

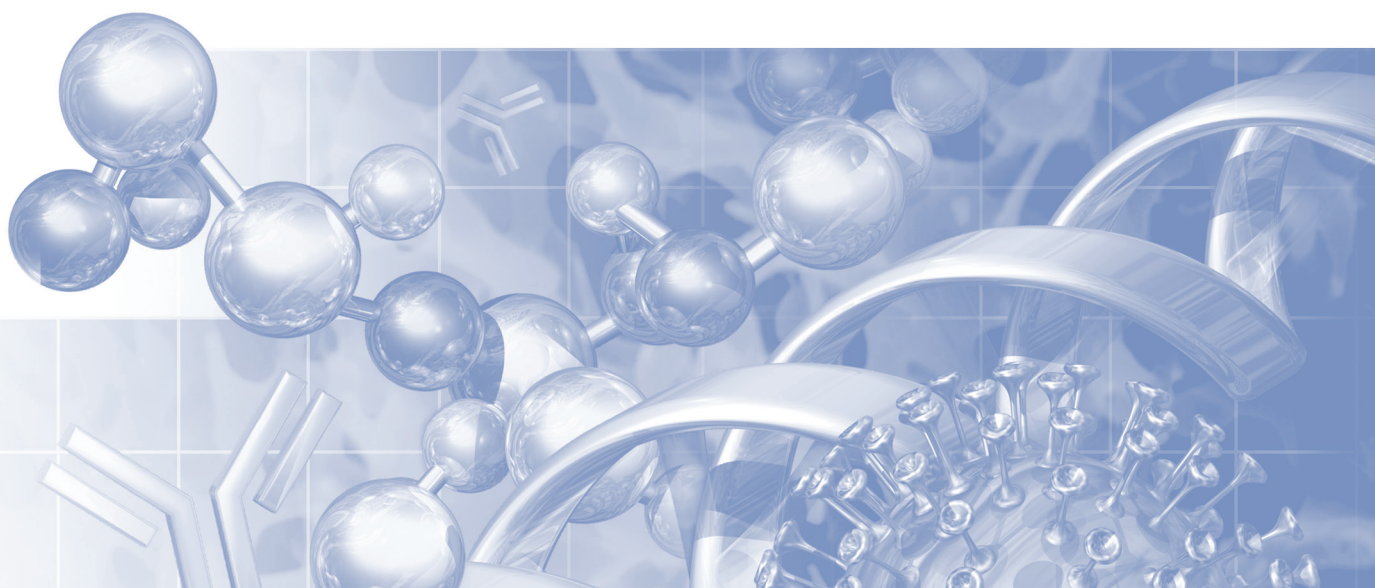


Biotech

Application Note

USD3384

Scalability Between the Allegro™ STR 50 and STR 500 Bioreactors in a CHO-S Fed-Batch Process



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1. Introduction

This application note will demonstrate scalability between the Allegro STR 50 L bioreactor and the Allegro STR 500 L bioreactor. A fed-batch cultivation of a CHO cell line expressing a recombinant monoclonal antibody, in a chemically defined, animal-derived component-free (ADCF) medium was performed. An Allegro STR 50 bioreactor was used as the n-1 bioreactor and seeded the Allegro STR 500 bioreactor at an initial working volume of 385 L. An Allegro STR 50 production bioreactor was then initiated by dropping out 35 L from the Allegro STR 500 bioreactor to ensure comparable culture.

Besides state-of-the-art design and usability features, scalability performance of the Allegro STR single-use stirred bioreactor family has been demonstrated. More information is available in application notes USD2980, USD3136, USD3180, USD2926, and USD3135 describing respectively engineering performance of the Allegro STR 200, 1000, and 2000 bioreactors and CHO cell culture performance in the Allegro STR 200 and 1000 bioreactors. These studies highlighted that the critical parameters to be kept constant from one scale to another are the power input per volume (P/V) for agitation and the superficial gas velocity (v_s) for aeration. This will successfully scale-up a process at constant $k_{L,a}$, keeping equivalent mixing time and shear stress environment in the bioreactor.

