Single-use technologies

Streamlining seed train scale-up through utilization of the turndown ratio of the DynaDrive S.U.B.

Keywords
DynaDrive S.U.B.,
single-use bioreactors,
scalability, turndown

Introduction

Upstream bioproduction has seen a substantial movement in the industry toward single-use systems. This has been driven primarily by the need to reduce contamination risk and cleaning requirements when compared to stainless steel systems, and to allow for faster changeover of equipment between batches. At the same time, bioprocessing manufacturing processes have matured significantly, and intensification of cell culture processes has pushed the limits of these legacy single-use systems. In recent years, Thermo Fisher Scientific has brought enhancements to the Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) platform with 5:1 S.U.B. and enhanced S.U.B. options, which have allowed for higher turndown ratios and optimized mixing and gassing strategies for intensified processes. Now Thermo Fisher Scientific has launched a next-generation bioreactor for the biopharmaceutical industry with the Thermo Scientific™ DynaDrive™ S.U.B. (Figure 1).

Figure 1. DynaDrive S.U.B.s in 50 L, 500 L, 3,000 L, and 5,000 L sizes.

Building on our extensive experience and nearly two decades of end users' feedback, the DynaDrive S.U.B. employs a new agitator drive technology with carefully engineered hardware that enables exceptional performance. The DynaDrive S.U.B. allows for a higher turndown ratio of at least 10:1 and up to 20:1 in the larger sizes, with a reliable power input of up to 80 W/m^3, and scalability through each size.

The innovative impeller design enables a higher turndown ratio than previously seen in single-use systems, offering working volumes as low as 5 L in the 50 L S.U.B. These higher turndown ratios open a new paradigm of what is possible with seed trains, potentially eliminating multiple vessels and reducing logistical and operating costs dramatically while increasing the efficiency of the seed train through reduced connections and transfer losses.

S.U.B. seed train options

During seed train expansion, cell cultures are sequentially increased in volume and cell population to provide enough starting material to enter batch, fed-batch, or perfusion production unit operations. Small-scale cell cultures, usually in less than 1 L of working volume, are typically maintained in flasks on an orbital shaking platform. Shake flask culture requires careful handling within a biosafety cabinet in a separate, higher-classification cleanroom to limit the risk of contamination. Due to the heightened risk associated with these open-unit operations, it is desirable for bioprocess engineers to move the cell culture into a closed system as early as possible in the scale-up process. A commonly chosen closed system for expansion steps after shake flasks is the rocking-motion bioreactor. This single-use closed system employs a platform to mix cells and gases to maintain viable cultures. After expanding in the rocker, cultures are transferred to stirred-tank S.U.B.s for further expansion prior to inoculating the N-stage production vessel (Figure 2A).

Enhancements to our legacy S.U.B. systems, with the launch of the 5:1 and the enhanced S.U.B. systems, allowed for working volumes as low as 20% of the final working volume (e.g., 10 L in a 50 L vessel), which has allowed for scale-up of the seed train within the vessel and elimination of some seed train vessels (Figure 2B). With the innovative design features of the 50 L DynaDrive S.U.B., cell cultures in working volumes as low as 5 L can be grown in a stirred-tank vessel, allowing for scale-up processes to happen at even earlier stages. This extremely high turndown ratio eliminates the need for rockers and extra steps in the seed train process further on, with the ability to seed the 5,000 L DynaDrive S.U.B. at 20:1 directly from the 50 L scale. This essentially means that the seed train process can be limited to two reactors, with each having scale-up steps that take place within them. For example, the 5 L minimum working volume of the 50 L DynaDrive S.U.B. enables cell culture scale-up to transfer directly from shake flasks to the S.U.B. After the 5 L culture reaches a viable cell density suitable for expansion, additional culture medium can be added to the vessel as a second passage within the 50 L DynaDrive S.U.B. Once that culture reaches a viable cell density suitable for expansion, it can then be used to seed the 5,000 L DynaDrive S.U.B. at a 250–500 L working volume, which subsequently can be passaged within the reactor for inoculation of the production run (Figure 2C).

