Efficient operation of the HyPerforma 5:1 Single-Use Bioreactor at low working volume

Introduction

The Thermo Scientific™ HyPerforma™ 5:1 Single-Use Bioreactor (S.U.B.) was developed in response to end-user interest in operating S.U.B.s at lower working volumes to realize improved seed-train utilization and mixing during harvest. These performance enhancements are available to customers on new systems, or as a retrofit kit for water-jacketed HyPerforma S.U.B.s. The changes allow customers to operate the bioreactor with as low as 20% working volume in 50 L—2,000 L vessels while maintaining sufficient O_2 and CO_2 mass transfer to attain high cell culture densities. The design improvements include the new cross-flow sparger (CFS), which reduces the buildup of CO_2 in the headspace during operation at low working volumes.

Testing objective

Design improvements were tested to evaluate the ability of the HyPerforma 5:1 S.U.B. to support operating conditions for optimal cell culture. Results were confirmed in fed-batch cell runs, which showed no loss in cell culture performance and homogeneous harvest during the drainage process.

S.U.B. design

To operate standard 50—2,000 L HyPerforma S.U.B.s at lower working volumes, the following design changes were made.

- **5:1 S.U.B. kits designed for 50 L—500 L systems:**
  - Color-coded agitator drive shaft (6 cm to 16 cm).
  - Agitator motor mounting block (angle decreased from 19.6° to 16.5°)
  - S.U.B. and Thermo Scientific™ BioProcess Container (BPC) designs to allow for longer shaft and additional port locations

- **5:1 S.U.B. kits designed for 1,000 L and 2,000 L systems:**
  - Use standard agitator shaft
  - Agitator motor mounting assembly (pneumatic-style position adjustment with handheld remote control unit)
  - S.U.B. and BPC designs to allow for port locations
- Addition of port locations to keep the sensors immersed in 20% working volume
- Incorporation of bottom water jacket in all bioreactors for improved heat exchange
- Addition of CFS for use at low working volumes
- Removal of microsparger (frit) in all vessels

ThermoFisher SCIENTIFIC

Figure 1 shows the illustration of motor and impeller positions when operating at 5:1 volume and at full volume in the 2,000 L S.U.B. The 1,000 L S.U.B. has a similar setup with a movable motor and drive shaft, while smaller S.U.B.s use a rigid motor block. The cross-flow sparger (CFS) and drilled-hole sparger (DHS) are noted to show relative positions with respect to vessel working volume and impeller position. The CFS is used at 5:1 volume to assist in mass transfer, while a standard overlay at the top of the BPC is used at full volume. The figure also highlights the conserved vertical position of the impeller with respect to the DHS when operating in either configuration.

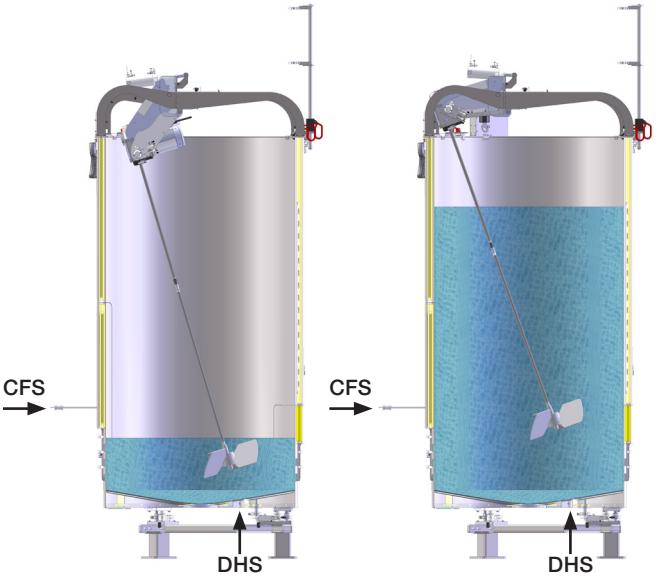

Figure 1. Illustration of the 2,000 L HyPerforma 5:1 S.U.B. Operating positions for 400 L using 5:1 mode (left) and 2,000 L using the standard mode (right) are shown. Note that at full volume, the standard overlay is used in place of the CFS.

