

Application enhancements for the HyPerforma S.U.B.

Introduction

Increased demand in the field of biotherapeutic manufacturing has resulted in the need for bioprocess developers to provide more product yield at lower production cost, to decrease the time required to bring new therapies to the patient, and to consistently manage operational risks. These trends are leading to increased focus on three key areas of advancement: continuous manufacturing, intensification of the bioprocess unit operations, and expanded utilization of single-use technologies. For animal cell culture processes, the reliance upon a bioreactor as the heart of the upstream operations has necessitated the improvement of these unit operations.

Because bioreactors are inherently time- and cost-intensive operations that are susceptible to contamination or control failures, the industry has sought to simplify operations and move toward single-use options to reduce cleaning and verification costs, as well as time lost between cultures. With the advent of robust polymer films, single-use bioreactors (S.U.B.s) were developed to primarily replace seed train operations and assist in process development. In 2006, the first 50 L and 250 L stirred-tank S.U.B.s were launched, more closely representing traditional stainless steel reactors and ultimately giving a design guide for further buildup to the current 2,000 L scale that most manufacturers strive toward having in their operations unit.

When S.U.B. designs were first developed, end users were only looking for the ability to replicate simple growth conditions seen in stainless steel operations, where cell densities, product titers, and overall system demands were much lower than those achieved today.



Niche operations such as low-turndown operation, perfusion, and adherent cell culture were dealt with on a case-by-case basis. However, with many end users now demanding streamlined operations, high-density culture in fed-batch modes, even higher production by perfusion cultures, and contrastingly gentle operations required in microcarrier applications, standard S.U.B. offerings must keep pace with the ever-evolving needs of the bioprocessing industry.

Legacy S.U.B. design

Developing the first stirred-tank S.U.B.s posed initial challenges associated with film robustness, chosen agitation method, and design, as well as designs for gassing, heating, and *in situ* sensing of key process parameters. Overcoming these challenges has led to a versatile, modular S.U.B. system available in sizes from 50 L to 2,000 L, suitable for most bioprocess applications.

Film

Polymer films had been used and well accepted as materials for 2D BioProcess Containers (BPCs) for several years prior to the development of the first S.U.B.s. Because of this, the same film type was used in the first S.U.B. iterations. The film was found to be robust, easily fitted to the application, simple to produce in a 3D format, and weldable to the porting needed to produce a S.U.B. BPC. Iterations of Thermo Scientific™ CX3-9, CX5-14, and Aegis™ 5-14 films have been implemented in most bioreactor processes with great success.

Bioreactor design

The S.U.B. design was chosen to mimic traditional stainless steel systems with similar aspect ratios, such as height to diameter (2:1), reactor to impeller diameter (3:1), impeller diameter to floor clearance (1:1), and impeller height to diameter (1:3). These ratios have been maintained across all current S.U.B. sizes from 50 L to 2,000 L. These designs have allowed users to operate the S.U.B. at operating volumes from 50% to 100% working volume.

The traditional overhead drive system was modified slightly to account for the lack of baffles in the S.U.B. To achieve optimal radial and axial mixing, the drive train was offset, angled to 19.6°, and a 45° pitched blade impeller was implemented. Use of the pitched blade impeller also reduced liquid shear compared to a Rushton impeller, which can sometimes be detrimental to cell growth. The length and angle of the drive train placed the impeller in an ideal location to provide sufficient mixing while maintaining sufficient clearance from the walls of the S.U.B. to avoid possible mixing interferences and liquid dead zones.

Use of an overhead drive system required development and verification of a bearing hub and impeller assembly, which was weldable to the BPC and allowed simple connection to a motor and drive shaft to impart mixing in the system. This bearing hub provides a robust, sterile barrier to the S.U.B., allowing the reactor to be used in nonsterile clean rooms.

The direct drive also allows consistent mixing power to with even highly viscous solutions. Other improvements have been implemented over time, resulting in a flexible design capable of meeting most customer needs [1,2].

Sparger design

To ensure proper growth conditions in the S.U.B., subsurface gassing was implemented to provide the cells with proper levels of O₂ and CO₂. Sparger types have evolved over time, from Tyvek™ membranes to sintered PVDF microspargers (frit), and open-pipe designs to precision laser drilled-hole spargers (DHSs). While the end goal of sparging seems simple (i.e., provide enough O₂ mass transfer while removing respired CO₂), the kinetics of this process require proper sparger design. To this end, the current DHSs have been specially designed to meet most process needs while maintaining acceptable gas flow rates and gas entrance velocities.

Heating

The first S.U.B.s were designed with resistive heating to maintain temperature at 37°C, the physiological condition. As culture demands were considered, such as fast heat-up times and chilling capabilities, the S.U.B. design was modified with a water jacket that could be connected to a temperature control unit. With standard 2:1 S.U.B.s, side-wall heating and cooling was sufficient to achieve and control temperature.

Reactor culture trains

The size scales of the legacy 2:1 S.U.B.s allowed end users to seed reactors at specific cell densities, grow to target cell densities, and transfer successively larger sizes (ultimately to production scale up to 2,000 L). The S.U.B. 2:1 aspect ratio coupled with cell splitting ratios allowed most processes to move from flask to production scale in as few as 4–5 vessels, which was sufficient for most processes. Driven by demand for both improved timelines and economics, experienced professionals also began deviating from traditional scaling approaches of using 10–15,000 L stainless steel bioreactors by coupling as many as six 2,000 L S.U.B.s in a modular format to achieve the same final production volume, and to minimize risk of potential losses at such large volumes. These measures have helped establish S.U.B.s as the go-to method for implementing single-use technologies into cell culture workflows.

