Single-use technologies

ThermoFisher SCIENTIFIC

Perfusion eBook

Unlock the power of perfusion with preconfigured hardware and disposable systems

thermo scientific

Contents

Overview

Perfusion is a cell culture process that requires two things: the exchange of medium, and a mechanism to retain cells. The goal of the perfusion process is to maintain high cell density and viability with increased control and faster development. This method allows for intensified production methods, and the seed train can be accelerated to enhance productivity within the bioproduction workflow. Perfusion has been used within biomanufacturing for years, although only recently has it been able to achieve high cell densities and stability in both product quality and operational processing.

What is perfusion?

Perfusion is a continuous cell culture process that uses a media feed source, bioreactor, cell retention device, and harvest technology to generate high product yields while reducing the overall working volume of upstream bioprocessing.

Why use perfusion cell culture processing?

Perfusion cell culture processing offers the benefit of automation in both process parameter monitoring and operational labor. The labor required for processing is shifted toward the front end, rather than the continuous monitoring and flow rate adjustments that must be performed to optimize fed-batch processing. After a run is set up, little work is required to keep it going. Using a stable and optimized cell line allows for increased consistency and confidence that product quality remains the same over a length of time. Robust sensing technologies offer the capability of continuously measuring cell density, glucose, and other pivotal process parameters. Combined with the ability to keep flow rates consistent across the entirety of a run, continuous measuring eliminates a substantial portion of the labor associated with traditional fed-batch cell culture processing.

Overview

Types of perfusion processes

Seed train	Used to achieve high cell densities while maintaining growth within a short duration. The goal is to create massive quantities of cells in the log phase to save and use as cell banks or to seed batch processes.
Concentrated fed-batch	Alternating tangential flow (ATF) filtration or tangential flow filtration (TFF) must be used as the cell retention method. Cells and the product are returned to the reactor throughout the run.
Intensified fed-batch	A seed train process that initially uses a high-density seed vessel and then seeds a larger vessel at a higher starting density than standard. This leads to increased cell densities in the N-stage reactor in a reduced timeframe and allows for faster turnaround of the production vessel.
Continuous	A process where products and waste are removed as fresh medium is added in regimented intervals across a specific cell growth period. The goal is to develop a process that maintains a steady state in which productivity and product quality can be sustained long-term with minimal variability.

Advantages of perfusion

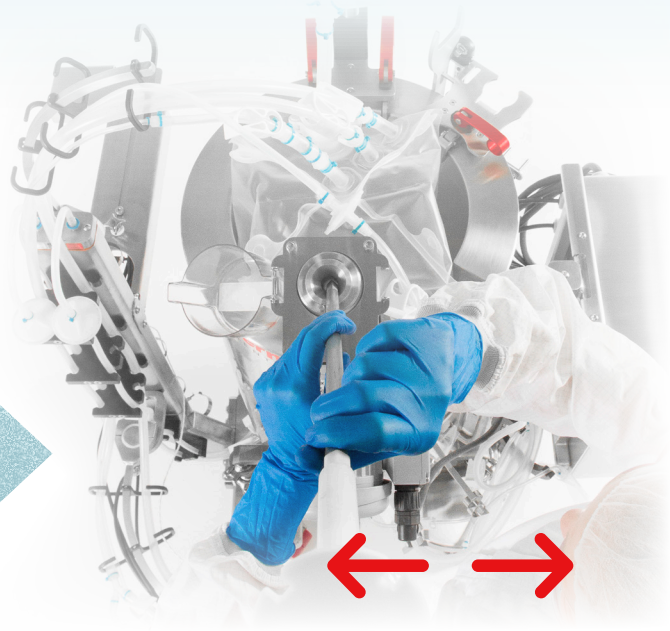
- Flexibility in the production train with the ability to use configurable single-use products
- Automation and digitization of operational procedures with improved monitoring capabilities
- Improved scalability to create higher quantities of protein faster once the process is optimized
- Efficiency for modular or multiproduct facilities that support a diverse biological portfolio

Single-use perfusion solutions

While it is estimated that only a small number of approved biologics products in the market are currently manufactured through perfusion or continuous processing, this method of production is gaining ground in its applicability for bioprocessing. Perfusion technology, the driving force for continuous manufacturing, was first used in the 1980s for the manufacture of sensitive proteins that, under traditional production, did not maintain their biological function. Later, users leveraged this method in the production of blood clotting factors, antibodies, and enzymes. The promise of greater productivity, high quality, and increased flexibility at more economical costs has renewed interest in this technology.

