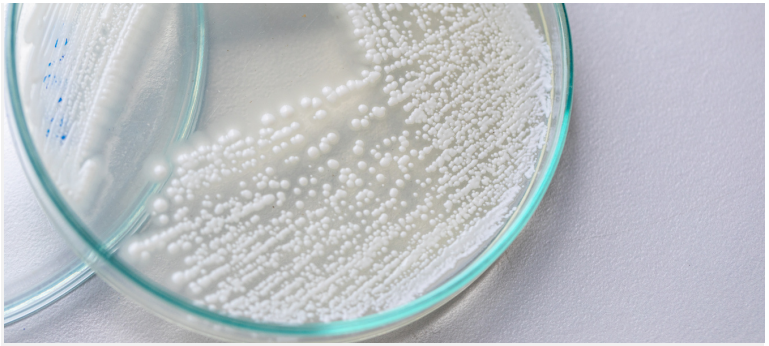
Improved Induction Screenings for a Successful Protein Production in *Pichia pastoris*

with sbi's Biomass-based Feeding Application

sbi SCIENTIFIC BIOPROCESSING

Introduction



Pichia pastoris

Pichia pastoris is a methylotrophic yeast species (it can use methanol as a source for energy) that has become a popular protein expression system. Several advantages make it an ideal host for protein expression, e.g.;

- The production of large amounts of recombinant protein
- Post-translational modifications of proteins
- The secretion of proteins into the culture medium

Pichia can be grown in minimal media to high cell densities and is easily genetically manipulated, making it an economical choice for protein expression. Overall, *Pichia pastoris* is a versatile, efficient, and reliable protein expression system.

Protein Expression in Biotechnology

Protein expression refers to the production of a functional protein from its genetic code. In microorganisms such as bacteria, yeast, and fungi, protein expression is an important tool for the production of therapeutic proteins, vaccines, enzymes, and other products. However, protein expression in microorganisms also presents several challenges that can affect the efficiency and yield of the process.

One major challenge is the optimization of growth conditions for the microorganisms, which can impact the expression of the target protein. In some cases, the target protein may also exhibit toxicity to the host microorganism, leading to reduced growth and yield. Depending on the microorganism, several other challenges can occur, including the production of misfolded proteins or the unwanted degradation of the protein by cellular enzymes.

Defining the most suitable expression system and procedure is crucial to optimize the yield and quality of the respective target protein.

The Challenge

Methanol Induction

Methanol induction is commonly applied and a critical step in the expression of recombinant proteins using the *Pichia pastoris* expression system. The used promotor is an inducible promoter that drives the expression of genes in the presence of methanol.

By inducing with methanol, the cultivation is divided into two phases:

1. **The cell growth phase, with ideal conditions for biomass production**
2. **The protein production phase, that focuses on efficient protein generation**

Methanol induction is usually performed after the yeast cells have reached a high cell density in the growth phase. The yeast cells are then exposed to a certain concentration of methanol and incubated for a period of time to induce the expression of the recombinant protein. The level of methanol in the induction medium and the timing of the addition can be optimized for different recombinant proteins to achieve the highest expression levels. Finally, the yeast cells are harvested, and the recombinant protein is purified using standard protein purification techniques.

