Application note | Single-use bioreactor

Single-use bioprocessing

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

Design improvements, performance characterization, modeling, and cell culture

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

Introduction

Improvements in single-use systems have allowed implementation of high-density cultures in emerging bioprocess workflows, while progressive advances in media optimization and improved clone genetic selection have underscored the perceived performance limitations of single-use bioreactors (S.U.B.s). Here we show 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. We investigated the following:

- Bioreactor characterization; Thermo Scientific™ TruBio™ Software and Emerson™ DeltaV™ controller optimization; online process analytics; and scalability analysis of the S.U.B. when targeting perfusion applications in working volumes for pilot scale (50 L) to production scale (500 L).
- High-density culture results (>260 x 10^6 cells/mL) while maintaining proper operating parameters. New data reveal how a 50 L S.U.B. equipped with a specialized precision drilled-hole sparger (DHS), single-use foam probe, and oversized impeller is able to improve overall S.U.B. operating efficiency. Results also include specific suggestions on how to maintain a nearly ideal dissolved carbon dioxide environment, reduce generation of foam in the headspace, and produce lower overall shear levels, thus yielding excellent cell viability.

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 one to two vessel volumes per day (VVD).

Goal

Enhancements to the HyPerforma S.U.B. allow large increases in mass transfer and ultimately enable cell cultures to reach extremely high densities. Enhanced S.U.B. systems for perfusion have been optimized at scales from 50 L to 500 L, where cell growth and high protein production can be economically balanced with media exchange rates and volumes. Perfusion within this volume range and standard media exchange rates of one to two VVD can lead to metric tons of product per year while utilizing smaller vessels. Additionally, perfusion in these smaller vessels has been shown to be highly effective for N-1 bioreactors as seed train to larger production reactors. The data show very high mass transfer rates in all enhanced S.U.B.s, ultimately leading to ultrahigh cell densities in the 50 L S.U.B.

S.U.B. design

Table 1 presents the enhancements made to legacy HyPerforma S.U.B.s to specifically convert them to perfusion systems where higher mass transfer and more automation are required.

Materials and methods

Mass transfer studies were performed in all vessels, using the standard dynamic method [2], where the transfer of oxygen from gas to liquid phase is represented by Equation 1:

$$\frac{dC_{O_2}}{dt} = k_L a \cdot \left(C^*_{O_2} - C_{O_2}\right)$$

Equation 1.

$k_L a$ is the volumetric mass transfer coefficient, C_{O_2} is the concentration of dissolved oxygen, and $C^*_{O_2}$ is the saturation concentration of dissolved oxygen. Oxygen mass transfer was measured as the $k_L a$ of oxygen (air) transferring into an N_{2-} saturated solution. Carbon dioxide mass transfer out of solution was measured as the $k_L a$ of oxygen (air) transferring into a solution saturated with $C_{O_{2-}}$.

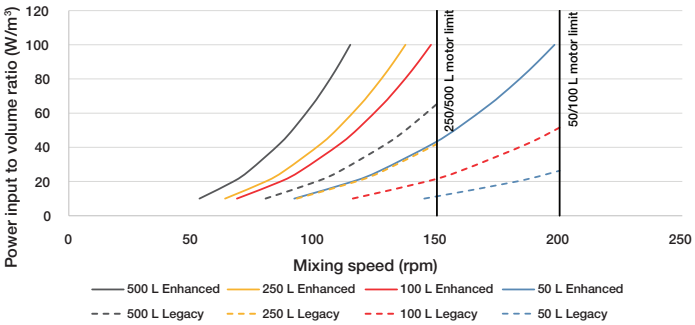


Figure 1. Power input to volume (PIV) ratio vs. mixing speeds for legacy and enhanced S.U.B.s for perfusion from 50 L to 500 L.

Table 1. Enhancements to HyPerforma S.U.B.s.

