



**ABSTRACT BOOK**

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## Playing with the methanol utilization pathway in *Pichia pastoris*

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### Motivation

Alcohol oxidase is the key enzyme for methanol utilization (Mut) in the methylotrophic yeast *Pichia pastoris*. However, also other enzymes along the Mut pathway play a crucial role especially when it comes to the metabolism of produced formaldehyde directing it either to the assimilation or dissimilation pathway. Overexpressing either of these enzymes should significantly affect methanol metabolism and thus probably also protein production of recombinant *P. pastoris* strains.

### Results

We recently developed a novel, generally applicable approach based on fast and easy to do batch cultivations with methanol pulses which enables a more rapid determination of strain specific parameters and thus allows a reliable and fast strain characterization and speeds up process development [1]. We applied this strategy (Figure 1) to compare a Mut<sup>+</sup> strain and a Mut<sup>s</sup> strain expressing the recombinant model-enzyme horseradish peroxidase. When the specific substrate uptake rate ( $q_s$ ) and the specific productivity ( $q_p$ ) were put in relation, the Mut<sup>s</sup> strain turned out to be much more efficient than the Mut<sup>+</sup> strain. Consequently we overexpressed the MUT pathway enzymes dihydroxyacetone synthase (DAS1), formaldehyde dehydrogenase (FLD1) and transketolase (TKL1) in recombinant *P. pastoris* Mut<sup>s</sup> strains expressing either *Candida antarctica* lipase B (CalB) or horseradish peroxidase (HRP). Unexpectedly, overexpression of the MUT pathway enzymes did not have any effect on biomass and off-gas yields - the energy balance of the cells remained unimpaired. However, dramatic changes in  $q_s$  and  $q_p$  were observed (Figure 2). Strains overexpressing DAS1 could produce 3 times more recombinant enzyme with respect to the consumed substrate methanol than the benchmark strain. Since the conversion from substrate into product was much more efficient for this strain, it could be interesting for industrial large-scale production processes, also in terms of a reduced risk management.

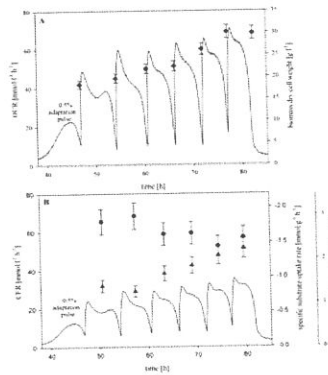


Figure 1. Batch cultivation with methanol pulses.

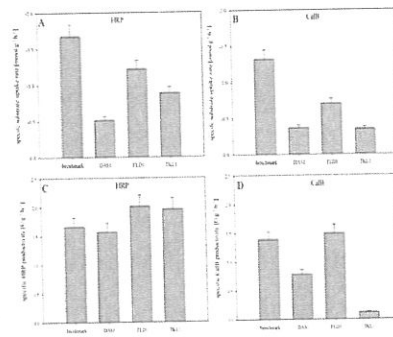


Figure 2. The specific substrate uptake rate and the specific productivity of recombinant *P. pastoris* strains.

### Reference

- [1] Dietzsch C, Spadiut O, Herwig C. 2011. A dynamic method based on the specific substrate uptake rate to set up a feeding strategy for *Pichia pastoris*. *Microb. Cell Fact.* 10(1):14.