S.U.B. enhancements for high-density fed-batch cultures

Design improvements, performance characterization, modeling, and cell culture

Introduction

Improvements in single-use systems have allowed implementation of high-demand 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). 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 4- to 5-fold improvement in mixing and mass transfer performance compared to legacy S.U.B. designs.

Importantly, improvements to the S.U.B. design revealed no loss in cell culture growth or bioreactor performance and can ultimately allow for simple scale-up and tech transfer among vessel sizes. An overall increase in oxygen mass transfer abilities can result in lower oxygen usage with appropriate gassing strategies and less foam generation in the reactor. When S.U.B.s are coupled with robust control systems and sensors, cell cultures were optimally controlled to obtain ideal cell growth.

Goal

Enhancements to the HyPerforma S.U.B. allow large increases in mass transfer and, ultimately, enable the S.U.B. to support high-demand cultures. Enhanced HyPerforma S.U.B. systems have been optimized from pilot to small-scale production bioreactors (50 L to 500 L) for aggressive fed-batch cultures. These S.U.B.s can be used in process development to obtain proper scale-up guidance or in scaling up cell quantities to seed larger production bioreactors. The data show very high mass transfer rates in all S.U.B.s, ultimately leading to successful cultures in each 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 enhanced systems, where higher mass transfer and more automation are required. Further guidance on S.U.B. hardware and consumables for this and other specific applications can be found in supplemental application studies [1].

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

Enhancement	Result
Improved mixing using larger agitator; all systems use: • 4:1 style shafts • 16.5° motor blocks Note: 250 L S.U.B. requires a special shaft/motor conversion kit for hardware.	Allows for higher power input-to-volume (PIV) within ratio motor limits (Figure 1) while maintaining slower impeller tip speeds (<1.5 m/s). Sufficient liquid coverage of impeller to operate as low as 25% working volume (4:1 ratio)
New precision-lasered DHS configured to meet higher oxygen uptake rates	Enhanced mass transfer: • Enhanced DHS in each standard Thermo Scientific™ BioProcess Container (BPC). Includes smaller pore sizes and higher pore quantities in each DHS • Sparge flux area increased to support up to 0.1–0.2 vessel volumes per minute (vvm) while minimizing gas bubble shear, targeting a maximum gas entrance velocity of 23 m/s (far below the literature-reported acceptable maximum of 30 m/s) [2]
Optional frit sparger recommendations	50 L: SV21967.02 100 L: SV21967.02 250 L: SV21967.02 500 L: SV21967.01
Cross-flow sparger (CFS) for low working volume	More efficient CO_2 mass transfer at low working volume, resulting in improved cell growth
Foam sensing	Automated foam control
Simple integration of conventional sensors, or easily customizable with single-use sensors	Flexible sensing options per customer needs

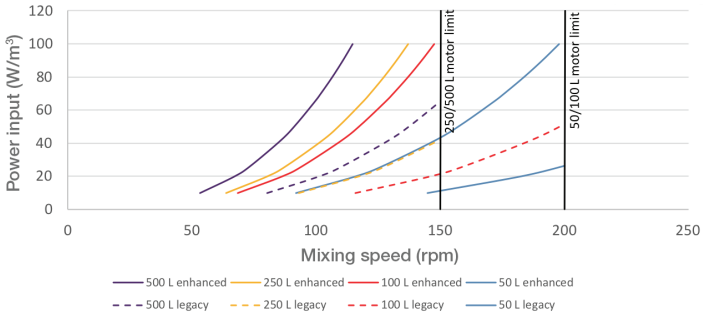

Figure 1. PIV versus mixing speeds for legacy and enhanced S.U.B.s for fed-batch from 50 L to 500 L.

Materials and methods

Mass transfer testing

Mass transfer studies were performed in all vessels using the standard dynamic method [3], where the transfer of oxygen from gas to liquid phase is represented by:

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

where $k_L a$ is the volumetric mass transfer coefficient, C_{O_2} is the concentration of dissolved oxygen (DO), and $C^*_{O_2}$ is the saturation concentration of DO. 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 CO_2-saturated solution.

