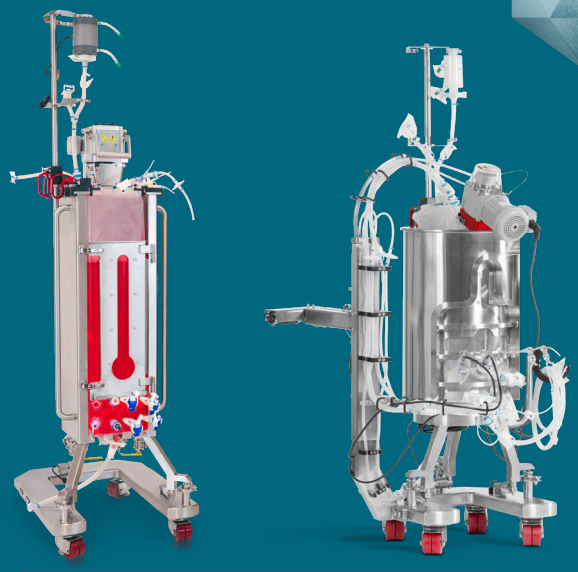

Single-use bioprocessing

Process performance comparison between the 50 L DynaDrive S.U.B. and legacy HyPerforma S.U.B. platforms

Evaluation across multiple production cell lines

Keywords
Single-use bioreactor, DynaDrive S.U.B., fed-batch, turndown, tech transfer

Introduction

As a molecule approaches commercial launch and more is known about the potential market demand, companies are often faced with the decision to scale up or scale out their manufacturing processes. Generally, when scale-up requires production vessels larger than 2,000 L, this decision also involves moving from single-use bioreactors (S.U.B.s) to traditional stainless-steel systems. An alternative approach to meeting market demand may be to improve batch volumetric productivity through process intensification. However, many traditional S.U.B.s, particularly at larger scales, are becoming excessively challenged in terms of oxygen transfer rate (OTR) for intensified processes, making scale-up unfeasible. A next-generation S.U.B., the Thermo Scientific™ DynaDrive™ S.U.B., is now enabling scale-up to 5,000 L and process intensification above previous limits while continuing to leverage benefits of S.U.B.s.

As OTR becomes limiting, most traditional S.U.B.s rely primarily on sparging flow to increase oxygen mass transfer and maintain dissolved oxygen (DO) in high-density cell cultures, due to limitations in the amount of power that can be delivered through their drivetrain. Sparging through a micro-sparger has become a widely used strategy to improve OTR in traditional S.U.B.s and typically requires a secondary sparger to facilitate removal of partial pressure of CO_2 (pCO_2). Some cell lines, however, are sensitive to the

higher shear produced by micro-sparging, and process scale-up cannot depend on this method to ensure sufficient O_2 delivery or pCO_2 removal. The DynaDrive S.U.B.s overcome many of these limitations in providing next-generation S.U.B. solutions at the 50–5,000 L scale.

The 50 L DynaDrive S.U.B. is multifunctional with a range of applications—an intermediate production–scale option for preclinical material, a scale-down model for the 5,000 L bioreactor, an accelerated option for seed-train expansion, and a solution for previous limitations halting the development of perfusion or intensified processes. The cuboid geometry, agitation design, and enhanced (laser-drilled) drilled-hole sparger mimic those of its larger 5,000 L counterpart, thereby making it an excellent scale-down model for process development. Improvements to the agitation shaft and impeller design have facilitated the use of a lower turndown ratio than ever seen before. With the ability to operate at a 10:1 turndown ratio at the 50 L scale, the next-generation S.U.B. provides the option to use one vessel for multiple seed expansion steps. Utilizing the 10:1 turndown ratio of the DynaDrive S.U.B. for your seed-train strategy ultimately enables fewer manipulations, tube connections, and bioprocess containers (BPCs), thus contributing to time and cost savings during the manufacturing run. With improved ergonomics, the installation time for the 50 L DynaDrive S.U.B. is half that of traditional 50 L Thermo Scientific™ HyPerforma™ S.U.B.s, making it easier to incorporate into your daily workflow. The implementation of a collapsible drivetrain keeps the packaging small to ensure minimal storage space is required.

