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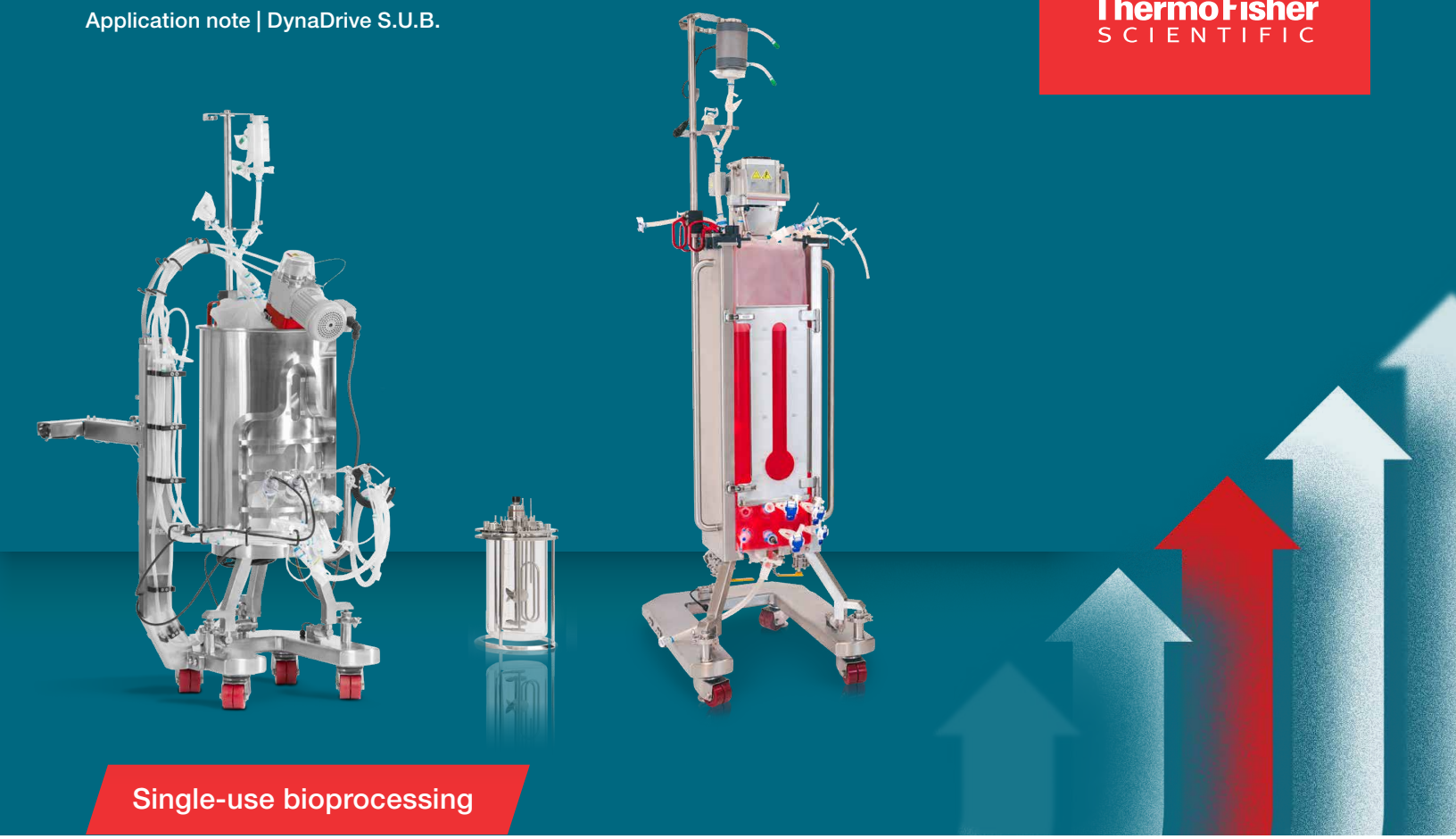

Single-use bioprocessing

Part 1: Intuitive bioprocess scale-up from bench scale to pilot scale

A comparative study of Thermo Scientific single-use bioreactors

Keywords
Single-use bioreactor, DynaDrive, CHO, fed-batch, scale-up, Efficient-Pro, bench scale, pilot scale

Introduction

The transition from bench scale to pilot scale is often viewed as a critical step in bioproduction process development, generally because of physical differences of the separate systems. The ability to generate scalable parameters is critical to enable confidence when progressing to clinical and production scales. An example of scaling up a CHO-based bioprocess from bench scale to pilot scale using the Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.) is outlined here, highlighting similarities and differences in control parameters and outcomes. In general, the culture carried out in the DynaDrive S.U.B. displayed key performance indicators that matched or exceeded those of the bench-scale bioreactor, including IgG titer, specific productivity, and cell density.

