

Process transfer of CHO cultivations using the Minifors 2 as an example

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Introduction

The scale-up of cell cultivations is frequently challenging, since process parameters cannot be adopted on a one-to-one basis. Initially, the systematic characterization of the bench-top bioreactor Minifors 2 is shown in this application note. Then a scale-up from the shake flasks to the Minifors 2 takes place, as well as a process transfer between the Multifors 2, Labfors 5 and Minifors 2, as an example.

Technical specifications

The technical specifications of the Minifors 2 (Fig. 1) can be summarized as follows:

- Culture vessels: 1.5 L, 3.0 L or 6.0 L total volume (V_t) with rounded, flat bottom
- Stirring speeds from 24 to 600 min^{-1} (corresponds to tip speeds of 0.065 to 2.670 m s^{-1})
- A 3-blade segment impeller with diameters of 50 mm (1.5 L vessel), 65 mm (3.0 L vessel) and 85 mm (6.0 L vessel)
- Five mass flow controllers for gassing with air, nitrogen, oxygen and carbon dioxide with up to 0.15 vvm
- Four freely configurable pumps with optional gravimetric feeding
- Gentle temperature control
- Recording of online parameters using the bioprocess platform software eve[®]
- Optional: Integration of sensors for exit gas analysis and online biomass measurement

Experimental specifications

a. Process engineering characterization

For the purpose of process-engineering characterization, the $k_L a$ value as well as the mixing time were determined by experiment and the volumetric power input was numerically determined. The experimental procedures used correspond to the recommendations of the DECHEMA (Society for Chemical Engineering and Biotechnology) expert group on single-use technologies, which are described in the guideline "Recommendations for process engineering characterization of single-use bioreactors and mixing systems by using experimental methods" [1].

For the mixing time measurements, the destaining method was used in which an iodine-potassium iodide-starch complex is destained through the pulsed addition of sodium thiosulfate. For the $k_L a$ determination, the bioreactors were initially degassed with nitrogen until the dissolved oxygen concentration was less than one percent. Gassing with process air was then performed with the selected process parameters. The measurement method was able to be performed automatically through the use of the bioprocess software eve[®].

The power input was determined by *Computational Fluid Dynamics* (CFD) [2]. Using the OpenSource software OpenFOAM (Version 5.0), the torque was determined in a single-phase simulation.



Fig. 1: Minifors 2 with 6.0 L glass vessel

b. Cultivation with Chinese Hamster Ovary (CHO) cells

The scale-up trials were performed with the CHO cell line DP-12 (clone 1934, subclone of the University of Bielefeld, Prof. Noll) in chemically defined TC-42 medium (Xell AG). The comparative cultivations between the INFORS HT bioreactors were performed with the CHO cell line XM 111-10 (CCOS 837, established by Prof. Fussenegger, ETH Zürich) in the chemically defined minimal medium CHOMaster[®] HP-1 (Cell Culture Technologies LLC). Both test series were performed as a batch at 37 °C with headspace gassing of 0.1 vvm, a pO_2 target value of $30 \pm 10\%$ (regulation via pure oxygen), and a pH target value of 7.2 ± 0.1 (regulation via CO_2). The cell count measurement was performed using NucleoCounter NC-200 and the metabolite determinations were performed using BioProfile 400 and Cedex Bio.

Process-engineering characterization

The process-engineering characterization was performed in all three vessels at 33 %, 50 % and 67 % of the total volume (V_t) and stirrer speeds between 50 min^{-1} and 300 min^{-1} . Gassing rates between 0.05 vvm and 0.15 vvm were selected for the $k_L a$ value determination.

a. Mixing time

For all three vessel sizes, very brief mixing times were able to be determined which were in the range of 2 s (300 min^{-1} , 33 % relative filling volume) to 2 min (50 min^{-1} , 67 % relative filling volume). With an increasing vessel size, the maximum mixing times decreased from 123 s in the 1.5 L vessel to 27 s in the 6 L vessel. Starting at a tip speed of 0.6 m s^{-1} , the mixing time in all systems was below 10 s (Fig. 2). The dependence of the mixing time on the stirring speed and filling volume is shown in Fig. 3 using the 1.5 L vessel as an example.

