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Parallel CHO Cell Cultivation in Eppendorf BioBLU® c Single-Use Bioreactors

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Abstract

In industrial bioprocess development time and cost pressure is high. A promising strategy to reduce the development time is to combine parallel experimentation with the use of single-use equipment. In this study we cooperated with Dieter Eibl and his team at Zurich University of Applied Sciences (ZHAW) to assess the reproducibility of parallel cell culture process runs in a DASbox® Mini Bioreactor System equipped with BioBLU Single-Use Vessels. We compared cell growth, viability, and the metabolic profile in four parallel bioprocesses and found that the processes developed highly similar. These results confirm the excellent suitability of this bioprocess system for efficient process development.

Introduction

Bioprocess development aims at maximizing the yield and the quality of the desired end product while minimizing the costs. Numerous process variables exist, including the producer strain, the medium composition, the process duration, the feeding strategy, and process parameters like temperature, pH, and DO. Researchers usually optimize many of them by empirical experimentation, which requires many process runs.

Parallel bioprocess systems have the great advantage that multiple experimental parameters can be tested simultaneously in one run, which ensures maximal comparability between runs and saves precious time. The development time can be further reduced by the use of single-use bioreactors. With single-use equipment lengthy cleaning and sterilization procedures are omitted and the time between runs can be reduced.

The aim of this study was to test whether cell culture processes run in a parallel DASbox Mini Bioreactor System equipped with BioBLU Single-Use Vessels perform comparably. To assess this, the Eibl team analyzed cell growth and viability, and the metabolic profile in four parallel CHO cell processes.

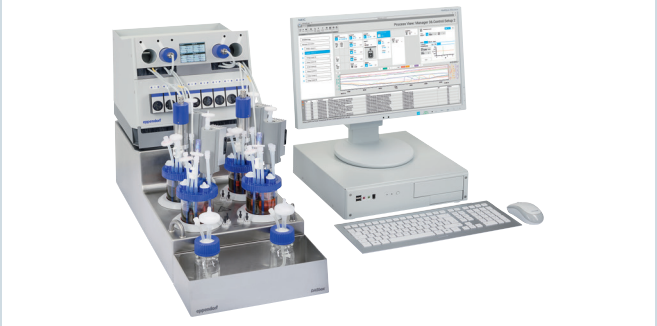

Fig. 1: DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Vessels.

Materials and Methods

Bioprocess system and vessel

The researchers carried out the processes in BioBLU 0.3c Single-Use Vessels with a maximum working volume of 250 mL. They controlled four vessels in parallel using a DASbox Mini Bioreactor System. The processes were controlled using DASGIP® Control software*.

Cell line and inoculation

The researchers used the suspension cell line CHO XM 111-10 (CCOS no. 837). To prepare the bioreactor inoculum the cells were expanded in single-use shake flasks in chemically defined ChoMaster® HP-1 medium (Cell Culture Technologies, Switzerland) at a shake rate of 120 rpm and an amplitude of 25 mm. The researchers inoculated all bioreactors to an initial viable cell density of 0.5 x 10^6 cells/mL.

