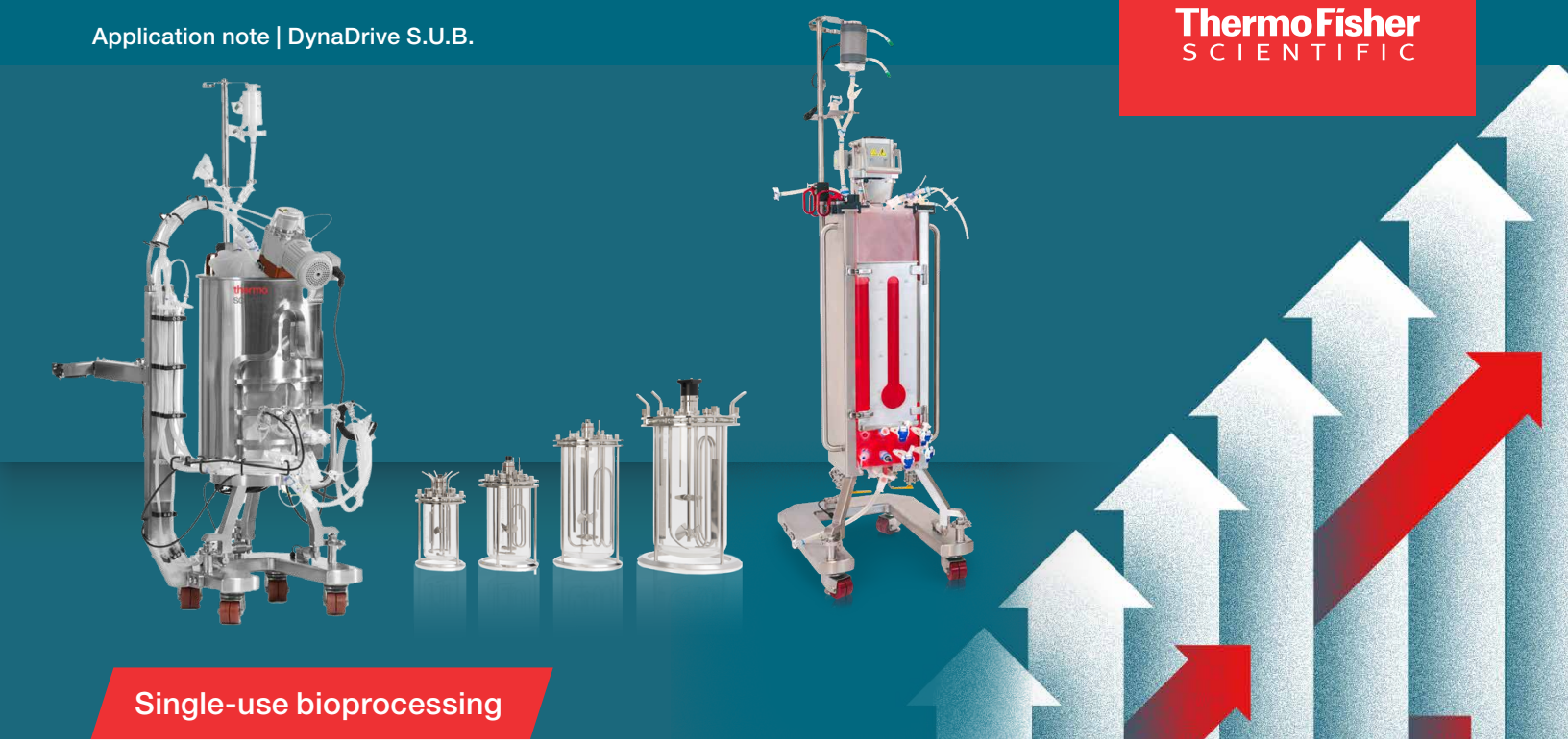

Single-use bioprocessing

Part 2: Intuitive bioprocess scale-up from bench scale to pilot scale

A comparative study of Thermo Scientific single-use bioreactors

Introduction

Process development and bioprocess scale-up present many challenges, typically including maintaining consistent and reproducible growth kinetics, productivity, cell health, and mass transfer. To meet these challenges, a critical need arises for effective control strategies for process parameters such as agitation, dissolved O_2 and CO_2, and nutrient supplementation to ensure optimal cell performance and product yield. The Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.) lineup facilitates typical scale-up activities for high-intensity processes through use of its laser-drilled hole sparge technology and an innovative drivetrain with multiple impellers. These features ultimately enable high mass transfer at relatively low tip speeds and allow for improved gas control and mixing, thereby enhancing culture performance.