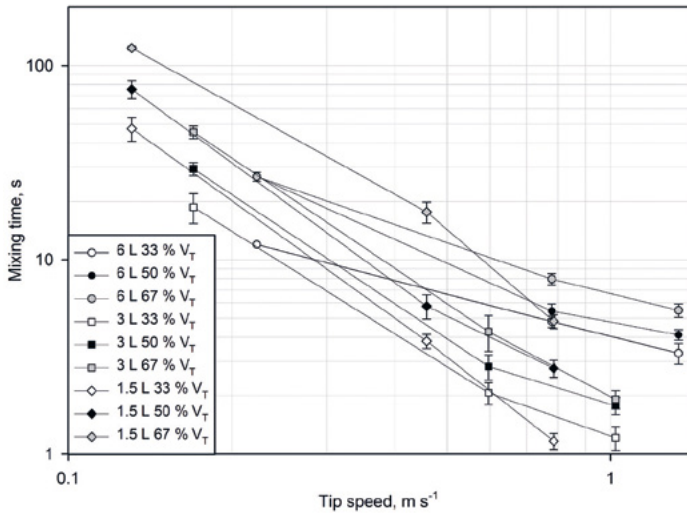


Fig. 2: Mixing time of the three vessel sizes at 33 % (white), 50 % (black) and 67 % (gray) of the total volume depending on the tip speed.

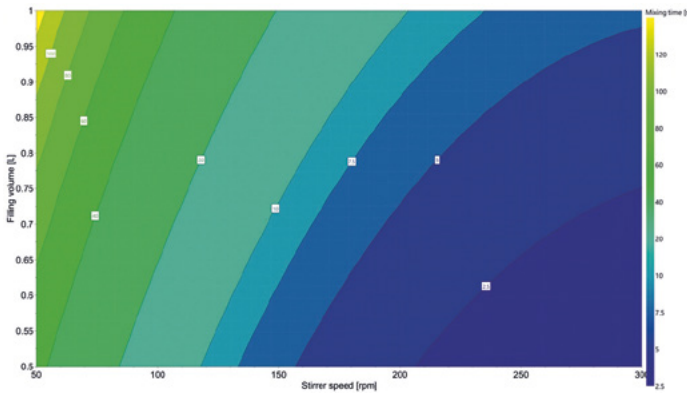


Fig. 3: Mixing times in the 1.5 L vessel with variation in stirring speed and filling volume, yellow (> 2 min) to blue (< 2.5 s).

b. $k_L a$ value

The measured $k_L a$ values were in the range of 2.5 h^{-1} (minimal working volume and gassing rate, all vessels) to 25 h^{-1} (1.5 L vessel), 45 h^{-1} (3.0 L vessel) and 70 h^{-1} (at 300 min^{-1} and maximal filling volume). At the same tip speed and gassing rate, the $k_L a$ values in all three vessels are highly comparable (Fig. 4). Higher stirrer speeds and filling volumes led to higher $k_L a$ values (Fig. 5).

