

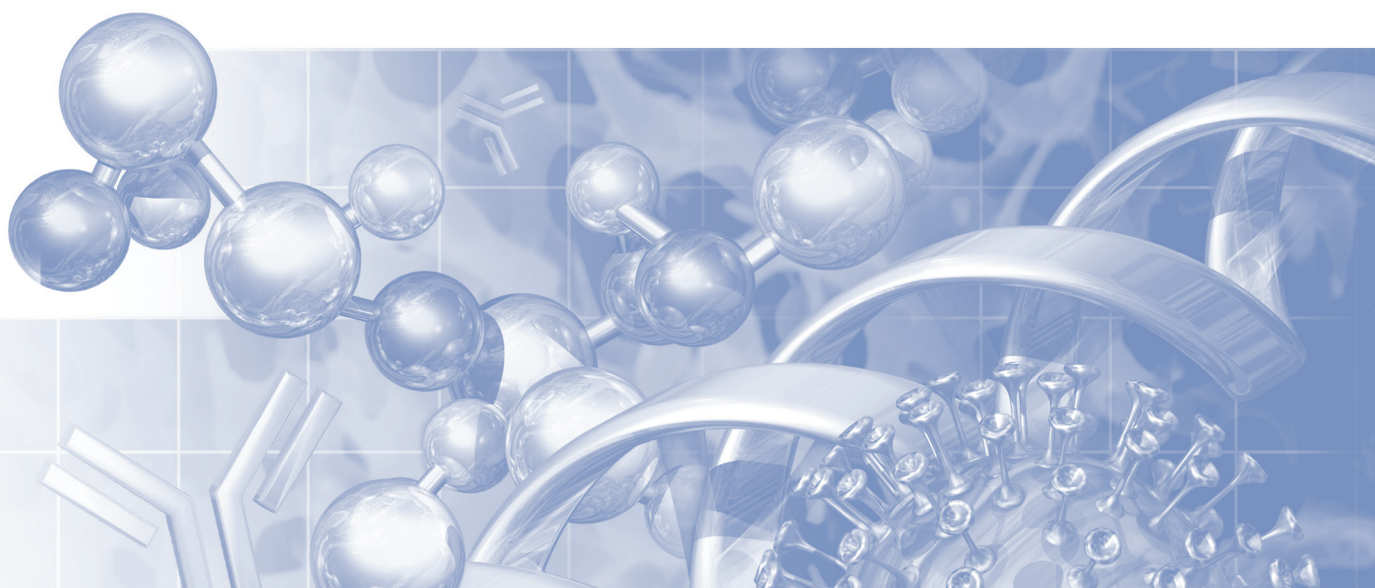


Life Sciences

Application Note

USD3100

CHO Cell Cultivation in the Allegro™ XRS 20 Bioreactor System with Working Volumes from 2 to 25 L



Introduction

The Allegro XRS 20 bioreactor system is a single-use bioreactor system suitable for applications ranging from general life sciences research to seed train operations and small scale production. It features a 3D rectangular biocontainer that is rocked across two independent axes perpendicular to one another. In combination, the improved biocontainer design and biaxial rocking allow for significant improvements in mixing and k_La when compared with a conventional rocking bioreactor that features a 2D “pillow” biocontainer that rocks across a single axis. As a result, the Allegro XRS 20 bioreactor can support higher maximum viable cell densities and increased culture duration as compared to a more conventional design (Pall application note reference USD 2898).

Single use bioreactors for the cultivation of mammalian cells have many established advantages including reduced validation costs, rapid turnaround times and ease-of-use benefits. Additionally, the Allegro XRS 20 bioreactor system features:

- Integrated tubing management, which allows sampling without the need to pause the agitation.
- Bottom mounted drain valve for simplified harvesting and maximal fluid recovery.
- Pall Emflon® II technology on inlet and outlet filters that have been generously sized to allow for increased gas flow rates.
- A comprehensive validation guide to accompany the system which details the testing on the biocontainer including robustness and extractables.
- Agitation control over a wide range of conditions. From gentle agitation required for shear sensitive cultures, to more vigorous conditions, generating higher oxygen transfer rates and decreased mixing times needed for highly productive processes seen in the biopharmaceutical industry today.
- Fully automated pH and DO control via the optical sensors supplied with the biocontainer.
- Three independent mass flow controllers allowing for fixed or sensor automated gassing strategies.
- Three integrated pumps for manual and automated fluid additions.
- User friendly touchscreen interface allowing for easy set up and trending.
- Compatibility with BioCommand™ control software ensures full batch record traceability, making the system ideal for use in GMP manufacturing.

To further expand the process flexibility of the Allegro XRS 20 system, a 25% increase in maximum working volume (25 L) was tested and compared to the current maximum fill volume of 20 L. Testing included a fed batch process using a CHO cell line producing monoclonal antibody (mAb) as well as engineering tests of the Allegro XRS 20 biocontainer and hardware. Additionally, a demonstration of cell culture at the minimum working volume of 2 L was performed with the same CHO cell line as part of a seed train expansion from 2 L to 6.25 L.

The 25 L and 20 L cultures were performed in parallel on duplicate Allegro XRS systems. It has been established in previous optimization studies that this cell line favors conditions where the mixing time is low and the k_La is high. If the engineering performance of the bioreactor was significantly impacted by the increase in fill volume, or the minimum working volume, for example a decrease in oxygen transfer or increase in mixing time, then different cell culture characteristics would be observed between each operating volume for this process.

All tests were performed using commercially available, chemically defined media. Samples were taken throughout each bioprocess to determine cell growth, antibody production, metabolite profiles and product quality for the larger scale work, whilst the low volume study focussed specifically on viable cell density.

In a separate test, the durability of the hardware and biocontainer was also proven at the higher fill volume. Two Allegro XRS 20 systems were run at the maximum agitation and angles (35 cpm, $X=15^\circ$, $Y=15^\circ$) at 40 °C, using water as the test fluid. The maximum volume in the bioreactor was also increased by 10% to 27.5 L, representing a worst case culture. The bioreactors were operated continually for 28 days.

Materials and Methods

Materials

- Allegro XRS 20 Bioreactor System (Pall Life Sciences)
- Allegro XRS 20 Single-Use Biocontainer (Pall Life Sciences)
- GIBCO® CD FortiCHO II® medium (Life Technologies®), 4 mM UltraGlutamine 1 Supplement (Lonza), 10 mM hypoxanthine (Sigma-Aldrich®), 1.6 mM thymidine (Sigma-Aldrich).
- EfficientFeed® C (Life Technologies)
- Antifoam C (Sigma-Aldrich)
- 1 M Carbonate solution – for automatic pH control (Sigma-Aldrich)
- 45% Glucose solution (Sigma-Aldrich)
- Cell line derived from CHO-S, producing a human IgG antibody

Methods

Inoculum Preparation

For the 20 and 25 L fed-batch cultures, the CHO clone was expanded in FortiCHO II chemically defined medium from a working cell bank into a series of shake flasks (Corning), then to an 11.5 L culture in an Allegro XRS 20 bioreactor system. This culture was used to inoculate the 4 Allegro XRS 20 bioreactors used in the test, each with an initial volume of 5 L. Cells were grown to mid-exponential phase at 37 ± 1 °C. The pH was maintained at pH 7.2 by sparging with a constant blend of 90% air/10% CO₂ at 1 L/min. For the 2 L demonstration, the culture was inoculated directly from the shake flasks.

