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Continuous process performance enhancements for 50 L to 500 L single-use bioreactors: A technical comparison of erformance characterization, cell culture, and scale-up modeling

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Application-Specific Enhancements to Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.)

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ABSTRACT

The rapid growth of bio-therapeutic manufacturing has created significant demand for workflow solutions featuring greater product yield, lower production costs, and accelerated development timelines. To address these demands, developers have moved away from “one-size-fits-all” approaches and are increasingly focused on solutions that address the specific needs of diverse bioproduction processes. Given this shift toward process-specific solutions, Thermo Scientific has introduced a series of application-specific enhancements to the HyPerforma™ Single Use Bioreactor (S.U.B.) product platform (Figure 1), each tailored to the unique requirements of perfusion, intensified fed-batch, and adherent cell cultures (Table 1).

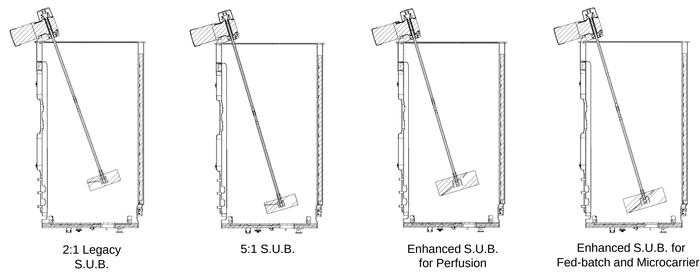
Table 1. Application-specific requirements for perfusion, fed-batch, and microcarriers

Perfusion	Fed-Batch	Microcarriers
• Greater mixing power • Greater mass transfer • ATF/TFF lines	• Greater mixing power • Greater mass transfer • High turndown ratio	• Lower tip speed • High turndown ratio • Decantation lines

INTRODUCTION

In 2006, Thermo Scientific released the HyPerforma™ S.U.B. – the first single-use stirred tank bioreactor on the market. Since 2006, Thermo Scientific has continued to innovate by releasing a number of improvements to the first generation S.U.B. including the first 2000 L S.U.B. (2010), a low-shear drilled hole sparger (2014), and the 5:1 HyPerforma™ S.U.B. allowing operation at a 5:1 turndown ratio (2016/2017). In this tradition, we introduce the enhanced Hyperforma™ S.U.B. for perfusion, fed-batch, and microcarriers

Figure 1. Cross section of each generation of HyPerforma™ S.U.B



Mixing power input per volume (PIV) is an important cell-culture parameter and can be described as:

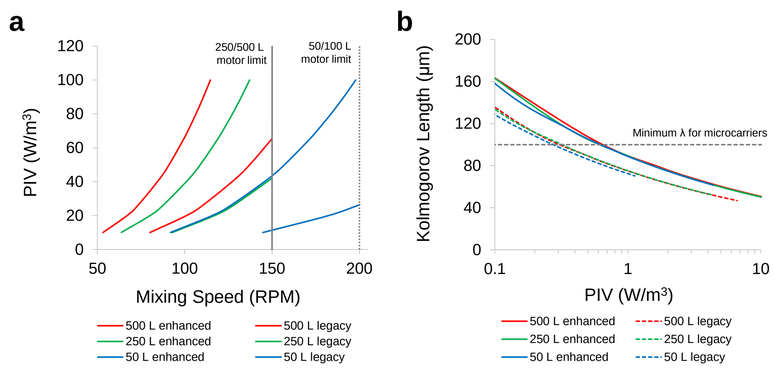
$$PIV = \frac{N_P \rho N_i^3 D_i^5}{V}$$

Where N_p represents the impeller power number, ρ represents the fluid density, N_i represents the impeller speed, D_i represents the impeller diameter, and V represents the vessel volume. At high PIV, the impeller tip speed can produce shear forces that damage cells and microcarriers. Shear forces on microcarriers can be described by the Kolmogorov length (λ), which characterizes the minimum eddy size of a given system:

$$\lambda = \left(\frac{\mu^3}{\rho^3 N_P {D_i}^2 N_i^3}\right)^{1/4}$$

where μ represents the fluid kinematic viscosity, and the remaining parameters have been described previously. The value of λ should be limited to approximately half the microcarrier diameter to avoid generating destructive eddies. Each enhanced S.U.B. features an upsized impeller that delivers greater mixing power and less destructive eddies than legacy S.U.B. designs (Figure 2a-b).