Legacy S.U.B. gaps

As single-use technology began taking hold of the bioproduction market, demands and expectations on performance and process rapidly shifted to more optimized procedures. The legacy S.U.B. was no longer sufficient to achieve some goals that end users specified, including operation at volumes as low as 20% (5:1 turndown ratio) and use in perfusion workflows, which ultimately require higher mixing and oxygen mass transfer. Therefore, the S.U.B. has undergone several redesigns to meet these performance needs.

5:1 redesign

In order to intensify seed-train and scale-up operations, and to implement better mixing through drain, the first S.U.B. iterations focused on operation at lower working volumes. This posed some initial challenges in regard to mixing, heat, and mass transfer, as well as process control. However, following initial research and simple design changes, the Thermo Scientific™ HyPerforma™ 5:1 S.U.B.s were launched to meet customer needs in this design space. While a brief overview of the design changes is presented here, we recommend that readers refer to other documents covering the 5:1 S.U.B. for more detailed information [3,4].

Hardware design modifications

Initial redesign work was performed on the smaller S.U.B. sizes (50–500 L) due to motor mount modifications needed for the 1,000 L and 2,000 L S.U.B.s. The largest adjustment needed to operate the S.U.B.s at 5:1 volume was repositioning the impeller within the S.U.B. to reach below the lowest liquid surface. This required changing the motor mount angle from 19.6° to 16.5° while adding 6 to 16 cm of length to the drive shaft for the 50–500 L S.U.B.s. Operation of these systems is done using all 5:1 hardware and positions for the entire culture. For the larger systems, an adjustable overhead mount and utilization of the flexible BPC were used to physically lower and raise the motor, drive shaft, and impeller assembly into and out of the S.U.B. hardware. Operation of the larger systems is performed at the 5:1 position while operating at volumes less than 50%, the motor is stopped, the motor mount is adjusted to the upper portion, and the motor is reengaged. Similarly, during culture harvest, the motor mount is adjusted from the upper position to the lower position when liquid volume drops below 50%. This ensures more homogeneous mixing through the drain and avoids any plug-flow situations that may be unfavorable to downstream processes.

Figures 1 and 2 display the motor mounting system changes, using the 500 L and 2,000 L S.U.B.s as examples. Other changes are also included, such as additional probe ports below standard positions (especially needed in the 50 L and 100 L S.U.B.s) and in the back window of the hardware (optional), and bottom jacketing for better heat transfer when operating at low working volumes.

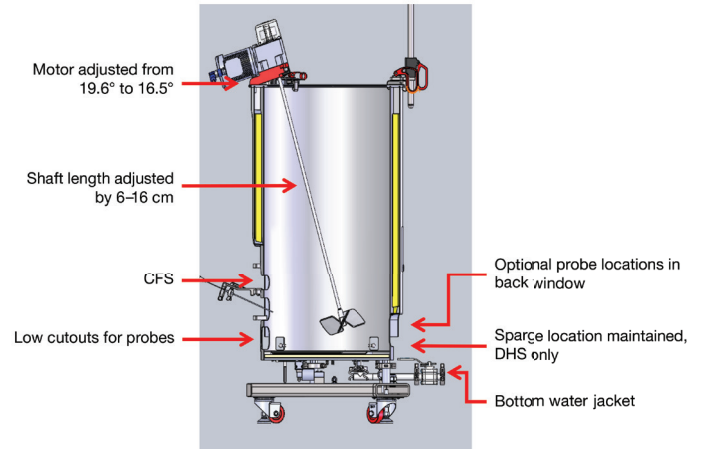


Figure 1. Hardware and BPC changes to the 500 L S.U.B.

Similar modifications were made to 50 L–250 L S.U.B.s. CFS: cross-flow sparger.

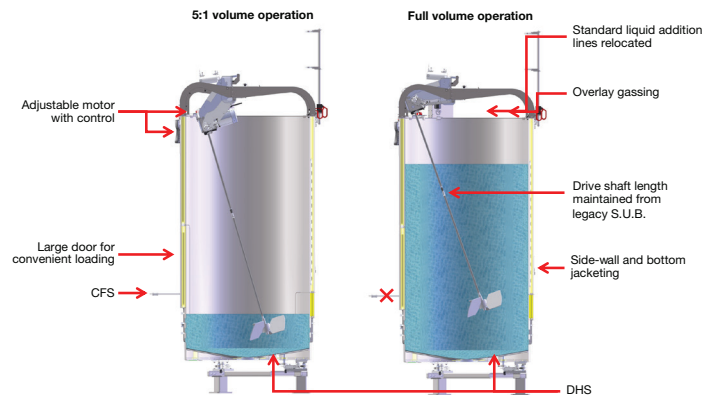


Figure 2. Hardware and BPC changes to the 2,000 L S.U.B.

Similar modifications were made to the 1,000 L S.U.B. CFS: cross-flow sparger.

Sparger and BPC modifications

With the redesigned motor drive system, it became necessary to consider sparging options, especially in the 50 L to 250 L S.U.B.s where the lowered impeller would make contact with the standard frit. The zero-profile DHS was thoroughly tested at 5:1 and full volumes to determine mass transfer for both O₂ and CO₂, and was found to yield sufficient performance for most applications across all S.U.B. sizes. This sparger allows for gas flow rates up to 0.1 vessel volumes per minute (vvm) while maintaining low gas-entrance velocities and scalable mass transfer

performance. Implementation of an O₂-heavy gas control is generally recommended, where ultimately O₂ is the primary gas sparged through the DHS at higher cell densities in order to maintain the dissolved O₂ setpoint. This requires properly sizing mass flow controllers (MFCs) and adjusting control strategies to accommodate the higher gas demand. Due to the contact of the impeller with the standard frit in the smaller S.U.B. designs, the frit was removed from all standard 5:1 BPCs. However, the frit can be added in custom configurations with guidance from a BPC design team.

