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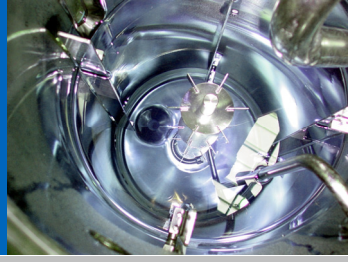

Autonomous multi-scale cascade of parallel stirred-tank bioreactors for fast protein expression studies

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Bioprocess development

Bioprocess development applying microbial expression systems usually includes many, labor-intensive experiments. Therefore, automation, parallelization and autonomous operation of standard lab equipment, usually applied for manual bioprocess development, is considered as the key for the reduction of bioprocess development time and costs.

Automated cascade of parallel stirred-tank bioreactors

An automated bioreactor system with 4 stirred-tank bioreactors on a L-scale was combined with a custom-made biomass transfer system to distribute the cell suspensions produced on the L-scale into 48 parallel stirred-tank bioreactors on a mL-scale, automated by a liquid handling system with integrated fluorescence reader (Fig. 1). Automated protein expression studies with *Escherichia coli* were chosen as a first application example.

Identification of optimum inducer concentrations

In a first automated study, isopropyl β-D-1-thiogalactopyranoside (IPTG) induced expression of the red fluorescence protein mCherry was studied in fed-batch processes with recombinant *E. coli*. IPTG concentrations were varied in 48 parallel fed-batch processes with *E. coli* cells produced at a growth rate of 0.1 h^{-1} on the L-scale by exponential feeding and automated transfer and distribution of the cells into the 48 parallel mL-scale stirred-tank reactors. The mCherry expression rate increased with increasing inducer concentration until the highest protein expression rate was observed at > 9 µM IPTG (Fig. 2).

In a second automated study, the growth rate of *E. coli* was varied between 0.1 - 0.2 h^{-1} in the parallel stirred-tank bioreactors on a L-scale. After automated cell transfer and distribution, the inducer concentration was varied in the 48 stirred-tank bioreactors on the mL-scale. An increased growth rate during cell production resulted in increased IPTG concentrations necessary to achieve identical expression rates compared to a growth rate of 0.1 h^{-1} with the exception of very low inducer concentrations and inducer concentrations in excess (data not shown) (Fig. 3).

Fig. 1 – Photo of the multi-scale cascade of parallel stirred-tank bioreactors for automated bioprocess development: Up to 4 L-scale stirred-tank bioreactors can be used automatically for cell production. An automated biomass transfer system ensures the subsequent transfer and distribution of the cells into up to 48 parallel stirred-tank bioreactors on a 10 mL-scale for performing individual parallel protein expression studies automated by a liquid-handling system with integrated fluorescence reader.

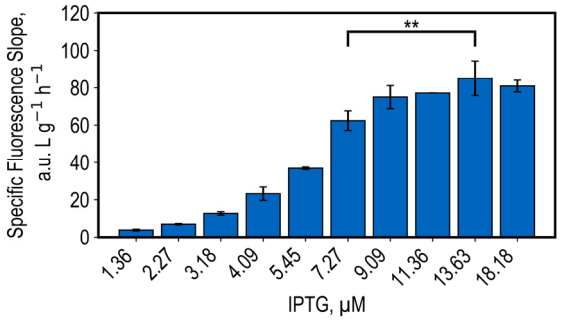

Fig. 2 - Results of the first automated study: Slopes of the at-line measured specific fluorescence with *E. coli* mCherry as an estimation of protein productivity in fed-batch-operated stirred-tank bioreactors on the mL-scale. ** indicates $p < 0.01$, Welch's T-test. (V = 11 mL, F_{in} = 2.4 g glucose L^{-1} h^{-1}, T = 37 °C, pH = 6.9, n = 3000 rpm)

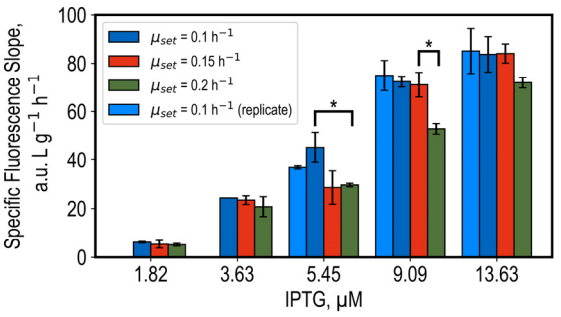

Fig. 3 – Results of the second automated study: Slopes of the biomass-specific fluorescence with *E. coli* mCherry in 48 parallel fed-batch-operated stirred-tank bioreactors on a mL-scale as function of IPTG concentrations added. Different μ_{set} were applied during the cell production phase on the L-scale. * indicate $p < 0.1$, Welch's T-test. (V = 11 mL, F_{in}= 2.4 g glucose L^{-1} h^{-1}, T = 37 °C, pH 6.9, n = 3000 rpm)

References

Von den Eichen et al. (2021): *J Biotechnol* **332:** 103-113

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