Application Note

Characterization and Engineering Performance of the Allegro™ STR Single-Use Stirred Tank Bioreactor Family Range
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1 Introduction

Stirred tank bioreactors (STRs) have become the mainstay for single-use large scale commercial manufacture of suspension cell lines and their respective therapeutics. The ability to provide a seamless scale up from a benchtop bioreactor to large scale manufacture requires careful thought in vessel design engineering modeling and computational fluid dynamics, as well as actual engineering studies guided by correlations and studies of conventional bioreactors (Nienow, 2015). The Allegro STR bioreactor range has been designed to provide predictable scale-up performance, therefore, the comparison of the mixing and mass transfer capabilities across the bioreactor range can be presented from reactor to reactor at their specified volumes. The range maintains scalable engineering performance while leveraging usability and process assurance features that have differentiated Pall’s Allegro STR bioreactor product range from all others in the single-use stirred tank bioreactor market.

The Allegro STR family range has been developed to provide excellent mixing performance through a direct drive agitation mechanism, coupled with a large elephant ear 45° pitched wide- blade bottom-mounted impeller, which generates excellent axial and radial flow inside the reactor, with baffles to maximize culture homogeneity with no dead zones (Nienow et al., 2016). In addition, the biocontainers feature a macro ring sparger to deliver gas directly to the culture fluid interface. The unique seating of the macro sparger below the large impeller distributes gas delivery directly into the rotating blades of the impeller to maximize mass transfer, as well as facilitating effective CO2 stripping. For more demanding high cell density applications, requiring additional carbon dioxide (CO2) stripping capabilities, an optional open pipe sparger is included in all designs.

With the use of an agitated bioreactor, there is always the perception of shear damage. The Allegro STR range has been designed to allow for a wide range of specific power inputs, the upper end of the range being higher than in other available single-use bioreactors (SUBs), in order to achieve high $k_a$ values, thus avoiding the use of a micro-sparger. Whilst the potential lethal damage to cells from the tiny bubbles emitted by microspargers can be prevented using Pluronic F68, they can also lead to high levels of pCO2 and excessive foaming. Both can be avoided by the specific power combined with the higher vvm values also available with the Allegro STR bioreactors. Roche and Lilly made use of higher power input and vvm to reduce CO2 levels in this way (Mostafa and Gu, 2003; Sieblist et al., 2011). Use of mean specific power levels up to 250 W.m⁻³ have been shown not to reduce culture performance for a wide range of animal and insect cells (Nienow, 2006). In addition, with CHO cells, Sieck et al., (2013), in collaboration with Novartis, and Nienow et al., (2013), in work undertaken in Genentech, have shown that even at 1000 W m⁻³, neither culture performance nor antibody quality, including glycosylation were compromised. Avoiding the build-up of pCO2 by high specific power and vvm also avoids low pH levels having to be mitigated by alkali addition, leading to high osmolality and again poor culture performance (de Zengotita et al., 2002). Thus, the design of the Allegro series of bioreactors can be used to avoid all these problems (Nienow et al., 2015).

Engineering performance characterization of the Allegro STR family range, in terms of mixing efficiency, mass gas transfer (oxygen mass transfer and CO2 stripping efficiency), and temperature control, demonstrates excellent performance at a wide range of agitation speed which are presented in this application note.
1.1 Materials and Methods

1.1.1 Equipment

- Allegro STR single-use bioreactor systems (50 – 2000 L) (Pall)
- InPro 6850i polarographic oxygen sensor (Mettler-Toledo*)
- InPro 3250i pH sensor (Allegro STR 500, 2000 L bioreactors) InPro3253i/SG/325i (Allegro STR 50, 200, 1000 L bioreactors) pH Sensor (Mettler-Toledo)
- InPro 5000i pCO2 sensor (Mettler-Toledo)
- M800 transmitter (Mettler-Toledo)
- National Instruments® CompactDAQ® multi-purpose compact RIO I/O module
- Probe bellows (Pall)
- Kaye® validator (GE)
- Pall Temperature control unit (TCU)
- Lauda® Integral T 10000 and 2000 (Lauda)

1.1.2 Materials

- Media simulant (Sigma-Aldrich®) (2 g/L Sodium Bicarbonate, 6.4 g/L Sodium Chloride, 1 g/L Pluronic F68, 0.0025% Antifoam C)
- 4 M NaOH (VWR)
- 5 M HCl (VWR)
- Water

1.2 Experimental Set-Up

Experiments involving dissolved oxygen, pH and partial pressure of CO2 (pCO2) measurements were carried out using two probes inserted in the standard probe ports, located at the front bottom of the biocontainer via probe bellow assemblies. Probes were wired to a Mettler Toledo M800 Ingold 4-Channel. The M800 transmitter was wired to a Compact DAQ (National Instruments) using a multi-purpose I/O module. Data was logged in LabVIEW® (National Instruments) using a program developed in house. Experiments involving temperature were carried out using external thermocouples, calibrated using the Kaye Validator (GE Healthcare) and inserted directly into the fluid inside of the biocontainer. Data was recorded using Kaye Validator software.

