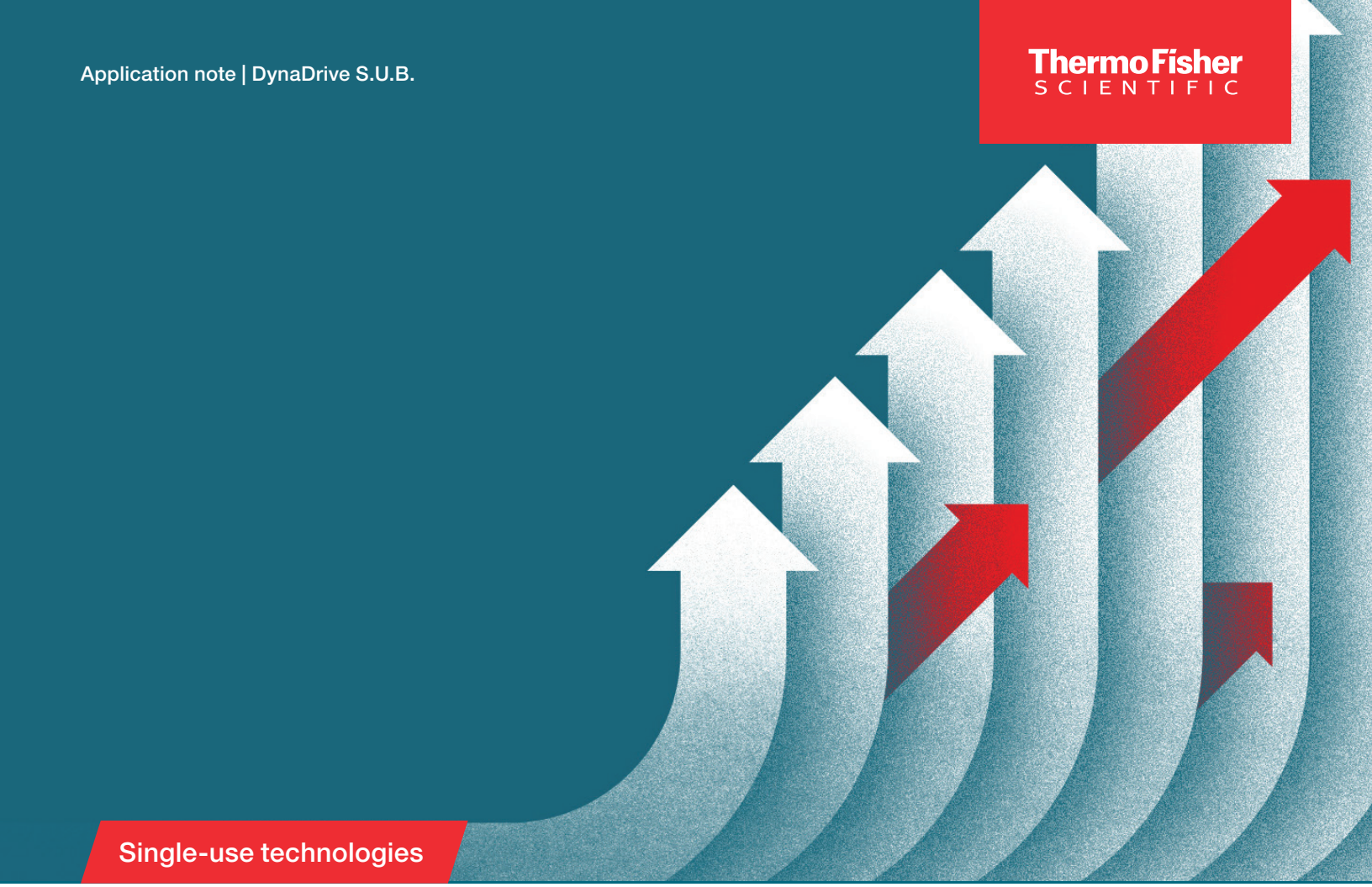
ThermoFisher SCIENTIFIC



Single-use technologies

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

Part 1: ExpiCHO-S Cells and CHO-S Cells (cGMP banked)

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

Additionally, more recently developed process intensification methods have allowed manufacturers 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. S.U.B. quality requirements, robustness, and functional performance can all become constraints, especially at scales 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 increase oxygen mass transfer.

thermo scientific

Maintaining a dissolved oxygen (DO) target in high-demand cell cultures can be increasingly difficult due to limitations in the amount of mixing power that can be distributed effectively through the drivetrain of traditional S.U.B.s. 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 or stripping of dissolved CO_2 (measured as the 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 CO_2 removal.

