Continuous Process Performance Enhancements for 50 L to 500 L Single-Use Bioreactors: A Technical Comparison of Performance Characterization, Cell Culture, and Scale-Up Modeling

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ABSTRACT

Improvements in single-use systems have allowed implementation of high-density cultures in emerging bioprocess work flows. Specifically, advantages of single-use bioreactors have been realized in perfusion applications in high-density seed train intensification or as a compact production-scale bioreactor system. Due to this and additional progressive advances in media optimization and improved clone genetic selection have increased stress on the perceived performance limitations of single-use bioreactors. This study shows integration of the Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) and how strategic enhancements to the sparger and agitation systems have revealed the potential for 3-4X improvement of mixing and mass transfer performance compared to legacy SUB designs. This work includes:

1) Bioreactor characterization and scalability analysis of the S.U.B. when targeting perfusion applications from 50 L pilot scale to 500 L production scale working volumes.
2) High-density culture results (>200E06 cells/mL) while maintaining proper operating parameters using a TruBio DeltaV controller and online process analytics. New data reveals specifically how a 50 L S.U.B. equipped with a specialized precision drilled hole sparger, single use foam probe, and oversized impeller is able to improve overall SUB operating efficiency. Coupled with best practices and the desirable process benefits achieved through automation and control of vital process parameters, evidence is provided as to the advantages of continuous processing in single-use systems.

INTRODUCTION

A continuous processing workflow allows cell growth to higher cell densities within the bioreactor as compared with traditional batch or fed batch processes. This is facilitated by the regular addition of fresh media and continuous removal of extra-cellular proteins and metabolic waste. Using perfusion, significant yield (as measured in grams of protein per cell per volume) is feasible at a smaller scale. Due to the continuous nature of the process, increased cell production can be achieved over a shorter period of time. Consistent replenishment of nutrients, combined with reduced build up of waste products in the bioreactor allows for a persistent homogeneity in the vessel and a higher degree of control over culture conditions, thus contributing to enhanced quality. Perfusion amplifies the benefits of single-use technologies, enabling more efficient use of production space and greater overall flexibility.

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

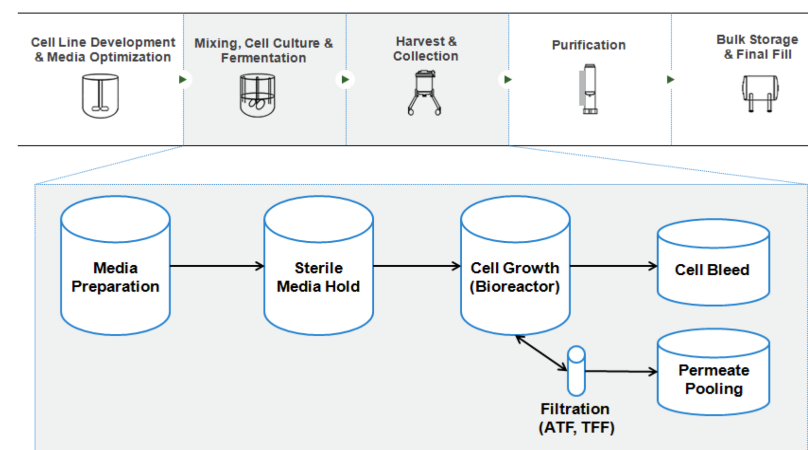

Figure 1. Continuous processing workflow highlighting media mixing, cell culture and primary harvest.

Thermo Fisher Scientific offers solutions for most points within the continuous bioproduction workflow including:

- Cell line development
- Media optimization
- Media hydration
- Sterile media hold
- Bioreactor production vessels
- Product pooling
- Waste containment
- Chromatography resins
- Bulk storage and final fill

S.U.B. AND CONTROLLER ENHANCEMENTS: 50-500 L

- Enhanced mixing systems using larger impeller allows for higher power input to volume within motor limits (Figure 3) while maintaining slower impeller tip speeds (<1.5 m/s)
- Scalable sparging solutions for enhanced mass transfer
  - DHS/frit combo in each BPC
  - Up to 0.2 VVM with drilled hole sparger
  - Minimized gas entrance velocity through DHS for reduced bubble shear; targeted maximum 15 m/s[1]
- Foam sensing for automated foam control
- Simple connection to either alternating tangential flow (ATF) or tangential flow filtration (TFF) technologies
- Scalable from 50 L pilot scale to 500 L production working volumes
- Simple integration of conventional sensors or easily customizable with single-use sensors
- Integration of S.U.B. load cells to target media addition rates

Figure 2. 250 L S.U.B. integration with Finesse G3Lite controller.

