ABSTRACT BOOK

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From Strain to Product: A fast approach in bioprocess development for recombinant protein expression in *Pichia pastoris*

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Motivation

Pichia pastoris is one of the most important host organisms for the recombinant production of proteins in industrial biotechnology. Beside the genetic engineering of strains methods for a fast early bioprocess development in order to increase the process understanding of the used strain are needed. To date, strain specific parameters, which are needed to set up feeding profiles for fed batch cultivations, are determined by time-consuming continuous cultures or consecutive fed batch cultivations, operated at different parameter sets.

Results

Here, we developed a novel approach based on fast and easy to do batch cultivations with methanol pulses enabling a more rapid determination of strain specific parameters, such as specific substrate uptake rate q_s, specific productivity q_p and the adaption time ($\Delta time_{adapt}$) of the culture to the inducer methanol [1]. The strategy was applied to several *Pichia pastoris* phenotypes (Mut^S and Mut^+) expressing different recombinant products [2]. Based on q_s, an innovative feeding strategy to increase the productivity of the recombinant product HRP was developed. Higher specific substrate uptake rates resulted in increased specific productivity, which also showed a time dependent trajectory. However, a dynamic feeding strategy, where the setpoints for q_s were increased stepwise until a $q_{s,max}$, resulted in the highest specific productivity (Fig. 1). Besides the application of this novel approach on a single substrate system, we additionally examined the impact of q_s on controlled mixed feed fed batch systems, where cell metabolism and product formation showed different mechanistic behaviors.

Conclusion

Our strategy describes a novel and fast approach to determine strain specific parameters of recombinant *Pichia pastoris* strains for the set up of fed batch regimes based on q_s. We furthermore show the potential of a novel dynamic feeding profile based on q_s to boost recombinant protein expression in comparison to conventional strategies. Upcoming studies focus on further optimization of the productivity by controlled q_s ramp feeding profiles and the modeling of the promoter kinetics to further enhance bioprocess understanding for recombinant protein production in *Pichia pastoris*.

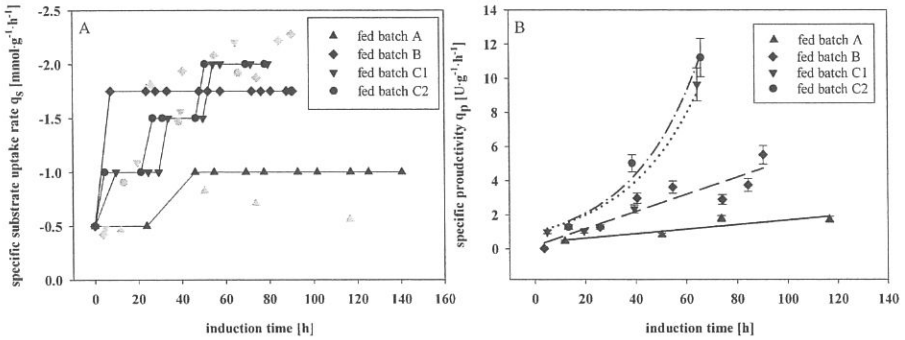

Figure 1: Fed batch cultivations of *Pichia pastoris* on the single substrate methanol: A, specific substrate uptake rate profiles; B, specific productivities.

References

[1] Dietzsch, C., O. Spadiut, and C. Herwig, *A dynamic method based on the specific substrate uptake rate to set up a feeding strategy for Pichia pastoris.* Microb. Cell Fact., **2011**. 10(1): p. 14.
[2] Dietzsch, C., O. Spadiut, and C. Herwig, *A fast approach to determine a fed batch feeding profile for recombinant Pichia pastoris strains.* Microb. Cell Fact., **2011**. 10(1): p. 85.

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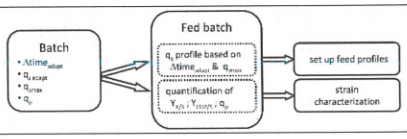
Background and Motivation

Pichia pastoris is one of the most important host organisms for recombinant protein production in industrial biotechnology. To date, strain specific parameters, which are needed to set up feeding profiles for fed batch production processes, are determined by time-consuming continuous experiments or consecutive fed batch cultivations, operated at different parameter sets. Due to the current advances in genetic manipulation and the resulting emerging number of various host strains, faster quantification methodologies for strain characterization and bioprocess development are needed.

The present study aimed at establishing a novel strain quantification method to set up efficient fed batch production processes for recombinant *Pichia pastoris* strains

Experimental strategy

Batch cultivations - Methanol pulses were used to determine the adaptation time, the specific substrate uptake rate during the adaptation and the maximum specific substrate uptake rate of the used strain.



Fed Batch cultivations - Data from batch cultivations were used to set up feeding strategies based on q_s. Different dynamic feeding profiles were tested to find an optimized operation point regarding productivity.