In addition to the DHS-only operation in 5:1 systems, a new cross-flow sparger (CFS) was implemented to provide mass transfer at low working volumes. It was found in initial development that, when operating a system at 5:1 volume, respired CO₂ blanketed the air-water interface, and the overlay sparger was ineffective at removing the CO₂ layer. This CO₂ blanket led to localized high dissolved CO₂ and lower pH, which led to greatly reduced cell growth. The CFS, an overlay sparger positioned just above the liquid-air interface when operating at 5:1 volume, provided the necessary gassing to remove the CO₂ blanket and maintain proper cell culture operating conditions.

Benefit and use of 5:1 S.U.B.s

The primary benefits of 5:1 S.U.B.s include the following, resulting in improved customer processes:

- Streamlining seed train and final volume production by requiring fewer vessels
- Homogeneous mixing through drain
- More robustly scaled sparging using only the DHS
- Higher BPC standardization at customer sites due to the reduced vessel sizes

Figure 3 demonstrates the use of 5:1 S.U.B.s in a process terminating at the 2,000 L scale. The ability to seed vessels at lower working volumes allows smaller vessels to seed subsequent vessels, resulting in removal of some vessels from seed trains. Depending on allowable cell-split ratios, one or two vessels may be removed from the process, resulting in a more streamlined process with fewer cell and liquid transfers, which can lead to fewer culture losses.

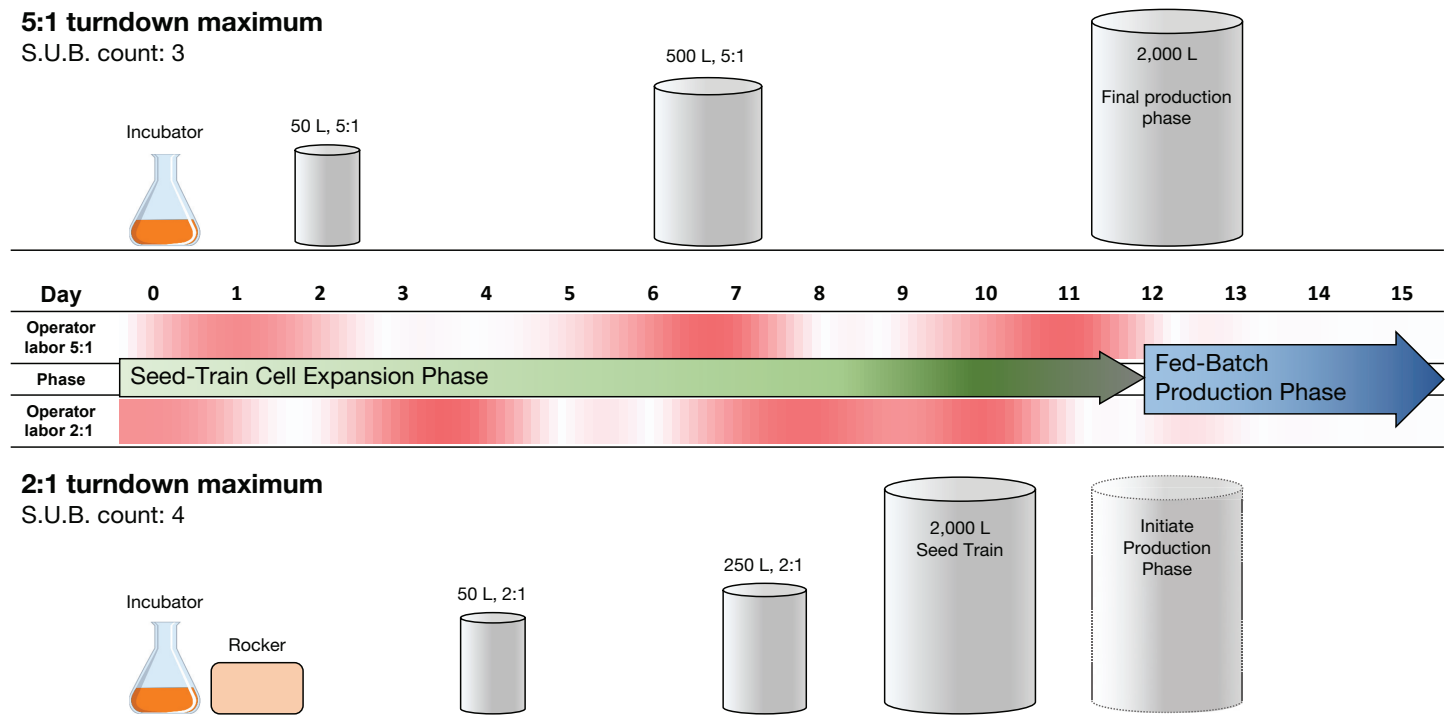


Figure 3. Improved seed train using 5:1 systems results in fewer seed train vessels and cell transfers.

The ability to mix more thoroughly through drain allows a more homogeneous mixture to move from one vessel to subsequent vessels. For example, a 2,000 L S.U.B. could be used to seed multiple 2,000 L S.U.B.s using this technique. Homogeneous mixing through drain would assist in seeding each reactor with a known cell count. Additionally, mixing through drain avoids potential plug-flow to downstream processes (centrifuge or depth filter), which could be problematic.

