

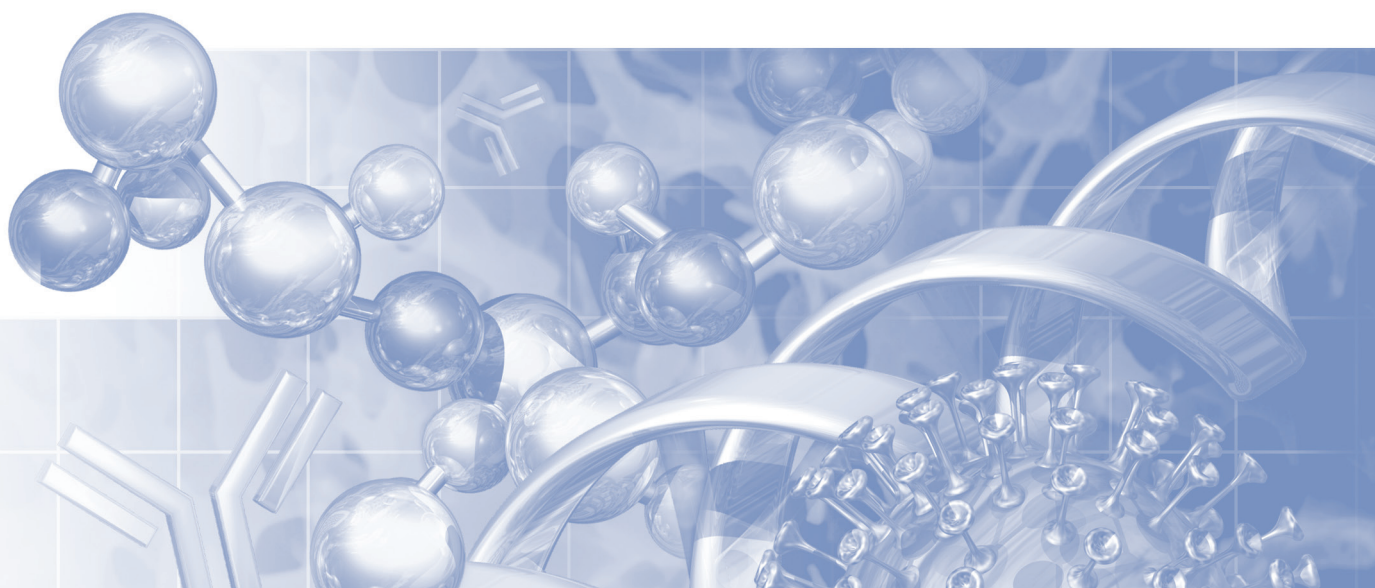


Life Sciences

Application Note

USD3180

Characterization and Engineering Performance of the Allegro™ STR 2000 Single-Use Stirred Tank Bioreactor



Introduction

Stirred tank reactors (STRs) have become the prevalent choice for the commercial manufacture of therapeutic proteins from suspension cell lines. Process performance in this scalable family of bioreactors 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 direct impeller driven agitation that can deliver a wide range of specific power inputs to the fluid. The Allegro range of single-use stirred tank bioreactors has adopted this direct driven impeller technology, which allows a wide range of specific power inputs to be achieved. In addition, modifications to current design features utilized with other single-use bioreactor technologies in the market place today, have been implemented for increased ease of use and process assurance.

Performance characterization of the Allegro STR 2000 bioreactor in terms of mixing, mass transfer (oxygen mass transfer and CO₂ stripping efficiency), and temperature control demonstrates good performance at a wide range of agitation speeds. The Allegro STR range has been designed to provide a predictable scale-up performance modeling, therefore, the comparison of the mixing and mass transfer capabilities across the bioreactor range is presented with consistency from reactor to reactor at specified volume sizes. This information can help significantly in the selection of operating parameters during process transfer, including scale-up and scale-down, from bench-scale and stainless steel reactors. This leads to a reduction in the time required for additional development that may be needed for internal engineering performance modeling, and aids with conceptual understanding of how the cell line may perform during the process.

