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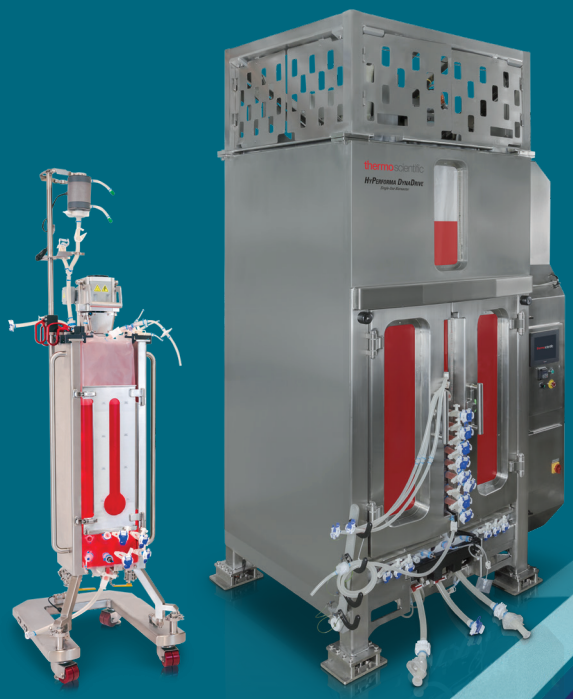

Single-use technologies

Scale-up evaluation of the DynaDrive S.U.B.s

Part 3: pilot and manufacturing scale comparison of DynaDrive S.U.B. capabilities

Keywords
Single-use bioreactor, DynaDrive S.U.B., scalability

Introduction

Commercialization of a drug is a monumental milestone that hinges on years of lifecycle management. As a product enters commercialization, product sponsors are positioned to derive demand from a fluid market. Analysis of supply will inevitably question the cost of goods sold (COGS) and whether it is a process of scale-out or scale-up. In some cases, the decision must be made on how to scale up the process, which may require leaving the existing 2,000 L single-use bioreactor (S.U.B.) systems and moving the process into larger stainless steel vessels. Up until this point, there is a volumetric gap separating the single-use technology and stainless steel bioreactors. Existing 2,000 L S.U.B.s are supply chain limiting, while stainless steel systems can pose other challenges when demand remains fluid.

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Modern process approaches have allowed increased product output, elevating titers past 10 g/L in some cases. These output achievements require increased production efficiency and/or input, pushing many bioreactor systems outside of their operating capabilities. For example, as oxygen transfer rate (OTR) becomes a limiting factor, most traditional S.U.B.s rely primarily on increased sparging flow to increase oxygen mass transfer. Maintaining a dissolved oxygen (DO) target in high-demand cell culture can be difficult due to limitations in the amount of power that can be delivered through the existing drivetrain. Delivering the gas through a micro-sparger has become a strategy that is widely used to improve OTR in traditional S.U.B.s and typically requires a secondary sparger to facilitate removal of dissolved CO_2 (measured as partial pressure of CO_2 or pCO_2). However, certain cell lines have been identified to be sensitive to the higher shear produced by micro-sparging, and thus process scale-up should not depend on this method alone to ensure sufficient O_2 delivery or pCO_2 removal.

The next generation of S.U.B.s, such as the Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.), which vastly improve mixing and mass transfer performances, can now enable scales of up to 5,000 L while remaining in the single-use format. Previous limits are easily addressed via the DynaDrive S.U.B. series, while the operating range is suitable to leverage an existing process using S.U.B.s and to allow for a smooth transfer to the new technology. The DynaDrive S.U.B.s are multifunction reactors with a range of applications including intermediate production scale for preclinical and clinical materials, scalable seed train vessels with wide turndown ranges (up to 20:1), and perfusion processes at 50 L and 500 L scales—both for production and for use in large-scale intensified fed-batch applications. Additionally, the DynaDrive S.U.B.s include improved features over previous and alternative S.U.B. options as noted below:

- Exceptional enhanced drilled-hole sparger (DHS) helps provide repeatable and reliable performance
- Flexible drive train with multiple impellers enables increased power input while offering reduced shear rates
- Cuboid design contributes to better Thermo Scientific™ BioProcess Container (BPC) fit, increased mixing efficiency, and enables more productive use of facility footprint
- Turndown capability (20:1) reduces facility requirements and increases flexibility in applications and scale-up
- Minimal hold-up volume (<1%) post-harvest and drain
- Each system is equipped with BPC-loading platforms, reducing handling and setup time, increasing safety, and providing consistent BPC loading; BPC loading can be accomplished in less time than with other 50 L and 500 L S.U.B.s and in less than 45 minutes at the 5,000 L scale
- Improved exhaust system for the 5,000 L S.U.B. enabling increased gas flow rates necessary for the increase in scale

Table 1. Comparison of pilot-scale S.U.B. capabilities.

Parameter	50 L DynaDrive	50 L HyPerforma	50 L XCellerex XDR	200 L XCellerex XDR	250 L HyPerforma
Maximum volume	50 L	50 L	50 L	200 L	250 L
Turndown ratio	10:1	5:1	5:1	5:1	5:1
k_La	50 hr^{-1}	8 hr^{-1}	60 hr^{-1}	24 hr^{-1}	27 hr^{-1}
T_{95} mixing times	<30 sec	<35 sec	<30 sec	<30 sec	<30 sec
Maximum P/V ratio	80 W/m^3	40 W/m^3	71 W/m^3	110 W/m^3	20 W/m^3

Table 2. Comparison of manufacturing-scale S.U.B. capabilities.