Application-specific needs: Next-generation modifications

While the legacy and 5:1 S.U.B. designs are effective for most bioprocessing applications, specific applications have required case-by-case engineered-to-order modifications to the S.U.B. These applications can generally be grouped into the following categories: perfusion, fed-batch, and microcarrier. By standardizing products and requests into each specific category, Thermo Scientific can better support customer needs and provide a more reliable product.

Table 1 outlines the application-specific requirements of each design modification. Generally these applications require higher power input, but have varying needs of mass transfer and turndown ratio. Relying on standard components and staying within the capabilities of existing manufacturing equipment, simple iterations of the S.U.B. have been created to achieve the demands outlined. These application-specific designs are described in the following sections, showing how each S.U.B. can be configured to meet the individual process demands.

Table 1. Application-specific needs for enhanced S.U.B.s.

	Perfusion	Fed-batch	Microcarrier
Mixing power	Higher	Higher	Higher
Tip speed	NA	NA	Lower
Mass transfer	Higher	Higher	NA
Turndown ratio	NA	High	High
Specific porting	ATF/TFF	NA	Media exchange

Enhanced S.U.B. for perfusion

Recently, perfusion cell culture, where cells are grown to high densities and maintained for long durations by utilizing a cell separator and constant media exchanges, has played a major role in advancing the concept of continuous processing. While standard S.U.B.s have been shown to sustain growth at relatively low perfusion demand, some customers have desired to increase mass transfer and mixing to extremely high levels. This has led some users to drive the system to its performance limits, while operating far above the recommended gassing and agitation limits of the systems. Therefore, further enhancements to the legacy S.U.B. were needed to achieve desired performance required by these high-demand cultures. Further details can be found in previously published literature [5,6].

The enhancements generally utilized the library of standard parts or slightly modified pieces, resulting in simple plug-and-play modifications to the HyPerforma S.U.B.s. These modifications are made available in 50–500 L S.U.B.s only. Perfusion cultures above this volume become technologically and logistically problematic due to media preparation and storage, quantity of ATFs required, and S.U.B. mixing and gassing limitations in larger S.U.B.s. With proper media and cell-line development, most innovative companies are driving to perfusion at smaller scales so as to lower operation costs.

Enhanced mixing

One of the limits of legacy systems was the relatively low power input ability with the standard impeller and the e-box. Power input per volume is calculated based on equation 1 below, where N_p is the impeller power number, ρ is the fluid density, N_i is the impeller speed, D_i is the impeller diameter, and V is the working volume.

$$P/V = \frac{N_p \cdot \rho \cdot N_i^3 \cdot D_i^5}{V} \quad \text{Equation 1}$$

Beyond simply increasing the impeller speed by either changing or reprogramming the variable frequency drive (VFD) to run outside its recommended ranges, the most logical method for imparting more mixing power into the system while using standard parts is to use the next-size impeller in the S.U.B., e.g., using the 100 L impeller in the 50 L S.U.B. This small increase in impeller size results in the ability to impart up to 100 W/m³ (2.5x greater power input than in all legacy systems), as displayed in Figure 4. This also effectively decreases the operating tip speed of the impeller, reducing the impeller shear while maintaining high power input.

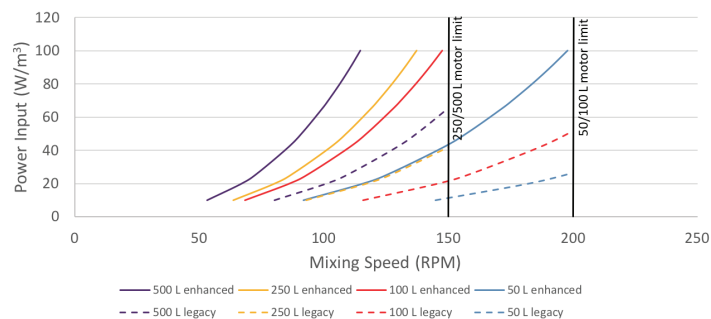


Figure 4. Power input to volume (PIV) vs. mixing speeds for legacy and enhanced S.U.B.s for perfusion, from 50 L to 500 L.

The challenge of increasing impeller size is avoiding impeller interference on the S.U.B. BPC and hardware while maintaining sufficient mixing capabilities and increasing mass transfer capabilities. It was found that using standard 2:1 drive shafts and 16.5° motor mounting hardware resulted in the impeller being positioned above the sparger and sufficiently away from the BPC wall to avoid any possible damage.

Enhanced sparging

Substantial increases in mass transfer were obtained by not only increasing agitation, but also by altering the sparging system in the S.U.B. Bubble sizes were reduced by reducing the DHS pore size in all S.U.B.s, effectively increasing the surface area of mass transfer. At the same time, DHS pore quantities were increased to maximize the gas flux area, allowing flow rates of up to 0.1–0.2 vvm while maintaining gas entrance velocities at or below 23 m/s [7]. Pore sizes and quantities for each vessel are listed in Table 2.

Table 2. Pore sizes and quantities for legacy and enhanced S.U.B.s.