Alternatively, because of the flexibility of the DynaDrive S.U.B. systems, the 500 L DynaDrive S.U.B. can be used in the seed train in place of the 5,000 L DynaDrive S.U.B., increasing the required reactors to three, which allows for increased overall bioreactor production and throughput. In this scenario, the seed train would still utilize the 5 L minimum working volume of the 50 L DynaDrive S.U.B., but after reaching a viable cell density suitable for expansion, these cells would be used to seed the 500 L DynaDrive S.U.B. at a 25 L or 50 L volume. When ready, this would be expanded within the 500 L vessel and then used to inoculate the 5,000 L DynaDrive S.U.B. production run (Figure 2D).

Since every additional aseptic connection is a potential vector for contamination, the high turndown ratios of the DynaDrive S.U.B. systems enable risk reduction for bioprocess scale-up operations. Performing expansions in the same vessel saves time in setup and reduces the quantity of sterile connections needed for the overall seed train expansion.

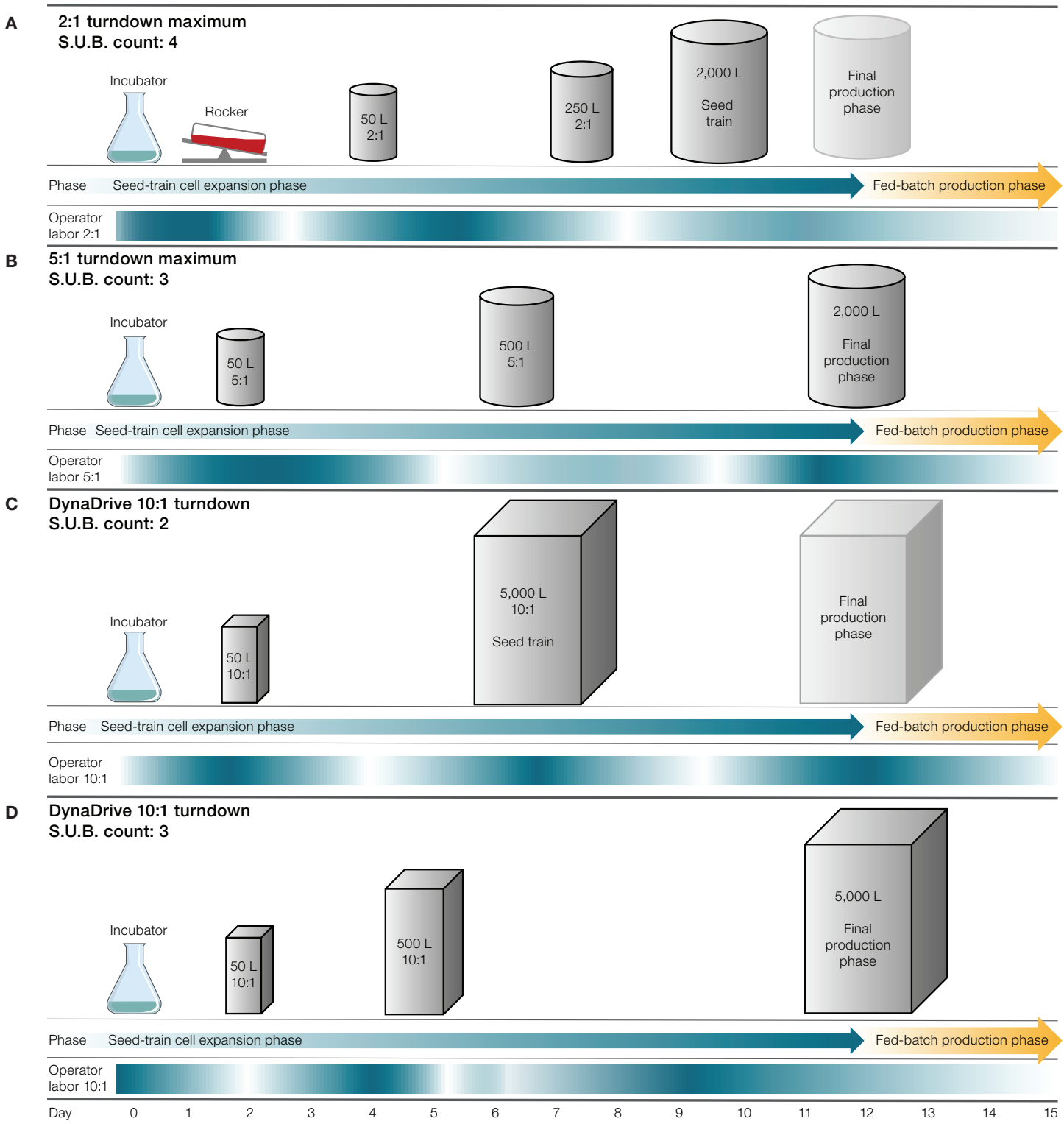


Figure 2. Improving facility efficiency with increased S.U.B. turndown ratios and careful logistical planning. Using bioreactors capable of high turndown ratios, such as the DynaDrive S.U.B.s, can enable a streamlined seed train for cell expansion. Benefits of high turndown ratios include increased facility space through reduction of bioprocess units, reduction of operator labor (e.g., setups, takedowns, and sterile or aseptic transfers), and reduction of logistical risks. **(A)** Example of a 2:1 turndown ratio operation that incorporates a rocking-motion bioreactor and stirred-tank S.U.B.s. **(B)** With the removal of at least one or more bioprocess units (e.g., rocker and S.U.B.), the cell expansion requires 20–25% less operator intervention and up to 50% fewer single-use Thermo Scientific™ BioProcess Containers, compared to a 2:1 turndown ratio operation. The high-intensity portions of operator labor (dark blue) of both the 5:1 and the DynaDrive S.U.B. seed trains are spaced at intervals of 2–4 days, reducing risk of operator error through fatigue or haste. **(C)** Using the 5,000 L DynaDrive S.U.B. at a turndown ratio of 10:1 as the N-1 bioreactor allows for a smaller footprint in the facility, less setup, and fewer sterile or aseptic transfers. **(D)** Alternatively, utilizing the 500 L DynaDrive S.U.B. in the seed train allows the 5,000 L DynaDrive S.U.B. to remain a dedicated production vessel. Maximum split ratio = 10; minimum cell density = 0.3 x 10^6 cells/mL.

Application and benefits

