Application Note

Characterization and Engineering Performance of the Allegro™ STR 50 Single-Use Bioreactor System
Introduction

Cell culture performance is strongly influenced by the efficiency of mixing to achieve culture homogeneity, supplemented with the effectiveness of gas delivery to support varying oxygen mass transfer coefficient \( (k_{l,a} O_2) \) demands. The ability to provide a seamless scale up from a benchtop bioreactor needs careful consideration including vessel design engineering modelling, CFD, engineering experimental studies, and live run performance from the bench. The success of traditional benchtop glass bioreactors systems lies in their ability to maintain desired precise control of multiple parameters in parallel to sustain a suitable monitored ‘in vitro’ environment to propagate, which are directly facilitated by type of vessel design, impeller type and size, leading to effectiveness of culture distribution in the reactor. Gassing dynamics from the gas to liquid interface are facilitated through the sparger type and location, which are also critical to cell culture success.

As efficient mixing represents a core key performance indicator of a bioreactor successful batch. Successful cell culture performance relies on a homogenous growth environment to sustain adequate gas enrichment and nutrient delivery in shortened time period. Poor mixing capabilities will result in solutes and temperature gradients across the culture fluid, as well as poor mass transfer, lack of effective gas delivery and nutrient uptake, which are detrimental to culture growth and viability. The Allegro STR 50 novel single-use bioreactor has been developed to provide excellent mixing performance through a direct drive agitation mechanism, coupled with a large 45 degree elephant ear bottom-mounted impeller, a novel shaped biocontainer with baffles to maximize culture homogeneity with no dead zones. The large impeller generates excellent axial and radial flow inside the reactor.

In addition, the Allegro STR 50 biocontainer features a macro ring sparger to deliver gas directly to the culture fluid interface. The unique seating of the macro sparge below the large elephant ear impeller distributes gas delivery directly through the rotating blades of the impeller to maximise mass transfer, as well as facilitates effective \( CO_2 \) stripping. For additional demanding applications requiring additional carbon dioxide (\( CO_2 \)) stripping capabilities, an optional open pipe sparger is included. Also understanding criticality of smaller bench scale volumes that will be needed to inoculate the Allegro STR 50 bioreactor at a lower process control volume, the design concept of the Allegro STR 50 bioreactor is a true 5:1 turndown to promote full process control at 10 L, providing flexibility seeding from a benchtop bioreactor with ease. This design flexibility allows for optimal use in a process development laboratory or a cGMP manufacturing environment for control volume flexibility.

With consideration to all of the performance benefits the Allegro STR 50 bioreactor bring to the single-use bioreactor industry. The Allegro STR 50 bioreactor also maintains direct scale-down engineering design concept from the Allegro STR 200, 1000, and 2000 L bioreactors, 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.

In this application note we demonstrate the excellent performance of the Allegro STR 50 bioreactor in terms of mixing (mixing time and oxygen transfer), temperature control and \( CO_2 \) stripping.

Material and Methods

Experimental set-up

Experiments involving dissolved oxygen, pH, partial pressure of \( CO_2 \) \( (pCO_2) \), or measurements were carried out using two probes. The probes were inserted in the standard probe ports, located at the front bottom of the biocontainer. Probes were purchased from Mettler Toledo\textsuperscript{*} (InPro5000i, InPro6850i, InPro3253i,) and wired to a Mettler Toledo M800 Ingold 4-Channel. The M800 transmitter was wired to a Compact DAQ (National Instruments\textsuperscript{*}) using a multi-purpose I/O module. Data was logged in LabVIEW (National Instruments) using a program developed in house. Thermocouples were calibrated using the Kaye Validator by GE Healthcare and inserted directly into the fluid inside of the biocontainer (See Figure 1).
Determination of mixing time
Mixing studies were conducted by performing a single shot addition of a 0.2% v/v of 4 M NaOH solution, then measuring the change in pH over time in 50 L of Media Simulant (2 g/L sodium bicarbonate, 6.4 g/L sodium chloride, 1 g/L Pluronic® F68, all purchased from Sigma-Aldrich®) at 37 ± 1 °C. pH was measured at two different locations within the bioreactor using two different pH probes (Inpro ISM, Mettler Toledo). The probes were located in the designated conventional probe ports, at the front of the bag. Mixing time was defined as the time required to reach 95% homogenisation characterised by a stable reading in the pH. Shot additions were made at an opening to the back right hand corner just above the fluid level.

