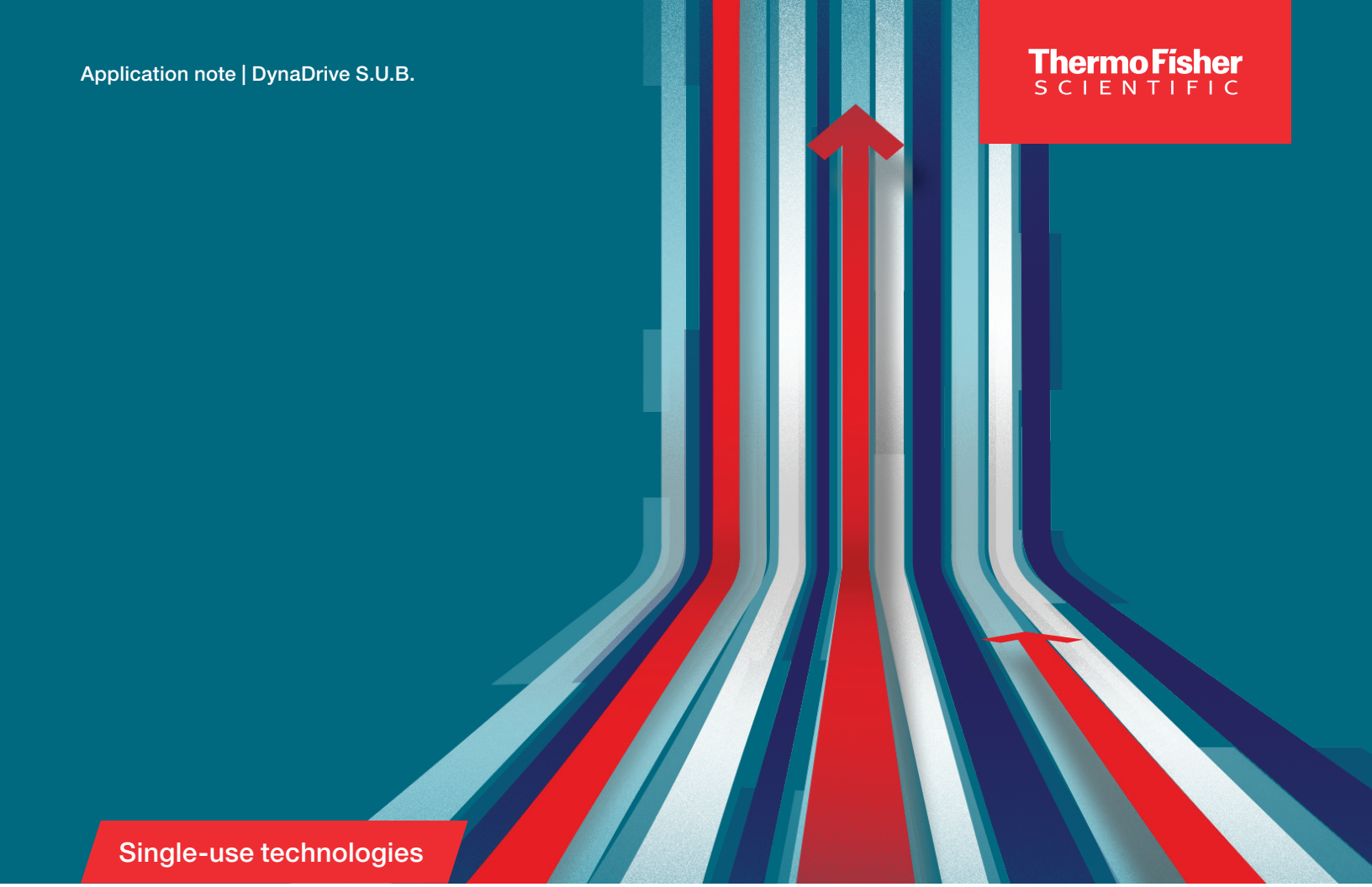

Single-use technologies

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

Part 2: CHO DG44 and CHO-M

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

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 the required volume for scale-up production vessels is more than 2,000 L, this decision also involves moving the scale-up process from single-use bioreactors (S.U.B.s) to traditional stainless-steel systems.

