- reoxidate with Tyr^+ then when reoxidate with water oxidation complex.

Figures 6; 7; 8; 9 - panels A, C (without DCMU) shows the relative fluorescence intensity.

The highest amplitude is observed at treated samples compared to the control samples at *Synechocystis* sp. PCC 6803, *Anabaena variabilis* sp. ATCC 29413, *Synechococcus* sp. PCC 7002 but at *Cyanothece* sp. ATCC 51142 the highest amplitude is observed at control sample compared to the treated sample.

In the cyanobacteria *Synechocystis* sp. PCC 6803 the highest amplitude for treated sample is observed in the third day of treatment and for control sample is observed in the fifth day; in *Anabaena variabilis* sp. ATCC 29413 the highest amplitude for treated sample is observed in the third day of treatment and for control sample is observed in the sixth day; in *Synechococcus* sp. PCC 7002 the highest amplitude for treated sample is observed in the the fourth day of treatment and for control sample is observed in the sixth day; in *Cyanothece* sp. ATCC 51142 the highest amplitude for treated sample is observed in the the second day of treatment and for control sample is observed in the sixth day. In those days the potential photochemical efficiency of acceptor side of PSII occurred with a better performance.

Figures 6; 7; 8; 9 - panels B, D (without DCMU) shows the normalized fluorescence intensity which indicates the electron transport. In the cyanobacteria *Synechocystis* sp. PCC 6803 there are no significantly differences during treatment between treated and untreated samples although it can be seen little differences in electron transport, so at treated samples electron transport occurs faster in the first three days but starting with day four electron transport is slowed down and at control sample electron transport is slowed down starting with day six. In *Anabaena variabilis* sp. ATCC 29413 5% CO_2 treatment has a positive effect by stimulating electron transport compared to the control sample. At *Synechococcus* sp. PCC 7002 graphs are indicating a fast electron transfer under 5% CO_2 treatment. On *Cyanothece* sp. ATCC 51142 treatment has a negative effect by slowing electron transport.

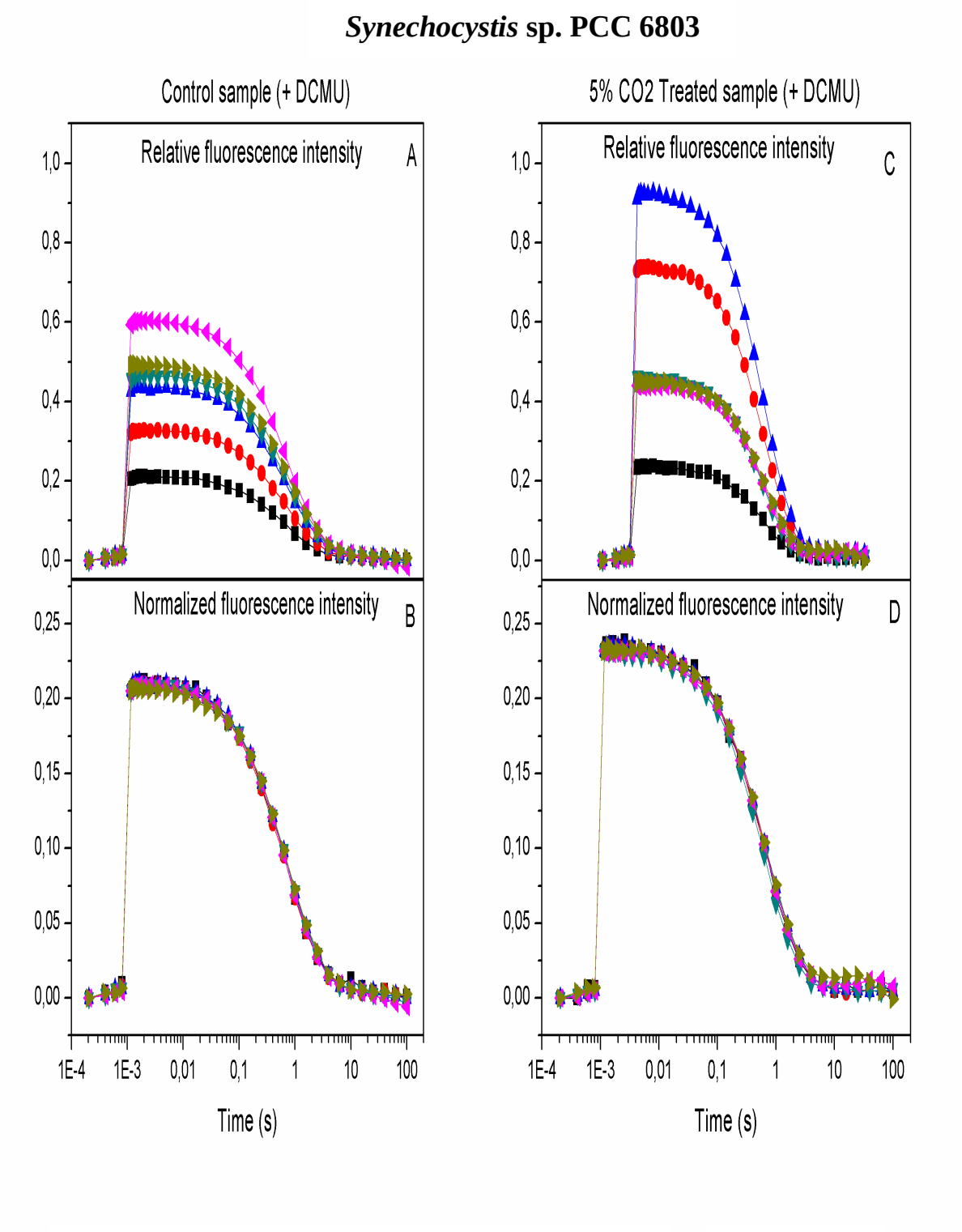


Fig. 2. Effect of 5% CO2 concentration on photosynthetic electron transport chain in *Synechocystis* sp. PCC 6803. This figure show the results with DCMU. Panels A and B are for the untreated cells; panels C and D are for the treated cells. Panels A and C show curves shift to „0" and panel B and D show curves normalized to the same amplitude.
Symbols: black squares day 1; red circles day 2; blue up - pointing triangle day 3; green down - pointing triangle day 4; pink left - pointing triangle day 5; olive right - pointing triangle day 6.

