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

Currently, stirred tank reactors (STRs) represent the gold standard for the large scale growth of suspension cell lines. Cell culture performance is strongly influenced by the efficiency of bulk fluid mixing and the oxygen mass transfer coefficient ($k_La$). The success of traditional stainless steel STR systems lies in their impeller driven agitation that can deliver a wide range of power inputs to the fluid. The Allegro range of single-use stirred tank bioreactors have a directly driven impeller, which allows a wide range of power inputs to be achieved.

In this application note, the performance data for the batch cultivation of a CHO recombinant monoclonal antibody (mAb) process, in the Allegro STR 1000 single-use stirred tank bioreactor, is presented. The scalability of the Allegro STR range is demonstrated from a 10 L, re-usable, bench-top bioreactor. As part of the study a seed train incorporating the Allegro XRS 20 and Allegro STR 200 bioreactors was used to generate the inoculum volume required. Additionally, the assessment of the robust performance of the Allegro STR 1000 single-use pH and DO sensors, relative to conventional re-useable sensors, is shown.

Materials and Methods

Bioreactors
- Allegro STR 1000 single-use bioreactor system (Pall)
- Allegro STR 200 single-use bioreactor system (Pall)
- Allegro XRS 20 bioreactor system (Pall)
- 10 L Celligen® 310 bioreactors (Eppendorf)
- 250 mL Erlenmeyer shake flask (Corning)

Analytics
- Polarographic oxygen sensor InPro® 6800 (Mettler-Toledo)
- pH sensor InPro 3250 (Mettler-Toledo)
- pCO$_2$ sensor InPro 5000 (Mettler-Toledo)
- M400 Transmitter (Mettler-Toledo)
- BioProfile® FLEX Analyzer (Nova Biomedical)

Cell Line, Medium and Supplements
- Cell line derived from CHO-S, producing a human IgG antibody
- IS CHO-CD® G10.3 medium (Irvine Scientific)
- L-Alanyl-L-Glutamine (Biowest)
- 1 M NaOH & HCL (Sigma Aldrich)
- 45% Glucose Solution (Sigma Aldrich)
- 25% Antifoam C Solution (Sigma Aldrich)

Inoculum Preparation

The CHO clone was maintained in G10.3 CHO-CD medium, supplemented with 4 mM L-Alanyl-L-Glutamine, for all bioreactors. Cells from the working cell bank were cultured in a series of shake flasks, which were expanded into a 25 L Allegro XRS 20 bioreactor system. An Allegro STR 200 bioreactor was seeded from the Allegro XRS 20 bioreactor to generate the inoculum for the Allegro STR 1000 culture. Seed train cells were grown to the mid-exponential phase at 37 °C and 8% CO$_2$. Cells were cultivated at 135 RPM in shake flasks, 95 RPM (100 P/V W.m$^{-3}$) in the Allegro STR 200 bioreactor and at 35 CPM in the Allegro XRS 20 system (X amplitude 15 degrees, Y amplitude 5 degrees).
Bioreactor Set-up

The pH, dissolved oxygen (DO) and partial pressure of CO₂ (pCO₂) sensors were calibrated as recommended by the manufacturer’s instructions. pCO₂ sensors were integrated into the extra sensor connection on the Allegro STR 200 and STR 1000 bioreactors with a 4-20 mA connection via the M400 transmitter, which allowed for the signal to be read by the controller to initiate feedback control. Single-use (SU) pH and DO sensors were integrated into the biocontainer and logged alongside the re-useable sensors. The sensors were fitted into probe bellows (Pall) and sterilized by autoclaving, before being inserted into the biocontainer via the Kleenpak® sterile connectors at the front of the biocontainer. The probes were then connected to the Allegro STR 200 and STR 1000 controller cabinet via the labelled transmitter cables. Using the 1 in. Kleenpak II sterile connector, 900 L of pre-prepared sterile medium was pumped into the biocontainer. The temperature probe was inserted into the biocontainer temperature sleeve before turning on the agitation and the temperature control unit (TCU); to allow the liquid to reach the set-point of 37 ºC overnight. Prior to inoculation the medium was allowed to equilibrate to the starting conditions.