Challenges of Methanol Induction

Certain levels of methanol (>1%) are toxic and reduce biomass formation

Methanol is also used as substrate and induces metabolic changes

The right time of induction is crucial to achieve optimal protein yields

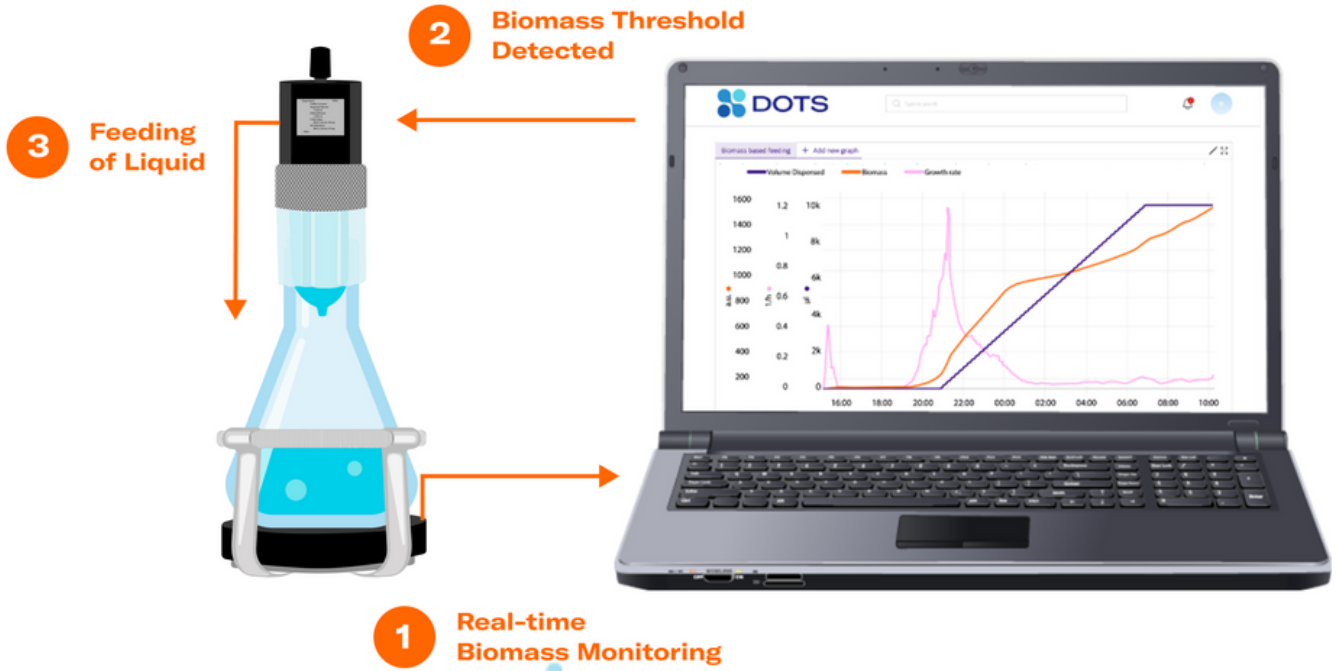
Low but constant methanol levels are desirable for ongoing expression

Methanol is harmful for humans and needs to be handled with care

The Technology

Biomass-based Feeding

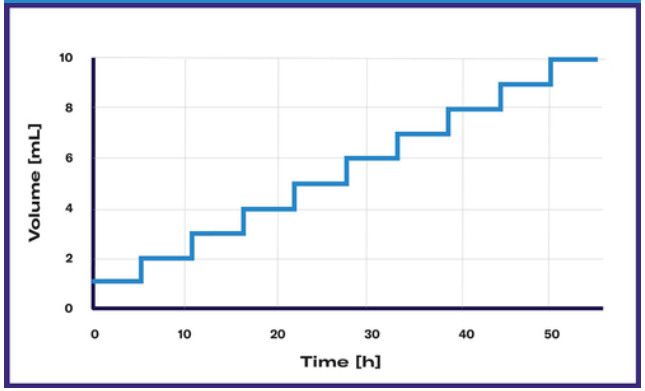
Biomass-based feeding in shake flasks increases the control options of bioprocesses carried out in this vessel type. The term describes the feeding of a substance to a growing microbial culture, initiated by the reach of a certain biomass level or growth rate. The automated substance release is enabled by the interconnectivity of the Cell Growth Quantifier (CGQ), a reliable biomass monitoring sensor, the state-of-the-art Liquid Injection System (LIS), and the powerful DOTS Software.