Materials and methods

Mixing studies

Mixing studies were performed by adding a 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 with the 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 to 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 1,000 L and <95 rpm for the 2,000 L).

Mass transfer studies

Mass transfer studies were performed at 5:1 volume and at full volume using the standard dynamic method [1], where the transfer rate of oxygen from the gas to liquid phase is represented by:

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

Where $k_L a$ 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 O_2 (air) transferring into an N_2-saturated solution. Carbon dioxide out of solution was measured as the $k_L a$ of O_2 (air) transferring into a CO_2-saturated solution.

The following conditions were tested:

- Full volume and at 5:1 volume
  - DHS air flow rates up to 0.1 vessel volume per minute (VVM)
  - Headspace sparging using either standard overlay (for full volume) or CFS (for 5:1 volume), targeting 50–70 L/m^2 liquid surface area per minute
- 40 W/m^3 mixing (n_P = 2.1)
- Test solution:
  - 1 g/L poloxamer 188
  - 3.5 g/L HEPES buffer
  - Adjusted to pH 7.25 at air saturation

Cell culture testing

The following materials and operating conditions (Table 1) were used in our test procedures:

- 50 L–2,000 L S.U.B.s
- Gibco™ Freedom™ CHO-S™ Cells, mAb producer
- Gibco™ Dynamis™ Advanced Granulation Technology™ (AGT™) Medium with 0.1% Gibco™ Anti-Clumping Agent
- Feeds:
  - Gibco™ EfficientFeed™ C+ AGT™ Supplement
    - 2X concentration
    - 15% constant feed over 8 culture days
  - 45% glucose constant feed as needed (maintain <5 g/L in culture)

Table 1. HyPerforma 5:1 S.U.B. operating conditions.

Parameter	Condition
Power input to volume (agitation)	20 W/m^3 Note: Assumes N_P = 2.1, 20 W/m^3 recommended at all scales; 40 W/m^3 may be desirable when above 80% working volume
Sparging	• Single DHS • Gassing: O_2 as primary; N_2 and CO_2 as needed
Air flow	Cross-flow and overlay sparging at 50–70 L/m^2 surface area per minute
Feed strategy	• Day 0: seed at 20% working volume • Day 2–3: feed to 85% working volume • Day 5–12: continue standard feed
Variable pH control	• Day 0–5: variable pH 7.2 to 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

Harvest mixing efficiency studies

Following the 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 to determine solution homogeneity during the drainage process.

Results

Mixing studies

Figures 2 and 3 illustrate the T95 mixing times for each vessel at various power inputs. At 5:1 volume, mixing times for all vessels were less than 1 minute, with similar mixing times for smaller sizes (50 L to 250 L), and slightly higher mixing times for the larger sizes (500 L to 2,000 L). Mixing times at full volume were greater than those in the 5:1 volume mode, but less than 1 minute for all vessel sizes.

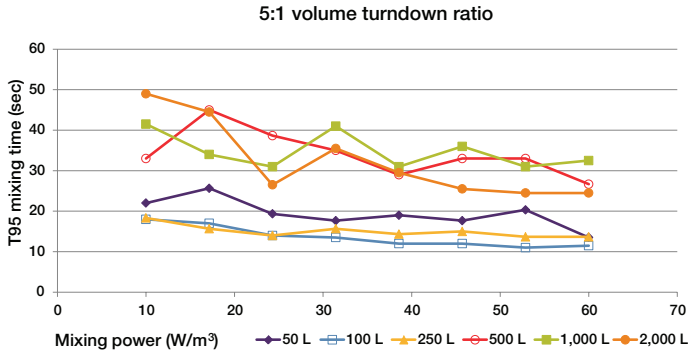

Figure 2. T95 mixing times for all 5:1 S.U.B.s at 5:1 volume.

