Simplifying Progress

Advantages of CHO cell process intensification in state-of-the-art single-use bioreactors

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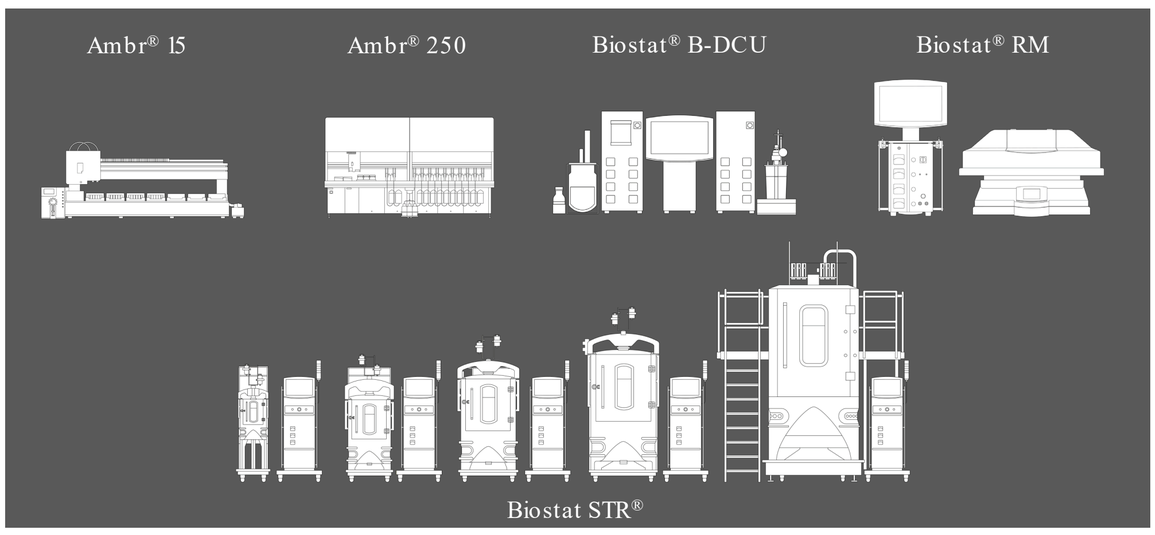
Introduction

Process intensification of mammalian biomanufacturing is a key approach to solve upcoming industrial challenges[1]. Cost pressure rises as average sales prices per drug decrease, whereas research and development costs of new therapeutics increase. Additionally, the ever-growing variety of low volume products requires flexible multi-product facilities and platforms. Thus, highly productive single-use (SU) manufacturing technologies accelerating process development and facilitating implementation at manufacturing scale are required.

In this study, we present the new Univessel® SU 10 L bridging the gap for (a) low-volume processes under GMP conditions and (b) facilitating the scale-up from process development (PD) to commercial manufacturing (CM) scale. Experimental batch data demonstrate the seamless transferability of both a CHO cell N-1 perfusion and fed-batch process from the Ambr® 250, over the Univessel® SU 10 L into the Biostat STR® 50.



