Automated Design of Experiments (DoE) in a multi-bioreactor system BIOSTAT® Qplus 6

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The bioreactor system BIOSTAT® Qplus (Sartorius Stedim Biotech GmbH, Göttingen) was established in the Laboratory of Bioprocess Automation at Hamburg University of Applied Sciences. This multi-reactor system enables the execution of parallel experiments with independent measuring and control of process parameters. Therefore it is a very powerful solution for the execution of optimization experiments following DoE.

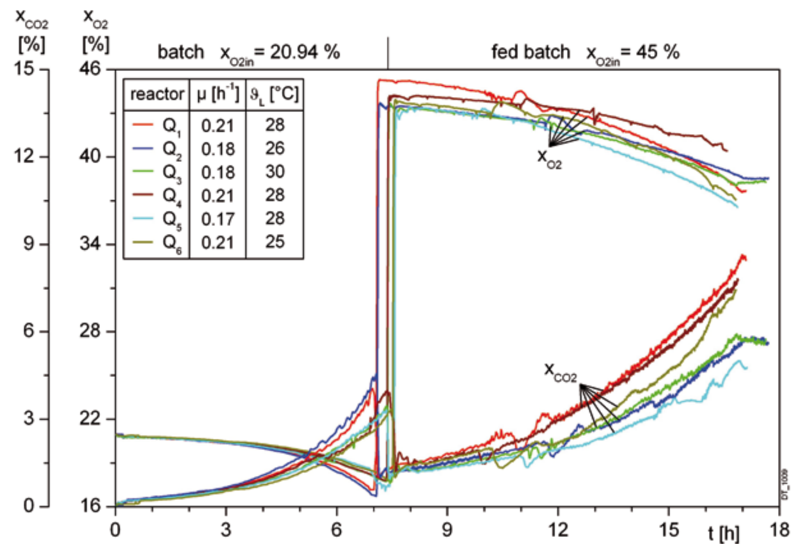
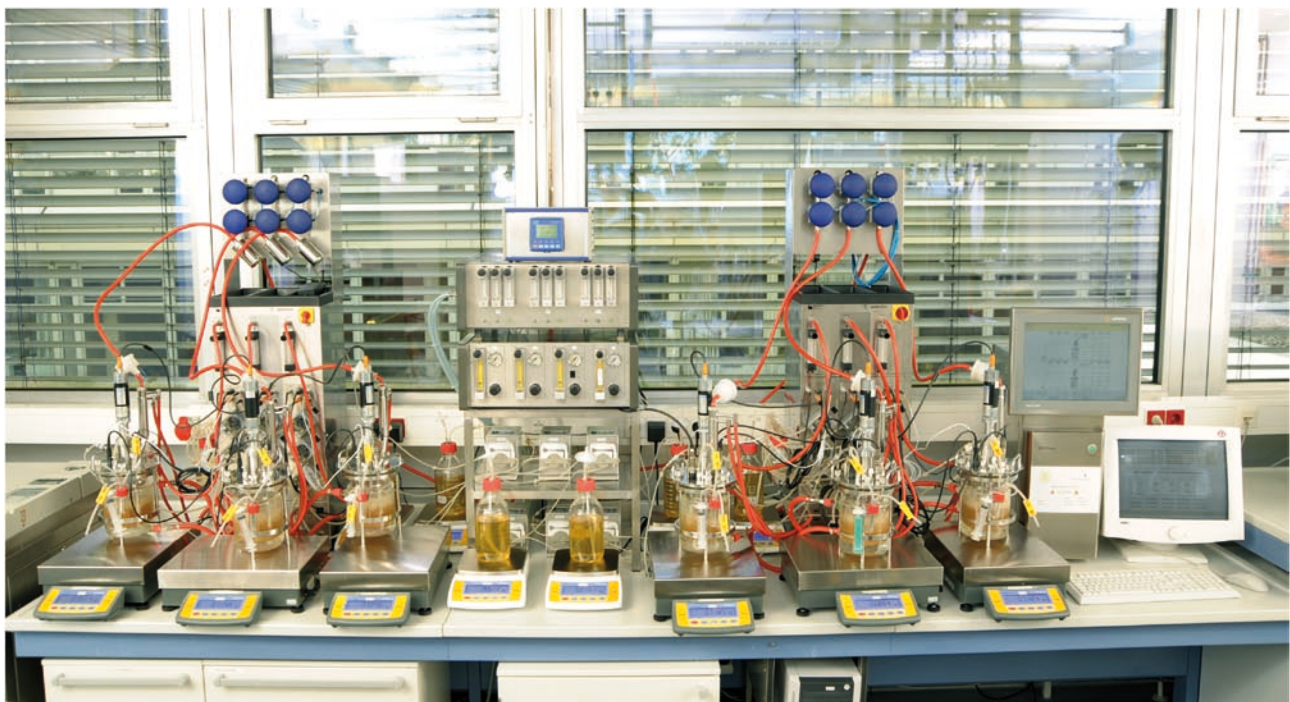


Figure 1: O_2- and CO_2-signals from one experiment showing all six vessels with batch phase followed by a fed batch phase

The system consists of two supply towers, a digital control unit DCU 4 and six autoclavable 1 l culture vessels. Each vessel is equipped with probes for measurement of pO_2, pH and foam. Two external gasmix stations with mass flow controllers are used for aeration up to 2 vvm. A pump station enables different substrate limited fed-batch operations with reduced cell specific growth rates.