A next generation of 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 and process intensification. Previous limits are no longer a burden for the DynaDrive S.U.B., and it continues to leverage known and acknowledged benefits of legacy units. DynaDrive S.U.B.s are multifunction reactors for a range of applications, including intermediate-scale production of preclinical, clinical, and commercial material, as well as perfusion for both production and N-1 seed processes. Additionally, each DynaDrive S.U.B. includes features that are improved over legacy and alternative S.U.B. options:

- Each system is equipped with a Thermo Scientific™ BioProcess Container (BPC) load assist device, reducing handling and setup time, increasing safety, and providing consistent BPC loading. BPC loading can be accomplished in less time at the 50 L and 500 L scales compared to legacy S.U.B.s, and in less than 45 minutes at the 3,000 L and 5,000 L scales.
- Best-in-class enhanced drilled-hole sparger (DHS) provides repeatable and reliable performance that users of S.U.B.s have embraced due to its linear scale-up benefits.
- Revolutionary drivetrain design with multiple impellers allows increased power input and efficiency while offering reduced shear rates.
- Cuboid design contributes to better BPC fit 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.

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 at least 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.

Goal

The goal of this study was to evaluate the performance of the DynaDrive S.U.B. across 50 L–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 a 2,000 L scale. These experiments were designed to demonstrate that the DynaDrive S.U.B. could be successfully implemented for use with multiple cell lines across scales with standard scale-up criteria. Both cell lines were subjected to a 14-day fed-batch run at full working volume for each scale.

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	>50 hr^{-1}	>50 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

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™ ExpiCHO-S™ Cells	Gibco™ CHO-S™ Cells (cGMP banked; part of the Gibco™ Freedom™ CHO-S™ Kit)
Production medium	Gibco™ ExpiCHO™ Stable Production Medium (SPM)	Gibco™ Dynamis™ Medium
Feed supplement	Gibco™ EfficientFeed™ C+ AGT™ Supplement	
Titer range	Medium: ~3 g/L	Low: ~1 g/L
Cell line characteristics	Platform cell line	Legacy cell line
Case study	1	2

Case study 1

Scale evaluation using ExpiCHO-S Cells in a 14-day fed-batch run

Methods

Cells were expanded in shake flasks or pilot-scale S.U.B.s through the N-2 stage. The N-1 stage for each seed train was performed at a 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 are described in Table 3. Daily bolus feeds of 2X concentrated EfficientFeed C+ AGT Supplement were added from day 3 to 13 via 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 addition of EfficientFeed C+ AGT Supplement to bring the final glucose concentration to >4 g/L. Cell counts, viability, dissolved gases, nutrients, and metabolites were measured offline daily. Titer samples were filtered and frozen daily starting on day 6 for batch testing at the culmination of the run.

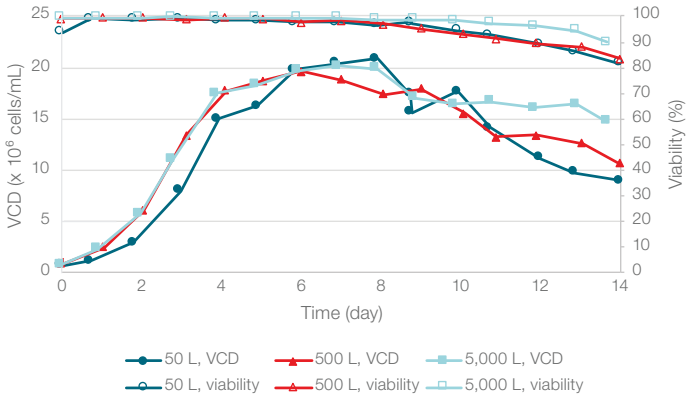


Figure 1. VCD and viability comparison of ExpiCHO-S Cells in the 3 DynaDrive S.U.B.s.

