Simplifying Progress

Advantages of Intensified CHO Cell Culture Using State-of-the-Art Single-Use Bioreactors

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Introduction

Process intensification is a key approach to solving upcoming challenges in mammalian biomanufacturing.[1] Cost pressures are growing as average sales prices per drug decrease, while research and development costs for new therapeutics continue to rise. Additionally, the ever-growing variety of low volume products requires flexible multi-product facilities and platforms. Thus, highly productive single-use (SU) manufacturing technologies are required to accelerate development and support the implementation of robust processes at manufacturing scale. In this study, we present the new Univessel® SU 10 L, which bridges the gap between (a) low-volume processes under GMP conditions and (b) scale-up from process development (PD) to commercial manufacturing (CM). Experimental batch data demonstrate the seamless transferability of an N-1 perfusion-based and a fed-batch CHO cell culture from the Ambr® 250, through the Univessel® SU 10 L into the Biostat STR® 50 (Figure 1).

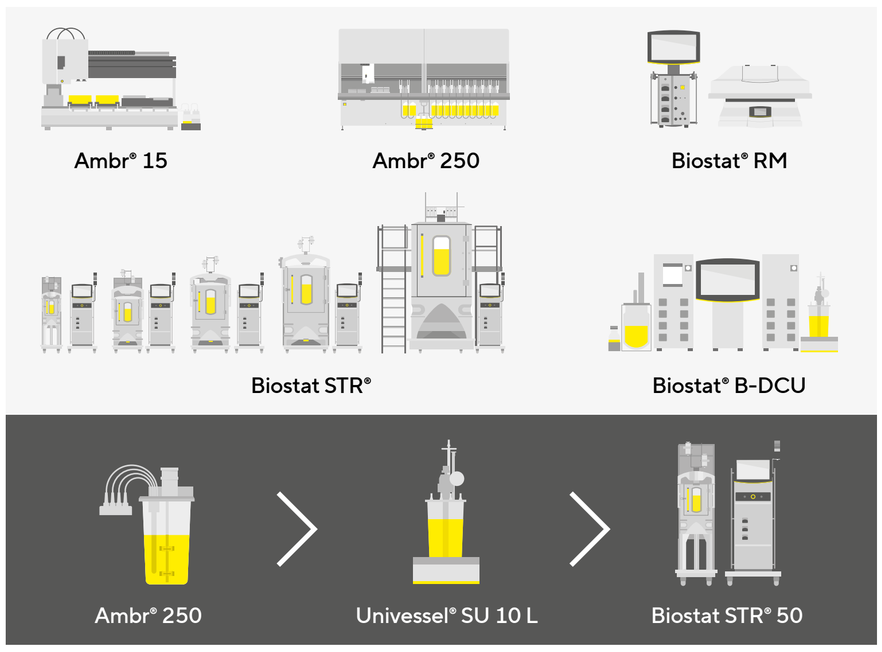


Figure 1: *Sartorius scalable single-use bioreactor portfolio from PD to CM scale. The focus of this study is on the transfer of N-1 perfusion-based and fed-batch CHO cell culture processes between Ambr® 250, Univessel® SU 10 L, and Biostat STR® 50 systems.*

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). 4Cell® SmartCHO medium (Sartorius) was used in both cases. Additional nutritional requirements for the perfusion process were identified by design of experiments.[2]

N-1 perfusion process
Perfusion in the Ambr® 250 high throughput, Univessel® SU 10 L (XCell® ATF2, Repligen) and the Biostat STR® 50 (XCell® ATF6, Repligen) was conducted at 36.8 °C, 40% dissolved oxygen (DO) and a pH of 7.1. A k_La of 36 h^{-1} (achieved using a microsparger) was applied 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 vessel volumes per day (vvd) at a viable cell concentration (VCC) of 2.5 million cells/mL. Perfusion was controlled online using BioPAT® Viamass at a cell-specific perfusion rate (CSPR) of 40 L/cell/day. The process is operated for six days in perfusion mode to obtain 80 million cells/mL.

