

Bi-directional promoter systems facilitate expression of unspecific peroxygenase in *K. phaffii* (known as *P. pastoris*) BSYBG11

Hypothesis

- Using a bi-directional promoter system for the expression of an **unspecific peroxygenase (=POX)** and a **chaperone** (protein disulphide isomerase, **PDI**) we can boost enzymatic activity

Goals

- Compare the expression of the target enzyme using mono- (POX) and bi-directional (POX/PDI) promoter systems
- Comparison of specific productivity using mixed feed and derepressed feeding

Step 1: Strain development

- Generation of strains with different promoter combinations and screening for best producers
- POX expression regulated by PDF promoter (strong derepressible and methanol inducible)
- PDI expression regulated by PDC19 promoter (strong derepressible and methanol inducible)

Bidirectional promoter

Step 2: Strain screening

- Application of different feeding rates for identification of derepressed $q_{s\ Gly}$
- Pulsed induced fed-batch with Methanol for identification of $q_{s\ max\ MeOH}$
- Comparison of mixed-feed (Methanol + Glycerol) and derepressed feeding (Glycerol only)

Step 3: Real time PCR

- Analysis of time dependent gene expression during mixed-feed and derepressed feeding
- Comparison of gene activation based on induction method

Results

Strain screening

- Best $q_{s\ Gly}$ and $q_{s\ MeOH}$ were identified (resulted in highest enzymatic activity, data not shown)
- Best process parameters were used for comparison of derepressed feed and mixed-feed cultivations, shown in Figure 1
- Derepressed feeding resulted in higher productivity compared to mixed-feed cultivation
- PDI co-expression improved enzymatic activity