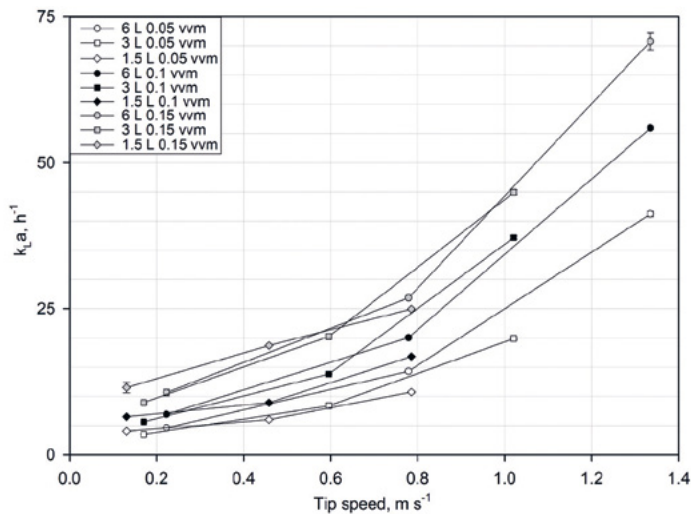


Fig. 4: $k_L a$ values in the 1.5 L (diamond), 3.0 L (square) and 6.0 L vessel (circle) at 0.05 vvm (white), 0.1 vvm (black) and 0.15 vvm (gray) at 67 % total volume.

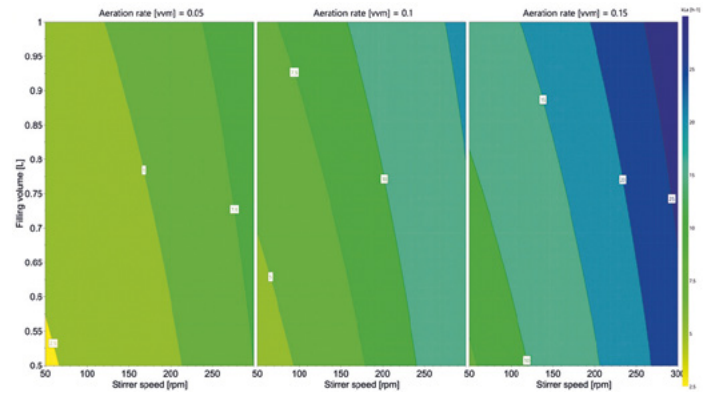


Fig. 5: $k_L a$ values in the 1.5 L vessel with variation in stirrer speed and filling volume, from yellow (< 2.5 h^{-1}) to blue (> 25 h^{-1}).

c. Specific power input

The CFD simulations (Figure 5) in the test chamber yielded maximum power inputs of 523 W m^{-3} (1.5 L), 574 W m^{-3} (3.0 L), and 385 W m^{-3} (6.0 L vessel) at 33 % of the nominal volume and a tip speed of 1335 m s^{-1} (corresponds to stirrer speeds of 510 min^{-1} in the 1.5 L vessel, 390 min^{-1} in the 3.0 L vessel and 300 min^{-1} in the 6.0 L vessel). At a stirrer speed of 600 min^{-1} in the 6.0 L vessel (system limit), power inputs of more than 2 kW m^{-3} are possible (Fig. 7), however these are rather unsuitable for cell cultures and cause increased foaming due to the funnel formation. The power input can be estimated using equations 1 to 3.

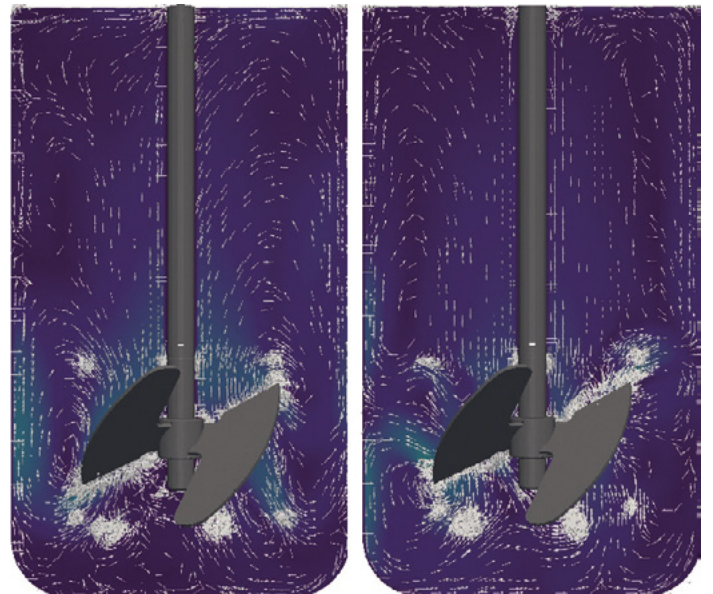


Fig. 6: Comparison of downwards (left) and upwards (right) flow in the Mini-forms 2, 6.0 L vessel.