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Material and Methods

Equipment

- Allegro STR 2000 single-use bioreactor system (Pall)
- Allegro STR 1000 single-use bioreactor system (Pall)
- Allegro STR 200 single-use bioreactor system (Pall)
- Polarographic oxygen sensor InPro[®] 6850i (Mettler-Toledo)
- pH sensor InPro 3250i (Mettler-Toledo)
- pCO₂ sensor InPro 5000i (Mettler-Toledo)
- M800 transmitter (Mettler-Toledo)
- National Instrument[®] CompactDAQ[®] using a multi-purpose Compact RIO[®] I/O module
- Probe bellows (Pall)
- Kaye Validator[®] (GE)
- Temperature control unit (TCU) (Pall)
- Chiller (MTA)

Materials

- Media simulant (Xing *et al*, 2009)
- 4 M NaOH (Sigma-Aldrich)
- 5 M HCl (Sigma-Aldrich)

Allegro STR Design

The Allegro STR range of bioreactors has been designed to provide scalable cell culture performance by incorporating the principals of successful scale up from traditional stainless steel reactor vessels, mainly by maintaining similar geometries across the range. The only exception to this is for the Allegro STR 2000 bioreactor, where the impeller size was maintained the same as the Allegro STR 1000 bioreactor, thus improving manual handling, operation and disposal of biocontainer, as well as achieving target $k_L a$ and power with the smaller impeller aspect ratio. Despite the reduction in the size of the Allegro STR 2000 impeller relative to the volume, a high specific power input is still achieved, resulting in similar engineering performance parameters between the Allegro STR 2000, 1000 and 200 bioreactors. The key dimensions of the Allegro STR range are shown in Table 1.

Figure 1

Allegro STR Biocontainer Dimensions

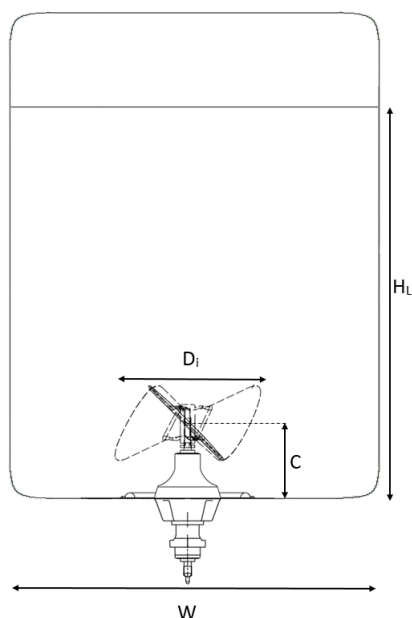


Table 1

Allegro STR Dimensions

Allegro STR	200	1000	2000
Working Range (L)	60 - 200	300 - 1000	400 - 2000
Biocontainer Dimensions (mm)	585 x 585	1025 x 975	1270 x 1220
Liquid Height, H_L (mm)	≈600	≈1000	≈1300
Impeller Diameter, D_i (mm)	290	490	490
Sparger Hole Size (mm)	1	2	2
No. Sparger Holes	6	28	28
H_L/W	1	1	1
D_i/W	0.5	0.5	0.4
C/H_L	0.25	0.25	0.2

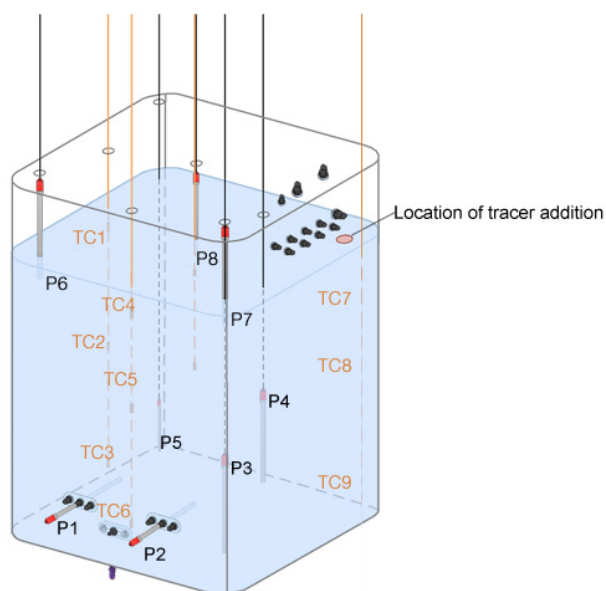
With the use of any agitated bioreactor system the potential risk of shear damage to cells needs to be considered. The Allegro STR range has been designed to allow for a wide range of agitator speeds, being able to achieve high agitation speeds for high mass transfer demanding processes, and able to provide gentle mixing for low mass transfer demanding processes. In addition to this, much of the shear sensitivity of cells is alleviated by the addition of protectants to the media, such as serum or Pluronic[®] in chemically defined processes (Neinow, 2014). Therefore, when transferring a cell culture process currently operated in stirred tank reactors to the Allegro STR range, the risk of shear stress negatively impacting performance is low.