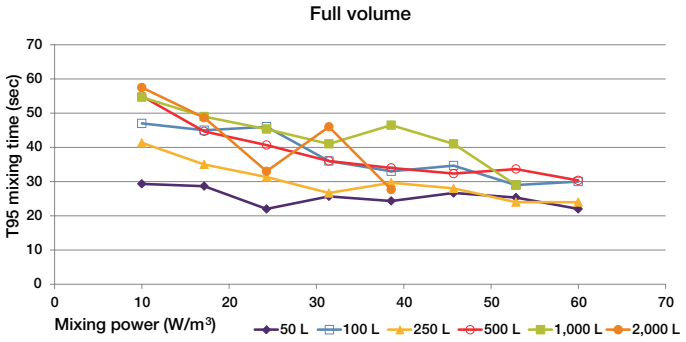

Figure 3. T95 mixing times for all 5:1 S.U.B.s at full volume.

Mass transfer studies

Figure 4 displays the O_2 mass transfer results for the 250 L S.U.B. at 5:1 volume, at 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 k_La across flow rates when using either the CFS or the overlay sparger, when compared to adding no headspace gas. No difference was seen between the CFS and the standard overlay sparger. Figure 5 illustrates the carbon dioxide (CO_2) mass transfer results for the 250 L S.U.B. at 5:1 volume, at 40 W/m^3, and various DHS air flow rates up to the rated limit of 0.1 VVM. Whereas O_2 mass transfer was comparable using either the CFS or the overlay sparger, these results show a substantial increase in CO_2 removal when using the CFS. Other vessel sizes demonstrated similar trends. Figures 6 through 9 illustrate the results of mass transfer tests in all vessel sizes at 5:1 volume (Figures 6 and 7) and at full volume (Figures 8 and 9). The data show good scalability of systems for both O_2 mass transfer into and CO_2 transfer out of the bioreactors. While CO_2 k_La was nearly identical among all vessel sizes at both 5:1 and full volumes, O_2 k_La showed proportional increases with increasing vessel size. The ratio of CO_2 to O_2 k_La, as an indicator of bubble mass transfer efficiency, ranged from 0.8–0.9 for the 50 L S.U.B. to 0.4–0.6 for the 2,000 L S.U.B.

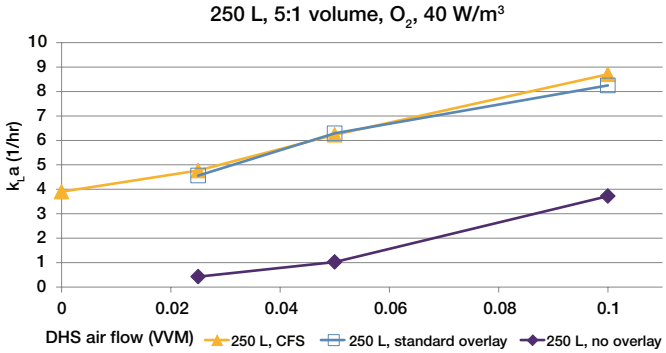

Figure 4. O_2 mass transfer results for the 250 L S.U.B. at 5:1 volume.

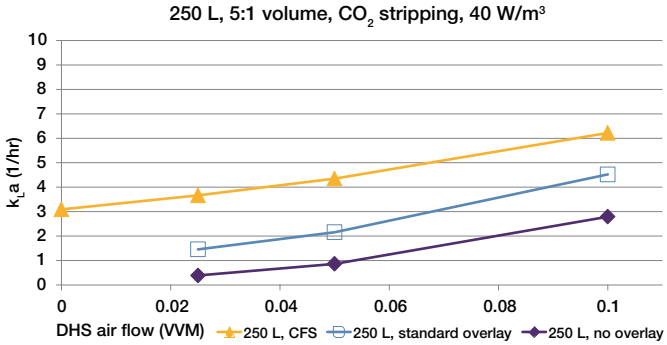

Figure 5. CO_2 mass transfer results for the 250 L S.U.B. at 5:1 volume.

