

# Identifying bottleneck reactions and developing a systemic fed-batch feeding strategy of *Pichia pastoris* through fine-tuning of methanol utilization pathway

Mohammad Amin Boojari<sup>1</sup>, Fatemeh Rajabi Ghaledari<sup>1</sup>, Ehsan Motamedian<sup>1</sup>, Mehdi Soleimani<sup>1</sup>, and Seyed Abbas Shojaosadati<sup>1</sup>

<sup>1</sup>Tarbiat Modares University

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## Abstract

*Pichia pastoris* as an efficient host for the production of recombinant proteins is mostly cultivated in fed-batch mode in which the cell's environment is continuously changing. Therefore, to fine-tune bioreactor performance in respect to the associated metabolic changes of the microorganism, it is crucial to understand the influence of feeding strategy parameters on the intracellular reaction network. In this study, dynamic flux balance analysis (DFBA) integrated with transcriptomics data was used to simulate the recombinant *P.pastoris* (Muts) growth during induction phase for 3 fed-batch  $\mu$ -stat strategies. The induction phase was divided into equal time intervals and the correlated reactions with protein yield were identified in the 3 fed-batch strategies using the Pearson correlation coefficient. Subsequently, Principal Component Analysis was applied to cluster induction phase time intervals and identify the role of correlated reactions on metabolic differentiation of time intervals. It was found that increasing fluxes through the methanol dissimilation pathway increased protein yield. By adding a methanol assimilation pathway inhibitor (HgCl<sub>2</sub>) to the shake flask medium containing 10% (v/v) glycerol, the protein titer increased by 60%. Using the DFBA revealed that the higher the dimensionless flux of methanol, the higher amounts of protein yield. Finally, a novel feeding strategy was developed so that the dimensionless methanol flux increased compared to the performed cultivations. Protein titer increased by 16% compared to the optimally performed cultivation, while production yield increased by 85%.

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