Experimental set-up

Mixing time experiments were carried out using eight probe locations, shown in Figure 2. The probe locations were selected to map as much of the bioreactor volume as possible. This included the assumed worst areas of mixing; the corners, directly above impeller and the furthest area from the point of the tracer addition. These locations were determined by bioreactor design, visual inspection by experienced operators, from bench-top and Allegro STR 1000/200 bioreactor performance mapping and CFD mixing simulations. Results indicated a homogenous mixture; therefore, for k_La O_2 and CO_2 evacuation rate experiments, only two probes were assumed to be necessary. For k_La and CO_2 evacuation rate experiments, two probes in the standard probe inserts (P1 & P2, Figure 2) were used.

Figure 2

Arrangement of probes (P) and thermocouples (TC) within the Allegro STR 2000 Biocontainer.



The probes were connected to an M800 Ingold 4-Channel transmitter which was wired to a National Instrument CompactDAQ, using a multi-purpose CompactRIO I/O module. Data was logged using a LabVIEW[®] (National Instruments 9.0.1) program developed by Pall Corporation.

The k_La 2080 and CO_2 evacuation rate were mapped using a face centered central composite design and analyzed using Minitab[®] Statistical Software (Minitab 16.2.1). All experiments were performed in upflow mode and performed using a media simulant.

Determination of the specific power input

The power dissipated by the rotating impeller was measured for the Allegro STR 200 bioreactor and the ungassed power number (P_0) calculated from this, which was used to determine the power dissipated by the Allegro STR 2000 bioreactor. As the power dissipated is dependent on the impeller type and not the volume, and the Allegro STR 2000 impeller is a scale-up from the Allegro STR 200 impeller, the ungassed power number is assumed to be the same as the Allegro STR 200 bioreactor. P_0 was determined from the specific power input relationship:

$$P = P_0 \rho N^3 D_i^5$$

Where P_0 is the ungassed power number, ρ the fluid density in $kg.m^{-3}$, N the rotation speed in s^{-1} and D_i the impeller diameter in meters.

Determination of mixing time

Mixing times were determined by measuring the change in pH over time, following a bolus addition of 2% v/v of 4 M sodium hydroxide solution to the bioreactor, at the location (red circle) shown in Figure 2. The mixing time was defined as the time required in seconds to reach $100 \pm 5\%$ of the final measured pH value.

Determination of oxygen mass transfer coefficient (k_La 2080)

The volumetric mass transfer coefficient for oxygen (k_La) was determined using the gassing out method with nitrogen and air, carried out at 37 °C. k_La 2080 values were calculated between 20 and 80% of dissolved oxygen using the equation:

$$k_La \text{ 2080} = \ln \left(\frac{DO^* - DO_{20}}{DO^* - DO_{80}} \right) \times \frac{1}{t_{80} - t_{20}}$$

Where DO^* , DO_{80} and DO_{20} are the DO values at 100, 80 and 20% air saturation, respectively. t_{80} and t_{20} are the times at which DO_{80} and DO_{20} were reached.

The k_La 2080 was mapped using a face centered central composite design and analyzed using Minitab Statistical Software (Minitab 16.2.1). All experiments were performed in upflow mode and performed using a media simulant.

Determination of CO₂ stripping rate

The CO₂ stripping rate was determined by measuring the change in pH of a CO₂ rich media simulant while sparging 100% air. The pH of the media simulant was maintained at pH 6.5, by sparging CO₂ gas and temperature was kept constant at 37 °C. A pCO₂ probe recorded the initial pCO₂ once a steady pH value of 6.5 was achieved. Air was then supplied through the ring sparger of the bioreactor at various airflow rates and at different agitation speeds until the pH reached a value of 7.2. The CO₂ concentrations were derived from pCO₂ values using the equations detailed by Goudar *et al* (2011).