$$33 \% V_T \quad 251.07 \cdot u_{\text{Tip}}^{2.5167} \quad (1)$$

$$50 \% V_T \quad 177.60 \cdot u_{\text{Tip}}^{2.7027} \quad (2)$$

$$67 \% V_T \quad 131.79 \cdot u_{\text{Tip}}^{2.7670} \quad (3)$$

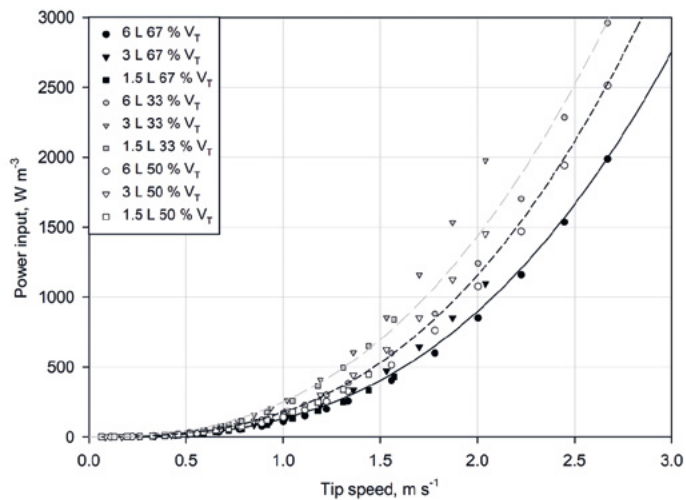


Fig. 7: Volumetric power input in the 6.0 L (circle), 3.0 L (triangle) and 1.5 L vessel (square) at 67% (black), 50% (gray) and 33% (white) filling volume.

Scale-up tests

a. Scale-up from the 250 mL shake flask to the Minifors 2, 1.5 L vessel

For the scale-up, the growth and IgG formation of CHO DP-12 cells in TC-42 medium were investigated over 8 days at a power input of 19 W m^{-3} (internal standard condition). The control worked smoothly within the dead band range ($30 \pm 10\%$) (Fig. 9).

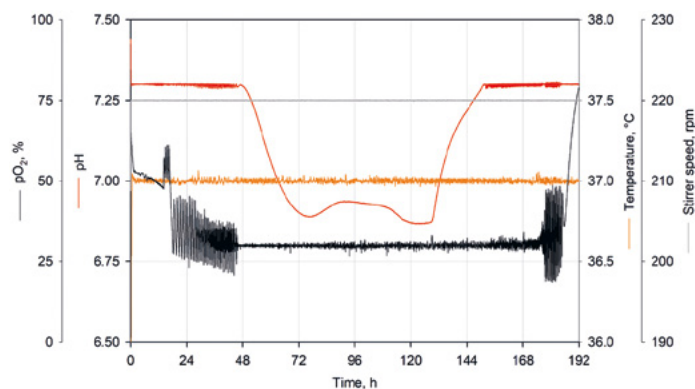


Fig. 8: Temperature (orange), stirrer speed (gray), pH value (red) and dissolved oxygen concentration (black) during the cultivation of CHO DP-12 cells in the 1.5 L vessel with a 1 L working volume.

In this test, the viable cell density was able to be increased within 6 days from 0.3 to $15.5 \cdot 10^6 \text{ cells mL}^{-1}$, whereby the maximum growth rate was 0.05 h^{-1} (Fig. 10). In parallel to this, the IgG concentration increased until full consumption of the glucose after 160 hours to 260 mg L^{-1} .

