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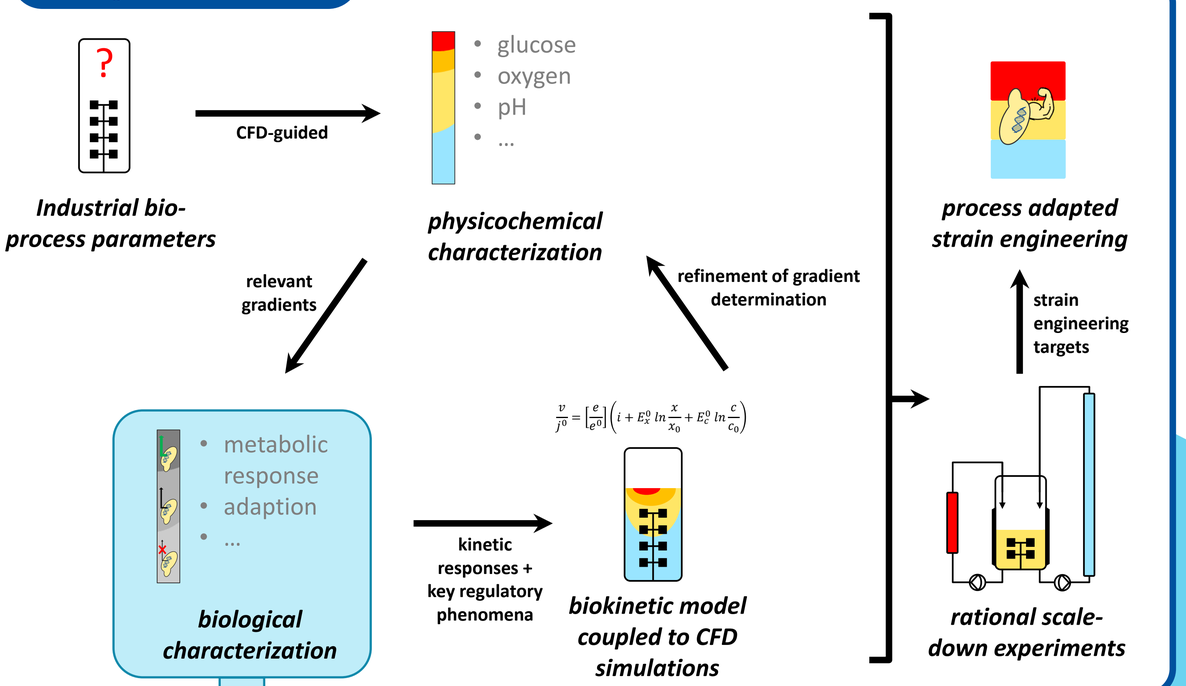
Adaption efforts of eukaryotic host systems experiencing industrial-scale substrate gradients (P 62)

Introduction

Performance loss of eukaryotic host systems on the large *versus* development scale is a fundamental problem attached to most bioprocesses. Thus, understanding how cells adapt or why they might struggle to do so in a heterogeneous environment can deliver vital information for optimizing the genetic background of a producer system. In this work, the model organism *Saccharomyces cerevisiae* is exposed to industrially relevant perturbations, which are transitions between carbon limitation and starvation realized with a substrate gradient of 100 µM in a two-compartment scale-down system. Initial exposure to the scale-down environment causes a growth-arrest type of behavior, whilst adapted cells seem to cope with dynamic substrate availability *via* internal resource reallocation.

