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Single-use technologies

500 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 previous S.U.B.s. Its cuboid-shaped tank offers several key advantages over legacy S.U.B. designs. The most important features of the DynaDrive S.U.B. are its superior mixing capabilities, mass transfer capabilities, and improved scalability.

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

The work presented here showcases the improved mass transfer performance seen in the 500 L DynaDrive S.U.B. and associated cell culture work in continuous perfusion mode. We demonstrate the ability of the DynaDrive S.U.B. to easily support cell densities in excess of 100 x 10^6 cells/mL while utilizing automation with the Thermo Scientific™ G3Pro™ Bioprocess Controller with online single-use sensors that enable easy and accurate control of a continuous culture. This success was enabled by a new perfusion medium, Gibco™ High Intensity Perfusion CHO (HIP CHO) Medium, ultimately creating high productivity cultures even at low medium-exchange rates.

Materials and methods

Mass transfer testing

Mass transfer studies were performed in all vessels using the standard dynamic method, where the transfer 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. $k_L a$ 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 500 L DynaDrive S.U.B. integrated with two parallel XCell ATF™ 10 systems (Repligen). 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 1 vessel volume per day (VVD) to allow constant replenishment of nutrients and removal of waste and product. A 45% glucose solution was added as a supplement to achieve a target glucose concentration of approximately 2–5 g/L. Operating conditions for the DynaDrive S.U.B.s and XCell systems are listed in Table 1. Measurements of cell count, nutrients, metabolites, and protein were taken daily.

The results of this study were compared against results obtained with similar cultures grown in a 50 L DynaDrive S.U.B. and a 3 L glass bioreactor, all of which were controlled using either a G3Pro or G3Lab Bioprocess Controller running Thermo Scientific™ TruBio™ DV12.3 software. Cells were initially seeded and perfused at a 350 L working volume to match filter flux conditions tested at bench and 50 L scales. After steady-state conditions were demonstrated, the reactor volume was increased to 500 L to demonstrate performance at full volume.

Table 1. Cell culture parameters for the studies performed at several bioreactor scales.

Parameter	3 L glass bioreactor	50 L DynaDrive S.U.B.	500 L DynaDrive S.U.B.
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	Days 0–45: 350 L; Days 47–60: 500 L; automated via load cells
Temperature	37°C		
Agitation	300 rpm (~40 W/m^3 power input)	110 rpm (~20 W/m^3 power input)	74–80 rpm (~30 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	6 slpm
Antifoam	10,000 ppm Antifoam C in phosphate buffered saline, automated via foam sensor		Gibco™ FoamAway™ Irradiated AOF Antifoaming Agent, 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%	
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 0.2–1.0 VVD; Days 5–end: 1 VVD
ATF unit	XCell ATF 2	XCell ATF 6	XCell ATF 10 (2 units)
ATF rate (shear)	0.9 L/min (2,037 s^{-1})	17.3 L/min (2,037 s^{-1})	50.0 L/min (1,263 s^{-1})
ATF flux	0.64 LMH	0.67 LMH	0.67–0.95 LMH

Results

Mass transfer improvements

Mass transfer in the DynaDrive S.U.B. was compared with testing performed in a legacy S.U.B. with a standard DHS and impeller and a S.U.B. with an 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 160–190% and 20% were achieved compared to the legacy and enhanced S.U.B.s, respectively. Importantly, the DynaDrive S.U.B. offered better mixing at lower tip speeds than the legacy and enhanced S.U.B.s at a similar power input, resulting in better culturing conditions and less shear. The consistent scalability of mass transfer performance with increased gas flow rates and agitation (Figure 2) ensured easy and consistent control to support a broad range of cell densities and mass transfer requirements.

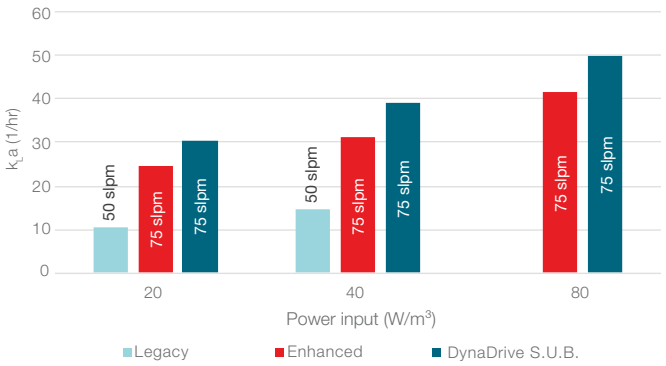


Figure 1. O_2 mass transfer coefficients (air through DHS) in 500 L legacy, enhanced, and DynaDrive S.U.B.s.

Continuous perfusion process in the DynaDrive S.U.B.

Cells were grown to a target viable cell volume (VCV) of 100 mm^3 cells/mL, after which a cell bleed was enabled to limit culture cell mass and assist with viability control (Figure 3). VCV (Equation 2) was used as the primary culture measurement for cell density (as opposed to viable cell density, VCD) because it made it easier to maintain proper nutrient feed targets when dealing with high-density cultures where cell size, and thus cell volume, could vary.

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

Equation 2

VCV in the 500 L culture was allowed to increase steadily by adjusting the daily cell bleed target to allow the viability to settle to approximately 95% for the duration of the culture. The target bleed rate was maintained at approximately 22% (volume/volume) for the duration of the culture. Fueled by HIP CHO Medium and supported by the DynaDrive S.U.B., VCV reached a peak of 161 mm^3 cells/mL (124 x 10^6 viable cells/mL) at a 1 VVD perfusion rate with near constant nutrient and metabolite levels and protein production rates.

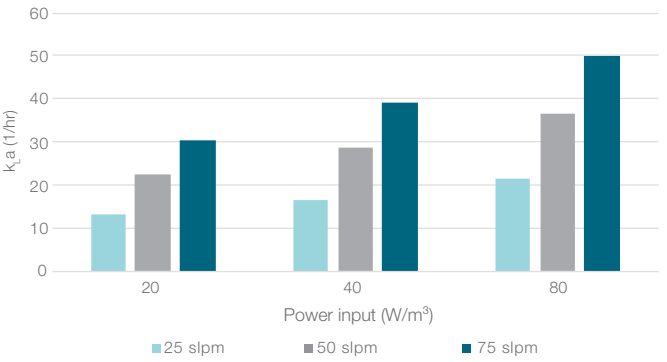


Figure 2. O_2 mass transfer coefficients (air through DHS) in the 500 L DynaDrive S.U.B. for listed power inputs and gas flow rates.

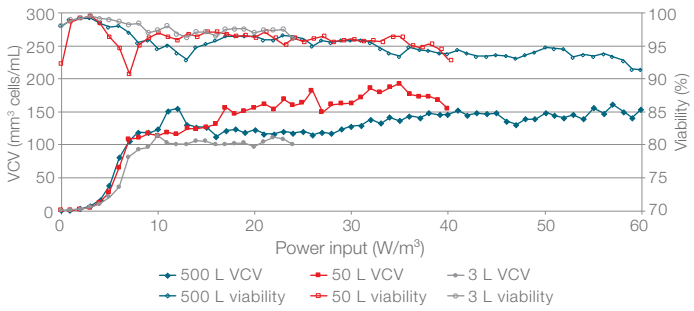


Figure 3. VCV and viability for the continuous perfusion cultures at various scales. After day 5, perfusion rates among the reactors were maintained at 1 VVD for the duration of all cultures.

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 between 80 and 120 mmHg from days 10 to 50.

Protein production rates (qPs) were comparable between the DynaDrive S.U.B.s and glass reactor across cell densities and operating conditions. All cultures showed an initial production spike, followed by a drop, and finally a steady state protein production rate as the cells adapted to steady-state conditions. The 500 L DynaDrive S.U.B. displayed the highest relative protein production rate among the vessels tested, but all were comparable and maintained expected values between 8 and 12 pg/mm^3 cells/day (Figure 5).

The 500 L run was performed at 350 L working volume for the first 45 days with the aim of achieving consistent protein production along with near-constant nutrient and metabolite profiles. This nearly perfect steady-state condition was achieved from days 35 to 45 with constant cell density, viability, and ammonium, lactate, glucose, and protein production. After the steady-state condition was demonstrated, the working volume was slowly increased to 500 L over 2 days by reducing the cell bleed while maintaining the medium feed and perfusion rates at the 350 L volume exchange rates. After reaching full volume, the bleed rate was readjusted to 22%, while the medium and permeate flow rates were adjusted to reach 1 VVD perfusion for the 500 L working volume. Perfusion at 500 L was then maintained for the remaining 13 days. Conditions were generally maintained at the higher volume, but a renewed steady state was not achieved.

The DO set point was maintained in the 500 L DynaDrive S.U.B. by sparging air through the DHS initially up to 6 slpm. It was then reduced to 2 slpm for most of the rest of the culture. The O_2 flow rate increased up to a maximum of 30 slpm initially (Figure 6), then settled during steady state near 15 slpm (20% of rated capacity of the DHS). This low gas flow rate coupled with low agitation (approximately 30 W/m^3) showcases the ability to support yet 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, which helped enable proper growth of the cell clone.

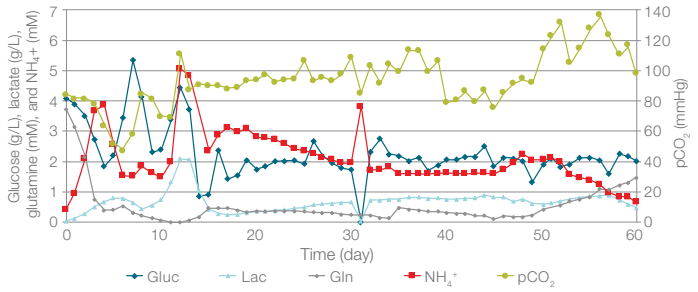


Figure 4. Key nutrient and metabolite data for the 500 L continuous perfusion culture.

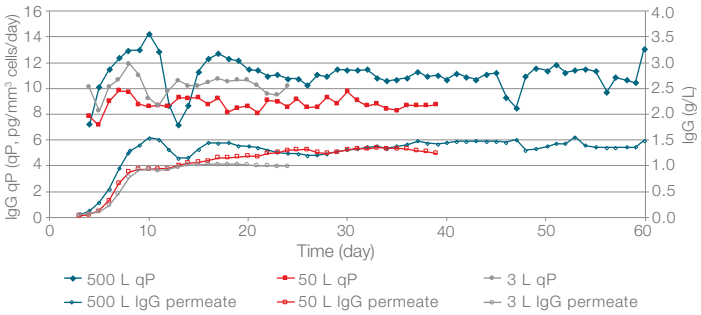


Figure 5. Protein concentrations and production rates (qP) for the continuous perfusion cultures.

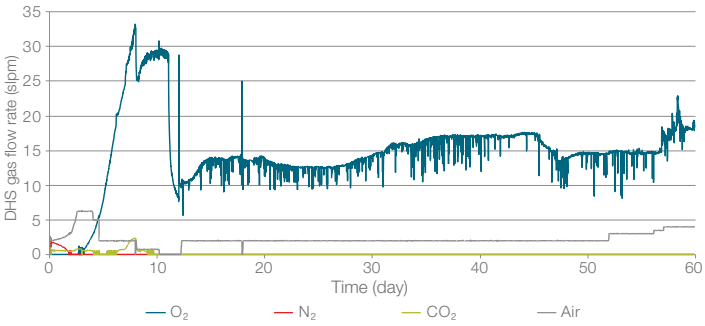


Figure 6. DHS gas flow rates for the 500 L continuous perfusion culture.

It is important to note the gassing differences and effects seen as the working volume changed from 350 to 500 L. A steady-state O_2 flow rate was noted at approximately 17 slpm (0.047 vvm) at 350 L. Maintaining cell density at the higher volume actually required less gas (15 SLPM O_2, 0.027 vvm) at a similar power input of 30 W/m^3. This is primarily attributed to the greater column height and residence time for the bubbles to transfer O_2 into the culture. A side effect of this was an increase in pCO_2 toward the end of the culture. This could have been mitigated by adjusting the air ballast to allow more CO_2 stripping, which likely would have resulted in slightly higher O_2 flow rates. Because such low gas flow rates and mixing speeds were required to maintain the DO set point, this could have been easily achieved within the gassing and agitation limits of the system.

The total oxygen flow rates through the DHSs for the 50 L and 500 L cultures relative to the working volumes and cell concentrations were analyzed (Figure 7). After the initial growth phase and reaching a balance of gases that supported proper oxygen mass transfer and CO_2 stripping, both DynaDrive S.U.B.s stabilized near the same relative gas flow rate. This gave further credence to the inherent scalability of the DynaDrive S.U.B.s and the ease of technology transfer between vessel sizes.

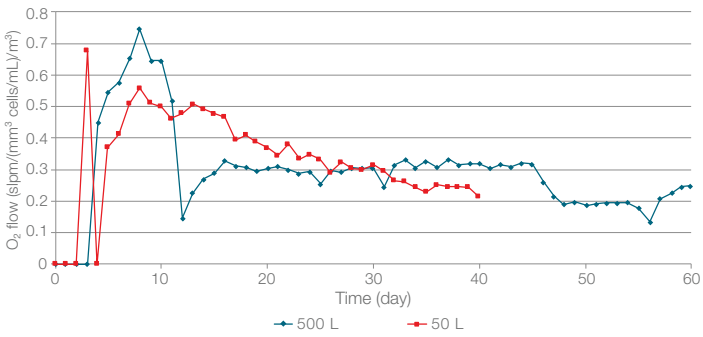


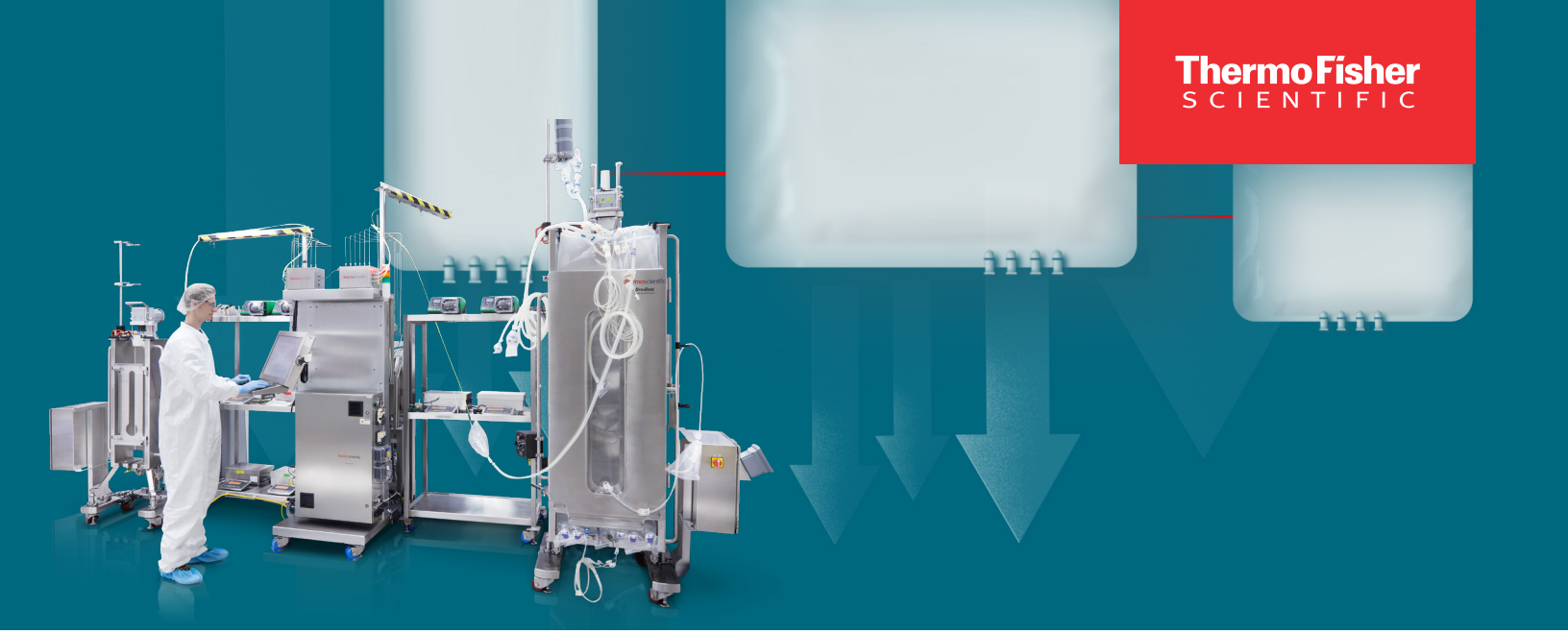
Figure 7. Oxygen gas flow through the DHSs relative to working volume and cell concentration for the 50 L and 500 L continuous perfusion cultures.

Discussion

The increase in k_La for the DynaDrive S.U.B. is attributed to both the enhanced DHS with associated smaller and increased quantity of pores, as well as the superior mixing ability of the S.U.B. The optimized off-center impellers coupled with the cuboid shape of the reactor allowed high power input with a vertical drivetrain while avoiding vortexing, which could occur with non-baffled mixers. The use of multiple impellers enabled high bulk-fluid cycling both radially and axially along with lower impeller tip speeds at equivalent power inputs (20–30% less than the enhanced S.U.B.). Together, these features provided a homogeneous and gentle cell culture environment. All of this increases predictability and simplifies scale-up, taking the guess work 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 161 mm^3 cells/mL (120 x 10^6 cells/mL) at less than 15 slpm and 30 W/m^3 agitation, which was approximately 20% of the device's performance capacity. With a rated k_La of >40/hr for the 500 L DynaDrive S.U.B. at maximum mixing (80 W/m^3) and gassing rates (up to 75 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. HIP CHO Medium is specifically formulated to be a complete medium at balanced pH and osmolality to support high high-density cultures at relatively low medium-exchange rates. Using the balanced medium in the continuous perfusion study led to low levels of lactate and ammonium waste byproducts, which allowed the pH to be balanced slightly higher to 7.10–7.20 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 showed that higher glucose levels in cell culture could 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 is 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 500 L DynaDrive S.U.B. provided superior mixing and mass transfer performance to easily support cell densities in excess of 120 x 10^6 cells/mL in perfusion workflows. Thus, the DynaDrive S.U.B. is a great choice for mid-volume continuous 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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Paula Decaria, Staff Engineer,
Katelyn Parkinson, Systems Design Engineer,

Reference

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