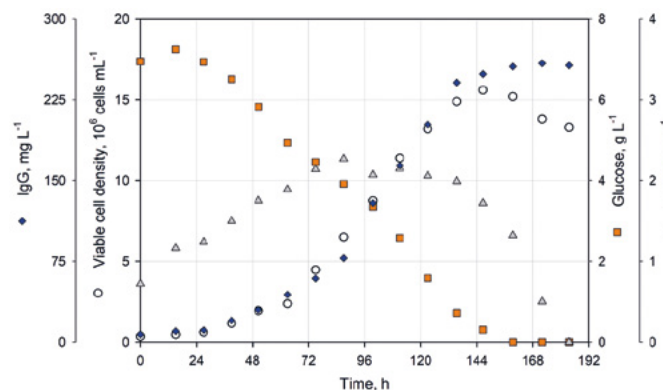


Fig. 9: Progression of the viable cell density (white circles) as well as the IgG (blue diamonds), glucose (orange squares) and lactate concentrations (gray triangles) in the CHO DP-12 batch trial.

In the direct comparison with the shake flask (250 mL nominal volume, 80 mL filling volume), comparable maximum growth rates, viable cell densities, and IgG concentrations were determined (Fig. 11). In the subsequent course of the cultivation, a slightly higher maximum viable cell density (15.5 for $14 \cdot 10^6 \text{ cells mL}^{-1}$) and IgG concentration (260 for 249 mg L^{-1}) were noted in the Minifors 2.

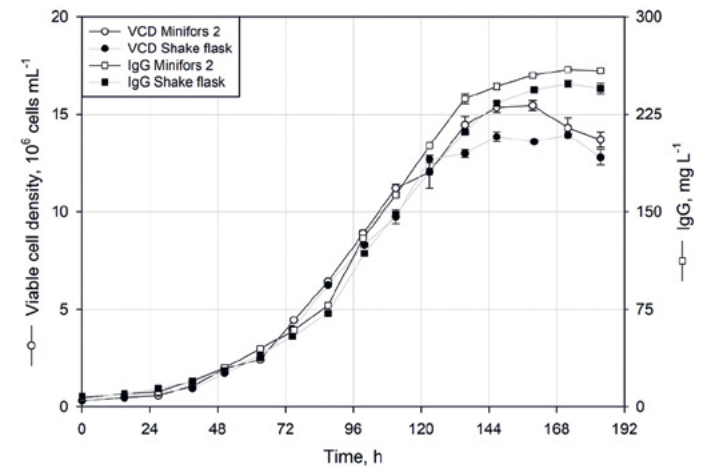


Fig. 10: Comparison of the viable cell densities (circles) and IgG concentrations (squares) between Minifors 2 (white) and Corning 250 mL Erlenmeyer flask (black) at a power input of 19 W m^{-3} .

b. Comparison with the bench-top bioreactors Multifors 2 and Labfors 5

The comparison trials in the Multifors 2, Labfors 5 and Minifors 2 were performed with the CHO XM 111-10 cell line. The focus was on the mass reproduction of the cells in the CHOMaster HP-1 growth medium in the batch mode. The good controllability of the dissolved oxygen concentration and pH value is evident in Fig. 7; the decrease in pH value between 36 h and 60 h can be explained by the increased formation of lactate.

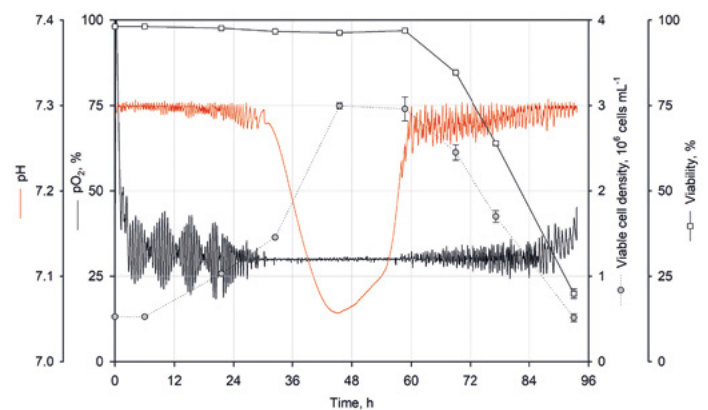


Fig. 11: CHO XM 111-10 cultivation in the Minifors 2, 1.5 L vessel. The viable cell densities (gray circles), viability (white squares), dissolved oxygen concentrations (red line) and pH value (black line) are shown.