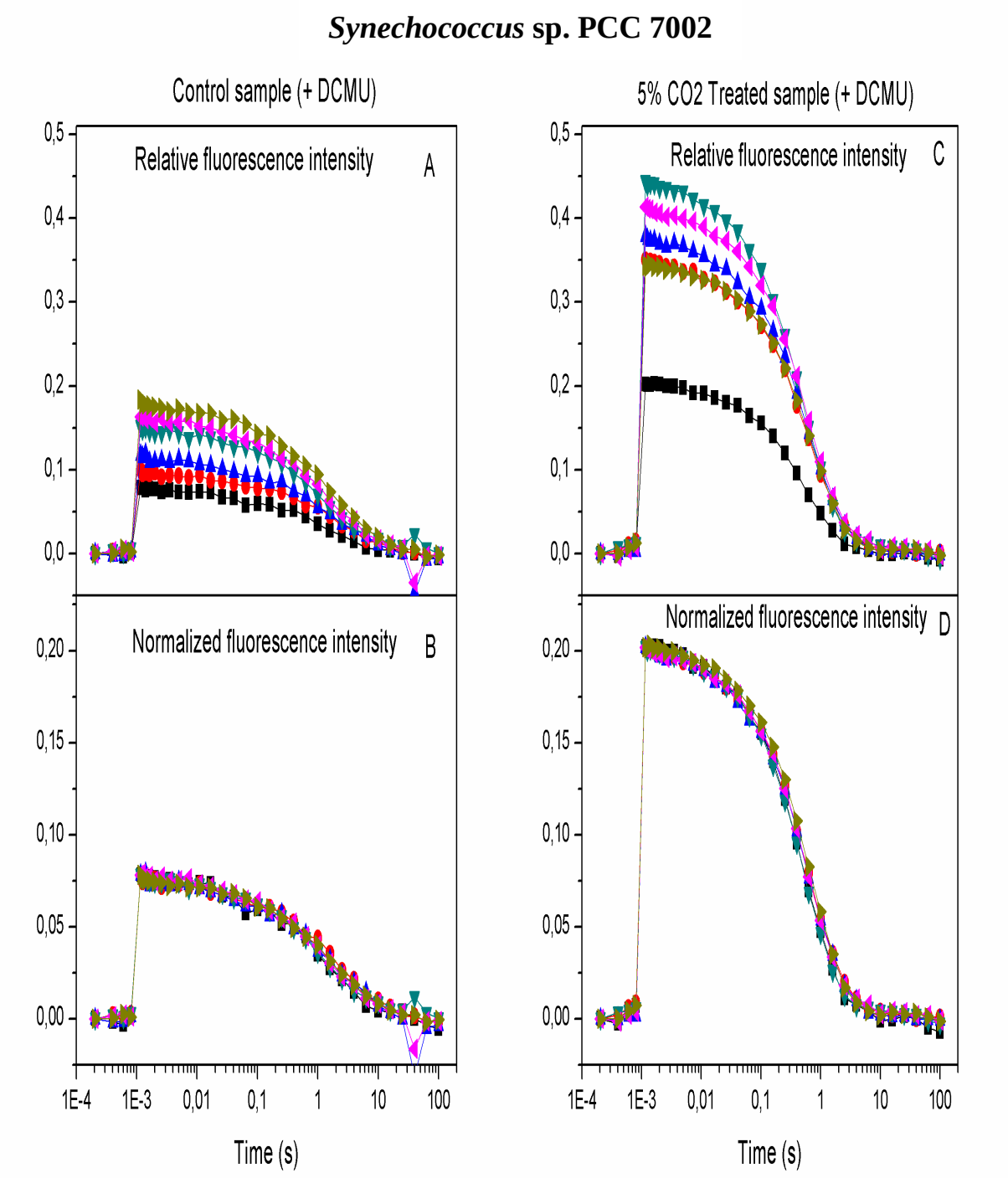


Fig. 3. Effect of 5% CO2 concentration on photosynthetic electron transport chain in *Synechococcus* sp. PCC 7002. This figure show the results with DCMU. Panels A and B are for the untreated cells; panels C and D are for the treated cells. Panels A and C show curves shift to „0" and panel B and D show curves normalized to the same amplitude.
Symbols: black squares day 1; red circles day 2; blue up - pointing triangle day 3; green down - pointing triangle day 4; pink left - pointing triangle day 5; olive right - pointing triangle day 6.

