

COMMENTARY TO HABILITATION THESIS

Bacteria, their enzymes, and biochemical routes find use in diverse biotechnological processes. Modern biotechnologies are being recognized as essential weapon for coping with some of the global challenges of the 21st century. One pronounced challenge is the accumulation of waste – gaseous, solid, and liquid - generated in unprecedented amounts by the growing population of men. The poorly regulated waste release results in well-known global consequences including climate change, plastic pollution, or contamination of soils and waters with toxic anthropogenic chemicals. With the increasing pressure on economic transformation towards circular economy and sustainable development, lignocellulosic residues, dumped petroplastics, atmospheric CO₂, certain halogenated hydrocarbons, and other abundant types of organic waste attract attention not only as troublesome pollutants that need to be removed but also as potential new resources of carbon and energy for bioproduction of valuable compounds. Environmental bacteria and their enzymes used in such biotechnological conversions are sometimes limited by insufficient substrate scope, poor productivity, and low robustness (bacteria) or by suboptimal activity, selectivity, or stability (enzymes). The encountered bottlenecks can be mitigated or completely removed by the means 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.

This work aims to summarize my contribution to the study and engineering of molecular and whole-cell bacterial biocatalysts for biodegradation of and value-adding to two types of organic waste compounds – halogenated hydrocarbons and lignocellulosic residues. The first part of the thesis describes the adoption of engineering principles in the knowledge-aided designs of better molecular and whole-cell bacterial catalysts for the biodegradation and potential valorization of anthropogenic waste chemicals that are harmful to the environment: halogenated hydrocarbons and namely to the synthetic toxic chemical 1,2,3-trichloropropane (TCP). Our studies on the designer catabolic pathway for TCP have contributed to the invasion of synthetic biology into the field of biodegradation and bioremediation of environmental pollutants. We have expanded the understanding of biocatalysis of anthropogenic halogenated hydrocarbons and introduced several new

concepts. We demonstrated that: (i) the combination of protein engineering and kinetic modeling of *in vitro* reconstructed biochemical cascade can greatly reduce the total enzyme load needed for the substrate conversion, (ii) a mathematical model assembled from parameters collected *in vitro* (enzyme kinetics) and *in vivo* (substrate and metabolite toxicity, plasmid copy number) can be used for reliable computer-driven modular design of orthogonal biodegradation pathway in a suitable heterologous host, (iii) widely used synthetic inducer molecule IPTG can exacerbate substrate toxicity in popular bacterial host *E. coli* BL21(DE3), the use of natural inducer lactose can alleviate this effect, (iv) and that *in vitro* immobilized catabolic cascades can offer an alternative solution to microbial degraders for removal of toxic chemical waste.

The second part of the thesis highlights the role of microbial bioengineering in the preparation of enhanced bacterial cell factories that can valorize substrates from waste plant biomass in environment-friendly bioprocesses. Our recent attempts to upgrade soil bacterium *Pseudomonas putida* for this purpose are featured as an example. This research enabled: (i) the expansion of *P. putida*'s substrate scope towards abundant lignocellulose-derived pentose sugars and oligosaccharides and presented this organism as a suitable bacterial chassis for the parallel valorization of multiple lignocellulosic substrates, (ii) the development of a system for cell surface attachment of recombinant proteins that may expand the utility of *P. putida* towards numerous new applications including facilitated degradation of organic polymeric waste, (iii) the preparation and validation of new structural model of *P. putida*'s transcriptional regulator XylR which will be instrumental for the designs of molecular and whole-cell bacterial biosensors for lignin-derived aromatics.

This thesis is a commented collection of 15 peer-reviewed articles published between years 2009 and 2021. My contribution to the 15 selected studies in terms of experimental work, supervision of students, manuscript preparation, and research direction is summarized in the tables below. Eight key first-author and corresponding-author research articles that significantly contributed to the studied field are underlined. The papers are listed in the order that corresponds to their citation in the introductory text of the thesis.

[1] Dvořák, P., Nickel, P.I., Damborský, J., and de Lorenzo, V. (2017) Bioremediation 3.0: Engineering pollutant-removing bacteria in the times of systemic biology. *Biotechnology Advances*. 35, 845-866. (2017 IF = 12.451)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
-	-	85	-

[2] Koudelakova, T., Bidmanova, T., **Dvorak, P.,** Pavelka, A., Chaloupkova, R., Prokop, Z., and Damborsky, J. (2013) Haloalkane dehalogenases: Biotechnological applications. *Biotechnology Journal*. 8, 32-45. (2013 IF = 3.237, most cited article in Biotechnology Journal in 2014).