Here we provide an example for scaling a high-demand fed-batch process from bench scale to pilot scale. This example highlights differences and similarities in control parameters for the process at the 50 L and 500 L scales using both the Thermo Scientific™ 5:1 HyPerforma™ S.U.B. and the DynaDrive S.U.B. product lines. Furthermore, it shows consistent growth profiles and product titers despite differences in gassing and agitation controls.

Methods

The reactor platforms used in this study included the 5:1 HyPerforma S.U.B. (50 L and 500 L HyPerforma S.U.B.), and the DynaDrive S.U.B. (50 L and 500 L DynaDrive S.U.B). Thermo Scientific™ HyPerforma™ G3Lab™ or G3Pro™ Bioprocess Controllers, running Thermo Scientific™ TruBio™ automation software, were used for control of each of these vessels. A benchtop-scale glass bioreactor was also inoculated as a control condition to facilitate comparisons to small-scale processes. For this control condition, a 3 L Thermo Scientific™ HyPerforma™ Glass Bioreactor was used (operated at the standard nominal working volume of 2 L).

Standard thaw and expansion of IgG-producing CHO-K1 cells was performed in shake flasks as needed to inoculate the various vessels at the following volumes for the n-stage processes: 360 L in the 500 L DynaDrive S.U.B. and 500 L HyPerforma S.U.B., 36 L in the 50 L DynaDrive S.U.B. and 50 L HyPerforma S.U.B., and 1.7 L in the 3 L HyPerforma Glass Bioreactor.

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For each of the reactors, the process control parameters used for the n-stage operation are documented in Tables 1–3. Gibco™ Efficient-Pro™ Medium was used as the basal medium from thaw through the final n-stage process. Gibco™ Efficient-Pro™ Feed 1 was used to supplement the n-stage cultures beginning on day 3, at a daily addition of 2.25% of the current vessel volume (Equation 1). A 2 M glucose solution was also used to supplement the cultures as needed, targeting a 3 g/L glucose concentration upon starting the fed-batch phase of the process. Gibco™ FoamAway™ Irradiated AOF Antifoaming Agent was employed to regulate excess foam buildup during operation via use of the foam probe and pump triggered by TruBio software's "Foam Hi Lim Out" as a remote set point for the pump.

Equation 1: Daily feed rate

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

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

Table 1. Operation and control parameters.

Parameter		3 L HyPerforma Glass Bioreactor	50 L HyPerforma S.U.B.	50 L DynaDrive S.U.B.	500 L HyPerforma S.U.B	500 L DynaDrive S.U.B.
Target initial/final volume		1.7/2 L	36/50 L	36/50 L	360/500 L	360/500 L
Seed density (x 10^6 cells/mL)		0.3	0.3	0.3	0.3	0.3
Temperature set point (°C)		37	37	37	37	37
Agitation (rpm)		350	183	105	101	69
Power input per volume (W/m^3)		100*	20	20	20	20
Tip speed (m/sec)		1.01	1.06	0.59	1.33	0.83
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	DHS 980 x 368 µm pores	DHS 2,610 x 233 µm pores
Target glucose conc. (g/L)		3	3	3	3	3
Foam control	High-threshold output	45	45	45	45	45
	Foam alarm delay (sec)	60	60	60	60	60
	Splash delay (sec)	5	5	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.	500 L HyPerforma S.U.B.	500 L DynaDrive S.U.B.
DO set point (%)		40	40	40	40	40
DO PID	Gain	0.07	0.10	0.10	0.10	0.10
	Reset (sec)	200	200	200	200	200
O_2	Controller output	15 → 100%	15 → 100%	20 → 100%	15 → 100%	30 → 100%
	MFC scaling	0 → 0.5 slpm	0 → 5 slpm	0 → 3 slpm	0 → 40 slpm	0 → 25 slpm
N_2	Controller output	0 → 40%	0 → 30%	0 → 30%	0 → 30%	0 → 30%
	MFC scaling	0.15 → 0 slpm	1 → 0 slpm	1 → 0 slpm	5 → 0 slpm	5 → 0 slpm
Air	Overlay	0.2 slpm	5 slpm	5 slpm	10 slpm	10 slpm
	Sparge controller output	–	–	15 → 30 → 45%	–	15 → 30 → 45%
	MFC scaling	–	–	0 → 0.5 → 0 slpm	–	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.	500 L HyPerforma S.U.B.	500 L DynaDrive S.U.B.
pH set point		7.15	7.15	7.15	7.15	7.15
pH PID	Gain	0.04	0.04	0.04	0.04	0.04
pH PID	Reset (sec)	200	200	200	200	200
pH deadband		Not enabled	Not enabled	Not enabled	Not enabled	Not enabled
CO_2	Controller output	–100 → 0%	–100 → 0%	–100 → 0%	–100 → 0%	–100 → 0%
CO_2	MFC scaling	0.1 → 0 slpm	1 → 0 slpm	1 → 0 slpm	5 → 0 slpm	5 → 0 slpm
Base		Not enabled	Not enabled	Not enabled	Not enabled	Not enabled