By utilizing DynaDrive S.U.B.s in seed trains, not only are there ideal turndown ratios of scaling within vessels, but the system has been optimized to provide ideal mixing and mass transfer, even at low turndown ratios, as shown in Table 1.

Also shown in Table 1 is a comparison of the monitoring capabilities of each system. In comparing the single-use closed system options for seed train expansion, stirred-tank S.U.B.s allow for better monitoring and control than rocker systems.

Due to design limitations of the rocker bioreactors, there are limited online process monitoring capabilities, whereas the 50 L DynaDrive S.U.B. can support temperature, pH, and DO probes as well as an additional probe at the 10:1 turndown ratio, with space for more probes at higher volumes (Table 1). This capability provides greater design space for bioprocess monitoring and enhanced process analytical technology (PAT) capabilities for quality by design (QbD) approaches.

Table 1. Highlighted performance comparisons and monitoring capabilities of the HyPerforma, enhanced HyPerforma, and DynaDrive S.U.B. systems.

S.U.B.	Rocker	HyPerforma		Enhanced HyPerforma		DynaDrive
Generation	Rocker	2:1	5:1	Enhanced fed-batch	Enhanced perfusion	DynaDrive
Vessel size	10 to 50 L	50 to 2,000 L		50 to 500 L		50 to 5,000 L
Scalable volumes	1 to 25 L	25 to 2,000 L	10 to 2,000 L	12.5 to 500 L	25 to 500 L	5 to 5,000 L
Turndown ratio	5:1	2:1	5:1	4:1	2:1	10:1 or 20:1
Sparge	Overlay only	Frit and drilled-hole sparger (DHS)		Enhanced DHS		
k_La at 20 W/m^3 with DHS only	No data	≤10 hr^{-1}		≤20 hr^{-1}	≤25 hr^{-1}	20–30 hr^{-1}
Max k_La	No data	≤15 hr^{-1}		30–40 hr^{-1}		>40 hr^{-1}
Max P/V	No data	40 W/m^3		100 W/m^3		80 W/m^3
Dissolved oxygen (DO) sensor	Must be single-use, limited options available	Standard or single-use available, multiple options				
pH sensor	Must be single-use, limited options available	Standard or single-use available, multiple options				
Temperature control	Electric, heat only	Jacket, temperature control unit (TCU)				
Additional sensor port options	None available	2 probe belts, no options below first probe belt	2 probe belts plus 1 DO and 1 pH option at low turndown ratio			Additional port options at low turndown ratio

Case study

Seed train evaluation using ExpiCHO-S cell line in 14-day fed-batch run

The following case study was done to evaluate the performance of the DynaDrive S.U.B.s across all scales from 50 to 5,000 L in a fed-batch process using CHO cells (Table 2), as well as to evaluate the performance of the cells during the seed train steps within these bioreactors.