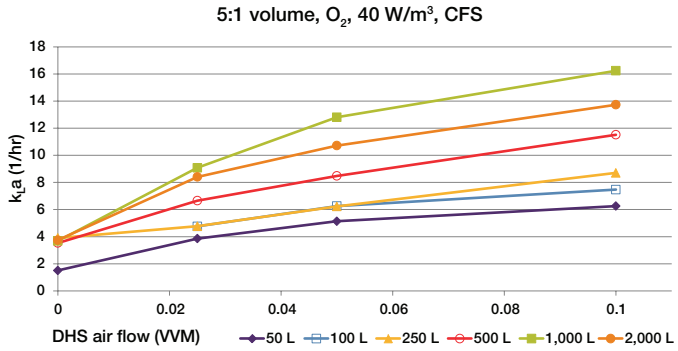

Figure 6. O_2 mass transfer results for all 5:1 S.U.B.s at 5:1 volume.

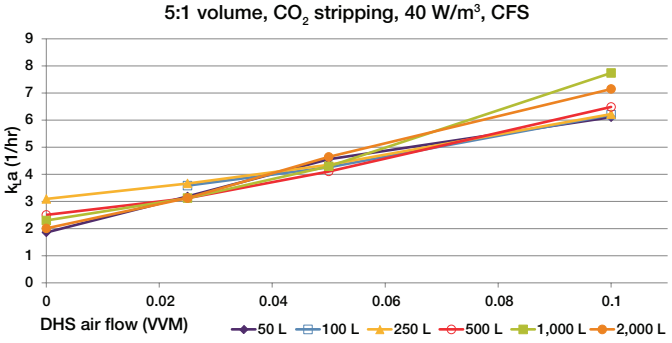

Figure 7. CO_2 mass transfer results for all 5:1 S.U.B.s at 5:1 volume.

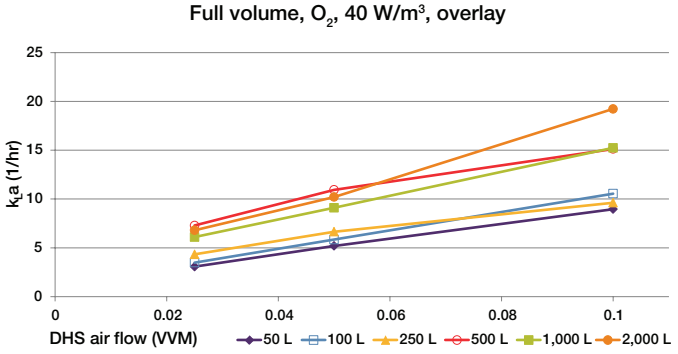

Figure 8. O_2 mass transfer results for all 5:1 S.U.B.s at full volume.

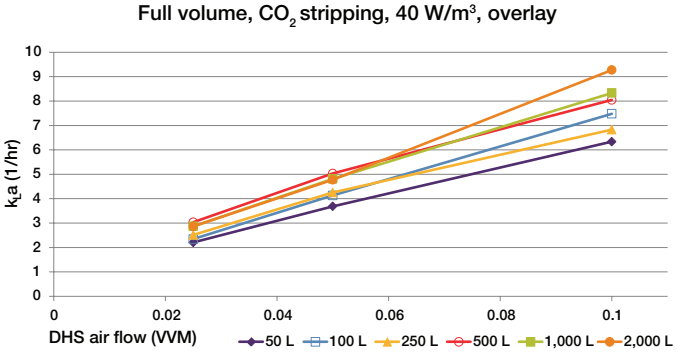

Figure 9. CO_2 mass transfer results for all 5:1 S.U.B.s at full volume.

Cell culture testing

Cell culture was performed in each vessel, starting at 20% working volume. Depending on viable cell density (VCD), 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 (Figures 10 and 11), with nearly identical growth profiles including growth rates and peak cell densities.

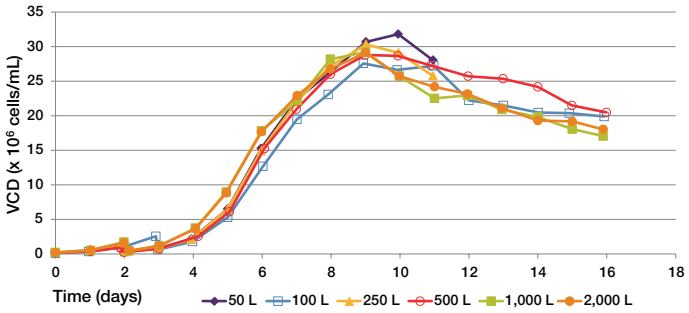

Figure 10. VCD results for the 5:1 S.U.B.s.