In a bicarbonate buffered medium (at a pH of 6.5 to 8.0), the partial pressure of CO₂ (pCO₂) is proportional to the buffer pH as follow (Bowers 2008):

$$pCO_2 = (pH_t - pH_{initial}) \times \log(pCO_{2 \text{ initial}})$$

Where pCO₂ and pCO_{2 initial} are the calculated and initial measured CO₂ concentration and pH_{initial} and pH_t the initial and current pH values.

Temperature Mapping

Twelve thermocouples were used for temperature mapping experiments in the Allegro STR 2000 bioreactor. Ten thermocouples were placed at various locations within the biocontainer, nine in the fluid and with one between the biocontainer film and the water jacket. Two additional thermocouples were used to measure the ambient room temperature, with one of the thermocouples directly above the reactor (Figure 1). Thermocouples were calibrated using a KAYE HTR400 Hot Box with a calibrated IRTD-400 temperature probe. Thermocouples were connected to a KAYE validator X2010E. Temperature data was logged using a Kaye Validator.

Temperature was regulated by the temperature control unit (TCU, Pall) with the cooling function provided by a chiller set to 10 °C, connected to the cooling side of the heat exchanger within the TCU. The PID settings for the temperature control are shown in Table 2.

Table 2

Control Settings for Temperature Study

P (Proportional)	I (integral)	D (Derivative)
500	480	0

Results and Discussion

Mixing times in the Allegro STR 2000 Bioreactor

Rapid mixing times are essential for good cell culture performance. Most cultures require the addition of nutrients or other chemicals to maintain viability and high productivity. These are usually highly concentrated fluids that must be mixed rapidly to limit cell exposure to potentially harmful concentration gradients and allow for adequate consumption of the provided carbon source added. At the small scale, good mixing can be achieved with relative ease. However, at larger scale this is much more challenging.

Figure 3 shows that a mixing time as low as 47 seconds is achieved at the lowest agitation speed in the Allegro STR 2000 bioreactor. The fastest recorded mixing time measured was 16 seconds at 105 RPM.

No significant difference was seen in the mixing time measured between the eight probe positions. The variability seen was mainly due to experimental and measurement error, as opposed to the heterogeneity in the bioreactor. The study did not detect the presence of any mixing dead zones and indicates a homogenous system.

Figure 3

Mixing times at 2000 L for various agitation speeds. Error bars represent standard deviation from three replicate runs (n=3) in which the mixing time was averaged from eight probes within a single run.

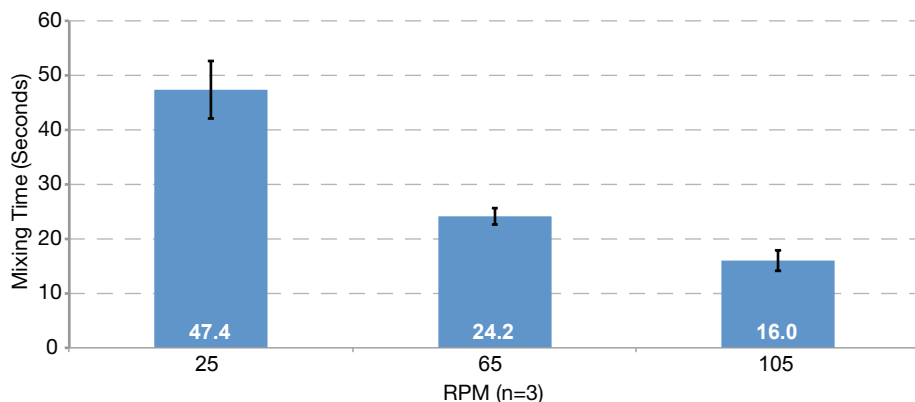
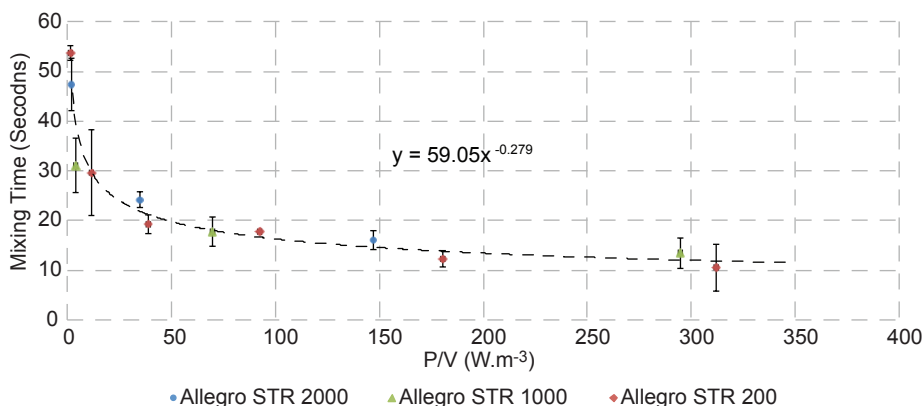