Methods

Cells were expanded in shake flasks, the 50 L DynaDrive S.U.B., or both through the N-2 stage. The N-1 stage for each seed train was carried out at a 10:1 turndown ratio within the N-stage vessel. Fresh production medium was added to the S.U.B. after 3 days, resulting in the culture starting at the proper N-stage production volume and initial seed density. Operating conditions for both the N-1 and N-stage production vessels are described in Table 3. During the production run, daily bolus feeds of 2X concentrated EfficientFeed C+ AGT Supplement were added from days 3 to 13 through either a subsurface (5,000 L S.U.B.) or top feed (50 and 500 L S.U.B.) line. Following addition of EfficientFeed C+ AGT Supplement, glucose was supplemented in the same way on an as-needed basis after taking glucose measurements to bring the final glucose concentration to >4 g/L. Cell counts, viability, gases, nutrients, and metabolites were measured offline daily. All three DynaDrive S.U.B. volumes were tested in N-stage production runs. The 500 L and 5,000 L production runs came from the same seed train, with the 500 L N-1 being inoculated with cells from the 5,000 L N-1 stage reactor, prior to the start of that 5,000 L N-stage production run.

Table 2. Cell line evaluated in 50–5,000 L DynaDrive S.U.B.s.

Cell type	Gibco™ ExpiCHO-S™ Cells
Production medium	Gibco™ ExpiCHO™ Stable Production Medium
Feed supplement	Gibco™ EfficientFeed™ C+ AGT™ Supplement
Titer range	~3 g/L

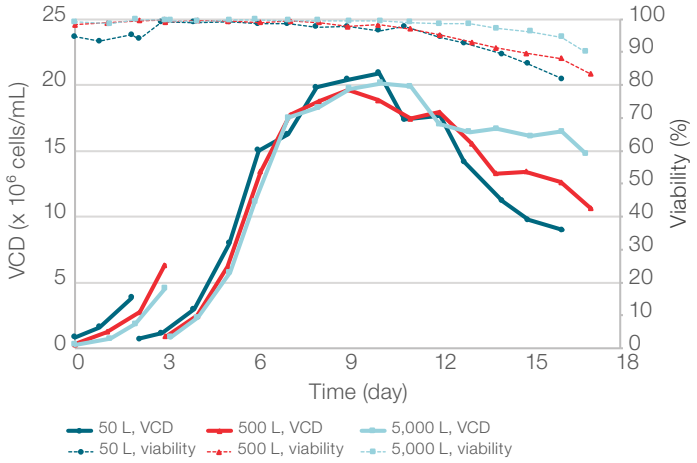
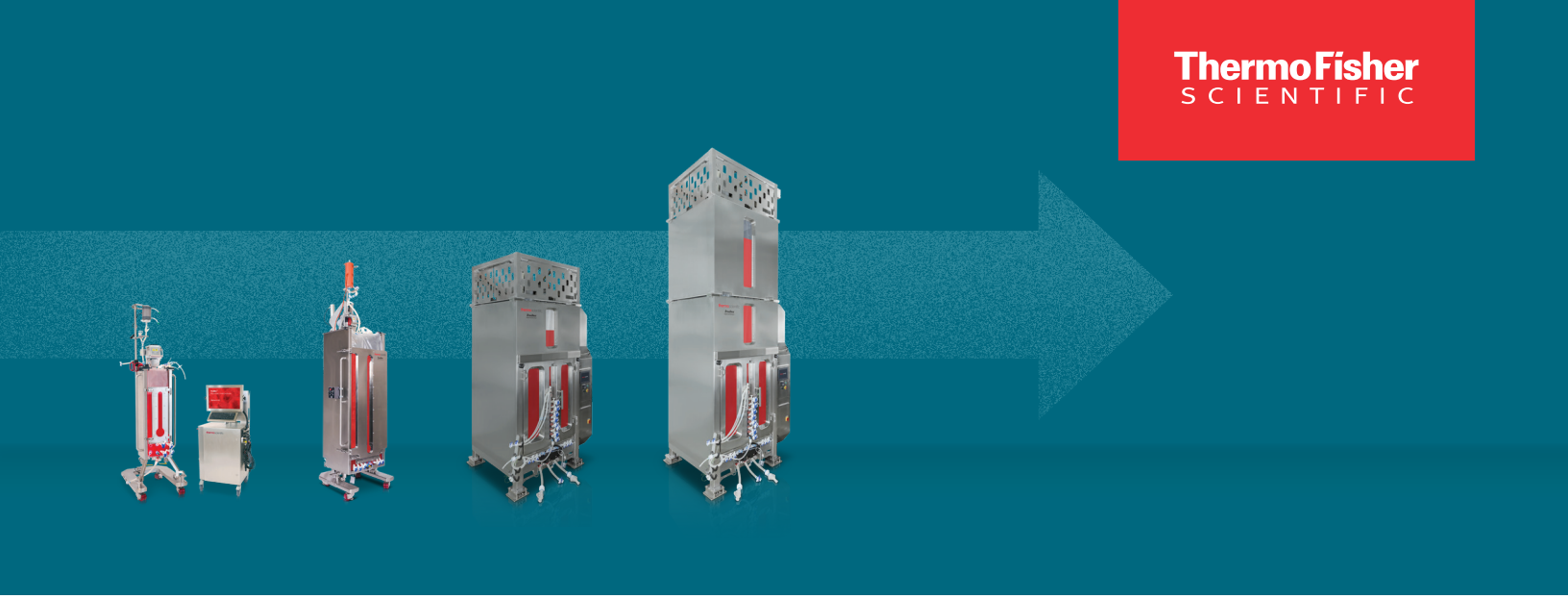