Parameter	500 L DynaDrive	2,000 L HyPerforma	2,000 L XCellerex XDR	5,000 L DynaDrive
Maximum volume	500 L	2,000 L	2,000 L	5,000 L
Turndown ratio	20:1	5:1	2:1	20:1
k_La	40 hr^{-1}	8 hr^{-1}	10 hr^{-1} (MSP 2 hr^{-1})	40 hr^{-1}
T_{95} mixing times	<32 sec	<60 sec	<50 sec	<45 sec
Maximum P/V ratio	80 W/m^3	40 W/m^3	32.6 W/m^3	80 W/m^3

These major design changes have enabled a power-to-volume (P/V) ratio of up to 80 W/m^3 in all sizes, T_{95} mixing times of less than 45 seconds, and k_La performance of at least 40 hr $^{-1}$ at all scales (Tables 1–2).

Additionally, the DynaDrive S.U.B. allows for process scale-up and transfer from Thermo Scientific™ HyPerforma S.U.B.s offering benefits of consistent BPC film, assurance of supply, robust quality controls, and BPC integrity. End users can continue using previously qualified traditional and single-use probe options as well as inlet and exhaust filters and other peripheral components integrated through high-strength porting and line sets.

Goal

The goal of this study was to evaluate the performance of the DynaDrive S.U.B., HyPerforma S.U.B., and Cytiva™ XCellerex™ XDR S.U.B. using four different cell lines (Table 3) with an XCellerex XDR S.U.B.–specific process developed in either the HyPerforma or XCellerex XDR systems for each cell line. These experiments were designed to demonstrate that these processes could be successfully transferred across different systems without impacting the process performance or product quality. A range of host cell platforms and processes was selected to demonstrate robustness and applicability across the diverse landscape in upstream bioprocessing.

Table 3. Cell lines used to evaluate performance across the different platforms.

Parameter	Cell line 1	Cell line 2	Cell line 3	Cell line 4
Cell type	ExpiCHO-S	Freedom CHO-S	CHO DG44	CHO-M
Titer output	Medium ~3 g/L	Low ~1 g/L	Medium ~3 g/L	High ~7 g/L
Cell line characteristics	Medium oxygen demand	Low oxygen demand	Shear sensitivity to micro-sparging	High oxygen demand Shear sensitivity to micro-sparging
Product	mAb	mAb	mAb	Non-mAb
Case study	1	2	3	4

Case study 1

Vessel evaluation using ExpiCHO-S cell line in 14-day fed-batch run

Methods

Standard vial thaw, resuscitation, and propagation procedures were used in initiating the cell train. Once cells were successfully thawed, they were expanded in a stepwise manner through a series of shake flasks and single-use vessels up to and including the N-2 stage. The N-1 stage for each seed train was performed in either a WAVE™ bioreactor or stirred-tank seed bioreactor for the XCellerex XDR and HyPerforma S.U.B.s. For the N-1 step feeding the DynaDrive S.U.B. arm, the same vessel was used for both the N-1 and N stage. Specifically, a 10:1 turndown was used in the 50 L scale and a 20:1 turndown was used in the 500 L and 5,000 L scales at N-1 stage. After 3-day growth as N-1 culture, fresh production medium was added to the S.U.B. to reach the initial volume and seed density of the N-stage. Operating conditions used across all experimental arms are described in Table 4. Specifically, a daily bolus feed of 2X concentrated Gibco™ Efficient Feed™ C+ was added from day 3 to day 13, and glucose was added as needed. Daily samples were collected for cell counts, viability, dissolved gases, metabolites, and titer. Titer samples were filtered and frozen starting on day 6 for ProA-UPLC analysis.

Results and conclusions

Viable cell density (VCD) and viability for the cultures (Figure 1) show consistent growth profiles among the cultures with peak cell densities between 17.26 x 10^6 and 21.25 x 10^6 viable cells/mL and viability >74% at harvest. Metabolite data collected offline indicated some fluctuations with glucose (Figure 2) and levels of metabolic byproducts, including lactate (Figure 3) and ammonium (Figure 4). As observed in Figure 2, there were some fluctuations in glucose feeding, especially in the 50 L, 200 L, and 250 L vessels as the glucose feed trigger was initiated on different days. However, lactate trends in Figure 3 remained consistent except for the 50 L DynaDrive S.U.B. vessel where lactate accumulation increased on day 7 onwards. This was coupled with a drop in ammonium from 9.03 to 5.36 mM (Figure 4), while all other vessels followed a consistent upward trend.

Table 4. Basic operating parameters for evaluation of ExpiCHO-S cells in the S.U.B.s.

Parameter	50 L DynaDrive	200 L XCellerex XDR	200 L HyPerforma	500 L DynaDrive	5,000 L DynaDrive
Seeding density (x 10^6 cells/mL)	0.7				
Temperature (°C)	Day 0–5: 37.0, Day 5–14: 34.0				
pH	6.8—7.2				
pH control	Acid control: sparged CO_2, Base control: 1 N NaOH				
Agitation (rpm)	120	113	113	60	26 rpm (days 0–3) 33 rpm (days 3–14)
DO (%)	40				
Air headspace (sLPM)	1	6	6	6	10—20
DO cascade	Air supplemented with O_2 through DHS (80 µm)	Air supplemented with O_2 through the 20 µm sparger	Air supplemented with O_2 through the 20 µm sparger	Air supplemented with O_2 through DHS (233 µm)	Air through both DHSs. O_2 supplemented through micro DHS (233 µm)
Feeding strategy	Daily bolus of EfficientFeed C+ and glucose (as needed)				