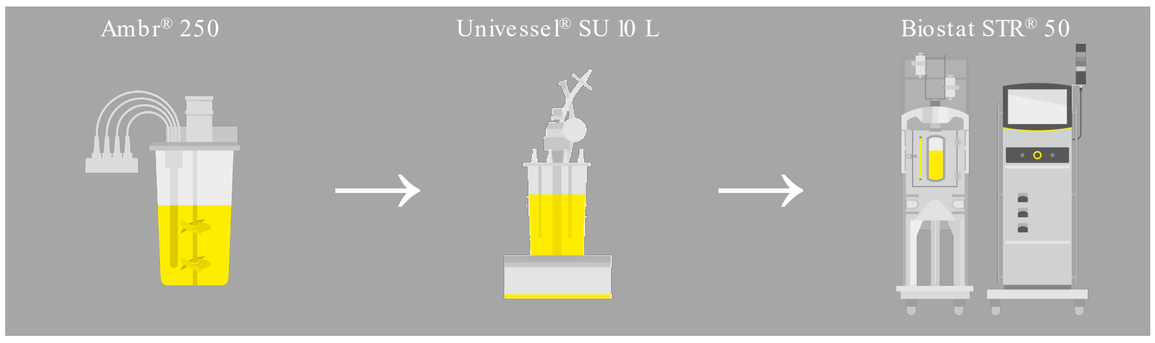


Figure 1: Scalable single-use bioreactor portfolio from PD to CM scale. Focus of this study is on process transfer of CHO cell N-1 perfusion and fed-batch processes between Ambr® 250, Univessel® SU 10 L and Biostat STR® 50.

1. Experimental Approach

Both processes were selected from the Sartorius upstream model process platform using the same CHO DG44 cell line (Sartorius) expressing a monoclonal antibody (mAb, IgG1). SmartCHO cell culture medium (Sartorius) was used in both cases, the perfusion media was identified by design of experiments[2].

N-1 Perfusion Process

Perfusion in the Ambr® 250 high throughput (HT), Univessel® SU 10 L (XCell® ATF2, Repligen) and the Biostat STR® 50 (XCell® ATF6, Repligen) was conducted at 36.8°C, 40 % DO and a pH of 7.1. A k_La of 36 h^{-1} (micro-sparger) was used to scale-up the process from the Univessel® SU 10 L into the Biostat STR® 50. After an initial batch phase of three days, perfusion was started at 0.25 vvd at a VCC of 2.5M c·mL^{-1}. Perfusion was controlled via on-line CSPR-control using BioPAT® ViaMass at 40 pL·(c·d)$^{-1}$. The process is operated for six days in perfusion mode to obtain 80 M c·mL^{-1}.

Fed-Batch Process

An industrially relevant, well-characterized CHO cell fed-batch process was used[3]. Scalability from Ambr® 250 upwards was ensured using tip speed of 1.2 m·s^{-1} to have turbulent flow as scaling criteria. Golden Batch modelling using SIMCA® 18 allows to compare new batch data sets of this process against historical data across various Sartorius bioreactors (mL – kL) on a multi-variate level, ensuring process transferability.

2. Results: N-1 Perfusion Process

The same N-1 perfusion process was conducted in the Ambr® 250 HT, Univessel® SU 10 L and in the Biostat STR® 50. In all three scales, a similar process performance was obtained. In the Ambr® 250 HT and the Biostat STR® 50 about 80 M c·mL^{-1} were reached in six days. This was also accomplished one day later in the Univessel® SU 10 L (data only shown until day 6), the delay being caused by a malfunction of the classical DO probe during day 4. A constant glucose concentration of about 4 g/L indicated a steady-state perfusion process using a constant CSPR of 40 pL·(c·d)$^{-1}$ controlled on-line. Intensified processes are demanding regarding the oxygen supply. Highlighted for the Univessel® SU 10 L and the Biostat STR® 50, the DO setpoint of 40 % was well maintained at controller output values of max. up to 80 % required for 80 M c·mL^{-1} (day 7 for UV-SU 10 L, not shown). Thus, even more challenging processes would potentially be feasible.