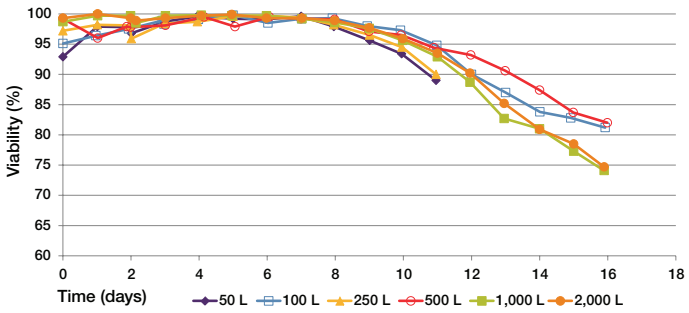

Figure 11. Cell viability results for the 5:1 S.U.B.s.

Figure 12 compares results of the 50 L and 250 L HyPerforma 5:1 S.U.B.s against both a standard 250 L HyPerforma S.U.B. at full volume and a 50 L HyPerforma 5:1 S.U.B. run entirely at 5:1 volume. Results show equivalent VCD and cell viability among all vessels and culture strategies.

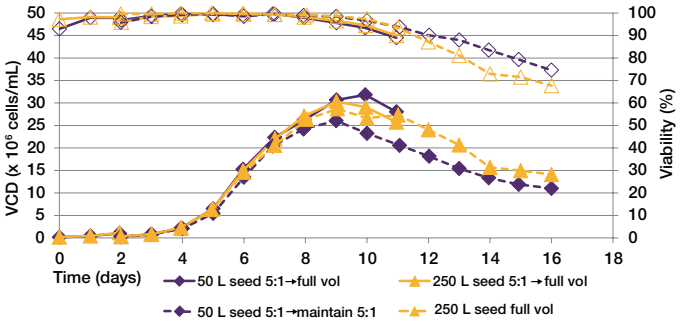

Figure 12. VCD (lower plot lines) and viability (upper plot lines) of 50 L and 250 L HyPerforma 5:1 S.U.B.s operating at 20% to 100% working volumes.

Harvest mixing efficiency studies

Following the 50 L and 250 L HyPerforma 5:1 S.U.B. runs, cell mass was measured during harvest to determine homogeneity of the culture throughout drainage (Figure 13). For the 50 L culture, mixing speed was constant during drainage until working volume reached 20%, at which point 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 working volume dropped to 20%. Both tests indicate only a 10% increase in cell mass over a 1.4—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.

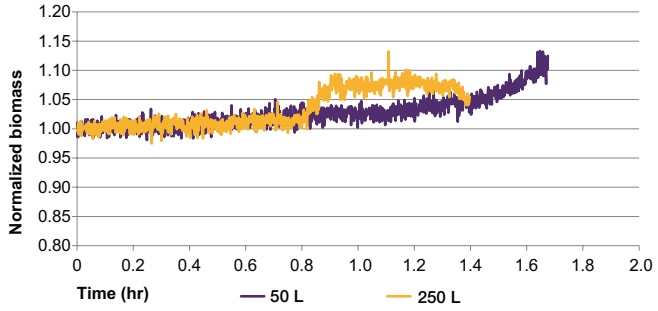

Figure 13. Mixing efficiency of 50 L and 250 L 5:1 S.U.B.s during drainage.

Discussion

High levels of dissolved CO_2 (dCO_2) in liquid cultures have been shown to inhibit cell growth and protein production [3,4]. When S.U.B.s operate 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 4 shows the ability of either the standard overlay or the CFS to create equal O_2 mass transfer into the system, while Figure 5 shows the benefit of proper gas mixing at the liquid-air interface when operating with gases 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.

