



Review of Ph.D. Thesis

Title: Growth and division of microalgae in highly deuterated media

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Reviewer: Mgr. Petr Maršík, Ph.D., Institute of Experimental Botany of the Czech Academy of Sciences

The presented doctoral thesis aimed at the effect of highly deuterated media to the growth and proliferation of microalgae with regard to the production of main energy-storage metabolites. Since there is only limited knowledge about the impact of a deuterium-rich environment on the life processes of autotrophic microorganisms, the work is focused on changes in the cell cycle and biomass production of algal cultures on media with a high D₂O content. For this purposes, three microalgae species (*Chlamydomonas reinhardtii*, *Parachlorella kessleri* and *Desmodesmus quadricauda*) with specific cell cycle pattern characterized by multiple fission were used as model organisms. In combination with the deuterium effect, the influence of two key abiotic factors, light intensity and temperature, was investigated. Finally, the utilization of optimized conditions for biotechnological production of biomass and highly deuterated compounds by algal cultures is discussed.

The thesis is presented as a set of four articles published in peer reviewed scientific journals and one chapter in a book framed by a joint Introduction and Conclusion with future prospects. All of the articles deal with affecting of growth, reproduction and accumulation of energy storage molecules as a response to the abiotic stress factors in three model algae. All published experiments are methodically consistent, well designed and it is obvious that used workflow is well established. I consider the choice of model organisms to be very appropriate - all investigated algae belong to eukaryotes with a relatively unusual way of cell proliferation (multiple fission), which provides the used model organisms wide flexibility in dynamic environmental changes and, under suitable conditions, a rapid increase in biomass. All of them include testing of deuterium as stress factor affecting wide scale of cellular processes including grow, cell division or energy management. The first article mainly deals with the principal questions of deuterium impact on cell cycle, the remaining four articles rather focus on the factors leading to optimal production of biomass with the potential for use in biotechnological applications. However, although the theoretical applicability of the results is obvious, I'm not sure about the profitability of producing for example deuterated standards by such system (e.g. the cost of purification steps, yield etc.).

In general, the thesis is well written, I have only minor complaints regarding the introduction, where I lack a more detailed description of the effect of temperature and light on the cell cycle and growth of algae. These factors are quite essential in most of the mentioned articles and therefore deserve at least a short chapter in the introduction.



Regarding of the publishing activity of the Ph.D. candidate - Mgr. Veronika Kselíková is the first author of three publications involved in the thesis and three other papers on related topics, and co-author of two others.

Questions:

- 1) What is the isotopic homogeneity (same number of D in all molecules of a given chemical compound) of deuterated compounds produced by cultures of microalgae cultivated in highly deuterated media? Is it possible to manipulate the number of D atoms in desired molecules by culture conditions?
- 2) How does the deuterated environment affect the biosynthetic pathways of algal secondary metabolism? Are there any metabolomic studies on this topic?
- 3) According to your findings, the production of neutral lipids is significantly affected by heat and light intensity. Does their qualitative composition (e.g. ratio of saturated and unsaturated fatty acids in lipids, number of double bonds, etc.) change with their intensity?

As overall evaluation of the thesis, I consider the presented work to be of very high quality in term of formal processing as well as obtained results. The high level of the thesis was confirmed by publication of the results in impacted journals and in the chapter of scientific monography.

In my opinion, the presented work meets all requirements for Ph.D. thesis, and therefore I recommend it for defense.



In Čisovice, 15th October 2023

Petr Maršík