# High-density culture strategies for improved scalability

## For single-use systems

### Introduction

High-density culture (>40 x $10^6$ cells/mL) strategies reduce processing time and include perfusion, concentrated fed-batch, and intensified seed trains—whereas an N-1 perfusion bioreactor is used to seed a production bioreactor. In this study, the integration of the Thermo Scientific™ HyPerforma™ Single-Use Bioreactor Turnkey (S.U.B. TK) and the perfusion XCell™ ATF 6 system was used to achieve high-density cultures, without modifications to standard components. Integration of the HyPerforma S.U.B. TK and the ATF 6 system is capable of being used for a high-density seed train or as a production vessel system. Scalability criteria were also generated using standard components.

### Goal

In this study, high-density cultures, theoretical scale-up, integration hurdles, and the effects of high-density cultures on growth and processing parameters were explored on the integrated bioreactors and perfusion ATF systems.

### Materials and methods

- 50 L S.U.B. TK integrated with the ATF 6 system
- 3 L Applikon benchtop bioreactor integrated with the ATF 2 system

- ATCC™ CRL-12445™ CHO-DP-12 clone #1934 cells adapted to LONG™ $R^3$IGF-1 Sigma growth factor
- Cultured in Gibco™ CD OptiCHO™ AGT™ medium with 100 ng/mL R3 IGF-1 and 4 mM Gibco™ GlutaMAX™ supplements
- Cells were seeded in the S.U.B. at 4 x $10^5$ cell/mL
- Operating conditions for the S.U.B. are listed in Table 1
- Operating conditions for the ATF 6 system are listed in Table 2

**Table 1. Bioreactor operating conditions.**

| Parameter | Setting conditions | |
|---|---|---|
| | Thermo Fisher | Repligen |
| Working volume | 40 L | 1.5 L |
| Temperature | 37°C | 37°C |
| pH | 7.0 ($CO_2$ / 1 N NaOH) | 6.8 ($CO_2$ / 0.5 N NaOH) |
| Agitation | 172 RPM | 200 RPM |
| DO setpoint | 50% | 40% |
| DO cascade | Oxygen through standard drilled-hole sparge | Oxygen through standard micro sparge |
| Air sparge | 3 L/min air, headspace sweep | 250 mL/min air, through drilled hole sparge |
| Antifoam<br>Based on visual observation | 20 ppm, daily | 4 ppm, hourly (daily equivalent is 96 ppm) |

**Table 2. ATF conditions.**

| Parameter | Setting conditions | |
|---|---|---|
| | Thermo Fisher | Repligen |
| ATF System | ATF 6 system | ATF 2 system |
| ATF filter | F6:RF02PES 0.2 µm PES hollow fiber | F2:RF02PES 0.2 µm PES hollow fiber |
| Filter surface area | 2.53 $m^3$ | 0.13 $m^3$ |
| Perfusion rate | • Day 0–3: None<br>• Day 3–4: 1 BRV (40 L/day)<br>• Day 4–5: 1.5 BRV (60 L/day)<br>• Day 5–end: 2 BRV (80 L/day) | • Day 0–3: None<br>• Day 3–5: 1 BRV (1.5 L/day)<br>• Day 5–end: 2 BRV (3 L/day) |
| Permeate flux | 1.67 LMH | 1.67 LMH |
| ATF rate | 12 LPM | 1 LPM |
| Shear rate | 1,415 $s^{-1}$ | 2,264 $s^{-1}$ |
| Cell bleed | Constant | Daily |
| Target cell density | 4 x $10^6$ cells/mL | 4 x $10^6$ cells/mL |

## Results

### Cell growth

Viable cell density of >4 x $10^6$ cells/mL was achieved in all cell runs with similar growth rates and viabilities (Figure 1). Viability stayed above 88% for both 50 L runs while viability dropped to near 80% in the benchtop culture by day 11. Cell bleed for the 50 L run was constant based on growth rates and target cell density while pulsed bleeds were performed on the 3 L bioreactor.