Determination of oxygen mass transfer $k_La O_2$ (2080 method)
The volumetric mass transfer coefficient for oxygen ($k_La$) was determined using a gassing out method with nitrogen and air. Experiments were conducted using a cell culture media simulant (2 g/L sodium bicarbonate, 6.4 g/L sodium chloride, 1 g/L Pluronic F68, 50 ppm of Antifoam A, all purchased from Sigma-Aldrich) 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). $k_La$ values were then calculated between 20 and 80% of dissolved oxygen using the equation:

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

Where DO* refers to DO at saturation, DO$_{20}$ and DO$_{80}$ refers to 20% and 80% of DO* respectively, and t$_{20}$ and t$_{80}$ the times at which DO$_{20}$ and DO$_{80}$ were reached respectively. The values measured in this study are dependent on this specific method and simulate formulation used, and should be not be directly correlated directly to other compared studies using different simulant formulation composition or a different DO Oxygen mass transfer $k_La O_2$ method, such as max DO.

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:

$$pCO_2 = (pH_f - pH_{initial}) \cdot \log (pCO_{2\,initial})$$

(Bowers 2008).

A volume of 50 L of media simulant (2 g/L sodium bicarbonate, 6.4 g/L sodium chloride, 1 g/L Pluronic F68, all purchased from Sigma-Aldrich) heated up at 37 ± 1 °C was brought down to pH 6.5 using CO$_2$ gas and maintained stable for a duration of 10 minutes. A pCO$_2$ probe recorded the initial pCO$_2$. Air was then sparged through the ring sparger of the 50 L bioreactor at the desired flowrate and pH measurements recorded via a program in LabVIEW (National Instruments) until the pH of the media reached a value of 7.2. CO$_2$ concentrations were derived from pCO$_2$ values using the equations extracted from Goudar et al. (2011).
Temperature mapping

A series of eight thermocouples were placed at various locations within the biocontainer. 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 the Kaye Validator software.

The PID controller of the bioreactor received the signal from the Allegro STR 50 bioreactor PT100 temperature probe located at the bottom back of the 50 L tote in direct contact with the biocontainer. Temperature control was carried out in a cascade control manner, with the fluid temperature as master control, and the temperature control unit (TCU) temperature as slave control loop.

Temperature mapping was performed using a Lauda Integral T2200 W (Lauda-Brinkman) with the settings shown in Table 1.

Table 1
Temperature PID controller settings for Allegro STR 50 bioreactor and Lauda T2200.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Allegro STR 50 Bioreactor Temperature Controller</th>
<th>Lauda Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>I</td>
<td>500</td>
<td>50</td>
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<td>D</td>
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Results and Discussion

A wide range of P/V values can be achieved via agitation

Energy dissipation by the impeller to the fluid is the driving force for mixing, but is also regarded as one source of hydrodynamic stress on mammalian cells known as shear. As different bioreactors come with different impeller diameters, agitation speed is not regarded as a relevant indicator of the hydrodynamic forces generated in the bioreactor. Instead, power input per volume (P/V) is widely accepted as good indicators (Godoy-Silva et al. 2009).

The power delivered to the fluid by the Allegro STR 50 bioreactor was converted for different agitation speeds, based on characterisation work performed in the Allegro STR 200 bioreactor. Results show that a wide range of P/V values could be obtained, with a maximum of 300 W/m³ at maximum agitation speed. A bioreactor system, such as the Allegro STR 50 bioreactor, able to generate a wide range of P/V values, highlights a significant advantage to the system with scalability being achievable in Pall’s Allegro STR bioreactor range.

Figure 2
Calculated power input per volume for different agitation speeds.

Values were calculated from measured torque in the Allegro STR 200 bioreactor and mathematically converted to the Allegro STR 50 bioreactor. In house CHO cell culture processes were carried out at a P/V of 71 W/m³ (agitation speed of 112 rpm), which correlates with to a tip speed of 1.1 m/s.
Mixing times below 11 seconds can be achieved

The times required to homogeneously mix fluid inside a bioreactor is a simple way to assess the mixing capabilities of a bioreactor system. Long mixing times are not desirable, 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/toxic concentrated chemicals that have been dosed. Although mixing time may be less of a concern at small scale, the difficulty to mix larger volumes of media make it a critical one at large scale.