Enhancement	Result
Improved mixing using larger agitator; all systems use:* • 2:1-style shafts • 5:1-style motor blocks	Allows for higher power input to volume (PIV) ratio within motor limits (Figure 1) while maintaining slower impeller tip speeds (<1.5 m/s)
New precision-lasered DHS configured to meet higher oxygen uptake rates	Enhanced mass transfer:** • Enhanced DHS in each standard Thermo Scientific™ BioProcess Container (BPC) • Sparge flux area increased to support up to 0.2 vessel volumes per minute (VVM) while minimizing gas bubble shear, targeting a maximum gas entrance velocity of 15 m/s (far below the literature-reported accepted maximum of 30 m/s) [1]
Foam sensing	Automated foam control
Simple connection to either alternating tangential flow (ATF) or tangential flow filtration (TFF) technologies	Reliable and high-capacity (1 inch ID) aseptic connection to autoclaved or irradiated filters Flexibility of perfusion processes for either N-1 perfusion or true perfusion
Simple integration of conventional sensors, or easily customizable with single-use sensors	Flexible sensing options per customer needs; low-drift sensors will benefit longer-duration processes
Integration of S.U.B. load cells	Target media addition
Inclusion of separate cell bleed port	Achieve working cell density

* 250 L S.U.B. requires a special shaft/motor conversion kit for hardware.
** Frit spargers are optional but not recommended for perfusion applications, because of their high propensity for fouling, leading to high levels of variability in performance.

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 at 37°C. Tests were performed at varying gas flow rates through the DHS and several calculated power inputs (n_P = 2.1).

Cell culture testing

Cell culture was performed in a 50 L HyPerforma S.U.B. at 40 L working volume integrated with a Repligen™ XCell™ ATF 6 system. CHO DP-12 cells (ATCC, Cat. No. CRL-12445) adapted to Repligen™ LONG™ R^3 IGF-1 growth factor were seeded at 0.4 x 10^6 cells/mL in 40 L Gibco™ CD OptiCHO™ AGT™ Medium with 100 ng/mL LONG R^3 IGF-1 and 4 mM Gibco™ GlutaMAX™ Supplement. Operating conditions for the S.U.B. are listed in Table 2. Operating conditions for the XCell ATF 6 system are listed in Table 3. Cell counts, nutrients, metabolites, electrolytes, and protein yields were measured offline.

It was desirable to automate and control most parameters throughout the culture run to ensure a high probability of success. To that end, the following components were integrated into the DeltaV controller:

- Standard single-use DO sensor, pH sensor, and temperature sensor.
- Foam sensor to automate antifoam delivery and control foam levels. The built-in foam control was linked to the antifoam pump, and antifoam was delivered, as needed, to minimize total antifoam usage.
- Aber™ cell density sensor. The Aber transmitter signal was integrated into the DeltaV controller, and a bleed pump was linked to the measured cell density to cycle on and off, as needed, to maintain target cell density.
- S.U.B. load cells to maintain target volume. A calculation block was built using a simple Heaviside equation and linked to the media addition pump to avoid over- or underfilling the S.U.B. with new medium.

Medium for the culture was formulated in a 1,000 L Thermo Scientific™ HyPerforma™ Single-Use Mixer (S.U.M.) and sterile-filtered into a 1,000 L S.U.B. maintained at <8°C with a TCU. Spent medium from the process was transferred to a 1,000 L ALLpaQ™ container.

Oxygen was used as the primary gas for mass transfer through the DHS. Using this cascade, the dissolved oxygen set point was easily maintained with scalable mass transfer performance across gas flow rates while achieving sufficient levels of CO_2 stripping.

Table 2. 50 L HyPerforma S.U.B. operating conditions.

Parameter	Condition
Controller	Thermo Scientific™ G3Pro™ controller, running TruBio software powered by DeltaV distributed control system v12.3
Working volume	40 L, automated via load cells
Temperature	37°C, maintained with temperature control unit (TCU)
pH	7.0 ± 0.2 (CO_2 control only)
Dissolved oxygen (DO) set point	30%
DO cascade	Oxygen through DHS
Headspace sparge	2 L/min air
Antifoam	10,000 ppm Sigma-Aldrich™ Antifoam C Emulsion, automated via foam sensor
Glucose feed	45% glucose as needed (maintain 1–3 g/L in culture)
Cell bleed	Automated via cell density sensor
Target cell density	Variable setpoints, monitored and controlled via capacitance probe integrated into DeltaV controller

Table 3. XCell ATF 6 system operating conditions.

Parameter	Condition
Alternating tangential flow (ATF) system	XCell ATF 6 SU
ATF filter	ATF F6:RF02PES 0.2 µm PES hollow fiber
Filter surface area	2.53 m^2
Perfusion rate	Days 0–3: none Days 3–26: variable depending on cell density/nutrient demands, 1–4 VVD (40–160 L/day)
ATF rate	19.2 L/min
Shear rate	2,264 s^{-1}

Results

Mass transfer testing

Figure 2 displays O_2 and CO_2 mass transfer results for the enhanced S.U.B. and O_2 mass transfer for legacy S.U.B.s using only the DHS at maximum recommended impeller speeds (30 W/m^3 for legacy systems, 100 W/m^3 for enhanced S.U.B.s). Whereas legacy systems tend to reach a limit near 10–13/hr for k_La, results for the enhanced S.U.B.s show three- to four-fold increases in k_La using only the DHS.