Recent process intensification has enabled manufacturers to achieve increased product output, pushing titers past 10 g/L in some cases. These output achievements require increased production efficiency and input, pushing many bioreactor systems past their limits. Quality requirements, robustness, and functional performance of traditional S.U.B.s can all become constraints, especially up to 2,000 L. For example, as oxygen transfer rate (OTR) becomes a limiting factor, most traditional S.U.B.s rely primarily on increased sparging flow to raise oxygen mass transfer.

Maintaining a dissolved oxygen (DO) target in high-demand cell cultures can be difficult due to limitations in the amount of mixing power that can be distributed effectively through the drive train of traditional S.U.B.s. Sparging through a microsparger has become a widely used strategy to improve OTR in traditional S.U.B.s and typically requires a secondary sparger to facilitate removal or stripping of dissolved CO_2 (measured as partial pressure of CO_2 or 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 alone to ensure sufficient O_2 delivery or pCO_2 removal.

A next-generation S.U.B., the Thermo Scientific™ DynaDrive™ S.U.B., with vastly improved mixing and mass transfer performance, is now enabling scale-up to 5,000 L as well as process intensification. Previous limits are no longer a burden for the DynaDrive S.U.B., and it continues to leverage known and accepted benefits of legacy units. The DynaDrive S.U.B.s are multifunctional reactors for a range of applications, including intermediate-scale production of preclinical, clinical, and commercial materials, as well as perfusion for both production and N-1 seed processes.

Additionally, each DynaDrive S.U.B. includes features that are improved over previous Thermo Scientific and alternative S.U.B. options:

- Revolutionary drive train design with multiple impellers allows increased power input and efficiency while offering reduced shear rates
- Best-in-class enhanced drilled-hole sparger (DHS) provides repeatable and reliable mass transfer performance that users of DynaDrive S.U.B.s have embraced due to its linear scale-up benefits
- Cuboid design contributes to better fit with the Thermo Scientific™ BioProcess Container (BPC) and increased baffled-like mixing efficiency, and allows more productive use of facility footprint
- 10:1 or better turndown ratio reduces facility requirements and investment costs while increasing flexibility in seed train applications and all aspects of scale-up
- Continuous mixing during harvest and minimal hold-up volume (<1%) after drain
- Improved exhaust system for the 3,000 L and 5,000 L S.U.B.s allowing for increased gas flow rates and utmost reliability typically required for production-scale cultures
- Each system is equipped with Thermo Scientific BPC load-assist device, reducing handling and setup time, increasing safety, and providing consistent BPC loading that can be accomplished in less time at the 50 L and 500 L scales compared to previous S.U.B.s and in less than 45 min at the 3,000 L and 5,000 L scales

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 60 sec, and k_La performance of 40 hr $^{-1}$ at all scales (Table 1).

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

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

Parameter	50 L S.U.B.	500 L S.U.B.	5,000 L S.U.B.
Maximum volume	50 L	500 L	5,000 L
Turndown ratio	10:1	20:1	20:1
k_La	>40 hr^{-1}	>40 hr^{-1}	40 hr^{-1}
t_{95} mixing times	<30 sec	<40 sec	<60 sec
Maximum P/V ratio	80 W/m^3	80 W/m^3	80 W/m^3

Goal

The goal of this study was to evaluate the performance of the DynaDrive S.U.B. across 50–5,000 L scales using two different cell lines (Table 2) together with previously developed processes specific to those cell lines for manufacturing up to 2,000 L scale.

These experiments were designed to demonstrate that the DynaDrive S.U.B. could be successfully implemented for use with multiple, high-demand CHO cell lines across scales with standard scale-up criteria [1]. Both cell lines were subjected to a 14-day fed-batch run at full working volume for each scale.