Improvements in single-use systems allow the implementation of high-density cultures through continuous manufacturing in emerging workflows. While progressive advances in media optimization and improved clone genetic selection increase stress on continuous single-use systems, strategic enhancements to the bioreactors are integrated to counter the perceived limitations. Coupled with best-in-class media hydration, hold tanks, and bioreactor control systems, a continuous bioprocessing workflow is established.

The resurgence in the popularity of continuous manufacturing has led to an increased focus on technologies that support perfusion as a complete workflow solution. With the appropriate hardware in place, those who wish to accomplish continuous manufacturing in the near future must augment their process with accessory products from various sources. Thus, selecting consumable products that both deliver high performance and connect seamlessly within a process remains a crucial decision.



Single-use perfusion solutions

Large-scale solutions

Upstream bioproduction has experienced a substantial movement toward single-use systems. This has been driven primarily by the need to reduce contamination risk and cleaning requirements when compared to stainless steel systems, and to allow for faster changeover of equipment between batches. At the same time, bioprocess manufacturing operations have matured significantly, and the intensification of cell culture processes has pushed the limits of legacy single-use systems. With a continued focus on large-scale monoclonal antibody production and tremendous growth of gene therapy and viral vector vaccines, companies are exploring how to scale manufacturing processes efficiently.

DynaDrive S.U.B.

The Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.) is the latest advancement based on our history of proven innovation, offering superior performance to accommodate larger volumes as programs progress to commercialization.

Key features:

- Flexibility and economy of scale
- Greater productivity with unsurpassed performance
- Superior power input per volume and mixing times
- Innovative cubical geometry and ergonomic design

Brochure
DynaDrive Single-Use Bioreactor (S.U.B.)
June 2022

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

Single-use perfusion solutions

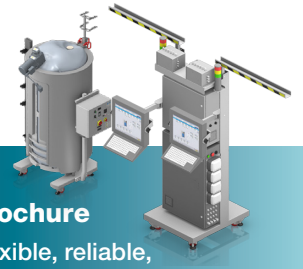
Large-scale solutions

HyPerforma G3Pro Bioprocess Controller

The Thermo Scientific™ HyPerforma™ G3Pro Bioprocess Controller enables an additional layer of versatility by allowing mobility and flexibility in terms of reconfiguration and application expansion. Consistent, accurate data management is essential for organizations in process development and throughout bioprocessing scale-up.

Key features:

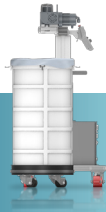
- Scalability from 50 L to 5,000 L
- Open-architecture capabilities to integrate with vessels from other suppliers
- Redundant sensor control mechanism, built-in circuit for optional stack light, and alarm relay for building alarm
- Utilizes Thermo Scientific™ TruBio™ Bioprocess Control Software powered by the Emerson™ DeltaV™ platform, with a touchscreen National Electrical Manufacturers Association (NEMA) interface for data entry and control



Brochure
Flexible, reliable, and configurable bioprocessing solutions
February 2021

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

Single-use perfusion solutions



Brochure
Single-use mixing technologies
August 2020

Large-scale solutions

Mixers

Our mixing products are designed for current good manufacturing practice (cGMP) bioproduction applications, both upstream and downstream, and feature companion Thermo Scientific™ BioPprocess Container (BPC) products designed specifically for superior performance in our systems.

Mixing technology platforms:

- Thermo Scientific™ HyPerforma™ Single-Use Mixer (S.U.M.)
- Thermo Scientific™ imPULSE™ Single-Use Mixer (S.U.M.)
- Thermo Scientific™ HyPerforma™ Single-Use Mixer (S.U.M.) DS 300
- Thermo Scientific™ HyPerforma™ Mixtainer™ System

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

Single-use perfusion solutions

Large-scale solutions

3D BioProcess Containers

Thermo Scientific™ 3D BPCs are cost-effective and diverse alternatives to conventional stainless steel systems. They employ a novel design approach that is highly valued for its versatility and utility. BPC components are readily integrated into a variety of high-performance systems for all steps in the production of biologics.

