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Process optimization made easy: design of experiments with multi-bioreactor system BIOSTAT® Qplus

Design of experiments (DoE) is one of the most important techniques for systematic planning, execution and statistical evaluation of experiments. Although a DoE investigation is executable in one bioreactor, multi-bioreactor systems designed for parallel operation provide the optimal basis to realize a series of experiments in an economical way. The BIOSTAT® Qplus with up to 12 culture vessels represents the basis for a professional and time-saving process optimization.

Reduced time to market and decreased production costs by improving productivity are central issues in pharmaceutical bioprocessing. Within this context the combined application of process analytical technology (PAT) tools and reliable but also flexible bioprocess equipment is the basis for an efficient optimization of existing production processes as well as the development of new ones.

Sartorius Stedim Biotech supplies numerous customers from industry and academia with the BIOSTAT® Qplus, a new generation of bioreactor systems designed for parallel operation (**Fig. 1**). Application-driven, pre-configured packages for microbial fermentation and cell culture provide everything to get started immediately. Besides a fully independent control of up to 12 scalable culture vessels, the system additionally offers a broad range of measurement and automation features ideally suited to the purpose.

The efficient approach—design of experiments

Pushed forward by the FDA initiative for process analytical technologies, the implementation of DoE into the development and design phase of products and processes is frequently applied. Bearing in mind the main optimization target of maximized yield and productivity, one can apply the BIOSTAT® Qplus for several bioprocess cultivation applications.

For instance, the optimization of growth- and production-culture media for growth studies of microbial, mammalian, insect and plant cells becomes feasible with the least number of experiments to find the most favorable mix of nutrient factors. Other examples include the optimization of protein-expression conditions by investigating basic process-state variables such as temperature, pH and dissolved oxygen, or the combined investigation of the latter with PAT-based software sensors for procedural or even biological variables such as volumetric or cell-specific rates.

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The basic idea of DoE is to vary all relevant variables simultaneously over a set of planned experiments and then connect the results by means of a mathematical model. This model can subsequently be used for interpretation, predictions and optimization. In contrast to the intuitive but inefficient way of experimental work by changing one factor at a time, the DoE procedure delivers high information content with a minimum number of experiments.

Figure 1 | The BIOSTAT® Qplus designed for fast-track parallel operation is available with working volumes of 0.5 l (left) and 1 l (right). A minimal three-fold system can easily be extended to a six-fold, nine-fold or 12-fold system.

The case study

The following case study shows the successful use of a BIOSTAT® Qplus six-fold system for the optimization of recombinant protein expression via DoE. It was of primary interest to maximize the amount of soluble protein using two derivates of *Escherichia coli* BL21 (DE3).

For advanced process monitoring, the BIOSTAT® Qplus was equipped with O_2– and CO_2–off-gas analyzers. The bioprocess management software BioPAT® MFCS/win was used for data storage and supervisory control. Because of its open-system architecture, additional equipment, such as balances for exact mass balancing and a numerical computing environment for online estimation of cell-specific growth rate, could easily be connected through standard interfaces. State-of-the-art DoE software was employed for design generation and statistical evaluation of raw data as well as model construction and evaluation.