Results

Utilizing the control strategies described above, each of the cultures had similar growth profiles. All cultures reached peak viable cell densities (VCDs) of at least 55 x 10^6 cells/mL (Figure 1) and maintained robust cell viability of above 70% at the time of harvest (Figure 2). The 500 L HyPerforma S.U.B. was terminated on day 11, but its culture performance up to that point closely aligned with that of the companion 500 L DynaDrive S.U.B.

In this particular experiment, the 50 L DynaDrive S.U.B. culture had lower initial cell viability as well as a lower peak and final VCD. This can likely be attributed to the vessel being used during cell expansion, then having an idle period prior to being reused for n-stage culture. Residual culture material left in the bioprocess container after draining during cell expansion would have been of high cell density relative to n-stage seed material, and would have died during the idle period. Thus, it is likely that extra cell death signaling factors were present from the onset of the n-stage culture, relative to what would be expected for a reactor that had no break in usage and controls, ultimately resulting in slightly lower peak net growth kinetics. For reference, the same process and controls were used for a 50 L DynaDrive S.U.B. culture referenced in a companion document [1], which yielded a culture with a peak VCD of 64.5 x 10^6 cells/mL and final VCD of 44.3 x 10^6 cells/mL, both metrics being more analogous to the results for the remaining reactors in this experiment.

These results demonstrate the capacity of the HyPerforma and DynaDrive S.U.B.s to enable stable growth kinetics and maintain healthy cultures at various scales, even when utilizing simple control strategies.

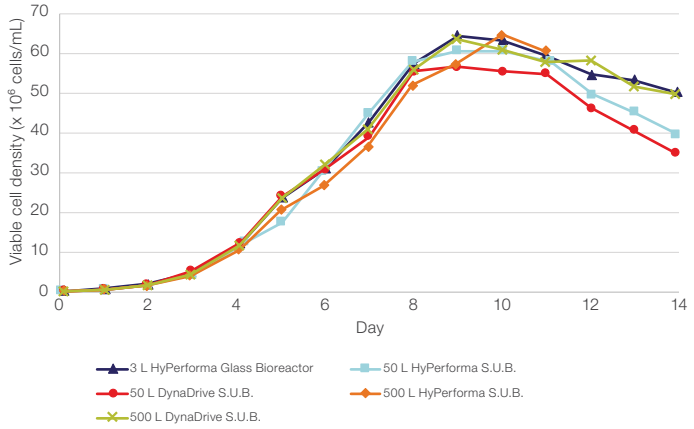

Figure 1. Viable cell density profiles for CHO-K1 cultures in each bioreactor.

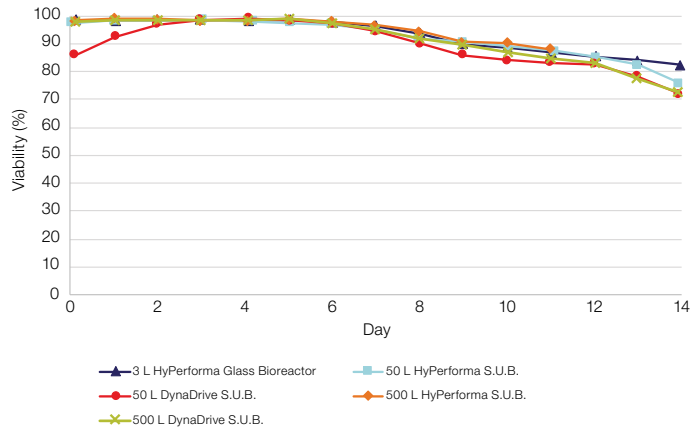

Figure 2. Cell viability profiles for CHO-K1 cultures in each bioreactor.