Key features:

- Consistent cGMP manufacturing helping to produce high-performing solutions
- Assisting with supply assurance, with stocking strategies for components and robust high-quality film
- Design flexibility allowing you to have standard, configurable, or custom solutions

Brochure
Single-use
BioProcess Containers
July 2021

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

Single-use perfusion solutions

Benchtop solutions

Scaling processes from bench to pilot to manufacturing scale remains a challenge for many in the bioprocessing industry. The cost and time of bench-scale process optimization contribute to making the scale-up and scale-down of bioprocessing a challenge. Our benchtop bioprocessing solutions offer flexible, reliable bioreactor and control systems for laboratory-scale applications.

Brochure
Benchtop bioprocessing solutions
January 2021

HyPerforma G3Lab Controller

The Thermo Scientific™ HyPerforma™ G3Lab Bioprocess Controller is compatible with most brands of single-use or autoclavable bioreactors or fermentors that are ≤50 L, including stirred-tank and rocking models. The enclosure contains state-of-the-art transmitters along with power supplies, pumps, I/O modules, and the hardware required to connect to the control network, providing maximum control capacity.

Key features:

- Open-architecture capabilities to integrate with vessels from other suppliers
- Data transfer and scalability from R&D to production to manufacturing
- The ability to build and manage complex, multifeed dosing strategies

HyPerforma Glass Bioreactors

Thermo Scientific™ HyPerforma™ Glass Bioreactors are available in 1 L, 3 L, 7 L, and 15 L total volume sizes. They offer straightforward operation and rapid assembly and are manufactured with the highest standards for materials and surface finish.

Key features:

- Motor adapter with coupling windows and alignment marker for easy assembly
- Ergonomic head plate design for easy assembly and disassembly

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

Single-use perfusion solutions

Benchtop solutions

2D BioProcess Containers

This design is used for small, simple BPCs and is produced from two sheets of film that are heat-sealed around the perimeter to form a pillow-shaped chamber. The ports are heat-sealed into the end seal or onto one of the faces of the chamber.

Key features:

- Produced in cGMP facilities, with common processes for manufacturing redundancy
- Automated lines for producing BPCs
- Engineering support to design and maintain products and processes
- Constructed of a 5-layer Thermo Scientific™ Aegis™ 5-14 film

Brochure
Single-use
BioProcess Containers
July 2021

Single-use perfusion solutions

Storage containers

Our rigid support containers hold closed-system BPCs and open-top tank liners for harvest and storage, or process liquid transportation. Our reusable support containers accommodate a variety of sizes and processes, including plastic for in-house and transport applications, and stainless steel for in-house, large-volume liquid-handling operations.

Our multiple design options help determine the ideal container solution for your process needs, taking into consideration application, batch size, filling and emptying, number of process steps, and locations of the process steps.

Brochure
Storage and transport containers
May 2020

Cylindrical Drums

Thermo Scientific™ drums provide a qualified shipping system for BPCs.

Key Features:

- Bottom- and top-drain options with a locking lid
- Multiuse for shipping and storage applications
- Ergonomic design for ease of use
- Matching dolly for ease of mobility

Cylindrical Tanks

Thermo Scientific™ cylindrical tanks are convenient, cost-effective containers for BPCs used for internal unit operations, and are not qualified for shipping.

Key Features:

- Low-cost option
- Top-drain access for in-house use

HyPerforma Smartainer 3.0 Systems

Thermo Scientific™ HyPerforma™ Smartainer™ 3.0 systems are stainless steel support containers used for in-house, large-volume, liquid-handling operations. The 3.0 systems have an updated design and offer multiple sizes up to 3,000 L.

Key Features:

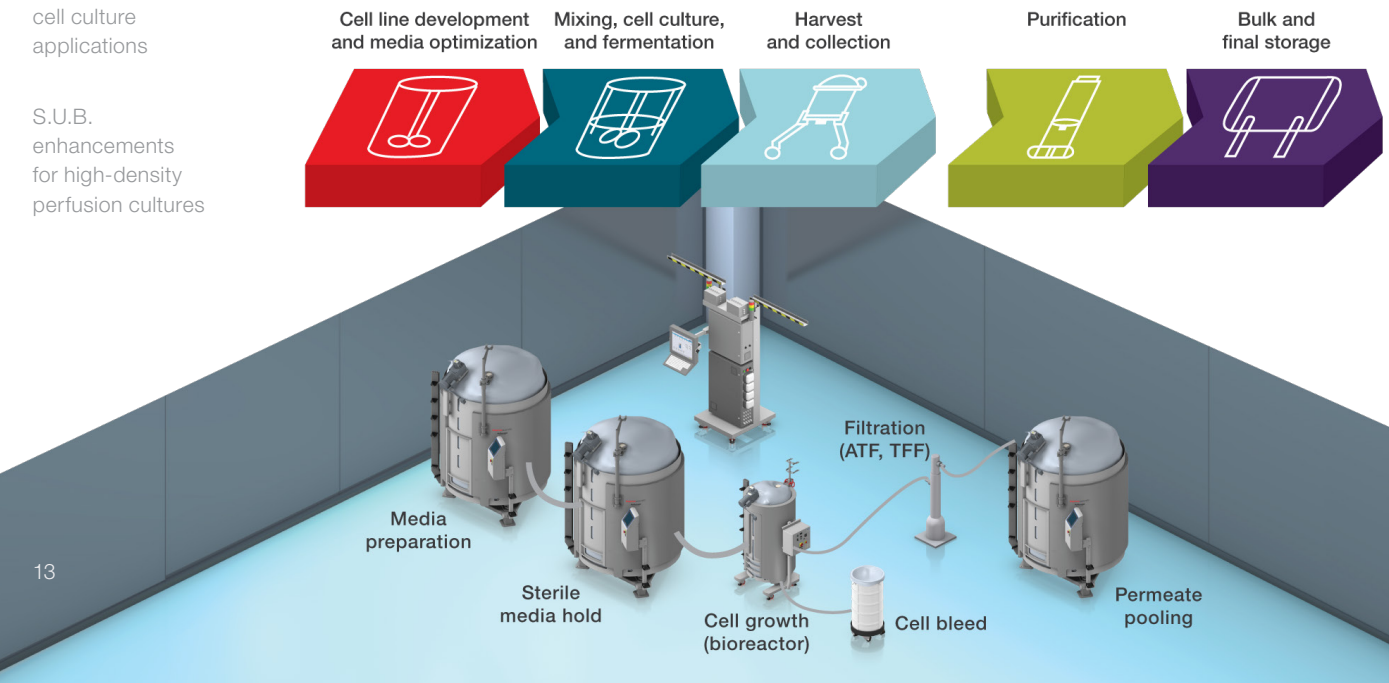
- 304-grade stainless steel construction, with smooth surfaces to help simplify clean-in-place processes
- Ergonomic design enhancements including top and bottom port access, and integrated load cells with enterprise control systems for accurate filling
- Jacketed versions available in all sizes for applications where heating or cooling is required
- Compatible with vaporized hydrogen-peroxide

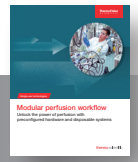
Learn more at **thermofisher.com/sutstoragetransport**

Modular perfusion workflow

Workflow explanation

A continuous processing workflow allows cells to grow to higher cell densities within the bioreactor, in contrast to traditional batch or fed-batch processes. This is facilitated by the regular addition of fresh media and the continuous removal of extracellular proteins and metabolic waste. Using perfusion, significant yields (measured in grams of protein per cell per volume) are feasible at a smaller scale, meaning more product is produced in reduced facility space. Due to the continuous nature of the process, increased cell production can be achieved over a shorter time. Regular replenishment of nutrients, combined with reduced buildup of waste products in the S.U.B., allows for consistent homogeneity in the vessel and a higher degree of control over culture conditions, contributing to enhanced quality. Perfusion amplifies the benefits of single-use technologies, enabling more efficient use of production space and greater overall flexibility.