Cell-specific growth rate, cultivation temperature and inducer

APPLICATION NOTES

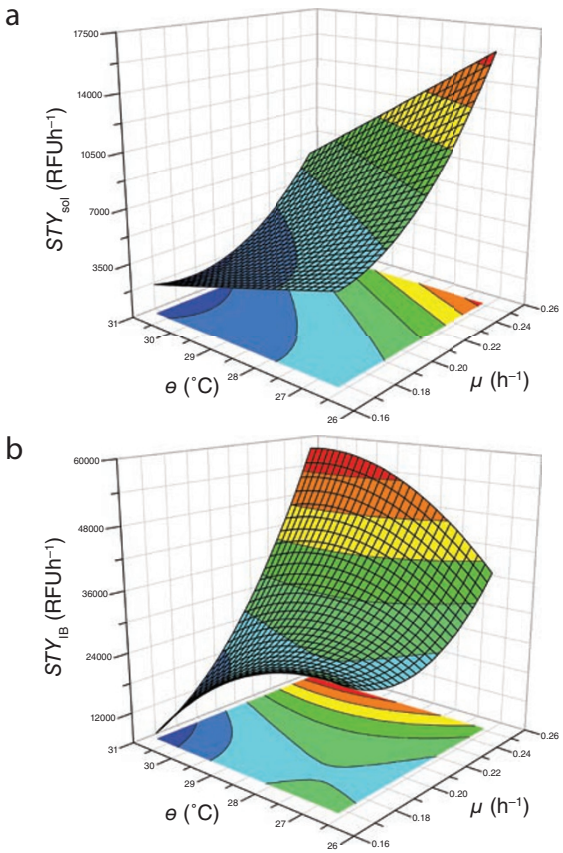

Figure 2 | Response surface plots for soluble (**a**) and insoluble (**b**) space-time yield. STY_k = soluble (k = sol) and insoluble (k = IB) space-time yield; θ = cultivation temperature; μ = cell-specific growth rate.

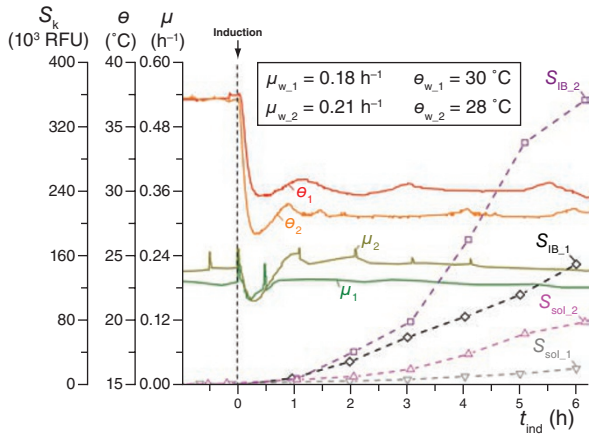

Figure 3 | Comparison of two protein-production phases with different factor settings 1 and 2. S_k = fluorescence signal soluble (k = sol) and insoluble (k = IB) of internal target protein; θ = cultivation temperature; μ = online observed cell-specific growth rate.

concentration were investigated for each *E. coli* strain. The effect of the studied factors on the space-time yield of soluble protein and inclusion bodies was used for process evaluation. The expressed protein was tagged with a green fluorescent protein, allowing simple and rapid post-experimental detection of cell internal fluorescence intensity with a common fluorescence reader.

The work flow to success

The experimental procedure began with a screening for the identification of variables with a major effect on the space-time yield. Considering the functional dependence of growth rate on temperature, we designed a carefully selected set of experiments. The investigated factors were varied simultaneously on both low and high levels, each level corresponding to a single experiment. The design was complemented with four additional runs on a centered level to achieve information about the system variability.

With only twelve screening runs for each *E. coli* strain, it was possible to identify the most promising cell strain. In addition, the inducer concentration was revealed not to be significant, so that the lowest investigated concentration could be used for further studies. Good prediction models gave a reliable direction to obtain high space-time yields, that is a low temperature in combination with a high growth rate.

After screening, the experimental procedure was continued with a more in-depth optimization design. Based on the good screening models for the prediction of high space-time yields, the experimental region was moved toward higher levels for growth rate in combination with lower levels for temperature. In comparison to the screening design, the level ranges were reduced, resulting in a smaller experimental domain.

Twelve optimization runs led to a substantial increase of soluble space-time yield as well as an increased understanding of the processes involved. Trustworthy models could be used for identification of optimal conditions for both soluble and insoluble protein expression. **Figure 2** displays the predicted space-time yields as a response surface spanned by the two factors.

Figure 3 shows the optimization potential of the DoE approach with simultaneously varying factor levels. Using the same inducer concentration, different set-points for growth rate (μ_w) and temperature (θ_w) resulted in a higher soluble and insoluble protein yield for the high/low factor level combination subscripted with 2.

Finally the experimental procedure was finished with a robustness design. Six experimental runs were performed to investigate the system's sensitivity to small factor changes. A safe region of operability could be achieved in which the desired space-time yields were met and robustness for the tested procedure could be claimed.

Conclusions

The case study clearly illustrates the beneficial use of DoE for process optimization. Including and fusing the DoE capabilities into the BioPAT® MFCS/win software enables the user to scout for best process conditions while simultaneously reducing expensive and time-consuming experiments to a minimum.

ACKNOWLEDGMENT

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