Bioreactor Scale-Up

As translating scaling parameters directly from the 10 L to Allegro STR 1000 bioreactor is difficult due to the non-linear relationship of critical scaling factors, a step-wise scaling strategy was adopted. For this, the operation of the 10 L STRs were scaled-up to the Allegro STR 200 bioreactor which in turn was scaled up to the Allegro STR 1000 bioreactor. The parameters for scale-up were determined from a bench-scale characterization study of the process and from the scale-up of the biochemical engineering parameters. In the bench scale study, the interaction of the agitator speed and airflow rate (i.e. the $k_L a$) had a greater impact on the cell culture performance than either parameter individually; $k_L a$ is known to be important in mammalian cell culture. Therefore, $k_L a$ was maintained during scale-up. However, due to their effect on the fluid dynamics, and hence the potential to impact on process performance, power input per volume (P/V), tip speed (Vt), superficial gas velocity ($v_{SG}$) and aeration (VVM, gas volume flow per unit of liquid volume per minute) were also taken into consideration during scale-up. This more holistic approach to scale-up, can minimize large differences across scales. Due to the non-linear relationships between engineering parameters it is not possible to maintain a linear scale-up for all parameters. Therefore, a constrained optimization was conducted to obtain the agitation and airflow parameters. The output variables normalized to the Allegro STR 200 bioreactor are shown in Figure 2. In this way a more balanced approach to maintaining the parameters could be adopted. The operational parameters are listed in Table 1.
Table 1
Control set-points for the Allegro STR 1000 bioreactor, Allegro STR 200 bioreactor and 10 L system

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>10 L</th>
<th>STR 200</th>
<th>STR 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agitator Speed</td>
<td>RPM</td>
<td>190</td>
<td>105</td>
<td>71</td>
</tr>
<tr>
<td>Power Input per Volume</td>
<td>P/V (W.m(^{-3}))</td>
<td>103</td>
<td>106</td>
<td>95</td>
</tr>
<tr>
<td>Tip Speed</td>
<td>(s(^{-1}))</td>
<td>1.1</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Aeration Rate</td>
<td>L.min(^{-1})</td>
<td>1.33</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>Aeration</td>
<td>vvm</td>
<td>0.133</td>
<td>0.075</td>
<td>0.035</td>
</tr>
<tr>
<td>(k_{La})</td>
<td>hr(^{-1})</td>
<td>7.4</td>
<td>7.0</td>
<td>7.6</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>7.1</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td>DO</td>
<td>%</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>pCO(_2)</td>
<td>mmHg</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

Inoculation and Batch Culture

Once the Allegro STR 200 bioreactor cell density reached the desired harvest count, the harvest line from the Allegro STR 200 bioreactor was connected to the Allegro STR 1000 bioreactor, and cells pumped directly into the Allegro STR 1000 bioreactor. Simultaneously, cells were taken from the harvest line on the Allegro STR 200 bioreactor via Kleenpak sterile connectors for inoculation of 10 L bench scale STRs. Kleenpak sterile disconnectors were used to make a sterile disconnect from the Allegro STR 200 bioreactor.

Once the required inoculum for the Allegro STR 1000 bioreactor and 10 L STRs had been transferred, the Allegro STR 200 bioreactor was topped-up to a fill volume of 200 L, ensuring the same starting cell density for each of the systems on test. All bioreactors were seeded to a starting density of 5 x 10\(^5\) cells.mL\(^{-1}\) at a cell viability of greater than 99%. The cell culture set-up is shown in Figure 3.
The glucose concentration was checked daily and the concentration maintained at $4 \pm 2 \text{ g.L}^{-1}$ by regular feed additions of a 45% solution when required. The generation of foam was reduced by adding antifoam when required. The cultures were stopped when culture viability decreased to $60 \pm 10\%$.

**Analytics**

Cell growth, nutrient/metabolites and gas/electrolytes were monitored via manual sampling using a BioProfile FLEX Analyzer. Online sensors were checked for drift by comparing to off-line sample analysis and offsets made if required. Culture samples were taken daily for offline mAb quantity and quality analysis, carried out at Pall.

**Results and Discussion**

**Comparison of Batch Culture Cell Growth**

**Seed Train**  
Figure 4 shows the viable cell growth of the seed train for the study. An Allegro XRS 20 bioreactor was inoculated at 5 L, expanded to 25 L and used to inoculate a Allegro STR 200 bioreactor to provide the final inoculum for the study.

**Figure 4**  
Allegro STR 1000 bioreactor seed train, showing the culture from Allegro XRS 20 bioreactor to Allegro STR 200 bioreactor to Allegro STR 1000 bioreactor
Viable Cell Growth

Cell growth and culture viability were equivalent across scales. The cells followed an exponential growth phase to a peak viable cell concentration of approximately $1.4 \times 10^7$ cells.mL$^{-1}$, with less than 3% difference between scales. For all bioreactor scales, the cells entered the decline phase at the same point (140 hours), with a similar death rate observed for all scales. The similar death rate is reflected in the similar cell viability.

Figure 5
Comparison of cell growth profiles (solid lines) and cell viability (dashed lines) between the Allegro STR 1000 and STR 200 bioreactor and 10 L glass vessel bioreactor. Dashed lines represent the calculated cell viability determined by the current viable cell count divided by the previous maximum cell count.

Figure 5 indicates highly similar exponential growth phases shown by the overlap in growth profiles between scales. The specific growth rate, the growth rate over the exponential phase, of 0.028 – 0.029 hr$^{-1}$ (doubling time of 24 to 25 hours) was observed for all cultures, shown in Table 2.

Table 2
Maximum viable cell concentration and specific growth rate across bioreactor scales

<table>
<thead>
<tr>
<th>Bioreactor Scale</th>
<th>Maximum Viable Cell Concentration (Cells.mL$^{-1}$)</th>
<th>Specific Growth Rate (hr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allegro STR 1000</td>
<td>$1.35 \times 10^7$</td>
<td>0.028</td>
</tr>
<tr>
<td>Allegro STR 200</td>
<td>$1.39 \times 10^7$</td>
<td>0.029</td>
</tr>
<tr>
<td>10 L - 1</td>
<td>$1.36 \times 10^7$</td>
<td>0.029</td>
</tr>
<tr>
<td>10 L - 2</td>
<td>$1.38 \times 10^7$</td>
<td>0.028</td>
</tr>
</tbody>
</table>
Comparison of Monoclonal Antibody Production

Product Titer

Figure 6
Normalized mAb titers across bioreactor scales. Note: mAb quantity was determined by protein-A affinity chromatography on a Prominence HPLC (Shimadzu, Kyoto, Japan) using PDA (UV) detection.

Monoclonal antibody titers were normalized with respect to the maximum titer measured in the Allegro STR 200 culture, as Allegro STR 200 bioreactor was the mid-point for scale-up method. Less than 11% difference in final mAb titer was seen across the bioreactor scales (Figure 4), within the expected variation for the process.

Product Quality
A similar glycan fingerprint for all bioreactor scales was seen, Figure 7, with only minor differences between bioreactors scale; mainly an increase in G0F for the Allegro STR 200 and STR 1000 bioreactors. Figure 7 indicates a predominantly fucosylated species with the main peak being G0F, as is normal for this IgG antibody. Glycan analysis did not detect the presence of mannose-5 species which can cause concern due to uncertainty about its impact on clearance, immunogenicity, and efficacy.²

Figure 7
Harvest mAb glycan analysis across bioreactor scales. Note: N-linked glycan profiling was determined by Hillic chromatography (H-Class Bio Acquity UPLC, Waters, Milford, USA) with detection by fluorescence (FLR) Quadropole time-of-flight mass spectrometer (Waters)
Charge variant analysis indicates the composition of mAb in terms of acidic and basic variants with the target mAb being the unmodified. Figure 6 and Table 3 demonstrate that there was less than 5% variation in target mAb between the scales, indicating a similar product quality, in terms of charged variants. The amount of aggregates was very similar between scales and Table 3 shows less the 0.2% difference between the aggregates and low molecular weight species (LMW).

