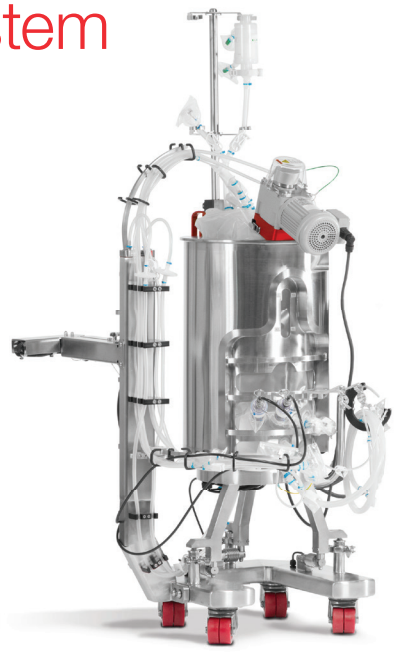
# High-density 50 L perfusion cell culture using the XCell ATF 6 Single-Use System



## Introduction

Improvements in single-use systems have allowed implementation of high-density cultures in standard workflows. This study shows the integration of the Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) and the Repligen XCell ATF™ 6 Single-Use System to achieve a stable, high-density perfusion cell culture, at 40 x $10^6$ cells/mL over 20 days, without modifying the standard systems. The data show that an integrated HyPerforma S.U.B. and XCell ATF 6 system can be used in a high-density seed train or as a production vessel system.

### Goal

In this study, the performance of the 50 L HyPerforma S.U.B. was studied with respect to achieving and maintaining a high-density perfusion cell culture. Further scale-up suggestions were also studied and are presented here.

### Materials

- 50 L HyPerforma S.U.B. integrated with XCell ATF 6 system
- CHO DP-12 cells (ATCC, Cat. No. CRL-12445) adapted to LONG™ $R^3$ IGF-1
- Cells cultured in Gibco™ CD OptiCHO™ AGT™ Medium with 100 ng/mL LONG $R^3$ IGF-1 and 4 mM Gibco™ GlutaMAX™ Supplement

### Methods

Cells were seeded in the HyPerforma S.U.B. at 0.4 x $10^6$ cells/mL in a 40 L working volume. Operating conditions for the HyPerforma S.U.B. are listed in Table 1. Operating conditions for the XCell ATF 6 system are listed in Table 2. Cell counts, nutrients, metabolites, electrolytes, and protein yields were measured offline.

**Table 1. 50 L HyPerforma S.U.B. operating conditions.**

| Parameter | Condition |
|---|---|
| Working volume | 40 L |
| Temperature | 37°C |
| pH | 6.8–7.2 (no base) |
| Agitation | 172 rpm (20 W/$m^3$) |
| Dissolved oxygen set point | 30% |
| Dissolved oxygen cascade | Oxygen through standard drilled-hole sparger |
| Headspace sparge | 3 L/min air |
| Antifoam | 6.5 ppm/day; automated using Thermo Scientific™ foam probe |

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**Table 2. XCell ATF 6 system operating conditions.**

| Parameter | Condition |
|---|---|
| Alternating tangential flow (ATF) filter | ATF F6:RF02PES 0.2 µm PES hollow fiber |
| Filter surface area | 2.53 $m^2$ |
| Perfusion rate | Day 3–5: 1 VVD (40 L/day)<br>Day 5–20: 2 VVD (80 L/day) |
| Flux | 1.67 LMH |
| ATF rate | 17.2 L/min |
| Shear rate | 2,028 $s^{-1}$ |
| Cell bleed | Constant, based on cell growth rate |
| Target cell density | 40 x $10^6$ cells/mL |

## Results

A viable cell density (VCD) of 40 x $10^6$ cells/mL with high viability (>96.8%) was achieved and maintained for 13 days during the cell culture run (Figure 1). Perfusion was initiated on day 3 at 1 VVD (volume of media per bioreactor volume per day) and increased to 2 VVD on day 5. A constant cell bleed was started on day 6 based on the target cell density and calculated growth rates, according to the following equation:

$$F_b = [\mu - (\ln X_2 - \ln X_1)]V_R$$

where $F_b$ is the bleed flow rate, µ is the average growth rate for the previous 3 days, X is the cell density, and $V_R$ is the bioreactor volume.