Oxygen mass transfer with the DHS is shown to be higher in larger S.U.B.s, thereby allowing oxygen and air flow rates to be balanced against agitation rates to achieve desired O_2 and CO_2 mass transfer across all vessel sizes. Importantly, the CO_2:O_2 mass transfer ratio of the DHS across vessel sizes, gas flow rates, and mixing speeds remains between 0.3 and 0.5. This ratio is well-suited to balance oxygen demand while maintaining a sufficient level of CO_2 stripping to keep dCO_2 levels in physiological ranges (30–80 mm Hg) [3,4].

Cell culture testing

Cell culture was performed in a 50 L S.U.B. to test system capabilities, including the S.U.B. and controller with an XCell ATF 6 single-use system to achieve ultrahigh cell densities. The reactor was seeded at 0.4 x 10^6 cells/mL and grown to target cell densities of 40, 100, 150, and 200 x 10^6 cells/mL. Following two days of culture at 200 x 10^6 cells/mL, the final objective was to stress-test the perfusion system. Thus, the cells were purposely allowed to grow without constraint, reaching >260 x 10^6 cells/mL. Perfusion rates were increased incrementally to support cell growth. Glucose was supplemented as needed to maintain 1–3 g/L in culture. The run was finally terminated after 26 days when the ATF unit fouled completely. Viable cell density (VCD) and viability for the culture are shown in Figure 3.

Culture controller set points were maintained throughout the run, including DO at 30 ± 4%, temperature at 37 ± 0.05°C, and pH at 7 ± 0.20. Additionally, other culture parameters were measured offline and used to determine proper media exchange and glucose feed rates. These values are plotted in Figure 4, showing glucose levels between 1 and 3 g/L, and minimal buildup of lactate and ammonia.

Bench-scale testing was performed separately, showing robustness of this cell line to perform in extreme mixing conditions with tip speeds in excess of 1.5 m/s and power input greater than 400 W/m^3. Therefore, agitation was occasionally adjusted between 20 and 100 W/m^3 while observing oxygen flow rates and effects on dCO_2 (Figure 5). The data show a correlation between power input, gas flow rate, VCD, and dCO_2, which will be explored further.

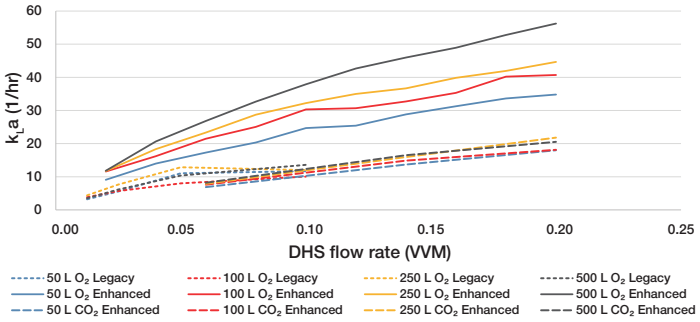


Figure 2. Oxygen and carbon dioxide mass transfer for enhanced S.U.B.s compared to legacy systems.

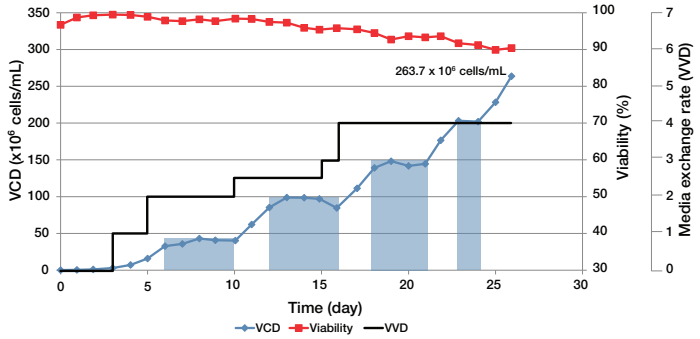


Figure 3. VCD and viability for enhanced 50 L S.U.B. operating at 1–4 VVD. Steady-state cell density zones are highlighted.