Mixing time determination was performed using two pH probes placed within the biocontainer. pH was measured following a single-shot addition of 4 M NaOH to 50 L of media simulant at 37 ± 1 °C. Results show that homogenisation could be achieved in less than 11 seconds at maximum speed.

Figure 3

Mixing time for various agitation speeds, at two different locations within the biocontainer.

Mixing time was defined at the time required to reach 95% homogenisation/stable pH following a single shot addition of 4M NaOH. Error bars represent the calculated standard deviation for three experiments. The graph includes results from mixing studies.

Oxygen transfer rate 2080 \( (k_L a) \) as high as 24 h\(^{-1} \) is achievable using a ring sparger and air

Oxygen mass transfer is a critical task for aerobic cultures, and can be challenging as oxygen is sparsely soluble and is quickly consumed by the cells. Therefore, determination of the volumetric oxygen mass transfer coefficient \( (k_L a \ O_2 \ 2080) \) is regarded as standard benchmarking method for assessing the ability of the bioreactor system to transfer oxygen from air to liquid phase. \( k_L a \) was determined for various agitation speeds, with 50 L of media simulant heated up at 37 °C ± 1 °C. During the experiment, air was sparged through the ring sparger.
Experimental $k_a$ vs predicted at 50 L working volume.

$\text{Experimental } k_a = 3600A(P/V)^{\alpha} \cdot v^{\beta}$

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.

**Precise and homogenous temperature control**

Cell culture performance is highly dependent on the capacity of generating a homogenous temperature across the biocontainer, and tight control held over a defined temperature heat up and cooldown range.

The Allegro STR 50 bioreactor system’s ability to heat up media, and maintain a desired temperature within ± 0.1 °C was tested. The biocontainer was fitted with 8 thermocouples located at different positions. A volume of 40 L of water was pumped inside the biocontainer, then topped up with ice to bring the temperature down to 4 °C, and the volume to 50 L. Temperature was set at 37 °C using the controller (see Table 1 for configuration), agitation set at 200 rpm and temperature data logged.

It was possible to heat up the media from 4 to 37 °C in 90 minutes once the temperature controller was actuated. Temperature fluctuations remained than 0.1 °C variation at the setpoint logged in the bioreactor at 37 °C. The independent process temperature values recorded for each thermocouple were all within 0.1 °C difference to the RTD element on the base of the bioreactor. No temperature gradient could be observed, suggesting that temperature was homogenous within the biocontainer across all 8 points in the reactor.
Temperature of the fluid within the biocontainer was lowered to 4 °C using ice additions. Temperature control was then set at 37 °C. Data from 8 thermocouples calibrated by an IRTD Kaye Validator probe was then recorded and plotted in Figure 6. This shows that heat up time from 4 °C to 37 °C is achieved within 95 minutes.

Due to the low heat transfer coefficient, an offset of +0.1 °C was observed between the reading of the PT100STR and the actual fluid temperature within the biocontainer. This offset should be applied to temperature settings when running bioreactor at maximum working volume of 50 L.

CO₂ can efficiently be stripped from the media using the ring sparger in the 50 L bioreactor.
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 generate as a metabolic by product water and CO₂. If in small scale bioreactors, the majority of the CO₂ can be stripped via surface aeration, the liquid to surface volume ratio generally decreases with an increase of scale, and can lead to CO₂ accumulation in large scale bioreactors.

Here we present the CO₂ stripping abilities of the Allegro STR 50 bioreactor using the ring sparger for different air flow rates. Up to 4 mol.L⁻¹.d⁻¹ could be stripped out using a maximum air flowrate of 5 L/min.

**Figure 8**

CO₂ stripping capabilities at various air flowrates.

Media pH was brought down to 6.5 using CO₂ gas, before sparging air at various flowrates and agitation speeds. Increase in pH was recorded, then pCO₂ and CO₂ removal rate calculated using the equations presented in material and methods.

**Conclusion**

The unique features of the Allegro STR 50 bioreactor lead to excellent engineering performance:

- Homogenous mixing across the biocontainer can be obtained in less than 11 seconds.
- Oxygen transfer rate as high as 24 h⁻¹ can be achieved using the ring sparger and air.
- Precise and homogenous temperature control across the bioreactor volume specifications within ± .1 °C at 50 L working volume.
- For most demanding applications, an optional open pipe sparger can be used for efficient CO₂ stripping however the ring sparger alone, proves to be efficient.
- Consistent scalability across Pall’s Allegro STR bioreactors portfolio.
References