These major design changes in the S.U.B. have enabled a power-to-volume (P/V) ratio of >80 W/m^3, T95 mixing times of <45 sec, k_La performance of >40 hr^{-1}, and a turndown ratio of 10:1. As a result, the system can deliver substantially greater oxygen transfer rates compared with traditional HyPerforma S.U.B.s, as seen in Table 1, leading to more flexibility for strenuous and oxygen-demanding cell lines or intensified processes. Alternatively, with lower shear introduced by the updated impeller design and gentler aeration with the enhanced drilled-hole sparger, the DynaDrive S.U.B. offers solutions for manufacturing with sensitive cell lines and perfusion applications as well. This technology opens the doors for scale-up in single-use manufacturing by addressing many of the limitations of the traditional S.U.B.s and broadens the design space to accommodate a wider variety of processes.

Goal

The goal of this study was to evaluate the performance of the 50 L DynaDrive S.U.B. using four different cell lines, together with processes that were previously developed, specific to those cell lines, for manufacturing at the 2,000 L scale using other single-use technology. The data generated from the 50 L DynaDrive S.U.B. runs were compared against the traditional HyPerforma S.U.B. runs (developed for the S.U.B.s from 50 L to 2,000 L volume) to better understand the scalability and capabilities of the new system. These experiments were designed to demonstrate that the DynaDrive system could be successfully implemented for use with a wide range of cell lines and processes using both the DynaDrive and Xcellerex™ XDR (Cytiva) single-use systems. Cell lines with varying expression levels and characteristics were used to challenge the system.

Table 1. Comparison of S.U.B. capabilities.

Parameter	50 L DynaDrive S.U.B.	50 L HyPerforma S.U.B.
Maximum volume	50 L	50 L
Turndown ratio	10:1	5:1
k_La	>40 hr^{-1}	8 hr^{-1}
T95 mixing time	<35 sec	<35 sec
Maximum P/V ratio	80 W/m^3	40 W/m^3

Table 2 shows a comparison of the cell lines used. All cell lines were subjected to a 14-day fed-batch run at full volume. To further evaluate the DynaDrive S.U.B. capabilities, some cell lines were subjected to the 10:1 turndown ratio at the N-1 stage. The goal of these evaluations was to demonstrate sufficient scale-up within the 50 L DynaDrive S.U.B. system to inoculate the N-stage production reactor.

Here we describe 5 case studies (Table 3).

Table 2. Cell lines to evaluate 50 L DynaDrive S.U.B.

Parameter	Cell line 1	Cell line 2	Cell line 3	Cell line 4
Cell type	Gibco™ Freedom™ CHO-S™	Gibco™ ExpiCHO-S™	Gibco™ CHO DG44	CHO-M
Titer range	Low (~1 g/L)	Medium (~3 g/L)	Medium (~3 g/L)	High (~7 g/L)
Cell line characteristics	Legacy cell line	Platform cell line	Shear sensitivity to micro-sparger	High oxygen demand

Table 3. Case studies.

Case study	Description
1	10:1 turndown ratio evaluation for the ExpiCHO-S, CHO DG44, and CHO-M cell lines
2	50 L DynaDrive S.U.B. evaluation of the Freedom CHO-S cell line in a 14-day fed-batch run
3	50 L DynaDrive S.U.B. evaluation of the ExpiCHO-S cell line in a 14-day fed-batch run
4	50 L DynaDrive S.U.B. evaluation of the CHO DG44 cell line in a 14-day fed-batch run
5	50 L DynaDrive S.U.B. evaluation of the CHO-M cell line in a 14-day fed-batch run