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Methods

IgG-producing CHO-K1 cells were thawed and propagated following standard procedures to establish a suitable seed train. The reactors used in this study included the 3 L Thermo Scientific™ HyPerforma™ Glass Bioreactor, the Thermo Scientific™ 5:1 HyPerforma™ S.U.B. (50 L), and the DynaDrive™ S.U.B. (50 L). Cell expansion was performed in a series of shake flasks until a sufficient number of cells were generated to inoculate the bioreactors. Control parameters used for the n-stage operation of each bioreactor are displayed in Tables 1–3.

Table 1. Operation and control parameters.

Parameter	3 L HyPerforma Glass Bioreactor	50 L HyPerforma S.U.B.	50 L DynaDrive S.U.B.
n-1 stage seed volume	—	10 L	5 L
Target initial/ final volume	1.7/2 L	36/50 L	36/50 L
Seed density ($x10^6$ cells/mL)	0.3	0.3	0.3
Temperature set point (°C)	37	37	37
Agitation (rpm)	350	183	105
Power input per volume (W/m^3)	100*	20	20
Tip speed (m/sec)	1.01	1.06	0.59
Impeller configuration	2 down-pumping pitched-blade impellers	1 down-pumping pitched-blade impeller	3 down-pumping pitched-blade impellers
Sparger configuration	Drilled pipe sparger 7 x 800 µm holes	Drilled-hole sparger (DHS) 360 x 178 µm pores	DHS 1,448 x 80 µm pores
Target glucose conc. (g/L)	3	3	3
Foam control			
High-threshold output	45	45	45
Foam alarm delay (sec)	60	60	60
Splash delay (sec)	5	5	5

* Approximate. Power number for the impeller configuration used has not been determined at time of publication.

Table 2. Dissolved oxygen (DO) control gassing strategy.

Parameter	3 L HyPerforma Glass Bioreactor	50 L HyPerforma S.U.B.	50 L DynaDrive S.U.B.
DO set point (%)	40	40	40
DO PID			
Gain	0.07	0.10	0.10
Reset	200	200	200
O_2			
Controller output	15 → 100%	15 → 100%	15 → 100%
MFC scaling	0 → 0.5 slpm	0 → 5 slpm	0 → 3 slpm
N_2			
Controller output	0 → 40%	0 → 30%	0 → 30%
MFC scaling	0.15 → 0 slpm	1 → 0 slpm	1 → 0 slpm
Air			
Overlay	0.2 slpm	5 slpm	5 slpm
Sparge controller output	—	—	15 → 30 → 45%
MFC scaling	—	—	0 → 0.5 → 0 slpm

Table 3. pH control strategy.

Parameter	3 L HyPerforma Glass Bioreactor	50 L HyPerforma S.U.B.	50 L DynaDrive S.U.B.
pH set point	7.15	7.15	7.15
pH PID			
Gain	0.04	0.04	0.04
Reset	200	200	200
pH deadband	Not enabled	Not enabled	Not enabled
CO_2			
Controller output	–100 → 0%	–100 → 0%	–100 → 0%
MFC scaling	0.08 → 0 slpm	2 → 0 slpm	2 → 0 slpm
Base	Not enabled	Not enabled	Not enabled

