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Design of Experiments for optimal production of malaria vaccines with *Pichia pastoris*

Application Note #01

#09
#02
#03
#04

turning science into solutions

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Application background

The aim of a joint project of the HAW Hamburg and the Biomedical Primate Research Center (BPRC) was to identify optimal protein expression conditions for high yields of potential malaria vaccines in the methylotrophic yeast *Pichia pastoris*.

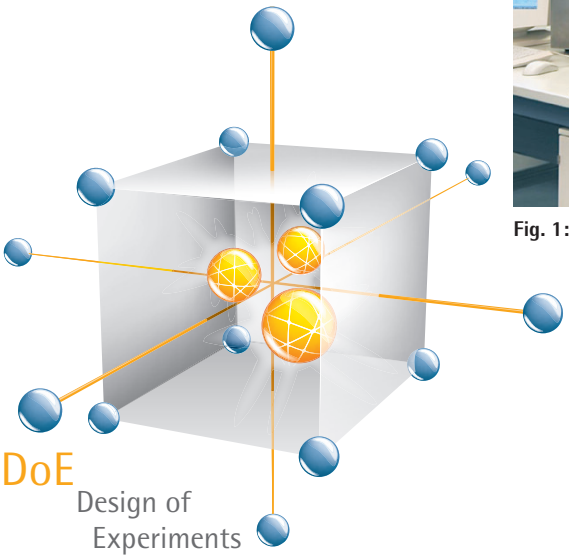
Optimal expression conditions vary in accordance to the kind of *Pichia pastoris* strain used for the foreign protein expressed. As strain characterization and target protein expression optimization need a large number of cultivation runs, a fully automated multi-bioreactor plant was designed and built up for the implementation of investigations via Design of Experiments (DoE).

Enabling technology for future process development

Figure 1 shows the fully automated multi-bioreactor plant used for the executed DoE investigations. The plant design enables a reproducible inoculation of the six-fold parallel parameter screening reactor system BIOSTAT® Qplus with cell broth from the pre-cultivation bioreactor BIOSTAT® Bplus.

The process is operated with the SCADA system BioPAT® MFCS/win. Recipe structures following ANSI | ISA-88.01 permit automated cultivations including harvest, refresh and inoculation operations. The plant concept envisages DoE procedures, planned and carried out by the developed MFCS/win DoE module networked with the DoE software MODDE® by MKS Instruments AB.

Fig. 1: Multi-bioreactor DoE-plant with BIOSTAT® Bplus and Qplus



Investigated expression factors and quality responses

As a first step a DoE screening procedure focused on the impact of three parameters, the methanol concentration in the media phase c_{S2M}, the pH-value, and the liquid temperature ϑ_L during expression. By varying these process parameters with DoE it is expected to improve target protein yield with minimum proteolytic degradation.

Figure 2 shows the optimization potential of the chosen screening parameters by comparing two experiments. Experiment 1 with low pH-value and low expression temperature ϑ_L and a higher c_{S2M} level has a much lower target protein production c_{P1M} than experiment 2 with higher pH-value and ϑ_L and a lower c_{S2M}.

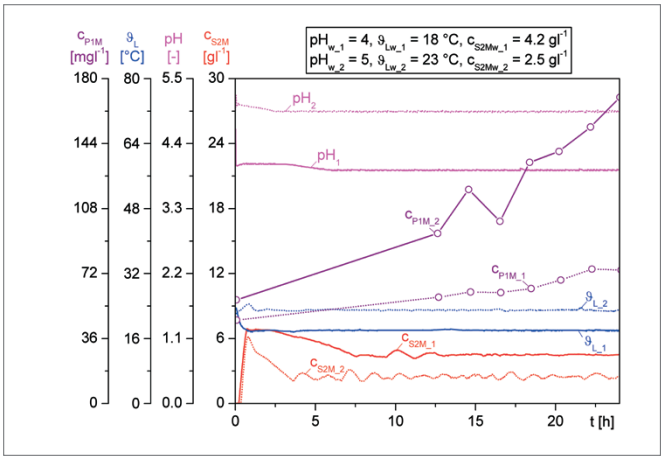


Fig. 2: Time course of two *Pichia pastoris* cultivations with different operation parameters reveals optimization potential

Results of the DoE-screening

Figure 4 shows the results of the DoE-screening which was accomplished by using a full factorial design. The lower response surface represents the productivity at pH 4, the upper at pH 6. The response surface plot indicates a higher productivity in the upper right corner with a low methanol concentration c_{S2M}, a higher temperature ϑ_L and a high pH-value.