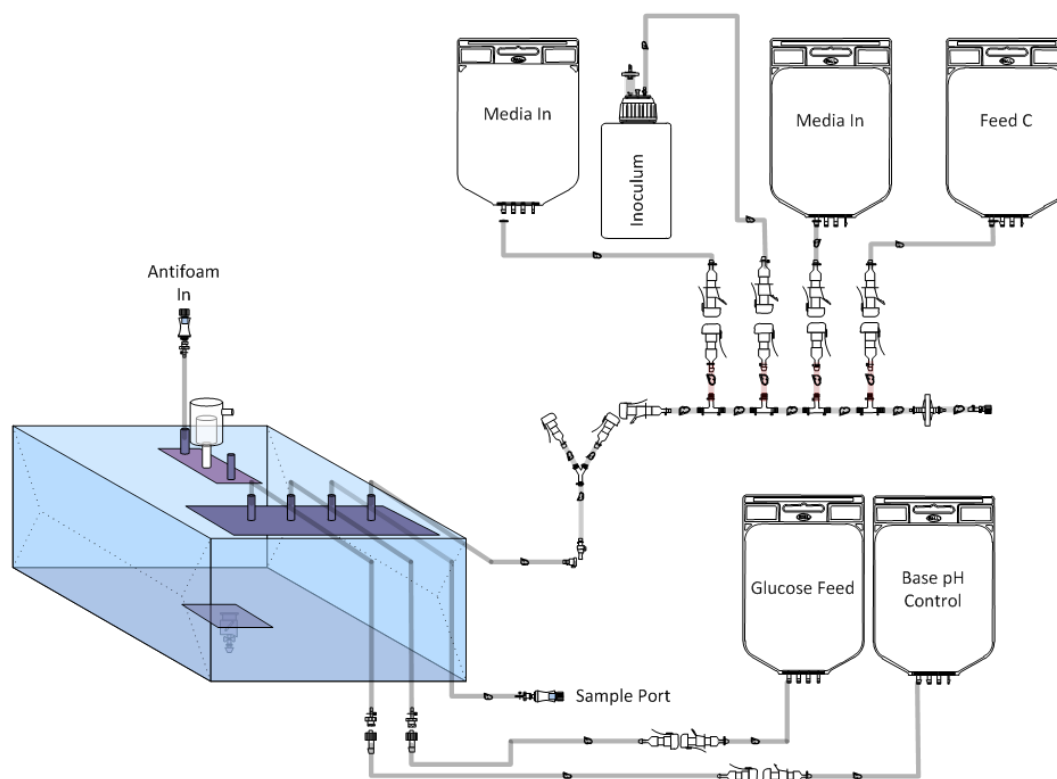
Allegro XRS 20 Bioreactor Set-up

Prior to inoculation, the biocontainers were removed from their protective packaging and placed into a laminar flow hood. The biocontainers were prepared for use according to the manufacturer's instructions and fluid transfer connections were added to the existing ports as required by the process. The closed biocontainers were then placed into the Allegro XRS 20 bioreactor, secured and inflated with an air and CO₂ mix. The filter heaters were secured to the vent filters to prevent condensation. The calibration settings for the optical pH and DO sensors were entered into the controller software.

The required volume of CD FortiCHO II medium was aseptically transferred to each biocontainer. An initial working volume of 14 L was needed for the 20 L and 17.5 L for the 25 L maximum working volume once inoculated. The seed train bioreactor, at the minimum fill was inoculated at 2 L. The air flow to each bioreactor was supplemented with 8% CO₂ to maintain a stable media pH. Bioreactors were set to operate at 37 ± 1 °C with a gentle agitation overnight. This ensures that the medium reaches the temperature set point and the optical sensors have sufficient time to stabilize. Two Allegro XRS 20 bioreactors were set up at each starting fill volume for comparison, with the exception of the 2 L.

Figure 1

Schematic of the Culture Operation.



Schematic illustration of fed-batch culture operation in the Allegro XRS 20 system. Associated single-use technology (single-use storage biocontainers and Kleenpak™ sterile connectors) shown above can be purchased from Pall Life Sciences

Inoculation and Fed-Batch Strategy

A sample from each bioreactor was obtained to measure the offline pH. This was used to verify the online pH reading on each controller. An offset was applied if necessary. pH and DO control was activated on all tests and set to 7.2 ± 0.05 and 40% respectively. Culture conditions were applied as detailed in Table 1.

Table 1

Summary of bioreactor and process conditions

Parameter	Maximum Fill 20 L Final Working Volume	25 L Final Working Volume	Minimum Fill 2 L Seed Expansion	6.25 L Seed Expansion
Start Agitation (<15 L)				
• Rate (CPM)	30	30	20	25
• X Angle (Deg)	10	10	2	5
• Y Angle (Deg)	5	5	2	5
Agitation (>15 L)				
• Rate (CPM)	35	35	N/A	N/A
• X Angle (Deg)	15	15	N/A	N/A
• Y Angle (Deg)	5	5	N/A	N/A
Aeration Rate (SLPM)	1.0	1.0	1.0	1.0
Seed Density (10^6 VC)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
pH Set Point	7.20 ± 0.05	7.20 ± 0.05	7.20 ± 0.05	7.20 ± 0.05
DO Set Point (%)	> 40	> 40	> 40	> 40
Glucose Concentration (g/L)	> 4.0	> 4.0	> 4.0	> 4.0

For the fed-batch cultures, an initial feed of either 1.2 L or 1.5 L (for the 20 L and 25 L cultures respectively) of EfficientFeed C was added to each when the viable cell density reached $8.0 \pm 2.0 \times 10^6$ cells/mL. Four further bolus additions of 1.2 L or 1.5 L were added at 24 hour intervals from the time of the initial feed. Concentrated glucose solution was added when required to maintain each culture above 4 g/L of glucose. Each bioreactor culture was inspected visually for foam accumulation. A 25% suspension of Antifoam C was added when necessary.

Analytics

Fed batch Cultures (20 & 25 L final volume) were sampled daily and process using a BioProfile FLEX[♦] analyser to determine viable cell density (VCD), Viability (%), pH, glucose, lactate and ammonium.

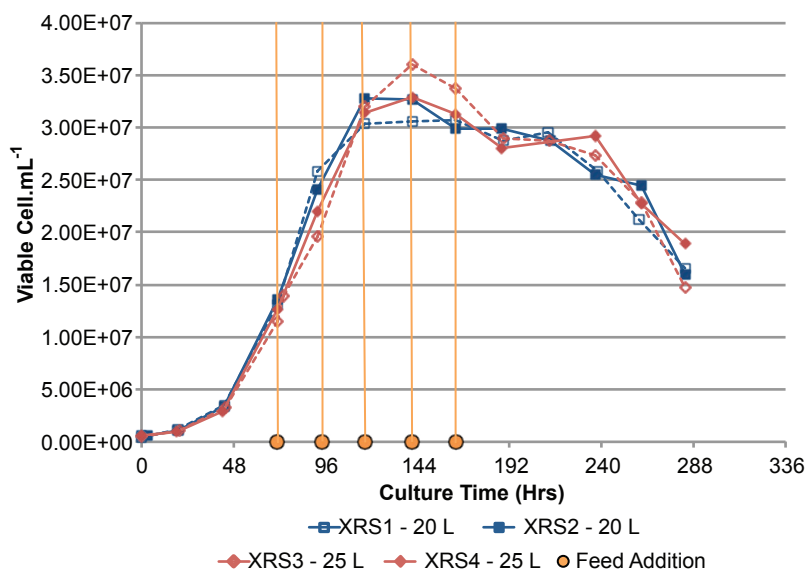
VCD and % viability for the low volume culture was determined using a Vi-Cell automated cell counter. Culture samples were also taken on a routine basis for offline mAb quantification by Bio-Layer Interferometry (BLI), charged variant analysis by UPLC cation exchange chromatography and glycoform profiling by mass spectrometry.

Results and Discussion

Cell Growth

Figure 2

Comparison of viable cell density and culture viability at 20 L and 25 L maximum working volumes. Data plotted for each process from daily and post feed FLEX analysis.

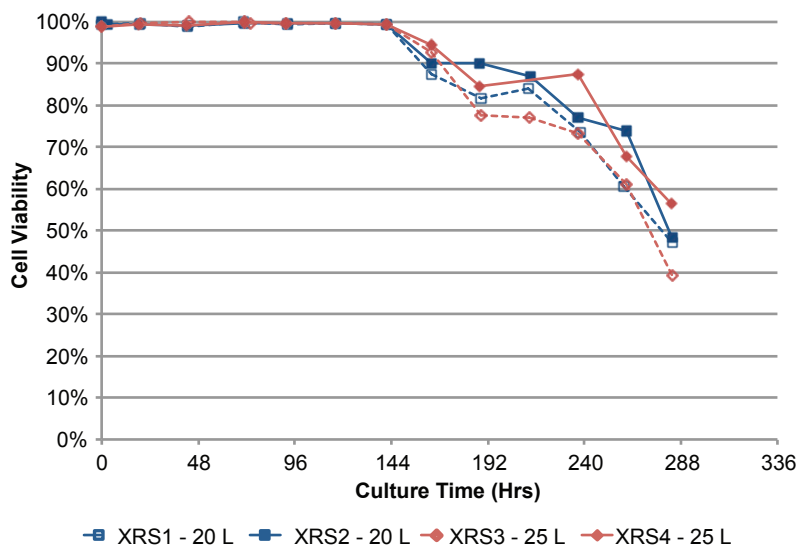


Cultures at both starting volumes grew at similar rates with all feeds being initiated at the same time. Peak cell counts were all seen between 120 and 144 hours culture duration, as shown in Figure 2.

As is typically seen in the Allegro XRS 20 bioreactor, a high culture viability was maintained until stationary phase was entered. The viability trend for all Allegro XRS 20 cultures was similar, independent of fill volume as shown in Figure 3.

Figure 3

Percentage viability of each Allegro XRS 20 culture in relation to time.



The peak cell densities were 31 to 36 million viable cells/mL, within 10% of the mean maximum VCD, as shown in Figure 5. The culture duration is also the same for each bioreactor, 12 days post final expansion. These data indicate that there is no difference in CHO growth patterns for fed batch cultures of 20 L and 25 L, supporting the expectation that the Allegro XRS 20 bioreactor design is compatible with the higher working volume.

Figure 4

Cell growth data from the first 90 hours of the low volume seed train bioreactor.

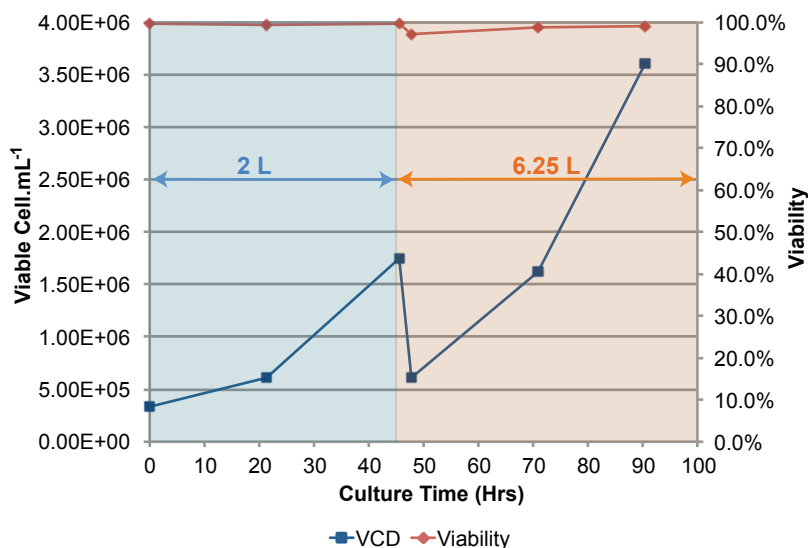
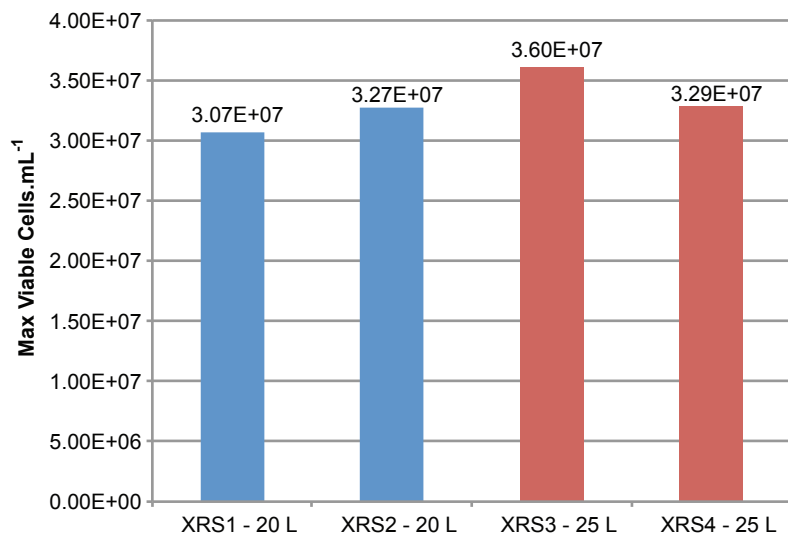


Figure 4 shows the cell growth and viability profile from the minimum working volume culture. Doubling times and viability are comparable to the expansion phase of all the Allegro XRS 20 cultures including those reported here.

Figure 5

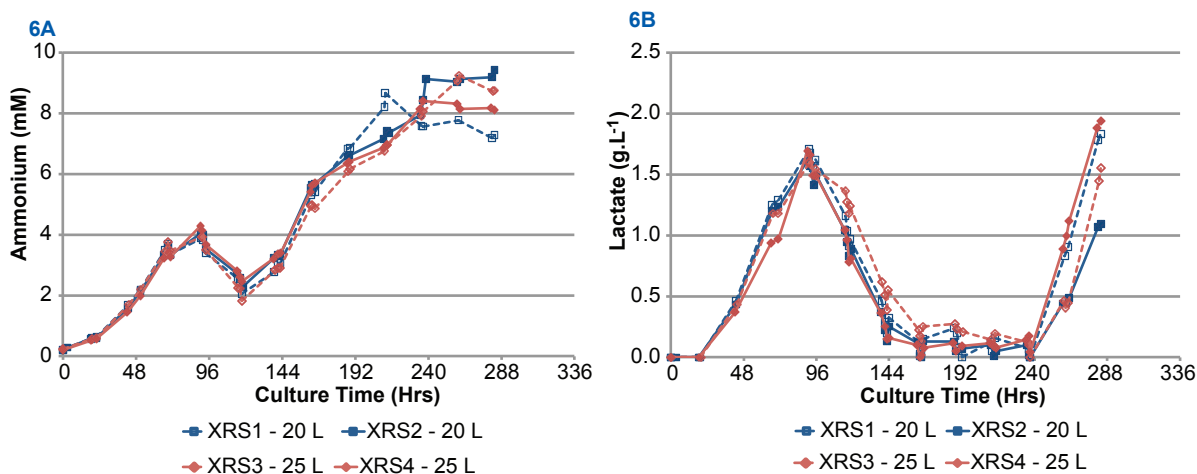
Maximum viable cell density for the fed-batch cultures in the Allegro XRS 20 System at 20 L and 25 L maximum working volume.



Metabolite Profiles

Figure 6

Comparison of lactate (6a) and ammonium (6b) concentration in Allegro XRS 20 bioreactor at 20 L and 25 L maximum working volumes. Data plotted for each process from daily and post feed FLEX samples



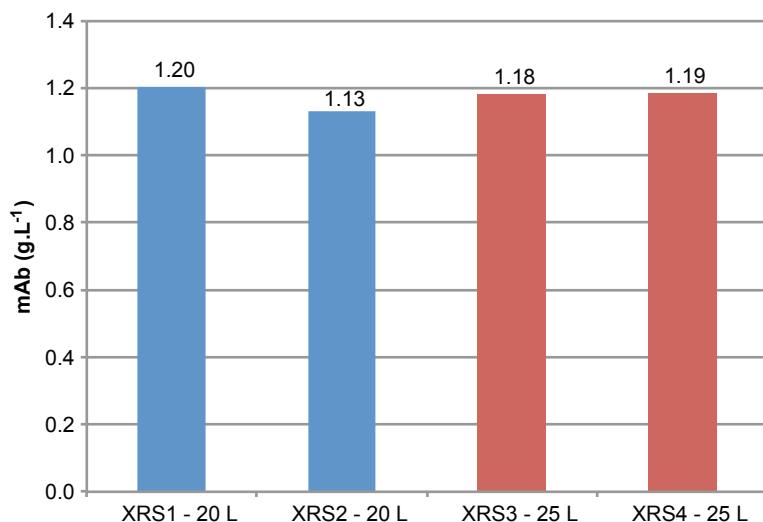
Lactate was produced at a similar rate in the initial stages of each culture (Figure 6a). The switch from net lactate production to reuse corresponds with the onset of the stationary phase and mAb production phase.

Ammonium production shown in Figure 6b again indicates a similar trend for all culture volumes during the exponential growth phase, with reuptake observed at the switch to stationary phase. This trend continues until harvest, and is typical for this cell line fed batch process in the Allegro XRS 20 bioreactor. The results from both volumes show similar trends for lactate and ammonium production.

Antibody Production

Figure 7

Antibody titer for the fed-batch cultures in the Allegro XRS 20 System at 20 L and 25 L maximum working volume. Analysis done using Octet® RED384 (Fortebio)

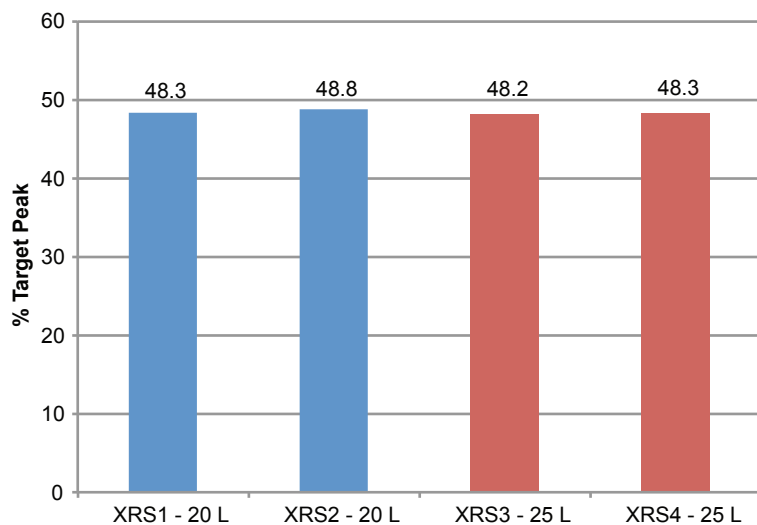


Antibody production (Figure 7) in these 4 parallel cultures is similar at 20 L and 25 L maximum fill volumes indicating no negative impact on mAb productivity due to the increased culture volume.

Product Quality

Figure 8

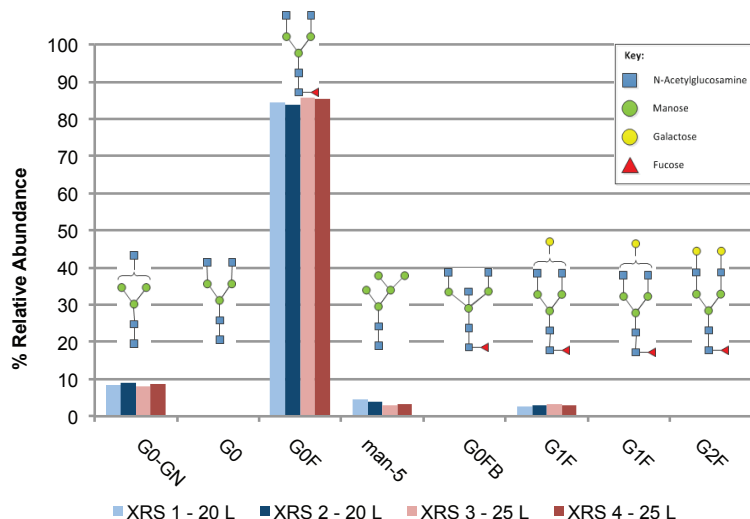
Comparison of target antibody in the Allegro XRS 20 System at 20 L and 25 L maximum working volume
Process conditions in the bioreactor can have an impact on the quality of the therapeutic protein produced.
Analysis done using ACQUITY UPLC H-Class Bio (Waters)



As process conditions in the bioreactor have an impact on the quality of the therapeutic protein produced, it was also important to demonstrate that increasing the maximum fill volume had no significant impact on product quality. Figure 8 shows results of the antibody charge variant analysis of mAb purified from the final harvest clarified supernatant. A similar target mAb value is observed at both reactor volumes, showing no impact on the target species of the increase in maximum working volume. Figure 9 shows data comparing the Glycan profiles of the same antibody samples. A similar profile was observed for all bioreactors at each volume, with G0F being the dominant glycoform in all cultures. The charge variant and glycan data is also in line with historical data (not shown) for this process. Taken together, these data show no differences in mAb quality from 20 L compared to 25 L culture volumes.

Figure 9

Results of glycosylation profiling for harvest samples in the Allegro XRS 20 System at 20 L and 25 L maximum working volume. Analysis done using Xevo G-2 QToF (Waters).



Biocontainer Durability

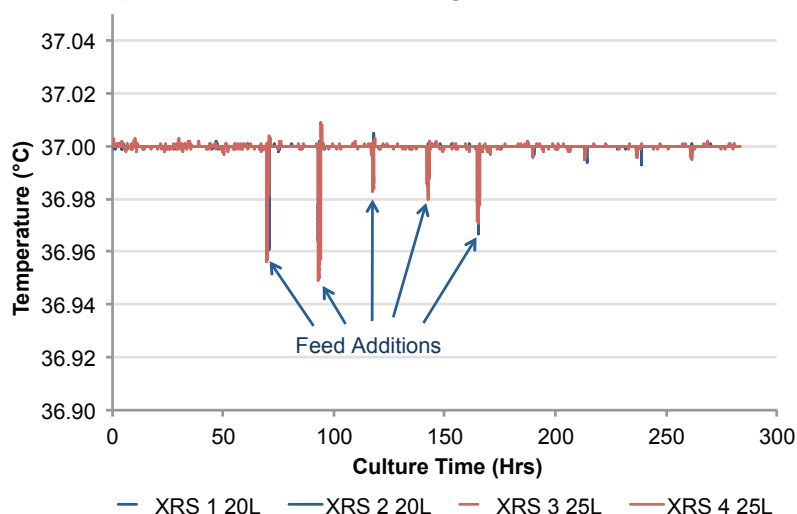
The Allegro XRS 20 Bioreactor was designed for a 20 L maximum fill volume, but with a very wide safety margin. To demonstrate the biocontainer and hardware could accommodate the 25 L fill volume, the test volume in the biocontainer was increased a further 10% to 27.5 L. The system was run at its maximum rock rate and angles (35 cpm, 15° Y, 15° X) and maximum temperature (40 °C) to mimic the most extreme use conditions for 28 days. The biocontainer and hardware showed no signs that the additional volume was in anyway compromising the system. These results are consistent with earlier data testing the durability of the Allegro XRS 20 biocontainers and system and are expected based on the safety margins built into the system design.

Temperature Control

The temperature profiles from each fed-batch bioreactor are shown in Figure 10. The data from all 4 units overlapped, confirming that all units effectively control temperature with volumes from 5 L to 25 L. Temperature in all 4 bioreactors was set to and maintained at 37 °C. There were slight temperature deviations with feed additions as the feed solution was at room temperature (note spikes on the temperature profiles), however the system recovered quickly.

Figure 10

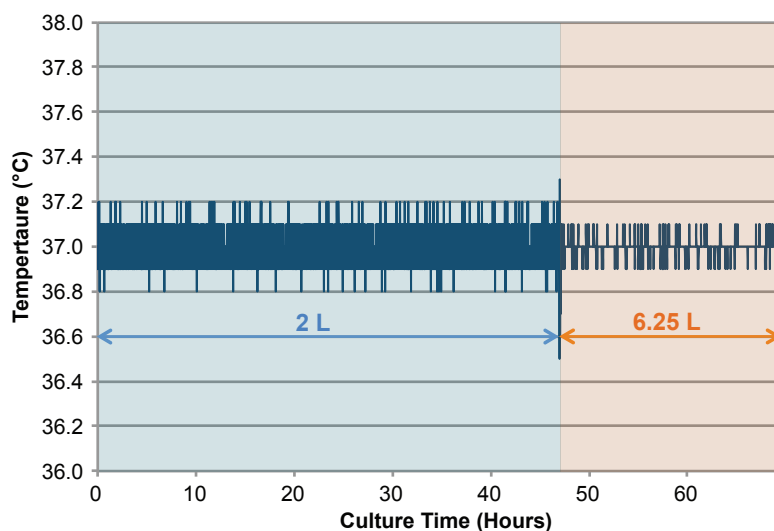
Online temperature data from each Allegro XRS 20 bioreactor



The online temperature data from the low volume seed expansion is shown in Figure 11. Small fluctuations in ambient temperature can have a greater effect on low volume cultures due to the high surface area to volume ratio. However accurate temperature control was achieved at the minimum working volume. The temperature control at the 2 L fill volume remained within ± 0.2 °C of the set point. Temperature dropped slightly at hour 48 of the culture as media was added to bring the volume up to 6.25 L.