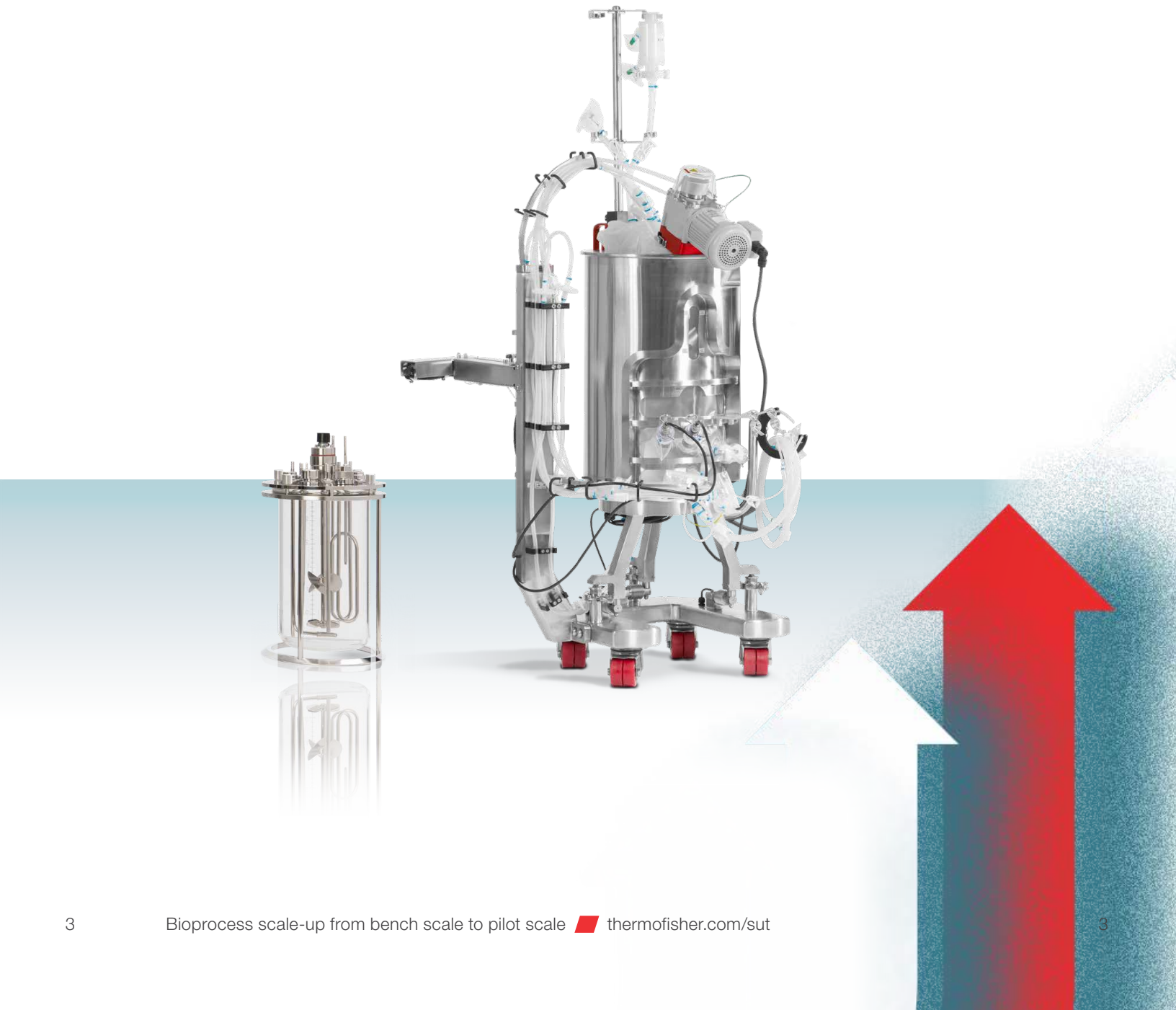
Although a power input per volume (P/V) of 20 W/m^3 was targeted for the 50 L S.U.B.s, the P/V was approximately 100 W/m^3 in the 3 L glass bioreactor culture. While the difference in P/V between scales is large, this is not a significant concern as P/V is frequently an impractical or unmeaningful scaling parameter at the benchtop scale. In this case, an agitation rate was selected for the 3 L glass bioreactor culture that provided for tip speed comparable to that of the 50 L HyPerforma S.U.B.

Gibco™ Efficient-Pro™ medium was used throughout the expansion and in the bioreactors for the n-stage production process. Gibco™ Efficient-Pro™ Feed 1 was used to supplement the production run cultures from day 3 onward, feeding 2.25% of the current vessel volume daily (see Equation 1). A 2 M glucose solution was also used to supplement the cultures, targeting a 3 g/L glucose concentration upon starting the fed-batch phase of the process. Gibco™ FoamAway™ Irradiated AOF Antifoaming Agent was used to control excess foam during operation via the use of the foam probe and pump triggered by Thermo Scientific™ TruBio™ automation software “Foam Hi Lim Out” as a remote set point for the pump.