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, 37°C. Tests were performed at varying gas flow rates through the DHS and several calculated power inputs (n_P = 2.1). Tests were conducted at 25%, 50%, and 100% working volume. All tests at 25% working volume utilized the CFS for overlay gas, while tests at 50% and 100% working volume used the standard overlay sparger.

Cell culture testing

Cell culture was performed in 50 L, 250 L, and 500 L HyPerforma S.U.B.s; Gibco™ Freedom™ CHO-S™ cells were seeded in Gibco™ Dynamis™ Advanced Granulation Technology™ (AGT™) Medium with 0.1% Gibco™ Anti-Clumping Agent at 25% working volume. Following two days of growth, more Dynamis medium was added to bring the working volume to 85%. Gibco™ EfficientFeed™ C+ AGT™ supplement at 2X concentration was fed to the culture on days 5–12, supplemented with a 45% glucose solution as needed to maintain glucose levels above 1 g/L. Further cell culture conditions are listed in Table 2. Cultures were controlled by Thermo Scientific™ HyPerforma™ G3Pro Controllers running Thermo Scientific™ TruBio™ software.

In addition to cultures in each individual vessel, a final cell run was performed to assess any differences or similarities between the enhanced S.U.B. and the legacy S.U.B. targeting identical growth between the vessels. This was carried out using enhanced and legacy 250 L S.U.B.s using similar protocols as those outlined, except that both vessels were seeded at 85% working volume and continued in a standard fed-batch culture.

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

Parameter	Condition
PIV ratio (agitation)	20 W/m^3
Sparging	• Single DHS • Gassing: O_2 as primary; N_2 and CO_2 as needed
Air flow	CFS and overlay sparging at 50–70 L/m^2 surface area per minute
Temperature	37°C, maintained with temperature control unit (TCU)
Culture strategy	Day 0: seed at 25% working volume Day 2: feed to 85% working volume Days 5–12: continue standard feed
Variable pH control	Days 0–5: variable pH (7.2–7.0) targeting dissolved CO_2 levels at 30–80 mmHg Day 5–end: pH 7.0 (no base required) Overall: pH varied from 6.8 to 7.2
Dissolved oxygen (DO) set point	30%
Antifoam	10,000 ppm antifoam C, automated via foam sensor

Results

Mass transfer testing

Figures 2 and 3 display O_2 and CO_2 mass transfer results for the enhanced S.U.B. and comparative data for the legacy S.U.B.s using the DHS at designated power inputs at full volume. Whereas legacy systems tend to limit O_2 k_La near 10/hr, enhanced systems increase to 35–48/hr (4–5x increase). Similarly, CO_2 stripping in legacy systems limit to 5–6/hr, while enhanced systems increase to 17–22/hr (3x increase). Mass transfer results at half volume and 4:1 volume (Figures 4–7) were also elevated above legacy systems (5:1), and were sufficiently high to support aggressive cell cultures.

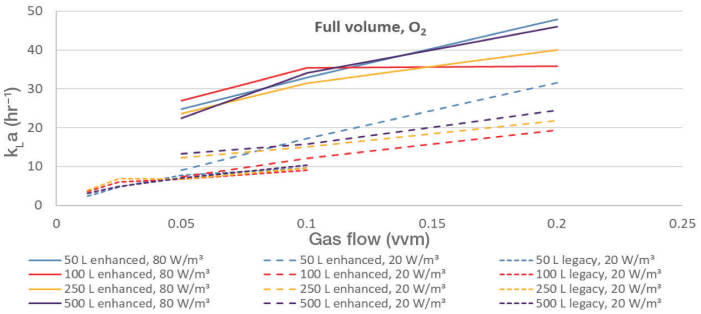

Figure 2. O_2 mass transfer results for enhanced S.U.B.s compared to legacy S.U.B.s at full volume.