Table 2. Cell lines evaluated in 50 L–5,000 L DynaDrive S.U.B.s.

Parameter	Cell line 1	Cell line 2
Cell type	Gibco™ CHO DG44	CHO-M
Production medium	Sartorius Stedim™ Cellca™ Production Medium	Proprietary production medium
Feed supplement	Sartorius Stedim™ Cellca™ Feed Medium A and B	Proprietary feeds A and B
Titer range	~3 g/L	~7–8 g/L
Cell line characteristics	Shear sensitive to microsparger	High oxygen demand
Case study	1	2

Case study 1

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

Methods

Cells were expanded in shake flasks or pilot-scale DynaDrive S.U.B.s through the N-2 stage. The N-1 stage for the 50 L S.U.B. was performed at 10:1 turndown, while the N-1 stage for the 500 L and 5,000 L S.U.B.s were performed at 15:1 turndown. Fresh production medium was added to the S.U.B. after 3 days, resulting in the culture starting at proper N-stage production volume and initial seed density. Operating conditions are described in Table 3. Daily bolus feeds were added from days 3 to 13 through either a subsurface (5,000 L S.U.B.) or top feed line (50 L and 500 L S.U.B.s). Glucose was supplemented in the same manner on an as-needed basis after taking a glucose measurement following standard daily feeds to bring the final glucose concentration to >8 g/L. Cell counts, viability, dissolved gases, nutrients, and metabolites were measured off-line daily. Titer samples were filtered and frozen daily starting on day 6 for batch testing at the culmination of the run.

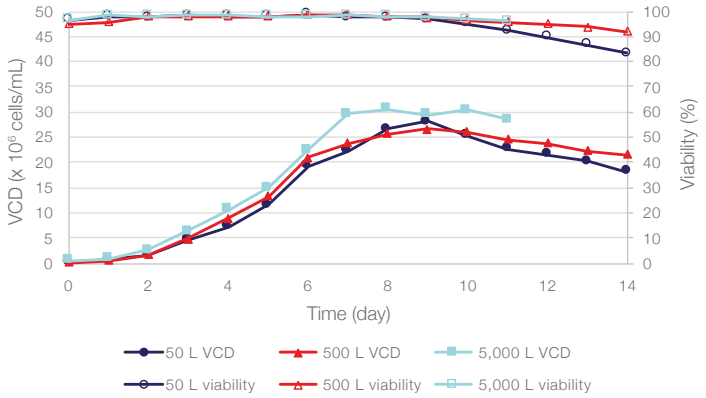

Figure 1. VCD and viability comparison of CHO DG44 cells in the 3 DynaDrive S.U.B.s.

Table 3. Operating parameters for evaluation of CHO DG44 cells in 3 scales of DynaDrive S.U.B.s.

S.U.B.	50 L	500 L	5,000 L
Target starting volume	35 L	320 L	3,000 L
Seeding density	0.3 x 10^6 cells/mL		
Temperature	37°C (days 0–5) 34°C (days 5–14)		
pH	6.9–7.1		
pH control	Acid control: sparged CO_2 Base control: 1 M sodium carbonate	Acid control: sparged CO_2 Base control: 1 M sodium carbonate	Acid control: sparged CO_2 through the macro DHS Base control: 1 M sodium carbonate
Agitation	185 rpm	83 rpm	60 rpm
DO	30%		
Air headspace	0.5 slpm	6 slpm	20 slpm
DO cascade	Air supplemented with O_2 through DHS	Air supplemented with O_2 through DHS	Air through the macro DHS; O_2 supplemented through the micro DHS
Feeding strategy	Daily bolus: 4.2% Feed A (based on inoculation volume), 0.42% Feed B, and glucose (as needed)		

Results

Viable cell density (VCD) and viability (Figure 1) show consistent growth profiles among the cultures, with similar cell densities and viability trends. While the 5,000 L culture terminated early, trending for critical parameters through peak cell density was similar in all cases. Peak VCDs were similar at ~30 x 10^6 cells/mL and the end-of-run viability was above 80% in all systems.