Anabaena variabilis sp. ATCC 29413

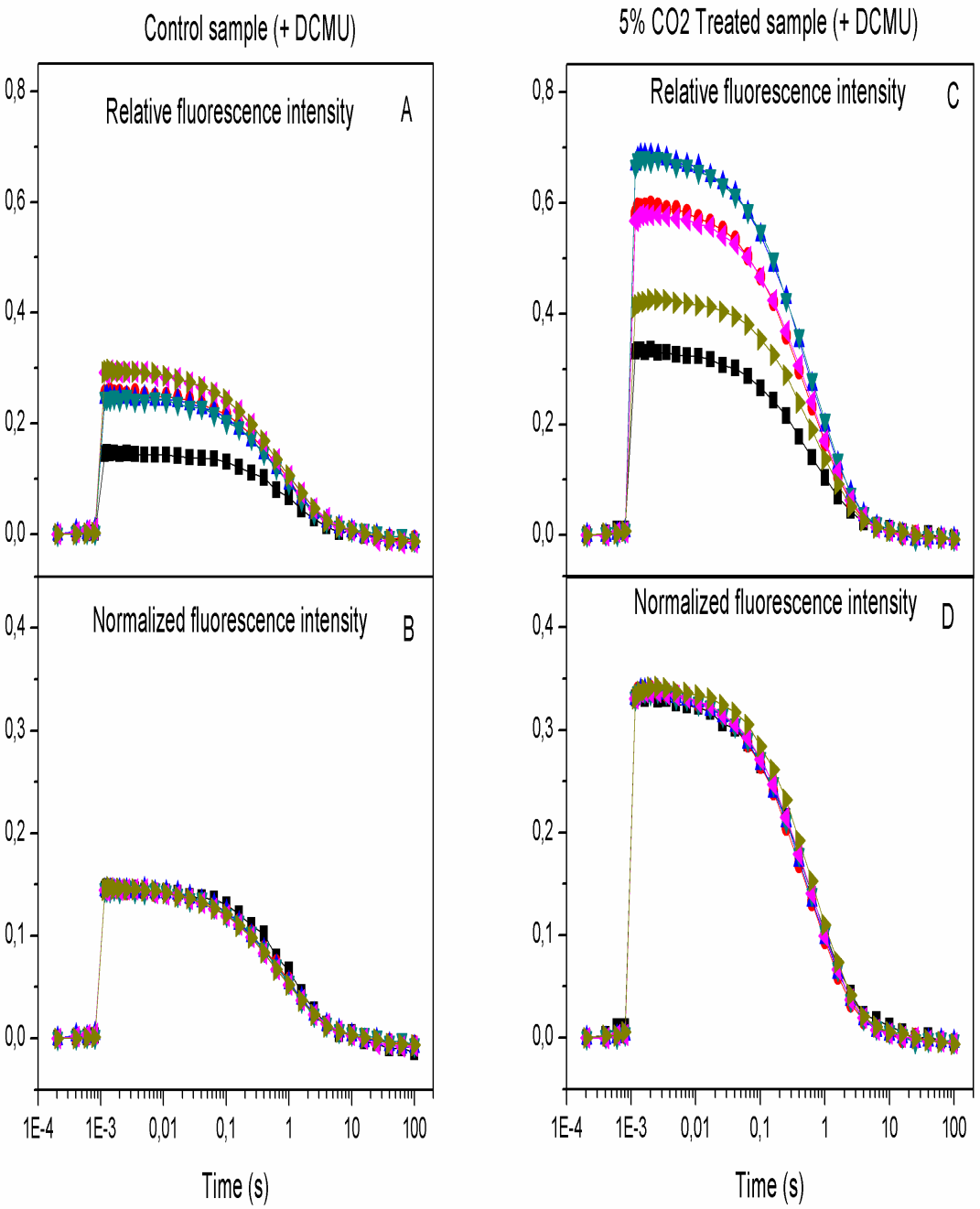


Fig. 4. Effect of 5% CO2 concentration on photosynthetic electron transport chain in *Anabaena variabilis* sp. ATCC 29413. This figure show the results with DCMU. Panels A and B are for the untreated cells; panels C and D are for the treated cells. Panels A and C show curves shift to „0" and panel B and D show curves normalized to the same amplitude.
Symbols: black squares day 1; red circles day 2; blue up - pointing triangle day 3; green down - pointing triangle day 4; pink left - pointing triangle day 5; olive right - pointing triangle day 6.

Cyanothece sp. ATCC 51142

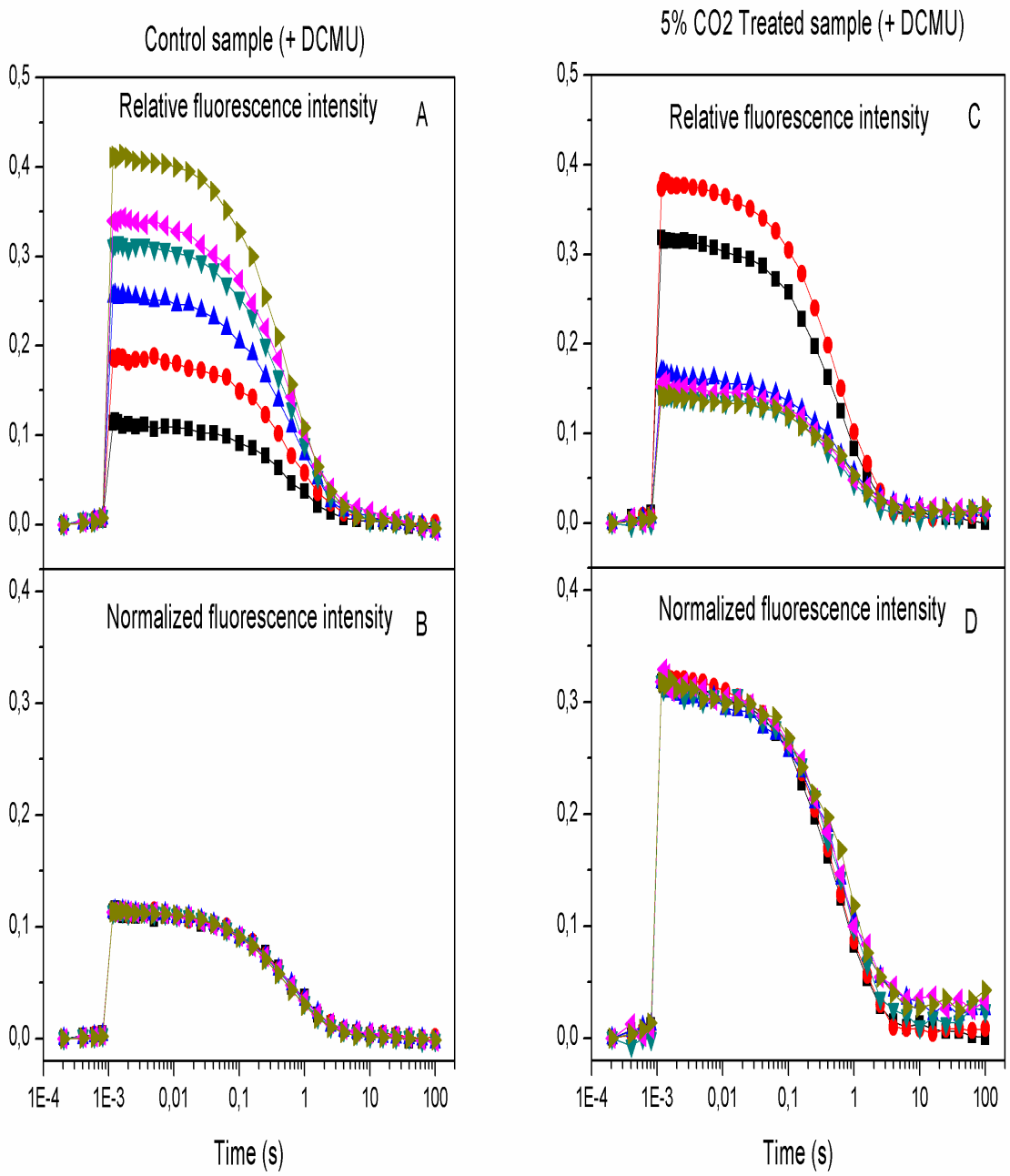


Fig. 5. Effect of 5% CO2 concentration on photosynthetic electron transport chain in *Cyanothece* sp. ATCC 51142. This figure show the results with DCMU. Panels A and B are for the untreated cells; panels C and D are for the treated cells. Panels A and C show curves shift to „0" and panel B and D show curves normalized to the same amplitude.
Symbols: black squares day 1; red circles day 2; blue up - pointing triangle day 3; green down - pointing triangle day 4; pink left - pointing triangle day 5; olive right - pointing triangle day 6.