Project outline



Approach

Assessing carbon limitation - starvation transitions

strain: *Saccharomyces cerevisiae* CEN.PK 113-7D

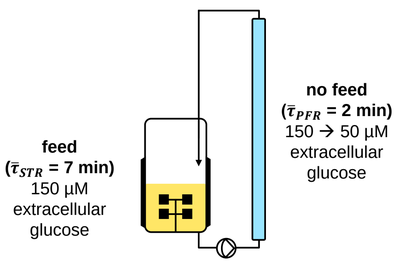
operation mode: aerobic, carbon-limited chemostat (D = 0.1 h^{-1})

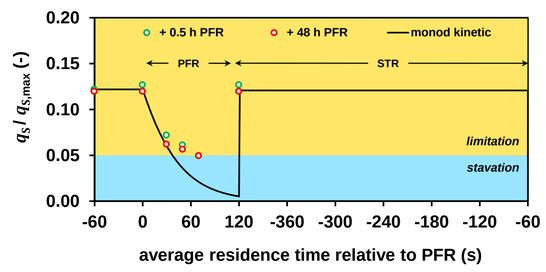
perturbation: uptake-driven transition to substrate starvation in a two compartment stirred tank reactor - plug flow reactor (STR-PFR) system

sample times: reference steady-state, 0.5 h - 48 h scale-down time

sample locations: 1 x STR (long-term response) and 5 x PFR (instant response)

analysis: extracellular glucose, adenylate energy charge ($\frac{c_{ATP}+0.5\,c_{ADP}}{c_{ATP}+c_{ADP}+c_{AMP}}$), biomass, ammonia uptake, intracellular glycogen and trehalose, transcriptomics





limitation: $0.05 < q_S / q_{S,max} < 0.2$

starvation: $q_S / q_{S,max} < 0.05$

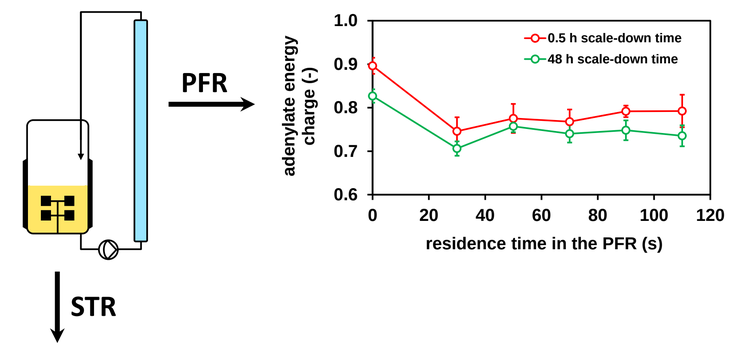
reference: 5 residence times unperturbed carbon limitation in STR

scale-down time: active STR-PFR cycling for another 5 residence times

This approach yields a 100 µM glucose gradient causing an 80 s starvation scenario, which can be regarded as industrially relevant according to simulations [1] and measurements [2] derived from the 22 m^3 scale

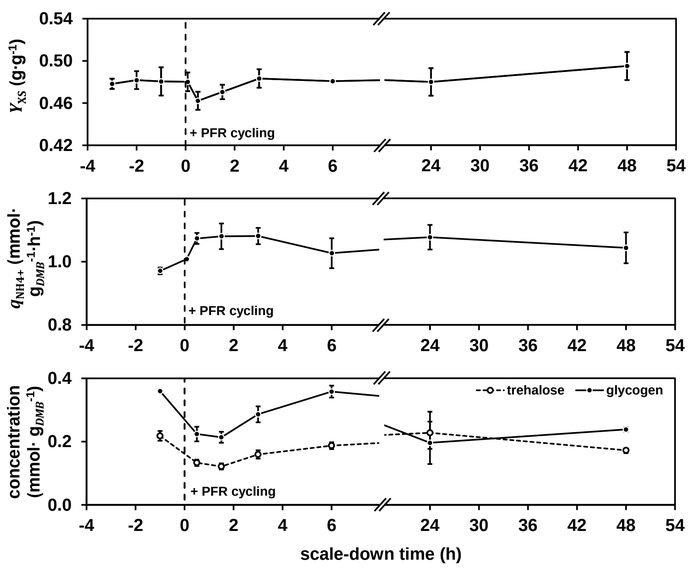
Results

Characterization of perturbation



Instant perturbation of the intracellular energy status by 12 - 15 % within 30 s

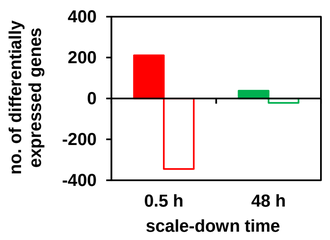
Phenotypic response to repeated perturbations