**Table 3**
Charge variant and antibody composition at the harvest point. Values in brackets represent the standard deviation. Note: Charge variants were analyzed using weak cation exchange chromatography on a Classic Acquity UPLC® (Waters, Milford, USA).

<table>
<thead>
<tr>
<th>Scale</th>
<th>Acidic Variant (%)</th>
<th>Target mAb (%)</th>
<th>Basic Variant (%)</th>
<th>Aggregate (%)</th>
<th>Monomer (%)</th>
<th>LMW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allegro STR 1000</td>
<td>17.4</td>
<td>66.4</td>
<td>16.2</td>
<td>0.7</td>
<td>99.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Allegro STR 200</td>
<td>17.9</td>
<td>67.3</td>
<td>14.8</td>
<td>0.9</td>
<td>98.9</td>
<td>0.2</td>
</tr>
<tr>
<td>10 L (n=2)</td>
<td>22.5</td>
<td>59.9</td>
<td>19.6</td>
<td>0.9</td>
<td>98.9</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Metabolites Profiles**

Similar lactate profiles can be seen between bioreactor scales (Figure 8). Lactate levels only deviated once the cells entered the decline phase, at which point lactate is released. Little difference was seen in the ammonium trend between bioreactors, shown in Figure 8.

**Figure 8**
Lactate (solid lines) and ammonium (dashed lines) profiles during the cell culture across scales

Glucose consumption was similar across the bioreactor scales, Figure 9, suggesting little difference between the cell metabolism across the different scales.
Online Control

The control response of the online parameters, DO, pH and pCO$_2$ achieved for the Allegro STR 1000 bioreactor are shown in Figure 10. Proportional, Integral and Derivative (PID) control was implemented using feedback from the Allegro STR 1000 controller via Mettler Toledo probes.

Figure 10
DO, pCO$_2$ and pH time series plots from the Allegro STR 1000 bioreactor, data points have been down sample for data handling purposes

DO was allowed to drift down from the initial value of 100% at inoculation to the set-point of 40%, at which point the controller varied the flow rate of oxygen, via the sparger, to maintain the DO at set-point. As the culture viability dropped, oxygen requirements decreased and the DO increased above the 40% set-point.

The pH was maintained at 7.1 ± 1.0, with the use of deadband control. Deviations early in the culture for the pH and pCO$_2$ were due to offsetting the online values against measured offline values determined via the BioProfile FLEX Analyzer. A deviation in the measured pH occurred at 6-15 hours due to an incorrect offset for the pH probe.

A pCO$_2$ sensor was integrated into the Allegro STR 1000 bioreactor with the use of one of the extra sensor connections. CO$_2$ gas was used to control the pCO$_2$ level to 60 mmHg using feedback control on the Allegro STR 1000 controller.
Single-Use Sensors
The single-use and re-useable sensor data is highly comparable, with little real difference seen between the values, as shown in Figure 11. An incorrect offset was applied to the re-useable pH sensor at 6-12 hours, which was not applied to the SU sensor, explaining the straight line for the SU sensor data between these time points.

Figure 11
Comparison of re-useable and single-use sensors

Conclusion
The study results demonstrated that the batch cultivation of a CHO mAb process in the Allegro STR 1000 single-use bioreactor was scalable when compared to the Allegro STR 200 bioreactor and a 10 L bench-top STR. Cell growth, mAb production and metabolite profiles were very similar and within the expected performance for this process. The Allegro XRS 20 and Allegro STR 200 bioreactors were successfully incorporated into the process to provide sufficient inoculum for production at the 1000 L scale. The single-use pH and DO sensors supplied with the Allegro STR 1000 biocontainer demonstrated excellent performance and were strongly aligned to the conventional sensor readings. The magnitude of drift seen in the re-useable and single-use sensors was comparable.

References