The process scale-up was also shown to be highly successful with regard to scalability of protein production and titer. For all cultures, day 14 protein titers ranged between 3.42 and 3.51 g/L (Figure 3). Inspection of specific productivity (Q_P) also shows that production was maintained for each phase of the cultures (Figure 4). Specific productivity is affected heavily by VCD, which was measured in our experiment using a Vi-CELL™ XR analyzer (Beckman Coulter), reported to have a counting accuracy of ±10%. Thus, a 3-point simple moving average was used to smooth the specific productivity data for each culture. The moving average highlights the overall trend, which is nearly equivalent for each of the cultures. These results suggest that processes were highly scalable, with the scale-up processes matching or exceeding the efficiency of protein production observed at benchtop scale.

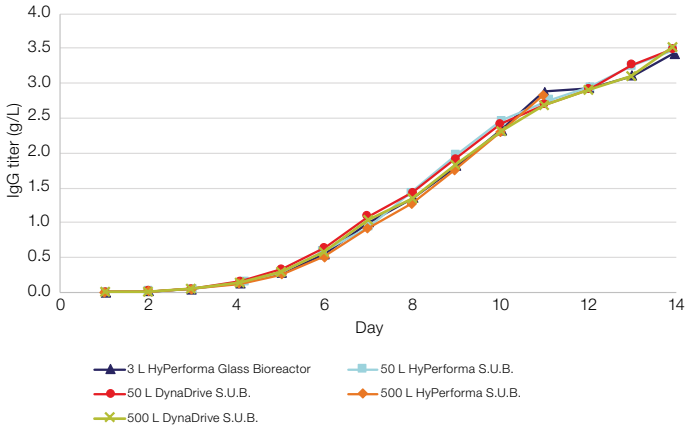

Figure 3. IgG titer profiles for CHO-K1 cultures in each bioreactor.

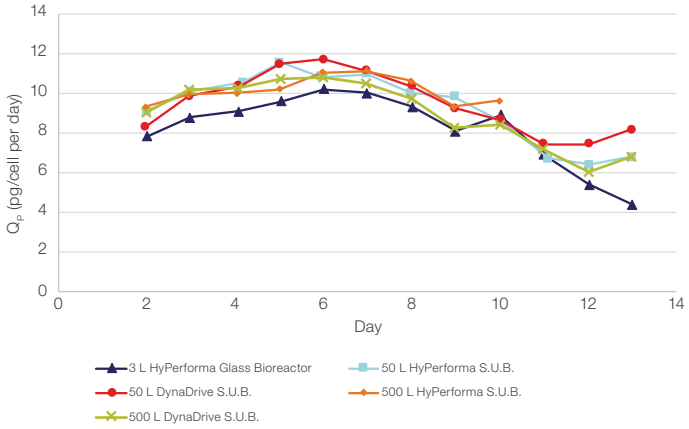

Figure 4. Specific productivity (Q_P, 3-point moving average) for CHO-K1 cultures in each bioreactor.

Lactate had an initial accumulation period for the first 4–5 days before decreasing for the next 4–5 days, followed by a secondary accumulation period lasting until process termination (Figure 5). While the secondary accumulation was relatively faster in the 3 L HyPerforma Glass Bioreactor and 500 L DynaDrive S.U.B. cultures, lactate was maintained below 1.5 g/L in all cultures. As there was no positive pH control (base was not enabled in this experiment), lactate accumulation at the end of the process correlated with a slight pH decline (Figure 6). However, with pH being maintained above 7.0 at all times in each culture, the lactate accumulation was not enough to drop pH far enough from the set point to become a concern. If the pH drops to a concerning level, controls can easily be employed to adjust pH and pCO_2 as needed, such as by addition of base, or addition or removal of an air ballast to decrease or increase pCO_2.

For this process, pCO_2 data were collected from a daily sample, as no pCO_2 probe was used (Figure 7). For pCO_2, corrective control actions were determined to be unnecessary provided that the pCO_2 did not accumulate beyond 120 mmHg for consecutive measurements. In each of the 5 instances where pCO_2 measurements did exceed 120 mmHg, the concentration dropped below 120 mmHg by the next day without intervention to the controls. While the culture in the 500 L DynaDrive S.U.B. did have higher pCO_2 concentrations than other cultures for the majority of the process, they were not high enough to be cause for concern for this process. In the event of excess pCO_2, the DynaDrive S.U.B. could have mitigated the accumulation of CO_2 while easily staying within the gassing and agitation constraints of the reactor. This can be achieved most simply by increasing CO_2 stripping via reintroduction of air or nitrogen to supplement the O_2 sparge.