Based on the screening results a more thorough DoE-procedure with optimization and robustness analyses are currently in progress. In the upper right corner of Fig. 4 the proposed CCC optimization is already shown.

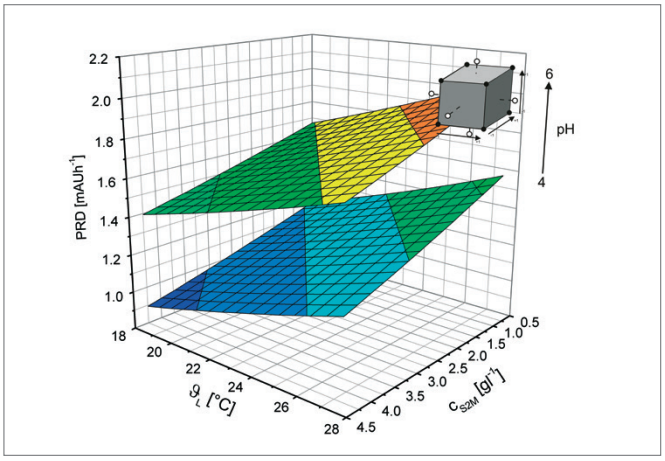


Fig. 4: Response surface plot of DoE-Screening of productivity (PRD) as a function of methanol concentration (c_{S2M}) and cultivation temperature (ϑ_L)

Creating a seamless experimental workflow

The wizard functionality of the MFCS/win DoE module was used to generate a set of screening experiments which were then automatically transferred into the already existing S88 recipe structure. The designed experiments were started simultaneously from within the DoE module and once triggered ran like a standard MFCS/win batch.

Following completion of the process, the DoE module extracts and stores the specified response data and automatically puts them into a MODDE® worksheet for further analysis, so that no typing errors and hence no odd anomalies hinder subsequent statistical data analysis.

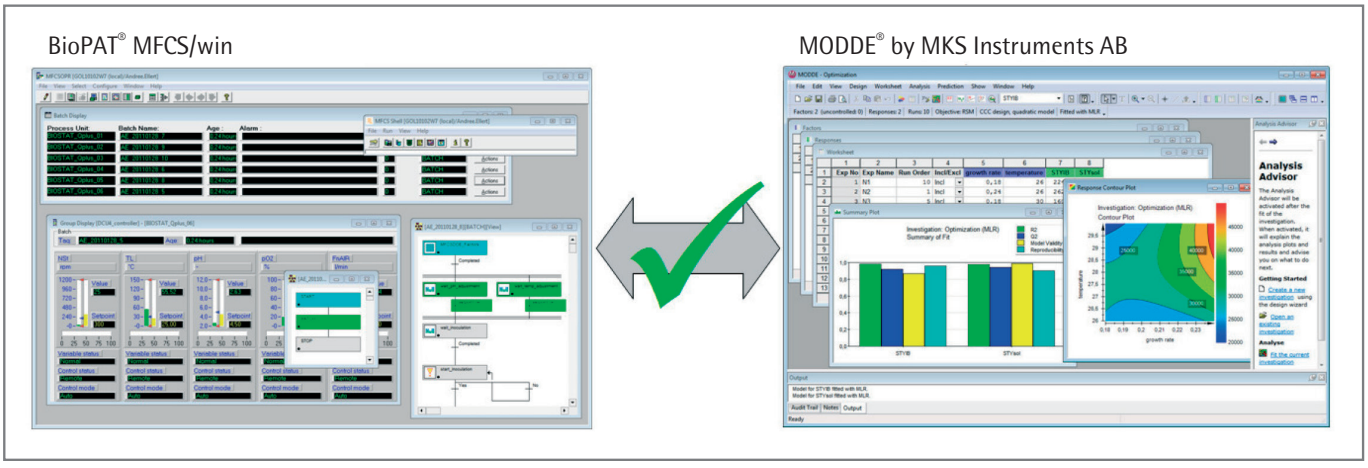


Fig. 3: Integration of DoE into the bioprocess management software BioPAT® MFCS/win

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