IgG titer was measured to be >3.0 g/L on day 14 (Figure 2) and was within expected range. CO_2 levels in the 5,000 L culture were slightly higher than those seen in the smaller vessels, but all were within expected thresholds (Figure 3).

Metabolite data collected off-line indicated healthy cultures with maintained glucose concentrations, and levels of metabolic byproducts, including lactate and ammonium, staying within expected thresholds (Figure 4). Minimum glucose concentrations were maintained according to the culture protocol with daily bolus feeds, and a glucose solution when needed.

The gas flow rates were controlled based on culture oxygen demand (Figure 5). For the 50 L and 500 L cultures, DO was maintained by sparging air first, then supplemented with O_2 through the single DHS. For the 5,000 L culture, DO was maintained by sparging air first through the macro DHS up to a maximum flow rate and O_2 through the micro DHS. Using these strategies, DO was maintained at 30 ± 5% with no major deviations during the duration of the run.

Importantly, O_2 gas flow requirements for the cultures remained very low at all scales, reaching maximums of 0.011 vessel volume per minutes (VVM) for the 50 L, 0.019 VVM for the 500 L, and 0.0064 VVM for the 5,000 L. These low gas flow rates in conjunction with the relatively low power inputs represent only about 30% of the available performance capacity of the system. The sparge strategy of the 5,000 L was adjusted on day 1 to balance O_2 demand with expected CO_2 stripping needs.

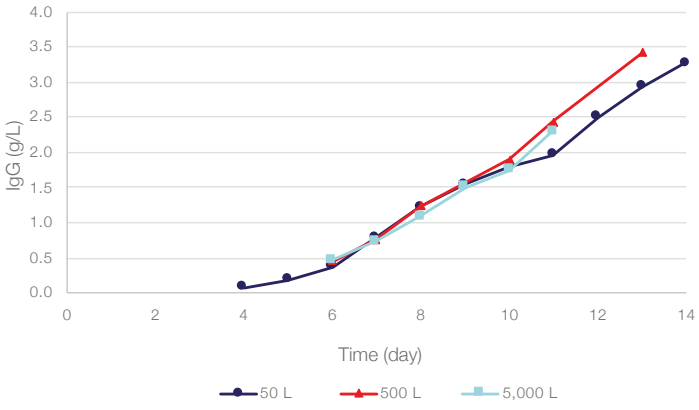

Figure 2. IgG titer results for CHO DG44 cells in the 3 DynaDrive S.U.B.s.

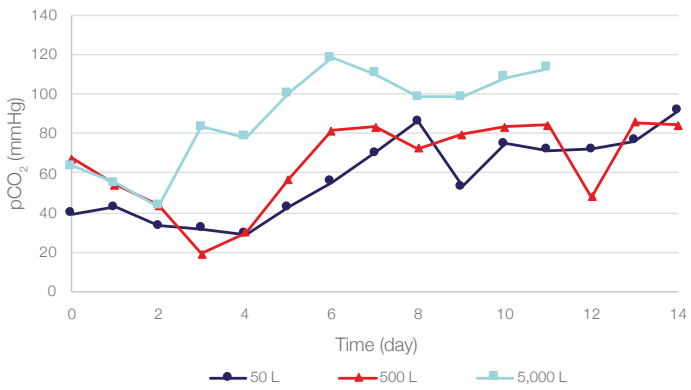

Figure 3. pCO_2 profile measurements for CHO DG44 cells in the 3 DynaDrive S.U.B.s.