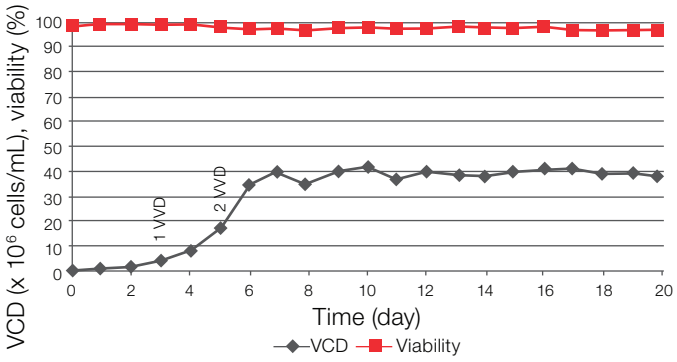


**Figure 1. Viable cell density and viability for the 50 L HyPerforma S.U.B. cell culture run.**

Proteins (IgG and LDH) were measured in both the bioreactor and the permeate (Figure 2). Lower IgG and LDH concentrations in the permeate stream were due to both steric hindrance to flow within the 0.2 µm membranes and minimal protein adsorption to the hollow-fiber membranes. The cumulative total protein compared to the integral of viable cell count (IVCC) displayed in Figure 3 shows constant protein production throughout the cell culture run.

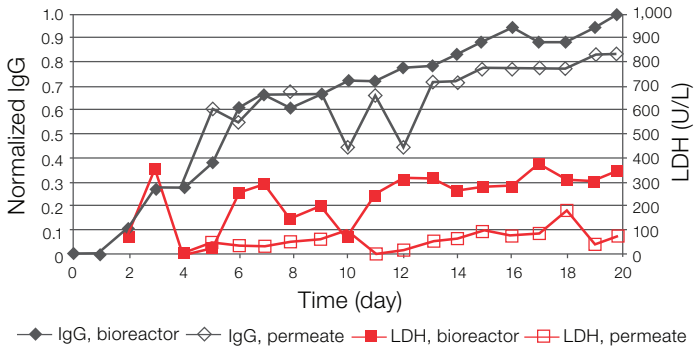


**Figure 2. Normalized IgG and LDH in the bioreactor and permeate stream of the XCell ATF 6 system for the 50 L HyPerforma S.U.B. cell culture run.**

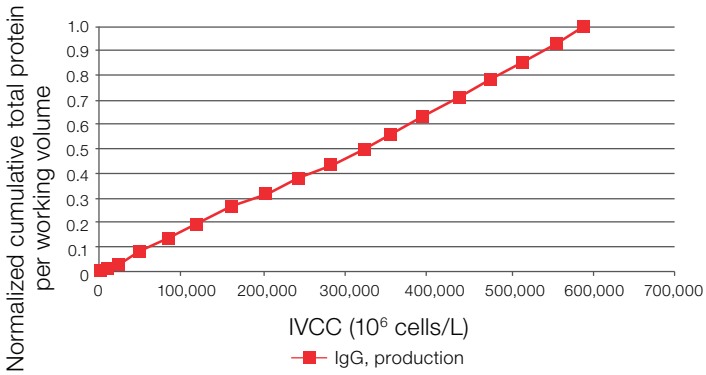


**Figure 3. Normalized cumulative total protein per working volume versus the integral of the viable cell count (IVCC) per working volume for the cell culture run.**

Oxygen, nitrogen, and carbon dioxide flow were data-logged by the controller for the cell culture run and plotted in Figure 4 (nonsteady oxygen flow from day 5 to day 9 was due to oxygen bubbles adhering to the dissolved oxygen sensor). Oxygen demand was low compared to the maximum rated flow rate for the drilled-hole sparger (DHS) (0.1 VVM, 5 L/min), resulting in gentle gas flow within the system. Constant dissolved $CO_2$ ($dCO_2$) levels (Figure 5) during steady-state cell density along with constant pH (Figure 5) and near-constant oxygen flow rates (Figure 4) showed a good balance of oxygen to carbon dioxide mass transfer while using oxygen as the primary gas through the DHS.