Figure 11

Online temperature data for the low volume seed train expansion

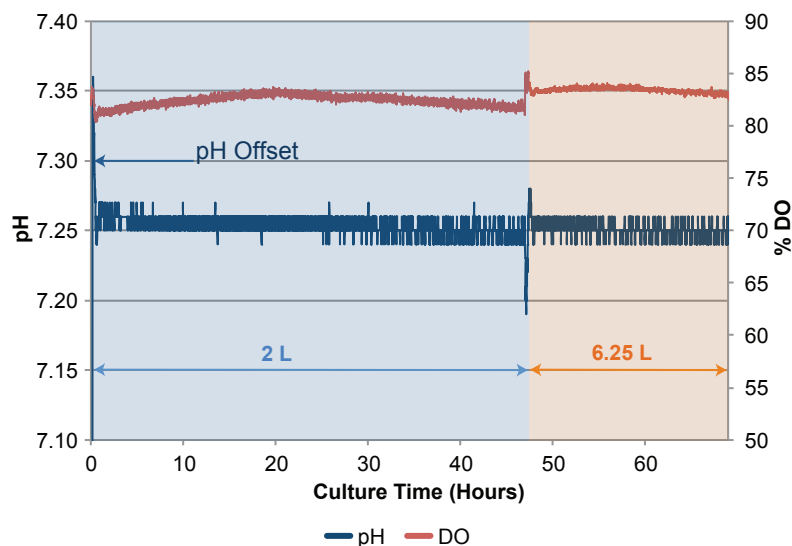


Sensor Control at Minimum Volume

In addition to accurately controlling temperature, automated control over pH and DO levels was also demonstrated at the minimum fill volume. Figure 12 shows that pH was controlled at the upper deadband limit of 7.25. The DO remained high as the cell density was low; however stable sensor data was obtained operating at the low angles and cpm appropriate for low volume cultures (Table 1).

Figure 12

Online pH and DO sensor data from seed train Allegro XRS 20 bioreactor at 2 L and 6.25 L volume.



Conclusion

Cell culture testing has confirmed internal engineering data that the Allegro XRS 20 bioreactor system can be used at volumes from 2 L to 25 L. The cell growth, mAb production and quality are similar at 20 L and 25 L final volumes for the fed-batch process tested. The increase in volume is also well within the tolerances of both the hardware and biocontainer.

As the fed batch cell culture performance was equivalent for 20 L and 25 L final volumes, the biochemical engineering parameters such as $k_L a$ and mixing time were sufficient for the requirements of this culture at both 20 and 25 L volumes.

The data presented in this Application Note has also demonstrated the suitability of the Allegro XRS 20 bioreactor for performing cell culture operations at volume of 2 to 25 L and is therefore suitable for seed train expansion and low volume applications.

The data comparing cell culture performance at 20 and 25 L combined with the biocontainer and hardware testing at 27.5 L confirms that the Allegro XRS 20 bioreactor working volume range can be extended – 2 L to 25 L. We believe this will help our customers improve their process design allowing for a greater level of flexibility.



Life Sciences

Corporate Headquarters

Port Washington, NY, USA
+1.800.717.7255 toll free (USA)
+1.516.484.5400 phone
biopharm@pall.com e-mail

European Headquarters

Fribourg, Switzerland
+41 (0)26 350 53 00 phone
LifeSciences.EU@pall.com e-mail

Asia-Pacific Headquarters

Singapore
+65 6389 6500 phone
sgcustomerservice@pall.com e-mail

Visit us on the Web at www.pall.com/bioreactors

E-mail us at bioreactors@pall.com

International Offices

Pall Corporation has offices and plants throughout the world in locations such as: Argentina, Australia, Austria, Belgium, Brazil, Canada, China, France, Germany, India, Indonesia, Ireland, Italy, Japan, Korea, Malaysia, Mexico, the Netherlands, New Zealand, Norway, Poland, Puerto Rico, Russia, Singapore, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, the United Kingdom, the United States, and Venezuela. Distributors in all major industrial areas of the world. To locate the Pall office or distributor nearest you, visit www.pall.com/contact.

The information provided in this literature was reviewed for accuracy at the time of publication. Product data may be subject to change without notice. For current information consult your local Pall distributor or contact Pall directly.

© 2016, Pall Corporation. Pall, , Allegro, the Allegro design, Emflon, Kleenpak and Octet are trademarks of Pall Corporation. © indicates a trademark registered in the USA and TM indicates a common law trademark. Filtration.Separation.Solution. is a service mark of Pall Corporation. ♦BioCommand is a trademark of Eppendorf Inc; EfficientFeed GIBCO, FortiCHO and Life Technologies are trademarks of Life Technologies; Bioprofile FLEX is a trademark of Nova Biomedical Corporation, and Sigma-Aldrich is a trademark of Sigma-Aldrich Corporation.

Filtration. Separation. Solution.SM