Case study 1

10:1 turndown ratio evaluation of the ExpiCHO-S, CHO DG44, and CHO-M cell lines

Methods

Cells were expanded in shake flasks and rocker bioreactors up through the N-2 step. At the N-1 stage, the cells were seeded into the 50 L DynaDrive S.U.B. targeting a 10:1 turndown ratio. Viable cell density (VCD) and cell viability measurements were taken on a daily basis. The cells were cultured in batch mode until the cells grew to a target VCD, after which fresh production medium was added to the 50 L DynaDrive S.U.B. to commence the fed-batch production phase. Operating conditions for the turndown ratio evaluation are shown in Table 4.

Table 4. Operating parameters for 10:1 turndown evaluation.

Parameter	Cell line		
	ExpiCHO-S	CHO DG44	CHO-M
Target starting volume	5 L		
Seeding density	0.3×10^6 cells/mL		
Temperature	37°C	36.8°C	37°C
pH	6.8–7.2		
Agitation	140 rpm		
Dissolved oxygen (DO) set point	40%	30%	30%
DO cascade	Air and oxygen through the enhanced drilled-hole sparger		
Headspace strategy	1 L/min air through the crossflow sparger		

Results

The VCD trends for the 10:1 turndown ratio in the DynaDrive S.U.B. for the ExpiCHO-S, CHO DG44, and CHO-M cells compared to traditional rocker bioreactor performance at the N-1 stage are shown in Figure 1. The VCD values were normalized for each cell type to the day 3 rocker bioreactor values and plotted for comparison. The cell growth in the DynaDrive S.U.B. was found to be comparable to the traditional N-1 stage growth. The normalized VCD for the CHO DG44 and CHO-M cell lines were slightly higher in the DynaDrive S.U.B. when compared to the rocking bioreactors. While the ExpiCHO-S cell line had a lower normalized VCD in the DynaDrive S.U.B. compared to the rocker bioreactor, there was enough cell mass generated to initiate the production phase after 3 days, thereby achieving a successful passage. The average viability for all cell lines at the 10:1 turndown ratio was >98% for the duration of the N-1 phase (data not shown). The 10:1 turndown ratio can be implemented as a scale-up strategy with minimal risk as this case study demonstrated suitable process performance for three cell lines.

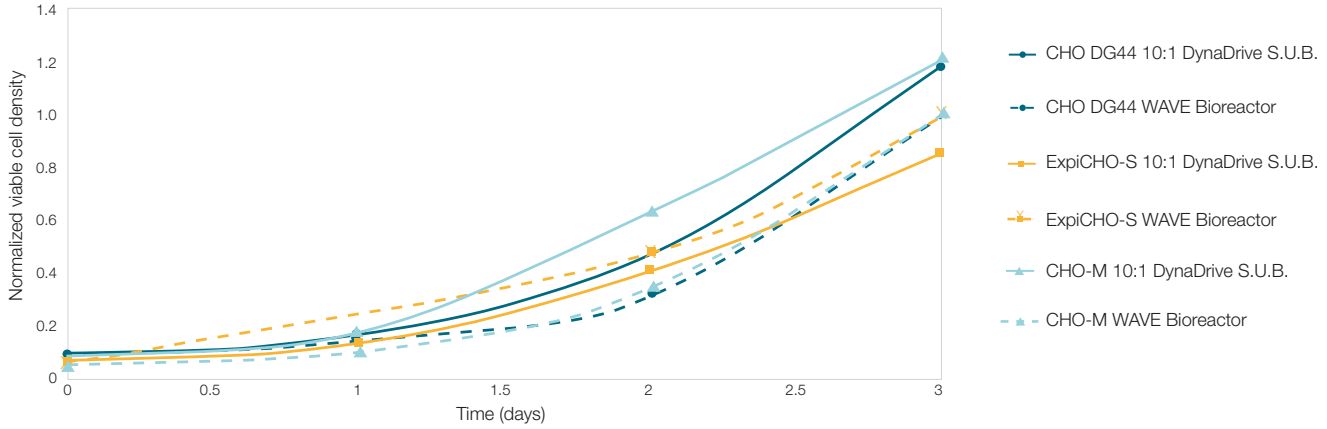

Figure 1. Growth comparison for three cell lines in 50 L DynaDrive S.U.B. (10:1 turndown ratio) vs. traditional rocker bioreactor.