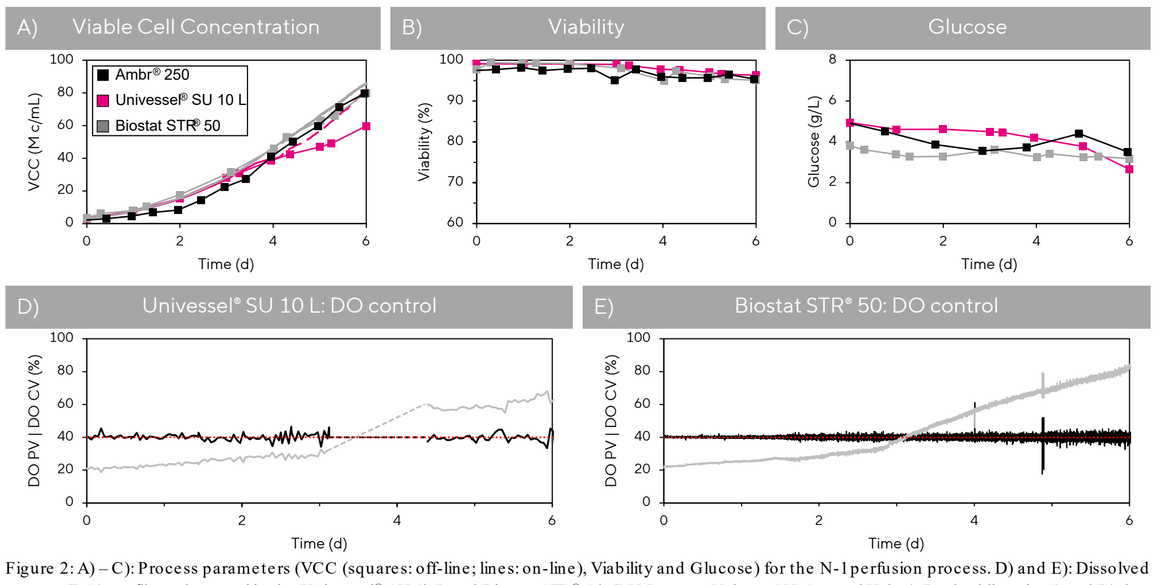


Figure 2: A) – C): Process parameters (VCC (squares: off-line; lines: on-line), Viability and Glucose) for the N-1 perfusion process. D) and E): Dissolved oxygen (DO) profile and control in the Univessel® SU 10 L and Biostat STR® 50 (PV: Process Values; CV: Control Value). Dashed lines in A) and D) due to malfunctioning of the classical DO probe used between day 3 and 4.

3. Results: Fed-Batch Process

The same CHO cell fed-batch process was conducted in all the Ambr® 250 (n=3), Univessel® SU 10 L (n=3) and the Biostat STR® 50 (n=1). Identical process performance was obtained when comparing individual parameters with our Golden Batch indicated by the three important critical process parameters VCC, viability and IgG titer (Fig. 3A – C), of which especially the values of the process end are relevant.
On a multi-variate level, it can be assessed that all batch trajectories are consistent over the entire process duration of 12 days (BLM) and cluster well with the historical Golden Batch data (BLM). With the Univessel® SU 10 L being centric for investigation of scalability, it can be clearly seen that the three batches from Fig. 3D &E) align very well with the existing bioreactor systems. This demonstrates and facilitates the ease of scalability from PD to CM scale.