Size	Legacy S.U.B.s		Enhanced S.U.B.s	
	Pore size	Pore quantity	Pore size	Pore quantity
50 L	0.178 mm	360	0.080 mm	1,448
100 L	0.178 mm	570	0.080 mm	1,448
250 L	0.233 mm	760	0.178 mm	2,244
500 L	0.368 mm	980	0.233 mm	2,610

While frit spargers can be included in perfusion processes, experience has shown that high cell densities and protein concentrations obtained in perfusion processes often lead to fouling of the frit surface. Buildup of this cell material can lead to large deviations in mass transfer performance and eventually to nearly total loss in performance. While slight fouling of the DHS pores may occur, no effective loss in mass transfer has been observed. For this reason, use of the DHS is recommended for all perfusion applications.

Perfusion-specific BPC modifications

In order to accommodate perfusion at all scales, ports for either alternating tangential flow (ATF) or tangential flow filtration (TFF) are included on standard drawings. For ATF connections, these generally include one or two large 1-inch ports and a length of thick-wall C-Flex™ tubing, terminating in a 1-inch CPC™ AseptiQuik™ X sterile connector. This makes for a simple and reliable connection to either an ATF6 or ATF10 filter. For TFF connections, port and tubing size are built per vessel size, minimizing tubing length and hold-up volume. These connections terminate in CPC AseptiQuik G connectors in standard drawings. As with most parts on standard drawings, these can be changed to fit customer needs.

A dedicated bleed port is also included for pulling cells from the culture, as a means of achieving the target cell densities during perfusion processes. Often these reactor bleeds are performed when cells grow past the target cell densities and can be controlled either via cell density sensors or by mathematically determining the bleed rates based on target cell densities and cell growth rates.

It is important during a perfusion process to automate as much as possible to avoid loss of the culture. Sensor choices (temperature, dissolved oxygen (DO), pH, foam, pressure) are important, and must be considered. Standard enhanced S.U.B.s include foam sensors and several ports for inclusion of any process analytical technologies the end user needs to include.

Porting for perfusion systems in S.U.B.s

Port placement within the S.U.B. BPC and hardware are important considerations for perfusion processes. Specifically, it is recommended that ATF systems be connected to the S.U.B. while maintaining a near-horizontal flow path between the two systems. This is done primarily to limit the overall length of tubing and the liquid hold-up volume used, and to assist in purging air from the ATF prior to operation. For this reason, the new style of S.U.B. hardware has been modified to include optional port placement at higher positions. For those with existing hardware, porting can still be achieved by using the standard upper probe belt. For TFF systems, porting within the standard probe belts is considered sufficient to provide a closed flow path.

When implementing a perfusion process in an existing HyPerforma S.U.B., port locations must be chosen to match existing constraints in the hardware; thus, the dimensions listed in Table 3 are recommended. For new build-outs, updated S.U.B. hardware that includes new ATF port cutouts provides a more ideal option.

Enhanced S.U.B. for fed-batch cultures

The enhanced S.U.B. for fed-batch culture was configured based on a combination of 5:1 and perfusion designs [8]. To achieve higher mixing input, the larger impeller used in the perfusion application was implemented, allowing power input up to 100 W/m³. At the same time, the requirement of a lower turndown ratio for lower seed volume and mixing through drain with the larger impeller required a redesign of the impeller drive shaft to maintain a minimal impeller clearance from the bottom of the S.U.B. Because the new impeller is slightly larger than the legacy configuration, the impeller isn't fully covered by liquid below a 4:1 volume (e.g., 12.5 L in a 50 L S.U.B.). Figure 5 depicts the impeller position within the 500 L S.U.B. for each design iteration.

Similar to the enhanced S.U.B. for perfusion, the fed-batch version required higher mass transfer. The same DHS is used to achieve gas flow rates up to 0.1–0.2 vvm while maintaining minimal gas entrance velocity. However, due to the impeller position in the fed-batch S.U.B., gas dispersion and mixing at full volume are less effective, providing slightly lower $k_L a$ values in the fed-batch S.U.B. compared to the perfusion design.

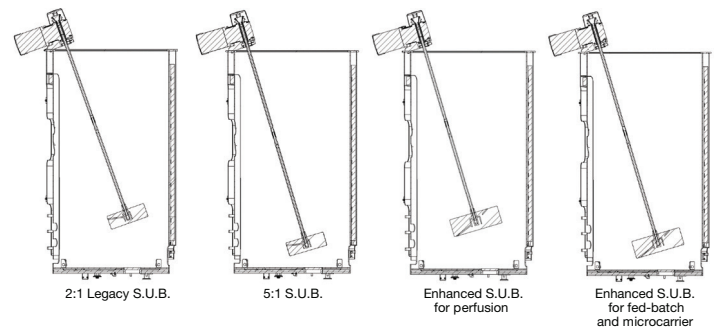


Figure 5. 500 L S.U.B. versions including 2:1 legacy, 5:1, and enhanced S.U.B.s for perfusion, fed-batch, and microcarrier.

Table 3. ATF port locations for both the hardware and BPC in legacy and redesigned standard S.U.B.s.

	50 L S.U.B.	100 L S.U.B.	250 L S.U.B.	500 L S.U.B.
Recommended XCell™ ATF system	1 ea XCell™ ATF 6	1 ea XCell ATF 6	1 ea XCell ATF 6	1 ea XCell™ ATF 10
Legacy hardware port location (from floor)	76.20 cm (30 in.)	64.77 cm (25.5 in.)	58.42 cm (23 in.)	NA
Legacy BPC port location (from V-seal)	45.57 cm (17.94 in.)	50.01 cm (19.69 in.)	57.63 cm (22.69 in.)	NA
New hardware port location (from floor)	91.44 cm (36 in.)	91.44 cm (36 in.)	91.44 cm (36 in.)	104.14 cm (41 in.)
New BPC port location (from V-seal)	61.54 cm (24.23 in.)	77.88 cm (30.66 in.)	92.68 cm (36.49 in.)	115.06 cm (45.30 in.)