Case study 2

50 L DynaDrive S.U.B. evaluation of the Freedom CHO-S cell line in a 14-day fed-batch run

Methods

Cells were expanded in shake flasks for 6 passages and were seeded in the DynaDrive S.U.B. at 0.3 x 10^6 cells/mL in a 42.5 L working volume. Operating conditions for the bioreactor process are listed in Table 5. Cell counts, viability, gases, and metabolites were measured offline on a daily basis. Titer samples were measured on a daily basis from day 6 onward.

Table 5. Operating parameters to evaluate the Freedom CHO-S cell line.

Parameter	Set point
Target starting volume	42.5 L
Seeding density	0.3 x 10^6 cells/mL
Temperature	37°C
pH	6.8–7.2
Agitation	120 rpm
DO set point	30%
Air headspace	1 L/min
DO cascade	Oxygen through the enhanced drilled-hole sparger
Feeding strategy	• Bolus additions of a single feed were added on a daily basis starting on day 3 • Glucose was supplemented as needed to maintain levels in the bioreactor

Results

Trends of VCD and viability of the CHO-S cell line in the 50 L DynaDrive S.U.B. vs. in the 50 L HyPerforma S.U.B. are shown in Figure 2; the trends are very similar. Peak VCD was achieved on day 7 at approximately 24 x 10^6 cells/mL for both vessels. The viability trends were almost identical for the two bioreactors until day 8. From day 8 to day 14, the 50 L HyPerforma S.U.B. had slightly lower viability than the 50 L DynaDrive S.U.B. with day 14 viability being 84% and 89%, respectively. Similarly, as seen in Figure 3, the titer profiles of the cell culture runs in the two types of bioreactors were similar. While the titer production in the 50 L HyPerforma S.U.B. was lower than in the 50 L DynaDrive S.U.B., the overall titer range achieved was within the expected range of 0.7–0.8 g/L on day 14. The similarity between fed-batch results suggests that there is minimal risk in transferring processes from the HyPerforma S.U.B. to the DynaDrive S.U.B.

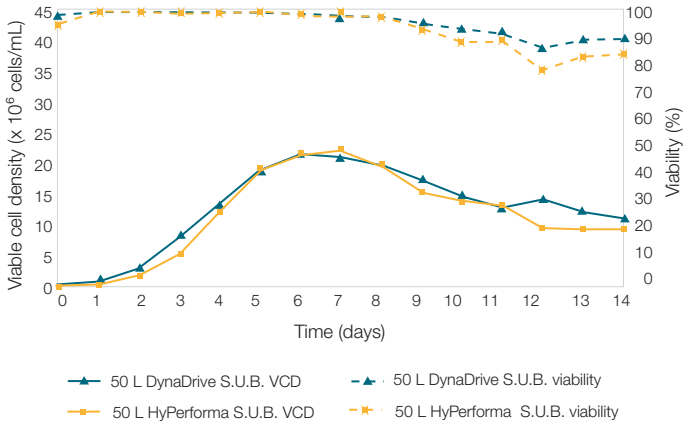

Figure 2. VCD and viability of Freedom CHO-S cell culture in the 50 L DynaDrive and 50 L HyPerforma S.U.B.s.