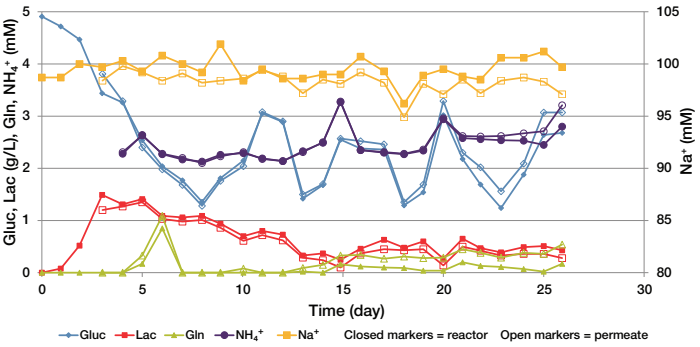


Figure 4. Glucose (Gluc), lactate (Lac), glutamine (Gln), ammonium (NH_4^+), and sodium (Na^+) levels in the reactor during the culture run.

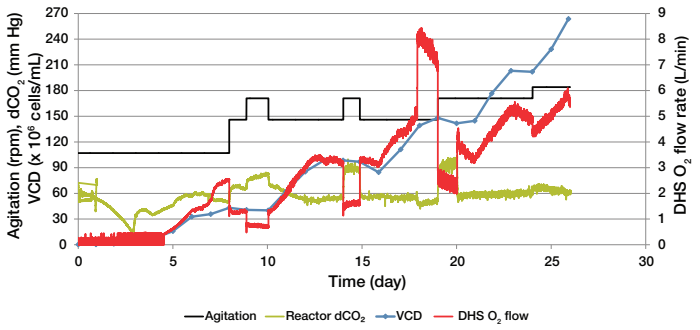


Figure 5. Agitation, dCO_2, VCD, and oxygen flow rates for the 50 L cell culture run.

Mass balance models

Oxygen mass balance

The literature has established empirical and theoretical models for cell culture in stirred-tank reactors using oxygen transfer rates (OTRs), cell density, mixing power, gas flow rate, and partial pressures in sparged gases [2,5]. To this end, a model from the culture data was developed relating k_La to power input per volume, gas flow rate, and basic metabolic and mass transfer equations, as shown in Equation 2.

$$OTR = OUR \therefore C_x = a \cdot k_La \cdot \left(C^*_{O_2} - C_{O_2}\right)$$

Equation 2.

Briefly, in a steady-state culture, OTR is equal to the oxygen uptake rate (OUR) of the cells. Therefore, cell density is proportional to the oxygen mass transfer coefficient (k_La) multiplied by the difference in partial pressures of the sparged gas and the operating DO set point in the culture. Here k_La is further modeled as a product of PIV and gas flow rate (VVM) with model factors as shown.

From this model (Figure 6), there is a direct correlation between the predicted and observed VCDs, meaning culture conditions (gas flow rate, mixing speed, and concentration of sparged gases) can be dialed specifically to process requirements to achieve the desired cell density. This establishes the S.U.B. as an ideal platform for obtaining predictive culture conditions for optimal growth and productivity.

CO_2 mass balance

Similarly, dCO_2 and pH can be modeled from the culture data to demonstrate the effectiveness of balancing process parameters. pH can be calculated using Equation 3:

$$pH = \text{baseline pH} - \text{acid buildup} + \text{base buildup}$$

Equation 3.

Essentially, each culture medium has a baseline operating pH and is affected by production and consumption of waste products such as lactate, ammonium, ammonia, and carbon dioxide.

Additionally, the pH of the medium can be adjusted through reactor settings such as gas flow rate and agitation to increase or decrease the amount of CO_2 stripping occurring in the process. Equation 3 was used to generate a model based on dCO_2 buildup (component of acid buildup) to show predictability of culture dCO_2 (Figure 7), demonstrating the ability to dial in gas flow rates and agitation to assist in dCO_2 (and thus pH) control. Measured pH is plotted against measured dCO_2 as well to show that pH stayed within process set points but varied depending on other culture conditions.

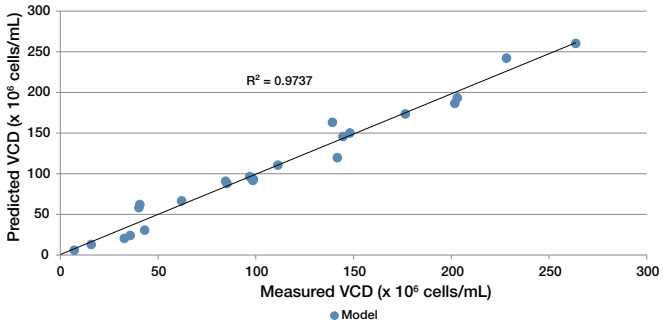


Figure 6. Model-predicted VCD vs. measured VCD.