2. Materials and Methods

Table 1

Equipment	Manufacturer	Model/Part/Serial No.
Allegro STR50-JC110 bioreactor	Pall	M3082080M
Lauda♦ Integral T 2200	Lauda	L002242
Allegro STR500-JC110 bioreactor	Pall	M6020913M
Lauda VC 10000	Lauda	S190003372
Nova♦ FLEX2 bioanalyzer	Nova Biomedical	T08310040
Vi-Cell♦ XR	Beckman Coulter♦	30527950
Octet RED96 system	ForteBio♦	FB-50090
pH probe InPro3253/225/pt1000	Mettler Toledo♦	8455038
DO probe InPro 6800/12/220	Mettler Toledo	8391365
50 L biocontainer	Pall	6412-0927L
500 L biocontainer	Pall	X6412-0891S
Materials	Manufacturer	Model/Part/Serial No.
CD FortiCHO medium	Gibco♦	A1148301
GlutaMAX 100X	Gibco	35050061
HT supplement 100X	Gibco	11067030
Antibiotic-Antimycotic 100X	Gibco	15240112
EfficientFeed♦ C+ AGT supplement	Thermo Fisher Scientific♦	A2503101
Glucose solution	Sigma-Aldrich♦	Ref G7528
Antifoam C	Sigma-Aldrich	A8011
Puromycin dihydrochloride	Thermo Fisher Scientific	A1113803

2.1 Inoculum Preparation

CHO-S cells were thawed from cryopreservation and cultivated in a 125 mL shake flask (30 mL working volume). Passages were performed in CD-FortiCHO media supplemented with 1x HT-Supplement, 4 mM GlutaMAX, and 1.25 mL/L Puromycin. Shake flasks were incubated in a humidified atmosphere at 37 °C containing 8% CO₂ in air on an orbital shaker with agitation 120 rpm until inoculation into the Allegro STR 50 L bioreactor.

2.2 N-1 Process Parameters

The seed train was expanded every 2-3 days in shake flasks until there were enough cells to inoculate an n-1 Allegro STR 50 bioreactor at a 40 L working volume. The n-1 was seeded at an initial density of 0.2 x 10⁶ cells/mL and allowed to grow for four days where it was then transferred to the Allegro STR 500 bioreactor for production. The n-1 parameters can be found in Table 2 below.

Table 2

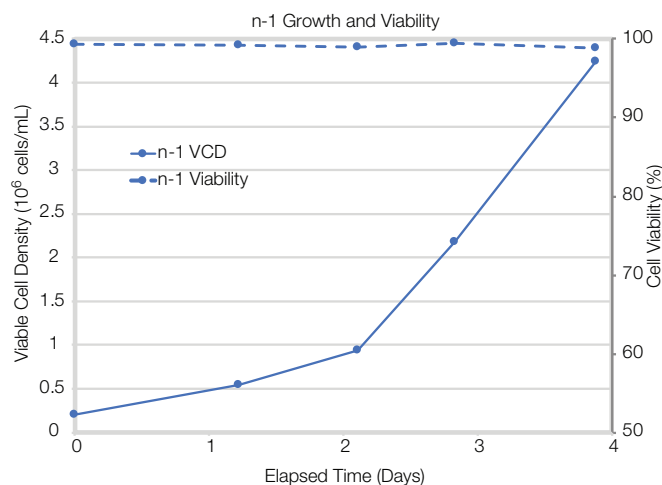
n-1 process parameters

Parameter	Allegro STR 50 Bioreactor
Basal medium	CD FortiCHO + 4 mM Glutamax +1x HT + 1x Anti-Anti
Initial working volume (L)	40
Power input P/V (W/m ³)	70
Agitation (rpm)	106
Air sparge flow rate (vvm)	0.0025
Air sparge flow rate (L/min)	0.1
Overlay flow rate (vvm)	0.005
Overlay flow rate (L/min)	0.2
pH	7.1 +/- 0.3
Dissolved oxygen (%)	40
Temperature (°C)	37
pH control	CO ₂ /Sodium carbonate
Inoculation cell concentration (cells/mL)	0.2 x 10 ⁶

2.2.1 N-1 Viable Cell Density and Viability

The n-1 cell density reached 4.2 x 10⁶ cells/mL with a viability of 98.8% on Day 4 when a portion of the culture was transferred to the Allegro STR 500 bioreactor. The doubling time was within the expected range for the cells with an average of 21 hours and the viability remained > 98.5% over the duration of the n-1 culture.