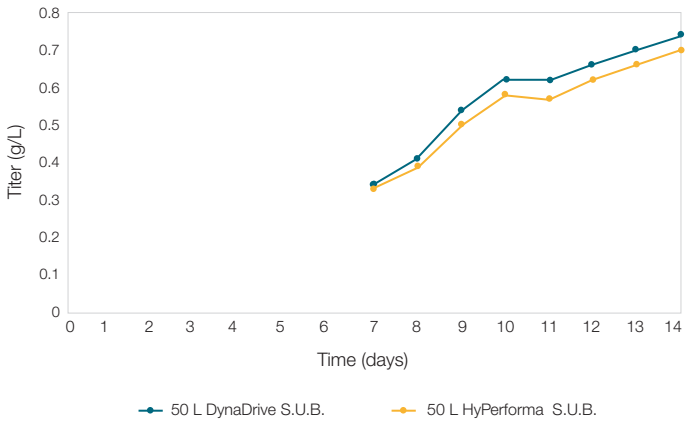

Figure 3. Titer production of Freedom CHO-S cell culture in the 50 L DynaDrive and 50 L HyPerforma S.U.B.s.

Case study 3

50 L DynaDrive S.U.B. evaluation of the ExpiCHO-S cell line in a 14-day fed-batch run

Methods

Cells were expanded in shake flasks until the N-1 stage. The N-1 stage was performed in the DynaDrive bioreactor at a 10:1 turndown ratio. Fresh production medium was added to the N-1 stage after 3 days to bring the initial seeding density to 0.7 x 10^6 cells/mL for the 14-day fed-batch process. Operating conditions for the bioreactor are described in Table 6. Cell counts, viability, gases, and metabolites were measured offline on a daily basis. Titer samples were filtered and frozen daily starting on day 6. Titer samples were batch tested at the end of the run.

Results

The comparison of VCD and viability between a 50 L DynaDrive S.U.B., 200 L Xcellerex XDR, and 250 L HyPerforma bioreactor is shown in Figure 4. The VCD profiles varied slightly, with peak VCD ranging from 18 x 10^6 cells/mL to 22 x 10^6 cells/mL on day 6 (200 L Xcellerex XDR and 250 L HyPerforma S.U.B.s) and day 8 (50 L DynaDrive S.U.B.), respectively. The viability profile remained consistent between the 50 L DynaDrive S.U.B. and 250 L HyPerforma S.U.B.s; however, the 200 L Xcellerex XDR cell run exhibited an earlier decline starting on day 5. Overall, the end-of-run viability was around 80% for the DynaDrive S.U.B. and 75% for the 200 L Xcellerex XDR and 250 L HyPerforma S.U.B.s. Despite the differences in VCD and viability profiles, productivity was comparable across all the runs with titers in the range of 2.7–2.8 g/L on day 14, as seen in Figure 5. The updated DynaDrive BPC design did not impact cell performance, as there were minimal differences in the results produced across the three bioreactor systems.

Table 6. Operating parameters to evaluate ExpiCHO-S cells.

Parameter	Set point
Target starting volume	35 L
Seeding density	0.7 x 10^6 cells/mL
Temperature	37°C
Temperature shift	34°C
pH	6.8–7.2
Agitation	120 rpm
DO set point	40%
Air headspace	1 L/min
DO cascade	Air and oxygen through the enhanced drilled-hole sparger
Feeding strategy	• Bolus additions of a single feed were added on a daily basis starting on day 3 • Glucose was supplemented as needed to maintain levels in the bioreactor

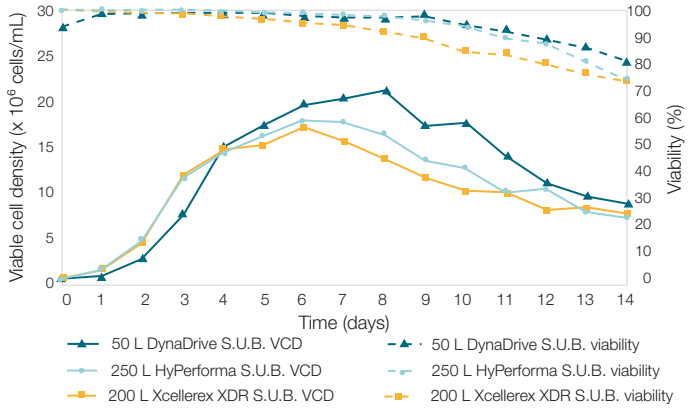

Figure 4. VCD and viability of ExpiCHO-S cell culture in the 50 L DynaDrive, 200 L Xcellerex XDR, and 250 L HyPerforma S.U.B.s.

