Reviewer's Dissertation Thesis Report

Charles University **Faculty of Science**	
Study Program	Ecology
Student	***Antonín Střížek***
Dissertation Thesis Title	Glycerolipids and carotenoids in microalgae: the implications in ecophysiology and applied phycology
Reviewer	**Luísa Barreira**
Reviewer's Institution	**University of Algarve, Faculty of Sciences and Technology**

Report

The thesis of Mgr. Antonín Střížek is a cumulative dissertation that contains five published peer-reviewed articles and one submitted publication, which is currently under review, and a manuscript draft. The candidate is the first author of one of the published papers and of the manuscripts submitted and in draft.

The thesis consists of two documents, one containing: the Introduction, where the candidate describes the theoretical background, the Motivation, Aims and Approaches used for the thesis, a summary of the main Results, Author Contribution Statements and the References cited, and a second document containing the published and submitted publications, and the manuscript draft as well as several Appendixes describing the development of a prototype, samples and verified technologies. The latter are written in Czech and could not be evaluated by the Reviewer.

The PhD thesis does not follow the common structure of a Monograph, but the candidate made an effort to summarize the most important results in the main document submitted. The thesis is generally well written in clear and concise manner. The language is comprehensive and coherent, and no major errors or inaccuracies were found.

Context and Scope of the Thesis

Microalgae are considered to be a sustainable source of several compounds in which polyunsaturated fatty acids (PUFA) and carotenoid pigments are included. These compounds have high market value because of its functional importance for human

health but also for feed. However, although over 30,000 microalgae species have been identified, only a few are currently produced at industrial scale and the production of the compounds mentioned above by microalgae remains a niche market. It is therefore crucial to continue the effort of isolation of new strains, to perform a thorough chemical characterization and establish optimum cultivation conditions to improve the productivity of the target compounds and decrease production costs.
The work reported in this thesis aims to identify and characterize new microalgal strains as sources of PUFA and fucoxanthin and develop methods to improve their productivity. For this, different parameters were tested, including light intensity, temperature, life strategy (autotrophy and mixotrophy), and salinity.

Specific comments and questions
Questions and comments will be focused on the papers in which the candidate is first author. The following points will be addressed during the discussion with the candidate.

1. The large majority of DHA supplements in the market comes from fish oil and those of microbial origin are generally produced by *Schizochytrium* sp.. How do the microalgal strains studied compare with these sources? Can any of the studied species, or other known to be good DHA producers, compete with the current sources, now or in the future?

Chapter 1

2. *Hibberdia magna* was presented as a concomitant producer of fucoxanthin and PUFA. However, the conditions to obtain both are divergent: low light and moderate temperatures for fucoxanthin and high light and low temperature for PUFA. Does this mean that we must favour one in detriment of the other? Is there an alternative?
3. How can the conditions tested in this study be implemented in an outdoor system?

Chapter 2

4. *Chlorochromonas danica* was only grown in mixotrophic conditions? Different light intensities but always with an organic carbon source? Would it be expected that the fucoxanthin content be the same in autotrophic cultivation?
5. The concept of biorefinery is several times mentioned but a scheme for the valorisation of both PUFA and fucoxanthin is never proposed. Could we obtain both products in a cascade approach?
6. How could the polysaccharides produced by *Hibberdia magna* participate in a biorefinery concept?

Chapter 3

7. Figure 2A: Biomass obtained has no error bars. Were replicates of the growth performed? Figure 2B: Values presented are the average and standard deviation of all polar strains, temperate, etc?

8. Why grow all strains at 17ºC during the 1st growth stage?
9. Was it unexpected to find that polar strains performed better at lower temperatures?

Appendix I

The development of this prototype was seen with great interest as temperature control is indeed one of the major bottlenecks of these types of experimentation with microalgae.

10. In addition to the temperature and light control already developed, a CO_2 injection system controlled by pH with be a nice addition to this prototype.
11. Regarding the colour LEDs, how precise is the wavelength control? Spectral light quality, in addition to light intensity, has been pointed as promoters of biomass and specific compounds productivity. Is it possible to apply different ratios of specific wavelengths?

In summary, the PhD thesis represents original and high level of scientific work. The conducted experiments are well organized, and methods are clearly described. The results are well presented, and the explanations are reasonable as well as suitable and focused on the relevant topics. Mgr. Antonín Střížek shows in his thesis that *Hibbardia magna* is a suitable candidate for industrial production of biomass containing PUFA and fucoxanthin although it is not clear if the concentrations obtained at latger scale are the result of the implementation of the induction methods described in the thesis.
I would like also to congratulate Mgr. Antonín Střížek for the successful development of the prototype for microalgal experimentation which reveals a very creative and strong hands-on character, much needed in research in biotechnology.

In my opinion, the thesis fulfils the requirements for obtaining a PhD degree.

Faro, 14th March 2024

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