

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

Petra Patáková, prof. Dr. Ing.

Reviewer's home unit, institution

Department of Biotechnology, University of Chemistry and Technology Prague

[Review text]

I read with great interest the habilitation thesis of Dr. Pavel Dvorak. The introductory text is clear, readable and gives enough information for the referee to get an idea of the candidate's research which is highly topical. Further, 15 appendices are included in the full text, in the form of articles in impacted journals. Regarding the thesis topics, I appreciate that someone in the Czech Republic specializes in the molecular biology of bacteria useful in bioremediation or the production of valuable substances from waste. It sometimes seems to me that these fields are not considered worthy of benefiting from advances in molecular biology and genetics. Dr. Dvorak's personal contributions to the research on the biodegradation of 1,2,3-trichloropropane (TCP) by recombinant *E.coli* bacteria and immobilized enzymes of the TCP biodegradation pathway are indisputable and of worldwide importance. Also the second part of the thesis focused on construction of different *Pseudomonas putida* mutants with improved ability to use lignocellulose as a substrate is meritorious and original. It is clear that the author did not work alone, but his authorial contribution to the publications is clearly defined. The importance of his contribution to these publications is also evident from the fact that he was often a corresponding or first author and collaborated with foreign researchers. The quality of the journals, in which the candidate has published, ensures that the articles have been peer-reviewed properly and thus have in fact already been opposed.

The thesis inspired me to a lot of questions, from which I chose the following ones:

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

- 1) Regarding TCP removal system based on enzyme immobilization, have you tested also multi-column system with immobilization of individual enzymes in different columns? Theoretically, such a system might take into account that individual enzyme reactions proceed at different rates and might enable further optimization.
- 2) Regarding genetically modified organisms and their potential application for bioremediation *in situ*, what do you think about application of suicidal genetically

engineered bacteria? Would this be a chance to apply them in compliance with GMO laws?

- 3) I did not understand whether the *P.putida* mutant with surface-displayed β -glucosidase was able to decompose β -glucans? (If not, still the work is remarkable but what was the problem and how to overcome it.)
- 4) The main problem of lignocellulose substrates utilization seems to be that bioprocess parameters, namely the product yield, its concentration and productivity, are not sufficiently high to enable efficient competition with standard, established processes. The yield is usually the least problem however the product concentration, which is of key importance for efficient separation and purification is frequently neglected as well as productivity. As a biotechnology teacher, I would like to know whether there is any emerging lignocellulose utilizing technology based on *P.putida* which is ready/close to industrial scale-up?

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: 26.7.2022

Signature: