Report of a thesis

submitted at the Faculty of Science, University of South Bohemia

❑ supervisor's report	☑ opponent's report
❑ bachelor thesis	☑ master thesis

Author: Anežka Terezie Míšková
Thesis Title: Comparison of photophysiological characteristics of two strains of the cyanobacterium *Trichodesmium* in relation to nitrogen fixation
Study program:
Year of submission: 2022

Opponent: Dušan Lazár
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Scientific/professional quality:
❑ excellent ☑ very good ❑ average ❑ below average ❑ unsatisfactory

Factual errors:
❑ almost none ☑ a reasonable number ❑ frequent minor errors ❑ serious

Results:
☑ original ❑ original and adopted ❑ non-trivial compilation ❑ cited from the literature
❑ copied

Extent of the work:
❑ large ☑ standard ❑ sufficient ❑ insufficient

Graphic, linguistic and formal quality:
❑ excellent ❑ very good ☑ average ❑ below average ❑ unsatisfactory

Typographic errors:
❑ almost none ☑ a reasonable number ❑ numerous

Overall quality of the thesis:
❑ excellent ☑ very good ❑ average ❑ below average ❑ unsatisfactory

Verbal evaluation, comments of the opponent:

The master thesis of the candidate, Anežka Terezie Míšková, aims to check correctness of original proposal by Hendrik Küpper and co-workers that nitrogen fixation by cyanobacteria is related to uncoupling of phycobilisome from PSII. To do this, two strains of marine filamentous diazotroph cyanobacterium *Trichodesmium erythraeum* were studied under diazotrophic (standard growth condition, nitrogen fixation occurs) and non-diazothropic (growth in nitrate-containing media, which inhibits nitrogen fixation) conditions. Fluorescence lifetime measurements at bulk level, using time-resolved fluorescence spectroscopy, and at single-cell level, using fluorescence-lifetime imaging microscopy, were applied together with staining of the cells by the Lugol's solution to determine nitrogen fixation. DCMU treatment of the cells and high light stress have been also used in the study.

From formal point of view, the thesis can be considered as a standard type with an introduction (includes sections 1 - 4), materials and methods, results, discussion, and conclusion. The introductory parts includes description of photosynthesis, nitrogen fixation, the experimental object, i.e., cyanobacterium *Trichodesmium erythraeum*, and fluorescence. The cultivation of the cyanobacterial cells, extraction of the pigments, measurement and evaluation of the lifetimes by the two fluorescence methods, and Lugol staining method are described in the materials and methods section. Then the results are described, which is followed by their discussion with respect to present literature knowledge. The thesis is finished by the conclusion.

The outcome of the thesis is that, in fact, it confirms the original proposal by Küpper and co-workers.

I have several comments to the thesis text listed below, which mostly deals with possible mistakes in the text (it is not necessary the candidate answer these comments):

1) In section 1.2, as well as in the title of section 1.2.2, the second phase of photosynthesis is called as dark reactions, which in not used in present literature, since the phase depends on light indirectly via its substrates, ATP and NADPH. Thus, the term carbon reactions is used in the literature / should be preferred for the second phase of photosynthesis.

2) In section 1.2.1 the term non-cyclic electron transfer is used, which is not a common name of the process and the term linear electron transport is rather used.

3) Figure 4.1. – even if the figure is a part of the section devoted to fluorescence, there is no mention about fluorescence in the figure legend, which is strange.

4) Section 5.4.1 - diazotrophic and nondiazotrophic conditions are mentioned in the text but it is not explained what the conditions are.

5) Section 6.1, the first sentence (and also the first sentence on page 31) states that a figure show a spectra of diazotrophic and non-diazotrophic filaments of a cyanobacteria. It would be better to write "spectra of the cyanobacerium in the absence or presence of NO_3^-.

6) Page 29, second paragraph from the bottom – statement "... lifetime of PBS is longer ..." is not correct. Probably lifetime of excitation on PBS should be used.