Equation 1: Daily feed rate

$$\text{Feed rate}\left(\text{mL/min}\right) = (\text{Current volume (L)}) \left(\frac{1{,}000\ \text{mL}}{1\ \text{L}}\right)\left(\frac{0.0225}{\text{day}}\right)\left(\frac{1\ \text{day}}{1{,}440\ \text{min}}\right)$$

Reactors were sampled daily, and measurements were recorded for cell count, cell size, cell viability, metabolites, and protein titer.



Results

Culture growth was similar among all reactors, with peak viable cell densities (VCD) between 60.4 x 10^6 cells/mL and 72.0 x 10^6 cells/mL (Figure 1). Comparing cell viability, some discrepancies between scales were apparent (Figure 2). The 50 L HyPerforma and DynaDrive S.U.B. cultures had comparable cell viability profiles, with the viability of the 3 L glass bioreactor culture trending slightly higher throughout the latter half of the process. Protein production was also similar among the conditions tested, with peak protein titer reaching between 3.48 and 3.73 g/L (Figure 3). Productivity behavior is further elucidated by evaluating specific productivity (Q_P), which is shown in terms of pg/cell per day (Figure 4). Excluding a couple of minor deviations, the specific productivity lies within a similar range for each of the vessels during the fed-batch portion of the process, with the overall trend being similar across all conditions.

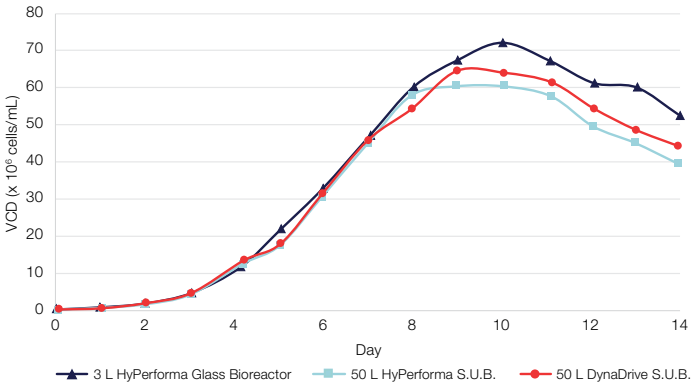


Figure 1. Viable cell density (VCD) profiles for CHO-K1 cells in the 3 L glass bioreactor, 50 L HyPerforma S.U.B., and 50 L DynaDrive S.U.B.

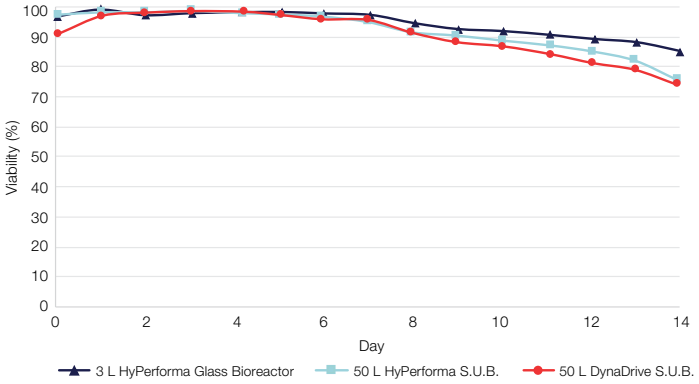


Figure 2. Viability profiles for CHO-K1 cells in the 3 L glass bioreactor, 50 L HyPerforma S.U.B., and 50 L DynaDrive S.U.B.