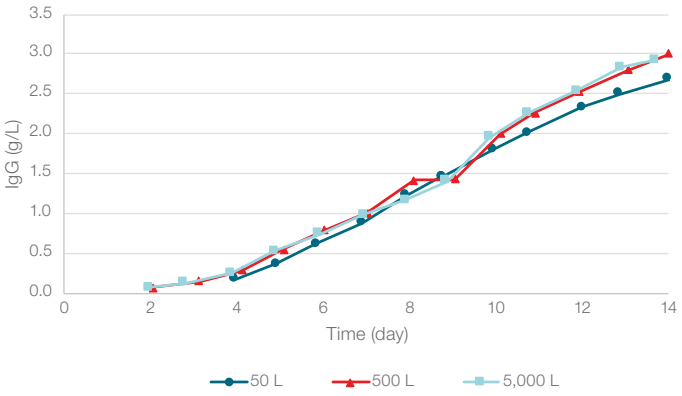


Figure 2. Titer results for ExpiCHO-S Cells in the 3 DynaDrive S.U.B.s.

Table 3. Operating parameters for evaluation of ExpiCHO-S 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	350 L	3,500 L
Seeding density		0.7 x 10^6 cells/mL	
Temperature		37°C (days 0–5) 34°C (days 5–14)	
pH		6.8–7.2	
pH control	Acid control: sparged CO_2 Base control: 1 N NaOH	Acid control: sparged CO_2 Base control: 1 N NaOH	Acid control: sparged CO_2 through macro DHS Base control: 1 N NaOH
Agitation	120 rpm	60 rpm	26 rpm (days 0–3) 33 rpm (days 3–14)
DO		40%	
Air headspace	1 slpm	6 slpm	10–20 slpm
DO cascade	Air supplemented with O_2 through DHS	Air supplemented with O_2 through DHS	Air supplied to both macro and micro DHS; O_2 supplemented through micro DHS
Feeding strategy	Daily bolus of 1.05 L EfficientFeed C+ AGT Supplement, and glucose (as needed)	Daily bolus of 10.5 L EfficientFeed C+ AGT Supplement, and glucose (as needed)	Daily bolus of 105 L EfficientFeed C+ AGT Supplement, and glucose (as needed)

Results

Viable cell density (VCD) and viability for the cultures show consistent growth profiles among the cultures, with similar cell density and viability trends (Figure 1). Peak VCDs were similar at ~20 x 10^6 cells/mL, and the end-of-run viability was above 80% in all systems. IgG concentration measured 2.7–3.0 g/L on day 14 and is within ranges observed historically (Figure 2). While CO_2 levels in the 5,000 L culture were within expected conditions, levels in the 50 L and 500 L cultures were slightly lower than anticipated, indicating possible over-stripping of CO_2 (Figure 3).

Metabolite data collected offline indicated healthy cultures with maintained glucose and low levels of metabolic byproducts, including lactate and ammonium, staying within ranges observed historically (Figure 4). Minimum glucose concentrations were maintained according to the culture protocol by feeding with EfficientFeed C+ AGT Supplement daily, 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 first sparging air, then supplementing with O_2 through the single DHS. For the 5,000 L culture, DO was maintained by sparging air through the macro DHS at a constant flow rate and sparging an air–O_2 mix through the micro DHS. Using these strategies, DO was maintained at 40 ± 5% with no major fluctuations during the duration of the run. Importantly, gas flow requirements for the cultures remained very low at all scales, never reaching past 0.035 vessel volumes per minute (VVM) for either the 50 L or 500 L S.U.B. For the 5,000 L S.U.B., gas flow requirements were below 0.01 VVM for the micro DHS with a constant 0.005 VVM for the macro DHS. These low gas flow rates in conjunction with the relatively low power inputs used at low rpm represent only about 30% of the available performance capacity of the system. The sparge strategy of the 5,000 L S.U.B. was adjusted on days 3–6 to successfully keep pCO_2 levels below 80 mm Hg for the balance of the cell culture run.

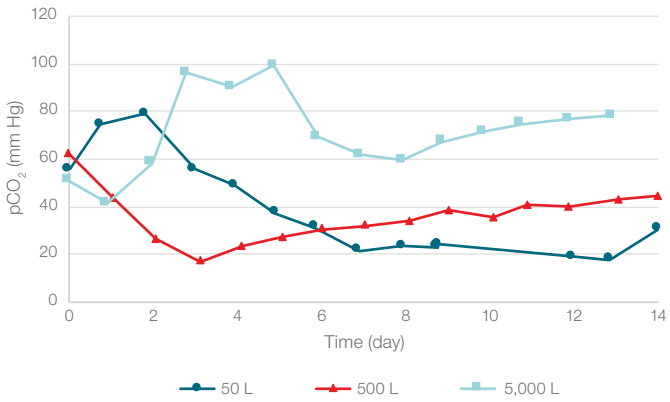


Figure 3. pCO_2 profile measurements for ExpiCHO-S Cells in the 3 DynaDrive S.U.B.s.