Figure 4 highlights similar mixing times achieved across the Allegro STR range when operated at the same specific power input per volume (P/V), which also holds true at the lower fill volumes. The mixing times for the Allegro STR 200 and 1000 bioreactors were generated using the same method as for the Allegro STR 2000 bioreactor, and the results are described in the Allegro STR 1000 and 200 bioreactors application notes (document reference numbers USD 2980 and USD 3136, please contact Pall for a copy).

Figure 4

Mixing times for the Allegro STR 2000, 1000 and 200 bioreactors across the agitation range (shown as specific power input per volume). Error bars represent the standard deviation for the Allegro STR 2000 and 1000 bioreactors (n=3) and the variance for the Allegro STR 200 bioreactor (n=2).



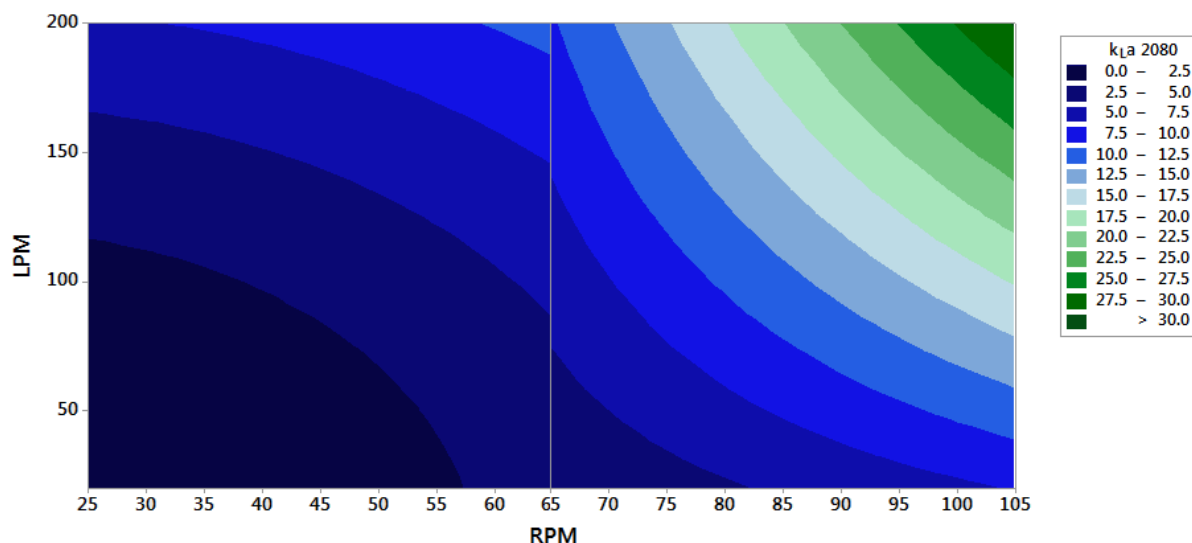
Oxygen Transfer Rate ($k_L a$ 2080) in the Allegro STR 2000 Bioreactor

Efficient oxygen mass transfer is critical for aerobic cultures, and can be challenging as oxygen is sparsely soluble and rapidly consumed by the cells. It is typically the rate limited factor in cell culture high density cultures. Determination of the volumetric oxygen mass transfer coefficient ($k_L a$ O₂) is a standard benchmarking method for assessing the ability of the bioreactor system to transfer oxygen from air to liquid phase.

The full design space for $k_L a$ 2080 was mapped, with respect to agitation speed and aeration rate. The contour plot from this data is shown in Figure 5. The ability of the Allegro STR 2000 bioreactor to deliver higher agitation rates and hence a higher P/V allows for a wide range of $k_L a$ values to be achieved.

