

Single-use technologies

50 L DynaDrive S.U.B. for perfusion cell culture applications

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

Summary

The Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.), the latest advancement in S.U.B. technology, offers better performance and scalability to far larger volumes than legacy S.U.B.s. Its cuboid-shaped tank offers several key advantages compared to legacy S.U.B. designs. The most important features of the DynaDrive S.U.B. are its superior mixing, mass transfer capabilities, and further improved scalability.

These are enabled by a unique stirred-tank design utilizing a novel drive train with multiple impellers, coupled with our next generation of drilled-hole sparger (DHS), allowing simple scalability and improved gas control. Coupled with standard large ports that perfusion systems typically require for connection to cell retention devices, the DynaDrive S.U.B. was specifically designed and is ideally suited for high-demand applications such as those seen in perfusion cell culture workflows.

The work presented here showcases the increased mass transfer performance seen in the 50 L DynaDrive S.U.B. and associated cell culture work, in both continuous and N-1 perfusion modes. This work demonstrates the ability of the DynaDrive S.U.B. to easily support cell densities in excess of 150 x 10^6 cells/mL while utilizing automation through the Thermo Scientific™ G3Pro™ Bioprocess Controller with online single-use sensors enabling easy and accurate control of a continuous culture.

Additionally, operation of an N-1 perfusion seed train culture shows the system easily supporting log growth to cell densities greater than 200 x 10^6 cells/mL. This work was enabled by utilizing a new perfusion medium, Gibco™ High-Intensity Perfusion (HIP) CHO Medium, at very low medium exchange rates, ultimately creating high-productivity cultures.

Materials and methods

Mass transfer testing

Mass transfer studies were performed in all vessels using the standard dynamic method, where the transition of oxygen from gas to liquid phase is represented by:

$$\frac{dC}{dt} = k_L a \cdot (C^* - C)$$

Equation 1

$k_L a$ is the volumetric mass transfer coefficient, C is the concentration of dissolved oxygen, and C* is the saturation concentration of dissolved oxygen (DO). kLa was measured by sparging air into an N_2-saturated solution. The test solution consisted of 1 g/L poloxamer 188 and 3.5 g/L HEPES buffer titrated to pH 7.25 at air saturation and 37°C.

Cell culture testing

Cell culture was performed in a 50 L DynaDrive S.U.B. integrated with a Repligen™ XCell ATF™ 6 system. CHO-K1 cells with good production rate stability were seeded at 0.3 x 10^6 cells/mL in HIP CHO Medium and perfused at rates defined for each study to allow for constant replenishment of nutrients and removal of waste and product. A 45% glucose solution was added as a supplement. Operating conditions for the DynaDrive S.U.B. and XCell ATF system are listed in Table 1. Measurements of cell count, nutrients, metabolites, and protein were taken daily.

Both a continuous and an N-1 log growth study were performed in the DynaDrive S.U.B. A satellite continuous perfusion run was performed in a 3 L glass bioreactor with the Repligen™ XCell ATF™ 2 system to compare culture performance against the 50 L DynaDrive S.U.B. continuous run.

Table 1. Cell culture parameters for the studies performed in the 50 L DynaDrive S.U.B. and XCell ATF system.

Parameter	3 L glass continuous perfusion	50 L continuous perfusion	50 L N-1 perfusion
Controller	G3Lab running TruBio DV12.3 software	G3Pro running TruBio DV12.3 software	
Working volume	2 L, automated via scale	40 L, automated via load cells	
Temperature	37°C		
Agitation	300 rpm	110 rpm (~20 W/m^3 power input)	
pH	7.0 ± 0.2 (CO_2 control only)		
Dissolved oxygen (DO)	40% air saturation, N_2/air/O_2 cascade		
Headspace air sparge	0.1 slpm	3 slpm	
Antifoam	10,000 ppm Antifoam C in phosphate buffered saline, automated via foam sensor		
Glucose feed	45% w/v glucose as needed to maintain 2–5 g/L in culture		
Target cell behavior control	Constant viable cell volume (VCV) at 100 mm^3/mL	Variable set points targeting cell viability near 95%	Log growth maintained
Perfusion rate	Days 3–4: 1 VVD, 67% medium concentration; Days 4–5: 1 VVD, 80% medium concentration; Days 5–end: 1 VVD, 100% medium concentration; automated via permeate tank load cells		100% medium concentration Days 3–5: ramped from 0.2–1.25 VVD; Days 5–end: 1.25 VVD
ATF rate (shear)	0.9 L/min (2,037 s^{-1})	17.3 L/min (2,037 s^{-1})	

Results

Mass transfer improvements

Mass transfer in the DynaDrive S.U.B. is compared with testing performed in a legacy HyPerforma S.U.B. (with standard DHS and impeller) and an enhanced HyPerforma S.U.B. (with enhanced DHS and larger impeller). Testing was performed at similar power inputs (Figure 1). Maximum recommended gas flow rates were tested for each S.U.B. test condition.

Oxygen mass transfer increases of 100–160% above that of the legacy S.U.B. were achieved, while performance was very similar between the enhanced 50 L HyPerforma S.U.B. and the DynaDrive S.U.B. The DynaDrive S.U.B. allows for support of even higher–cell density cultures by providing further improved mixing including lower impeller tip speeds and shorter mixing times. Additionally, the consistent scalability of mass transfer performance with increased gas flow rates and agitation (Figure 2) ensures easy and consistent control to support a broad range of cell densities and mass transfer requirements.

Continuous cell culture process in DynaDrive S.U.B.

Cells (Figure 3) were grown to a target viable cell volume (VCV) of approximately 100 mm^3/mL, after which a cell bleed was enabled to limit culture cell mass and assist with viability control. VCV was used as the primary control target (as opposed to viable cell density, VCD) because it assists with proper nutrient feed targets when dealing with high-density cultures where cell size, and thus cell volume, can vary.

$$VCV = \frac{\frac{4}{3}\pi \cdot (average\ cell\ radius)^3 \cdot (viable\ cell\ count)}{(reactor\ working\ volume)}$$

Equation 2

VCV in the 50 L culture was allowed to increase steadily by adjusting the daily cell bleed target to allow the viability to settle near 95–96% for the duration of the culture. Fueled by HIP CHO Medium and supported by the DynaDrive S.U.B., VCV reached a peak of 190 mm^3/mL, or 160 x 10^6 viable cells/mL, at a perfusion rate of 1 VVD with nearly constant nutrient and metabolite levels and protein production rates.

Vital nutrients and important metabolomic byproducts were monitored and controlled each day (Figure 4). In conjunction with the properly formulated HIP CHO Medium, reactor pH levels and DHS gas flow rates were balanced to keep pCO_2 levels in the physiological range.

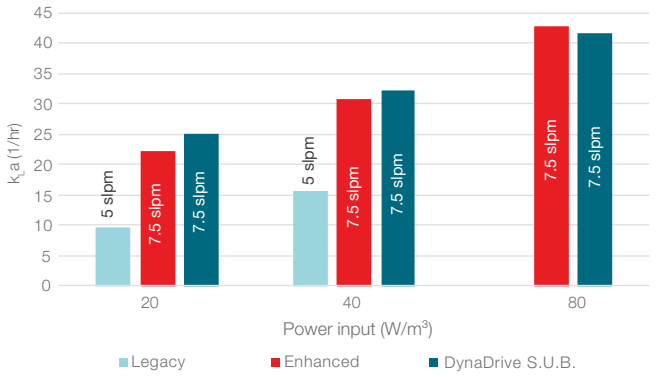


Figure 1. O_2 mass transfer coefficients (air through DHS) in 50 L legacy, enhanced, and DynaDrive S.U.B.s.

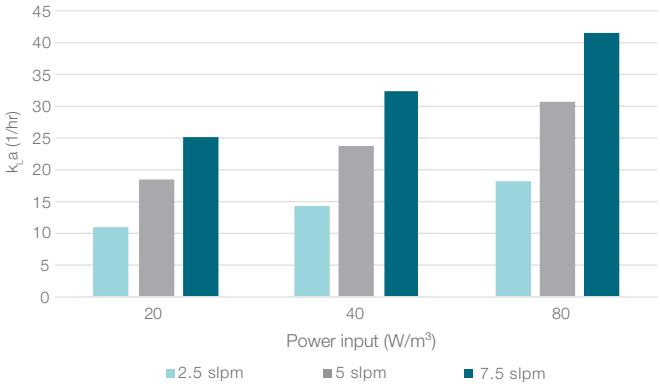


Figure 2. O_2 mass transfer coefficients (air through DHS) in the 50 L DynaDrive S.U.B. for listed power input and gas flow rates

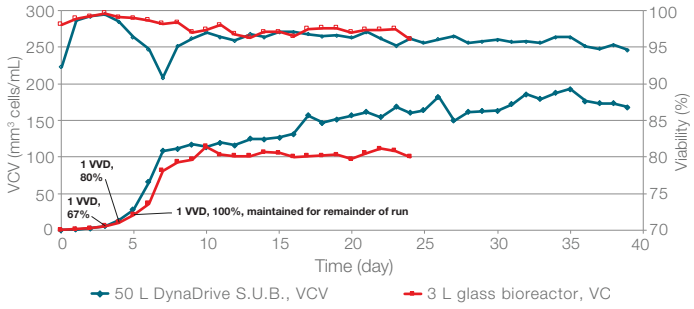


Figure 3. VCV and viability for the continuous perfusion cultures. Perfusion rates were maintained at 1 VVD for the duration of both cultures.

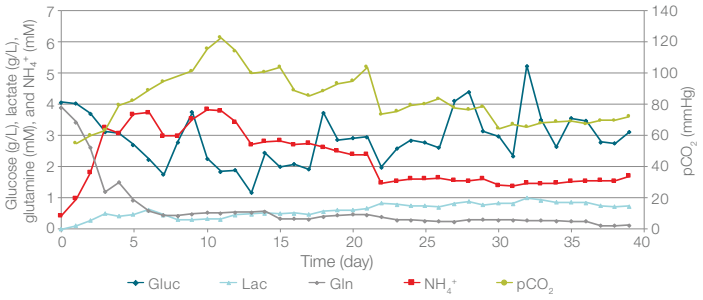


Figure 4. Key nutrient and metabolite data for the 50 L continuous perfusion culture.

Protein production rates (qP) were comparable between the DynaDrive S.U.B. and 3 L HyPerforma Glass Bioreactors across cell densities and operating conditions. While protein in the 50 L S.U.B. diminished the sieving performance of the ATF filter, this had little impact on the performance of the clone at perfusion rates similar to those tested at the bench scale (Figure 5).

The DO set point was maintained in the 50 L DynaDrive S.U.B. by sparging air through the DHS up to 0.6 slpm and then supplementing with O_2 as needed to support O_2 uptake rates of the cells (Figure 6). The O_2 flow rate increased up to a maximum of 2.5 slpm (33% of rated capacity of the DHS). This low gas flow rate coupled with the low agitation (approximately 20 W/m^3) showcases the ability to support higher cell densities than those achieved in this study. Additionally, the low gas flow rates and mass transfer scalability allowed control of DO to within 5% of the set point helping to ensure proper growth of the cell clone.

N-1 perfusion culture

The cells were maintained in exponential growth through day 7 by maintaining a minimum cell-specific perfusion rate (CSPR) up to a maximum overall perfusion rate of 1.25 VVD (Figure 7). Previous experience at the bench scale with this clone has shown that the perfusion filter does not maintain consistent performance at medium exchange rates higher than this threshold with this clone, due to the high cell densities achieved. Cells grew to a maximum VCV of 230 mm^3/mL (230 x 10^6 cells/mL) by day 10 of the culture, at which point the ATF had fouled and flow to and from the ATF could not be maintained by the diaphragm pump.

A similar gas strategy was used in this study compared to the continuous perfusion study. O_2 gas flow rates increased steadily to approximately 3 slpm until day 8, at which point gas flow maintained despite increases in cell density (Figure 8). While this ultimately could have been due to minor fouling of the DHS pores with the high cell concentration, the DO set point was easily maintained at a steady 40% with the steady flow provided through the DHS. Additionally, pCO_2, NH_4^+, and lactate levels were maintained at or below those seen in the continuous perfusion culture.

Discussion

The increase in k_La for the DynaDrive S.U.B. is attributed to the enhanced DHS with associated smaller and increased quantity of pores, as well as the increased mixing ability of the S.U.B. The optimized off-center impellers, coupled with the cuboid shape of the reactor, allow for high power input with a vertical drive train while avoiding vortexing, which can occur with non-baffled mixers. Additionally, the use of multiple impellers provides high bulk fluid cycling both radially and axially while providing lower

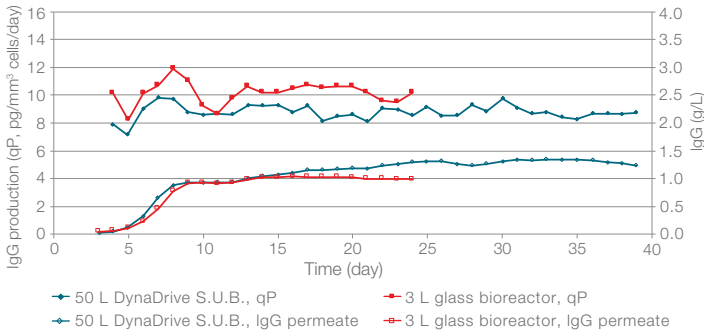


Figure 5. Protein concentration and production rates (qP) for the continuous perfusion cultures.

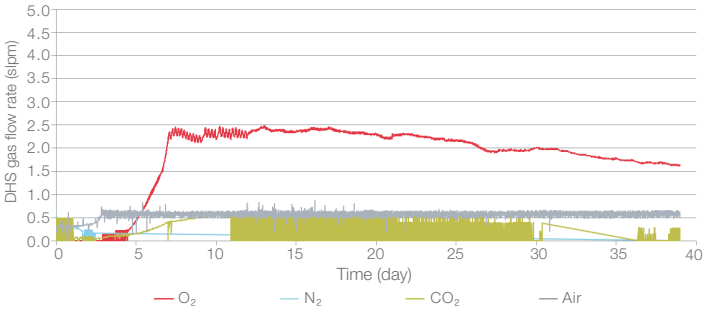


Figure 6. DHS gas flow rates for the 50 L continuous perfusion culture.

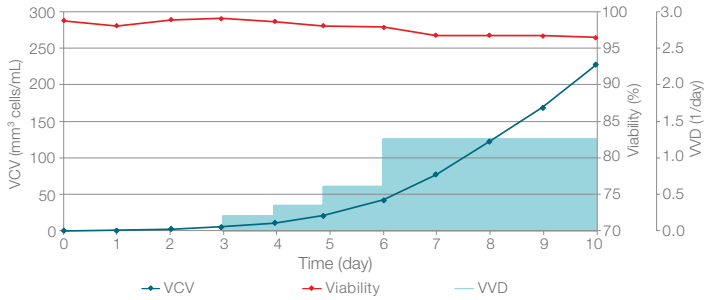


Figure 7. VCV and viability for the 50 L N-1 perfusion culture.
Perfusion medium concentration was 100% throughout run.

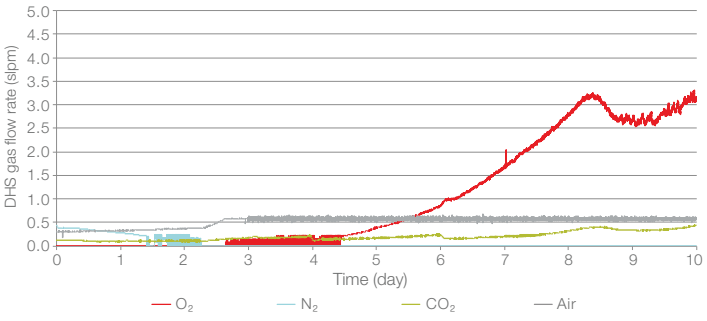
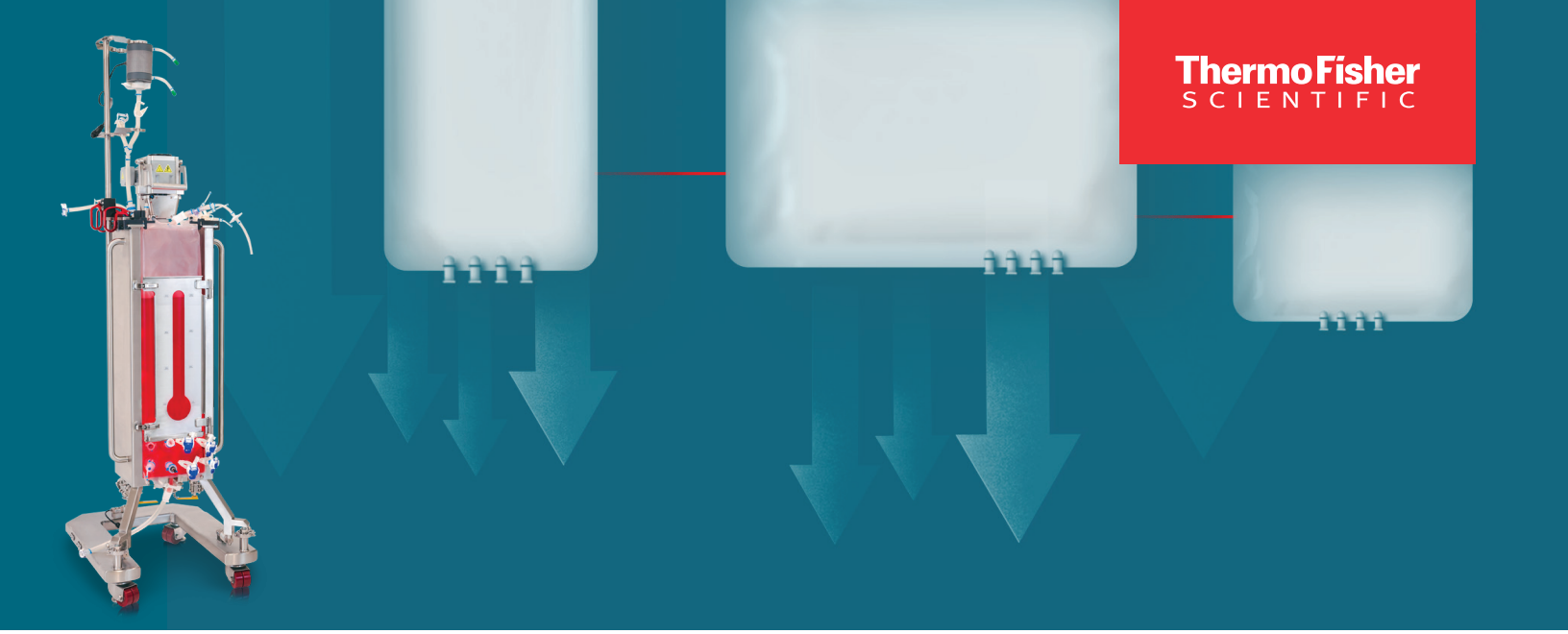


Figure 8. DHS gas flow rates for the N-1 perfusion culture.



impeller tip speeds at equivalent power inputs (30% less than with the enhanced S.U.B.), further improving on reactor goals to generate a better-scaling homogeneous and gentle cell culture environment. All of this allows for better predictability and simple scale-up, taking the guesswork out of performance and bioreactor controls. The continuous perfusion process showed the ability of the DynaDrive S.U.B. to support cell densities up to 160 x 10^6 cells/mL at only 2.5 slpm and 20 W/m^3 agitation, approximately 30% of the device's performance capacity.

The N-1 perfusion culture supported a cell density of 230 x 10^6 cells/mL, approximately 40% of the device's performance capacity. With a rated k_La of >40/hr for the 50 L DynaDrive S.U.B. at maximum mixing (80 W/m^3) and gassing rates (up to 7.5 slpm), higher cell densities could be easily supported while still allowing tuning for CO_2 stripping and pH management.

Critical to the success of a perfusion cell culture is balancing pH, pCO_2, DO, osmolality, glucose, lactate, and other metabolomic profiles. The HIP CHO Medium is specifically formulated to provide a complete medium at a balanced pH and osmolality to support high–cell density cultures at relatively low medium exchange rates. In the continuous perfusion study, the formulation balance led to low levels of lactate and ammonium waste by-products, allowing for the pH to be balanced slightly higher to 7.15 with minimal pCO_2 accumulation. This was further aided by the ability to control the glucose concentration in the culture with a separate glucose feed. Previous testing has shown that higher glucose levels in cell culture can lead to stunted early cell growth as well as an increase in lactate and a drop in the baseline pH of the culture. Balancing the glucose flow rate kept the lactate concentration low and allowed a gassing strategy that minimized CO_2 use, and the pH set point could be maintained without the addition of base.

Conclusion

With more biomanufacturing production processes moving toward perfusion, it becomes important to investigate new technologies to support processes that may stretch the limits of biology and engineering in legacy single-use systems. In these studies the DynaDrive S.U.B. has been shown to provide best-in-class mixing and mass transfer performance to easily support cell densities in excess of 200 x 10^6 cells/mL in perfusion workflows. Thus the DynaDrive S.U.B. becomes an ideal choice for small-volume continuous and N-1 perfusion. With ergonomic improvements, easy scalability, native high performance without customization requirements, and flexibility to integrate peripheral process analytical technology into the controller and BPC, the DynaDrive S.U.B. excels as a ready-made system for tomorrow's processes.

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