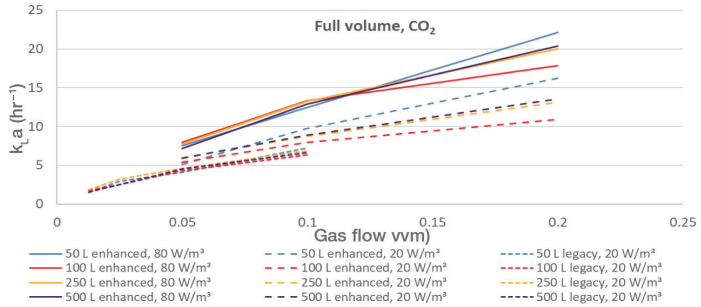

Figure 3. CO_2 mass transfer results for enhanced S.U.B.s compared to legacy S.U.B.s at full volume.

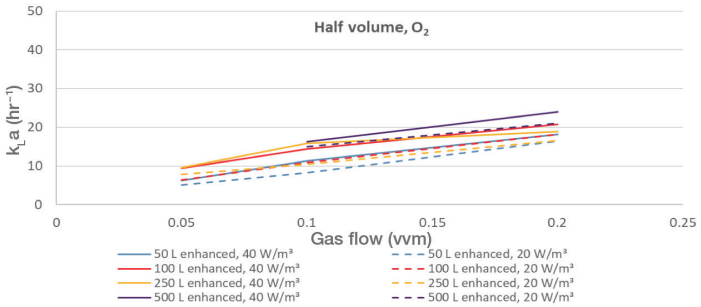

Figure 4. O_2 mass transfer results for enhanced S.U.B.s at half volume.

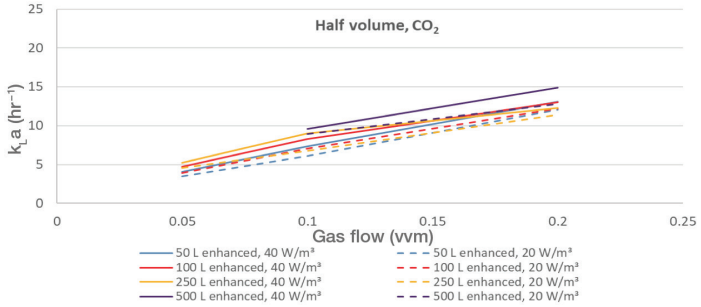

Figure 5. CO_2 mass transfer results for enhanced S.U.B.s at half volume.

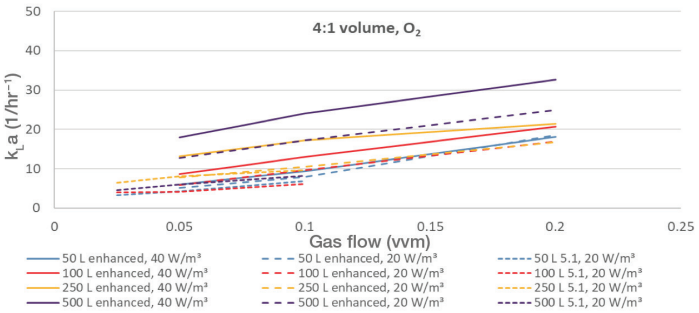

Figure 6. O_2 mass transfer results for enhanced S.U.B.s compared to 5:1 S.U.B.s at minimum working volume.

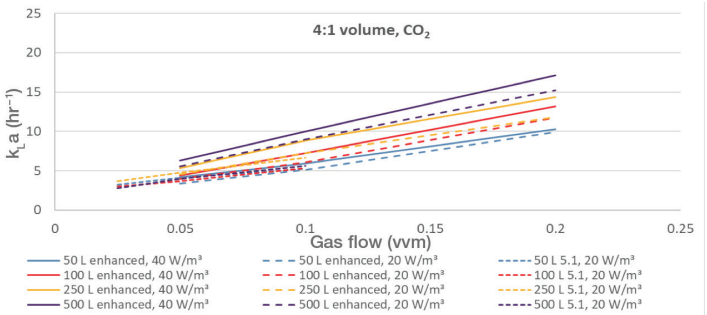

Figure 7. CO_2 mass transfer results for enhanced S.U.B.s compared to 5:1 S.U.B.s at minimum working volume.