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
-	-	15	-

[3] Klvana, M., Pavlova, M., Koudelakova, T., Chaloupkova, R., **Dvorak, P.,** Prokop, Z., Stsiapanava, A., Kutý, M., Kuta-Smatanova, I., Dohnalek, J., Kulhanek, P., Wade, R.C., and Damborsky, J. (2009) Pathways and mechanisms for product release in the engineered haloalkane dehalogenases explored using classical and random acceleration molecular dynamics simulations. *Journal of Molecular Biology*. 392, 1339-1356. (2009 IF = 4.031)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	-	10	-

[4] Dvorak, P., Kurumbang, N.P., Bendl, J., Brezovsky, J., Prokop, Z., and Damborsky, J. (2014) Maximizing the efficiency of multi-enzyme processes by stoichiometry optimization. *ChemBioChem*. 15, 1891-1895. (2014 IF = 3.157)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	25	90	50

[5] Kurumbang, N.P.*, **Dvorak, P.*,** Bendl, J., Brezovsky, J., Prokop, Z., and Damborsky, J. (2014) Computer-assisted engineering of the synthetic pathway for biodegradation of a toxic persistent pollutant. *ACS Synthetic Biology*. 3, 172-181. (*shared first author, 2014 IF = 4.433)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
50	50	50	50

[6] Dvorak, P., Chrast, L., Nickel, P.I., Fedr, R., Soucek, K., Chaloupkova, R., de Lorenzo, V., Prokop, Z., and Damborsky, J. (2015) Exacerbation of substrate toxicity by IPTG in *Escherichia coli* BL21(DE3) carrying a synthetic metabolic pathway. *Microbial Cell Factories*. 14, 201. (2015 IF = 4.151, among the most influential articles in MCF in 2015 and 2016 based on Altmetric.com)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
65	50	90	50

[7] Demko, M., Chrást, L., **Dvořák, P.**, Damborský, J., and Šafránek, D. (2019) Computational modelling of metabolic burden and substrate toxicity in *Escherichia coli* carrying a synthetic metabolic pathway. *Microorganisms*. 7, 553. (2019 IF = 3.864)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	20	10	15

[8] **Dvorak, P.**, Bidmanova, S., Prokop, Z., and Damborsky, J. (2014) Immobilized synthetic pathway for biodegradation of toxic recalcitrant pollutant 1,2,3-trichloropropane. *Environmental Science and Technology*. 48, 6859–6866. (2014 IF = 5.478, ACS Editors' Choice May 24th 2014, the best Technology article out of 1,500 published papers in 2014 in Environmental Science and Technology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
95	10	90	50

[9] Brezovsky, J., Babkova, P., Degtjarik, O., Fortova, A., Gora, A., Iermak, I., Rezacova, P., **Dvorak, P.**, Kuta Smatanova, I., Prokop, Z., Chaloupkova, R., and Damborsky, J. (2016) Engineering a de novo transport tunnel. *ACS Catalysis*. 6, 7597-7610. (2016 IF = 10.720)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	-	5	-

[10] Vanacek, P., Sebestova, E., Babkova, P., Bidmanova, S., Daniel, L., **Dvorak, P.**, Stepankova, V., Chaloupkova, R., Brezovsky, J., Prokop, Z., and Damborsky, J. (2018) Exploration of enzyme diversity by integrating bioinformatics with expression analysis and biochemical characterization. *ACS Catalysis*. 8, 2402–2412. (2018 IF = 12.025)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	5	5	5

[11] **Dvořák, P.** and de Lorenzo, V. (2018) Refactoring the upper sugar metabolism of *Pseudomonas putida* for co-utilization of cellobiose, xylose, and glucose. *Metabolic Engineering*. 48, 94–108. (2020 IF = 8.115)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
100	-	90	90

[12] **Dvořák, P.***, Kováč, J., and de Lorenzo, V. (2020) Biotransformation of D-xylose to D-xylonate coupled to medium-chain-length polyhydroxyalkanoate production in cellobiose-grown *Pseudomonas putida* EM42. *Microbial Biotechnology*. 13, 1273-83. (*corresponding author, 2020 IF = 4.181)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
85	100	100	90

[13] Dvořák, P.*, Bayer, E.A., and de Lorenzo, V. (2020) Surface display of designer protein scaffolds on genome-reduced strains of *Pseudomonas putida*. *ACS Synthetic Biology*. 9, 2749–64. (*co-corresponding author, 2020 IF = 5.229)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
100	-	90	90

[14] Espeso, D.R., Dvořák, P., Aparicio, T., and de Lorenzo, V. (2020) An automated DIY framework for experimental evolution of *Pseudomonas putida*. *Microbial Biotechnology*. 14, 2679-2685. (2020 IF = 4.181)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	10%	20	20

[15] Dvořák, P., Alvarez-Carreño, C., Ciordia, S., Paradela, A., and de Lorenzo, V. (2021) An updated structural model of the A domain of the *Pseudomonas putida* XylR regulator poses an atypical interplay with aromatic effectors. *Environmental Microbiology*. 23, 4418-4433. (2021 IF = 5.491)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
50	-	90	40