Figure 2. PIV plotted with respect to mixing speed and (a) Kolmogorov length plotted with respect to PIV (b) for the Enhanced (solid) and Legacy (dashed) S.U.B.s.

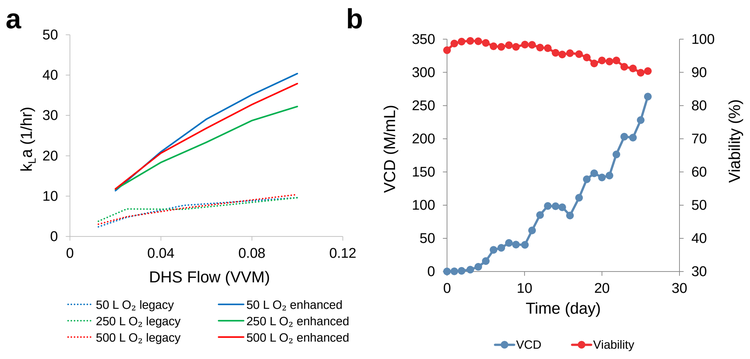


RESULTS

Enhanced S.U.B. for Perfusion

Perfusion cell culture conditions are especially demanding and require high mixing PIV, high mass transfer, and ATF/TFF connectivity. To address the unique demands of perfusion cell culture, the enhanced Thermo Scientific HyPerforma™ S.U.B. features an upsized impeller, improved DHS design, and user-friendly port configuration for ATF.[1] These enhancements resulted in up to a 4.3-fold increase in k_La (Figure 3a). When used in perfusion cell culture, the enhanced S.U.B. was able to support a viable cell density (VCD) of greater than 50 M/mL for more than 2 weeks, reaching a maximum VCD of nearly 264 M/mL on day 25 (Figure 3b).

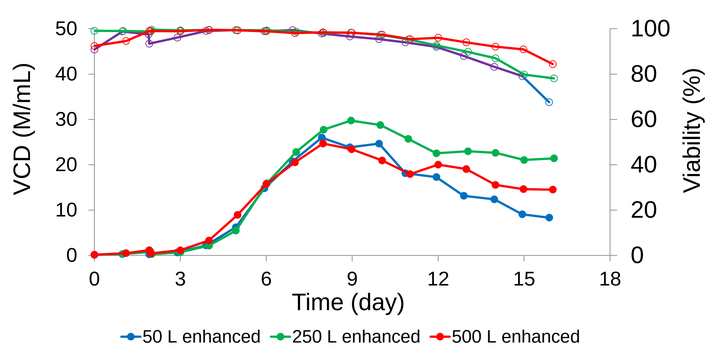
Figure 3. (a) Apparent k_La plotted with respect to O_2 flow rate in vessel volumes per minute (VVM) for enhanced (solid) and legacy (dashed) S.U.B.s. (b) Performance of enhanced S.U.B. throughout 25-day perfusion cell culture.



Enhanced S.U.B. for Fed-Batch

Increasing demand for high-product yields and greater risk mitigation in bioproduction processes has pushed legacy S.U.B. performance to its technological limits. To address the growing intensification of fed-batch bioproduction processes, the enhanced Thermo Scientific HyPerforma™ S.U.B. features an upsized impeller and improved DHS design, which sustains high cell viability in culture (Figure 4). In addition, the enhanced S.U.B. can operate at a 4:1 turndown ratio.[2] The ability of the enhanced S.U.B. to operate at low liquid volumes reduces the number of vessels required to reach production scale culture (e.g. 2000 L). Fewer vessels in a process seed train reduces risks associated with transferring high-density cell cultures between vessels while simplifying bioprocess development.

Figure 4. VCD (solid markers) and cell viability (hollow markers) plotted over two week fed-batch cell culture with the enhanced S.U.B.



Enhanced S.U.B. for Microcarriers

Adherent cell culture is an increasingly attractive strategy for producing vaccines and viral vectors for gene and cell therapies.[3] However, reliable scale up of adherent cell cultures with microcarriers is difficult due to the sensitivity of both the cells and microcarriers to shear (Figure 5a-b). To address this sensitivity limitation, we engineered an enhanced Thermo Scientific HyPerforma™ S.U.B. featuring the same upsized impeller and turndown ratio as the enhanced fed-batch, as well as optimized port configuration for media decantation, allowing high cell densities to be achieved on microcarriers (Figure 6).