Each DHS was designed to increase oxygen mass transfer by reducing pore size compared to legacy DHS designs while increasing the pore quantity to allow for higher mass flow rates. The end effect is the creation of a high quantity of small bubbles, effectively increasing the overall area of mass transfer. The DHS used in the 50 L and 100 L S.U.B.s was designed to perform optimally up to about 10 slpm, resulting in no net increase in mass transfer up to 20 slpm, as seen in Figure 2. Ultimately, sufficient mass transfer up to 35/hr is seen at only 10 slpm in the 100 L S.U.B.

Both O_2 and CO_2 mass transfer were highly scalable at full working volume, resulting in easier tech transfer and scalability among systems. Scalability among vessel sizes diminishes slightly at lower volumes, but mass transfer is still sufficiently high in these vessels to support high-demand cultures. Importantly, the CO_2:O_2 mass transfer ratio at full volume of the DHS across vessel sizes, gas flow rates, and mixing speeds remains near 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 mmHg) [4,5].

Cell culture testing

Cell culture was performed in the 50, 250, and 500 L S.U.B.s to ensure proper and scalable growth among the systems. Each S.U.B. was seeded from separate cell thaws, resulting in some inherent variability among the cultures. However, overall growth, including peak viable cell density (VCD) and viability trends, were similar among all systems (Figure 8). All vital culture parameters (DO, pH, temperature, and glucose) were maintained at or near setpoints for the duration of the culture.

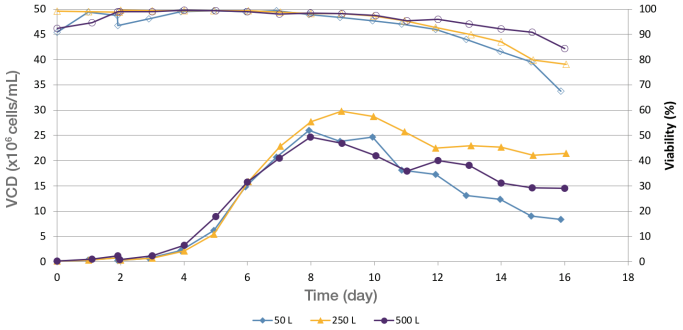

Figure 8. VCD and viability for the individual S.U.B. cell runs.

Due to the chosen gas strategy (O_2, N_2, and CO_2 cascading), O_2 flow was normalized as the effective O_2 flow rate relative to the overall gas flow rate, divided by the vessel working volume. The trends in Figure 9 show similar effective gas flow rates, resulting in good scalability among the systems. The higher effective gas flow rate in the 250 L S.U.B. can be predicted based on the k_La data in Figure 2, where k_La is shown to be slightly higher in the 50 L and 500 L S.U.B.s.

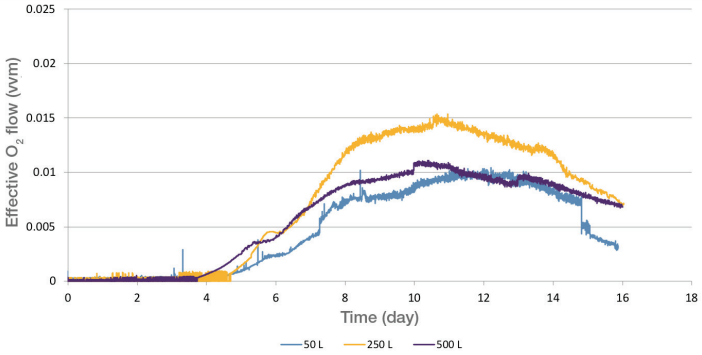

Figure 9. Effective O_2 flow for the individual S.U.B. cell runs.

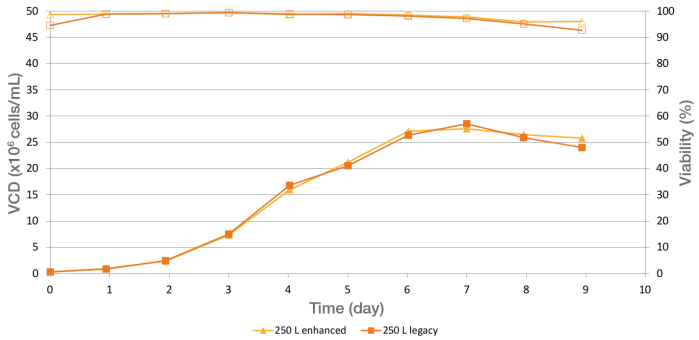

Figure 10. VCD and viability for the 250 L side-by-side cell run.