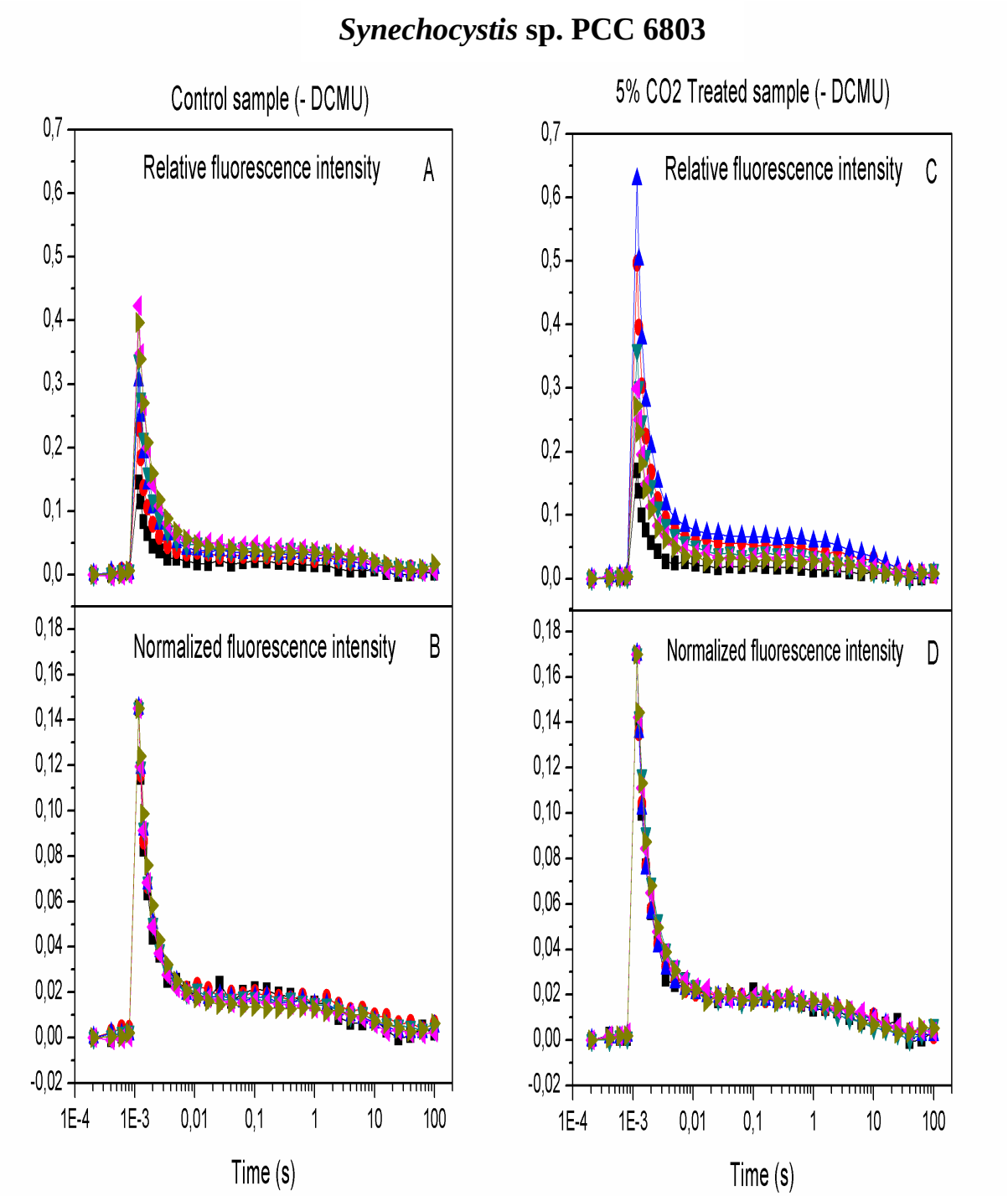


Fig. 6. Effect of 5% CO2 concentration on photosynthetic electron transport chain in *Synechocystis* sp. PCC 6803. This figure show the results without DCMU addition. Panels A and B are for the untreated cells; panels C and D are for the treated cells. Panels A and C show curves shift to „0" and panel B and D show curves normalized to the same amplitude.
Symbols: black squares day 1; red circles day 2; blue up - pointing triangle day 3; green down - pointing triangle day 4; pink left - pointing triangle day 5; olive right - pointing triangle day 6.