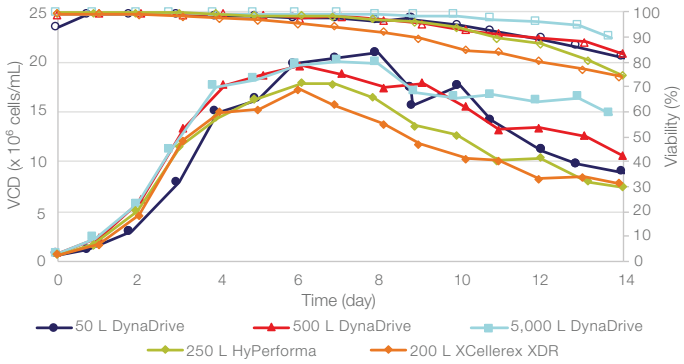

Figure 1. Viable cell density (VCD) and cell viability comparison of ExpiCHO-S (cell line 1) cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

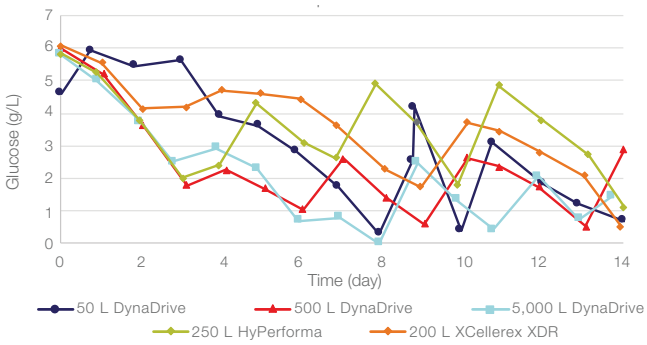

Figure 2. Glucose profile for ExpiCHO-S (cell line 1) cell culture in the the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

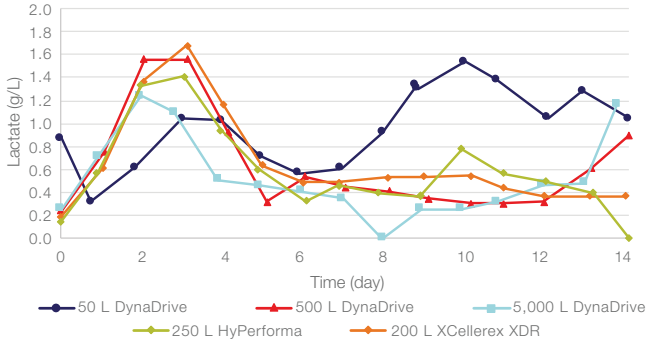

Figure 3. Lactate profile for ExpiCHO-S (cell line 1) cell culture in the the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

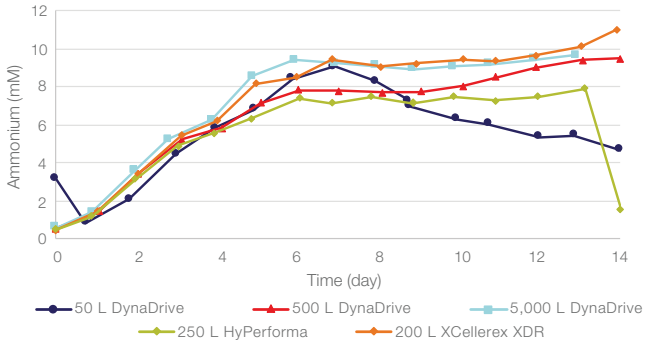

Figure 4. Ammonium profile for ExpiCHO-S (cell line 1) cell culture in the the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

Volumetric productivity across the different platforms (Figure 5) measured 2.70–3.09 g/L on day 14 and showed a consistent trend from day 6 to 14 across all vessels. Product quality (PQ) samples were miniprepped and evaluated by qualified capillary gel electrophoresis (CGE) and size exclusion chromatography (SEC) methods. Assessment of the charge species across the different vessels showed some differences in acidic, main, and basic species (Table 5). For example, the 250 L HyPerforma and 50 L/500 L/5,000 L DynaDrive S.U.B.s remained consistent, while 200 L XCellerex XDR S.U.B. showed a slight shift towards the basic species. Assessment of product impurities via SEC showed consistency across all bioreactors above the 50 L bioreactor scale, while the 50 L showed a slightly higher monomer content due to lower molecular weight (LMW) aggregate species. Based on the productivity and quality results, for the ExpiCHO-S cells, the data show strong consistency on productivity across all scales. A similar trend was also observed in the 250 L HyPerforma, and the 500 L and 5,000 L DynaDrive S.U.B.s.

While some minor PQ changes were observed at the 50 L DynaDrive and 200 L XCellerex XDR S.U.B.s, more work is required to determine connection to a process or vessel change.

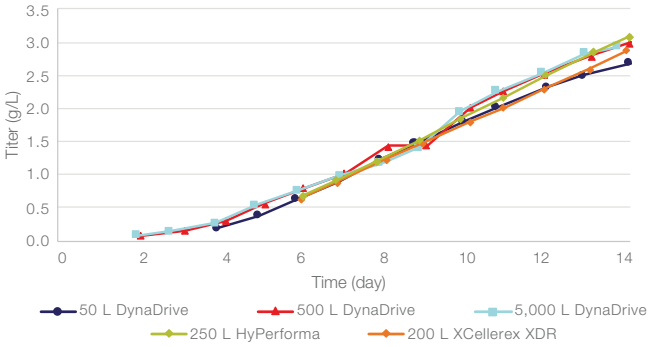

Figure 5. Titer results for ExpiCHO-S (cell line 1) cell culture in the the HyPerforma, DynaDrive, and XCellerex XDR vessels starting from day 2 to 14.