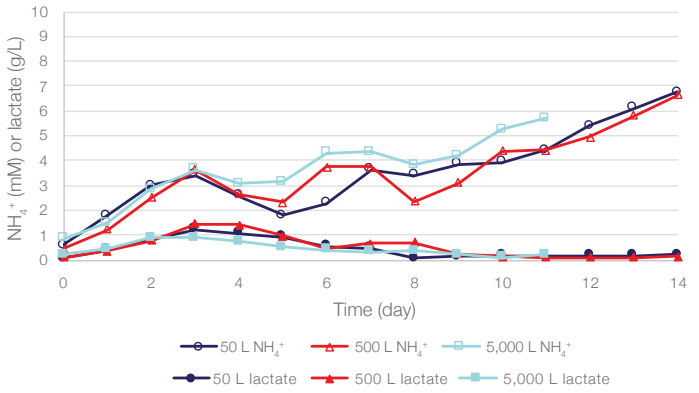

Figure 4. Lactate and NH_4^+ profiles for CHO DG44 cells in the 3 DynaDrive S.U.B.s.

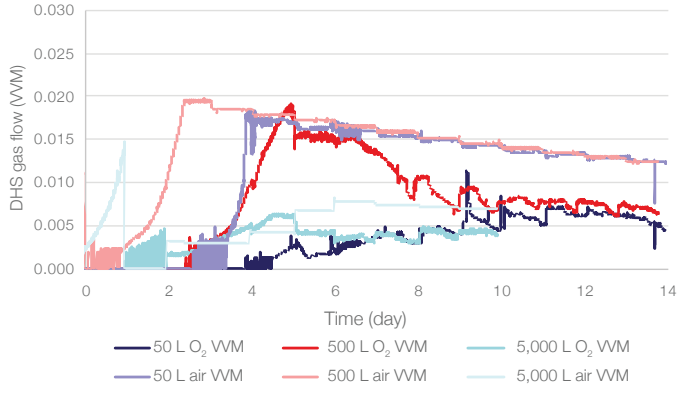

Figure 5. DHS gas flow rates for CHO DG44 cells in the 3 DynaDrive S.U.B.s.

Case study 2

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

Methods

Cells were expanded in shake flasks or pilot-scale DynaDrive S.U.B.s until seeding into the production vessels at 10:1 turndown ratio. Fresh production medium was added to the S.U.B. after 3 days, resulting in the culture starting at proper N-stage production volume and initial seed density. Operating conditions for each reactor are described in Table 4. Daily bolus feed quantities were based on approximate cell densities according to a defined schedule from days 3 to 11 through either a subsurface (5,000 L S.U.B.) or top feed line (50 L and 500 L S.U.B.s). Glucose was supplemented in the same manner on an as-needed basis. Cell counts, viability, dissolved gases, metabolites, and nutrients were measured off-line daily. Titer samples were filtered and frozen daily starting on day 6 for batch testing at the culmination of the run.

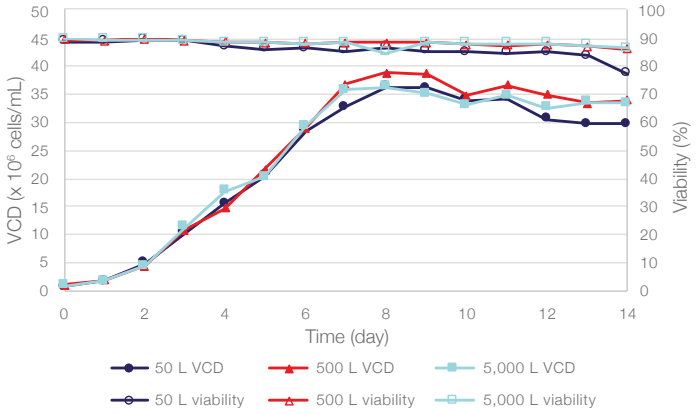

Figure 6. VCD and viability comparison for CHO-M cells in the 3 HyPerforma DynaDrive S.U.B.s.