Brochure
Modular perfusion workflow
October 2022

Enabling high-performing perfusion cell culture

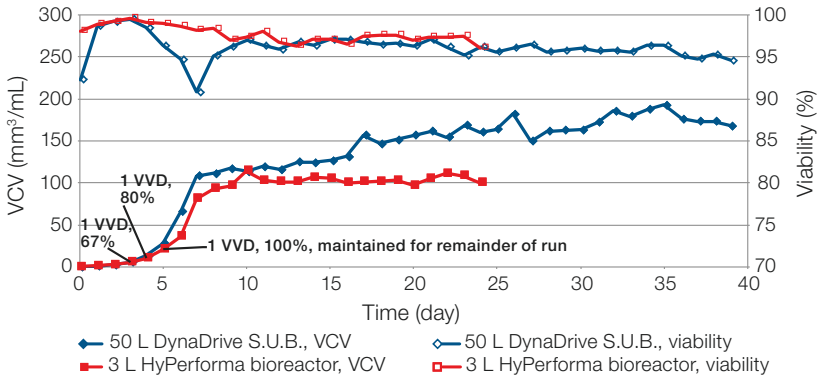
The number of biologics manufactured using methods that move in the direction of perfusion-based bioprocessing is increasing year to year. Advancement of the biopharma industry in this direction means there will be significantly higher demands placed upon both the equipment and consumables to drive these processes forward. This increased interest in performance creates an opportunity for improvement in consistent scalability as well as process design and management.

Combining two innovations, the DynaDrive S.U.B. and Gibco™ High-Intensity Perfusion (HIP) CHO (Chinese Hamster Ovary) Medium, we have created the basis for robust perfusion production while offering three key factors required to help you efficiently reach successful production: scalability, performance, and ease of use.

White paper
Enabling high-performing perfusion cell culture
October 2022

Data snapshot

These data illustrate the benefit of using the DynaDrive S.U.B. hardware and BPC with the HIP CHO Medium in terms of cell viability. The initial perfusion can be run at a lower concentration and increased in stages to a higher concentration as osmolality decreases. These combined tools help to simplify an array of perfusion workflows while easily supporting high performance and providing a clear path toward production scaling.



Viable cell volume (VCV) and viability data for the HyPerforma 3 L Glass Bioreactor and 50 L DynaDrive S.U.B. cell runs. Both cultures achieved stable viable cell volumes >100 mm³/mL while maintaining high viability at 1 vessel volume per day (VVD) perfusion rate.

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

Application note
500 L DynaDrive S.U.B. for perfusion cell culture applications
July 2022

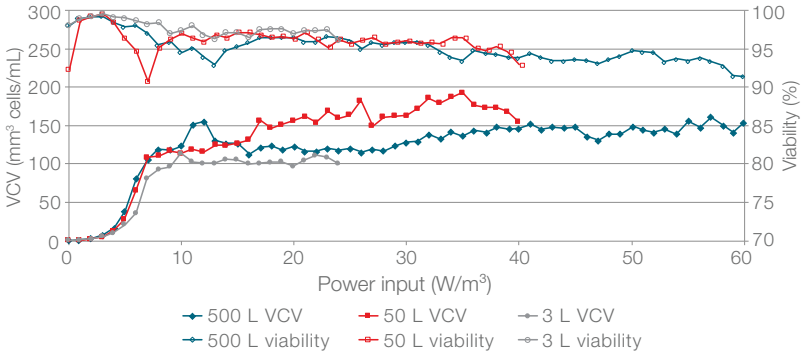
The DynaDrive S.U.B., featuring the latest advancement in S.U.B. technology, offers better performance and scalability to far larger volumes than previous S.U.B.s. Its cuboid-shaped tank offers several critical advantages over legacy S.U.B. designs. The essential features of the DynaDrive S.U.B. are its superior mixing capabilities, mass transfer capabilities, and improved scalability.

The work presented here highlights the improved mass transfer performance seen in the 500 L DynaDrive S.U.B. and associated cell culture work in continuous perfusion mode.

Data snapshot

The continuous perfusion process in the DynaDrive S.U.B. exhibited cells that had been 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. Perfusion rates were maintained, and viability settled at approximately 95% for the duration of the culture.

The DynaDrive S.U.B. exhibits increased k_La, superior mixing capabilities, and optimized cuboidal shape with impellers allowing for a high-power input without vortexing. Together, these features provided a homogeneous and gentle cell culture environment. All this increases predictability and simplifies scale-up, taking the guesswork out of performance and bioreactor controls.



VCV and viability data 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.