Table 5. ExpiCHO-S cells (cell line 1) product quality from bioreactor day 14 sample.

	cLEF			SEC		
Vessel	Acidic species (%)	Main species (%)	Basic species (%)	Aggregate species (%)	Monomer species (%)	Fragment species (%)
200 L XCellerex XDR	41.3	48.0	10.8	3.8	94.8	1.4
250 L HyPerforma	49.0	45.7	5.3	4.0	94.3	1.7
50 L DynaDrive	53.1	39.9	7.0	1.6	97.1	1.3
500 L DynaDrive	46.6	46.0	7.4	3.9	94.5	1.6
5,000 L DynaDrive	47.8	47.1	5.1	4.2	94.4	1.4

Case study 2

Vessel evaluation using Freedom CHO-S cell line in 14-day fed-batch run

Methods

The methods used for cell resuscitation, propagation, and cell mass accumulation were outlined in case study 1. Changes in cell line, and thus process and operating conditions for each bioreactor, are described in Table 6. A 2X concentration of EfficientFeed C+ feed was added continuously from day 3 through day 11, and glucose was supplemented as needed. Daily samples were collected for cell counts, viability, dissolved gases, metabolites, and titer. Titer samples were filtered and frozen starting on day 6 for ProA-UPLC analysis or immunoturbidimetric analysis via Roche Cedex™ Bio HT Analyzer.

Results and conclusions

The peak cell density for this cell line ranged from 22.42 x 10^6 to 29.12 x 10^6 viable cells/mL and viability at harvest ranged from 63.5 to 81.1% (Figure 6). The basic metabolites of glucose, lactate, and ammonium showed good consistency in trends across the different scales, especially lactate and ammonium (Figures 7–9). A comparison of productivity across all vessel types showed a range of 0.58–1.02 g/L on day 14 (Figure 10). The instrumentation used to measure productivity for all the 50 L runs was different compared to the ≥200 L runs, likely contributing to the observed productivity differences between the bioreactor scales.

Table 6. Operating parameters for Freedom CHO-S evaluation in different S.U.B.s.

Parameter	50 L DynaDrive	50 L HyPerforma	200 L XDR	2,000 L HyPerforma	5,000 L DynaDrive
Seeding density (x 10^6 cells/mL)	0.3				
Temperature (°C)	37.0				
pH	6.8–7.2				
pH control	Acid control: sparged CO_2, Base control: N/A				
Agitation (rpm)	120	161	113	72	31—41
DO (%)	30				
Air headspace (sLPM)	3	3	10	15	10—20
DO cascade	N_2 and O_2 through the DHS				
Feeding strategy	EfficientFeed C+ supplement added on continuous drip from days 3–11 and glucose as needed				

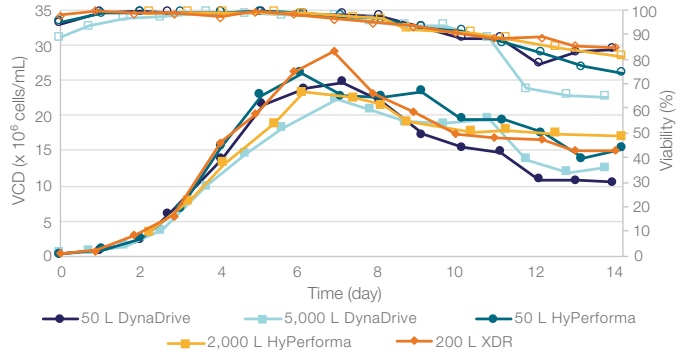

Figure 6. VCD and viability comparison of the Gibco™ Freedom™ CHO-S™ cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process. 50 L DynaDrive and 50 L HyPerforma VCD and viability were measured by Roche Cedex HiRes™ Analyzer, while 200 L XCellerex XDR, 2,000 L HyPerforma, and 5,000 L DynaDrive VCD and viability were measured by Beckman Coulter Vi-CELL™ XR.

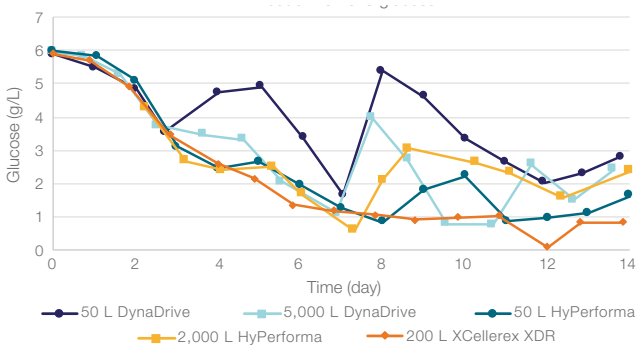

Figure 7. Glucose profile for the Freedom CHO-S cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

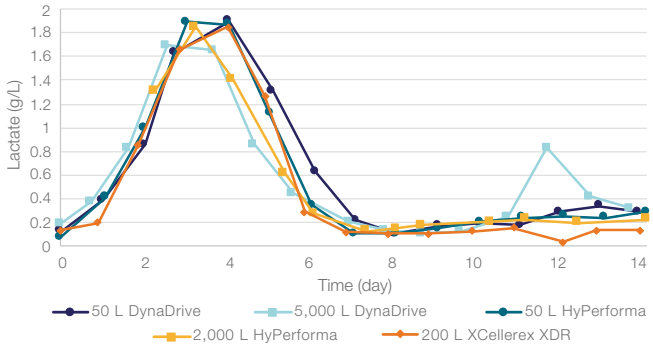

Figure 8. Lactate profile for the Freedom CHO-S cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