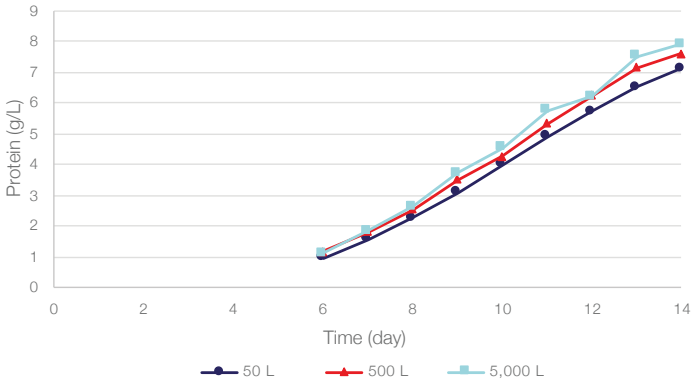

Figure 7. Protein titer results for CHO-M cells in the 3 HyPerforma DynaDrive S.U.B.s.

Table 4. Operating parameters for evaluation of CHO-M cells in 3 scales of DynaDrive S.U.B.s.

S.U.B.	50 L	500 L	5,000 L
Target starting volume	32.5 L	325 L	3,250 L
Seeding density	<1 x 10^6 cells/mL		
pH control	Acid control: sparged CO_2 Base control: 1.5 M sodium carbonate	Acid control: sparged CO_2 Base control: 1.5 M sodium carbonate	Acid control: sparged CO_2 through micro DHS Base control: 1.5 M sodium carbonate
Agitation	<130 rpm	<100 rpm	<60 rpm
DO	30%		
Air headspace	1x slpm	12x slpm	40x slpm
DO cascade	Air supplemented with O_2 through the DHS	Air supplemented with O_2 through the DHS	Air with O_2 supplemented through the macro DHS
Feeding strategy	Daily bolus feeds of Feed A and Feed B from days 3—11		

Results

VCD and viability for the cultures (Figure 6) show similar growth profiles among the cultures with similar peak VCD near 35–40 x 10^6 cells/mL and viability trends. Productivity (Figure 7) throughout the run was within expected range, with the 5,000 L HyPerforma DynaDrive S.U.B. exhibiting the best performance with protein titers near 8 g/L. Metabolite data (Figures 8 and 9) were near expected values for all parameters, including very wide pCO_2 ranges seen across all vessel sizes. Glucose concentrations were also consistent compared to legacy cultures in other S.U.B.s with daily bolus feeds (data not shown).

Gas flow rates for the culture were controlled based on oxygen demand (Figure 10). For all cultures, DO was maintained by sparging air first, then supplementing with O_2 through the same DHS. For the 5,000 L culture, the macro DHS was used for all DO control, while the micro DHS was used for pH control with CO_2 gas. Similar to the CHO DG44 culture, gas flow requirements among the vessel sizes were relatively low with peak O_2 demand of 0.014, 0.022, and 0.017 VVM for the 50, 500, and 5,000 L cultures, respectively.

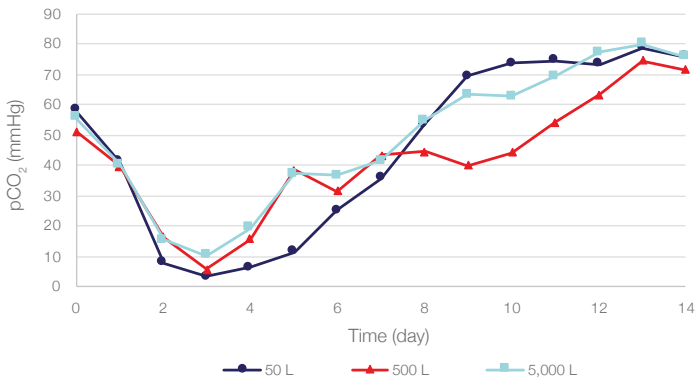

Figure 8. pCO_2 profile measurements for CHO-M cells in the 3 HyPerforma DynaDrive S.U.B.s.