Figure 1
n-1 viable cell density and viability



2.3 Bioreactor Set-Up and Parameters

This run is designed to demonstrate the scalability between the Allegro STR 50 bioreactor and the Allegro STR 500 bioreactor. The Allegro STR 500 bioreactor engineering parameters were scaled-up from the Allegro STR 50 bioreactor process using a strategy that maintains identical air sparge vvm, air overlay vvm and P/V. Agitation was operated in downflow in both scales. The operating parameters can be found in Table 3, additionally the PID values for control loops can be found in Table 4.

Table 3

Fed-batch operating parameters

Parameter	Allegro STR 50 Bioreactor	Allegro STR 500 Bioreactor
Basal medium	FortiCHO + 4 mM L-GlutaMAX + 1x HT Supplement + 1x Anti-Anti	
Initial working volume (L)	35	350
Power input P/V (W/m ³)	70	70
Agitation (rpm)	104	106
Agitation direction	Downflow	Downflow
Air flow rate (vvm)	0.0025	0.0025
Air flow rate (L/min)	0.0875	0.875
Overlay flow rate (vvm)	0.005	0.005
Overlay flow rate (L/min)	0.175	1.75
pH	7.1 ± 0.3	7.1 ± 0.3
Dissolved oxygen (%)	40%	40%
Temperature (°C)	37 °C	37 °C
Antifoam	Antifoam C (30,000 ppm)	Antifoam C (30,000 ppm)
pH control	CO ₂ and 1 M sodium carbonate	CO ₂ and 1 M sodium carbonate
Initial cell density	0.3 x 10 ⁶ cells/mL	0.3 x 10 ⁶ cells/mL
Nutrient feed	Efficient feed C+	Efficient feed C+
Glucose feed (g/kg)	500	500
Glucose action limit (g/L)	3	3
Glucose supplement target (g/L)	5	5
Cell concentration for feed initiation	8 – 12 x 10 ⁶ cells/mL	8 – 12 x 10 ⁶ cells/mL
Daily feeding volume (5 doses)	6% Final volume	6% Final volume

Table 4*PID settings for fed-batch production*

	Parameters	Setpoint	P	I	D	Dead-Band
Allegro STR 50 bioreactor	pH	7.1	5	50	0	0.3
	DO	40	5	500	0	0
	Temperature	37	20	500	0	N/A
TCU Lauda 2200	Temperature	N/A	TN = 50	XP = 8	N/A	N/A
Allegro STR 500 bioreactor	pH	7.1	5	50	0	0.3
	DO (Day 0-3)	40	3	5	0	0
	DO (Day 3-10)	40	5	500	0	0
	Temperature	37	20	500	0	N/A
Lauda VC10000	Temperature	N/A	TN = 80	XP = 10	N/A	N/A

**Note – PID values for DO were changed from the factory default values on Day 3 in the Allegro STR 500 bioreactor to improve control. The factory values have been updated since this run to the improved settings.*

2.4 Fed-Batch Process

The fed-batch was inoculated from the Allegro STR 50 bioreactor n-1 to an initial cell density of 0.3×10^6 cells/mL. The culture was allowed to grow in batch mode until the cell density was in the $8\text{-}12 \times 10^6$ cells/mL range where the first feed was initiated. The production reactor is fed 5 times in 24 hour intervals throughout the run with Efficient Feed C+ at a volume of 6% of the final reactor volume (3 L per feed in the Allegro STR 50 bioreactor and 30 L per feed in the Allegro STR 500 bioreactor). The glucose is also monitored as to not deplete glucose levels and supplemented back to 5 g/L as needed.