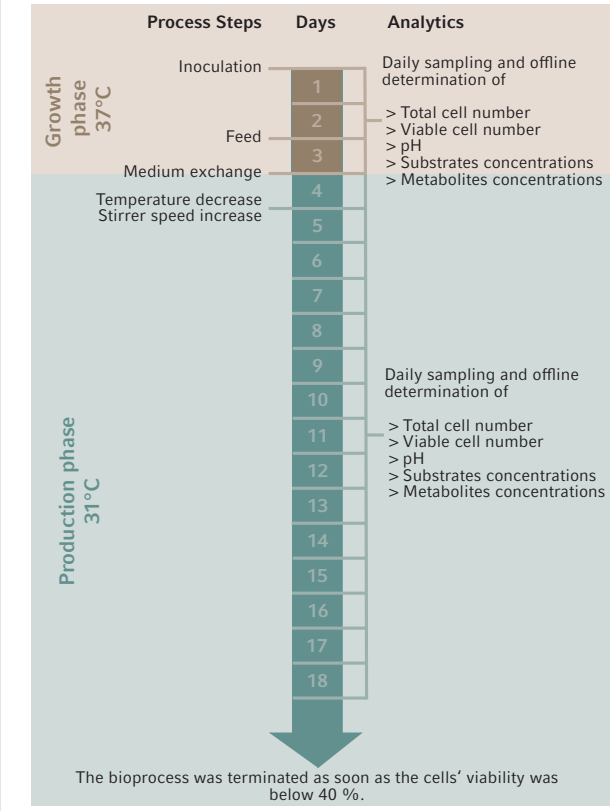

Fig. 2: Process of CHO cell cultivation in a DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Vessels.

*DASGIP Control is now DASware® control 5.

Process parameters and cultivation procedure

Figure 2 illustrates the cultivation procedure and Table 1 summarizes the cultivation parameters.

Table 1: Parameters for the cultivation of CHO suspensions cells in BioBLU 0.3c Single-Use Vessels

Parameter	Growth phase	Production phase
Initial viable cell density	0.5 x 10^6 cells/mL	
Culture medium	ChoMaster HP-1 and HP-5 medium (+ 0.2 % Pluronic F-68)	ChoMaster HP-5 medium (+ 0.2 % Pluronic F-68)
Culture volume	170 - 240 mL	250 mL
Temperature	37°C	31°C*
Stirrer speed	180 - 290 rpm	340 rpm*
Mixing time	5 s	4 s
Dissolved oxygen	30 %	30 %
pH	7.2	7.2
Gasflow	0.05 vvm	0.05 vvm
Gas destination (air, O_2, N_2 and CO_2)	submerse	submerse

* Temperature and stirrer speed were adjusted 24 hours after the medium exchange.

To mimick a typical protein production process, the process was carried out in two phases. During the initial phase the researchers cultivated the cells at 37°C to provide optimal conditions for growth. Subsequently they reduced the temperature to 31°C, as it is typically done in the protein production phase of a bioprocess. During the initial growth phase the researchers cultivated the cells in 170 mL ChoMaster HP-1 medium. Two days after inoculation they fed the culture by addition of 70 mL ChoMaster HP-5 medium.

Three days after inoculation the medium was exchanged with ChoMaster HP-5 medium. For the removal of the growth medium the bioreactor control was switched off so that the cells settled down. After medium removal the bioreactors were re-filled with ChoMaster HP-5 medium to the maximum working volume of 250 mL. All media contained 0.2% Pluronic® F-68 BioChemica (PanReac AppliChem, USA) as the protective agent against mechanical stress.

The cultures in the BioBLU 0.3c vessels were fed and harvested using the system's integrated pumps. Anti-foam agent was added by pumping.

The temperature was measured using Pt100 sensors and controlled by the heater integrated into the DASbox Mini Bioreactor System. The BioBLU 0.3c vessels were equipped

with DASGIP DO sensors and potentiometric pH probes. The pH was controlled at 7.2 by addition of CO_2. DO was controlled at 30 % by addition of pure oxygen. All gasses were introduced through a submerged sparger. To minimize foaming the researchers set the total gas flow (flow air + flow O_2 + flow CO_2) to a low rate of 0.05 vessel volumes per minute (vvm).

In the course of the process the researchers adjusted the agitation speeds to achieve mixing times of 5 seconds during the growth and of 4 seconds during the production phase.

The researchers terminated the cultivation processes as soon as the cell viability decreased to less than 40 %.

Analytics

The researchers analyzed samples of each culture daily. During the growth phase they determined total and viable cell densities, cell viability, pH, as well as substrate and metabolite concentrations offline.