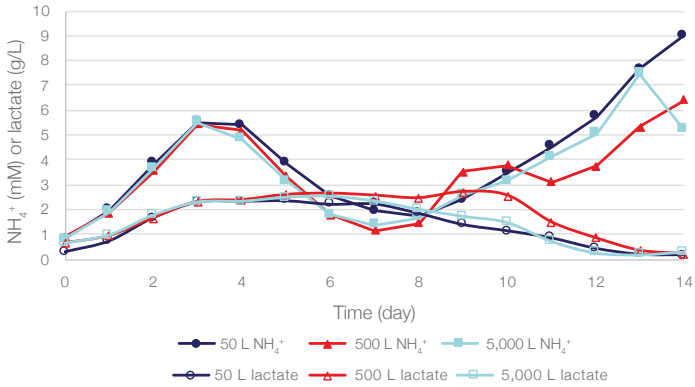

Figure 9. Lactate and NH_4^+ profiles for CHO-M cells in the 3 HyPerforma DynaDrive S.U.B.s.

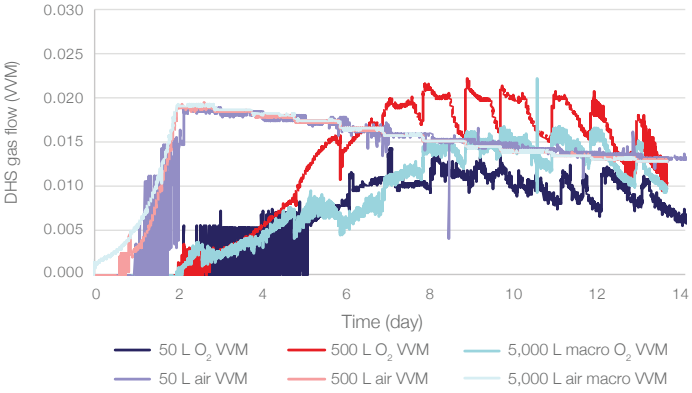

Figure 10. DHS gas flow rates for CHO-M cells in the 3 HyPerforma DynaDrive S.U.B.s.

Discussion

As cell cultures are scaled to large-volume S.U.B.s, several factors can become more difficult to manage and control. Often, decisions related to culture parameters and operation thresholds must be balanced to provide the culture with the best opportunity for success. The DynaDrive S.U.B. assists with ensuring culture success due to specifically designed aspects of the S.U.B., including the geometrically scalable agitator drive train and the uniquely linear sparge performance of the DHS, which both directly benefit mass transfer and mixing abilities.

The agitator drive train of the DynaDrive S.U.B. with multiple impellers at optimal locations allows for optimal and scalable power input. Most of the culture runs in these case studies were operated at a power input of approximately 20 W/m^3, far below the maximum recommended 80 W/m^3. The exception was the 50 L CHO DG44 culture which was run near the maximum of 80 W/m^3. Mass transfer in this design is more proportional to the power input of a system due to the mixer's ability to both disperse and retain sparged gas. Therefore, if a culture were to demand more mass transfer, increased mixer speeds above those tested here would be an option for improved performance.

The gas flow rates observed in these studies were far below the maximum rated gas flow rates for each system. As with power input for each system, mass transfer is shown to be linearly proportional and scalable to gas flow rate in the DynaDrive S.U.B.s. For the 50 L and 500 L DG44 cultures, total gas flow rate through the DHS was never higher than 0.037 VVM, less than 25% of the maximum 0.15 VVM limit. For the 5,000 L DG44 culture, total gas flow rate through the micro DHS reached only 0.0064 VVM (32 slpm). Additionally, the 5,000 L culture used a minimal macro DHS flow rate of only 30 slpm of air to maintain CO_2 stripping, and to provide additional O_2 mass transfer. These chosen gas flow rates resulted in easily maintained O_2 mass transfer to support the light-to-moderate demand cultures with cell densities of 25–30 x 10^6 cells/mL. Additionally, the CHO-M culture required only slightly higher gas flow rates while supporting even higher cell densities up to 40 x 10^6 cells/mL. Because the gassing strategies were markedly different between the 5,000 L cultures, gas flow requirements for the CHO-M culture were higher but still far less than can ultimately be delivered through the DHS and control system. Interestingly the gas flow rates observed here were only marginally higher than those observed in previous testing [1], with mainly longer durations of peak gas flow compared to lower-demand clones.