3. Results and Discussion

3.1 Comparison of Batch Culture Cell Growth

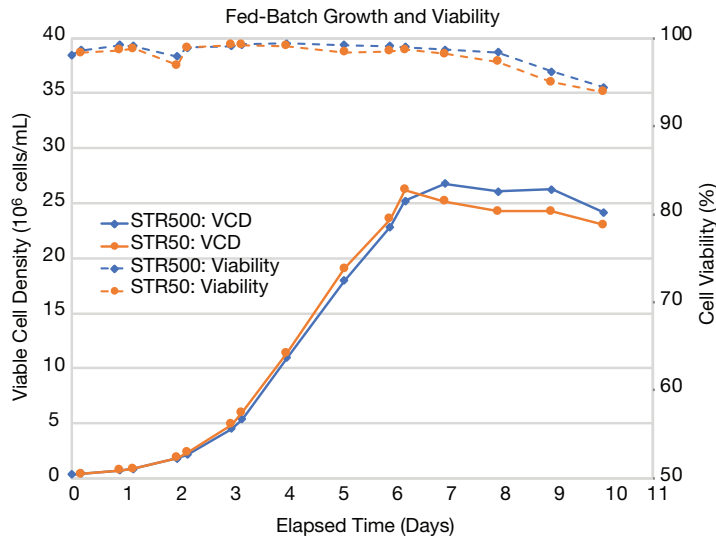
Both cultures grew very similarly during their exponential phase and peaked at approximately 26×10^6 cells/mL (Figure 2). After the peak on day 6/7, the VCD in the Allegro STR 50 bioreactor dropped slightly more than the Allegro STR 500 bioreactor. The Allegro STR 50 bioreactor and the Allegro STR 500 bioreactor were harvested at a viable cell density of 23.0×10^6 cells/mL and 24.2×10^6 cells/mL respectively.

The cell viability remained > 95% for the first 9 days and were very similar between both scales, the cell viability profile can be found in Figure 2. The first two day's viabilities are misleading as the Vi-Cell falsely detected dead cells due to a reagent issue, the slightly lower viabilities on days 0-2 should be ignored. The cultures were harvested on day 10 with viabilities at 93.9% in the Allegro STR 50 bioreactor and 94.3% in the Allegro STR 500 bioreactor.

Table 5*Maximum viable cell concentration, specific growth and doubling time rates in both bioreactors.*

Bioreactor Type	Maximum Viable Cell Concentration (cells.mL⁻¹)	Specific Growth Rate (h⁻¹)	Doubling Time (h)
Allegro STR 500 bioreactor	26.8×10^6	0.036	19.3
Allegro STR 50 bioreactor	26.2×10^6	0.036	19.1

Figure 2
Fed-batch viable cell density and viability



3.2 Comparison of Metabolic Data

The metabolic profiles all show very similar trends between the two runs. Slight variations can be observed between the runs but can be expected in a cell culture process.

Figure 3
Allegro STR 50 bioreactor metabolic profiles

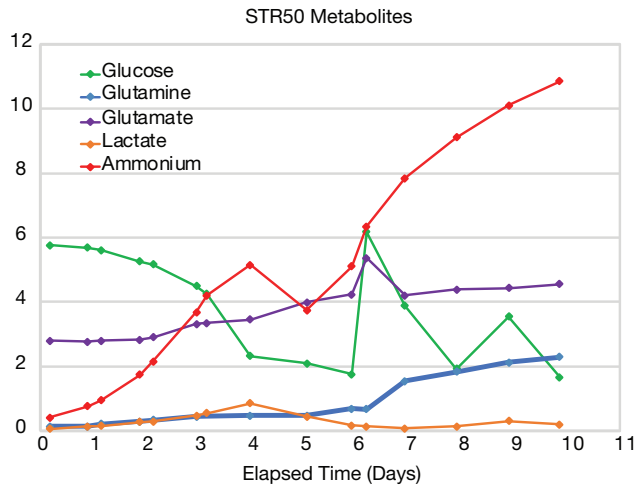
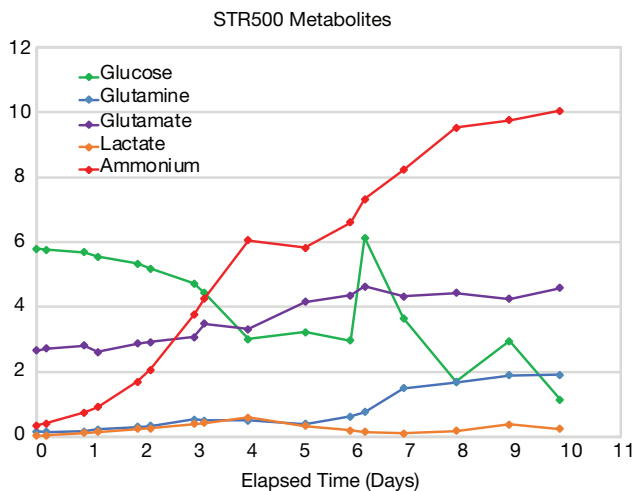