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

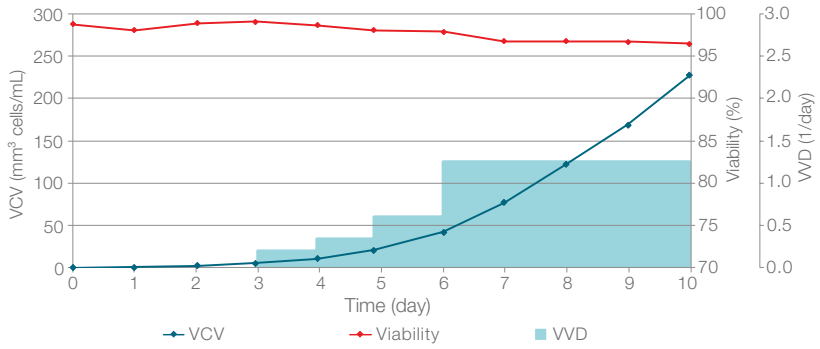
Application note
50 L DynaDrive S.U.B. for perfusion cell culture applications
October 2022

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. Superior mixing and mass transfer capabilities are enabled by a unique stirred-tank design utilizing a novel drive train with multiple impellers and a next-generation drilled-hole sparger.

This application note highlights 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. These data demonstrate the ability of the DynaDrive S.U.B. to easily support cell densities more than 150 x 10^6 cells/mL while utilizing automation through the HyPerforma G3Pro Bioprocess Controller with online single-use sensors, enabling easy and accurate control of a continuous culture.

Data snapshot

With more biomanufacturing production processes moving toward perfusion, it becomes crucial to investigate innovative 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 provided best-in-class mixing and mass transfer performance to easily support cell densities above 200 x 10^6 cells/mL in perfusion workflows. Thus, the DynaDrive S.U.B. is an ideal choice for small-volume continuous and N-1 perfusion.



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

S.U.B. enhancements for high-density perfusion cultures

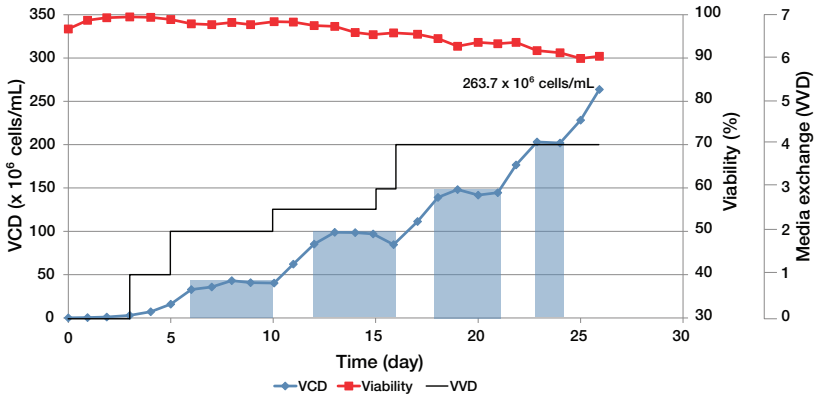
Application note
S.U.B. enhancements for high-density perfusion cultures
November 2022

Improvements in single-use systems have allowed the implementation of high-density cultures in emerging bioprocess workflows. In contrast, progressive advances in media optimization and improved clone genetic selection have underscored the perceived performance limitations of S.U.B.s. This study presents how strategic enhancements to the sparge and agitation systems of Thermo Scientific™ HyPerforma™ S.U.B.s have revealed the potential for a three- to four-fold improvement in mixing and mass transfer performance compared to legacy S.U.B. designs.

The work also demonstrates best practices and the desirable process benefits that can be achieved through reduced technical risk, lower labor, and simplified technical transfer of a completely disposable processing assembly. Further evidence is presented on the advantages of continuous processing when used in high-density seed train intensification or as a compact production-scale bioreactor system operating at reasonable media exchange rates of 1 to 2 VVD.

Data snapshot

A highly automated cell run was performed in a 50 L S.U.B., yielding cell densities in excess of 260 x 10^6 cells/mL while maintaining proper reactor conditions, including DO, pH, and dCO_2 levels at perfusion rates up to 4 VVD. Results demonstrate the effectiveness of the S.U.B. and controller in maintaining proper cell culture settings, even under demanding conditions of ultrahigh cell density with additional capacity above those conditions tested.



VCD and viability results for enhanced 50 L S.U.B. operating at 1–4 VVD. Steady-state cell density zones highlighted.

Maximize flexibility and facility utilization through continuous processing

To achieve a smooth and efficient perfusion process, having the right equipment is vital. Process optimization can be time-consuming, and the long duration represents an increased risk of operational errors. Let's reimagine what a true supplier-manufacturer partnership looks like and what innovative, optimized perfusion processing is capable of.

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