1.3 Determination of Mixing Time

Mixing studies were conducted by performing a bolus addition of 0.2% v/v of 4 M NaOH solution at an opening to the back centre of the biocontainer just above the fluid level, then measuring the change in pH over time, in the maximum working volume of the respective bioreactor, of media simulant, heated to 37±1 °C. pH was measured at two or more different locations through the designated conventional probe ports, at the front of the biocontainer. Mixing time was defined as the time required to reach 95% homogenization, characterized by a stable pH reading.
1.4 Determination of Oxygen Mass Transfer $k_L a$ O$_2$ (2080 Method)

The volumetric mass transfer coefficient for oxygen ($k_L a$) was determined using a gassing out method with nitrogen and air. Experiments were conducted using media simulant heated to 37±1 °C. The signal of two polarographic DO probes (Mettler Toledo) was acquired from a M800 transmitter and logged via a program written in LabVIEW (National Instruments). $k_L a$ values were then calculated between 20% and 80% of dissolved oxygen using the equation:

$$k_L a = \ln\left(\frac{D O^* - DO_{20}}{D O^* - DO_{80}}\right) \cdot \frac{1}{t_{80} - t_{20}}$$

where $D O^*$ refers to DO at saturation, $D O_{20}$ and $D O_{80}$ refers to 20% and 80% of $D O^*$ respectively, and $t_{20}$ and $t_{80}$ the times at which $D O_{20}$ and $D O_{80}$ were reached respectively.

The values measured in this study are dependent on this specific method and simulant formulation used and should not be compared to studies using different simulant formulations or different oxygen mass transfer $k_L a$ O$_2$ measurement methods.

1.5 Determination of CO$_2$ Evacuation Rate

In bicarbonate buffer (pH 6.5 to 8), partial pressure of CO$_2$ (pCO$_2$) is proportional to the buffer pH as follow:

$$pC O_2 = (pH_t - pH_{initial}) \cdot \log(pC O_{2initial}) \quad (Bowers \ 2008).$$

Each respective bioreactor was filled to maximum working volume with media simulant and heated to 37±1 °C. Fluid was brought to pH 6.5 using additions of CO$_2$ gas by sparging. A pCO$_2$ probe, inserted into a conventional probe port on the front of the biocontainer, recorded the initial pCO$_2$. Agitation speed was set give the desired specific power input and air was then sparged through the ring sparger at the desired flow rate. pH measurements were recorded via a program in LabVIEW (National Instruments) until the pH of the media reached a value of at least 7.2. CO$_2$ concentrations were derived from pCO$_2$ values using the equations extracted from Goudar et al. (2011).

1.6 Temperature Mapping

A series of ten thermocouples were placed at various locations directly into the biocontainer fluid. The thermocouples were calibrated using a Kaye HTR-400 Hot Box with a calibrated IRTD-400 temperature probe. Thermocouples were connected to a Kaye validator X2010E. Temperature data was logged using the Kaye Validator software. Bioreactors were filled to maximum working volume with water. Temperature was set at 37 °C using the controller and agitation set to maximum agitation. Temperature was data logged at various setpoints over the course of several hours. Two probes were placed outside the bioreactor to record ambient temperatures.

The PID controller of the bioreactor received the signal from the Allegro STR bioreactor PT100 temperature probe. Temperature control was carried out in a cascade control manner, with the fluid temperature as master control, and the TCU temperature as slave control loop. A Lauda Integral T10000 (Lauda-Brinkman) was used for temperature control with the settings shown in Table 1 for the Allegro STR 200, 500 L bioreactors. A Lauda Integral T2200 was used for the Allegro STR 50 L bioreactor. A Pall TCU was used for control of the Allegro STR 1000, 2000 L bioreactors.
Table 1.

*Temperature PID controller settings for Allegro STR bioreactor family range.*

<table>
<thead>
<tr>
<th>Bioreactor Size</th>
<th>Temperature Control Unit</th>
<th>P (Proportional)</th>
<th>I (Integral)</th>
<th>D (Derivative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 L</td>
<td>Lauda Integral T2200</td>
<td>20</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td>500 L</td>
<td>Lauda Integral T10000</td>
<td>20</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td>1000 L</td>
<td>Pall TCU</td>
<td>500</td>
<td>480</td>
<td>0</td>
</tr>
<tr>
<td>2000 L</td>
<td>Pall TCU</td>
<td>500</td>
<td>480</td>
<td>0</td>
</tr>
</tbody>
</table>

2 Results and Discussion

Mean specific energy dissipation rate \( \varepsilon \) W kg\(^{-1}\) from the impeller to the fluid is the driving force for mixing. As impeller diameters and shapes vary between different bioreactors, neither impeller speed nor tip speed is regarded as a relevant indicator of the hydrodynamic forces generated in the bioreactor. Instead, power input per unit volume, \( P/V \) and \( \varepsilon (P/V) W m^{-3} = 10^{-3} \varepsilon W kg^{-1} \) are widely accepted as more accurate indicators (Nienow, 2006).

2.1 Mixing Times

Short mixing times are essential for a successful cell culture. Long mixing times are undesirable, as, in the case of frequent chemical additions such as base for pH control, or concentrated nutrient feeds, they imply longer period of contact between cells and possibly harmful concentrations of chemicals. Although good mixing can be achieved relatively easily at smaller scale cultures, when scaling to larger volumes, rapid mixing can become more of a challenge as fundamental turbulence theory shows it increases with scale. However, mixing time is much longer if baffles are not present as in many other single-use bioreactors (Pogal and Kehn, 2018). The small aspect ratio (AR= 1 or less) of the Allegro STR range is also an important design feature because it enables mixing time \( \theta_m \) secs to be estimated from the correlation for baffled cylindrical bioreactors (Nienow, 2015);

\[
\theta_m = 5.3 (\varepsilon_T)^{-1/3} (T_{eq})^{2/3} (D/T_{eq})^{-1/3}
\]

where \( \theta_m \), is the mixing time in seconds, \( \varepsilon_T \) is local specific energy dissipation rate, W kg\(^{-1}\), \( g \) is gravitational constant, 9.81 m.s\(^{-2}\), \( D \) is agitator diameter in meters, \( T_{eq} \) is the diameter of a cylinder of equal cross sectional area to that of the Allegro STR bioreactor, i.e., \( T_{eq} = 1.13 \) times the side length of the Allegro STR bioreactor of the same volume. A similar equation has been proposed by Kresta et al., (2006). Aspect ratios above 1, as found in many other SUBs, show greatly increased mixing time (approximately proportional to AR\(^{2.5}\)) (Nienow, 2015).

Mixing time was defined as the time required to reach 95% fluid homogenization, characterized by a stable pH reading following a quick bolus addition of 4 M NaOH. Figure 1 highlights how short and predictable mixing times can be achieved across the entire Allegro STR range when operated at similar power input per volumes (P/V).
Figure 1.

Measured mixing times for the Allegro STR 50, 200, 500, 1000 and 2000 bioreactors across a range of power input per volumes alongside the predicted mixing times for the STR1000 based on the literature in Nienow, 2015. Error bars represent the standard deviation for the Allegro STR 2000 and 1000 bioreactors (n=3) and the variance for the Allegro STR 500, 200 and 50 bioreactors (n=2).

2.2 Oxygen Transfer Rate 2080 ($k_{La}$) in the Allegro STR Bioreactor Family

Determination of the volumetric oxygen mass transfer coefficient ($k_{La}$ O$_2$) is regarded as a standard benchmarking method for assessing the ability of the bioreactor system to transfer oxygen from air to liquid phase. Oxygen mass transfer is critical for aerobic cultures and can be challenging as oxygen is sparsely soluble and quickly consumed by the cells, therefore, typically this becomes the rate limiting factor in high cell density cultures.

Figure 2.

Contour plots for $k_{La}$ 2080 values at various agitation and airflow rates for the Allegro STR 50, 200, 500, 1000 and 2000 bioreactors. Two DOE models were required to capture change in curvature with increasing rpm due to changes in bubble entrainment and fluid dynamics between lower and higher agitation speeds. *Allegro STR 200 bioreactor was only tested with one DOE model at the higher airflow rates.
The Van’t Riet correlation was regressed to all the collected $k_{La}$ 2080 data using the inputs of superficial gas velocity and power input per volume, predicted values were then generated using these inputs. The Van’t Riet correlation predicts measurement data with a precision of 15-20%, variation is due to differing coefficients of the equation corresponding to low and high agitation speeds within the bioreactor and inherent experimental variability. Figure 3 shows predicted values versus measured experimental data demonstrating a good fit and good scalability across the bioreactor range. The higher values at every scale are significantly greater than those available in many other SUBs thus eliminating the need for oxygen enrichment or microspargers.