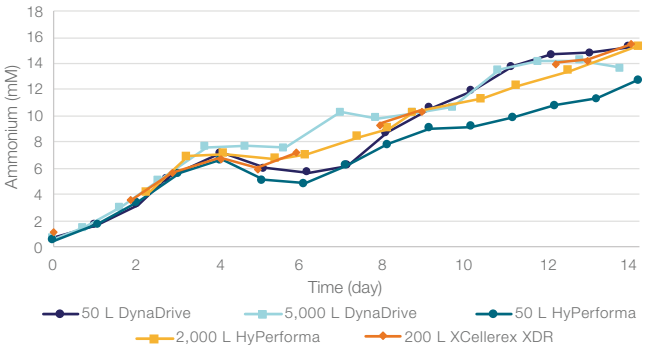

Figure 9. Ammonium profile for the Freedom CHO-S cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

Table 7. Freedom CHO-S cell line (cell line 2) product quality from bioreactor day 14 sample.

	cLEF			SEC		
Vessel	Acidic species (%)	Main species (%)	Basic species (%)	Aggregate species (%)	Monomer species (%)	Fragment species (%)
200 L XCellerex XDR	39.4	54	6.6	1.2	97.4	1.4
5,000 L DynaDrive	38.8	55.7	5.5	1.7	96.9	1.4

Day 14 samples from the 200 L XCellerex XDR and 5,000 L DynaDrive vessels were miniprepped, and product quality of charge variants, and high and low molecular species were evaluated. It was clearly observed that potential critical quality attributes such as charge variant species across the two samples were less than 2% different while SEC established a less than 0.5% difference in product impurities (Table 7). This consistency in product quality further highlights the opportunities and benefits in transferring existing processes based on Freedom CHO-S cells into the DynaDrive S.U.B. when searching for a larger vessel size.

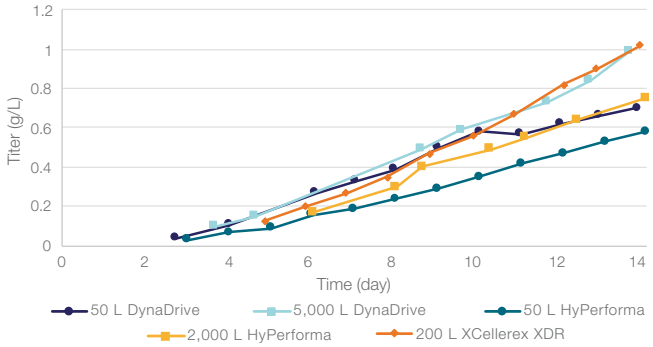

Figure 10. Titer results for the Freedom CHO-S cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process. 50 L DynaDrive and 50 L HyPerforma titers were measured by Cedex analyzer, while 200 L XCellerex XDR, 2,000 L HyPerforma, and 5,000 L DynaDrive titers were measured by ProA-UPLC.

Case study 3

Vessel evaluation using CHO DG44 cell line in 14-day fed-batch run

Methods

The methods used for cell resuscitation, propagation, and cell mass accumulation were outlined in case study 1. Changes in cell line, process, and operating conditions for each bioreactor are described in Table 8. Specifically, feed A and feed B were added as a bolus feed from day 3–14, and glucose was supplemented as needed. Daily samples were collected for cell counts, viability, dissolved gases, metabolites, and titer. Titer samples were filtered and frozen starting on day 6 for ProA-UPLC analysis.

Table 8. Operating parameters for CHO DG44 cell line evaluation in the pilot-scale S.U.B.s.

Parameter	50 L DynaDrive	50 L XDR	200 L XDR	250 L HyPerforma	500 L DynaDrive	2,000 L XDR	5,000 L DynaDrive
Seeding density (x 10^6 cells/mL)	0.3						
Temperature (°C)	Day 0–5: 36.8, Day 5–14: 34.0						
pH	6.9–7.1						
pH control	Acid control: sparged CO_2, Base control: 1 M sodium carbonate						
Agitation (rpm)	185	77	122	113	83	115	60
DO (%)	30						
Air headspace (sLPM)	0.5	0.4	4	4	6	5	20
DO cascade	O_2 through 80 µm sparger	O_2 through 20 µm sparger	O_2 through 20 µm sparger	O_2 through 20 µm sparger	O_2 through 233 µm sparger	O_2 through 20 µm sparger	O_2 through 233 µm sparger
Feeding strategy	Feed A and Feed B added as a bolus feed starting from day 3 and glucose as needed starting from day 6						

Results and conclusions

Case study 3 has the greatest distribution in vessel size and culture volume, covering the DynaDrive family of 50 L, 500 L, and 5,000 L and the XCellerex XDR family of 50 L, 200 L, and 2,000 L S.U.B.s while also including the 50 L and 250 L HyPerforma vessels (Table 8). Peak VCD of the CHO DG44 cell line shows similar trending across the different vessels with a range of 16.71 x 10^6 to 30.50 x 10^6 cells/mL (Figure 11). The 200 L XCellerex XDR culture had the lowest peak VCD of 17 x 10^6 cells/mL and the 5,000 L DynaDrive culture had the highest VCD, peaking at 30 x 10^6 cells/mL. The differences observed in growth are also reflected in the fluctuations observed in the various metabolites. While glucose was closely maintained post day 6 at 4.0 g/L and lactate remained below 1.65 g/L, there was significant metabolite fluctuation with this cell line even within the same bioreactor family (Figures 12–14). Regardless of the different growth and metabolite levels from different vessel types and scales, the productivity only showed two separate trending patterns (Figure 15). For the cultures ≤200 L, a range of 3.11 to 3.33 g/L was observed, while cultures ≥250 L showed a range of 4.23 to 4.76 g/L.

To further elucidate the impact of process parameters on product quality, cIEF and SEC (Table 9) were performed on day 14 samples from all vessel types and volumes. Charge variant distribution was more consistent across cultures ≥250 L compared to cultures ≤200 L. Specifically, the charge variants from the 250 L HyPerforma, 500 L DynaDrive, 2,000 L XCellerex XDR, and 5,000 L DynaDrive S.U.B.s were all within 6% of each other whereas the DynaDrive 50 L S.U.B. had at least 10% difference in acidic and main species when compared to XCellerex XDR 50 L and 200 L. In contrast to the charged species variability, product-related impurities, such as high and low molecular weight species, were more consistent across all vessel types and volumes. Specifically, there were ≤6% of aggregates and <3% of fragments in samples from each bioreactor. Overall, although different growth and metabolites were observed among different cultures, the productivity and product quality data showed the consistency with vessel volume regardless of the vessel type, suggesting the success of the process transfer from one type of vessel to another.

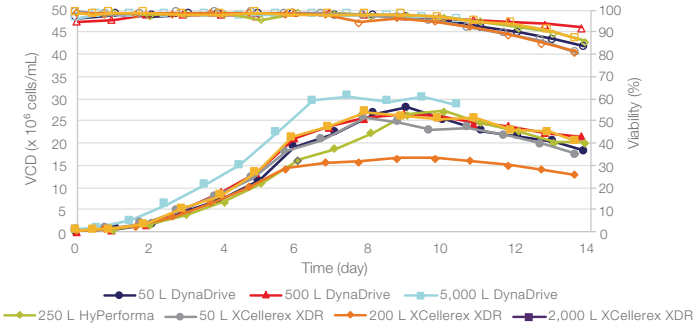

Figure 11. VCD and viability comparison of the CHO DG44 cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

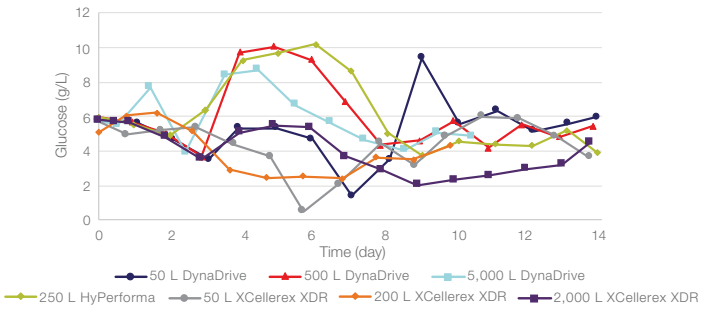

Figure 12. Glucose profile for the CHO DG44 cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

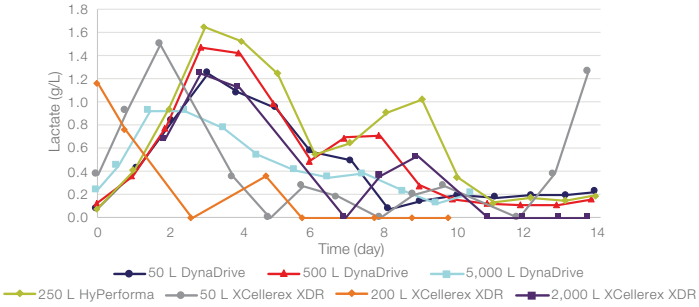

Figure 13. Lactate profile for the CHO DG44 cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

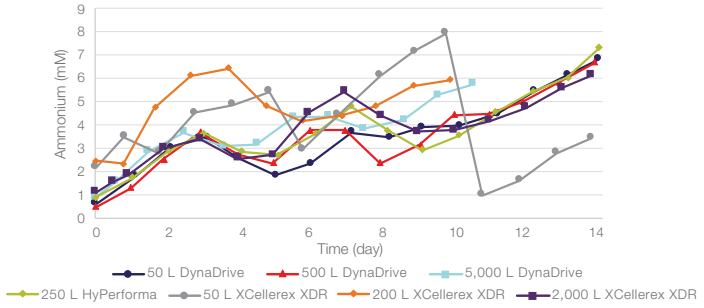

Figure 14. Ammonium profile for the CHO DG44 cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

To further elucidate the impact of process parameters on product quality, cIEF and SEC (Table 9) were performed on day 14 samples from all vessel types and volumes. Charge variant distribution was more consistent across cultures ≥250 L compared to cultures ≤200 L. Specifically, the charge variants from the 250 L HyPerforma, 500 L DynaDrive, 2,000 L XCellerex XDR, and 5,000 L DynaDrive S.U.B.s were all within 6% of each other whereas the DynaDrive 50 L S.U.B. had at least 10% difference in acidic and main species when compared to XCellerex XDR 50 L and 200 L. In contrast to the charged species variability, product-related impurities, such as high and low molecular weight species, were more consistent across all vessel types and volumes. Specifically, there were ≤6% of aggregates and <3% of fragments in samples from each bioreactor. Overall, although different growth and metabolites were observed among different cultures, the productivity and product quality data showed the consistency with vessel volume regardless of the vessel type, suggesting the success of the process transfer from one type of vessel to another.