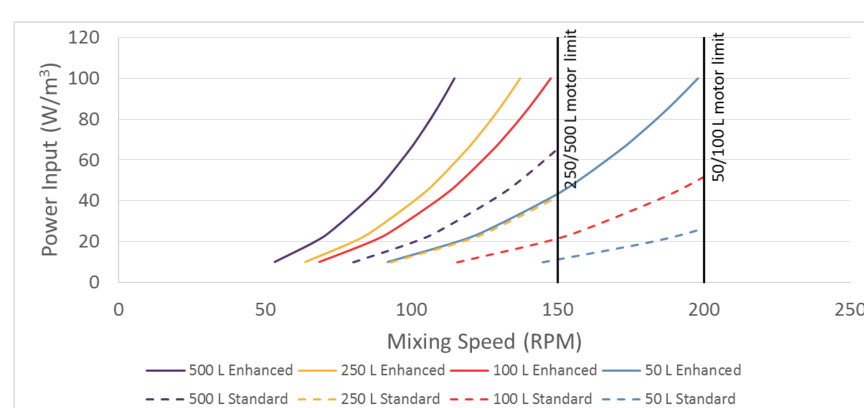

Figure 3. PIV versus mixing speeds for standard and enhanced S.U.B.s from 50 L to 500 L.

MATERIALS AND METHODS

Mass Transfer Testing

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

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

Where k_La is the volumetric mass transfer coefficient, C_{O2} is the concentration of dissolved oxygen, and C^*_{O2} is the saturation concentration of dissolved oxygen. Oxygen mass transfer was measured as the k_La of oxygen (air) transferring into an N_2-saturated solution. Carbon dioxide mass transfer out of solution was measured as the k_La of oxygen (air) transferring into a CO_2-saturated solution.

Test solution consisted of 1 g/L poloxamer 188 and 3.5 g/L HEPES buffer titrated to pH 7.25 at air saturation at 37°C. Tests were performed at varying gas flow rates through the frit and drilled hole sparger and several calculated power inputs (n_P = 2.1).

Table 1. Culture components and cells

Components	Description
Cell Line	CHO-DP12 (ATCC# CRL-12445, Adapted to LONG® R3 IGF-1)
Media Composition	CD OptiCHO Medium + 100 ng/mL LR3 + 4 mM Glutamax
Base	N/A
Antifoam	10,000 ppm Antifoam C as needed

Table 2. Bioreactor conditions

Components/ Conditions	Setting
Bioreactor	Thermo Fisher 50 L HyPerforma S.U.B.
Controller	Finesse™ G3Pro running TruBio DV 12.3
Bioreactor w.v	40 L, automated via load cells
Temp	37°C
pH	7.0 ± 0.2 (CO_2)
Agitation	20-100 W/m^3 (107-184 RPM)
DO Set point	30% (Pure O_2 through standard drilled hole sparger)
Air	Headspace sweep at 2 L/min air
Antifoam dosing	Automated via foam sensor
Glucose Feed	45% glucose as needed (maintain 1-3 g/L in culture)
Seeding Density	~0.4E6 Cells/mL

Table 3. ATF conditions

Components/ Conditions	Single-Use ATF
ATF System	XCell™ ATF 6 SU
ATF Filter	F6:RF02PES 0.2 µmPES HF
Filter SA	2.53 m^2
Perfusion rate	Day 0-3: None
	Day 3-end: variable depending on cell density/nutrient demands 1-4 VVD (40-160 L/day)
ATF Rate	19.2 LPM
Shear Rate	2264 s^{-1}
Cell Bleed	Constant
Target Cell Density	Variable setpoints, monitored and controlled via capacitance probe integrated into Finesse DeltaV controller

RESULTS

Mass Transfer Testing

Figure 4 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 and 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 limit near 10-13/hr for k_La, results for the enhanced S.U.B.s show 3-4 fold increases in k_La using only the DHS. Further k_La increases past 65/hr are achievable in all S.U.B. sizes when the microsparger is coupled with the DHS.

Oxygen mass transfer with 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 oxygen 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 0.3-0.5. 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)[3,4].