Figure 5

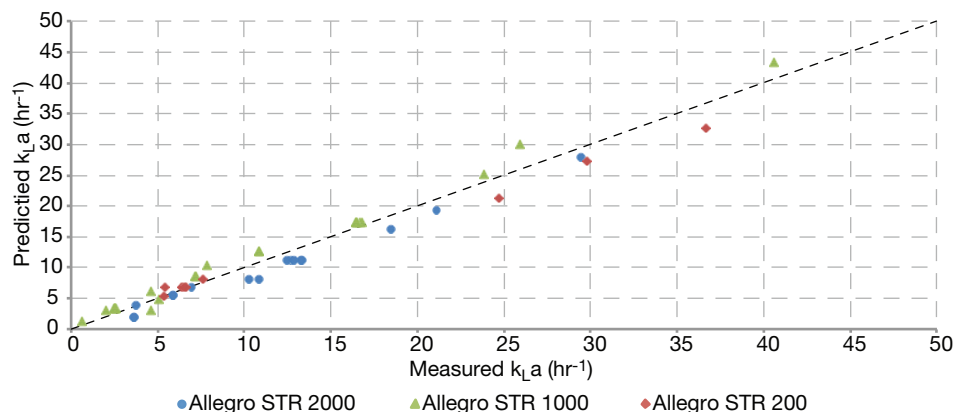
Contour plots for k_{La} 2080 values at various agitation and airflow rates. Two DOE models were required to capture change in curvature with RPM (25 to 65 RPM & 65 to 105 RPM).



The van't Riet correlation was fit to all the k_{La} 2080 data generated in the Allegro STR 2000, 1000 and 200 bioreactors, using the inputs of superficial gas velocity (v_s) and P/V. Predicted values were then generated at the same inputs as the measured data. Figure 6 shows the model fit for the model versus measured data, showing a good fit. This demonstrates that at the same P/V and v_s across the bioreactor range with different volume scales, the same k_{La} is achieved.

Figure 6

Model versus measured k_{La} across the Allegro STR range



CO₂ Stripping Rate in the Allegro STR 2000 Bioreactor

Carbon dioxide mass transfer plays an important role in the success of large scale cell culture processes. Animal cells naturally produce CO₂ as a by-product of aerobic respiration. In small scale bioreactors CO₂ is effectively removed via surface aeration and sparging air through the liquid. However as the bioreactor scale increases, the gas/liquid surface area to volume ratio decreases. This can lead to CO₂ accumulation in some processes. While the optimal amount of pCO₂ varies with the cell culture process, the ability to efficiently extract CO₂ from the culture allows for better control, potential conservation of needed base addition, and allows for more comparable pCO₂ concentrations across bioreactor scales.

Figure 7 shows the CO₂ stripping data from the Allegro STR 2000 bioreactor at different agitator rates at the maximum airflow rate, using the ring sparger. Up to 5 mol.L⁻¹.day⁻¹ was stripped from the fluid using a maximum airflow rate and agitation. Assuming a CO₂ production rate of 5.36 pmol.cell⁻¹.day⁻¹ for CHO cells, (Goudard *et al*, 2011) even for a fed-batch culture at 35 x 10⁶ cells.mL⁻¹ all the CO₂ produced by the cells can be stripped from the culture at the lowest agitation rate or airflow rate. Additional CO₂ stripping is available with an open pipe sparger capable of delivering 20 LPM of air. Figure 8 shows the contour plot for the CO₂ evacuation rate in relation to the full agitator and airflow range.

Figure 7

CO₂ evacuation rate at various agitation speeds at 0.1 vvm. Errors bars represent the variance between two probes in a single run (n=2)