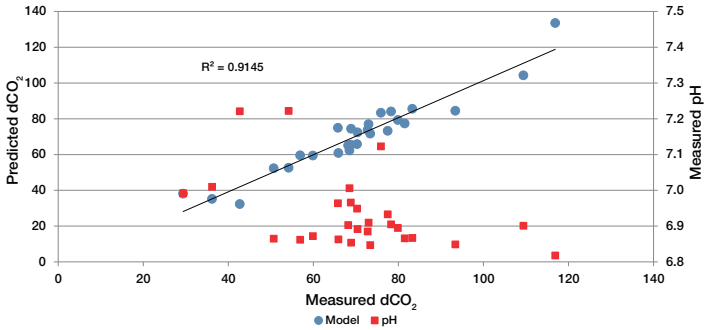


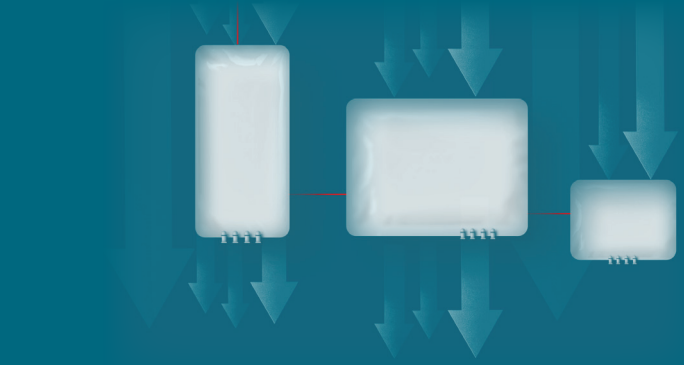
Figure 7. Model-predicted dCO_2 vs. measured dCO_2.

Discussion

Enhancements to the legacy HyPerforma S.U.B. have been implemented to achieve peak performance for high-demand cultures such as those seen in continuous processing applications. These enhancements, focusing on impeller and DHS configurations and designs, show improved mixing and mass transfer performance across vessel sizes of 50–500 L. Additionally, the improvements allow for easily scaling processes across these vessel sizes while maintaining scale parameters such as PIV and gas flow rate, especially when utilizing the enhanced DHS.

Importantly, the large increases in mass transfer were achieved using the DHS only without the frit as a mass transfer aid. Previous testing has shown the frit is prone to fouling in high-density or long-duration cultures, leading to highly variable performance and significant loss in performance due to changes in bubble formation on the frit surface. However, the DHS, with its benefits of low cell shear stress and high k_La, has shown robust performance under high-demand conditions due to high resistance to cell debris fouling.

The oxygen and CO_2 mass transfer data generated through extensive testing show predictability and scalability of these parameters within specific vessel sizes as well as across vessels.



These enhancements have shown increased performance that is three- to four-fold above legacy systems while maintaining mixing and sparging within the bounds of recommended reactor settings.

A confirmation cell culture run was performed to highlight the effect of these mixing and mass transfer enhancements, achieving stable cell densities at various set points up to 200 x 10^6 cells/mL, while achieving final growth to 260 x 10^6 cells/mL. While most processes would never require such high cell densities because of filter limitations and clone stability, the data demonstrate that the S.U.B. is capable of supporting very high demand cultures.

Conclusions

Strategic enhancements to mixing and gassing in 50–500 L S.U.B.s result in three- to four-fold increases in oxygen mass transfer to support ultrahigh demand cultures such as those seen in perfusion bioprocesses. Scalability of S.U.B.s is shown based on mass transfer data, allowing for perfusion processes to be achieved either as N-1 seed train to larger bioreactors, high cell density seed banking, or 500 L production-scale perfusion.

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 effectiveness of the S.U.B. and controller in maintaining proper cell culture settings even under demanding conditions of ultrahigh cell density with further capacity above those conditions tested.

Optimal media selection and operation at practical media exchange rates are certainly important logistical and financial considerations. Most importantly, this study confirms the potential for significantly improved process efficiency, rapid setup, and dramatically reduced technical risks, resulting in benefits that are achievable when implementing fully integrated and customizable disposable workflow solutions using Thermo Scientific S.U.M.s, S.U.B.s, bins, BPCs, and disposable flow paths.

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