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Improving single use bioreactor design and process development using the HyPerforma 5:1 S.U.B

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Efficient Operation of the HyPerforma 5:1 Single-Use Bioreactor at Low Working Volume

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ABSTRACT

Operating bioreactor vessels at low working volumes (high turn-down ratios) is often desirable but brings about challenges in regard to mixing, mass transfer, and process control. Research concerning the optimization cell culture has provided methods to improve performance and control when operating under low working volumes.

- Enhanced energy transfer – implements bottom heat exchange, alternate impeller positions, and considers agitation dissipation rates.
- Maximized platforms – implementation of the unique Thermo Fisher Scientific Drilled Hole Sparge (DHS) design and new Cross Flow Sparge (CFS) into the headspace have yielded reliable mass transfer and cell culture results from 10-2000 L working volumes.
- Improved bioprocess production – new technology improves equipment utilization, scheduling efficiency, inventory logistics, and reactor harvest consistency.
- 5:1 turn-down operation with the new 1000 L and 2000 L Thermo Scientific™ HyPerforma™ Single-Use Bioreactors (S.U.B.s) – considers cGMP operations, design features, implements enhanced processing tools.

INTRODUCTION – S.U.B. DESIGN

HyPerforma 5:1 S.U.B. design changes include:

- New 5:1 kits designed for 50-500 L systems include:
  - Increased drive shaft length by 6-16 cm
  - Decreased motor block angle from 19.6° to 16.5° in 50-500 L S.U.B.s
- New 5:1 kits designed for 1000 L and 2000 L systems include automated agitator/motor positioning for bimodal operation
- Added cutouts to the S.U.B. tank to allow for sensors below liquid levels at 20% working volume
- Bottom heat-exchange
- Added a new CFS for use at low working volumes
- Removed the microsparger (frit) in all vessels

MATERIALS AND METHODS

Mass Transfer Studies

Mass transfer studies were performed at 5:1 and full volume using the standard dynamic method[1], where the transfer rate of oxygen from the 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. Tests were performed at 20 and 40 W/m^3 mixing (n_p = 2.1). Drilled hole sparger flow rates were up to 0.1 VVM with overlay or CFS flow rates at 50-70 L/m^2 liquid surface area per minute.

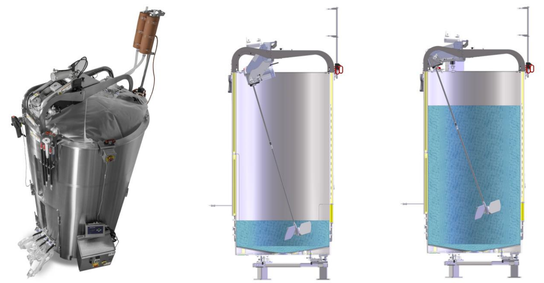
Mixing Studies

Mixing studies were performed by adding concentrated sodium chloride solution to the reactor and measuring the conductivity over time. Two conductivity sensors placed in opposite sides of the probe belt were used at 20% working volume while three conductivity sensors placed at three separate heights were used at full volume. Three separate tests were conducted at each working volume for each mixing speed. The overall mixing time was determined as the time taken for all three sensors to stabilize within 95% of the final conductivity (T95). Mixing speeds ranged from 10-60 W/m^3 for all vessel sizes but only within the boundaries of recommended maximum operating conditions for each S.U.B. (<120 RPM for the 1000 L and <95 RPM for the 2000 L).

Harvest Mixing Efficiencies

Following cell culture runs, a capacitance probe was placed in the reactor near the bottom drain port. Capacitance, which correlates to cell mass[2], was measured during drain to determine solution homogeneity during the drainage process.

Figure 1. HyPerforma 5:1 S.U.B. top view perspective of system as tested (left) with cross-section models showing motor/impeller in down position at 20% working volume (middle) and in up position at full volume (right).