Figure 5. (a) Impeller tip speed in the enhanced (solid) and legacy (dashed) S.U.B.s (b) Low tip speed of enhanced S.U.B. impeller reduces damage to cells and microcarriers.

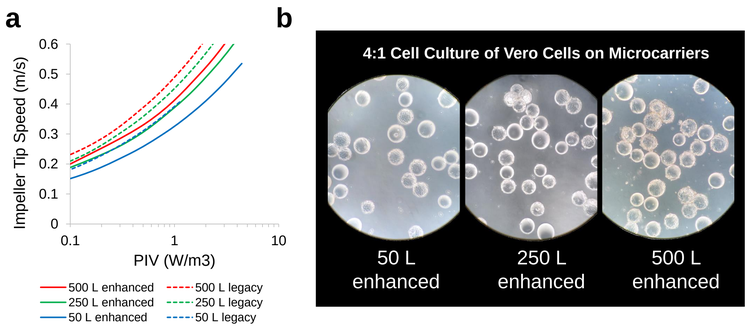
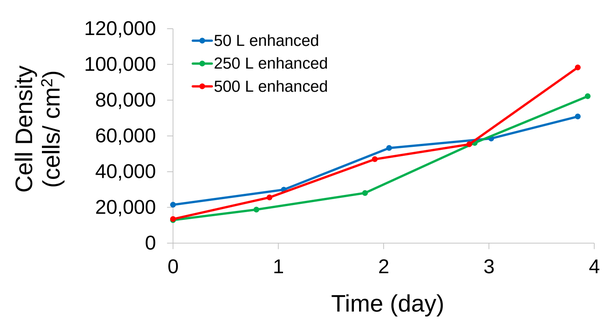


Figure 6. Adherent cell culture density on microcarriers using enhanced S.U.B.



MATERIALS AND METHODS

Enhanced S.U.B. for Perfusion

CHO-DP12 cells (ATCC CRL-12445™) were cultured in Gibco™ CD OptiCHO medium in an enhanced 50 L, 250 L, and 500 L HyPerforma™ S.U.B. for this study. Mixing power input per volume (PIV) throughout the culture varied from 20 – 100 W/m^3. Mass transfer was achieved by sparging O_2 through an enhanced DHS at rates up to 0.2 VVM. An antifoam sensor was used to monitor foam level. Alternating tangential flow (ATF) filtration was performed using a single-use XCell™ ATF 6. Culture conditions were controlled throughout using a HyPerforma™ G3Pro controller.

Enhanced S.U.B. for Fed-Batch

CHO-S cells were cultured in Gibco™ Dynamis medium and supplemented with Gibco™ EfficientFeed C+ for this study. To demonstrate scalability, cells were cultured in 50 L, 250 L, and 500 L HyPerforma™ S.U.B.s. Mixing PIV was maintained at 20 W/m^3 throughout the cultures. Mass transfer was achieved by sparging O_2 through an enhanced DHS at rates up to 0.05 VVM. Culture conditions were controlled throughout using a HyPerforma™ G3Pro controller.

Enhanced S.U.B. for Microcarriers

Vero cells (ATCC, CCL-81™) were cultured in Gibco™ VP-SFM with Cytodex® 1 microcarriers at a concentration of 3 g/L throughout the study. To demonstrate scalability, cells were cultured in 50 L, 250 L, and 500 L with average agitation rates of 42.8 RPM, 24.9 RPM, and 19.5 RPM, respectively. Cell culture media was exchanged at a rate of 0.5 VVD via decantation.

CONCLUSIONS

To address the trend toward specialization in bioproduction, Thermo Scientific™ has upgraded the HyPerforma™ S.U.B. product platform to feature application-specific enhancements for:

- Perfusion
- Fed-batch
- Adherent cell culture with microcarriers

Thermo Fisher Scientific is committed to developing best-in-class solutions and is proud to introduce the enhanced HyPerforma™ S.U.B. line in this tradition.

REFERENCES

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2. Madsen, B. Efficient Operation of the HyPerforma 5:1 Single-Use Bioreactor at Low Working Volume. Thermo Fisher Scientific. 2017.
3. Chatel, A. A Brief History of Adherent Cell Culture: Where We Come From and Where We Should Go. Bioprocess International. 2019

TRADEMARKS/LICENSING