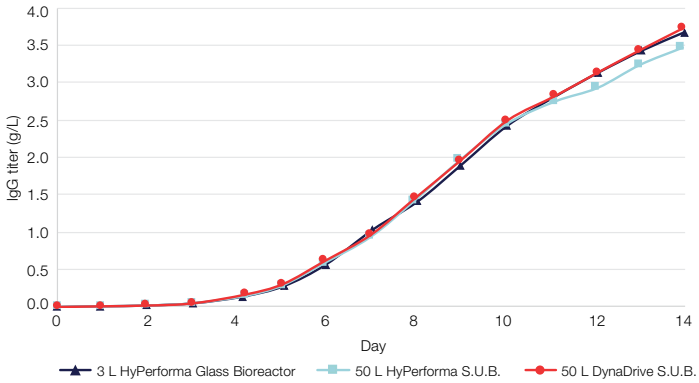


Figure 3. IgG titer profiles for CHO-K1 cells in the 3 L glass bioreactor, 50 L HyPerforma S.U.B., and 50 L DynaDrive S.U.B.

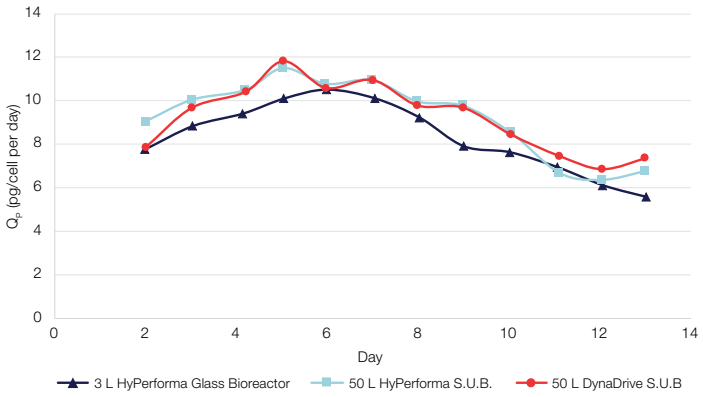


Figure 4. Specific productivity (Q_P, a 3-point moving average) profiles for CHO-K1 cells in the 3 L glass bioreactor, 50 L HyPerforma S.U.B., and 50 L DynaDrive S.U.B. Q_P is expressed in terms of picograms IgG per cell per day.

The lactate concentration in each reactor increased over the first few days, with a primary peak on day 4, after which the concentration dropped for 4–6 days before increasing again until process termination (Figure 5). While the lactate concentration in the 3 L culture was lower than that of both 50 L cultures during the primary peak, the lactate accumulation rate was higher in the vessel from day 10 and beyond, resulting in a higher final concentration. The relatively larger increase in lactate concentration in the 3 L glass bioreactor culture coincided with a drop in pCO_2 near the end of the process, falling as low as ~25 mmHg, whereas pCO_2 in both 50 L cultures was maintained at 40–80 mmHg from day 3 onward.

Scaled total gas flow rates (vessel volume per minute, VVM) show a lower O_2 sparge rate in the glass reactor and the DynaDrive S.U.B. compared to the HyPerforma S.U.B. (Figure 6). Nitrogen flows are also shown in the figure, and any sparged air flow rates were treated as 21% O_2 and 78% N_2. A direct comparison of the gassing requirements of the glass bioreactor and S.U.B.s is somewhat difficult due to the relatively high P/V in the glass reactor and varying rates of sparged N_2 in each condition. Even so, it can be seen from the similarity in scaled flow rates that the 50 L DynaDrive S.U.B. serves as a straightforward option when considering scalability from bench to pilot scale. Inspection of the CO_2 sparge rates (Figure 7) and referring to Figure 5 show that acceptable pCO_2 concentrations were maintained in the 50 L cultures even while the CO_2 flow rate was insignificant.

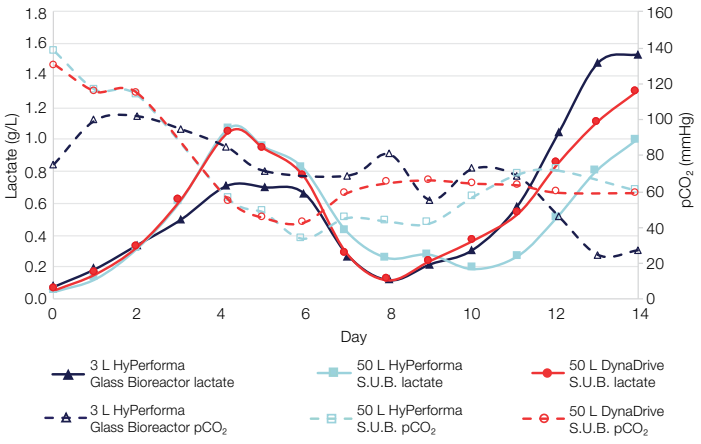


Figure 5. Lactate and pCO_2 profiles for CHO-K1 cells in the 3 L glass bioreactor, 50 L HyPerforma S.U.B., and 50 L DynaDrive S.U.B.