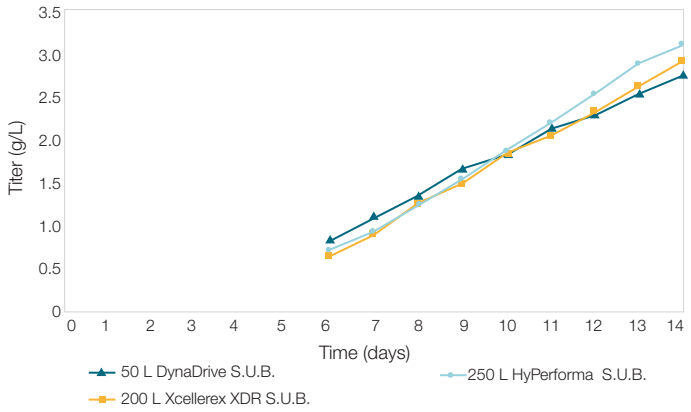

Figure 5. Titer production of ExpiCHO-S cell culture in the 50 L DynaDrive, 200 L Xcellerex XDR, and 250 L HyPerforma S.U.B.s.

Case study 4

50 L DynaDrive S.U.B. evaluation of the CHO DG44 cell line in a 14-day fed-batch run

Methods

Cells were expanded in shake flasks and rocker bioreactors to reach an adequate cell mass for the N-stage, 50 L bioreactor inoculation. The N-stage bioreactor was seeded at 0.3 x 10^6 cells/mL for the 14-day fed-batch process. Operating conditions for the bioreactor process are described in Table 7. Cell counts, viability, gases, and metabolites were measured offline on a daily basis. Titer samples were filtered and frozen daily starting on day 6. Titer samples were batch tested at the end of the run.

Results

The VCD and viability profiles for the CHO DG44 cell line are shown in Figure 6 for both the 50 L DynaDrive and 50 L Xcellerex S.U.B.s. The 50 L DynaDrive S.U.B. had performance comparable to the traditional 50 L Xcellerex XDR bioreactor's performance, with peak VCD of 28 x 10^6 cells/mL on day 9 in the 50 L DynaDrive S.U.B. and 25 x 10^6 cells/mL on day 8 in the 50 L Xcellerex XDR. Viability remained high for the duration of the run, ending at 80% on day 14. Titer profiles for the 50 L DynaDrive S.U.B. and the 50 L Xcellerex XDR bioreactor processes are shown in Figure 7. The titer production profile was very similar between the two runs, with day 14 titer at 3.4 g/L for both vessels.

When scaling up the traditional process from bench-scale reactors, the DO control strategy was modified to use a combination of a micro-sparger and drilled-hole sparger to maintain an adequate k_La for the duration of the run.

The DynaDrive system helped to overcome these manufacturing challenges by permitting the use of the enhanced drilled-hole sparger as the sole sparger for DO control and a higher allowable agitation rate than previously executed.

Table 7. Operating parameters to evaluate CHO DG44 cells.