Real time PCR

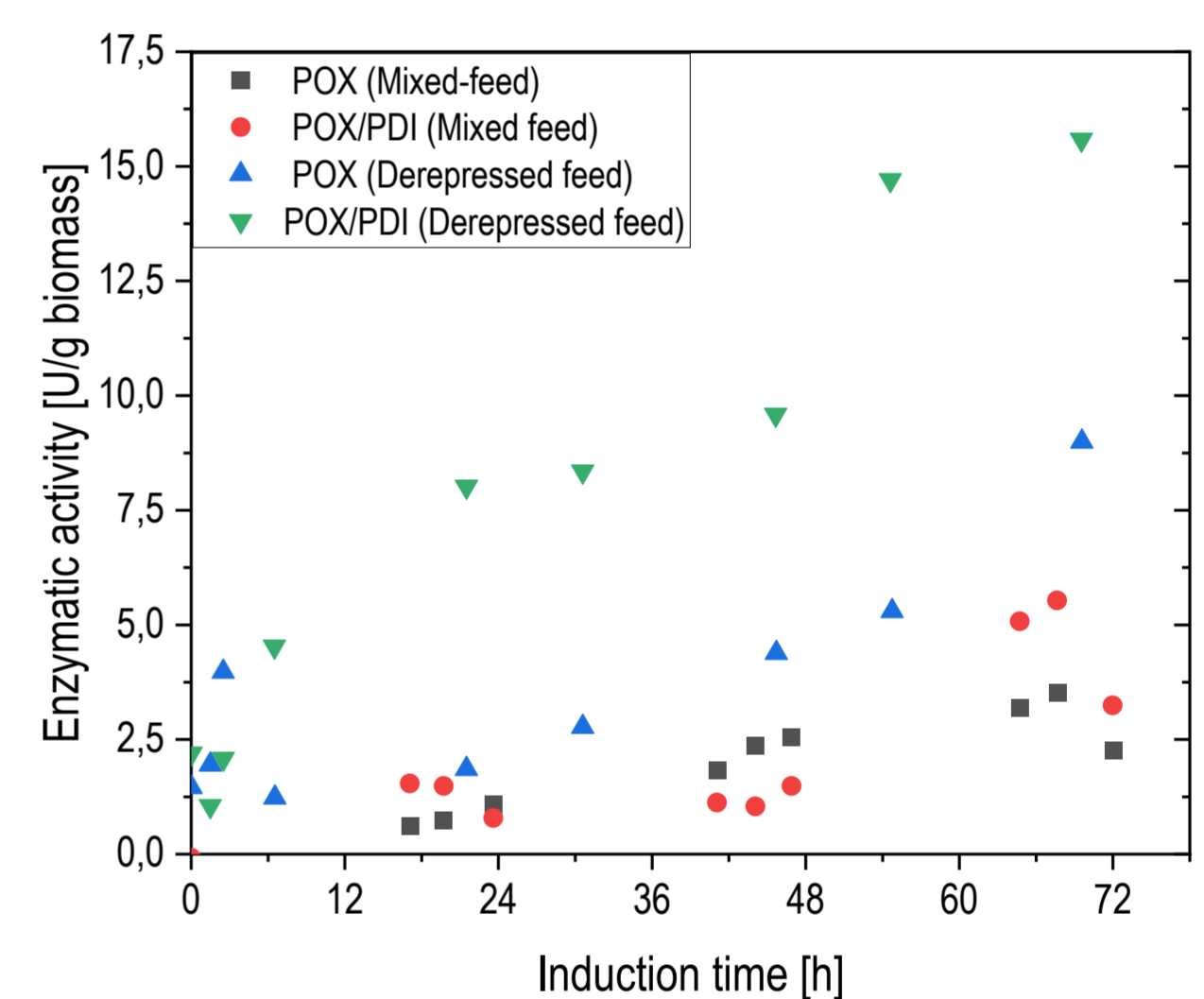
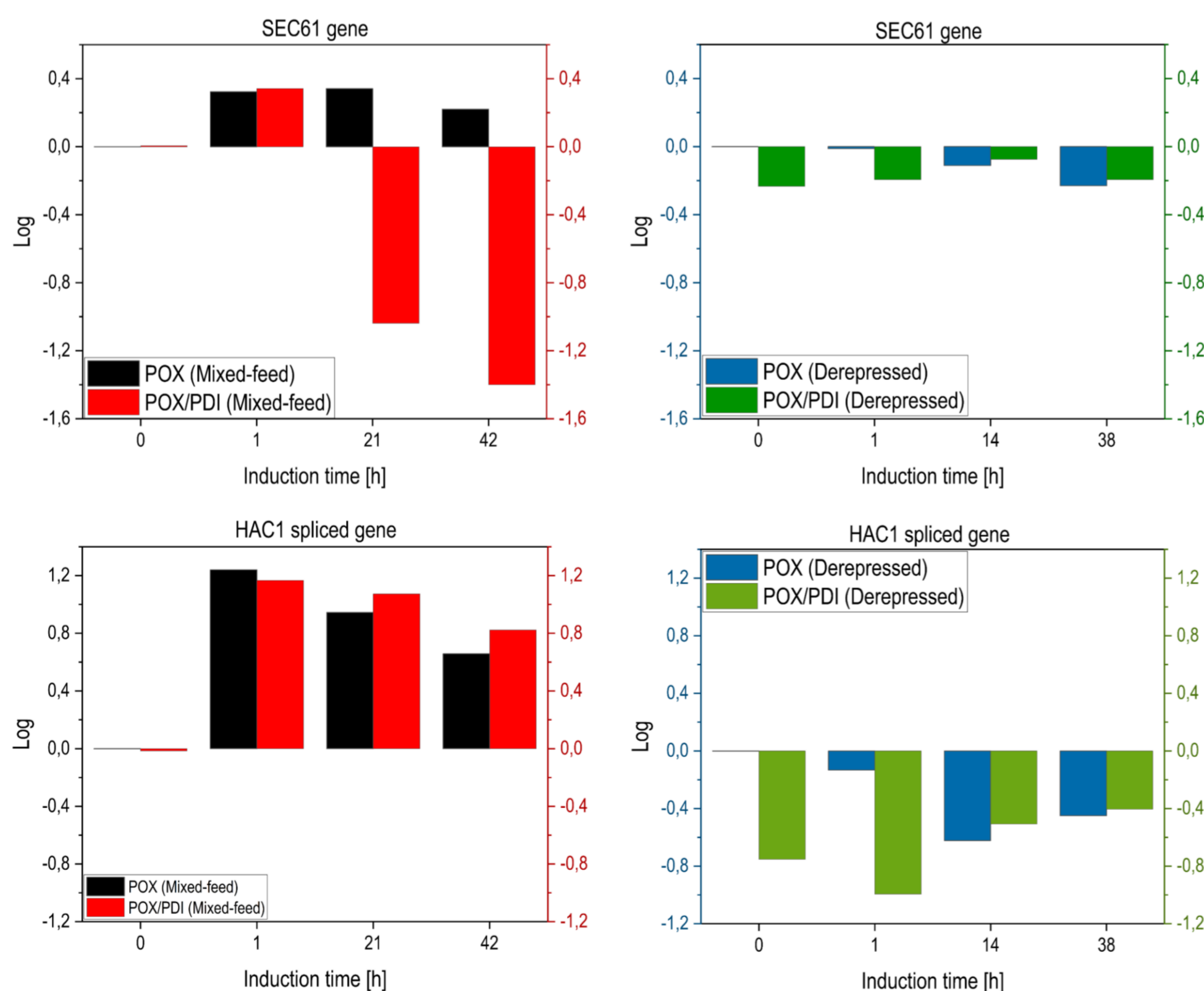


Figure 1. Comparison of enzymatic activity in derepressed fed-batch and mixed-feed cultivations

- SEC61 gene mediates translocation of proteins, while HAC1 spliced gene regulates the unfolded protein response (UPR)
- During mixed-feed cultivations, SEC61 expression was altered, apparently methanol induction promotes stress on vesicle transport system
- No significant alteration of SEC61 up-/downregulation (+/- 0.2) was found in derepressed cultivation
- Derepressed feeding did not overwhelm vesicle transport system resulting in higher productivity of POX and POX/PDI
- UPR stress can be seen right after MeOH addition: HAC1 spliced gene revealed a high activity of UPR system in mixed-feed cultivations
- Derepressed feeding showed that the HAC1 spliced gene was downregulated during induction time in cultivations

Take Home Message

- Methanol imposes high cell stress leading to lower productivity
- Derepressed feeding shows higher productivity compared to mixed-feed
- PDI helps to decrease UPR and increase product quality (higher specific enzymatic activity)

Outlook

- Exercising the potential of derepressed feeding in cascaded continuous cultivation (two reactors operated sequentially in a continuous mode)