Enhanced SUB for microcarrier

While adherent cell cultures in standard S.U.B.s with microcarriers have been demonstrated, they represent a much smaller percentage of use than their suspension counterparts. An increase in applications requiring adherent cultures, and a shift from traditional systems to single-use systems, are the impetus for enhancing the S.U.B. directly for microcarrier culture applications. The flexibility of S.U.B.s, as well as the fact that all moving drive parts are located outside of culture liquid, lend themselves to successful microcarrier cultures where particles or beads will not be compromised. As before, the enhancements utilized the library of standard parts or slightly modified pieces for simple modifications to HyPerforma S.U.B.s ranging from 50 to 500 L in volume. While work above this volume is possible, such enhancements would be evaluated on a case-by-case basis to ensure optimal performance. Further reading on the subject can be found in a supplemental application note [9].

Enhanced mixing

In microcarrier-based cultures, settling of adherent cells on beads at the bottom of the vessel is a key concern. Cells in such a state will experience hypoxia and dramatic pH deviations from the bulk culture. The implementation of a larger-size impeller provides greater power input per rotation, as explained above. This allows for overall reductions in operating rotational speeds while providing sufficient lift and bottom sweeping from the down-pumping impeller to prevent settling of particles. This decreased operation speed is key for maintaining low shear rates of the impeller, as well as preserving impeller-related Kolmogorov lengths above 1/2–2/3 the diameter of the microcarrier particles (see equation 2). By preventing small eddy lengths and impeller tip speeds (Equation 3), cells are less likely to be sheared off the microcarriers.

$$\lambda = \left(\frac{\mu^3}{\rho^3 \cdot N_p \cdot D_i^2 \cdot N_i^3} \right)^{1/4} \quad \text{Equation 2}$$

$$u_{tip} = \pi \cdot D_i \cdot N_i \quad \text{Equation 3}$$

where λ is the Kolmogorov length, μ is the fluid kinematic viscosity, ρ is the fluid density, N_p is the impeller power number, D_i is the impeller diameter, N_i is the impeller speed, and u_{tip} is the impeller tip speed.

The effects of these impeller modifications can be seen in Figures 6 and 7, depicting Kolmogorov lengths and tip speeds with respect to power input. At equivalent Kolmogorov length, higher power and lower tip speed are achievable when utilizing a larger impeller.

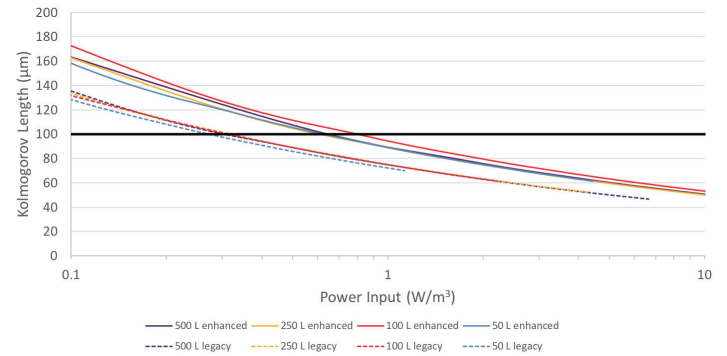


Figure 6. Kolmogorov length vs. power input for legacy and enhanced S.U.B.s for microcarrier from 50 L to 500 L.

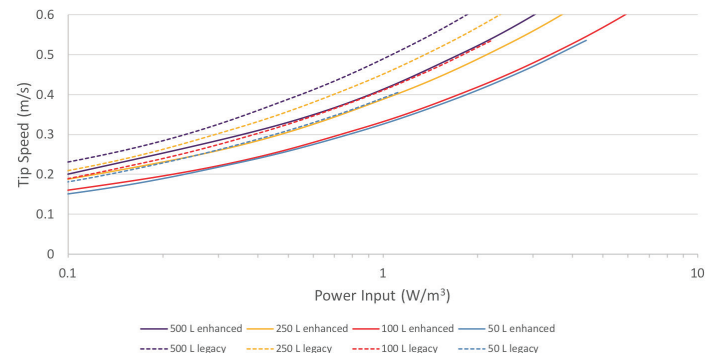


Figure 7. Impeller tip speed vs. power input for legacy and enhanced S.U.B.s for microcarrier from 50 L to 500 L.

Microcarrier BPC modifications

In most adherent cell culture workflows, the cell detachment process (such as with trypsin), microcarrier addition (such as for increasing bead concentration for scale-up), and media exchanges require the ability to add and remove solutions to and from the BPC while retaining microcarriers in the vessel. The simplest approach is allowing for temporary settling of reactor contents by stopping agitation and sparging, and then decanting the liquid from above the bead and cell pack. To this end, standard BPC configurations provide a decant line as well as multiple addition lines for these operations. Modification of these decanting levels (e.g., for different microcarrier concentrations) is possible through individual customization. In terms of sparging, in general the standard DHS provides higher mass transfer than will be required by adherent cultures, as they are relatively less demanding than the standard suspension cultures. Similarly, the standard sensor choices (temperature, DO, pH, foam, pressure) provide control of these adherent cultures.

S.U.B. specifications (all versions)

Table 4. 50 L S.U.B. options and design specifications.

