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Annex No. 10 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Applicant	Mgr. Pavel Dvořák, Ph.D.
Habilitation thesis	Engineering bacteria, their enzymes, and metabolic pathways for biotechnological processing of waste compounds
Reviewer	Ivana Márová, Prof. RNDr. CSc.
Reviewer's home unit, institution	Faculty of Chemistry, Brno University of Technology

I am writing this review based detailed study of above habilitation thesis. In this letter I would like to recommend the thesis to acceptation as a basis for Habilitation Procedure of Mgr. Pavel Dvořák, Ph.D. The thesis is focused on application of protein engineering, metabolic engineering, and synthetic biology that enable the preparation of more efficient biocatalysts for the degradation and valorization of diverse groups of organic waste.

The thesis is elaborated in the form of summary of author´s contributions and innovative approach by using the engineering of molecular and whole-cell bacterial biocatalysts for biodegradation of selected types of organic waste materials – lignocellulosic residues and halogenated hydrocarbons. Production of some high value-added enzyme systems by metabolic engineering and cell factory design was studied as well.

The text has been organized into two sections. The first section describes the adoption of engineering principles and synthetic biology into the field of biodegradation and bioremediation of environmental pollutants. The main objective of this part was to develop better molecular and whole-cell bacterial catalysts for the biodegradation and potential valorization of some toxic anthropogenic waste chemicals (such as degradation of TCP by genetically engineered *E. coli*). The second part of the thesis was focused on the role of microbial bioengineering in the preparation of enhanced microbial systems that can valorize waste plant biomass in environment-friendly bioprocesses. Soil bacterium *Pseudomonas putida* was used as a model system for this purpose. Some mutants capable to utilize lignocellulose as a nutrition source were selected and studied in detail.

Both sections are completed with 15 scientific publications published in the period of 2009 – 2021. Dr. Dvořák is the first (or corresponding) author of most of these papers. The quality and high impact factor of the journals, in which the papers were published, ensures that the articles have been per-reviewed very properly and have already been thoroughly opposed. Moreover, some of the articles are highly cited by scientific community.

This thesis can be considered as a set of consistent results of complex experimental study. Clearly demonstrates high degree of innovation and author´s invention in the field of structural biology and its interconnection with metabolic and protein engineering. The papers included into the thesis are of excellent scientific level and are complemented by a high-quality additional commentary text. The thesis confirmed both scientific and pedagogic skills of the author, broad knowledge and ability to combine inventively interdisciplinary approaches including computation modelling. Especially I would like to appreciate involvement of the author in international teams and his significant personal contribution to both studied topics.

In conclusion, I would highly recommend Habilitation thesis of dr. Pavel Dvořák as the high-quality basis for further procedure.

I have following questions to the author:

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

1. Biotechnological conversion of lignocellulosic materials seems to be one of the most promising strategies for the future. To author´s opinion: is economical and energetical effectiveness of these processes really so high that they can be considered as perspective for practical and industrial use? If so, in what time horizon?
2. What is the average long-term adaptation stability of genetically modified lignocellulose consuming microorganisms? Do you have constructed strains applicable for industrial production?
3. Have you described some specific regulatory mechanisms during simultaneous utilization of multiple sugars (hexoses and pentoses) in *P. putida* mutant? What are the main control factors of these metabolic pathways?
4. According to author´s experience: What is the perspective of targeted co-valorization of multiple lignocellulosic materials (or their components) to produce more than one value added product? Can we expect that it could serve predominantly as a complex research model system or for potential industrial application?

Conclusion

The habilitation thesis entitled “Engineering bacteria, their enzymes, and metabolic pathways for biotechnological processing of waste compounds” by Pavel Dvořák **fulfils** requirements expected of a habilitation thesis in the field of Molecular Biology and Genetics.

Date: 23.9.2022