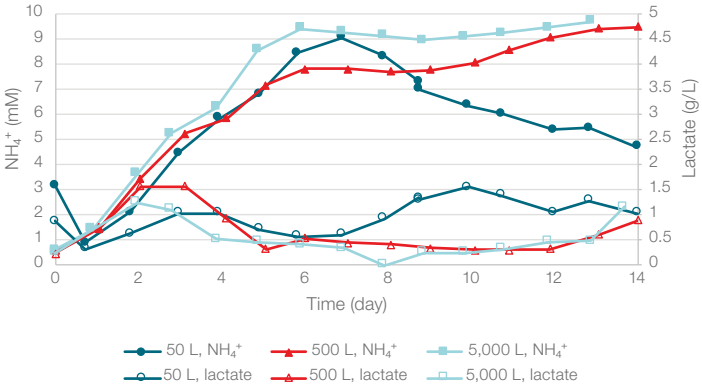


Figure 4. Lactate and NH_4^+ profiles for ExpiCHO-S Cells in the 3 DynaDrive S.U.B.s.

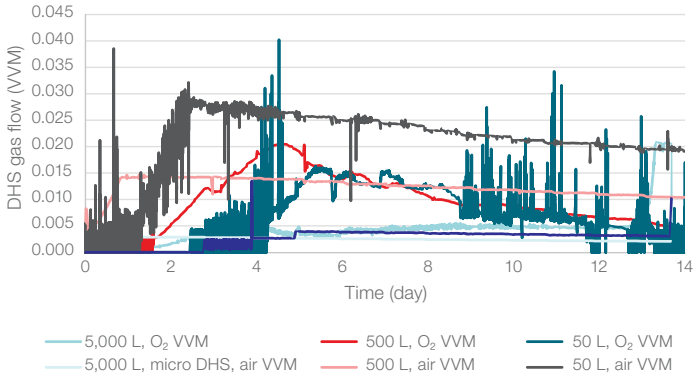


Figure 5. DHS gas flow rates for ExpiCHO-S Cells in the 3 DynaDrive S.U.B.s.

Case study 2

Scale evaluation using CHO-S Cells (cGMP banked) in a 14-day fed-batch run

Methods

Cells were expanded in shake flasks or pilot-scale S.U.B.s until seeding into the production vessels at either a 10:1 or 20:1 turndown ratio. The 50 L and 500 L S.U.B.s were brought to full volume with fresh production medium after 1–2 days, while the 5,000 L S.U.B. was filled to a 5:1 turndown ratio after 3 days and then full production volume after another day of growth. Operating conditions for each bioreactor are described in Table 4. A 2X concentration of EfficientFeed C+ AGT Supplement was added continuously from day 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 a continuous drip as needed depending on culture demands, to maintain glucose concentrations of 1–3 g/L. Cell counts, viability, dissolved gases, metabolites, and nutrients were measured offline daily. Titer samples were filtered and frozen daily starting on day 6. Titer samples were batch tested at the end of the run.

Results

VCD and viability for the cultures show similar growth profiles among the cultures, with similar peak VCD and viability trends (Figure 6). The 5,000 L culture exhibited slightly slower growth and lower peak VCD compared with the 500 L culture (VCD of 23 x 10^6 and 26 x 10^6 cells/mL, respectively) but was still within expected ranges. Productivity throughout the run was within expected ranges (Figure 7). The 50 L culture exhibited the lowest harvest titer, likely due to low pCO_2 in the latter half of the run.

Metabolites and gas flow demands were similar in concentration and magnitude compared to the ExpiCHO-S cell cultures. Glucose was maintained in the S.U.B.s at around 1–5 g/L, while lactate and ammonium concentrations were within ranges observed historically (data not shown).