Exposure to scale-down conditions causes transient growth reduction

Dynamic substrate availability leads to a new operation mode of the cellular machinery causing increased nitrogen demand and decreased glycogen pools

Transcriptome analysis



After 0.5 h, 599 differentially expressed genes underline strong adaption efforts after initial gradient exposure

No new transcriptional steady-state can be observed after 48 h, most likely due to non-homogeneous gene-expression profile in the STR as a consequence of constant triggering in the PFR

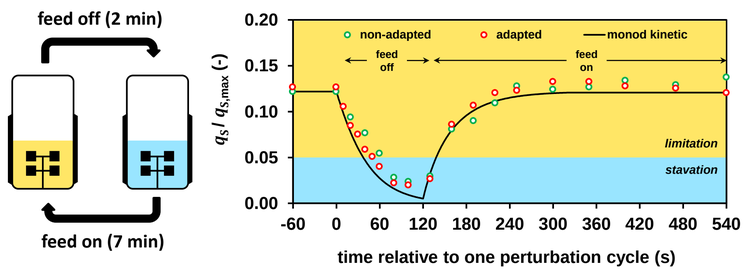
regulation	genes (no.)	GO enrichment	FDR	example genes
up (0.5 h)	214	ribonucleoprotein complex biogenesis	$7.8 \cdot 10^{-15}$	RPA190, RPA135
		rRNA processing	$1.1 \cdot 10^{-9}$	NOP7, DBP3
down (0.5 h)	385	carbohydrate catabolic process	$1.8 \cdot 10^{-2}$	ATH5, GPH1, TDH1

Conclusion

This experimental series indicates a 2-phase response to scale-down conditions (i) an instant growth-arrest-type of behavior and (ii) a coping strategy against dynamic conditions through a change in intracellular resource management.

To meet future modeling demands and to better understand the adaption efforts observed, a new experimental design in a one-compartment approach is proposed.

Outlook



A one-compartment fermentation series will be conducted to allow for an in-depth investigation on how cells react to a regime transition in the case of a non-adapted and an adapted culture. With an intermittent feeding strategy, the whole culture will be kept in the same status to allow the investigation of additional mid-term cellular responses, which could not be investigated so far. Furthermore, metabolome data will be generated to fit a biokinetic model, which is then linked to CFD simulations. By doing so, realistic gradients will be computed to drive the next experimental scale-down series. This new series might challenge the hypothesis that no true starvation scenario exists in the industrial-case scenario due to by-product formation close to the feeding point [3].

References

[1] Haringa, C., Deshmukh, A. T., Mudde, R. F., & Noorman, H. J. (2017). Euler-Lagrange analysis towards representative down-scaling of a 22 m^3 aerobic *S. cerevisiae* fermentation. *Chemical Engineering Science*, *170*, 653–669. https://doi.org/10.1016/j.ces.2017.01.014

[2] Larsson, G., Törnkvist, M., Ståhl Wernersson, E., Trägårdh, C., Noorman, H. J., & Enfors, S. O. (1996). Substrate gradients in bioreactors: Origin and consequences. Bioprocess Engineering, 14(6), 281–289. https://doi.org/10.1007/BF00369471

[3] Sarkizi Shams Hajian, C., Haringa, C., Noorman, H., & Takors, R. (2020). Predicting By-Product Gradients of Baker ' s Yeast Production at Industrial Scale : A Practical Simulation Approach. Processes, 8(1554), 19. https://doi.org/10.3390/pr8121554

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