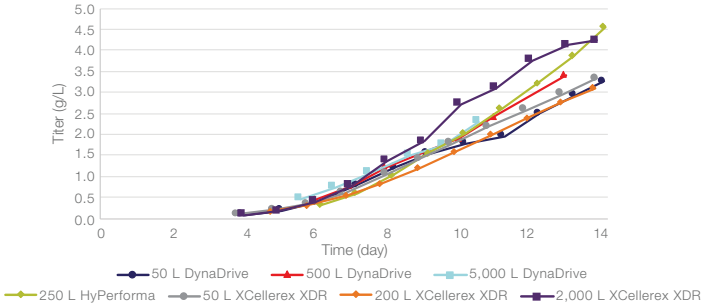

Figure 15. Titer results for the CHO DG44 cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

Table 9. CHO DG44 cell line (cell line 3) product quality from bioreactor day 14 sample.

	cLEF			SEC			R-CGE	NR-CGE
Vessel	Acidic species (%)	Main species (%)	Basic species (%)	Aggregate species (%)	Monomer species (%)	Fragment species (%)	Purity (%)	Peak (%)
50 L XCellerex XDR	57.7	34.5	7.8	2.11	95.62	2.27	NA	NA
200 L XCellerex XDR	60.5	36.1	3.4	4.43	93.06	2.51	73.9	94.1
2,000 L XCellerex XDR	53.5	40.0	6.4	2.12	95.06	2.82	NA	NA
250 L HyPerforma	52.5	40.8	6.9	5.29	92.26	2.46	78.8	94.2
50 L DynaDrive	44.9	47.4	7.7	3.95	94.08	1.98	92.0	93.9
500 L DynaDrive	58.5	36.5	6.0	4.43	93.12	2.44	86.7	90.5
5,000 L DynaDrive*	52.3	39.7	8.0	6.01	91.82	2.17	92.4	91.1

* Test results were from day 11 sample.

Case study 4

Vessel evaluation using CHO-M cell line in 14-day fed-batch run

Methods

The methods used for cell resuscitation, propagation, and cell mass accumulation are outlined in case study 1. The process and operating conditions for each bioreactor are described in Table 10. Specifically, feed A and feed B were added as a bolus feed from day 2 to 13, and glucose was supplemented as needed. Daily samples were collected for cell counts, viability, dissolved gases, metabolites, and titer. Titer samples were filtered and frozen starting on day 6 for titer measurement.

Table 10. Operating parameters for CHO-M cell line evaluation in different S.U.B.s.

Parameter	50 L DynaDrive	50 L XCellerex XDR	200 L XCellerex XDR	500 L DynaDrive	2,000 L HyPerforma	5,000 L DynaDrive
Seeding density (x 10^6 cells/mL)	<1					
pH control	Acid control: sparged CO_2, Base control: 1.5 M sodium carbonate					
Agitation (P/V) (W/m^3)	33*					
DO (%)	30					
Air headspace (sLPM)	1x	1x	4x	12x	10x	40x
DO cascade	O_2 through the DHS (80 µm)	O_2 through the DHS (500 µm)	O_2 through the DHS (500 µm)	O_2 through the DHS (233 µm)	O_2 through the DHS (582 µm)	O_2 through the DHS (457 µm)
Feeding strategy	Feed A and feed B added on as a bolus feed from days 2–13; glucose was added as needed					

* Agitation of the 2,000 L HyPerforma S.U.B. was set to the vendor-suggested maximum agitation of the vessel.

Results and conclusions

Results from case study 4 showed the peak VCD with a range of 29.08 x 10^6 to 38.53 x 10^6 cells/mL while maintaining over 82% viability on the final day of the process (Figure 16). Glucose, lactate, and ammonium profiles all followed their general trends and were consistent across vessels and scales (Figures 17–19). Specifically, lactate concentrations in the XCellerex XDR batches were below 1 g/L by day 7, while for both the HyPerforma and DynaDrive vessels, lactate reached below 1 g/L at later days. This change in lactate consumption was not observed to impact the titer range of 6.74 to 7.89 g/L (Figure 20), and the highest titer batch was the 5,000 L DynaDrive at 7.89 g/L.

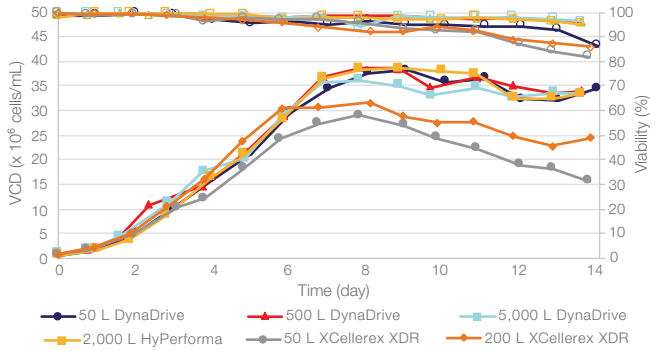

Figure 16. VCD and viability comparison of the CHO-M cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

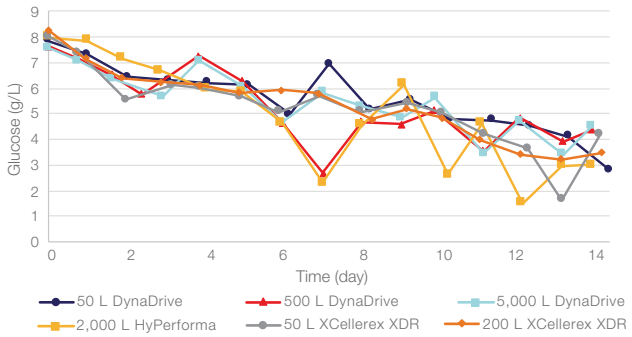

Figure 17. Glucose profile for the CHO-M cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

