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4-3-2022

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## IMPACT OF OXYGEN AVAILABILITY ON ORGANELLE-SPECIFIC REDOX POTENTIALS AND STRESS IN RECOMBINANT PROTEIN PRODUCING *PICHIA PASTORIS*

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**Key Words:** *Pichia pastoris*, chemostat cultivation, glutathione redox potential, hypoxia

The methylotrophic yeast *Pichia pastoris* is one of the most effective and established expression hosts for heterologous protein production. The redox balance of its secretory pathway, which is multi-organelle dependent, is of high importance for recombinant protein production. Redox imbalance and oxidative stress are two main factors that can influence protein production and secretion, especially the redox potential of the ER where protein folding and disulfide bond formation occur. Glutathione is the main redox buffer of the cell and its redox conditions can be determined by the status of glutathione redox couple (GSH-GSSG). *In vivo* measurements of the glutathione redox potential in different subcellular compartments can be achieved by genetically encoded redox sensitive fluorescent probes (roGFPs).<sup>1,2</sup> The aim of this study is to investigate the impact of oxygen availability on the redox potentials of different organelles in glucose-limited chemostat cultures of non-producing and producing *Pichia pastoris* strains. It was found that the switch from normoxic to hypoxic conditions affected the redox potentials of all investigated organelles, the specific oxygen uptake rate as well as the accumulation of reactive oxygen species (ROS). Also, as reported previously, hypoxic conditions led to increased recombinant protein secretion.<sup>3</sup> Consequently, a better understanding of oxidative stress and redox homeostasis of the *P. pastoris* strains can lead to improved production of therapeutic disulfide-bonded protein in industrial setups.

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