



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: MSc. Anushree BACHHAR	First name(s), surname, titles of supervisor: Mgr. Jiří Jablonský, Ph.D.
Title of PhD thesis: Role of isoenzymes in metabolic regulation of cyanobacteria	

REVIEWER:

Surname: Lazár	Institution: Univerzita Palackého v Olomouci, Přírodovědecká fakulta, Katedra biofyziky
Name: Dušan	
Titles: prof., RNDr., Ph.D.	E-mail: lazard@seznam.cz
Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: biophysics of photosynthesis, mathematical modelling

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (extent ¼ – ½ page):

The PhD thesis of the PhD candidate, Anushree Bachhar, deals with the role of isozymes in carbon metabolism of cyanobacterium *Synechocystis* sp. PCC 6803 under different conditions. Since the exploration is difficult to be realized in the wet laboratory, the Ph.D. candidate applied in silico approach, i.e., a mathematical modelling was used. This is a modern approach, which enables to get new results, and it is in line with what is used worldwide.

The thesis is based on two papers published in high-quality journals (Scientific Reports and Frontiers in Microbiology) with good impact factors. The PhD candidate is the first author in both the papers and the only other co-author is her supervisor, showing that all the work was done in their laboratory, and also showing the advantage of the in silico approach; not many dedicated experimentalists and expensive equipment are needed to get good results. The number of the papers and their quality is in agreement with what is expected from a successful PhD candidate in other Czech universities as well as abroad.

Having in mind that cyanobacterium *Synechocystis* sp. PCC 6803 is used as a host organism to synthesise various products, and considering that the papers present new results on metabolism of the cyanobacterium, the impact of this PhD thesis is expected to be high.



Elaboration of the PhD thesis, objectives of the work and deliverables Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (**extent ¼ – ½ page**):

The thesis has a modern form, where the results are not described in the thesis text but the published papers, which are part of the thesis, are used for this purpose. The thesis consists from 4 chapters. In the first chapter, general information about cyanobacteria is provided as well as a detailed description of known isozymes involved in the central carbon metabolism of *Synechocystis* sp. PCC 6803. Chapter two and three are reprints of the two published papers mentioned above. The last chapter provides general discussion/description of the used in silico tools, the used theoretical model and a short conclusion. However, from my point of view, description of the in silico tools and of the model, which is in fact the core of this PhD thesis, should be provided before presentation of the results.

In the first paper, which results are presented as chapter 2, the role of phosphoketolase pathway/isozymes was explored, since the pathway is thought to be inactive under ambient autotrophic CO₂ conditions. In the second paper, which results are presented as chapter 3, the role of recently established Entner-Doudoroff pathway of glycolysis in *Synechocystis* sp. PCC 6803 was explored in detail. Since the topics of the papers directly cover the topic of the thesis, the objectives of the thesis were fulfilled.

OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

Since the thesis is based on two papers published in good journals with impact factors, where the PhD candidate was the first author, scientific quality of the thesis is high. As for formal quality, I have found some minor problems with English language (I can provide a list of the problems upon request).

Further, although a scheme of the model used in the second paper is presented in the thesis, more details on the modelling should be provided. For example, initial concentrations of the reactants are not mentioned. Also units of the values of the parameters presented in Table 4.1 are missing.

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

- 1) Why there is so many isozymes in cyanobacteria?
- 2) Is it correct to consider pentose phosphate pathway and phosphoketolase pathway as types of glycolysis as used in the thesis?
- 3) What is the mechanism of FBA suppression by light pulses?
- 4) The thesis mentions that when performing the modelling, a single set of kinetic parameters, which could explain/fit all the growth condition, is identified. My question is whether the values of the fitted parameters obtained after the fitting are checked for their



correctness with respect to the values known from the literature. And what would be the workflow if the fitted values are very different from the values known from the literature?

5) The thesis and particular papers describe changes in fluxes through particular reactions based on the in silico experiments. Is it possible to verify these results by experimental measurements?

6) The PhD candidate mentions at the end of the thesis that she wants to include tricarboxylic acid cycle and amino acid metabolism in the future version of the model. My question is whether the conclusions based on model used in the thesis, which does not include these reactions, are still valid or what changes in the results can be expected when the mentioned reactions are included in the model.

At the end, I conclude that the PhD thesis of Anushree Bachhar meets common required criteria for a PhD thesis and I recommend the thesis for its defence.

Your sincerely,
prof. Dušan Lazár
Department of Biophysics
Faculty of Science
Palacký University
Olomouc, Czech Republic

FINAL RECOMMENDATION

- ☒ **PhD thesis can be recommended for defence**
- ☐ **PhD thesis can be recommended for defence with reservations**
- ☐ **PhD thesis cannot be recommended for defence**

December 9, 2022, Olomouc
Date and place

Dušan Lazár
Name and signature