Figure 3. VCD and viability of ExpiCHO-S cell culture from N-1 through N-stage production culture in the 50 L, 500 L, and 5,000 L DynaDrive S.U.B.s. Each N-1 culture was seeded at a 10:1 volume. The low viability seen in the N-1 culture in the 50 L S.U.B. occurred in a prototype BPC with known effects on cell health at low working volumes. Production BPCs have been shown to have reduced impact on cell health and growth across multiple cell clones tested.

Table 3. Operating parameters for evaluation of ExpiCHO-S cells in the 3 scales of DynaDrive S.U.B.s.

S.U.B.	50 L	500 L	5,000 L
Target starting volume	5 L, 35 L*	50 L, 350 L*	500 L, 3,500 L*
Seeding density	0.3 x 10^6 cells/mL, 0.7 x 10^6 cells/mL*		
Temperature	37°C (N-1 and days 0–5), 34°C (days 5–14)		
pH	6.8–7.2		
pH control	Acid control: sparged CO_2 Base control: 1 N NaOH	Acid control: sparged CO_2 Base control: 1 N NaOH	Acid control: sparged CO_2 through the macro DHS Base control: 1 N NaOH
Agitation	140 rpm, 120 rpm*	60 rpm	26 rpm for N-1 37 rpm (days 0–3) 33 rpm (days 3–14)
DO	40%		
Air crossflow/headspace (slpm)	1	6	10–20
DO cascade	Air supplemented with O_2 through DHS	Air supplemented with O_2 through DHS	Air supplied to both macro and micro DHS O_2 supplemented through micro DHS
Feeding strategy	Daily bolus of 1.05 L EfficientFeed C+ AGT Supplement and glucose (as needed)	Daily bolus of 10.5 L EfficientFeed C+ AGT Supplement and glucose (as needed)	Daily bolus of 105 L EfficientFeed C+ AGT Supplement and glucose (as needed)

* The N-1 culture conditions are listed first, followed by the N-stage culture conditions.



Results

Viable cell density (VCD) and viability (Figure 3) for the N-1 and production cultures show consistent growth profiles among the cultures, with similar cell density and viability trends at each step in the seed train. The viability at the end of the run was above 80% in all systems, which was within expectations.

Conclusion

Overall, the ability of the DynaDrive S.U.B. systems to operate at a 10:1 or 20:1 turndown ratio enables more efficient cleanroom utilization, reduces risk of contamination, and simplifies seed train expansion operations. This innovative product offering from Thermo Fisher Scientific enables consolidation of unit operations into fewer vessels and helps provide a more flexible manufacturing system for upstream bioprocessing.

Learn more at **thermofisher.com/dynadrive**