Figure 4
Allegro STR 500 bioreactor metabolic profiles



3.3 Comparison of Batch Productivity

The productivity between the two scales matched very well. The slightly lower productivity in the Allegro STR 50 bioreactor can likely be attributed to the slightly lower VCD in the latter part of the run and some slight variations in other measured parameters observed in the run. Monoclonal antibodies were quantitated using Octet RED96 system which uses bio-layer interferometry (BLI). Interferometric signals from the samples with unknown mAb concentrations were compared with the known concentrations of the standard mAb.

Figure 5

mAb titer

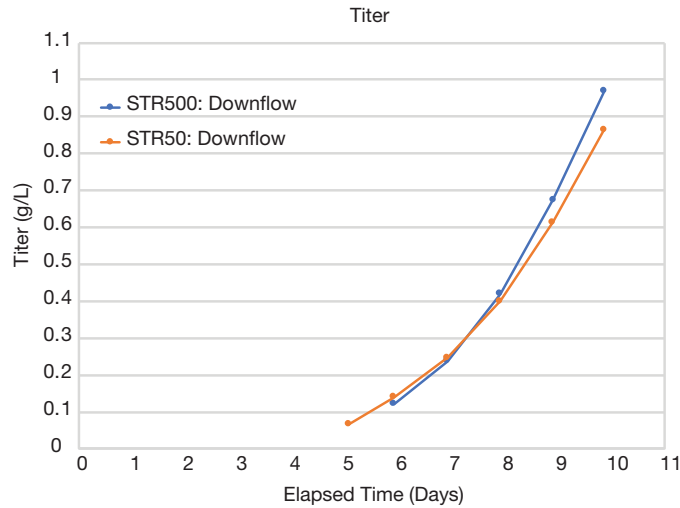
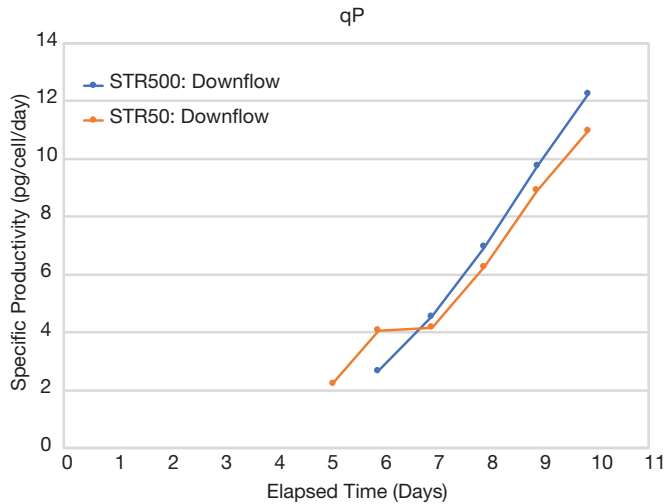


Figure 6

Specific productivity



4. Conclusion

This comparative study demonstrates similar cell growth, viability and monoclonal antibody productivity across Pall's Allegro STR 50 and 500 L bioreactors when operating at the same power input per unit volume, P/V, and same air sparge and overlay hold up (vvm). When operating under the recommended scaling conditions all metabolic profiles match very well indicating the cell culture is in a similar metabolic state across both scales. Overall, the cell culture performance matched well across both scales and can be successfully scaled up from the Allegro STR 50 to the Allegro STR 500 bioreactor.

5. Reference

Application Note USD2980: Characterization and Engineering Performance of Allegro™ STR 200 Single-Use Stirred Tank Bioreactor System

Application Note USD3136: Characterization and Engineering Performance of the Allegro™ STR 1000 Single-Use Stirred Tank Bioreactor

Application Note USD3180: Characterization and Engineering Performance of the Allegro™ STR 2000 Single Use Stirred Tank Bioreactor

Application Note USD2926: Cultivation of CHO Cells in Allegro™ STR 200 Single-Use Stirred Tank Bioreactor

Application Note USD3135: Cultivation of Chinese Hamster Ovary (CHO) Cells in Allegro™ STR 1000 Single-Use Stirred Tank Bioreactor System



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