**Figure 3.**

*Measured $k_{La}$ vs predicted $k_{La}$ in the Allegro STR bioreactor family at respective maximum working volumes.*

2.3 Precise and Homogenous Temperature Control

Cell culture performance and product quality is highly dependent on the capacity of the bioreactor to maintain a homogenous and tightly controlled temperature across the biocontainer around a defined setpoint, as well as quick heat up and cooldown times to maintain temperature during feed additions throughout a culture. The Allegro STR bioreactor systems ability to heat up, cool down and maintain a desired temperature within $\pm 0.1 \degree C$ of a given setpoint has been tested across the range using external thermocouples (Figure 4). All reactors achieve homogenous and constant temperature control at 37$\pm 0.1 \degree C$.

For processes which require setpoints below 30 $\degree C$, an offset should be applied due to the PT100 probes being calibrated for accuracy at 37 $\degree C$. This should be determined using an external temperature probe.
2.4 CO2 Stripping Rate Across the Allegro STR Bioreactor Family

Carbon dioxide mass transfer plays an important role in the success of large scale cell culture processes. CO2 concentration can affect cell culture performance and is a commonly overlooked parameter during scale-up. Animal cells naturally produce CO2 as a metabolic by-product of aerobic respiration. In small scale bioreactors, the majority of the CO2 can be stripped via surface aeration. However, the liquid to surface volume ratio decreases with an increase of scale and can lead to CO2 accumulation in large scale bioreactors, especially if the oxygen mass transfer requirements on scale up are met by enhancing the driving force or increasing kLa by using a microsparger (Sieblist et al., 2011). As a result of the high air flow rates (vvm) of the Allegro STR bioreactors compared to other SUBs, this problem can be overcome.

Figure 5 shows the CO2 stripping abilities of the Allegro STR family of bioreactors using the ring spargers at various air flow rates. Up to 5 mol.L⁻¹.d⁻¹ can be stripped out using a maximum air flow rate and maximum agitation speed in the Allegro STR 200, 1000 and 2000 bioreactor and up to 4 mol.L⁻¹.d⁻¹ in the Allegro STR 50 and 500 bioreactors.

Control of pCO2 can also be important as the optimum level varies with cell type (Irfan, 2017). At the smaller scale, vvm often has to be high in order to achieve the superficial gas velocity, vs to give the desired kLa value. This vvm may strip out CO2 and it is often introduced into the sparged air to get the desired pCO2 level. However, at large scale, if the same kLa is obtained by matching specific power and vs, the vvm will be lower, thus reducing the rate of CO2 stripping causing pCO2 to become too high. In the Allegro STR bioreactor range, the air flow can be increased to remove and control pCO2 levels via the use of a pCO2 probe. In addition, the dO2 can be controlled by adjusting kLa and lowering the specific power (Sieblist et al., 2011). These control strategies are not possible with other single-use bioreactors, which use microspargers and/or oxygen enrichment at lower vvm and specific power to achieve adequate oxygen transfer.
Figure 5.
Contour plot of CO₂ stripping capabilities at various air flow rates and agitation speeds across the Allegro STR bioreactor family range from 50 – 2000 L.
3 Summary

- Scalable and high oxygen transfer rates, in terms of kLa, can be observed across the whole Allegro STR family range.
- Stable control of temperature within ±0.1 °C of setpoint (when testing at 37 °C) can be achieved across the Allegro STR 50, 500, 1000 and 2000 bioreactors.
- Sufficient CO₂ evacuation rates can be achieved, across the entire Allegro STR bioreactor family, to successfully control the stripping of CO₂ produced even from a high cell density CHO cell culture to give similar levels of dissolved CO₂ by using the range of agitation speeds and airflow sparge rates available at all scales.
- Short and scalable mixing times can be achieved across the entire Allegro bioreactor family range providing efficient and quick homogenization of nutrient, pH control chemicals and the like.
- The use of the higher levels of specific power and airflow bring important advantages for all the Allegro STR bioreactors and are within the range that both theory and academic and industrial tests have shown do not damage a wide range of cell types.

4 References