7) According to the text to Fig. 6.8C a peak/wawe at about 570 and 650 nm of DAS for lifetime of about 1 ns is related to free PBS. Then, the amplitude of the 1 ns DAS at 570 nm is about 6 times higher (compare the black curves in Figs. 6.10 B and D at about 570 nm) not two times higher as mentioned in the last sentence on page 29.

8) Legend to figure 6.1 includes a mistake in "not NO_3^- consuming"

9) A very small p-value of a statistical test is referred as $p<0.001$ and not, e.g., by $8.43\ 10^{-8}$ as used in the first paragraph on page 40.

10) The last sentence in the second paragraph on page 46 is not exact since the lifetime is of fluorescence and it is fluorescence of free PE.

11) Next paragraph: the statement "DCMU, a specific inhibitor of photosynthesis, replaces the plastoquinone binding site of Q*B* of PSII RC." is not correct since DCMU does not replace the binding site but plastoquinone in the site.

12) 7.1.2, the last paragraph on page 47 and the same sentence in the conclusions "The fact that there are more filaments with long lifetime in N_2-fixers suggests that they are more susceptible to stress." is not a good statement, in my opinion, since different reasons for PBS uncoupling are mixed.

13) Page 48, first paragraph – it is not clear if chlorophyll content or lifetime of chlorophyll florescence is mentioned in the statement "it is relatively small in chlorophyll". Further, in the same place, "appearance of something" sound non-scientific.

14) Section 7.2.2 – "than" is missing in "…which is the opposite trend expected …"

15) Section 7.3 – there is something wrong in the text "the bulk pigment composition the comparison of the PUB:PEB ratio in both strains"

16) Generally, a legends to tables are usually written above, not below the tables.

Questions to the defence and suggestions for a discussion:

Apart to the issues mentioned above I have some questions and I ask the candidate to answer them. The questions are as follows:

1) In section 1.2.1 PSI and PSII are referred to as reaction centres. Is that correct? And similarly, is correct to name P680 and P700 in Fig. 1.1a as reaction centres?

2) Below Eq. 4.1 is written that "The value of the rate constant k (its reciprocal value is the time constant, τ) is determined by probabilities (rates) of all the possible deexcitation pathways" Is it correct to consider probability as rate?

3) Above Eq. 4.6 is mentioned that "the signal emitted from the sample is detected only from a certain direction, not from all the photons emitted from the sample." Might it affect the measured lifetime?

4) In the last sentence on page 20 is written: "It should be noted that the utility of the fitting approach to data analysis is limited by the number of photons recorded." What this sentence warm about?

5) Section 5.3.1 mentions average radiant power (of 16 μW). Can it be reported in micromoles of photons per square meter and per second? The point is, whether the energy of the laser is not too high so that is can close or even destroy the reaction centres.

6) Section 5.4. – Is it correct to write that glycogen is a form of starch?

7) The Fig. 6.8 shows DAS based on global lifetime analysis and origin of particular spectrum with given lifetime is judged based on the shape of the spectrum. Also the lifetimes are apparent lifetimes, which are not identical with time constants of particular molecular processes. On the other hand, an improved procedure of data analysis is the target analysis leading to species associated spectra, where a component of a model (also called state variable) describing the decay is meant by a species. Would it be helpful for the study to use the improved data analysis? If yes,

what additional information you could get from the improved analysis? In connection to that, for example, the DASs presented in Fig. 6.8B and 6.8C are qualitatively similar and the improved analysis might lead to a more exact conclusion, especially when presence of the three (uncoupled) PBSs is a key for the hypothesis tested in this thesis.

8) Three methods were used in the thesis: time resolved fluorescence spectroscopy, fluorescence lifetime imaging microscopy, and Lugol staining. However, the thesis does not mention, which of the experimental measurements have been done by the author of the thesis herself. Based on the acknowledgement, I guess that it was Lugol staining and partly the fluorescence lifetime imaging microscopy. Is that correct?

☑ **I recommend**
❑ **I do not recommend**
to accept the thesis as a master thesis.

I suggest that the thesis be graded:
❑ excellent ☑ very good ❑ good ❑ failed

Place, date and signature of the opponent: January 6, 2023,