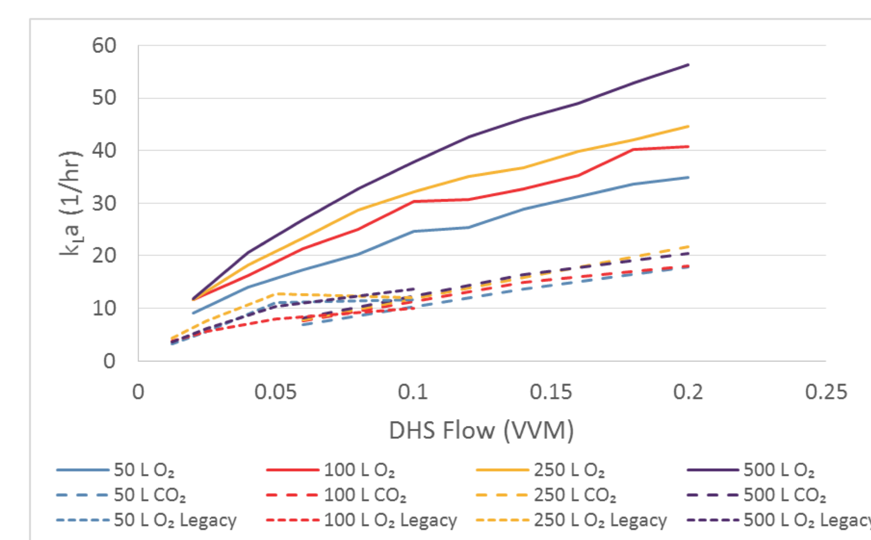

Figure 4. Oxygen and carbon dioxide mass transfer results for enhanced S.U.B.s compared to legacy.

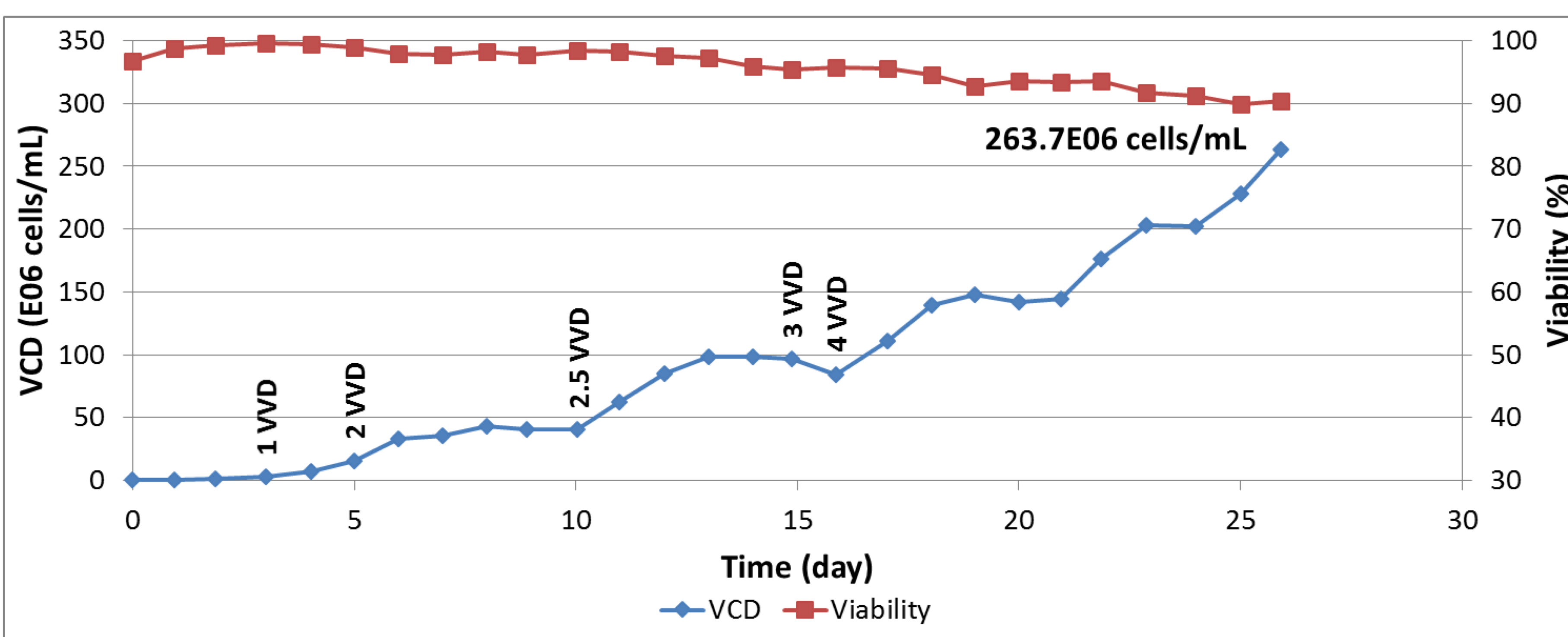

Figure 5. VCD and viability results for enhanced 50 L S.U.B. operating at 1-4 VVD.