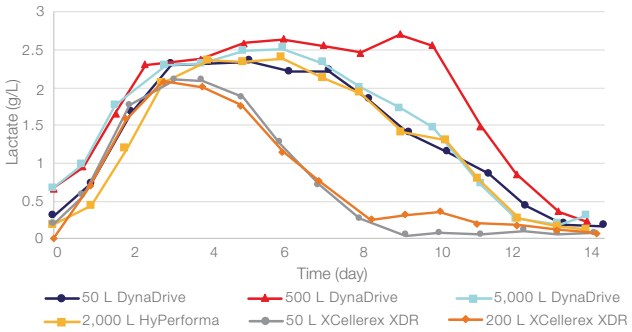

Figure 18. Lactate profile for the CHO-M cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

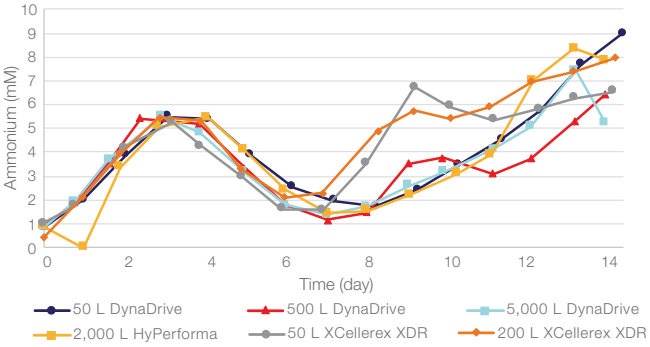

Figure 19. Ammonium profile for the CHO-M cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

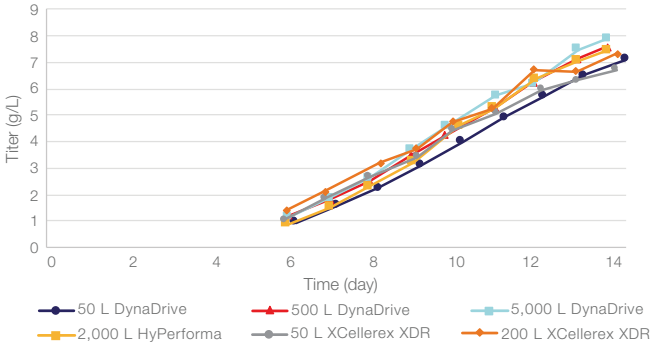

Figure 20. Titer results for the CHO-M cell culture in the HyPerforma, DynaDrive, and XCellerex XDR vessels over a 14-day process.

Using the CHO-M cell line, product quality from the 500 L and 5,000 L DynaDrive, 2,000 L HyPerforma, and 50 L and 200 L XCellerex XDR bioreactors was evaluated (Table 11). For all scales and bioreactors evaluated, the CHO-M cells product was purified using the same single-step chromatography purification method.

Some differences were observed across the charge variant species among the XCellerex XDR, HyPerforma, and DynaDrive vessels. The differences in product impurities are less pronounced, as the aggregate and fragment content show a difference of 1.2% or less across all vessels studied. Overall, the CHO-M cell line showed consistent productivity and similar product quality when the process was transferred from XCellerex XDR and HyPerforma S.U.B.s to DynaDrive S.U.B.s.

Table 11. CHO-M cell line product quality from bioreactor day 14 sample.

	cLEF			SEC			R-CGE	NR-CGE
Vessel	Acidic species (%)	Main species (%)	Basic species (%)	Aggregate species (%)	Monomer species (%)	Fragment species (%)	Purity (%)	Peak (%)
500 L DynaDrive	31	64.2	4.8	2.36	95.09	2.55	93.6	95.8
5,000 L DynaDrive	30.5	66.1	3.4	2.47	94.75	2.78	92.7	95.6
2,000 L HyPerforma	29.9–36.7	53.4–63.3	6.8–12.1	1.9–3.0	94.5–95.7	2.4–2.8	91.4–94.9	96.0–97.0
50 L XCellerex XDR	29.7	67.7	2.6	1.59	93.73	4.68	92.5	97.3
200 L XCellerex XDR	28.1–28.3	62.4–65.6	6.3–9.4	3.0–3.4	92.3–92.8	4.0–4.4	92.1–92.7	96.3–97.3

Conclusions

Preliminary runs with DynaDrive S.U.B.s suggest that the process transfer from XCellerex XDR or HyPerforma S.U.B. to DynaDrive S.U.B. is achievable. While there is not a fit-for-all strategy for a vessel change or scale-up, the P/V scale-up strategy for agitation worked quite well for our studies. In the case studies presented in this paper, we were able to successfully transfer processes that had been developed for either the 2,000 L XCellerex XDR or HyPerforma bioreactor to the 5,000 L DynaDrive S.U.B.

Through the studies with four cell lines, it was clear that the process defines the product. With the processes for for the CHO-S cell lines and the CHO-M cell line there was enough robustness within the process to overcome the vessel change and scale-up. Conversely, with the processes for CHO DG44 cells, the changes in vessel type and volume impacted cell growth, metabolites, and product quality. In cases like this, a risk-based approach may go hand in hand with the process transfer or scale-up. Understanding the process robustness and vessel capabilities are necessary for a seamless transfer from one system to another.

Overall, the design, scale, and operations of the 5,000 L DynaDrive S.U.B. can be key in the commercialization of a human therapeutic. As such, the case studies in this paper serve as the basis for future process transfer from existing systems into the DynaDrive family of S.U.B.s.

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