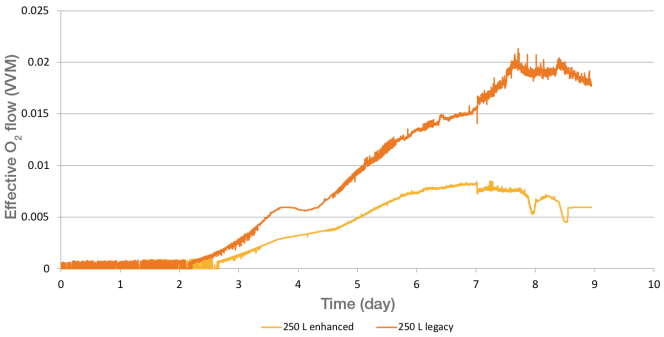

Figure 11. Effective O_2 flow for the 250 L side-by-side cell run.

Whereas the individual cell runs utilized a combination of O_2, N_2, and CO_2 gases to control DO and pH setpoints, the 250 L side-by-side run also used air in combination with the other gases to control dCO_2 levels. Specifically, dCO_2 levels were held between 60–80 mmHg in these runs in order to improve the health of the cultures. The gas strategy was adjusted to sparge air in as needed to improve CO_2 stripping capabilities of the DHS while maintaining O_2 mass transfer. Therefore, the effective O_2 flow rate decreased compared to the individual 250 L cell run. Importantly, the effect of the smaller pore size in the enhanced S.U.B. is pronounced in the overall lower effective O_2 flow rate compared to the legacy system, operating at approximately 40–60% of the total O_2 flow, especially toward peak growth from days 6–9.

Discussion

Enhancements to the legacy HyPerforma S.U.B. have been implemented to achieve peak performance, targeting high-demand cultures such as those seen in aggressive fed-batch cultures. These enhancements, focusing on impeller and DHS configurations and designs, show improved mixing and mass transfer performance across vessel sizes from 50 L to 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.

Mass transfer gains are highlighted by the improved DHS in each enhanced system, resulting in 4–5x increases in mass transfer. Importantly, these gains are seen using only the DHS, resulting in less reliability on the frit as a mass transfer aid. Previous testing has shown the frit is prone to fouling in high-density cultures, leading to highly variable performance, even significant loss in performance due to changes in bubble formation on the frit surface. The DHS, however, exhibits low cell-shear stress, high k_La, and robust performance under high-demand conditions while showing resistance to cell debris fouling.

Individual cell runs showed the ability to grow cells in the enhanced S.U.B.s, and similar peak cell densities compared to legacy data [6]. Additionally, a cell run directly comparing gassing strategies between an enhanced 250 L S.U.B. and a legacy 250 L S.U.B. showed no difference in cell growth, and 40–60% less O_2 usage in the chosen sparging strategy. Lower O_2 demand in the culture can result in lower operating costs for the run, less foaming and antifoam usage, and potentially healthier cultures.

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Conclusions

As customers create increasingly demanding processes, the legacy S.U.B.s have needed modification to accommodate higher mixing and mass transfer, supporting higher oxygen demands in aggressive fed-batch cultures. Strategic enhancements to mixing and gassing in 50–500 L S.U.B.s result in 4- to 5-fold increases in oxygen mass transfer to support ultrahigh-demand cultures. Scalability of S.U.B.s is shown based on mass transfer data, allowing for efficient fed-batch scale-up among pilot and small production-scale bioreactors.

Cell culture data showed no loss of cell health, and an overall decrease in total oxygen demand compared to legacy systems. Implementing proper gassing cascades leads to controllable DO and dCO_2 levels in the bioreactor, reduced foaming, and simple scale-up.

References

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