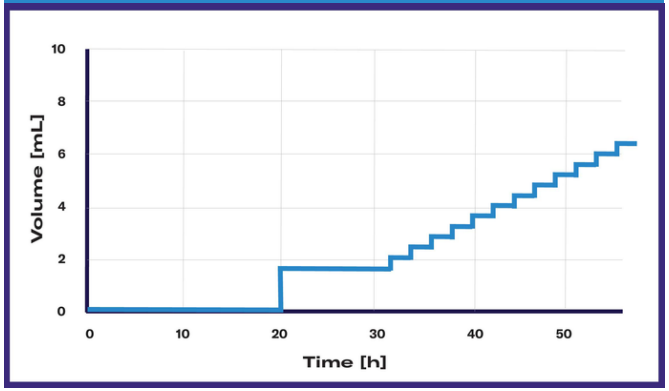
The Solution

Dr. Christina Dickmeis, a Senior Research Scientist at sbi, utilized biomass-based feeding to initiate methanol induction at the optimal time - once cell growth on the primary substrate (glycerol) was completed and sufficient biomass was generated, indicated by a reduction in growth rate. Once the growth rate fell below 0.002 h-1, the feeding process was initiated. She compared the outcomes of different profiles to find out which would lead to the most profitable result - the highest level of active protein.

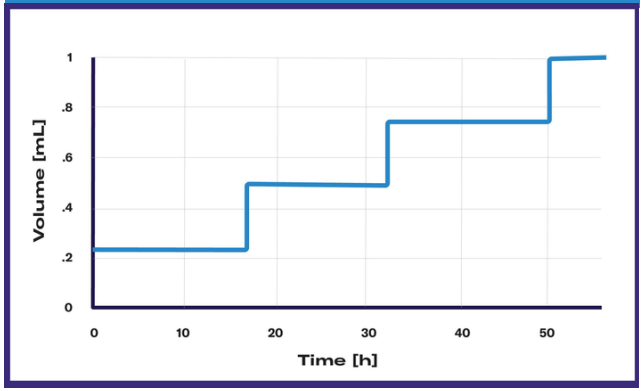
Feeding Profile 1: Constant Feeding



Feeding Profile 2: Single Shot, followed by a pause before a Constant Feed is started