The comparison between the bioreactors was performed via a multiparametric selection of the process-engineering parameters in all vessels (power input $< 50 \text{ W m}^{-3}$, mixing time $< 30 \text{ s}$, $k_L a > 5 \text{ h}^{-1}$) in order to achieve optimal growth. All bioreactors were inoculated with a cell density of $0.5 \cdot 10^6 \text{ cells mL}^{-1}$ and demonstrated comparable growth (Fig. 8). The maximal growth rates – with $\mu_{\text{max}} = 0.045 \text{ h}^{-1}$ (Minifors 2), $\mu_{\text{max}} = 0.045 \text{ h}^{-1}$ (Labfors 5) and $\mu_{\text{max}} = 0.042 \text{ h}^{-1}$ (Multifors 2) – can be compared equally well and correspond to the values in the literature [3].

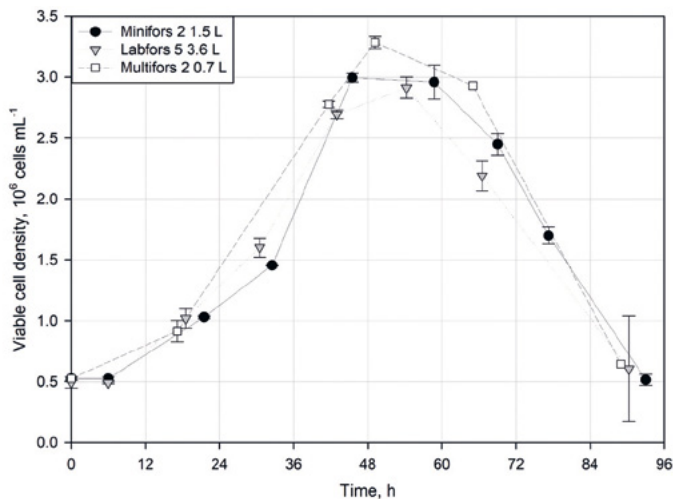


Fig. 12: Comparison of the viable cell density of Minifors 2 (1.0 L working volume, black circles), Labfors 5 (2.3 L working volume, gray triangles) and Multifors 2 (0.5 L working volume, white squares) in the case of a cultivation with CHO XM111-10 cells.

Summary

In the process-engineering characterization, the Minifors 2 demonstrated very short mixing times (2 s to 2 min) with simultaneously high $k_L a$ values (2.5 to 70 h^{-1}) and low power inputs (up to 385 W m^{-3} at 300 min^{-1} in the 6.0 L vessel), whereby the Minifors 2 is recommended for CHO cell culture processes but also other shear-sensitive cell types.

The scale-up from the shaker flask to the Minifors 2 yielded highly comparable growth curves, whereby a maximum cell density of $15.5 \cdot 10^6 \text{ cells mL}^{-1}$ and an IgG concentration of 260 mg L^{-1} were able to be reached in the batch test with CHO DP-12 cells, which fully met the expectations.

With the selection of suitable parameters, the scale-up between the various INFORS HT bioreactors is possible without any problems and thus a process transfer to the Multifors 2 and Labfors 5 with the desired results can be easily performed.

Literature

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- [3] J. López-Meza, D. Araíz-Hernández, L. M. Carillo-Cocom, F. López-Pacheco (2015) Using simple models to describe the kinetics of growth, glucose consumption, and monoclonal antibody formation in naive and infliximab producer CHO cells, DOI: 10.1007/s10616-015-9889-2