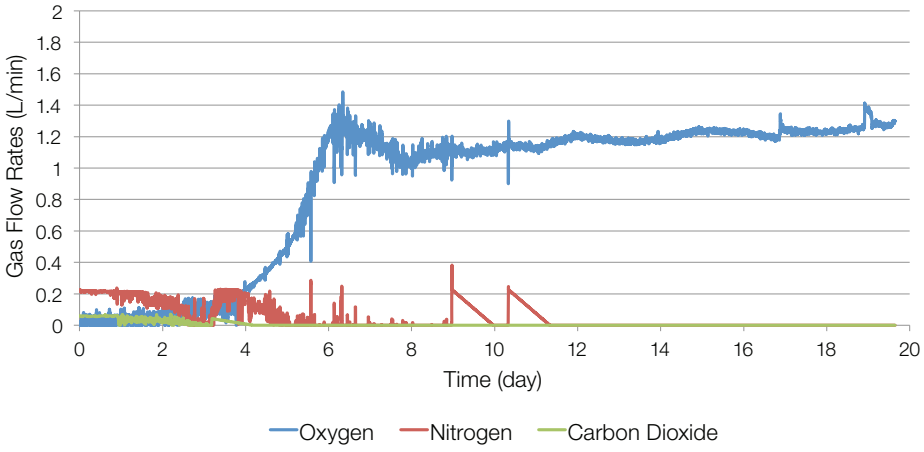


**Figure 4. Drilled-hole sparger gas flow rates recorded by the controller.**

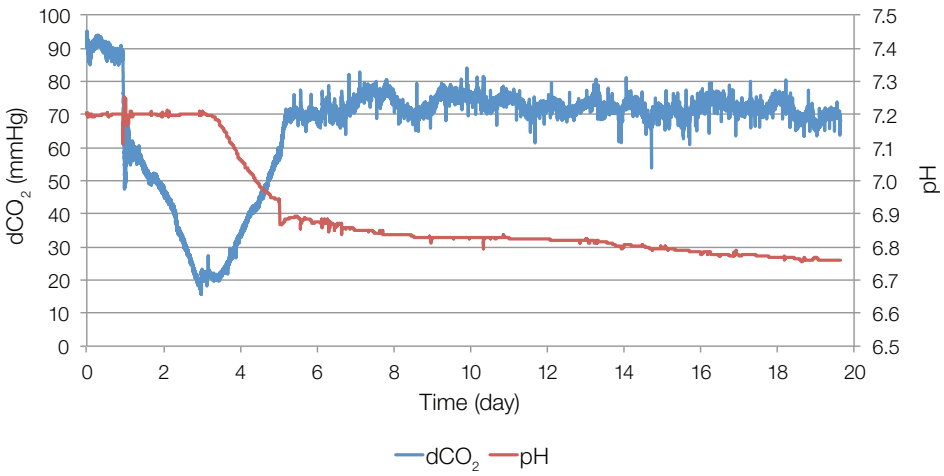


**Figure 5. Online $dCO_2$ concentration and pH for the 50 L S.U.B. cell culture run.**

## Discussion

The data show stability of the S.U.B. system through a full 20-day cell culture run, which included constant gas flow rates to maintain dissolved oxygen at the desired set point. Additionally, cell density was maintained in the bioreactor at the desired set point by utilizing the integrated load cell value and calculated growth rates.

A primary concern for scale-up of S.U.B.s is sparging performance and gassing requirements. This study showed that high-density cultures are easily sustained by the 50 L HyPerforma S.U.B. while maintaining oxygen gas flow rates well below the maximum recommended sparging rate of 0.1 VVM for the DHS. Based on this performance, it is expected that the HyPerforma S.U.B. theoretically can maintain cell densities greater than 100 x $10^6$ cells/mL. Previously generated data (see Thermo Scientific S.U.B. Validation Guide) have shown scalability from 50 to 2,000 L for both oxygen and carbon dioxide mass transfer.

The performance of the DHS is highlighted by a good balance of oxygen and carbon dioxide mass transfer into and out of the culture. Based on the constant oxygen flow rates, $dCO_2$ levels, and pH during steady-state cell density, oxygen mass transfer to the reactor is perfectly balanced with oxygen consumption, $CO_2$ respiration, and $CO_2$ stripping from solution. For a system that needs to be balanced with higher pH and lower $dCO_2$ levels, oxygen could be diluted with air or nitrogen to decrease oxygen mass transfer while maintaining a higher carbon dioxide stripping rate.

Scalability of the XCell ATF 6 system has already been proven through separate testing. The scalability of HyPerforma S.U.B. systems along with appropriate connections between the XCell ATF and HyPerforma S.U.B. systems offer confidence when working at larger scales.

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## Conclusion

Perfusion cell culture using the 50 L HyPerforma S.U.B. and the XCell ATF 6 system demonstrated a stable culture for 13 days of a 20-day culture. Growth limits in the study were due to nutrient limitations (glucose), as expected based on bench-scale data, and not due to bioreactor performance. During the steady-state culture, stable levels of gassing were used, protein production was constant, and cell bleed rates were highly predictable. These results demonstrate that the HyPerforma S.U.B. is capable of achieving cell culture at high density for extended periods of time. Based on performance and maximum gas flow rates, it is expected that very high cell densities are achievable depending on cell line stability, medium, and feed (>100 x $10^6$ cells/m).

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