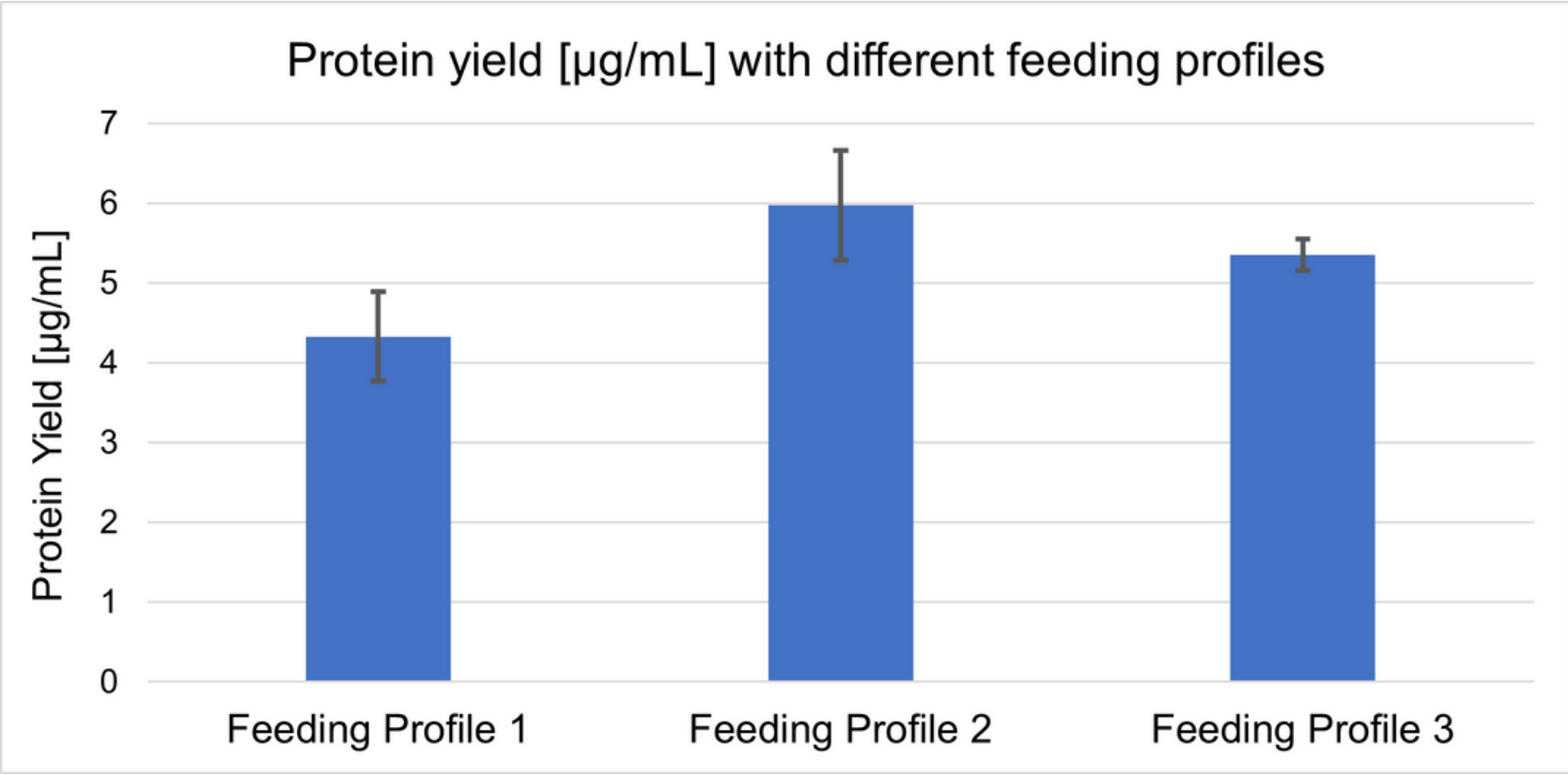


Feeding Profile 3: Multi Shot (one shot every 24 hours)