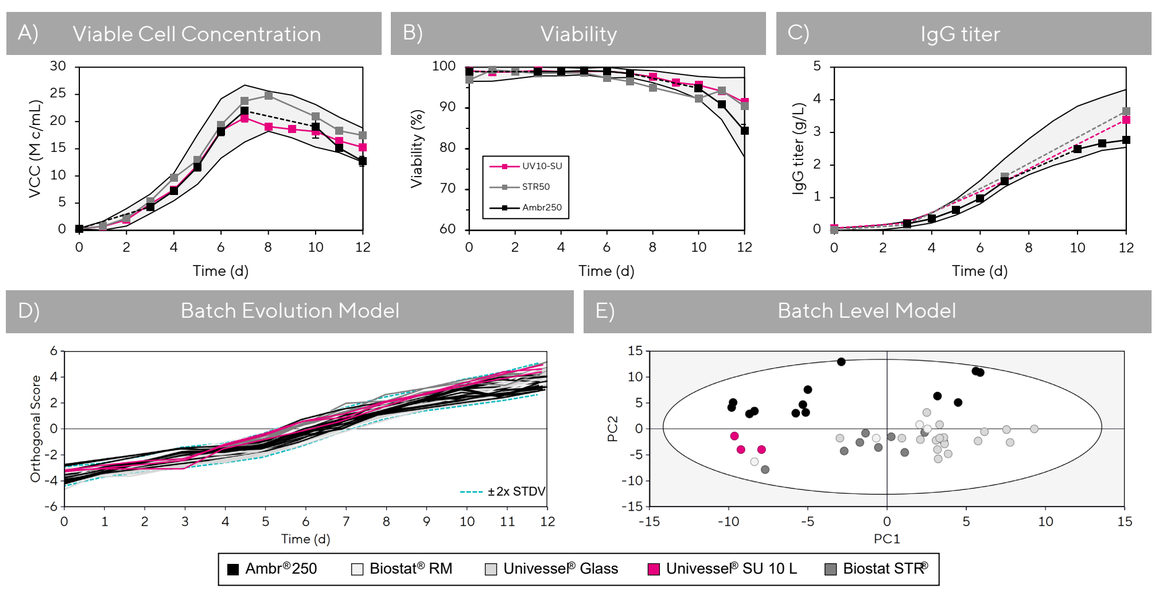


Figure 3: A) – C): Process parameters (VCC, Viability, IgG titer) for the CHO cell Fed-Batch process. Indicated in grey shading is the Golden Batch (Mean ± 2 x STDV) of this process across various bioreactor systems from Ambr® 250 up to Biostat STR® with a total of 45 batches included. D) and E): MVDA-based analysis using SIMCA® 18 for Golden Batch comparison via BEM and BLM (PC1 = 0.19, PC2 = 0.16). The Golden Batch comprising off-line parameters VCC, viability, cell diameter, IgG titer, glucose and lactate concentration, pO_2, pCO_2, pH and osmolality.

4. Conclusion

Nowadays, process intensification of mammalian biomanufacturing is a central tool for improving conventional production strategies, especially for upstream technologies. We showcase how important hurdles can be tackled:

1. The development of these upstream processes is challenging as it requires a combination of technological advancements and process understanding. High-throughput automated bioreactors, like the **Ambr® 250**, enable operation of multi-parallel experiments to explore the experimental design space and identify ideal process conditions.
2. The implementation and transfer of this process in the bench-top scale allows for further process optimizing and investigating the process, e. g. perfusion settings. Additionally, relevant product material can be obtained for clinical trials. Hereby, the **Univessel® SU 10 L**, a new addition to Sartorius' single-use bioreactor portfolio, minimizes time-consuming manual handling steps (preparation and post-processing) and mitigates potential contamination risks compared to multi-use bench-top systems. This reduces experimental downtimes while maximizing process knowledge gained.
3. Finally, intensified processes can be scaled-up into pilot-scale and beyond, a suitable system being the **Biostat STR® 50**. For the operation of perfusion processes, the full integration of the XCell® ATF Technology (Repligen) into the automation platform Biobrain® streamlines operational efforts and complexities whilst bundling batch record[4].

Concluding, the implementation of an intensified upstream process strategy, from development to manufacturing scales, relies on the scalability of the chosen bioreactor portfolio. A given and industrially relevant process strategy can be the intensification of the seed train by using N-1 perfusion for the subsequent inoculation of the production scale[5] (fed-batch). In this study, we demonstrated the scalability for both a CHO cell N-1 perfusion and fed-batch process between Ambr® 250, Univessel® SU 10 L and the Biostat STR®.

Univessel® SU 10 L

- Single-Use stirred tank bioreactor, 10 L maximum working volume
- Pre-sterilised &ready-to-use
- Available in three application-specific vessel designs: Essential, Perfusion &Cell Therapy
- Consistent bioreactor design with Sartorius' portfolio enabling vessel scalability: Ambr® 250 ↔ Univessel® SU 10 L ↔ Biostat STR®

Biostat STR®

- Biobrain® automation platform with stand-alone recipe and cGMP features
- Direct scalability: Ambr® 250 ↔ Biostat STR® 2000
- Perfusion ready: Fully integrated Repligen XCell® ATF Technology into Biobrain®
- Refined gassing control: 8 high-precision mass flow controllers and BioPAT® Pinch Valve
- Advanced cell and metabolite sensors for control of demanding processes

Acknowledgement

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References

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