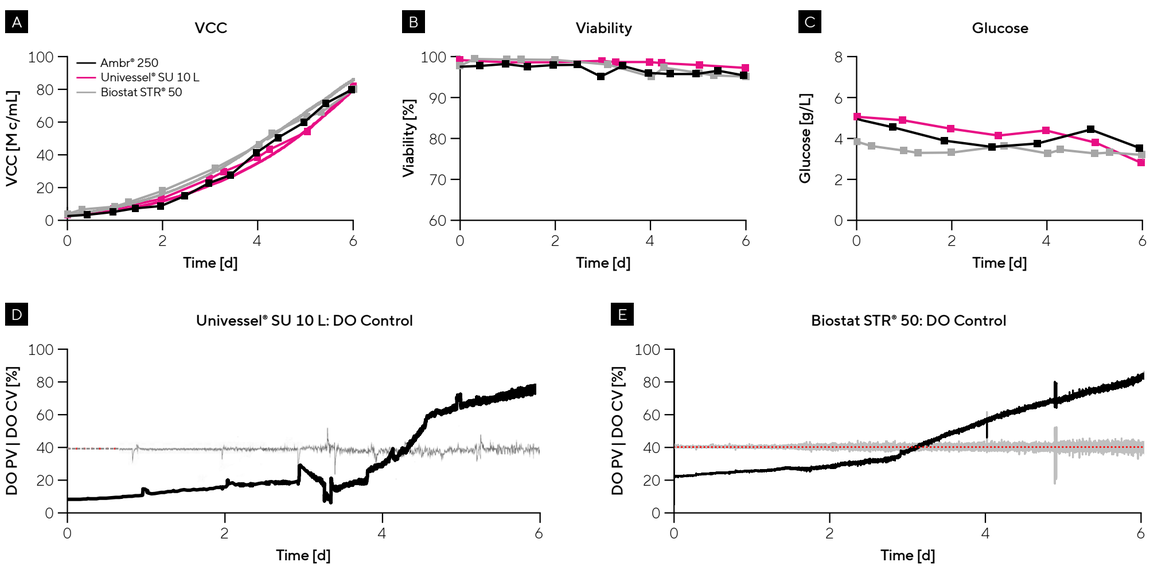
Fed-batch process
An industrially relevant, well-characterized CHO cell fed-batch process was used.[3] Scalability from Ambr® 250 upwards was ensured using a tip speed of 1.2 m/s as the scaling criterion to maintain turbulent flow. Golden Batch modeling using SIMCA® 18 allows comparison of new batch data sets from the current process with historical data across various Sartorius bioreactors (from milliliter to kiloliter scale) at a multivariate level, ensuring process transferability.

Results

1. N-1 perfusion process

The same N-1 perfusion process was conducted in the Ambr® 250 High Throughput, Univessel® SU 10 L, and Biostat STR® 50. At all three scales, a similar process performance was observed. In the Ambr® 250 High Throughput, Univessel® SU 10 L, and the Biostat STR® 50, a VCC of around 80 million cells/mL was reached in six days (Figure 2A). High viability was maintained across cultures (Figure 2B). A constant glucose concentration of around 4 g/L indicated a steady-state perfusion process using a constant CSPR of 40 L/cell/day controlled online (Figure 2C). Intensified processes place high demands on oxygen supply. For both the Univessel® SU 10 L, and the Biostat STR® 50, a DO setpoint of 40% was well maintained, with maximum controller output values of 80% required for cell densities of 80 million cells/mL (achieved on day 7 for Univessel® SU 10 L, data not shown).

