

Simple shake flask cultivation of *Komagataella phaffii* with constant glycerol feed



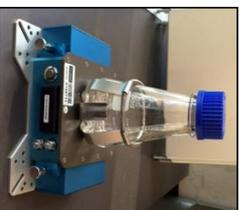
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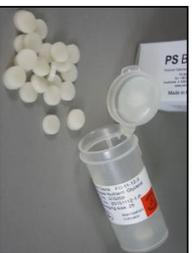
Introduction

Recombinant protein expression, driven by carbon source repressed promoters like P_{Gc} and P_{PF} in *K. phaffii*, is a promising alternative to the widely used methanol dependent expression system with P_{AOX1} . To characterize newly developed strains employing these promoters a bioreactor simulation with constant glycerol feed and online monitoring in shake flask scale is a useful tool to circumvent multiple expensive, time-consuming and laborious bioreactor cultivations.

Experimental Set-Up

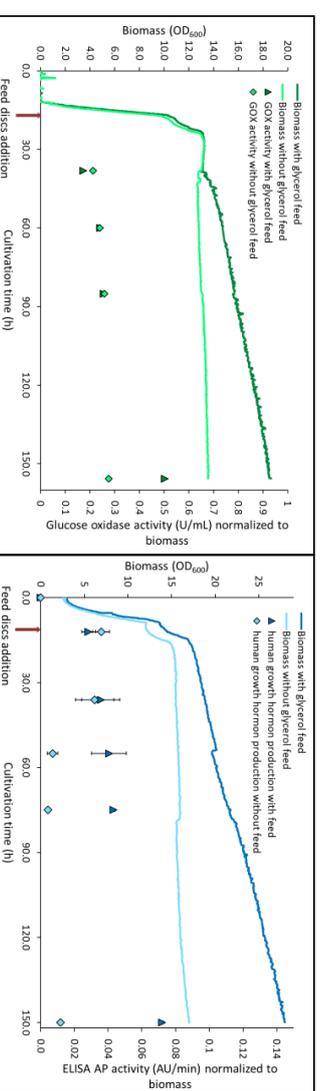


To successfully simulate bioreactor cultivations in shake flask scale including online monitoring of the cultivation parameters the Presens SFR Vario system is used, where pH, biomass, oxygen concentration, oxygen uptake rate and temperature can be monitored on-line.



To ensure constant glycerol feed after the batch, polymer feed discs releasing (spec. 2 mg/h) glycerol into the medium were used. Due to the exact online monitoring, the optimal timepoint for feed disc addition can be determined.

Analysis of reporter proteins



A. *niger* glucose oxidase production by $K. phaffii$

BSYBG11 driven by P_{Gc}
light green line and squares: no feed
dark green line and squares: 2 mg/h glycerol feed

Not only glucose oxidase expression per biomass unit could be increased by 100% but also the application of a constant feed could prevent the human growth hormone from degradation.

human growth hormone production by $K. phaffii$

BSYBG11 driven by P_{PF}
light blue line and squares: no feed
dark blue line and squares: 2 mg/h glycerol feed

Conclusion

Reliable derepressed protein expression by *K. phaffii* in microscale is realized by the application polymer discs (PS Biotech/Kühner) releasing a constant amount of glycerol to the medium in combination with exact observation of cultivation parameters by the Presens SFR Vario system. This experimental set-up offers the opportunity to characterize new methanol independent production strains and vector systems operably and at low cost prior to expensive upscaling.

Acknowledgements

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References

picture <http://www.psbiotech.com/images/gallery/produktbilder/PS-Glycerol-1200x900.jpg>
Vogl, T. et al. **A toolbox of diverse promoters related to methanol utilization – functionally verified parts for heterologous pathway expression in *Pichia pastoris***. ACS Synth. Biol. 2015).
doi:10.1021/acssynbio.5b00199