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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	Prof. Ing. Peter Šebo, CSc.
Reviewer's home unit, institution	Institute of Microbiology of the Czech Academy of Sciences, v.v.i., Prague, Czech Republic

Having myself obtained a master in bioengineering almost four decades ago and belonging to the first generation of recombinant DNA users, who witnessed the infancy of genetic engineering in this country, it was with a real pleasure and keen interest that I have read through this delightful habilitation thesis of Dr. Pavel Dvořák. It summarizes 15 publications of the author that report important and innovative contributions to the fields of enzyme engineering and bacterial cell factory design. The described work is of high scientific level and of high societal impact potential. It aimed at improvement of biodegradation processes of recalcitrant halogenated hydrocarbon pollutants and at degradation and value-adding to lignocellulosic waste. The high level of scientific competence and research skill of Dr. Dvořák is documented beyond any doubt by eight first authorships on high standing publications in prime biotechnology and chemistry journals. These papers report important contributions to the respective research fields.

Concerning the text of the thesis itself, the thorough introductory chapters are very well written and are easy to read and understand even for a non-specialist. These quality texts clearly document the scientific and pedagogic aptitudes of the author and discuss the problems rather objectively and with the necessary distance and outlook, thereby documenting the deep insight and broad scientific knowledge of the candidate. The texts provide a well-structured and captive reading, introducing the performed work by an initial review of the state-of-the art, definition of the problems, formulation of logical attack plans, explanation of the chosen approaches and discussion of the achieved results and of pitfalls of the chosen approaches, placing them into the context of recent advances of the field.

The section on design of an *in vitro* TCP degradation system using protein engineering and kinetic modelling and its translation into a genetically engineered TCP-degrader *E. coli* strain within the team of Prof. Jiří Damborský is highly innovative. The account of troubleshooting of the performance of the TCP-degrading recombinant bacterium by a set of comprehensive approaches is quite revealing and instructs on the bottlenecks of synthetic biology approaches to construction of biodegraders of toxic pollutants, such as TCP. This all is very adequately discussed in the text.

Along the same line, the section summarizing the postdoctoral research of the candidate with Víctor de Lorenzo on engineering bacterial cell factories derived from *Pseudomonas putida* strains, aiming for utilization and valorization of lignocellulosic waste pentose sugars and aromatic alcohols is quite impressive. So is the approach to generating engineered Gram-negative bacteria presenting on the surface designer scaffolds for immobilization of protein ligands of biotechnological interest, such as engineered cellulosome-like structures. This work further documents the technical and scientific skill of the author and his creativeness and ingenuity. It is a delightful reading through the description of how these systems were engineered.

Research is a never-ending process and the research aiming at understanding and engineering metabolic pathways and its regulatory circuits for generating potentially useful and applicable bioprocesses is particularly risky. This thesis documents beyond any doubt the scientific qualification of the author and the importance of his contribution to the two respective fields of oriented research on biodegradation and value adding to organic waste.

I have two questions to the author of this habilitation thesis:

1) In the end, the effort to generate an efficient TCP degrading *E. coli* did not succeed. Could you comment what do you personally consider as the currently most likely viable strategy to TCP degradation? Could the engineered *in vitro* system with immobilized enzymes be somehow useful for TCP removal also from complex environmental materials, such as soil? For example, upon extraction of the TCP from that material in some way? How did you imagine the use of an engineered *E. coli* for such purpose on environmental samples and would the use of such a GMO be practicable and possible, providing that the system was sufficiently efficient? On p. 13 you mention as promising the use of reductive conversion of TCP by zero-valent zinc. How practical could its application be for decontamination of environmental samples?

2) Both of the two biodegradation problems you worked on do still require quite some research and engineering, before individual microorganisms, or their synthetic consortia useful for industrial bioprocesses organisms can be developed. A major bottleneck in practical application of bioprocesses are the fixed capital costs of purchase of process equipment and running costs of its operation at the needed large volumes, limiting the efficacy and time horizons of the return on investment into such biotechnological processes. Are you aware of any studies discussing these crucial non-scientific aspects of the technologies under development for bioconversion of pentoses and aromatic lignin residues for lignocellulose degradation? Is there a chance that such processes could be energetically and economically viable? For example, aside of specialty applications, the production of biodegradable plastic based on microbially synthesized PHAs remains economically unviable on large scale, despite of decades of R&D, high level of process optimization and current high oil prices. How does the prospect of hemicellulose and lignin biodegradation and transformation into useful products position in this perspective? Would lignocellulosic residue waste biotransformation into useful chemicals be a valuable enough asset to justify its cost, as compared to using it as a fuel, for example?

Conclusion

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

Date: 21. 08. 2022 Signature: