White paper | DynaDrive S.U.B.

Single-use technologies

Enabling high-performing perfusion cell culture

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

Background

It is estimated that by 2025, 35% of biologics will be manufactured using process intensification methods, including perfusion-based bioprocessing [1]. Advancement of the biopharma industry in this direction means there will be significantly higher demands placed upon both the equipment and consumables to drive these processes forward. This increased interest in performance creates an opportunity for improvement in consistent scalability as well as process design and management. In this paper, two innovations to increase process performance were examined.

The first innovation, the Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.), is the latest advancement in S.U.B. technology and offers better performance and scalability to far larger volumes, up to 5,000 L, compared to previous S.U.B.s. This cuboid-shaped tank offers several key advantages compared to traditional designs: most importantly, optimal mixing, mass transfer capabilities, and higher-range scalability. Improvements are enabled by a unique stirred-tank design that utilizes a novel drive train with multiple impellers coupled with our next-generation drilled-hole sparger (DHS). This enhanced design allows for simple scalability and improved gas control. The DynaDrive S.U.B. is specifically designed with standard large ports that are typically required by perfusion systems for connection to cell retention devices, making this system ideally suited for high-demand applications such as those seen in perfusion cell culture

workflows. Paired with Thermo Scientific™ Bioprocess Controllers and Thermo Scientific™ TruBio™ Bioprocess Control Software (powered by the Emerson™ DeltaV™ Distributed Control Platform), the DynaDrive S.U.B. provides a flexible operational platform.

The second innovation, Gibco™ High-Intensity Perfusion CHO (HIP CHO) Medium, is formulated to provide exceptional performance and ease of use in perfusion processes. HIP CHO Medium is capable of sustaining more than 1 g/L/day of continuous productivity, density of more than 100 x 10^6 cells/mL, and 95% viability at 1 vessel volume per day (VVD). The HIP CHO Medium is available in Gibco™ Advanced Granulation Technology™ (AGT™) format, which allows it to be quickly reconstituted in a single step at varying concentrations, enabling tailored processing with a single product. With this high-performing medium supported by the largest global network, you can focus on getting your product to market.

Combined, these tools provide the basis for robust perfusion production while offering the three key factors required to help you efficiently reach successful production: scalability, performance, and ease of use.

Scalability

The DynaDrive S.U.B., with hardware availability at 50 L, 500 L, 3,000 L, and 5,000 L, was engineered to encompass scalability directly in the design to provide consistent operating parameters among vessel sizes. Each S.U.B. size can operate at extremely low working volumes (up to 20:1 turndown ratio) with consistent performance comparable to full-volume operation. This allows for simple technology transfer from volume to volume and vessel to vessel. Inclusion of multiple impellers reduces impeller tip speed, improves bulk mixing, and increases power input. The design assists with improved mixing in each size through scale-up, as well as providing consistent mixing through drain. This modeling and design effort helps provide significant reductions of risk in process scale-up and simplifies how to align process behavior.

The DynaDrive S.U.B. has been carefully modeled to provide exceptional consistency of scaling performance from 50 L up to 5,000 L, taking the effort out of matching scaling behaviors (Figure 1).

Additionally, computational fluid dynamic (CFD) modeling has shown exceptional mixing performance of the DynaDrive S.U.B. compared to the mixing performance of other large-scale stainless-steel systems [2]. CFD images show excellent mixing both radially and vertically throughout the vessel height, resulting in a homogeneous suspension both near the bottom as well as at the top of the DynaDrive S.U.B. (Figure 2).

To fuel production, medium handling must scale up in tandem. The AGT format is a significant boon for high-volume medium preparation, as it wets and goes into solution quickly and easily (Figure 3). Since the AGT format does not require pH adjustments, labor and time to prepare large volumes of medium is swift, consistent, and easy to manage.

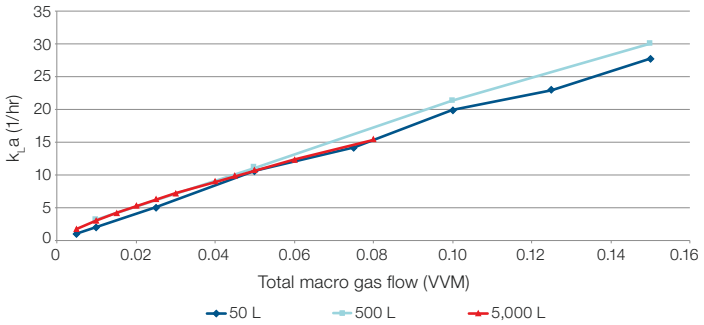


Figure 1. k_La data for the 50 L, 500 L, and 5,000 L DynaDrive S.U.B.s at 20 W/m^3 agitation and specified gas flow rates.

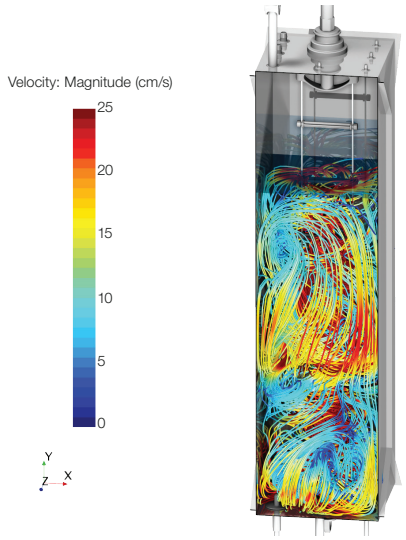


Figure 2. CFD modeling showing fluid streamlines in the 50 L DynaDrive S.U.B.

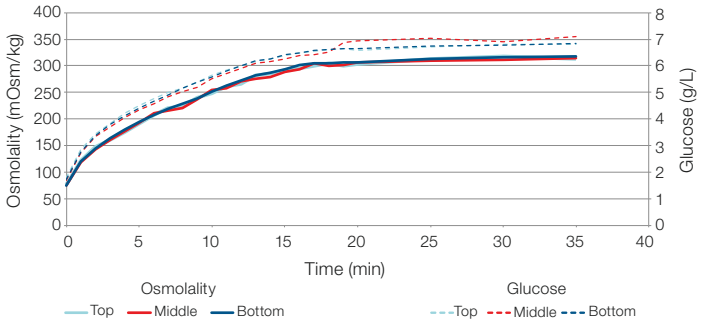


Figure 3. Mixing of AGT format medium in a 5,000 L imPULSE S.U.M. Samples were pulled from the top, middle, and bottom of the S.U.M. at various time points and measured offline.

Additionally, the HIP CHO Medium allows for flexible use in both seed trains and production. Stocking a single product, combined with labor savings, enables favorable cost of goods.

Even when formulating at 5,000 L with a Thermo Scientific™ imPULSE™ Single-Use Mixer (S.U.M.), after dispensing all of the AGT at once (simulating a suboptimal operation), the AGT medium can be completely dissolved in 37 minutes (Figure 4).

Performance

Studies using the HIP CHO Medium were performed in the 3 L Thermo Scientific™ HyPerforma™ Glass Bioreactor and 50 L DynaDrive S.U.B. IgG-producing CHO cells adapted to the medium were seeded at 0.3 x 10^6 cells/mL in each vessel and allowed to grow exponentially. Perfusion with either a Repligen™ XCell™ ATF 2 or ATF 6 Single-Use Device was enabled at 1 VVD medium exchange on day 3 of each culture. Initially, a lower-concentration medium formulation was used (67%), then ramped to 80%, and finally to 100% medium concentration at day 5 of the culture. Cells were grown to target cell volumes of approximately 100 mm^3/mL viable cell volume (VCV), then controlled to various targets by enabling cell bleeding. Operating parameters for the vessels were maintained, including temperature, dissolved oxygen, pH, and impeller power input.

The functional flexibility of the medium was demonstrated in side-by-side 3 L runs with 2 L working volume, where one vessel had the medium's concentration increased to 100% in the steps described above, and the other was kept at 67% concentration; bleeding was adjusted over time to bring both vessels to target and sustain approximately 95% cell viability (Figure 5).

It can be seen that high sustainable performance was achieved at 1 VVD with the HIP CHO Medium, stabilizing at approximately 1.2 and 1.7 g/L/day IgG for the 67% and full media concentrations, respectively.

Additionally, side-by-side cell runs using the 3 L HyPerforma Glass Bioreactor and 50 L DynaDrive S.U.B. were conducted (Figure 6).

The high cell viability, as well as nutrient and metabolite levels measured offline, indicated a healthy culture while operating at high VCVs. Bleeding was adjusted to target ~95% cell viability in the 50 L DynaDrive S.U.B., driving cell density to a peak cell volume of 190 mm^3/mL (164 x 10^6 cells/mL).

Importantly, the 50 L DynaDrive S.U.B. was operated at a low agitation rate of 20 W/m^3 and used only up to 3 slpm of oxygen to maintain cell densities, which is only ~30% of its performance capacity. This suggests higher cell densities and more demanding cell lines can be supported while still allowing tunability of gases or agitation to maintain ideal cell culture conditions and scalability.

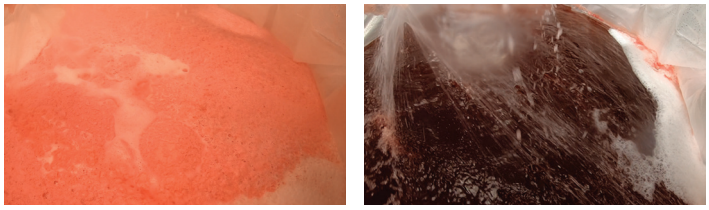


Figure 4. Pictures taken from the top of the 5,000 L imPULSE S.U.M. at 0 (left) and 37 (right) minutes. Note the complete dissolution of the AGT format medium at 37 minutes, consistent with the glucose and osmolality data in Figure 3.

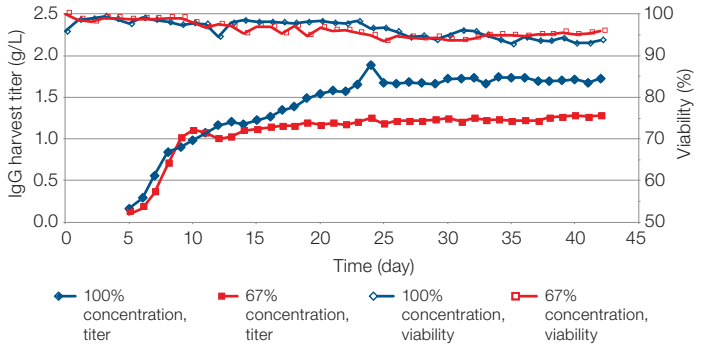


Figure 5. Side-by-side continuous perfusion in the 3 L HyPerforma Glass Bioreactor (2 L working volume) at two different medium concentrations, 67% and 100%.

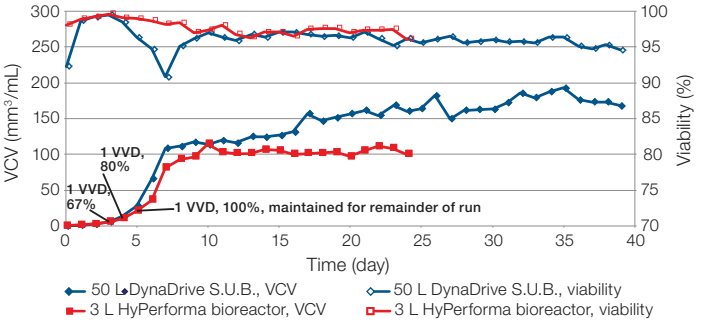


Figure 6. VCV and viability data for the 3 L HyPerforma Glass Bioreactor and 50 L DynaDrive S.U.B. cell runs. Both cultures achieved stable viable cell volumes of >100 mm^3/mL while maintaining high viability at a perfusion rate of 1 VVD.

Ease of use

The DynaDrive S.U.B. was specifically designed with multiple features to simplify use of operation compared to legacy systems, including:

- Large front doors that open completely on all systems, for easy DynaDrive S.U.B. BioProcess Container (BPC) loading
- Load-assist device for easy and consistent deployment of DynaDrive S.U.B. BPC
- Autosampling compatibility
- Low turndown ratio allows for intra-vessel scale-up, reducing required seed train vessels and fluid transfers, enabling standardized BPCs
- Ergonomic drain port for efficient and complete harvest of material

In addition to the benefits of the DynaDrive S.U.B. hardware and BPC, improved ease of use is realized when coupling the DynaDrive S.U.B. with exceptional sensing and controller options. Standard process analytical technologies (PAT) such as temperature, dissolved oxygen, and pH monitoring are included in most BPC offerings. Implementation of advanced PAT such as cell density measurement via a capacitance probe, glucose measurement via Raman spectroscopy, foam sensing, or dissolved carbon dioxide (dCO_2) measurement is accomplished with proper port and sensor pairings with the DynaDrive S.U.B. BPC and through simple integration with Thermo Scientific™ HyPerforma™ G3Pro™ Bioprocess Controllers. Thus, advanced control of media and feed flow rates as well as automated cell bleeding to maintain target cell densities can be achieved. Additionally, control of gassing to maintain proper culture conditions, including pCO_2 levels, as well as control of the resultant foam layer are accomplished through the same cGMP capable controller.

To accommodate a variety of perfusion needs and clone variation, the HIP CHO Medium can be formulated at a concentration as low as 67% and still provide high functionality (Figure 5). If a clone proves sensitive to a nutrient-dense medium, the operator can perform adaptation, cell banking, passaging, and seed train work at a lower nutrient concentration similar to that of a typical fed-batch medium. It also allows perfusion without the need to preassess cell-specific perfusion rate (CSPR) because the initial perfusion can be run at a lower concentration and increased in stages to higher concentrations as osmolality decreases (Figures 5 and 6).

While tracking the growth rate of an initial perfusion run at a static VVD medium exchange rate, a sudden drop can be observed, indicating the limit to sustaining maximum growth rate in a single perfusion run (Figure 7). Employing a bleed after this slows down the rate of cell density increase. Monitoring the percent cell viability and adjusting the bleed to a specific target to determine a sustainable CSPR for continuous perfusion provides the final details for operating needs. Alternatively, for intensified fed-batch perfusion or concentrated fed-batch perfusion, simply allowing the run to proceed without bleeding will immediately identify peak cell density and the behavior following the peak, providing an early assessment of practical run length.

In this manner, a more accurate performance profile of a given clone can be generated on a single, initial perfusion run to determine early CSPR, clone performance at higher density, and sustaining CSPR in the case of continuous perfusion operation. Further, the flexibility of the HIP CHO Medium allows perfusion processes to be run at very low VVD, making medium handling simpler and reducing labor and total medium volume per gram of product. However, if a toxic or unstable product is being manufactured, the HIP CHO Medium can be intentionally used at a lower concentration and higher VVD to quickly remove the product from the reactor before the product density goes above the intended range. With simple reconstitution and easy scaling, HIP CHO Medium can help maximize ease of use in designing a robust perfusion process.

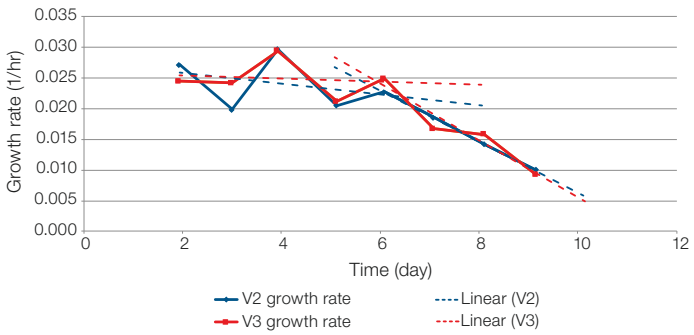
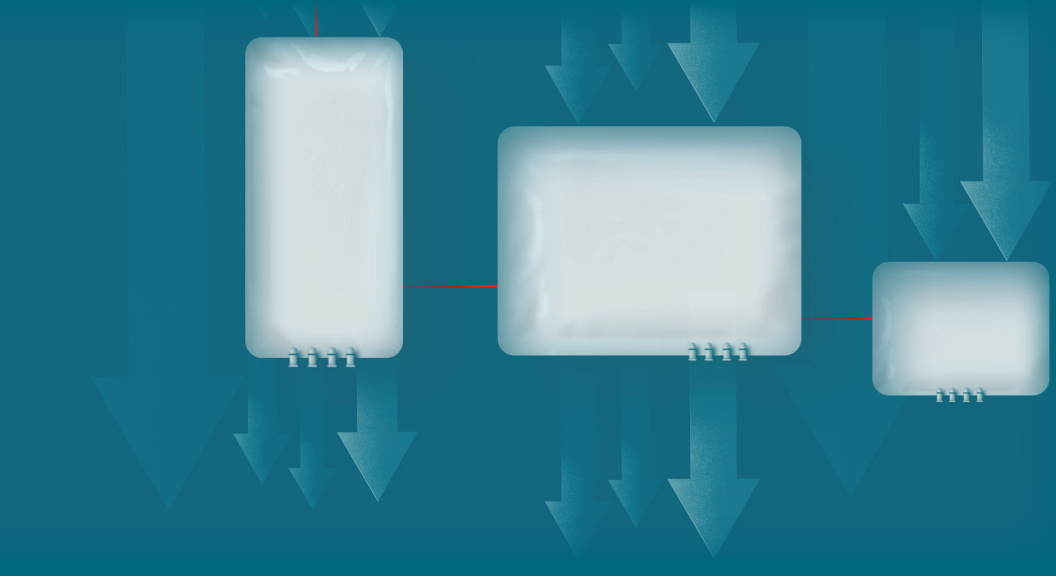


Figure 7. Growth rate in 3 L HyPerforma Glass Bioreactor replicates run side by side at 1 VVD. Linear regressions are applied pre- and post-growth rate change to mark the time in the process where approximate CSPR limits are reached to sustain logarithmic growth.



Conclusions

The DynaDrive S.U.B. and the HIP CHO Medium are efficient tools to help simplify an array of perfusion workflows while easily supporting high performance and providing a clear path toward production scale-up. Combined with a G3Pro Bioprocess Controller, they deliver a complete perfusion platform that is ready to bring the most from a clone, reducing labor and effort while flexibly adapting to process needs.

References

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