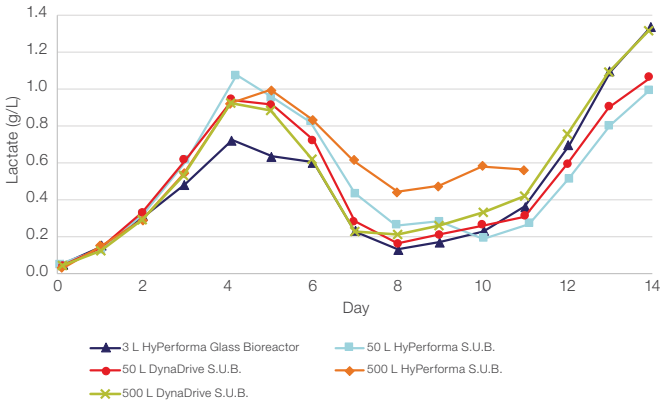

Figure 5. Lactate concentration for CHO-K1 cultures in each bioreactor.

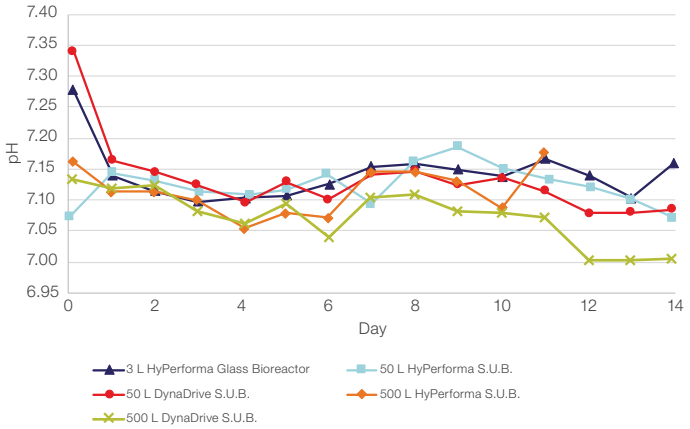

Figure 6. pH profiles for CHO-K1 cultures in each bioreactor.

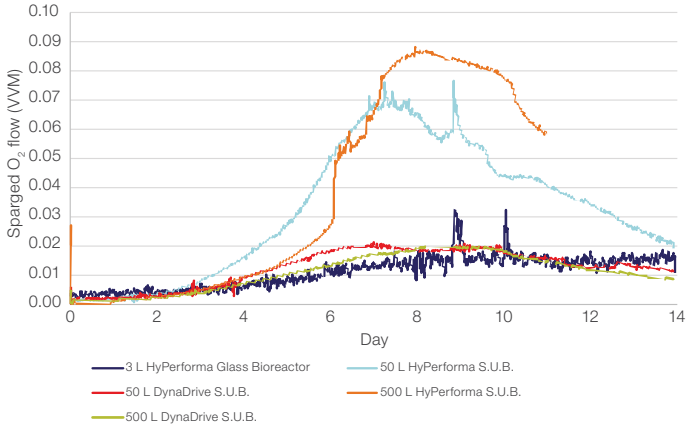

Figure 8. Oxygen gas flow rate through the drilled-hole sparger for CHO-K1 cultures in each bioreactor.

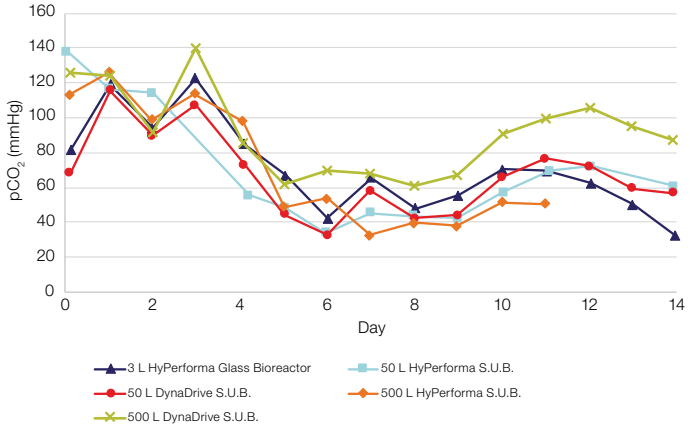

Figure 7. Dissolved carbon dioxide (pCO_2) for CHO-K1 cultures in each bioreactor.