Cell density and viability were determined using a NucleoCounter® NC-200™ (ChemoMetec A/S, Denmark). The specific growth rate and the doubling time were calculated using the following equations:

Specific growth rate: $$\mu_i = \frac{\ln(x_{i+1}) - \ln(x_i)}{t_{i+1} - t_i}$$

Doubling time: $$t_d = \frac{\ln 2}{\mu}$$

The concentrations of glutamine, lactate, and ammonium were measured with a BioProfile® 100 Plus analyzer (Nova Biomedical®, USA). Offline pH was determined with a standard laboratory pH meter (Mettler Toledo®, Switzerland).

Results and Discussion

The Eibl team compared four processes operated in parallel in BioBLU 0.3c bioreactors (Unit 1-4).

Viable cell densities

The viable cell densities developed comparably in all four bioprocesses (Fig. 3).

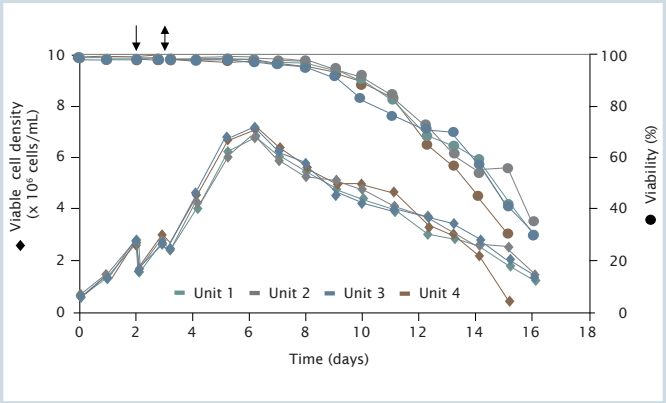

Fig. 3: Viable cell density and viability. The one-sided arrow indicates the time of growth medium addition. The two-sided arrow marks the medium exchange.

During the first two days the cells proliferated exponentially and reached viable cell densities of about 2.7 x 10^6 cells/mL. 48 hours after inoculation the cell densities dropped slightly because of the addition of feed medium. After medium addition the cultures reached viable cell densities between about 2.7 x 10^6 and 3.1 x 10^6 cells/mL. In the growth phase the cells multiplied with average specific growth rates of 0.030 h^{-1}, which corresponds to a doubling time of 24 hours. Some cells were lost when the growth medium was exchanged for production medium three days after inoculation. During the first 24 h after medium exchange, the cells grew with average specific growth rates of 0.024 h^{-1}. The cell densities reached their maximum six days after inoculation.

During the growth phase more than 98.6 % of the cells were viable in all bioreactors, and the cell viabilities remained nearly constant until the cultures reached their peak densities. The viabilities started to decrease nine days after inoculation.

Substrates and metabolites

Figure 4 shows the concentration profiles of the substrates glucose and glutamine, as well as their metabolites lactate and ammonium. The concentration of both substrates decreased equally in all units during the first 47 hours. The initial concentration of 3.64 g/L glucose dropped to 0.52 g/L glucose. Glutamine was already consumed after one day. As a consequence of medium addition on the second day, the glucose concentrations increased again. The medium exchange at day 3 led to similar glucose concentrations of 4.32 g/L in all four bioreactors. During the production phase glucose and glutamine concentrations dropped fast within the first 24 h. After the temperature shift from 37°C to 31°C on the fourth day after inoculation, glucose consumption decelerated. By then the glutamine was already depleted. The formation of the metabolites lactate and ammonium correlated with the uptake of glucose and glutamine. The processes started with lactate concentrations of 0.11 g/L and ammonium levels of 0.9 mmol/L. During the growth phase, the rates of lactate and ammonium production were comparable between all four units. Before the medium exchange after day three, the lactate concentration reached 2.22 g/L and the ammonium level was 2.35 mmol/L. During the first 24 h after the temperature shift to 31°C the lactate concentration increase from 0.62 g/L to 2.09 g/L. After the temperature shift on day 4, the glucose concentrations fell below 2 g/L while lactate was consumed continuously until the end of the bioprocess. The increase of ammonium concentration during the production phase was also comparable among the vessels. Final ammonium concentrations were about 6.0 to 6.5 mmol/L.