Parameter	Set point
Target starting volume	35 L
Seeding density	0.3 x 10^6 cells/mL
Temperature	36.8°C
Temperature shift	34°C
pH	6.9–7.1
Agitation	185 rpm
DO set point	30%
Air headspace	0.5 L/min
DO cascade	Air and oxygen through the enhanced drilled-hole sparger
Feeding strategy	• Two feeds were added as separate bolus additions on a daily basis starting on day 3 • Glucose was supplemented as needed to maintain levels in the bioreactor

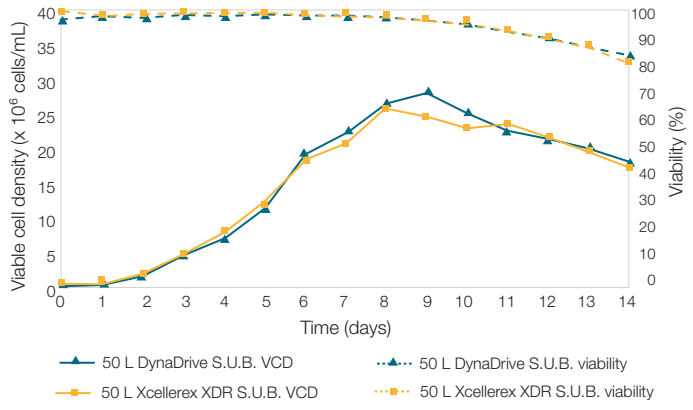

Figure 6. VCD and viability of CHO DG44 cell culture in the 50 L DynaDrive and 50 L Xcellerex XDR S.U.B.s.

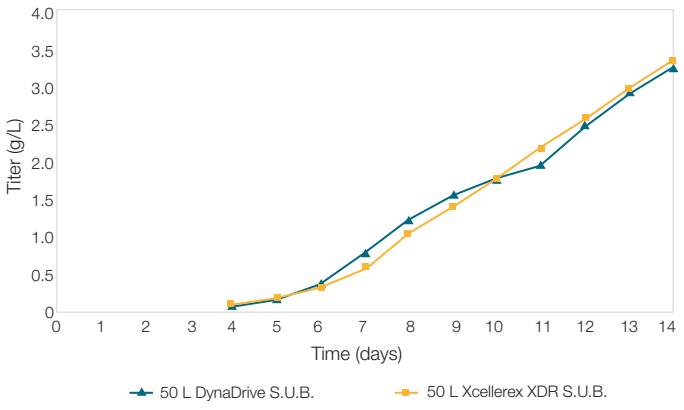

Figure 7. Titer production of CHO DG44 cell culture in the 50 L DynaDrive and 50 L Xcellerex XDR S.U.B.s.

Case study 5

50 L DynaDrive S.U.B. evaluation of the CHO-M cell line in a 14-day fed-batch run

Methods

Cells were expanded using shake flasks until the N-1 step. At the N-1 stage, the cells were seeded into the DynaDrive bioreactor at a 5 L working volume (10:1 turndown ratio). The N-1 stage was operated in batch mode for 3 days until the cells reached a certain VCD. The bioreactor was then topped up with medium at room temperature to bring the initial seeding density to 0.8 x 10^6 cells/mL for the 14-day fed-batch process. Operating conditions for the bioreactor process are described in Table 8. Cell counts, viability, gases, and metabolites were measured offline on a daily basis. Titer samples were retained and filtered daily starting on day 6. Titer samples were batch tested at the end of the run.

Results

The VCD and viability trends for the CHO-M cell culture runs in the 50 L DynaDrive and 2,000 L HyPerforma S.U.B.s are shown in Figure 8. The VCD profiles were comparable between the 50 L DynaDrive and 2,000 L HyPerforma S.U.B.s, with peak VCD in the range of 36–37 x 10^6 cells/mL. The viability remained high in both bioreactors, ending at 91% for the 50 L DynaDrive and 95% in the 2,000 L HyPerforma bioreactor. The productivity profiles, shown in Figure 9, were also similar, with day 14 titer in the range of 7.1–7.4 g/L. The 50 L DynaDrive S.U.B. was able to support the CHO-M cell production process while also meeting the high oxygen demand for this cell line, as growth profiles and titer production were comparable.

Table 8. Operating parameters to evaluate CHO-M cells.