Cell Culture Testing

Parameter	Condition
Cell Line	Gibco™ Freedom™ CHO-ST™, mAb producer
Medium	Gibco™ Dynamis™ AGT™ Medium with 0.1% Gibco™ Anti-Clumping Agent
Feeds	• Gibco™ EfficientFeed™ C+ AGT™ Supplement, 2X concentration • 45% glucose as needed
Culture Strategy	• D0: seed at 20% working volume • D2,3: feed to 85% working volume with Dynamis medium • D5-12: constant EFC+ and glucose feeds targeting <5 g/L
Variable pH control	• D0-5: variable pH 7.2 to 7.0 targeting dCO_2 levels 30-80 mmHg • D5-end: pH 7.0 (no base) • Overall: pH varied from 6.8 to 7.2, dCO_2 levels 30-80 mmHg
Sparging	• Standard DHS • Gassing: O_2 as primary, N_2 and CO_2 as needed
Headspace air flow	Cross-flow or overlay sparging at 50-70 L/m^2 surface area per min
Power input to volume (agitation)	20 W/m^3

RESULTS

Mass Transfer Studies

Figure 2. 250 L, 5:1 Volume, O_2, 40 W/m^3

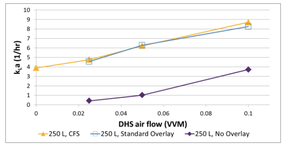
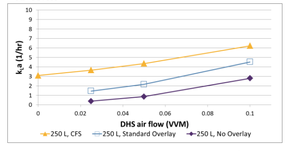


Figure 3. 250 L, 5:1 Volume, CO_2 stripping, 40 W/m^3



Figures 2 and 3 display O_2 and CO_2 mass transfer results for the 250 L S.U.B. at 5:1 volume, 40 W/m^3 and various DHS air flow rates up to the rated limit of 0.1 VVM. Other vessel sizes demonstrated similar trends. Results show a marked increase in oxygen k_La across flow rates when using either the CFS or overlay sparger, when compared to adding no headspace gas. However, there is a substantial increase in CO_2 removal when using the CFS compared to the overlay sparger.

High levels of dissolved CO_2 in solution have been shown to inhibit cell growth and protein production[3,4]. When operating S.U.B.s at low working volumes, CO_2 can build up at the liquid-air interface resulting in localized high concentrations of dCO_2 and lower pH levels in the solution. Due to gas density differences, CO_2 is also more difficult to remove from solution compared to oxygen and nitrogen (air). Figure 2 shows the ability of either the standard overlay or the CFS to create equal O_2 mass transfer into the system while Figure 3 shows the benefit of proper gas mixing at the liquid-air interface when operating with gasses of different densities. In this case, low gas velocities at this interface created by the standard overlay sparger are insufficient to move the denser CO_2 gas from the liquid surface. When using the CFS at low working volumes, these gas flows across the liquid-air interface are magnified, allowing for better gas mixing and creating a more homogeneous gas mixture in the headspace.

Figure 4. 5:1 Volume, O_2, 40 W/m^3, CFS

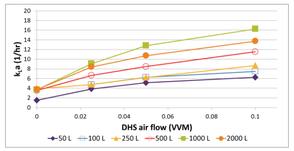
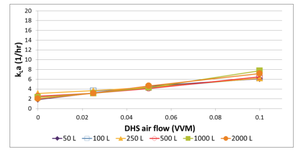


Figure 5. 5:1 Volume, CO_2 stripping, 40 W/m^3, CFS



Figures 4 and 5 display O_2 and CO_2 mass transfer results for all S.U.B.s at 5:1 volume, 40 W/m^3 and various DHS air flow rates up to the rated limit of 0.1 VVM. Data show exceptional scalability of CO_2 mass transfer among all vessels while O_2 mass transfer improves with increasing vessel size. Results suggest that sparging strategies can be configured to achieve sufficient O_2 mass transfer (with air and/or O_2 gas) while maintaining CO_2 stripping, which has traditionally been more difficult in larger bioreactor sizes.