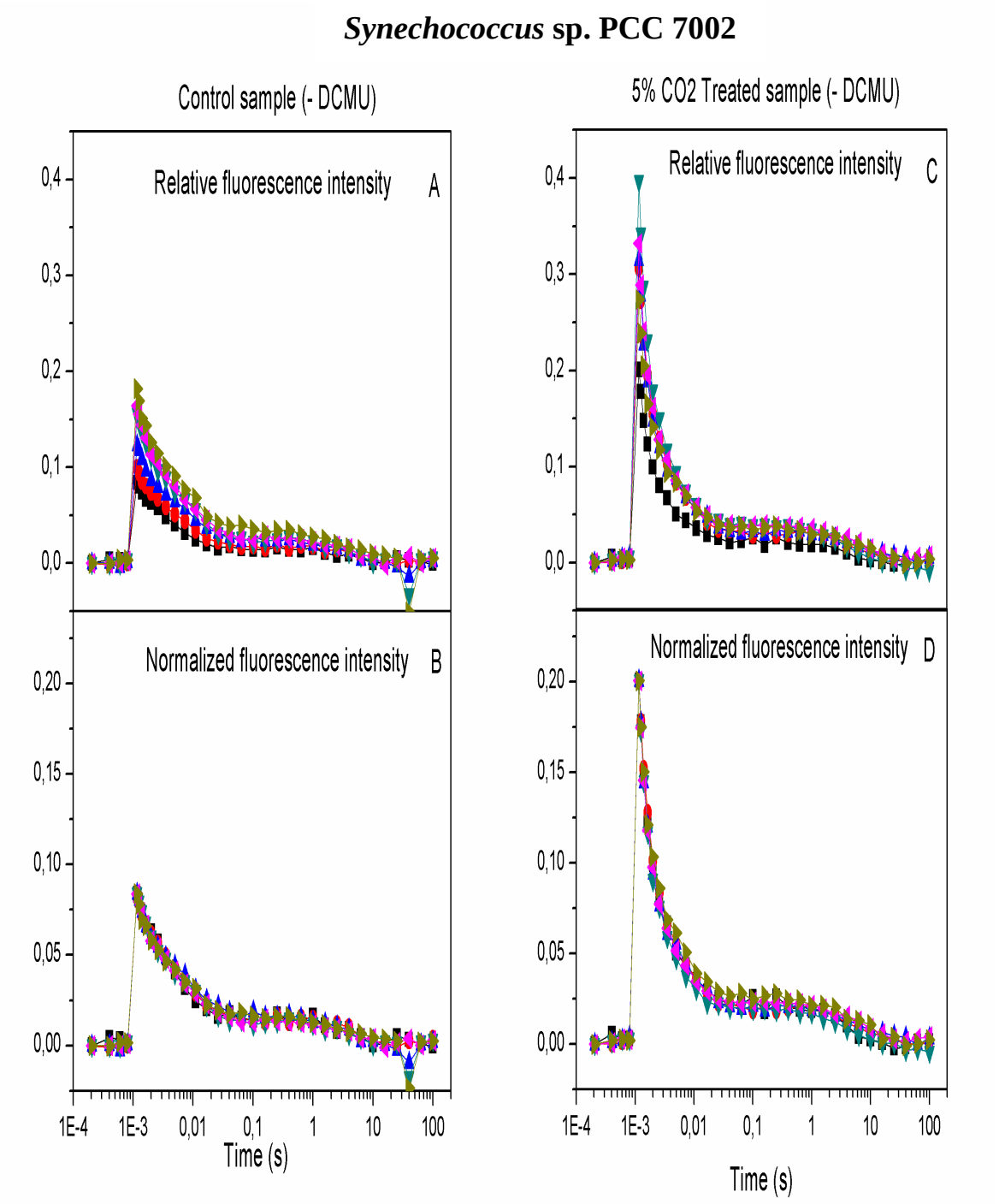


Fig. 7. Effect of 5% CO2 concentration on photosynthetic electron transport chain in *Synechococcus* sp. PCC 7002. This figure show the results without DCMU addition. Panels A and B are for the untreated cells; panels C and D are for the treated cells. Panels A and C show curves shift to „0" and panel B and D show curves normalized to the same amplitude.
Symbols: black squares day 1; red circles day 2; blue up - pointing triangle day 3; green down - pointing triangle day 4; pink left - pointing triangle day 5; olive right - pointing triangle day 6.

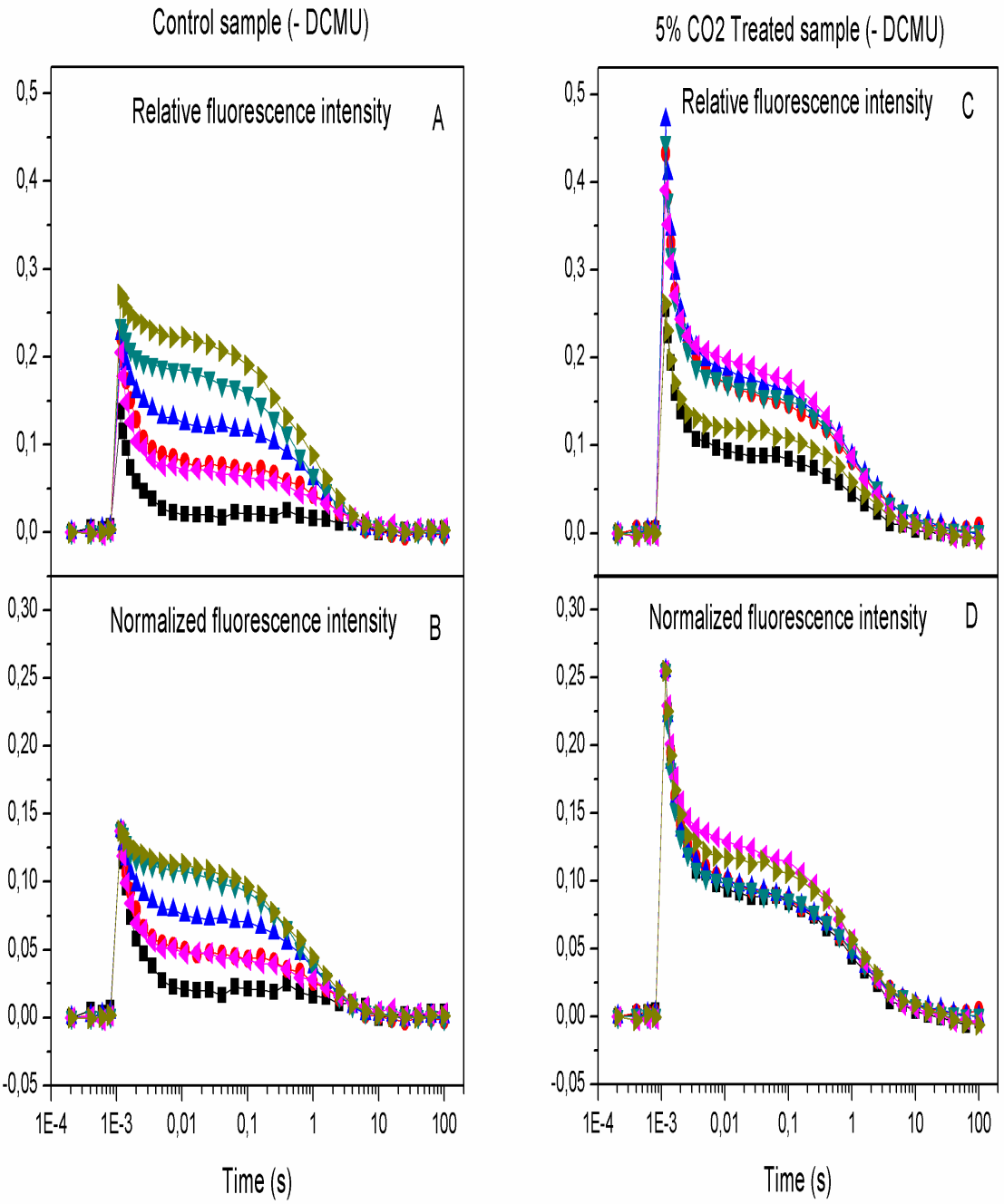


Fig. 8. Effect of 5% CO2 concentration on photosynthetic electron transport chain in *Anabaena variabilis* sp. ATCC 29413. This figure show the results without DCMU addition. Panels A and B are for the untreated cells; panels C and D are for the treated cells. Panels A and C show curves shift to „0" and panel B and D show curves normalized to the same amplitude.
Symbols: black squares day 1; red circles day 2; blue up - pointing triangle day 3; green down - pointing triangle day 4; pink left - pointing triangle day 5; olive right - pointing triangle day 6.