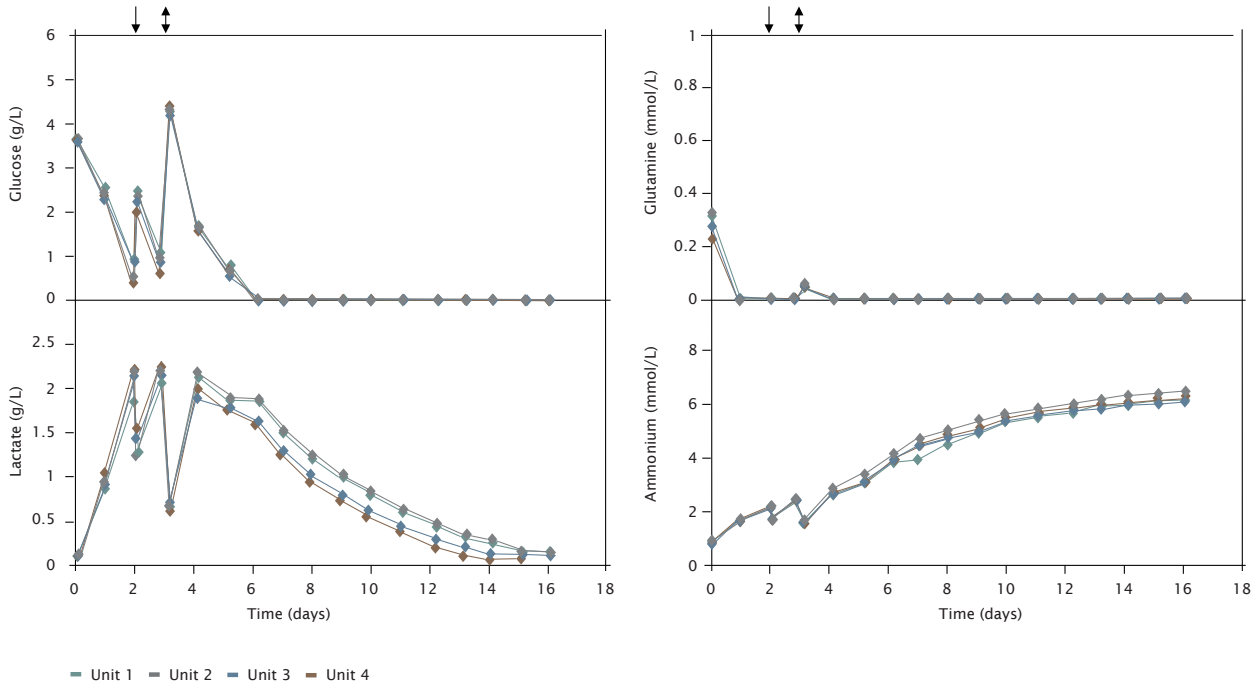

Fig. 4: Metabolic profiles. The concentrations of glucose, lactate, glutamine, and ammonium were determined offline.The one-sided arrow indicates the time of the growth medium addition. The two-sided arrow marks the medium exchange.

Conclusion

The team at ZHAW analysed four CHO cell culture processes, which they carried out in parallel. The aim of the study was to assess the reproducibility of process performance within the DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Vessels. The standard deviations of peak viable cell densities, and growth rates were only 7 % between the units 1-4. The metabolic profiles were almost identical.

In summary, this case study demonstrates that the system used is suitable for cell culture process development.

Parallel operation of multiple bioreactors and the use of single-use equipment can help reducing development times and ulimately costs.

Ordering information

Description	Order no.
DASbox® Mini Bioreactor System for Cell Culture Applications, max. 5 sL/h gassing	
4-fold system for single-use vessels	76DX04CC
8-fold system for single-use vessels	76DX08CC
16-fold system for single-use vessels	76DX16CC
24-fold system for single-use vessels	76DX24CC
BioBLU® c Single-Use Vessels	
BioBLU® 0.3c Single-Use Vessel, cell culture, 1 pitched-blade impeller, sterile, 4-pack	78903508

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