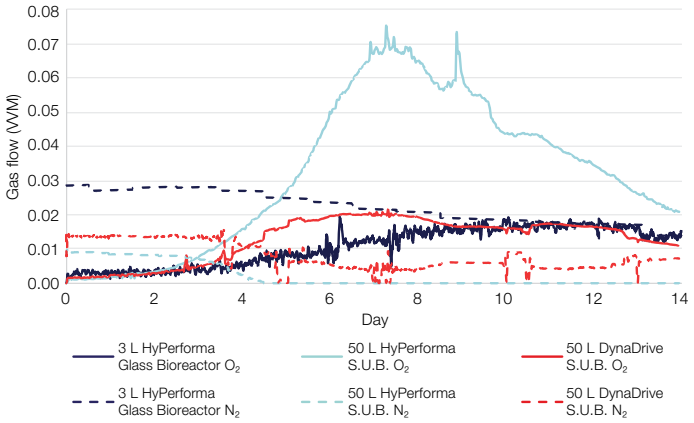


Figure 6. Oxygen and nitrogen gas flow through the drilled-hole sparger for each vessel in terms of vessel volume per minute.

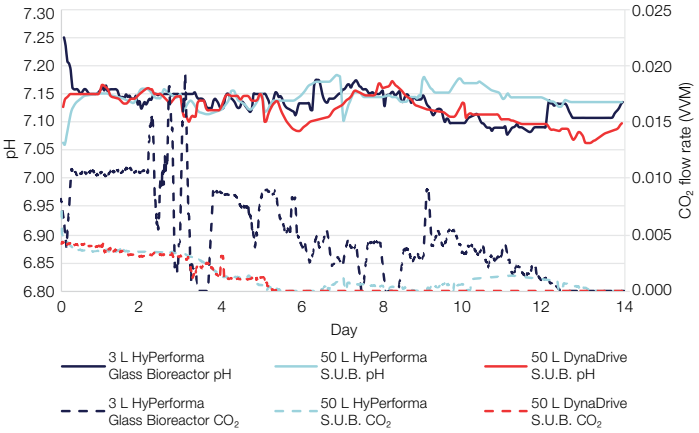
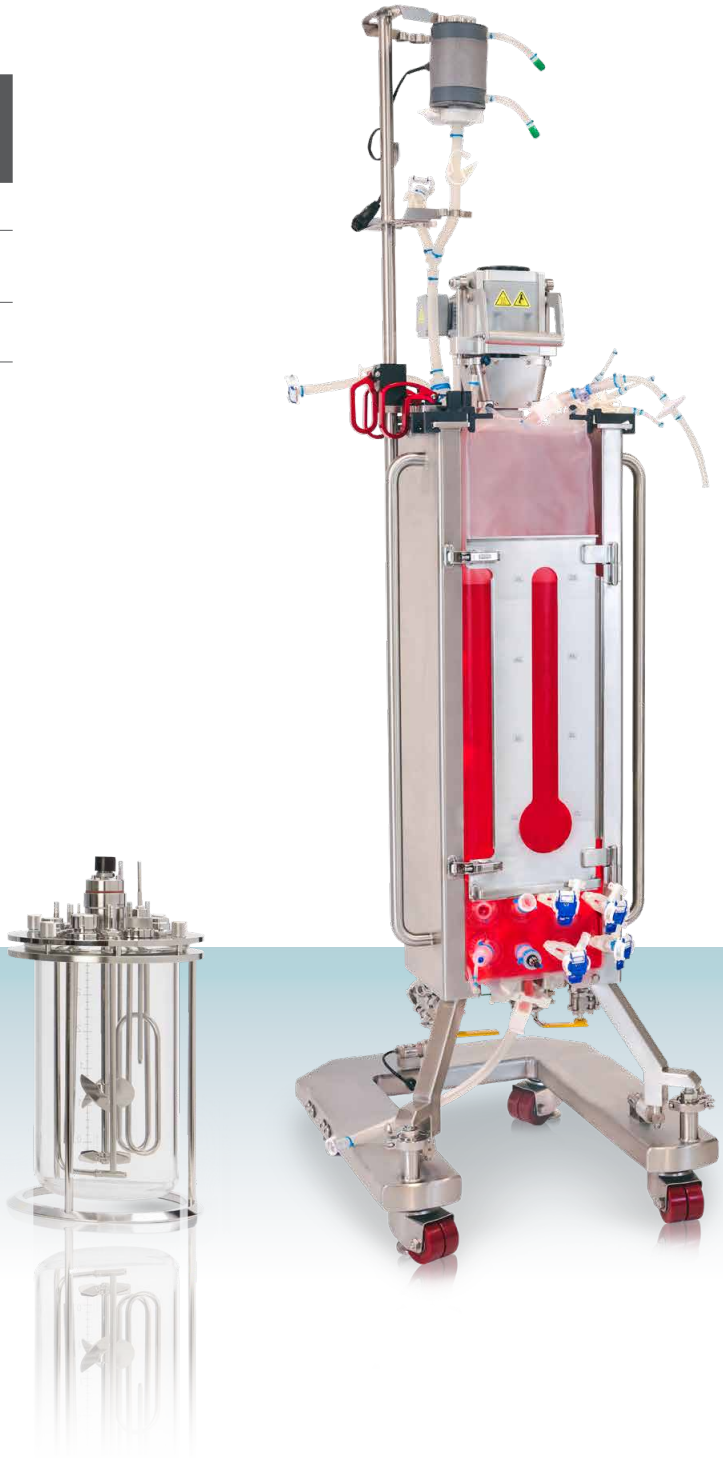