Cyanothece sp. ATCC 51142

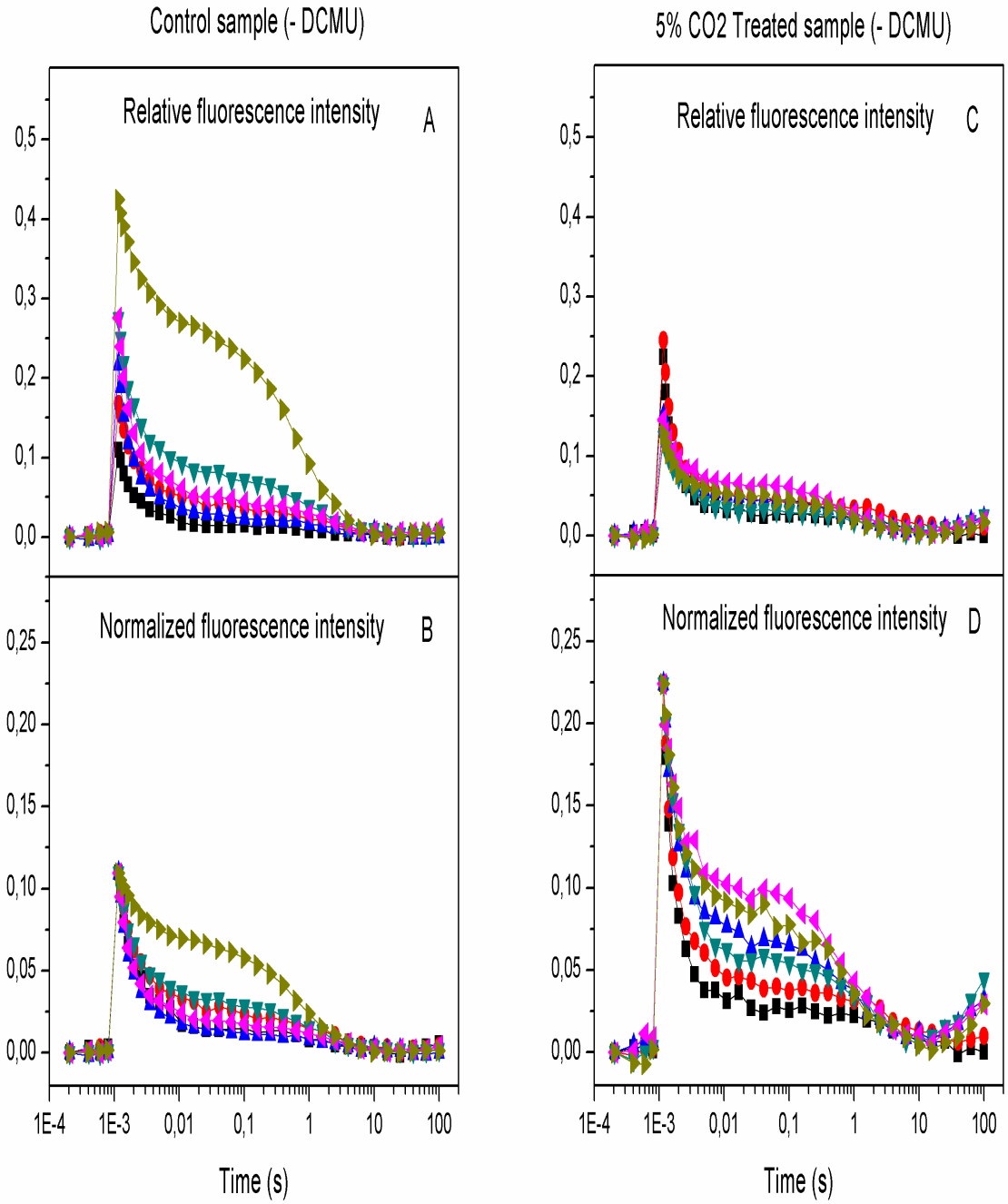


Fig. 9. Effect of 5% CO2 concentration on photosynthetic electron transport chain in *Cyanothece* sp. ATCC 51142. This figure show the results without DCMU addition. Panels A and B are for the untreated cells; panels C and D are for the treated cells. Panels A and C show curves shift to „0" and panel B and D show curves normalized to the same amplitude.
Symbols: black squares day 1; red circles day 2; blue up - pointing triangle day 3; green down - pointing triangle day 4; pink left - pointing triangle day 5; olive right - pointing triangle day 6.

Figures 10; 11 - shows the variation of maximum fluorescence (Fmax) under the action of 5% CO_2 treatment in presence and absence of DCMU. Fmax is the number of active reaction centers of photosystem II at the measurement time.

During treatment in *Synechocystis* sp. PCC 6803 there is an inhibition of active reaction centers at a rate of 50% for treated cells at samples with and without DCMU and about 5% for untreated cells at samples without DCMU and about 20% for samples with DCMU.

In *Anabaena variabilis* sp. ATCC 29413 there is an inhibition of active reaction centers at a rate of about 40 % at samples with and without DCMU and there is no inhibited centers at control sample with DCMU but at sample without DCMU (the acceptor side) there were a decrease in active reaction centers in day four but from day five photosynthetic production tends to recover showing the installation of repair mechanisms.

The proportion of inhibited active reaction centers in Synechococcus sp is about 30 % at treated samples in presence and absence of DCMU and there is no inhibited centers at control sample with and without DCMU.

At *Cyanothece* sp. ATCC 51142 treated samples there is an inhibition of active reaction centers at a rate of about 50% at samples with and without DCMU and there is no inhibited centers at control sample with and without DCMU.

From **figures 12; 13** it can be seen that by 5% CO_2 treatment cells reported positive growth rates both at 580 nm where chlorophyll absorbs and 730 nm where phycobilisomes absorbs to all four cyanobacteria studied strains. Chlorophyll register higher absorbance values than phycobilisomes therefore chlorophyll absorbance occurred with a better performance.

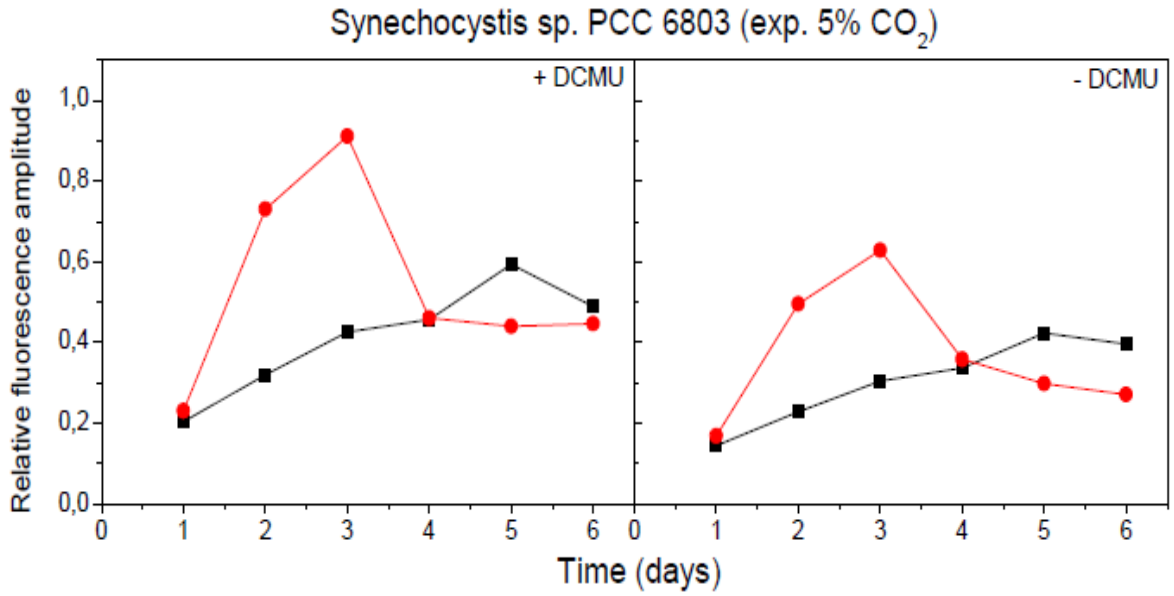


Fig. 10. Effect of 5% CO2 concentration on maximum fluorescence amplitudes in the presence and absence of DCMU.
Symbols: black squares control (- CO2); red circles treated cells (+ CO2).