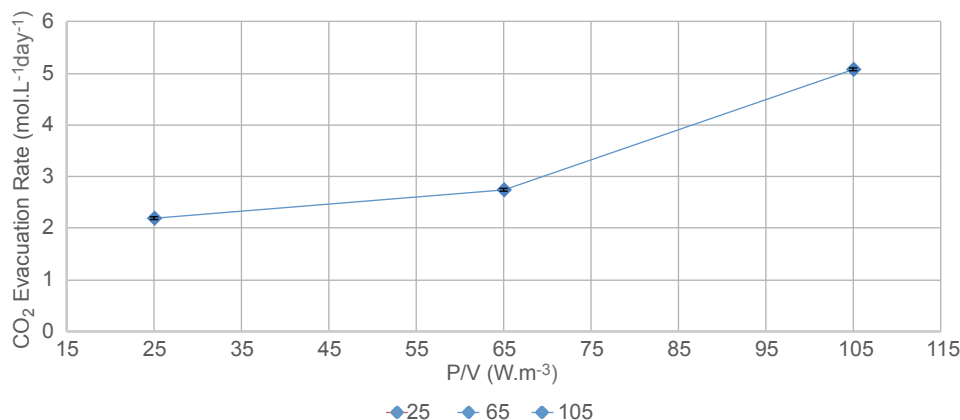
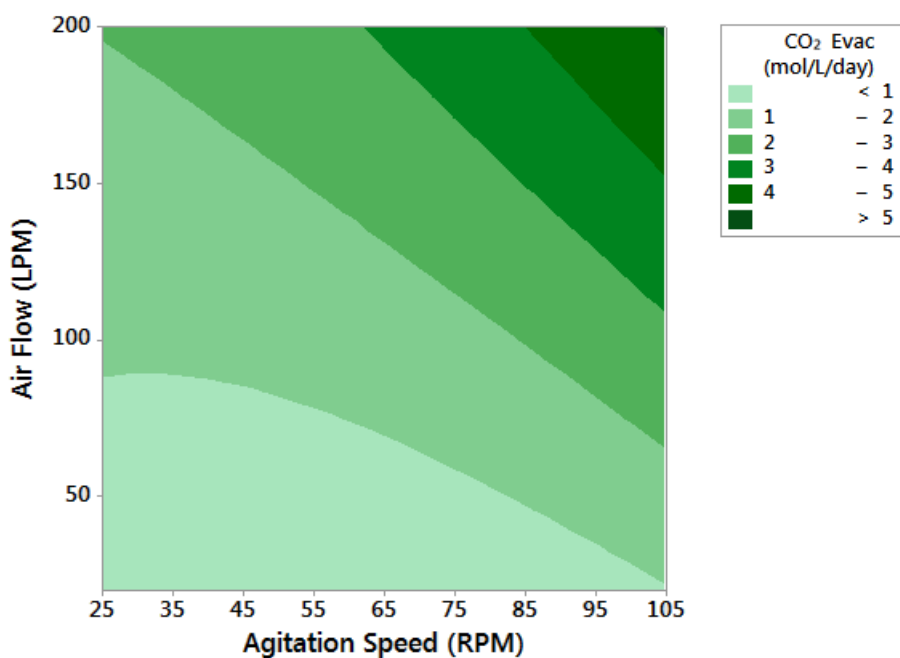


Figure 8

Contour plot for CO₂ evacuation rates at various agitation and airflow rates



Temperature Control in the Allegro STR 2000 Bioreactor

Cell culture performance is highly dependent on generating a uniform temperature profile throughout the culture fluid and precise control around a defined temperature set-point. Large scale bioreactors often experience poor heat transfer and heat loss that result in prolonged heat up times, temperature gradients and large temperature fluctuations. The Allegro STR 2000 bioreactor is designed to avoid these issues with an efficient water jacket and well established PID control. The ability of the Allegro STR 2000 bioreactor to heat-up, cool-down and maintain 2000 L of fluid at a desired temperature was tested.

Chilled water was heated from 4 to 37 °C in 9.5 hours, with no overshoot, as shown in Figure 9 and Figure 10. The temperature did not exceed the set-point and temperature fluctuations were minimal, with less than 0.1 °C variation around the set-point, shown in Figure 10. No temperature gradient was observed in the nine measurement locations, suggesting that temperature was uniform within the biocontainer. Less than 2% of the data points collected over the 12 hours exceeded 0.1 °C (and were within 0.15 °C) and are considered to be noise from the PT100 probe.

A temperature shift from 37 to 30 °C was induced at approximately 22 hours and the time to cool to 30 °C was less than 4.5 hours, shown in Figure 9.

The ability of the Allegro STR 2000 bioreactor to control temperature at low set-points, such as 25 °C was assessed. At 25 °C the temperature fluctuation was minimal, with deviations of ± 0.13 °C. As the PT100 was calibrated for accuracy around 37 °C, there is an offset of 0.25 °C at 25 °C. For processes which require temperatures below 25 °C an offset can be applied to give accurate temperature control.