One of the biggest concerns when scaling to larger S.U.B. sizes is the ability to control dissolved CO_2 concentrations within ideal physiological ranges (60–100 mm Hg). While spargers in smaller S.U.B.s typically provide sufficient CO_2 stripping capability due to their often oversized bubbles, shorter liquid column heights, and favorable overlay surface-to-volume ratios, spargers for larger S.U.B.s can be limited in providing sufficient CO_2 stripping while maintaining required O_2 mass transfer at reasonable gas flow rates. The 5,000 L DynaDrive S.U.B. is equipped with three separate DHSs to provide optimal gassing to drive mass transfer for both gases of interest. The two larger macro DHSs provide a measurable amount of O_2 mass transfer while also providing more CO_2 stripping capability due to the larger bubbles created, while the single micro DHS provides higher O_2 mass transfer due to the smaller bubbles created. Balancing these spargers in tandem with agitation in these cultures allowed pCO_2 to remain within desired ranges for each culture, thus providing a very generous operating design window in anticipation of future process intensification that may be requested by the end user.

Culture conditions in a S.U.B. are highly variable and must be balanced with multiple inputs and outputs. Through experience and tracking online and off-line readings, setpoints for gas flow rates, agitation, pH, DO, and feed flow rates can be balanced. In general, metabolite buildups such as lactate and respired CO_2 lead to acidic conditions in the reactor, requiring the addition of base to balance the culture pH. Additionally, especially in larger vessels, buildup of pCO_2 can be detrimental to cell health. The S.U.B.s in this study were able to maintain pCO_2 and pH conditions through the employed gassing strategies leading to pCO_2 levels maintained within physiological conditions, similar to those observed in legacy processes. While the pH in each culture was balanced sufficiently, more optimal gassing and pH control strategies could be employed in the future to provide more optimal growth and production conditions. Additionally, the differing gassing strategies employed in these and other studies show the versatility of the DynaDrive S.U.B. to support multiple cell demands, feed strategies, and gassing requirements.

Finally, each reactor was seeded at low working volume: 10:1 turndown in the 50 L DynaDrive S.U.B. and up to 15:1 turndown in the 500 L and 5,000 L DynaDrive S.U.B.s. This has been shown to reduce the complexity of seed train requirements by eliminating intermediate vessels, consumables, and operation space. While using a low turndown ratio in a production vessel such as the 5,000 L DynaDrive S.U.B. may not be ideal for some commercial applications seeking to maximize daily productivity, this feature allows for flexibility in manufacturing spaces not previously available.

Conclusions

The DynaDrive S.U.B.s were able to support both clones in this study and provided controlled conditions to achieve target VCDs and protein titers while maintaining high viability in both the shear-sensitive DG44 cell line and the high-density CHO-M cell line. This was achieved by choosing simple scale-up parameters among the HyPerforma DynaDrive S.U.B. sizes, including consistent power input and gas flow rates. The low gas flow rates and agitation rates tested provided sufficient mass transfer to maintain DO setpoints while maintaining pCO_2 concentrations at or below maximum limits. Additionally, the ability of each DynaDrive S.U.B. to be seeded at low volume allowed reduction in seed train complexity by using the N-reactor as the N-1 culture stage. Fewer manipulations, media preparations, fluid transfers, and less overall consumption of natural resources has the potential to noticeably mitigate risk, reduce waste, and lower operating costs.

Overall, the different scale-up processes demonstrated in these studies show the versatility of the DynaDrive S.U.B. to maintain culture setpoints compared to historical processes with simple scale-up criteria. The modified drive train and sparging compared to legacy S.U.B. products did not have adverse effects on the cell culture, thus enabling a much larger design space for process development and production than previously available.

Additionally, the DynaDrive S.U.B. provides consistent, scalable performance from 50 L to 5,000 L sizes with simple scale-up criteria. With this demonstrated consistency, the DynaDrive S.U.B. platform will enable users to stay within S.U.B.s with minimal process changes as they scale up their process to meet commercial therapeutic demand.

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