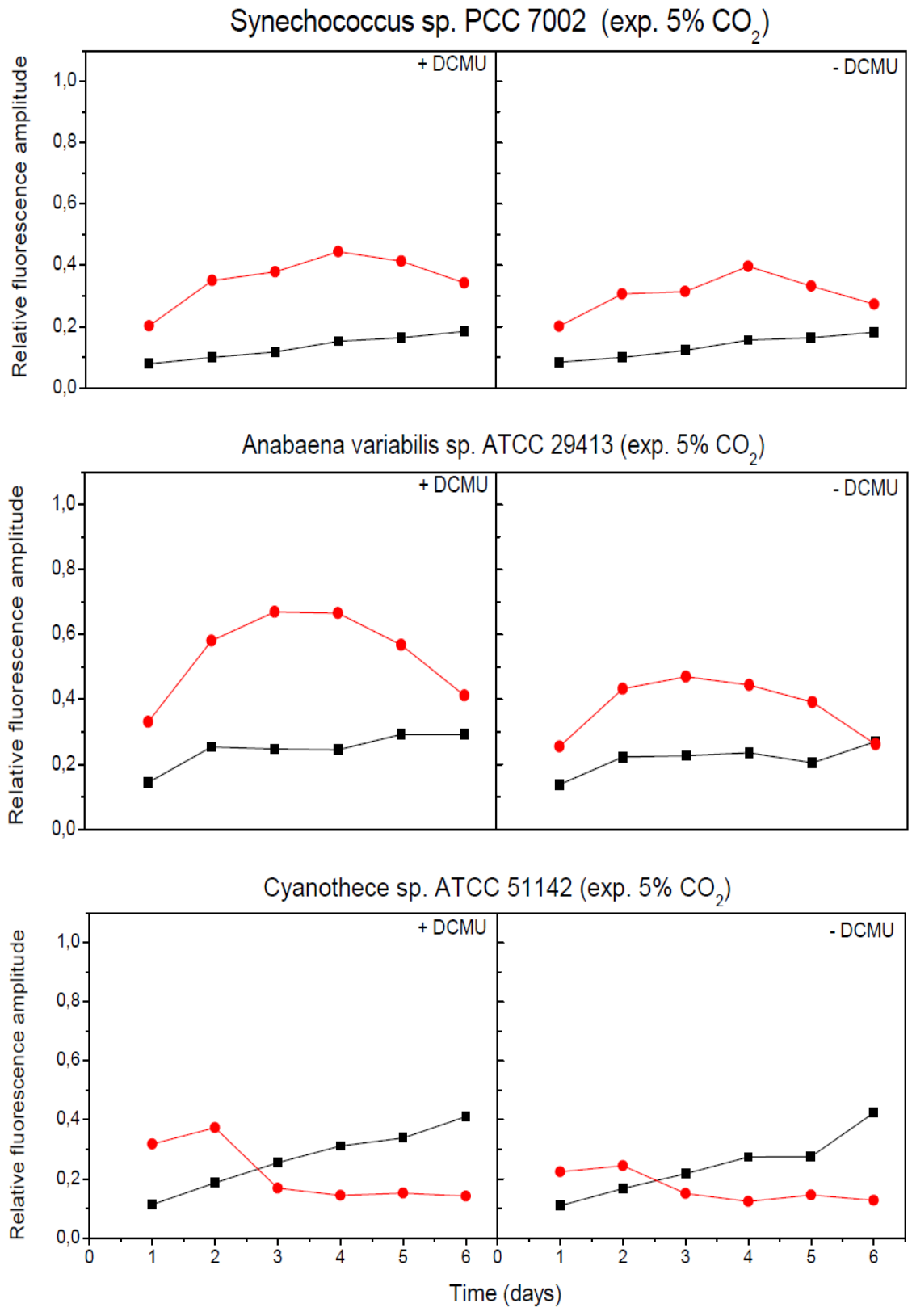


Fig. 11. Effect of 5% CO2 concentration on maximum fluorescence amplitudes in the presence and absence of DCMU.
Symbols: black squares control (- CO2); red circles treated cells (+ CO2).