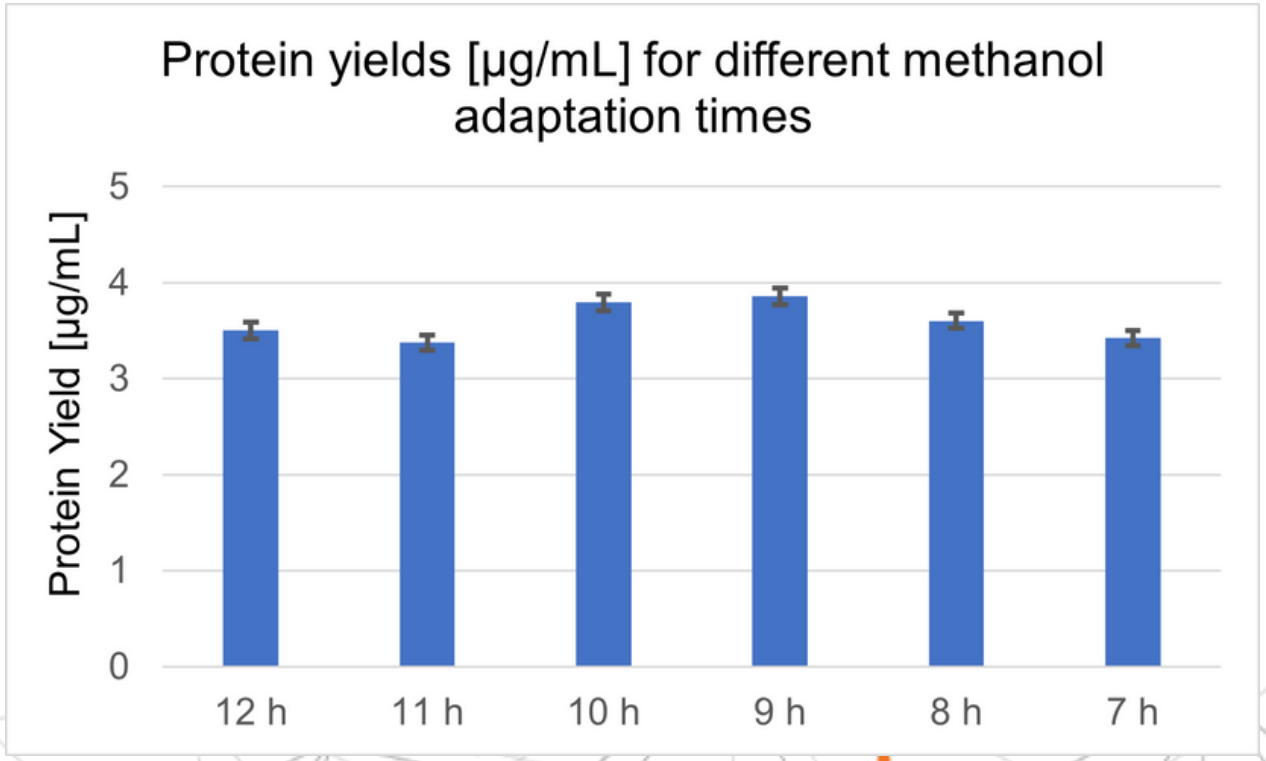
Results I

Upon examining growth curves and active protein yields, Christina discovered that Feeding Profile 2 (Single Shot, pause, Constant Feed) produced the most favorable outcome with the highest levels of active protein. The *Pichia pastoris* cells underwent metabolic adaptation to the new substrate following the initial methanol shot (during the pause). The subsequent constant feed maintained consistent low levels of methanol throughout the cultivation, which proved beneficial for optimal protein production.