Figure 7. pH profile and carbon dioxide gas flow through the drilled-hole sparger in terms of vessel volume per minute.

Totals for feeds and supplements at the time of run termination are provided in Table 4. Antifoam requirements are significantly lower in the 3 L culture compared to the 50 L cultures due to much smaller sparged gas flow rates, larger pore sizes in the drilled-hole sparger, and the foam sensor's proximity to the liquid level in the smaller culture.

Table 4. Total feed and supplement (from calibrated pump totalizer) added by the end of process.

Parameter	3 L HyPerforma Glass Bioreactor	50 L HyPerforma S.U.B.	50 L DynaDrive S.U.B.
Total feed	442 mL	10.0 L	9.9 L
Total 2 M glucose solution	160 mL	3.2 L	2.6 L
Total FoamAway agent	4.7 mL	350 mL	280 mL



Discussion and conclusion

Considerations for scale-up of cell culture processes frequently center around agitation and gassing strategies. The control parameters used in this study were found to produce comparable results when scaling from benchtop glass bioreactors to Thermo Scientific™ S.U.B.s at the 50 L scale. Cell growth profiles, metabolism, and productivity were maintained in similar ranges in each of the bioreactors. While an aggressive CHO-K1 clone was used, with VCD reaching 65 x 10^6 cells/mL in the DynaDrive S.U.B., the cell cultures were easily maintained within the design constraints of the reactors, with mixing and gassing demands being met in the glass bioreactor and the S.U.B.s without difficulty. The final protein titer at the time of harvest was 3.48 g/L in the glass bioreactor and 3.73 g/L in the DynaDrive S.U.B., with productivity being maintained during the duration of the culture.

Ordering information

Product	Cat. No.
Efficient-Pro AGT Medium	A5322303
Efficient-Pro AGT Feed 1	A5209102
FoamAway Irradiated AOF Antifoaming Agent	A1036902
HyPerforma G3 Lab Controller	F100-2695-001
HyPerforma G3 Lite Controller	F100-2701-001
HyPerforma G3 Pro Controller	F100-2961-001
HyPerforma Glass Bioreactor (3 L)	F100-2680-002
HyPerforma 5:1 Single-Use Bioreactor (50 L)	SUB0050.9500
Bioprocess Container for HyPerforma 5:1 S.U.B. (50 L)	SH31073.01
DynaDrive Single-Use Bioreactor (50 L)	DDB0050.1011
DynaDrive Bioprocess Container (50 L)	SH31192.01

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