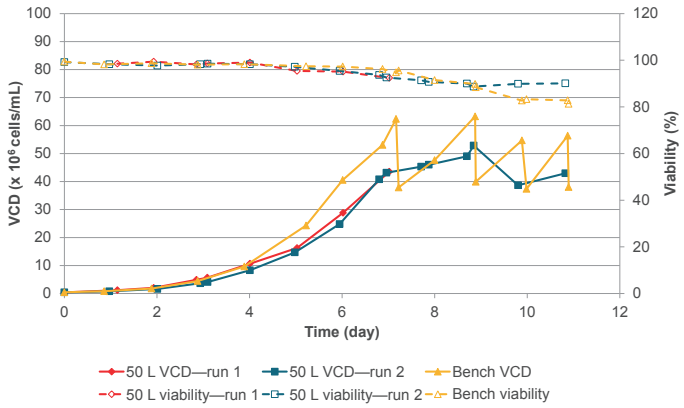


**Figure 1. Viability and viable cell density for the 50 L and 3 L bioreactors.**

### Protein production

Protein concentrations were similar between the 50 L and the 3 L bioreactors and in the ATF systems (Figure 2). Lower IgG concentrations in the ATF system permeate stream are due to both steric hindrance to flow within the 0.2 µm membranes and minimal protein adsorption to the hollow fiber membranes.

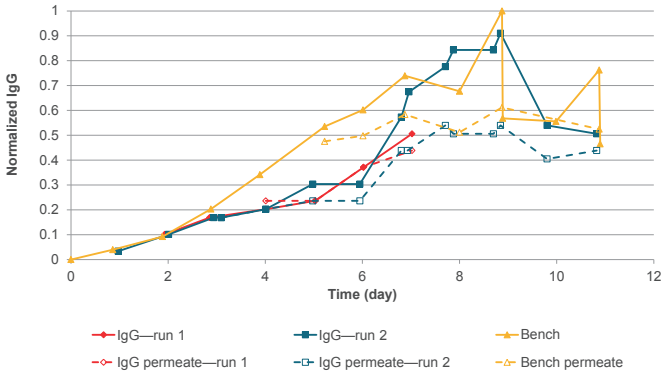


**Figure 2. IgG production in 50 L and 3 L bioreactors.**

Normalized cumulative protein per liter working volume versus integral of viable cell count per liter (IVCC/L) among the systems display nearly identical slopes, verifying similar protein production rates (Figure 3).

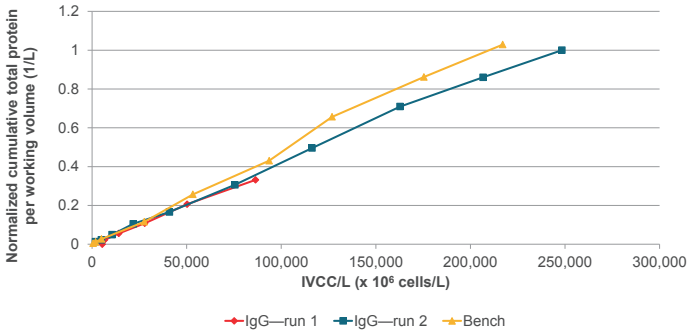


**Figure 3. Total cumulative protein versus IVCC among 50 L and 3 L bioreactors.**

## Oxygen flow

Oxygen flow was data-logged by the controller for the two 50 L cell runs and plotted with viable cell densities (VCD) (Figure 4). Oxygen demand between bioreactors was nearly identical with a definite correlation between oxygen demand and viable cell density. Previous testing has shown a linear relationship between the sparging, by the Thermo Scientific™ drilled-hole Sparger (DHS), and the volumetric mass transfer coefficient ($k_La$). Sparging also stayed well within the recommended 0.1 VVM gas flow rate for the 50 L bioreactor.

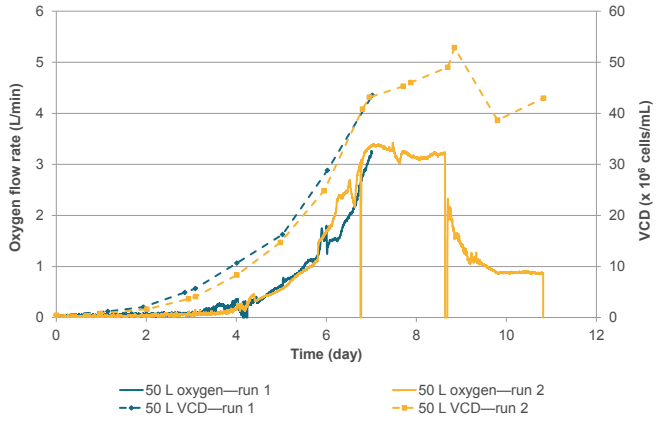


**Figure 4. Oxygen flow rates and viable cell densities (VCD) between 50 L and 3 L bioreactors.**

# Discussion

## System integration

The AseptiQuik™ X connector provided simple and fast connection between the ATF 6 system and the S.U.B. post-autoclave. The S.U.B. is equipped with an inlet port for the ATF system on the lower probe belt that allowed the AseptiQuik X connector to be fed through the hardware (Figure 5). No leaking was observed through the two 50 L runs while operating at low pressure and a slow flow rate (12 L/min).