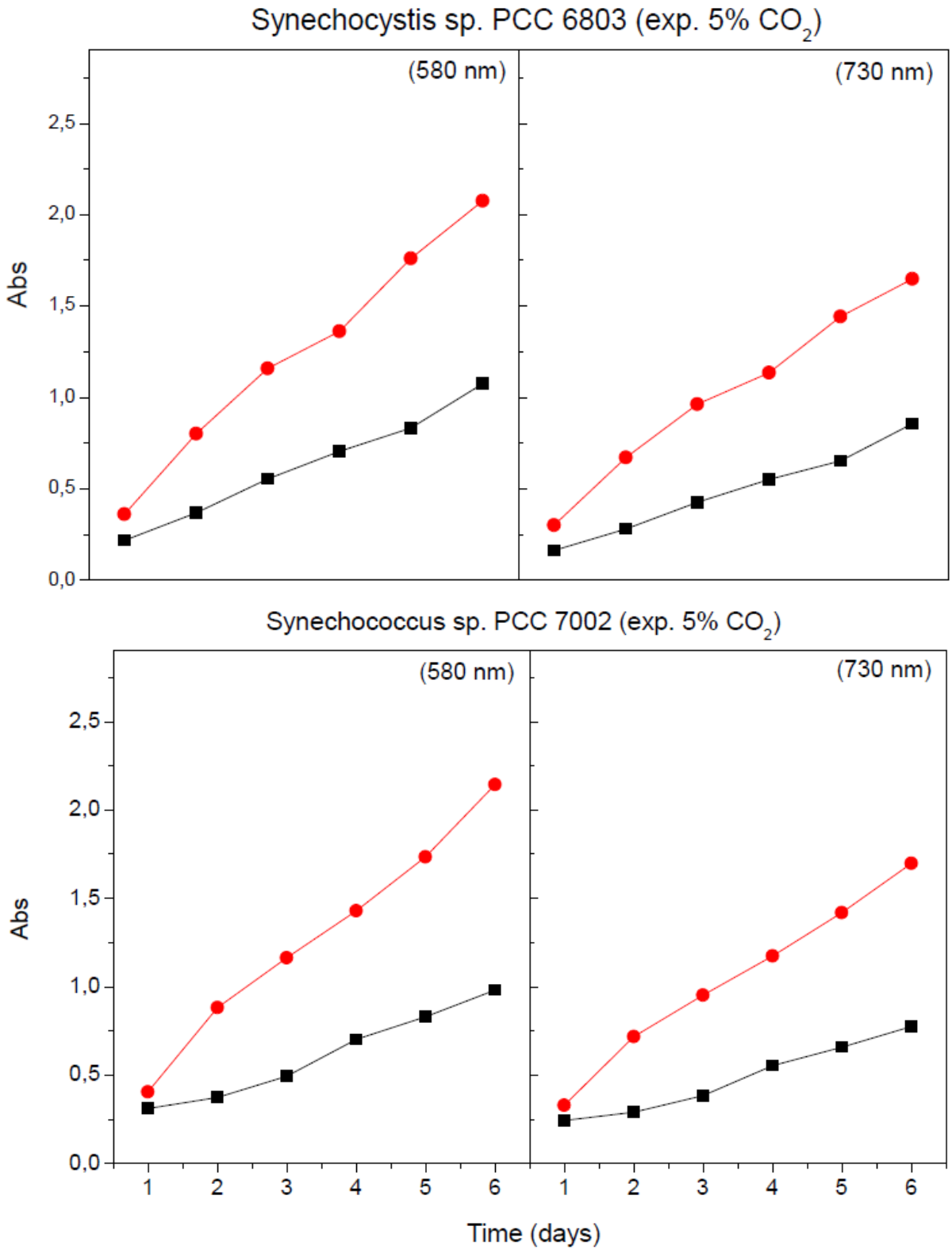


Fig. 12. Growth curves obtained on O.D. (λ=580 nm and 730 nm) in the presence and absence of CO2 (5%). Symbols: black squares control (- CO2); red circles treated cells (+ CO2).

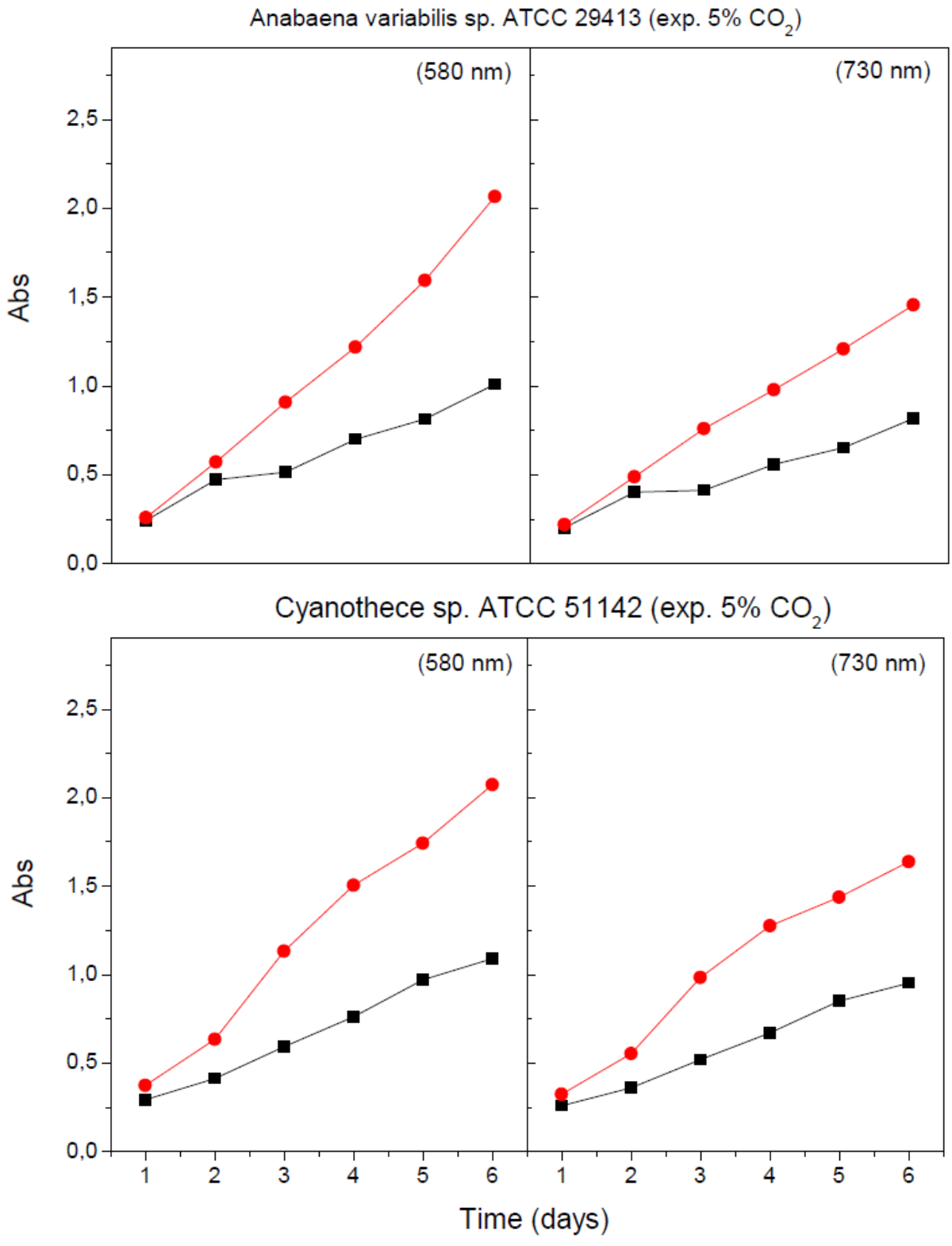


Fig. 13. Growth curves obtained on O.D. (λ=580 nm and 730 nm) in the presence and absence of CO2 (5%). Symbols: black squares control (- CO2); red circles treated cells (+ CO2).

Conclusions

1. In the cyanobacteria *Synechocystis* sp. PCC 6803 there are no significantly differences in electron transport between treated and untreated samples**.**
2. In *Anabaena variabilis* sp. ATCC 29413 and *Synechococcus* sp. PCC 7002 CO_2 treatment has a positive effect by stimulating electron transport compared to the control sample. On *Cyanothece* sp. ATCC 51142 treatment has a negative effect by slowing electron transport.
3. Although 5% CO_2 treatment drove partial reaction centre closure - no inhibition of the potential photochemical efficiency of PS II occurred over the course of the treatment.
4. The donor side of photosystems has not undergone major changes during treatment.
5. Fuorescence intensity shows that the addition of DCMU to the samples caused an increase of amplitude compared to samples without DCMU.

„Acknowledgments

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