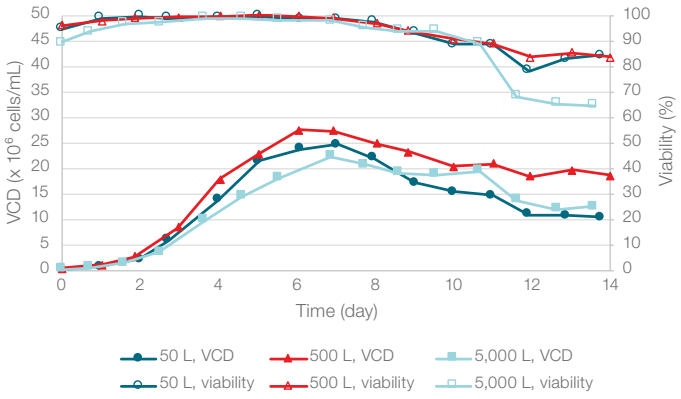


Figure 6. VCD and viability comparison of CHO-S Cells in the 3 DynaDrive S.U.B.s.

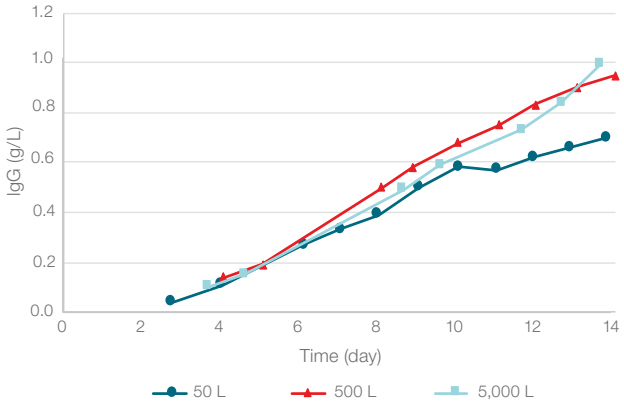


Figure 7. Titer results for CHO-S Cells in the 3 DynaDrive S.U.B.s.

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

S.U.B.	50 L	500 L	5,000 L
Target starting volume	42.5 L	425 L	4,250 L
Seeding density		0.3 x 10^6 cells/mL	
Temperature		37°C	
pH		6.8–7.2	
pH control	Acid control: sparged CO_2 Base control: not applicable	Acid control: sparged CO_2 Base control: not applicable	Acid control: sparged CO_2 through macro DHS Base control: not applicable
Agitation	120 rpm	68 rpm	31–41 rpm
DO		30%	
Air headspace	3 slpm	6 slpm	10–20 slpm
DO cascade	N_2 and O_2 through the DHS	N_2 and O_2 through the DHS	N_2 and O_2 through the micro DHS
Feeding strategy	7.5 L of EfficientFeed C+ AGT Supplement added on continuous drip from day 3 to 11, and glucose as needed	75 L of EfficientFeed C+ AGT Supplement added on continuous drip from day 3 to 11, and glucose as needed	750 L of EfficientFeed C+ AGT Supplement added on continuous drip from day 3 to 11, and glucose as needed

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 drivetrain and the uniquely linear sparge performance of the DHS, which both directly benefit mass transfer and mixing abilities.