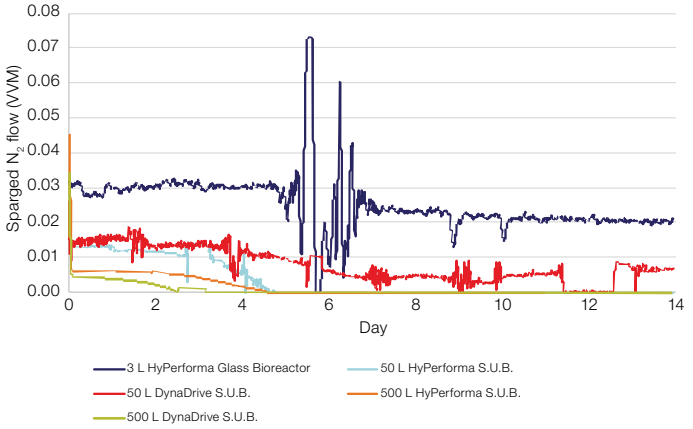

Figure 9. Nitrogen gas flow rate through the drilled-hole sparger for CHO-K1 cultures in each bioreactor.

The gas flow rates for both O_2 and N_2, normalized in terms of vessel volume per minute (VVM), are reported in Figures 8 and 9, with any sparged air addition being treated as 21% O_2 and 78% N_2. As expected, the O_2 sparge rate was much lower in the DynaDrive S.U.B.s than in the HyPerforma S.U.B.s. Notably, the required O_2 sparge rates for the 50 L and 500 L DynaDrive S.U.B.s were extremely comparable, each with a peak sparge rate of about 0.02 VVM. The consistency between the two is indicative of highly scalable mass transfer and gas exchange for the DynaDrive product line, which can considerably simplify use and process development.

Discussion and conclusion

Scalability should be a key consideration from the inception of process development projects. The innovative DynaDrive S.U.B. product line was designed to simplify and streamline common scalability challenges that process development labs encounter, including aeration, mixing and agitation, and heat and mass transfer. The straightforward control parameters for DO, pH, and agitation used in this study yielded results that were consistent across scales and highly analogous to bench-scale experiments.

In all bioreactors used in the experiment, key culture characteristics, including cell growth, metabolic rates, and productivity levels, were maintained within a similar band. A robust CHO-K1 clone with high-productivity medium and feed were utilized, with peak VCD reaching up to 65 x 10^6 cells/mL. Even with their intensive metabolic requirements, these cultures were managed easily without approaching the operational limits of the reactors.

The final titer at time of harvest reached between 3.42 g/L and 3.51 g/L for all cultures. The results showed not only sustained productivity throughout the culture process, but also noteworthy uniformity in culture performance and behavior across scales. Furthermore, the oxygen requirements were found to be extremely stable on a VVM basis for the 50 L and 500 L DynaDrive S.U.B. cultures, underscoring the scalability of the system with respect to mass transfer and mixing.

Author

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Ordering information

Item	Cat. No.
Efficient-Pro AGT Medium	A5322303
Efficient-Pro AGT Feed 1	A5209102
FoamAway Irradiated AOF Antifoaming Agent	A1036902
HyPerforma G3Lab Controller	F100-2695-001
HyPerforma G3Lite Controller	F100-2701-001
HyPerforma G3Pro Controller	F100-2961-001
HyPerforma Glass Bioreactor (3 L)	F100-2685-002
HyPerforma 5:1 Single-Use Bioreactor (50 L)	SUB0050.8100
HyPerforma 5:1 Single-Use Bioreactor (500 L)	SUB0500.8400
BioProcess Container for HyPerforma 5:1 S.U.B. (50 L)	SH31073.01
BioProcess Container for HyPerforma 5:1 S.U.B. (500 L)	SH31077.01
DynaDrive Single-Use Bioreactor (50 L)	DDB0050.1011
DynaDrive Single-Use Bioreactor (500 L)	DDB0500.1011
DynaDrive BioProcess Container (50 L)	SH31192.01
DynaDrive BioProcess Container (500 L)	SH31193.01

Reference

1. Part 1: Intuitive bioprocess scale-up from bench scale to pilot scale. Application note. Thermo Fisher Scientific, 2024.

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