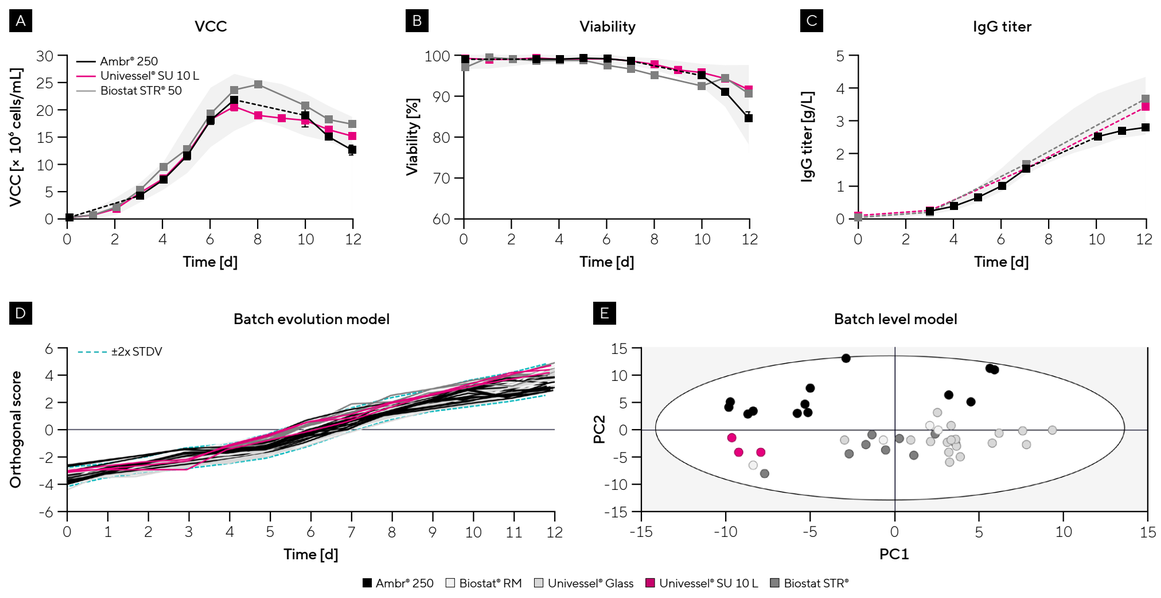
Figure 2: ***(A–C)*** *Process parameters (VCC, viability, and glucose) for the N-1 perfusion process.* ***(D and E)*** *DO profile and control in the Univessel® SU 10 L and Biostat STR® 50.*
Note. For VCC, squares represent offline measurements, lines represent online measurements. PV = process value, CV = control value. Dashed lines in A and D indicate data affected by the malfunction of the classical DO probe between days 3 and 4.



2. 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) systems. Identical process performance was observed when individual parameters were compared with the Golden Batch, as indicated by the three critical process parameters VCC, viability, and IgG titer (Fig. 3A–C), for which the process-end values are particularly relevant. Multivariate assessment showed that all batch trajectories are consistent across the process duration of 12 days (Batch Evolution Model, Figure 3D) and cluster well with the historical Golden Batch data (Batch Level Model, Figure 3E). The three batches from the Univessel® SU 10 L align closely with the existing bioreactor systems (Figure 3D and E), demonstrating seamless scalability from PD to commercial manufacturing CM scale.

Figure 3: ***(A–C)*** *Process parameters (VCC, viability, and IgG titer) for the CHO cell fed-batch process. Grey shading represents the Golden Batch (mean ± 2 × standard deviation) across various bioreactor systems from Ambr® 250 to Biostat STR®, including a total of 45 batches.* ***(D and E)*** *Multivariate data analysis–based Golden Batch comparison using SIMCA® 18 via batch evolution modeling and batch level modeling (PC1 = 0.19; PC2 = 0.16). The Golden Batch model includes offline parameters of VCC, viability, cell diameter, IgG titer, glucose and lactate concentrations, pO_2, pCO_2, pH, and osmolality.*



Conclusion

Process intensification is a central tool for improving conventional production strategies in mammalian biomanufacturing, especially for upstream operations. Here, we 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 the operation of multi-parallel experiments to explore the experimental design space and identify ideal process conditions.
2. The implementation and transfer of this process at the bench-top scale allows for further process optimization and investigation of, for example, perfusion settings. Additionally, relevant product material can be obtained for clinical trials. The **Univessel® SU 10 L**, a new addition to the Sartorius single-use bioreactor portfolio, minimizes time-consuming manual handling steps (preparation and post-processing) and mitigates potential contamination risks compared with multi-use bench-top systems. This reduces experimental downtimes while maximizing the process knowledge gained.
3. Finally, intensified processes can be scaled up to pilot scale and beyond, with the **Biostat STR® 50** serving as a suitable system. For the operation of perfusion processes, full integration of XCell® ATF technology into the Biobrain® automation platform streamlines operational efforts and complexity while bundling the batch record.[4]

In conclusion, the implementation of an intensified upstream process strategy, from development to manufacturing scales, relies on the scalability of the chosen bioreactor portfolio. An industrially relevant strategy is the intensification of the seed train using N-1 perfusion for subsequent inoculation of the production-scale fed-batch process.[5]
In this study, we demonstrated the scalability for both a perfusion-based and fed-batch CHO cell culture 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-sterilized and ready-to-use
- Available in three application-specific vessel designs: Essential, Perfusion and Cell Therapy
- Consistent bioreactor design with the 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® 2,000
- Perfusion-ready operation enabled by full integration of 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

Acknowledgments
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