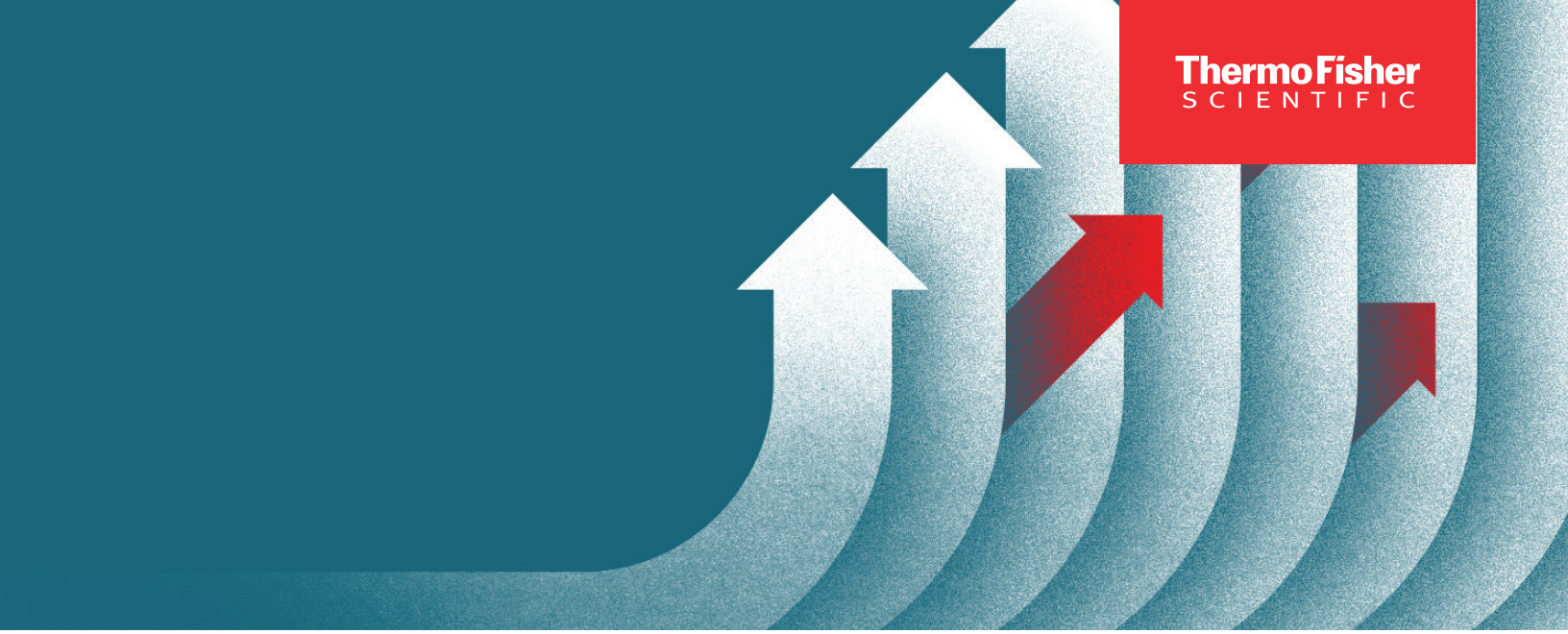
The agitator drivetrain of the DynaDrive S.U.B. with multiple impellers at optimal locations allows for optimal and scalable power input. The cultures performed in these case studies were operated at a power input of approximately 20 W/m^3, far below the maximum recommended 80 W/m^3. Mass transfer in 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 ExpiCHO-S cell cultures, total gas flow rate through the DHS was never higher than 0.035 VVM, less than 25% of the maximum 0.15 VVM limit. For the 5,000 L ExpiCHO-S cell culture, total gas flow rate through the micro DHS reached only 0.007 VVM (30 slpm) for most of the culture. Additionally, the 5,000 L culture used a minimal macro DHS flow rate of only 15 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 cultures of light to moderate demand with cell densities at 20–30 x 10^6 cells/mL. More demanding clones are expected to require higher gas flow rates or agitation but still remain within design limits of the DynaDrive S.U.B.s. It is important to note that the DHSs were designed with specific pore quantities and sizes to provide these high levels of mass transfer at low gas flow rates, removing the need for other sparger types such as the legacy sintered sparger.

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 3 separate DHSs to provide optimal gassing to drive mass transfer for both gases of interest. The two larger macro DHSs provide an 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 offline readings, setpoints can be balanced for gas flow rates, agitation, pH, DO, and feed flow rates. Generally speaking, metabolite buildup such as lactate and respired CO_2 lead to acidic conditions in the reactor that require 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. The 50 L and 500 L cultures actually showed a propensity to exhibit too much CO_2 stripping. 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.

Finally, each reactor was seeded at a low working volume: 10:1 turndown ratio in the 50 L S.U.B. and 20:1 turndown ratio in the 500 L and 5,000 L 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. is not 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 provide controlled conditions to achieve target VCDs and titers while maintaining high viability. This was achieved by choosing simple scale-up parameters among the 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 levels at or below maximum limits. Additionally, the ability of each S.U.B. to be seeded at low volume allowed reduction in seed train complexity by using the S.U.B. at the N-1 culture stage. Fewer manipulations, media preparations, and fluid transfers, and less overall consumption of resources have 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 drivetrain and sparging, when compared to legacy S.U.B. products, did not have adverse effects on the cell cultures, 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. With this demonstrated consistency, the DynaDrive S.U.B. will enable users to scale up their process with minimal process changes to meet demand for commercial therapeutics.

Authors: Jordan Cobia, Product Manager; Ben Madsen, R&D Manager

Learn more at **thermofisher.com/dynadrive**

thermo scientific