

Performance Comparison of CHO-S Cell Cultivation and mAb Production in the Allegro™ STR 200 Single-Use Bioreactor and in Conventional Single-Use Bioreactor

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INTRODUCTION

Today, the benefits of single-use (SU) technologies for upstream processing are much more prevalent and heavily adopted within biopharmaceutical industry. Pall has developed the Allegro™ STR stirred single-use bioreactor family which is currently available in 4 sizes: STR 50, STR 200, STR 1000, and STR 2000 (Figure 1). The direct-bottom mechanically driven impeller allows large range of power inputs from 0.002 W/kg up to 0.3 W/kg, while the macro sparger seated below the three 45-degree elephant ear blades results in high oxygen transfer rates (k_{La} up to 40 h⁻¹) and short mixing time (minimum $t_M \sim 10$ s).

Previous studies highlighted that the critical parameters to be kept constant from one scale to another are the volumetric power input (P/V) for agitation and the superficial gas velocity (v_s) for aeration. This will successfully scale up a process at constant k_{La} , keeping equivalent mixing time and shear stress environment in the bioreactor.

Figure 1

Allegro STR stirred single-use bioreactor family



From left to right: STR 50 (working volume 10–50 L), STR 200 (working volume 60–200 L), STR 1000 (working volume 300–1000 L) and STR 2000 (working volume 400–2000 L)

MATERIALS AND METHODS

Cell Line, Medium and Supplements

- ▶ Chinese hamster ovary (CHO)-S clone 38 cell line, producing a human IgG antibody
- ▶ ActiPro[♦] medium (GE Healthcare) supplemented with:
 - 4 mM UltraGlutamine[♦] 1 (Lonza)
 - 1x Gibco[♦] HT Supplement mixture (Thermo Fisher Scientific)
 - Puromycin (1.25 g/L, 10 mg/mL)
- ▶ Feed with HyClone[♦] Cell Boost 7a and Cell Boost 7b (GE Healthcare) start in day 3
- ▶ Glucose solution (45%, 450 g/L) for feed if glucose was below 4 g/L
- ▶ Antifoam C emulsion (Sigma Aldrich)

Bioreactors

- ▶ 125 and 500 mL Erlenmeyer shake flasks (Corning) for inoculum production
- ▶ Flexsafe[♦] RM wave bioreactor bag (Sartorius) for inoculum production
- ▶ Allegro STR 200 single-use bioreactor system with heat mat (Pall)
- ▶ Conventional single-use bioreactor

Operating parameters for the cultivation for Pall's Allegro STR 200 single-use bioreactor and for the conventional single-use bioreactor are shown in Table 1

Table 1

Operating parameters for the Allegro STR 200 SU bioreactor and the conventional SU bioreactor

Parameters	Unit	Allegro STR 200 SU Bioreactor	Conventional SU Bioreactor
Aeration rate	vvm	0.02	0.02
Volumetric power input	W/m ³	50	50
Agitation rate	rpm	73 to 77	132 to 139
Sparging aeration rate	L/min	3.2 to 3.7	3.2 to 3.7
Overlay aeration rate	L/min	5	5
pH	–	7.2 ± 0.2	7.2 ± 0.2
Dissolved oxygen	%	40 ± 10	40 ± 10
Temperature	°C	37	37