Attribute	Legacy	5:1	Enhanced S.U.B. for perfusion	Enhanced S.U.B. for fed-batch	Enhanced S.U.B. for microcarrier
Standard BPC Cat. No.	SH30999.01	SH31073.01	SH31170.01—ATF SH31173.01—TFF	SH31151.01	SH31150.01
Impeller Cat. No., size	SV20735.01 11.11 cm (4.37 in.)	SV20735.01 11.11 cm (4.37 in.)	SV20820.01 14.6 cm (5.75 in.)	SV20820.01 14.6 cm (5.75 in.)	SV20820.01 14.6 cm (5.75 in.)
Impeller wall clearance	5.80 cm (2.28 in.)	6.91 cm (2.72 in.)	7.04 cm (2.77 in.)	5.53 cm (2.18 in.)	5.53 cm (2.18 in.)
Impeller bottom clearance	11.78 cm (4.64 in.)	4.71 cm (1.85 in.)	9.82 cm (3.87 in.)	4.70 cm (1.85 in.)	4.70 cm (1.85 in.)
Drive shaft Cat. No., length, diameter, color	SV50177.34 78.7 cm (30.98 in.) 1.27 cm (0.5 in.) White	SV50959.10 85.1 cm (0.39 in.) 1.27 cm (0.5 in.) Black	SV50177.34 78.7 cm (30.98 in.) 1.27 cm (0.5 in.) White	SV50959.24 84.1 cm (33.11 in.) 1.27 cm (0.5 in.) Blue	SV50959.24 84.1 cm (33.11 in.) 1.27 cm (0.5 in.) Blue
Motor block Cat. No., designation	SV50957.25 19.6°	SV50957.26 16.5°	SV50957.26 16.5°	SV50957.26 16.5°	SV50957.26 16.5°
Motor Cat. No., power, gear ratio	SV50237.16 1/4 HP 10:1	SV50237.16 1/4 HP 10:1	SV50237.16 1/4 HP 10:1	SV50237.16 1/4 HP 10:1	SV50237.16 1/4 HP 10:1
Motor speed (min–max)	30–200 rpm	30–200 rpm	30–200 rpm	30–200 rpm	30–200 rpm

Table 5. 100 L S.U.B. options and design specifications.

Attribute	Legacy	5:1	Enhanced S.U.B. for perfusion	Enhanced S.U.B. for fed-batch	Enhanced S.U.B. for microcarrier
Standard BPC Cat. No.	SH30999.02	SH31103.01	SH31144.01—ATF SH31171.01—TFF	SH31152.01	SH31150.02
Impeller Cat. No., size	SV20820.01 14.6 cm (5.75 in.)	SV20820.01 14.6 cm (5.75 in.)	SV20733.01 20.0 cm (7.87 in.)	SV20733.01 20.0 cm (7.87 in.)	SV20733.01 20.0 cm (7.87 in.)
Impeller wall clearance	7.89 cm (3.11 in.)	9.23 cm (3.63 in.)	8.45 cm (3.33 in.)	6.78 cm (2.67 in.)	6.78 cm (2.67 in.)
Impeller bottom clearance	12.28 cm (4.83 in.)	3.88 cm (1.53 in.)	9.48 cm (3.73 in.)	3.89 cm (1.53 in.)	3.88 cm (1.53 in.)
Drive shaft Cat. No., length, diameter, color	SV50177.14 93.0 cm (36.61 in.) 1.27 cm (0.5 in.) White	SV50959.18 100.6 cm (39.6 in.) 1.27 cm (0.5 in.) Black	SV50177.14 93.0 cm (36.61 in.) 1.27 cm (0.5 in.) White	SV50959.25 98.8 cm (38.9 in.) 1.27 cm (0.5 in.) Blue	SV50959.25 98.8 cm (38.9 in.) 1.27 cm (0.5 in.) Blue
Motor block Cat. No., designation	SV50957.24 19.6°	SV50957.27 16.5°	SV50957.27 16.5°	SV50957.27 16.5°	SV50957.27 16.5°
Motor Cat. No., power, gear ratio	SV50237.16 1/4 HP 10:1	SV50237.16 1/4 HP 10:1	SV50237.16 1/4 HP 10:1	SV50237.16 1/4 HP 10:1	SV50237.16 1/4 HP 10:1
Motor speed (min–max)	30–200 rpm	30–200 rpm	30–200 rpm	30–200 rpm	30–200 rpm

Table 6. 250 L S.U.B. options and design specifications.

Attribute	Legacy	5:1	Enhanced S.U.B. for perfusion	Enhanced S.U.B. for fed-batch	Enhanced S.U.B. for microcarrier
Standard BPC Cat. No.	SH30999.03	SH31075.01	SH31143.01—ATF SH31172.01—TFF	SH31153.01	SH31150.03
Impeller Cat. No., size	SV20733.01 20.0 cm (7.87 in.)	SV20733.01 20.0 cm (7.87 in.)	SV21092.01 25.1 cm (9.88 in.)	SV21092.01 25.1 cm (9.88 in.)	SV21092.01 25.1 cm (9.88 in.)
Impeller wall clearance	11.67 cm (4.59 in.)	12.00 cm (4.72 in.)	13.22 cm (5.20 in.)	9.88 cm (3.89 in.)	9.88 cm (3.89 in.)
Impeller bottom clearance	20.17 cm (7.94 in.)	5.29 cm (2.08 in.)	16.25 cm (6.4 in.)	4.94 cm (1.94 in.)	4.94 cm (1.94 in.)
Drive shaft Cat. No., length, diameter, color	SV50177.40 106.7 cm (42.01 in.) 1.27 cm (0.5 in.) White	SV50959.11 120.9 cm (47.6 in.) 1.27 cm (0.5 in.) Black	SV50959.09 109.2 cm (43 in.) 1.9 cm (0.75 in.) White	SV50959.23 120.9 cm (47.6 in.) 1.9 cm (0.75 in.) Blue	SV50959.23 120.9 cm (47.6 in.) 1.9 cm (0.75 in.) Blue
Motor block Cat. No., designation	SV50957.23 19.6°	SV50957.28 16.5°	SV50957.36 16.5°	SV50957.36 16.5°	SV50957.36 16.5°
Motor Cat. No., power, gear ratio	SV50237.17 1/4 HP 10:1	SV50237.17 1/4 HP 12.5:1	SV50237.18 1/2 HP 5:1	SV50237.18 1/2 HP 5:1	SV50237.19 1/2 HP 15:1
Motor speed (min–max)	30–150 rpm	30–150 rpm	30–150 rpm	30–150 rpm	10–144 rpm