Mixing Studies

Figure 6. Mixing times at 5:1 volume

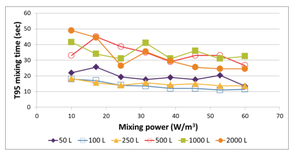
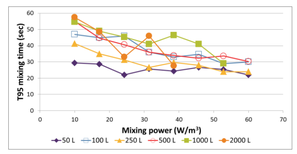


Figure 7. Mixing times at full volume



Figures 6 and 7 illustrate the T95 mixing times for each vessel at various power inputs for both 5:1 and full volume. At 5:1 volume, mixing times for all vessels were less than 1 minute, with similar mixing times for smaller sizes (50-250 L) and slightly higher mixing times for the larger sizes (500-2000 L). Mixing times at full volume were higher than at 5:1 volume, but less than 1 minute for all vessel sizes.

Cell Culture Testing

Cell culture was performed in each vessel, starting at 20% working volume. Depending on viable cell density, cultures were increased to 85% working volume on day 2 or 3. Standard feeds (EfficientFeed C+ supplement and glucose) were initiated on day 5 and continued through day 12. Cultures continued until termination on day 16. Results demonstrated consistent performance across all vessel sizes for both viable cell density (VCD) and cell viability (Figure 8) with similar growth profiles including growth rates and peak cell densities. Peak cell densities and growth rates were also conserved compared to culture runs performed at full volume (Figure 9). Figure 9 also demonstrates the ability of the S.U.B. to achieve high cell densities at 5:1 volume, resulting in the ability to remove specific vessels from seed trains or process development workflows.

Figure 8. VCD and viability results for HyPerforma 5:1 S.U.B.s

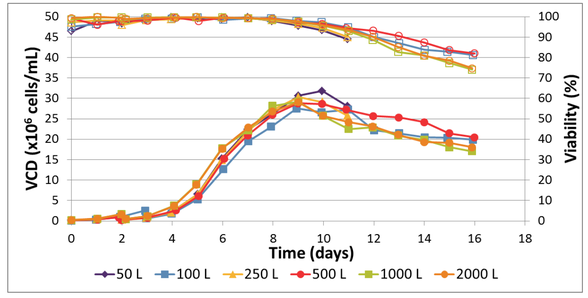


Figure 9. VCD and viability of 50 L and 250 L S.U.B.s operating at 20% and 100% volumes

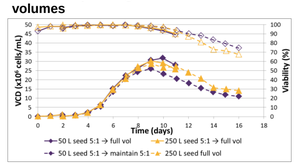
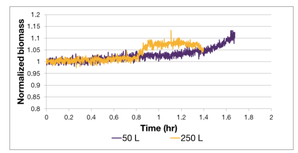


Figure 10. Mixing efficiencies of 50 L and 250 L 5:1 S.U.B.s during drainage



Harvest Mixing Efficiency

Following the 50 L and 250 L HyPerforma 5:1 runs, cell mass was measured during harvest to determine homogeneity of the culture throughout drainage (Figure 10). For the 50 L culture, mixing speed was constant during drainage until working volume reached 20%, whereupon agitation was disabled. For the 250 L culture, mixing speed was decreased when the vessel reached 50% working volume to reflect a reduction in power required to maintain 20 W/m^3. Agitation was again disabled in the 250 L vessel when the volume dropped to 20%. Both tests indicate only a 10% increase in cell mass over a 1.4- to 1.7-hour harvest. Despite the drop in agitation in the 250 L vessel, only a slight increase in cell mass at the bottom of the S.U.B. was measured.

CONCLUSIONS

The HyPerforma 5:1 S.U.B. provides a robust solution to customers seeking to improve their workflow through:

- Use of the new CFS, demonstrated and scalable method to remove CO_2 buildup both in the headspace and in the liquid culture fluid
- Improved utilization of floor space with:
  - Fewer vessels required for a single seed train
  - Concurrent cell runs possible in parallel vessels
- Significant reduction in required liquid transfers and sterile line connections
- Fewer required vessels, fewer BioProcess Container sizes, and more standardized parts
- Homogeneous mixing throughout drainage during harvest and scale-up
- Reduced overall process duration and cost of goods

REFERENCES

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TRADEMARKS