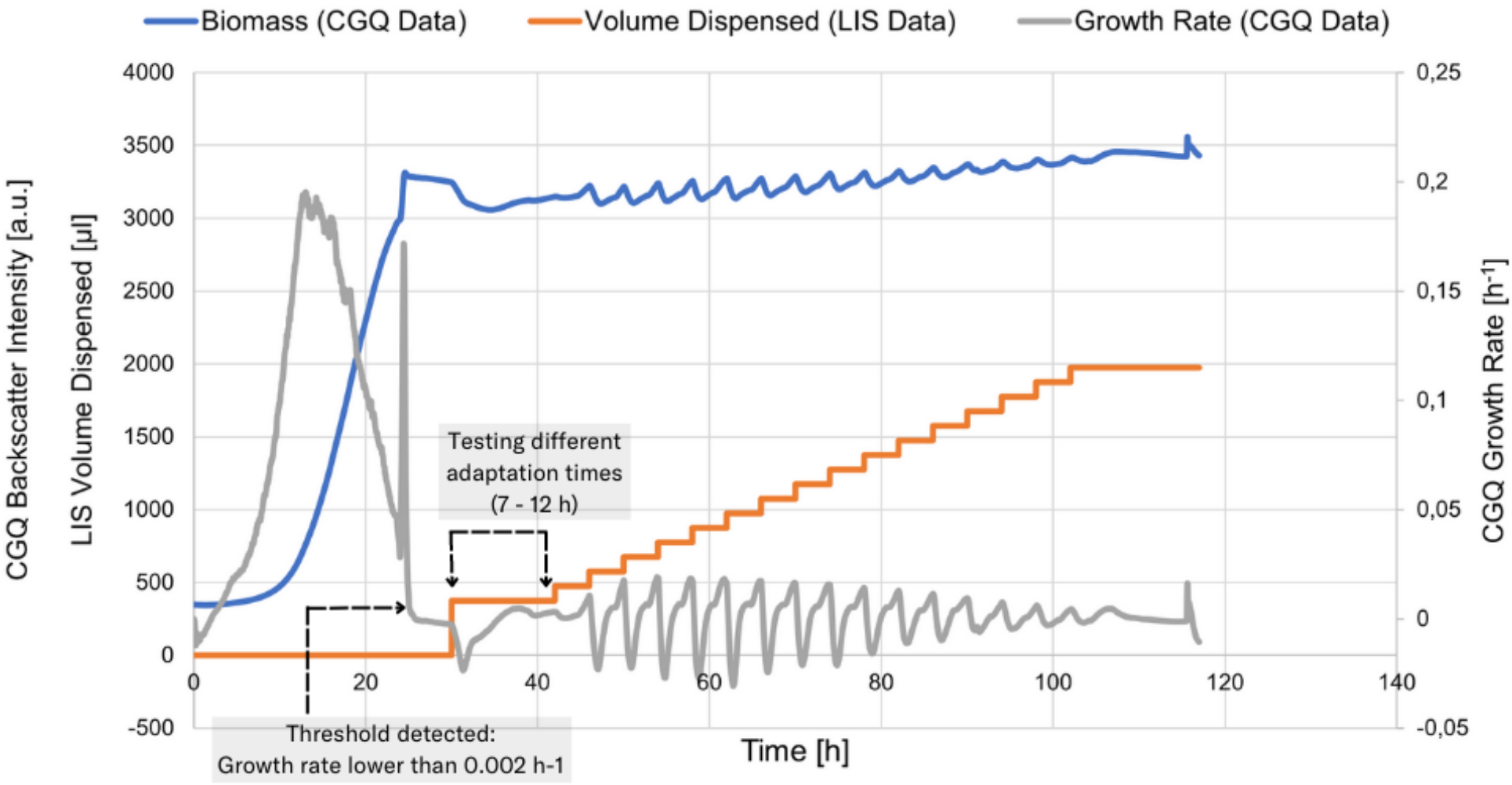
Results II

After establishing that Feeding Profile 2 yielded the best results, Christina proceeded to investigate the optimal adaptation time - the ideal duration between the initial methanol shot and the onset of constant feed. Experimenting with intervals ranging from 7 to 12 hours, she ensured that the total methanol fed to the culture and the expression time remained constant across all tests. Although a 9 hour adaptation time appeared to marginally improve protein yields, the differences observed were not significant enough to warrant a definitive conclusion. Hence, to save time, Christina chose 7 hours for the adaptation time.



Biomass-based Methanol Feeding

Optimization of Adaptation Times



The Results

Using biomass-based feeding in shake flasks allowed Dr. Christina Dickmeis, to start the methanol induction, once the cell growth phase was successfully completed and a sufficient biomass level was reached.

The automatized process and the availability of a range of feeding profiles made it possible for her to easily set up an induction screening process, screening for the ideal feeding profile and time intervals for her experiment. This data will help her to set up an informed bioprocess in bioreactors later on and ultimately increase her protein production yield.

Biomass-based Feeding Output

Improved Production Yields and Efficient Use of Time

By implementing automated procedures, including biomass-based feeding, and further optimizing process conditions, the yield of active protein could be improved by a factor of 20, compared to manual handling setups. Using preset feeding profiles that are automatically carried out and continuously monitored online, various conditions could be conveniently tested, including feeding profiles that necessitate automation such as Constant Feeding. When comparing an automated with a manually performed Multi Shot experiment, manual handling requires approximately 2 hours and 40 minutes more time per experiment than the automated biomass-based feeding setup.

20x Increase of Active Protein Yield

When compared to early experiments with manual feeding steps

0.3 mg/L in comparison to 6 mg/L

Improved Time Efficiency

Saving hours of manual handling and using nights and weekends

3 hours (manual handling) vs. 20 minutes (automated feeding) hands-on work per experiment

Get results like these for your bioprocess!

Let's work together to find a solution that works best for you.

Speak with an Expert

Design of Experiments (DoE)

To run successful bioprocesses, many parameters and conditions have to be taken into account. With the rising cost of media components and energy supply, experiments in large scales become more and more expensive. Having a profound knowledge of the bioprocess before moving into larger scales is therefore vital for economic planning of experiments.

Biomass-based feeding adds another dimension to shake flask experiments that will enhance the knowledge aquisition in smaller scales.

Shake Flask Experiments Enable Large Scale Screenings

More conditions, tested simultaneously and lower costs for media and nutrients

Online Monitoring Allows for Real-time Analysis

Convenient data analysis and documentation

Improved Imitations of Bioreactor Processes

Letting culture conditions - and not time lines - define process actions

Get results like these for your bioprocess!

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Outlook

What's Next?

The implementation of biomass-based feeding in shake flasks and the continuous online aquisition of data from different experiment runs provided critical information that helped Christina to further characterize her bioprocess with an improved outcome.

Having optimized the protein production phase, Christina wants to have a closer look on the cell growth phase next. Generating higher biomass levels of the yeast will likely enhance the final protein yields further. It has been described in literature that fed-batch feeding of the primary substrate (glycerol) might enhance biomass production in comparison to a batch process. Christina wants to use the liquid injection system (LIS) to collect data on different feeding strategies, including fed-batch.

Finally, Christina will consolidate the data from her shake flask experiments and identify the conditions that have shown to bring the most favourable outcome. She will then use this info to set up a defined bioprocess in a bioreactor, scaling up her experiments and protein yields.