Cell Culture Testing

Cell culture was performed in a 50 L S.U.B. to test system capabilities including the S.U.B., controller, and XCell ATF6 Single-Use System to achieve ultra-high cell densities. The reactor was seeded at 0.4E06 cells/mL and grown to target cell densities of 40, 100, 150, and 200E06 cells/mL. Following 2 days of culture at 200E06 cells/mL the final objective was to stress test the perfusion system. Thus the cells were purposefully allowed to grow without constraint, reaching >260E06 cells/mL at which point the ATF unit fouled completely and culture was successfully terminated. Perfusion rates were increased incrementally to support cell growth. Glucose was supplemented as needed to maintain 1-3 g/L in culture. Viable cell density and viability for the culture is shown in Figure 5.

Automation and control of most parameters was desired throughout the culture to ensure high possibility of success. To that end the following were integrated into the DeltaV controller:

- Standard dissolved oxygen, pH, and temperature sensors.
- 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.
- Cell density sensor (Aber™). The 4-20 mA signal from the Aber transmitter was integrated into the DeltaV and a bleed pump was linked to the cell density signal through a compare block 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/underfilling the S.U.B. with new media.

Media for the culture was formulated in a 1000 L Thermo Scientific HyPerforma S.U.M. and sterilely transferred to a 1000 L S.U.B. maintained at 8°C with a TCU. Spent media from the process was transferred to a 1000 L Thermo Scientific ALLpaQ container.

Oxygen was used as the primary gas for mass transfer through the DHS only. Using this cascade, dissolved oxygen setpoint was more easily maintained while achieving sufficient levels of CO_2 stripping. The frit was not utilized in this study to show the effectiveness of the DHS and to avoid effective losses in mass transfer performance by the frit due to fouling of the microsparge surface. Oxygen stayed well within the 0.2 VVD flow rate capacity of the DHS while maintaining dissolved oxygen setpoint, even to >200E06 cells/mL.

To demonstrate the effect of agitation on gassing, motor speeds were occasionally adjusted between 20-100 W/m^3 while observing oxygen flow rates and effects on dCO_2 (Figure 6). Bench-scale testing showed this clone was capable of running at tip speeds in excess of 1.5 m/s and > 400 W/m^3. To further automate the process in the future a CO_2 sensor could easily be integrated into the controller and air supplemented to the oxygen sparge to maintain target dCO_2 within the reactor.

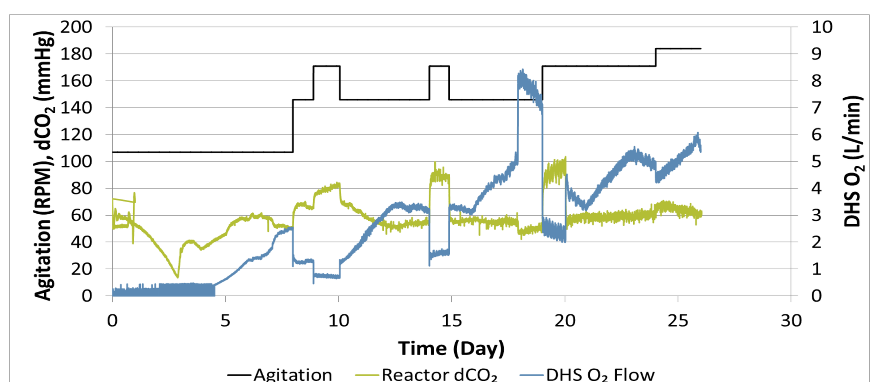

Figure 6. Oxygen flow rates, agitation, and dCO_2 for the 50 L cell run.

CONCLUSIONS

Strategic enhancements to mixing and gassing in 50-500 L S.U.B.s result in 3-4 fold increases in oxygen mass transfer to support ultra-high 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 bioreactor, 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 260E06 cells/mL while maintaining proper reactor conditions including dissolved oxygen, pH, and dCO_2 levels at perfusion rates up to 4 VVD. Results demonstrate effectiveness of the S.U.B. and controller to maintain proper cell culture settings even under demanding conditions of ultra-high cell density with further capacity above those conditions tested.

Optimal media selection and operating 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 real world benefits that are achievable when implementing a fully-integrated and customizable disposable flow workflow solution using Thermo Scientific S.U.M.s, S.U.B.s, bins, BPCs, and disposable flow paths.

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