Working in tandem with the CFS, the DHS provides O_2 mass transfer from 6 to 16 hr^{-1} at 5:1 volume and 9 to 19 hr^{-1} at full volume; and CO_2 mass transfer up to 6 to 8 hr^{-1} at 5:1 volume and 6 to 9 hr^{-1} at full volume. While the O_2 k_La increased with increasing vessel size at scaled gas flow rates, CO_2 mass transfer was generally maintained across vessel sizes. While oxygen mass transfer was generally less than that obtained using a typical microsparger, substituting air for oxygen in the process actually allows the user to tune gas concentrations to obtain more ideal cell growth conditions. Additionally, the DHS is a gentler sparger, with more predictable mass transfer across processes and vessel sizes.

It is important to note that while CO_2 k_La values were lower than O_2 k_La, relative gas partial pressure and gas solubility must be considered in assessing performance. While the ratio of dissolved CO_2 to O_2 is near 14 to 1 in a typical mammalian cell culture (30% dissolved oxygen), the partial pressure difference (i.e., mass transfer driving force) of gas in solution compared to the concentration in the sparged gas is in favor of O_2 mass transfer (0.06 atm partial pressure for CO_2, and 0.15 or 0.94 atm for O_2, using either air or O_2 gas, respectively). This means O_2 is readily added to solution, whereas CO_2 is more difficult to remove from solution despite the higher quantity of CO_2 molecules.

The balance of CO_2 and O_2 k_La and created by the DHS and CFS in tandem, results in ideal sparge conditions for consistent cell growth, as seen in cell culture processes.

Cell culture results indicate similar growth conditions among vessels, as shown by similar growth rates and peak cell densities. Importantly, similar growth rates are seen both at 5:1 volume and full volume, indicating no loss in culture performance despite the lower liquid volume. The data in Figure 12 indicate that high cell densities are achievable in the S.U.B.s at 5:1 volume, allowing the option to remove specific vessels from seed trains or process development workflows.

As previously discussed, the ratio of O_2 delivery to CO_2 stripping is seen in the cell culture data. When using O_2 as the primary gas through the DHS and air through the CFS, O_2 transfer to the culture was sufficient to support cell densities up to 3.2×10^7 cells/mL. The gassing strategy implemented in the culture provided enough CO_2 stripping to prevent the pH from dropping too low during culture (pH readings near 6.8 at peak lactate production) while maintaining dissolved CO_2 levels within the typical operating range.

Mixing studies have shown good liquid-to-liquid mixing times in all vessel sizes at both tested operating volumes. Similarly, mixing studies during harvest showed a homogeneous cell suspension through complete fluid drain. These results offer confidence in delivering a consistent cell culture fluid either when harvesting with a downstream system (centrifuge or filter) or when providing seed cultures to one or more larger production reactors.

thermo scientific

Conclusions

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

- Use of the new CFS to remove CO_2 buildup both in the headspace and in solution
- More optimal use of the DHS as a stand-alone sparger to obtain sufficient O_2 and CO_2 mass transfer for even demanding cell cultures
- Improved utilization of floor space with:
  - Fewer vessels required for a single seed train
  - Concurrent cell runs possible in parallel vessels
- Fewer solution transfers and sterile line connections
- Fewer required vessels, fewer BPC sizes, and more standardized parts
- Homogeneous mixing throughout drainage during harvest and scale-up

References

1. Doran PM (2013) "Mass Transfer" in *Bioprocess Engineering Principles*, 2nd ed., 416–25. Waltham, MA: Elsevier.
2. Davey CL, Davey HM, Kel DB et al. (1993) Introduction to the dielectric estimation of cellular biomass in real time, with special emphasis on measurements at high volume fractions. *Anal Chim Acta* 279:155–161.
3. Zhu MM et al. (2005) Effects of elevated pCO_2 and osmolality on growth of CHO cells and production of antibody-fusion protein B1: a case study. *Biotechnology Progress* 21:70–77.
4. deZengotita VM, Kimura R, Miller WM (1998) Effects of CO_2 and osmolality on hybridoma cells: growth, metabolism and monoclonal antibody production. *Cytotechnology* 28:213–227.

Find out more at **thermofisher.com/sub**

ThermoFisher SCIENTIFIC