Results and Discussion

fast strain quantification

scalable process parameter

specific productivity q_p

dynamic feeding profile

methanol

batch

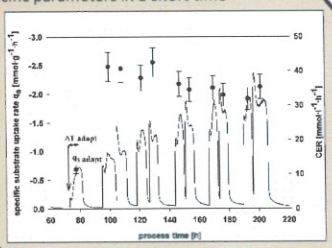
glycerol

Experimental strategy for fast strain screening

Parameter determination in batch mode

Pulses of methanol were used to estimate certain strain specific parameters in a short time [1,2]

- time for adaptation to the inducer and substrate methanol ($\Delta time_{adapt}$)
- specific substrate uptake rate during adaptation ($q_{s\,adapt}$)
- maximum specific substrate uptake rate ($q_{s\,max}$)



Data transfer from batch to fed batch

Successful transfer of strain specific parameters from batch to fed batch cultivations [1,2]

- $q_{s\,max}$ from batch were verified in fed batches without methanol accumulation
- product- and strain-specific interactions were identified
- equal or increased specific productivities in fed batches compared to batch experiments were found

strain / recombinant product phenotype	KM71H - Mut^S	KM71H HRP Mut^S	KM71H PDI HRP Mut^S	CBS7435 HRP Mut^S	SMD1168H GalOx Mut$^+$
$q_{s\,max}$ in batch experiments [mmol·g^{-1}·h^{-1}]	1.94	2.00	1.08	1.54	2.62
q_s without methanol accumulation in fed batch [mmol·g^{-1}·h^{-1}]	1.79	1.92	1.22	1.64	2.44
$q_{p\,max}$ in batch experiments [U·g^{-1}·h^{-1}]	-	2.50	6.30	4.25	200.80
$q_{p\,max}$ in fed batch experiments [U·g^{-1}·h^{-1}]	-	11.00	7.09	6.48	139.00

specific substrate uptake rate q_s

$\Delta time_{adapt}$

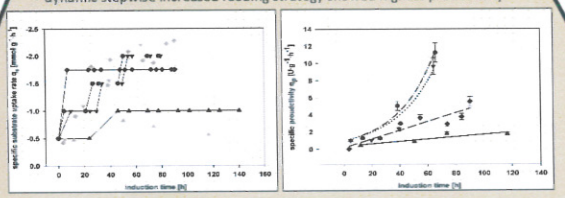
mixed feed

Increased process understanding by dynamic feeding profiles based on q_s

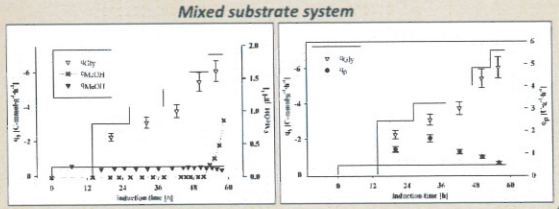
Single substrate system

Different feeding profiles based on q_s were tested with *P. pastoris* KM71H HRP Mut^S strain [1]

- feeding profiles for const. high q_s vs. low q_s vs. dynamically changed q_s
- time dependent increase of productivity detected
- dynamic stepwise increased feeding strategy showed highest productivity



Mixed substrate system



Glycerol uptake rates were adjusted dynamically in a fed batch environment with *P. pastoris* CBS7435 HRP Mut^S strain [3]

- methanol assimilation was repressed at critical specific glycerol uptake rate q_{Gly} of > 5 C-mmol·g^{-1}·h^{-1}
- repression effects on productivity were observed at q_{Gly} > 3 C-mmol·g^{-1}·h^{-1}

fed batch

Conclusion and Outlook

- The study presents a **fast** and **easy to do method** to determine **strain specific data** of *P. pastoris* expression systems
- The **scalable** and **novel process parameter q_s** was successfully transferred from batch into fed batch production systems for various strains
- A **dynamic feeding strategy** based on q_s in a single substrate system resulted in the highest specific productivity q_p compared to other strategies tested
- **Additional information** of **physiological interactions** during mixed substrate assimilation were revealed by applying the novel strategy to a mixed feed environment

→ **Dynamic feeding profiles** turned out to be a valuable tool to **boost specific productivity** and **increase process understanding**!

References: [1] Dietzsch C, Spadiut O, Herwig C: **A dynamic method based on the specific substrate uptake rate to set up a feeding strategy for *Pichia pastoris*.** *Microbial Cell Factories* 2011, **10**:14

[2] Dietzsch C, Spadiut O, Herwig C: **A fast approach to determine a fed batch feeding profile for recombinant *Pichia pastoris* strains.** *Microbial Cell Factories* 2011, **10**:85

[3] Zalai D, Dietzsch C, Herwig C, Spadiut O: **A dynamic fed batch strategy for a *Pichia pastoris* mixed feed system to increase process understanding.** submitted in revised form to *Biotechnology Progress*