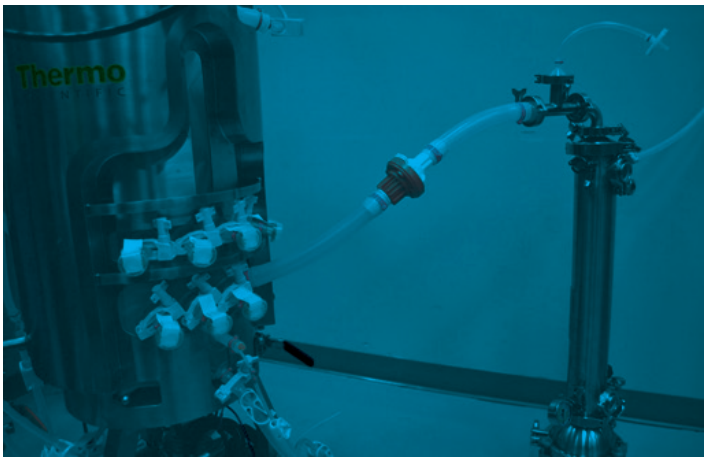


**Figure 5. 50 L Single-Use Bioreactor connected to an ATF 6 perfusion system with an AseptiQuik X connector.**

## Scale-up criteria

Scale-up criteria for the S.U.B. and ATF systems shown in Table 3 are based on constant shear, which has also been shown at smaller scales in separate tests [1].

System connection through a single 1-inch tube and AseptiQuik X connection leads to minimal pressure build-up due to flow up to the 1,000 L scale. To further reduce flow rates and pressure build-up, two parallel connections between the S.U.B. and the ATF system are required for the 2,000 L size and can be considered for use in the 50 L to 1,000 L sizes.

Pure oxygen sparging through the DHS is capable of achieving >60/hour $k_La$ in all S.U.B. sizes, which supports high cell-density culture up to 100 x $10^6$ cells/mL (data not shown) without the need for a separate microsparge.

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**Table 3. Scale-up criteria for S.U.B.** and ATF system at 1.5 VVD filtrate flow.

| | 50 L | 100 L | 200 L | 500 L | 1,000 L | 2,000 L |
|---|---|---|---|---|---|---|
| Working volume | 40 L | 100 L | 200 L | 500 L | 1,000 L | 2,000 L |
| ATF system | ATF 4 or 6 | ATF 6 | ATF 6 | ATF 10 | ATF 10 | ATF 10 (x2) |
| Surface area of theoretical filter ($m^2$) | 0.6 or 1.1 | 1.1 | 2.8 | 5.5 | 11 | 22 |
| Surface area of commercial filter ($m^2$) | 0.8 or 2.5 | 2.5 | 4.2 | 11 | 11 | 11 (x2) |
| ATF flow rate (L/min) | 8.4 or 14.9 | 14.9 | 25.1 | 68.4 | 68.4 | 68.4 |
| Shear ($s^{-1}$) | 2,021 | 2,021 | 2,021 | 2,021 | 2,021 | 2,021 |
| Permeate flux (LMH) | 2.5 or 1.25 | 2.5 | 3.0 | 2.84 | 5.7 | 5.7 |
| 1 in. Connection tubing Δp (psi) | <0.01 | <0.01 | 0.02 | 0.07 | 0.25 | 0.25 |
| DHS flow at 0.1 VVM (L/min) | 5 | 10 | 25 | 50 | 100 | 200 |
| $k_La$ ($hr^{-1}$) using $O_2$ gas | 58 | 59 | 60 | 68 | 67 | 76 |

## Conclusion

- Stable cell density of >40 x $10^6$ cells/mL achieved in both the 50 L S.U.B. and the 3 L bioreactors at 2 VVD flow rate
- Limited cell growth due to glucose consumption; cell growth independent of bioreactor and ATF system performance
- Identical protein production rates between the two 50 L cell runs, indicating equivalent performance
- Drilled-hole sparge in the 50 L S.U.B. capable of meeting oxygen demand as a stand-alone sparge for most high cell-density cultures
- System scale-up based on constant ATF shear and commercially available filters shown
- Relatively low antifoam requirement compared to bench-scale tests

### References


1. Sinani M, Erlandson T, Kudugunti S, Lin WR, Rusche J (2015) ATF perfusion technology: improved fed-batch throughput and reduced seed train expansion. Repligen.com. Retrieved May 18, 2016 from URL: https://www.repligen.com/download_file/view/315/426


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