

Abstract

For a sustainable future, there is a call to increase the market share of bio-based technologies and materials. Microbial-based technologies have the potential and the ability to contribute substantively on many levels to global efforts to achieve sustainability. Development and utilization of microbial technologies is, however, an extensive process involving numerous steps, including the discovery of novel technologies and the development of industrially viable production systems. In the presented thesis, individual steps of microbial biotechnology development were addressed.

In the first part of the study, a variety of methodological approaches were employed in order to study the effect of the anthropogenic activity (i.e., decades lasting production of penicillin G) on the structure of soil microbial communities. Moreover, both cultivable and non-cultivable fractions of populations were subjected to functional screening in order to unravel the biotechnological potential of the microorganisms in terms of production of enzymes involved in biotransformation of beta-lactam antibiotics: penicillin G acylase (PGA) and alpha amino acid ester hydrolase (AEH). Our results indicated that the impacted communities harbour a microbial community with increased diversity and richness. However, on the composition level, these communities differ significantly from the control samples, thus evidencing the grave impact of the industrial activity. Consequent analyses of biotechnological potential proved that this environment is a rich source of microorganisms with PGA- and AEH- like activity that could hold the potential to increase the portfolio of industrially important enzymes.

The second part of the study was concerned with the further steps of biotechnology development: the upstream development. The experimental plan aimed at the construction of the *Pichia pastoris* strain producing PGA with a particular focus on determining the optimum cultivation strategy leading to maximum extracellular concentrations of PGA, as well as on defining the physiological and genetic limitations of the production system. Fed-batch cultivations with the constructed strain showed a potential to extracellularly produce fully active PGA, however, a serious secretion bottleneck was also observed, as only around 40% of the produced enzyme was found outside of the cells. Consequently, it was revealed that the secretion limitations can be attributed to the cellular stress caused by intracellular accumulation of the produced enzyme that results in substantial up-regulation of the unfolded protein response pathway. This leads to translational arrest that on one hand relieves the cellular stress after which the system could reach its secretion maxima, although at the same time it significantly decreases the specific productivity of the system.

Overall, this unfortunately means that even after multiple cultivation-optimization trials the constructed strain failed to achieve the desired biotechnological potential, thus leading to the

conclusion that the strain construction process ought to be repeated while taking into account the knowledge established by this thesis.

To facilitate future research aimed at unravelling the true potential of PGA-*P. pastoris* system, an extra pilot study regarding rational strain design was performed. This study showed that the limitations of PGA production and secretion can be successfully overcome by rational design of the production strain and suitable cultivation strategy and evidenced that PGA-*P. pastoris* production platform indeed has a great potential for industrial biotechnology.