Analytics

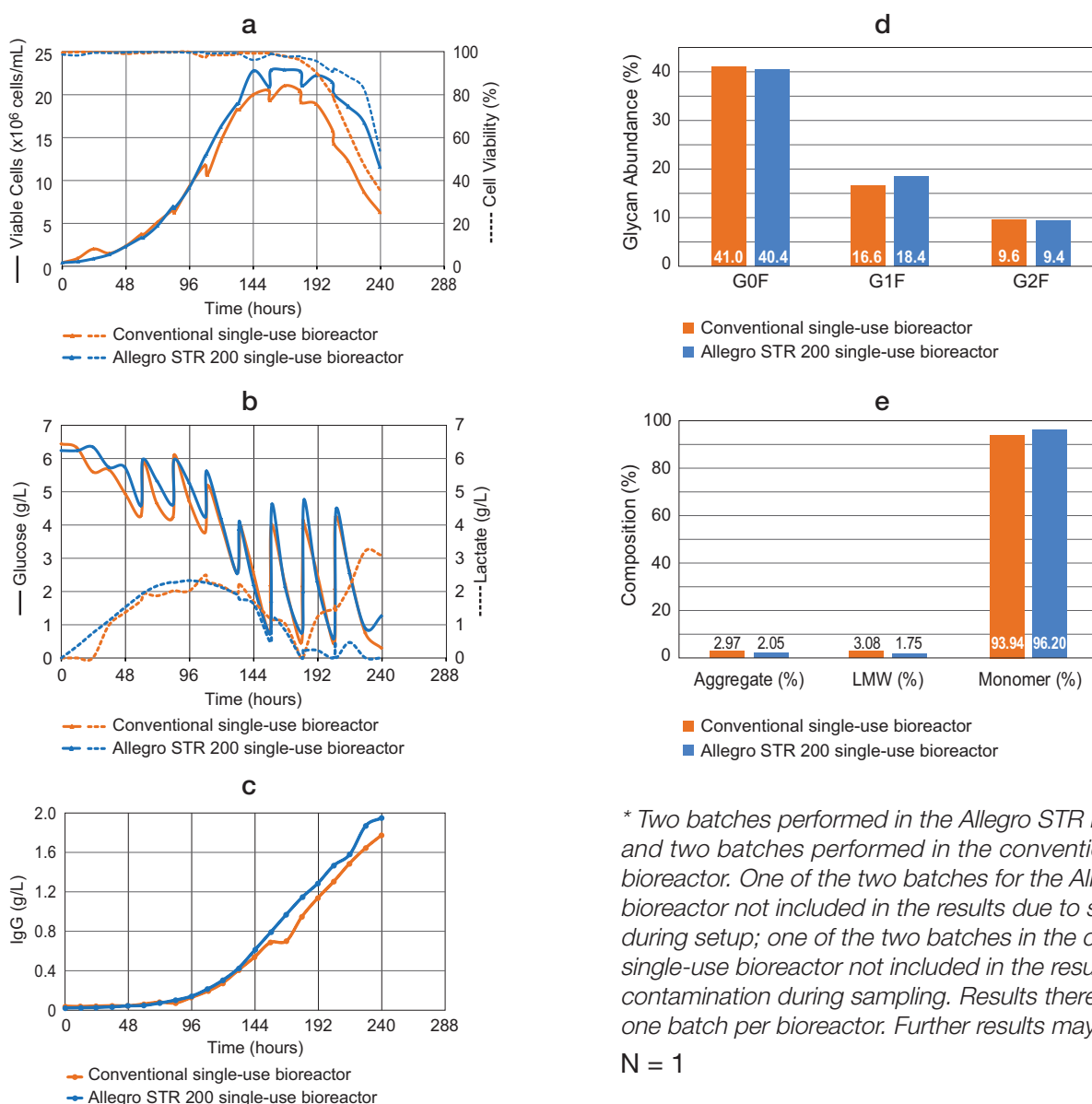
- ▶ Polarographic oxygen sensor InPro[♦] 6800 (Mettler-Toledo) for Allegro STR 200 single-use bioreactor
- ▶ pH sensor (EasyFerm Hamilton) for Allegro STR 200 single-use bioreactor
- ▶ Single-use pH and oxygen sensor incorporated in conventional single-use bioreactor
- ▶ BioProfile[♦] 100 Plus ID 2 (Nova Biomedical)
- ▶ Cedex HiRes and Cedex Bio Analyzer (Roche)
- ▶ Prominence HPLC System (Shimadzu)
- ▶ Xevo[♦] G2 LC-MS and Acquity H-Class Bio UPLC systems (Waters)

RESULTS

- ▶ Simplified handling of a novel bioreactor system
- ▶ Inoculum production with shake flask and wave-mixed bag is an appropriate method
- ▶ Average peak cell density in the Allegro STR 200 single-use bioreactor at the end of the growth phase was $2.15 \cdot 10^7$ cells·mL⁻¹ (Figure 2a)
- ▶ Cell metabolism in terms of glucose uptake and lactate formation is very comparable (Figure 2b)
- ▶ Average monoclonal antibody (mAb) titer of 2.18 g·L⁻¹ at the culture harvest day (day 10) (Figure 2c)
- ▶ The main glycan composition shows a similar finger print for both bioreactors for species G0F, G1F and G2F (Figure 2d)
- ▶ Aggregates in both systems and amount of molecular weight species (LMW) (Figure 2e)

Figure 2

Viable cell density and viability (a), glucose and lactate concentration (b) and IgG concentration (c) as well as IgG quality (d, e)*, number of runs: 1



* Two batches performed in the Allegro STR bioreactor and two batches performed in the conventional single-use bioreactor. One of the two batches for the Allegro STR bioreactor not included in the results due to software error during setup; one of the two batches in the conventional single-use bioreactor not included in the results due to contamination during sampling. Results therefore based on one batch per bioreactor. Further results may differ.

N = 1

CONCLUSION

- ▶ The Pall Allegro STR 200 stirred single-use bioreactor demonstrated excellent performance to successfully cultivate this CHO culture
- ▶ The large elephant ear impeller exhibits efficient mixing and effective oxygen transfer. No limitations could be detected during the cultivations
- ▶ The profiling of mAb quality and the mAb quantity show that the single impeller's shear profile does not influence the antibody
- ▶ Further, using one single impeller only, feeding processes can be applied with high degree of possible working volume

References

- Schirmer, Müller J., Steffen N., Werner S., Eibl R. & Eibl D., "How to produce mAbs in a cube-shaped stirred single-use bioreactor at 200 L scale". R. Pörtner, editor. *Animal Cell Biotechnology. Methods in Molecular Biology (Methods and Protocols), Fourth Edition.* New York, Springer Science+Business Media, LLC (Publication forthcoming)
- "Characterization and engineering performance of the Allegro STR 1000 stirred single-use tank bioreactor"
Pall Application Note USD 3136
- "Cultivation of chinese hamster ovary (CHO) cells in Allegro STR 1000 stirred single-use tank bioreactor system"
Pall Application Note USD 3135

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