Figure 9

Heat-up (4 to 37 °C), Cool-down (37 to 30 °C), and Temperature Stability (37 and 25 °C) in the Allegro STR 2000 Bioreactor

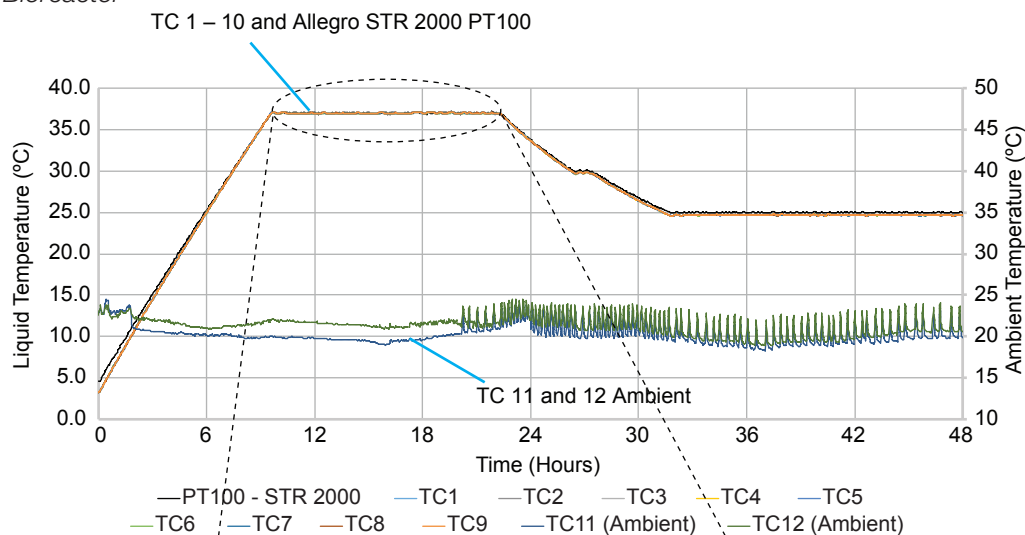
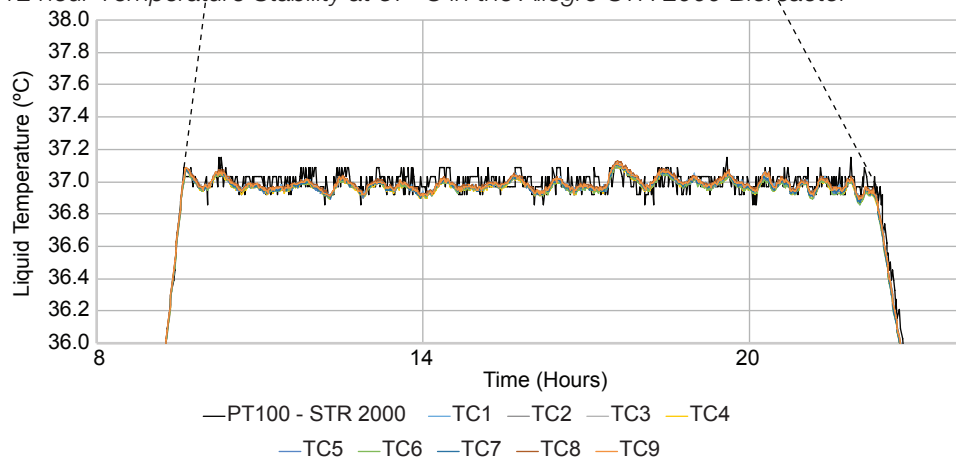


Figure 10

12 hour Temperature Stability at 37 °C in the Allegro STR 2000 Bioreactor



Summary

The design of the Allegro STR 2000 bioreactor is based on the understanding and control of the physical and engineering parameters that critically influence cell culture performance:

- A wide range of values of specific power input per unit volume of fluid can be obtained thanks to a large impeller and a direct drive agitation mechanism
- Homogenous mixing across the biocontainer containing 2000 L of fluid can be obtained in 16 seconds
- Oxygen transfer rate as high as 30 hr⁻¹ can be achieved using the ring sparger and air.
- The ring sparger can be used for stripping of up to 5mol.L⁻¹.d⁻¹ of CO₂
- Heat up time of 2000 L of media from 4 °C to 37 °C is under ten hours
- Cool down from 37 °C to 30 °C in under five hours using the Pall TCU with a chilled water supply at 10 °C on the cooling side of the heat exchanger
- Precise and homogenous temperature control across the bioreactor volume

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