Table 7. 500 L S.U.B. options and design specifications.

Attribute	Legacy	5:1	Enhanced S.U.B. for perfusion	Enhanced S.U.B. for fed-batch	Enhanced S.U.B. for microcarrier
Standard BPC Cat. No.	SH30999.04	SH31077.01	SH31142.01—ATF SH31169.01—TFF	SH31154.01	SH31150.04
Impeller Cat. No., size	SV21092.01 25.1 cm (9.88 in.)	SV21092.01 25.1 cm (9.88 in.)	SV21058.01 32.1 cm (12.64 in.)	SV21058.01 32.1 cm (12.64 in.)	SV21058.01 32.1 cm (12.64 in.)
Impeller wall clearance	13.39 cm (5.27 in.)	14.84 cm (5.84 in.)	15.66 cm (6.17 in.)	11.75 cm (4.63 in.)	11.75 cm (4.63 in.)
Impeller bottom clearance	24.93 cm (9.81 in.)	7.60 cm (2.99 in.)	20.75 cm (8.17 in.)	7.56 cm (2.98 in.)	7.56 cm (2.98 in.)
Drive shaft Cat. No., length, diameter, color	SV50177.36 139.6 cm (54.96 in.) 1.9 cm (0.75 in.) White	SV50959.12 155.7 cm (61.3 in.) 1.9 cm (0.75 in.) Black	SV50177.36 139.6 cm (54.96 in.) 1.9 cm (0.75 in.) White	SV50959.26 153.4 cm (60.39 in.) 1.9 cm (0.75 in.) Blue	SV50959.26 153.4 cm (60.39 in.) 1.9 cm (0.75 in.) Blue
Motor block Cat. No., designation	SV50957.22 19.6°	SV50957.29 16.5°	SV50957.29 16.5°	SV50957.29 16.5°	SV50957.29 16.5°
Motor Cat. No., power, gear ratio	SV50237.18 1/2 HP 5:1	SV50237.18 1/2 HP 5:1	SV50237.18 1/2 HP 5:1	SV50237.18 1/2 HP 5:1	SV50237.19 1/2 HP 15:1
Motor speed (min–max)	30–150 rpm	30–150 rpm	30–150 rpm	30–150 rpm	10–144 rpm

References

1. Smith, M.T. et al. (2018) Single-Use Bioreactors: Performance and Usability Considerations Part 1: Performance for Process Control. *Bioprocess International* 16: i15– i19.
2. Smith, M.T. et al. (2018) Single-Use Bioreactors: Performance and Usability Considerations Part 2: *Bioprocess International* 16:2–6.
3. Madsen, B. (2017) Efficient operation of the HyPerforma 5:1 single-use bioreactor at low working volume. Thermo Fisher Scientific. Application note.
4. Jones, N. (2017) Improving single use bioreactor design and process development. New research towards intensifying seed-train & scale-up methods using 5:1 turn-down. *La Vague* 53:31–36.
5. Madsen, B. et al. (2018) Continuous processing performance enhancements for perfusion applications in 50L to 500L single-use bioreactors: a technical comparison of performance characterization, cell culture & scale-up modeling. *La Vague* 56:25–28.
6. Madsen, B. et al. (2018) S.U.B. enhancements for high-density perfusion cultures, Thermo Fisher Scientific. Application note.
7. Zhu, Y. et al. (2008) NS0 cell damage by high gas velocity sparging in protein-free and cholesterol-free cultures. *Biotechnol Bioeng* 101:751–760.
8. Madsen, B. et al. (2019) S.U.B. enhancements for high-density fed-batch cultures, Thermo Fisher Scientific. Application note.
9. Hsiao, T. et al. (2019) S.U.B. enhancements for microcarrier cultures. Thermo Fisher Scientific. Application note.

Authors:

1. Ben Madsen, Staff Engineer, Process Development, Thermo Fisher Scientific
2. Jordan Cobia, Engineer II, Research and Development, Thermo Fisher Scientific
3. Paula Decaria, Senior Engineer, Process Development, Thermo Fisher Scientific
4. Tony Hsiao, Staff Engineer, Research and Development, Thermo Fisher Scientific
5. Kevin Mullen, Senior Product Manager, Thermo Fisher Scientific
6. Nephi Jones, Senior Manager, Research and Development, Thermo Fisher Scientific

Find out more at thermofisher.com/sub

ThermoFisher
SCIENTIFIC

For Research Use or Further Manufacturing. Not for diagnostic use or direct administration into humans or animals.

© 2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. AspetiQuik and CPC are trademarks of Colder Products Company. C-Flex is a trademark of Saint-Gobain. Tyvek is a trademark of DuPont. XCell is a trademark of Repligen. **COL010125 1019**