With the BlueSens sensors BCP-O_2 for oxygen and the



Multi-bioreactor system BIOSTAT® Qplus 6 for the execution of DoE optimization experiments

$BCP-CO_2$ sensors for carbon dioxide the measurement of these gases in the off-gas of each vessel is possible. The multiplexer unit BACCom transferring the off-gas values to the process control system MFCSwin, where data are recorded and further online calculations are carried out.

Experiments for the optimization of the space-time-yield of a recombinant fusion protein expressed in Escherichia coli are conducted. The process starts with a glucose batch, followed by a fed batch phase and the IPTG induced production phase.

Figure 1 shows the course of the off-gas measurement of all six vessels from the multi-reactor system. The initial conditions in every single reactor are the same. Also for the batch part all parameters are identical. This can be seen in an almost identical course of the six curves in the batch phase and the very small variation of the batch end time. In the fed batch phase the cell specific growth rate μ and the liquid phase temperature JL are changed to different values (see figure 1). Also the incoming oxygen mole fraction xO_2 was increased stepwise from 20.94 % (AIR) to 45 % (AIR/O_2) to avoid oxygen limited cell growth.

The production phase of two different DoE experiments is plotted in figure 2. For a better comparison of the two experiments the timeline of the chart is standardized onto the point of induction at the beginning of the production phase. The plot shows the observable cell specific growth rate , estimated online from the off-gas signals xO_2 and xCO_2, the fluorescence signal S48/53_sol of the soluble fusion protein measured in relative fluorescence units (RFU) and the cell density cXL determined from cell dry mass. The setpoint of the cell specific growth rate μw, realized with an open loop controlled glucose fed batch, was set to 0.18 h-1 in experiment 1 and 0.21 h-1 in experiment 2. After induction the growth rate is decreasing due to the change in metabolism and a reduced liquid phase temperature in the production phase, but it is increasing afterwards and shows an almost constant course.

The chosen parameters in experiment 1 yield in a much higher target protein concentration compared to experiment 2.

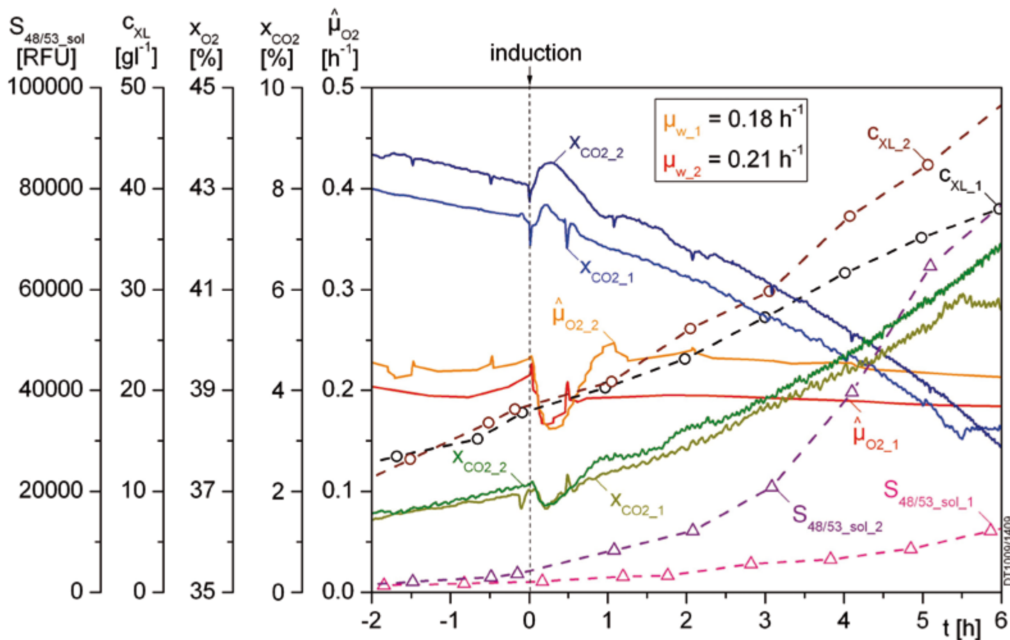


Figure 2: -estimation with off-gas measurement and O_2-balancing