Parameter	Set point
Target starting volume	30 L
Seeding density	0.8 x 10^6 cells/mL
Temperature	37°C
pH	6.8–7.2
Agitation	120 rpm
DO set point	30%
Air headspace	0.5 L/min
DO cascade	Air and oxygen through the enhanced drilled-hole sparger
Feeding strategy	• Two feeds were added as bolus additions on a daily basis starting on day 2 • Glucose was supplemented as needed to maintain levels in the bioreactor

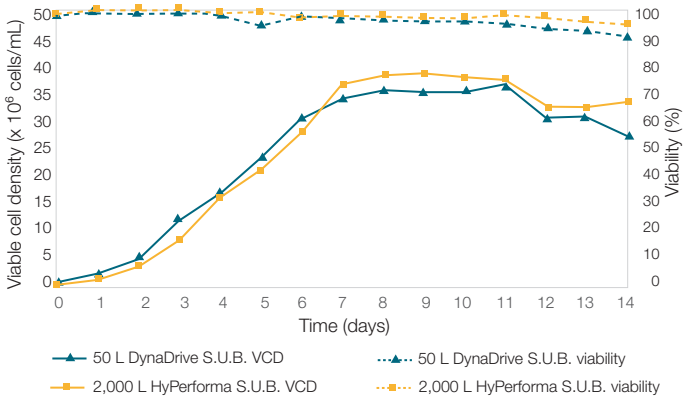

Figure 8. VCD and viability of CHO-M cell culture in the 50 L DynaDrive and 2,000 L HyPerforma S.U.B.s.

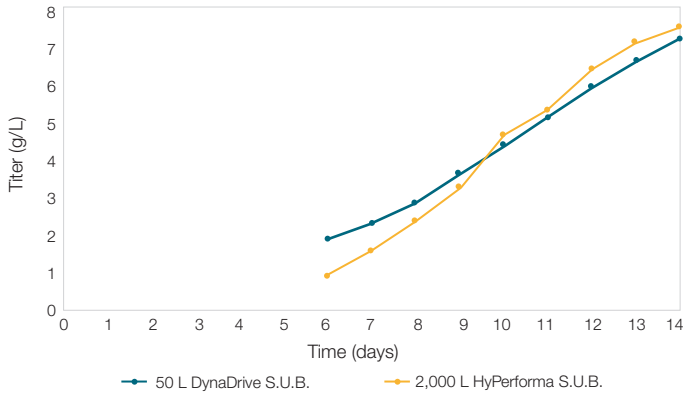

Figure 9. Titer production of CHO-M cell culture in 50 L DynaDrive and 2,000 L HyPerforma S.U.B.s.

Conclusions

The 50 L HyPerforma DynaDrive S.U.B. demonstrated equivalent performance across four different cell lines compared to the traditional processes developed using low turndown ratio and 14-day fed-batch experiments. Growth profiles and viabilities were maintained during the low-volume batch process for all three cell lines tested. The comparable cell growth profiles suggest ease of implementation in seed-train expansion, thus enabling the use of a single vessel for multiple passage steps. When scaling up, the seed-train scale-up can occur using the 10:1 turndown ratio in the 50 L S.U.B.s and 20:1 turndown ratio in the 500 L and 5,000 L S.U.B.s to eliminate the use of multiple shake flasks and rocker bioreactors or other intermediate vessels. Fewer manipulations, media preparations, and potentially even processing steps help to mitigate risk, reduce waste, and lower operating costs.

Overall, the different processes run in the 50 L HyPerforma DynaDrive S.U.B. were comparable to traditional processes for multiple cell lines. The updated agitator drivetrain, in combination with the enhanced drilled-hole sparger, did not have any adverse effects on the cell culture, thus enabling a much larger design space for process development than previously available when scaling up to the current S.U.B. systems. With this demonstrated consistency, the HyPerforma DynaDrive platform will enable users to stay within S.U.B.s with minimal process changes as they scale up their process for commercial demand.

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